Study of interactions of plants and endophytic microorganisms for characterizing active molecules for biocontrol and pharmaceutical applications

Munakata Yuka

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THESE

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par

Yuka MUNAKATA

<< Study of interactions of plants and endophytic microorganisms for characterizing active molecules for biocontrol and pharmaceutical applications >>

17 décembre 2021

Composition du Jury

Agata Ptak	Professeure à l'Université de Cracovie	Rapportrice
Aziz Aziz	Professeur à l'URCA	Rapporteur, Président du jury
Béatrice Baghdikian	Maîtresse de conférences à l'Université Aix Marseille	Examinatrice
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Abstract

Since the mid-21st century, the use of synthetic chemistry has provided a range of effective solutions for plant health (pesticides) and human health (medicines). However, the widespread use of some of these solutions has raised concerns for the environment and for human health and has led to the selection of resistant populations of pathogens. These effects call into question the use of these solutions to control plant and human pathogens. It is, therefore, necessary to identify innovative and more sustainable solutions for crop protection and drug development. One strategy is to explore new biological resources such as microbial resources.

My thesis focused on bacteria that realize part of their life cycle in plant tissues and are called endophytic bacteria. These micro-organisms have interesting potential for the development of biocontrol solutions for agriculture and for the production of pharmaceutical drugs. In the two first chapters of this thesis, I explored the taxonomic and functional diversity of the endophytic communities of two plant species with remarkable properties, *Chrysopogon zizanioides,* and *Leucojum aestivum*, using metabarcoding approaches and/or isolation of bacterial strains. In particular, I have established strain libraries whose activities against wheat pathogenic fungi and, in particular, against *Fusarium graminearum* have been analyzed. Some strains affiliated to the genera *Bacillus*, *Serratia*, *Janthinobacterium,* or *Streptomyces* showed excellent inhibition of the mycelial growth of the plant pathogen in *in vitro* co-culture. Specialized metabolites produced by these strains were also studied, targeting two groups of molecules known for their antimicrobial activities or their therapeutic interest, alkaloids, and lipopeptides. Thus, a precursor of indole alkaloids was identified in two bacterial strains isolated from *L. aestivum* 'Gravity giant'. Lipopeptides, already known for their antimicrobial activities, were also identified in *Bacillus subtilis* strains isolated from vetiver roots.

Both approaches led to the identification of numerous strains but only a few interesting candidates. In the third chapter, I investigated an innovative strategy to easier target microorganisms of interest, and I focused on the protection of wheat against *F. graminearum*. The preliminary results provided a fundamental part to test the concept of the host-mediated engineering of soil and endosphere microbiota toward a resistant microbial community, leading to more efficient isolation of functional endophytes.

This work, by exploring the endophyte diversity associated with plants with particular properties, provides very rich biological material that might be used as biocontrol solutions or for the production of bioactive compounds.

Keywords: endophytes, biocontrol agents, antifungal activity, alkaloids, lipopeptides, host-mediated-plant-microbiome engineering

Résumé français

Depuis le milieu du XX^{ième} siècle, le recours à la chimie de synthèse a permis de disposer d'un ensemble de solutions efficaces pour améliorer la santé des plantes (produits phytosanitaires) et la santé humaine (médicaments). Cependant, l'utilisation généralisée de certaines de ces solutions (produits phytosanitaires et antibiotiques), a eu des effets non intentionnels sur l'environnement et a conduit à la sélection de populations d'agents pathogènes résistants. Ces effets mettent en péril le recours à ces solutions pour maitriser les agents pathogènes des plantes et de l'homme. Il est donc devenu nécessaire d'identifier de nouvelles solutions, plus durables, pour la protection des cultures et le développement de médicaments, en explorant de nouvelles ressources biologiques et notamment des ressources microbiennes. Ma thèse s'est concentrée sur des bactéries qui effectuent tout ou partie de leur cycle dans les tissus de la plante et qui sont appelées des bactéries endophytes. Les bactéries endophytes présentent des potentialités intéressantes pour le développement de solutions de biocontrôle et production de bioactifs pharmaceutiques. Dans les deux premiers chapitres de cette thèse, j'ai exploré, par des approches de métabarcoding et/ou isolement des souches bactériennes, la diversité taxonomique et fonctionnelle des communautés endophytes de deux espèces végétales aux propriété remarquables, *Chrysopogon zizanioides* (le vétiver) et *Leucojum aestivum* (la nivéole d'été). J'ai ainsi constitué des banques de souches de bactéries endophytes dont les activités antifongiques vis-à-vis de champignons pathogènes du blé et, en particulier vis-à-vis de *Fusarium graminearum*, ont été analysées. Certaines souches affiliées aux genres *Bacillus*, *Serratia*, *Janthinobacterium* ou *Streptomyces* ont montré une excellente inhibition de la croissance mycélienne du phytopathogène en co-culture *in vitro*. Des métabolites spécialisés produits par ces souches ont également été étudiés, en ciblant deux groupes de molécules connues pour leurs activités antimicrobiennes ou leur intérêt thérapeutique : les alcaloïdes et les lipopeptides. Ainsi, un précurseur des alcaloïdes indoliques a été identifié dans deux souches bactériennes isolées *de L. aestivum* 'Gravity giant'. Des lipopeptides, déjà connus pour leurs activités antimicrobiennes, ont également été identifiés dans des souches de *Bacillus subtilis* isolées des racines de vétiver. Ce travail, en explorant la diversité microbienne associée à des plantes aux propriétés particulières, a fourni un matériel biologique très riche qui pourra être utilisé pour caractériser de nouvelles solutions de biocontrôle ou pour la production de composés bioactifs.

Les travaux réalisés dans les 2 premiers chapitres m'ont amené à isoler de nombreuses souches de bactéries dont seulement certaines sont potentiellement intéressante. Dans le troisième chapitre j'ai réalisé un travail préliminaire et innovant visant à enrichir un sol en

bactéries potentiellement intéressantes. Ce travail apporte la preuve de concept de l'intérêt de s'appuyer sur le génotype de l'hôte et la suppressivité du sol pour guider le recrutement de microorganismes bénéfiques par la plante. Cette thèse pourrait ainsi fournir de nouvelles perspectives en termes de stratégies de sélection de solutions microbiennes de biocontrôle.

Mots clés : endophytes, agents de biocontrôle, activité antifongique, alcaloïdes, lipopeptides, ingénierie microbiologique végétale à médiation par l'hôte.

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Table of contents

ABSTRACT 1

RÉSUMÉ FRANÇAIS 2

ACKNOWLEDGMENT 4

TABLE OF CONTENTS 7

FIGURES 9

TABLES 12

ABBREVIATIONS 13

THE BIOMOLECULE PROJECT 14

INTRODUCTION 14

STATE OF THE ART 18

A) Plant microbiota 18

B) Definition of endophytes 20

C) Evolution of the research field related to endophytes 20

D) Ecology of endophytes 21

E) Approaches to characterize endophytic bacteria 23

F) Endophyte functions: Goodwill practices 26

G) Plant growth promotion 27

H) Plant bioprotection 29

I) Plant-microbe interactions 30

J) Alkaloids 33

K) Lipopeptides and polyketides 33

L) VOCs 35

M) Other compounds 36

N)How to modulate the function of endophyte communities 40

References 43

OBJECTIVES 54

CHAPTER 1 56
"Isolation and Characterization of endophytic bacteria from *Leucojum aestivum* 'Gravity giant'"

CHAPTER 2 89
"Isolation and characterization of endophytes from *Chryzopogon zizanioides* （L.） Roberty roots"
Part 1 90
Part 2 104

CHAPTER 3 146
"Toward an innovative way to improve the selection of biocontrol agent candidates of endophytes: microbiome engineering"

GENERAL DISCUSSION AND PERSPECTIVES 164
A) Taxonomic composition of culturable endophytes and their functional analysis . 164
B)Investigation in the function of endophytic microbiota 166
C)Microbiome engineering 167

GENERAL CONCLUSION 169

RÉSUMÉ EN FRANÇAIS 172

ANNEX 185

Figures

Introduction and the state of the arts Page

Fig. 1	The 17 sustainable development goals of the United Nations	.....	16
Fig. 2	Plant microbiota compartments	.....	19
Fig. 3	Specific organ (nodules) for symbiosis of Lotus japonicus with rhizobia	.....	20
Fig. 4	Multi-dimensional analysis on 40 top differentiated words (except verbs) in the text mining of 9,913 research article titles regarding “endophyte” in Web of Science from 1953 to 2021 (August), analyzed by KH coder (Higuchi, Koichi, 2017)	.....	21
Fig. 5	Work flow of isolation and characterization of endophytic strains in the culture dependent way	.....	24
Fig. 6	Conserved and hypervariable regions of 16S rRNA genes and primer positions for amplicon sequencing analysis of bacterial communities	.....	25
Fig. 7	Beneficial traits of endophytic bacteria regarding plant growth promotion and plant protection from plant pathogens	.....	26
Fig. 8	Plant hormones that can be produced and modulated by endophytic bacteria	.....	29
Fig. 9	Alkaloids produced in endophytic bacteria	.....	33
Fig. 10	Lipopeptides produced in endophytic bacteria	.....	35
Fig. 11	VOCs in endophytic bacteria, with antifungal activities	.....	36

Chapter 1

Fig. 0-1	Representative Amaryllidaceae alkaloids and Amaryllidaceae plant family	.....	56
Fig. 0-2	Overview of the isolation of endophytic bacteria from L. aestivum plant tissues	.....	57
Fig. 1	A sampled plant (plant 2) and the definition of different tissues in this study and colony forming unit (CFU) per 1 g of each fresh materials 20 days after isolation	.....	64
Fig. 2	Principal coordinate analysis (PCoA) with Bray-Curtis distance of the L.aestivum bacterial collections at the genus level	.....	66
Fig. 3	Relative abundance of each phylum in the bacterial collection of the different plant tissues	.....	67
Fig. 4	Venn diagram of Shared and unique bacterial genera in L. aestivum plant tissues	.....	67
Fig. 5	Dual-culture test of representative strains tested.	.....	69
Fig. 6	Correlations between antifungal activities against *F. graminearum* and *M. nivale* , colored by taxonomical affiliation at the phylum level	.....	70
Fig. 7	Growth inhibition rate against *F. graminearum* (Fg) and *M. nivale* (Mn) by genus of bacterial isolates	.....	70
Fig. 8	Growth inhibition test of cell-free supernatant and methanol extracts of 3 endophytic bacteria, LaBFB3301, LaBFS1102, LaBFS2103, against mycelia of *F. graminearum* (Fg) and *M. nivale* (Mn) after 3-day co-culturing.	.....	73
Fig. 9	Venn diagram of shared and unique peaks of enriched metabolites	.....	74
Fig. 10	Fig. 10 | Ion chromatogram of m/z 162.0900 ± 0.02 for tryptophol found in LaBFB3301 and LaBFS2103	.....	74
Supplemental figure 1	Plant materials in this study	.....	77
Supplemental figure 2	Antifungal activities against *F. graminearum* were differed between the groups of shoot and root bacterial collections, but no difference in the antifungal activities for *M. nivale* .	.....	77
Supplemental figure 3	Ion chromatograms of m/z 288.1266 ± 0.01 for lycorine and 288.1564 ± 0.01 for galanthamine	.....	78

Supplemental figure 4	Identification of tryptophol in the extract of LaBFB3301 and LaBFS2103	.....	79

Chapter 2 page

Fig. 0	Overview of relationships between Vetiver root essential oil and endophytic bacteria	.....	89

Chapter 2 Part 1

Fig. 1.	PCoA plot based on weighted UniFrac distance of Vetiver samples. Points represent individual plants	.....	94
Fig. 2.	Taxonomical compositions of 13 phyla (A) and dominant 12 genera (B) in bacterial endophyte communities of Vetiver roots in 5 locations.	.....	94
Fig. 3.	Venn diagram of the number of shared genera over locations and specific genera for each location. The shown percentages are percentage of shared or specific genera against total number of genera found through all samples.	.....	94
Fig. 4.	Relative abundance of dominant genera in four phyla, in Vetiver root endophyte communities.	.....	95
Fig. 5.	Taxonomical compositions of 15 genera in functionally selected bacterial endophyte communities of Vetiver roots in 5 sites.	.....	96
Fig. 6.	Venn diagram of the number of common or unique genera in the metabarcoding data and the culturable communities.	.....	96
Fig. 7.	PCoA ordination of isolated endophyte communities with bioactivities against *F. graminearum* Points represent individual plants, and ellipses show a 95 % confidential zone of each locational group.	.....	97
Figure S1	Geographical origins of Vetiver roots used for the present study.	.....	101
Figure S2	Rarefaction curves of the bacterial community of Vetiver root samples	.....	101
Figure S3	Venn diagram of the number of shared or specific OTUs for each location	.....	102
Figure S4	Taxonomical compositions of dominant 14 families in bacterial endophyte communities of Vetiver roots in 5 locations	.....	102
Figure S5	Taxonomical compositions of 3 phyla in functionally selected bacterial endophyte communities of Vetiver roots in 5 sites	.....	103

Chapter 2 Part 2

Fig. 1	Dual culture test of Vetiver endophytes and *F. culmorum* (a) and *F. oxysporum* (b)	.....	111-112
Fig. 2	Growth inhibition rate of Vetiver endophytic strains against (a) *F. culumorum* (Day 6) and (b) *F. oxysporum* (Day 11)	.....	113
Fig. 3	Growth inhibition rate of the cell-free supernatant of Vetiver endophytic strains against *F. graminearum*	.....	115
Fig. 4	Growth inhibition rate of the cell-free supernatant of Vetiver endophytic strains against *E.coli* (a) and *S. cerevisiae* (b)	.....	116
Fig. 5	MALDI-TOF mass spectra of supernatant samples of *B. subtilis* (strain 22, 23 26, 28) and *B. tequilensis* (strain 25)	.....	120
Fig. 6	Primary structures of surfactins and plipastatins (Caulier et al., 2019)	.....	122
Supplemental figure 1	The layout of 96 well plates for growth inhibition test against *E. coli* and *S. cerevisiae*	.....	124

Chapter 3 page

Fig. 0	Global image of the wheat experiment	.....	148
Fig. 1	The spore production of *F. graminearum*	.....	150
Fig. 2	Measurement of the colored region of the primary root of the inoculated seedlings	.....	151
Fig. 3	Inoculation of wheat seedlings with *F. graminearum* macroconidia	.....	152
Fig. 4	Growing wheat plants in the artificial climate chamber	.....	152
Fig. 5	Visual indicators of SbSI and RSI	.....	153

Fig. 6 In vitro inoculation test of the primary root of wheat seedlings against *F. graminearum* 154
Fig. 7 The sampled plants inoculated with *F. graminearum* spore suspension (Fg inoculation) via root or soil 155
Fig. 8 Physical traits and FRR symptom indices of the samples plants 156
Fig. 9 Symptoms on stem and root 157
Fig. 10 Appearance of the plants inoculated with *F. graminearum* spore suspension via root 158
Fig. 11 Comparison of shoot and root dry weight between non inoculation (Mock) and Fg inoculation (Fusa) 159
Fig. 12 PCA of the measurement of Mock and Fg-spore inoculated plants. 160

General discussion and perspectives

Fig. 1 Strategies to identify modes of actions and antagonistic molecules from remaining isolates. 166

Tables

Introduction and the state of the arts			Page
Table 1	Bioactive compounds isolated from endophytic bacteria	.....	37-39

Chapter 1			
Table 1	Taxonomical compositions of the bacterial collection isolated from *L. aestivum* Gravity giant	.....	65
Table 2	List of strains of which extract (possibly) reacted to Dragendorff's reagent	.....	68
Supplemental Table 1	Bacterial isolates of *L. aestivum* endophytes	.....	80
Supplemental Table 2	Bacterial genera found as endophytes in Amaryllidaceae plants	.....	84

Chapter 2			
Chapter 2 Part 1			
Table 1	Alpha diversity of Vetiver bacterial communities in five locations using the indices of richness	.....	94
Table 2	Summary of isolation and antagonistic activities of endophytic isolates of Vetiver roots from 5 different locations.	.....	96
Table 3	Bacterial endophyte isolates that exhibited more than 70 % of inhibition activity against *F. graminearum* mycelia	.....	97
Table S1	OTU table of bacterial communities of Vetiver root samples from five different locations	.....	ANNEX
Table S2	Population of antagonistic Vetiver endophytic isolates against *F. graminearum*	.....	102
Table S3	A list of bacterial isolates exhibiting more than 50% of inhibition activity against *F. graminearum* mycelial growth	.....	ANNEX
Chapter 2 Part 2			
Table 1	Summary of antimicrobial activities of the Vetiver endophytes	.....	110
Table 2	Profile of peaks detected by MALDI-TOF	.....	117-119
Table 3	Profile of lipopeptide signals isolated by MALDI-TOF for the supernatant of 5 Bacillus subtilis strains after 48- or 72-hour cultivation	.....	121
Table 4	Possible assignment of lipopeptides detected in Bacillus subtilis (strain 22, 23 26, 28) and B. tequilensis (strain 25)	.....	122

Chapter 3			
Table 1	Chosen varieties of soft wheat and their resistant level against fungal diseases, summarized based on the database of ARVALIS	.....	149

Abbreviations

Abbreviation	Meaning	Abbreviation	Meaning
ACC	1-aminocyclopropane-1-carboxylate	NGS	Next Generation Sequencing
AHL	N-acyl homoserine lactone	NRP	Non-ribosomal peptide
BLAST	Basic Local Alignment Search Tool	NRPS	Non-ribosomal peptide synthase
CFU	Colony Forming Unit	PCoA	Principal Coordinate Analysis
DNA	deoxyribonucleic acid	PCR	Polymerase Chain Reaction
DW	dry weight	PDA	Potato Dextrose Agar
FCR	Fusarium Crown Rot	PERMANOVA	permutational multivariate analysis of variance
FHB	Fusarium Head Blight	Rf	Retention factor
FRR	Fusarium Root Rot	PK	Polyketide
FW	fresh weight	PKS	Polyketide synthase
HMPME	host-mediated-plant-microbiome engineering	RNA	Ribonucleic acid
		rRNA	ribosomal RNA
ISR	Induced Systemic Resistance	RSI	Root Symptom Index
JA	jasmonic acid	SA	salicylic acid
LC	Liquid Chromatography	SbSI	Stem-base Symptom Index
LP	Lipopeptide	TLC	Thin Layer Chromatography
MALDI	Matrix-Assisted Laser Desorption/Ionization	TOF	Time of Flight
		TSA	Tryptic Soy Agar
MBB	Mung Bean Broth	TSAC	Tryptic Soy Agar containing cycloheximide
MH	Mueller-Hinton		
MS	Mass spectrometry	VOCs	Volatile Organic Compounds
NB	Nutrient Broth	WHC	Water Holding capacity
		YPG	Yeast Extract-Peptone-Glycerol

The Biomolecule project

(https://lue.univ-lorraine.fr/fr/ressources-naturelles-et-environnement/projet-lue-impact-biomolecules)

This Ph.D. project was included in a large research program called LUE Biomolécules, in which different research departments of the Université de Lorraine were involved. The main goal of my project was to investigate the microbial diversity present in different plants. The endophytic microbiota of these plants can be considered as a vast and endless reservoir of small living factories able to produce an outstanding diversity of molecules. In the frame of the project, we focused on three different plants known to be of interest in the pharmaceutical field (Laboratoire Lorrain de Chimie Moléculaire, L2CM) and the agronomic field (Laboratoire Agronomie et Environnement, LAE).

Introduction

Microbes can be found everywhere on Earth, even in extreme environments. Some of them are known as pathogens for humans, animals, insects, and plants. However, many studies made evidence that they established complex and mutualistic interactions with plants and animals, which are helpful to provide nutrients and protection against bioagressors. Furthermore, microbes are outstanding cell factories able to produce an unlimited number of compounds. Therefore, they were used for diverse applications such as fermentation processes and, more recently, for producing natural molecules (like antibiotics) or original molecules through genetic engineering strategies. Nowadays, these later molecules are used as active ingredients or leading medicinal compounds [1].

Although many aspects were already studied during the last century, microbes remain largely unknown. They might be able to provide solutions to meet the major challenges of our society. In 2012, the European Commission adopted the strategy called Innovating for Sustainable Growth: Bioeconomy for Europe. The concept of bioeconomy, defined in this strategy, refers to industrial fields contributing by their processes to reduce our dependency on fossil fuels, limit climate change, manage sustainable natural resources, and ensure food security. In the bioeconomy concept, one key strategy relies on the biological production of useful compounds originating from plants or microbes to replace fossil-fuel-dependent processes. In this manuscript, I will approach this concept through two different application fields: agriculture

and pharmacy.

Agriculture

In 2015, the United Nations defined 17 sustainable development goals that we should reach to suppress poverty all over the world, protect the planet, and ensure prosperity for all (Fig. 1). These goals include the necessity to significantly raise the food production for a world population expected to increase and reach 10 billion human beings by 2075 (United Nations, 2020). This target is in contrast with the steady reduction of the agricultural land areas that were observed during the last centuries (http://www.fao.org/faostat/). To face this important concern, it is necessary to find innovative solutions to improve the productivity in agriculture and/or to increase arable areas.

The "Green Revolution" that occurred after the second world war provided some solutions that were widely used for more than fifty years. This revolution relies on using chemical inputs (such as pesticides) that significantly increase the productivity of the main agricultural crops. Although these approaches solved many problems, they also originated adverse effects, especially on ecosystems (on biodiversity, for example) and on human health. Awareness of these problems has prompted public authorities to consider new ways for significantly reducing the use of these chemical molecules. Movements to tighten the regulation of pesticides have accelerated, particularly in France through the Ecophyto plan (https://agriculture.gouv.fr/ecophyto). This project encourages the development of more sustainable farming systems that are less dependent on chemical inputs. This plan suggests reducing the use of these inputs by 1) setting up more adapted plants through breeding programs, 2) developing modern agronomical tools such as drones to precisely target areas that need to be treated, and 3) using more environmental-friendly solutions, including biocontrol strategies (BCs). Regarding this last point, it is important to note that many microbial-based biocontrol solutions have already been commercialized for facing disease and/or insect attacks but are restricted to a few microbial genera, including only *Bacillus* and *Trichoderma*[2]. These solutions are generally not used as they should be and are often less efficient than chemicals. Given the outstanding diversity of microbes that is described in the scientific literature, it might be of higher interest to investigate the potential of new microbial resources to set up original alternative solutions.

Fig. 1 |The 17 sustainable development goals of the United Nations
(https://www.un.org/sustainabledevelopment/)

Pharmacy

For thousands of years, humankind has healed itself by using various sources of medicines such as plants or microorganisms. The resources used are either organisms in their entirety (such as plant tissues) or extracts from these organisms (including various biomolecules). For example, a study carried out in 2005 listed 30,000 antibiotics (up to 2002), among which 16,500 were derived from microbes, especially actinobacteria, whereas 11,700 compounds were isolated from plants [3]. The discovery of those molecules to target pathogens like bacteria, fungi, and even viruses, provided an efficient weapon to fight infections such as tuberculosis by using streptomycin [4]. However, during the past century, these molecules were overused, which led to the occurrence of drug resistance against target pathogens and raised a significant problem in our society. Therefore, it is urgent to discover new antimicrobial compounds to diversify healing strategies and bypass the resistance that appears for commonly used molecules.

In addition to the antibiotic resistance concern, it is also noteworthy that human beings may also exploit some of these biomolecules for other applications. It has been described that some of these small molecules display antitumor or immune-suppressors properties. Some of them can be considered enzyme inhibitors or hypocholesterolemic drugs [5]. Our aging society increased the ratio of non-infectious diseases in the major global causes of death, such as ischaemic heart disease, cancers, Alzheimer's disease, and diabetes mellitus (World Health Organization, 2021). New drugs are, therefore, required, and all possible tracks should be investigated. For example, study rare and minor bacterial genera to find novel compounds [6,7].

Interesting reports obtained through studies on heart diseases, diabetes, and some cancers revealed that the diseases might be associated with human bacterial communities (especially gut microbiome) [8,9]. Such studies encourage many scientists to deeper investigate the relationships between the human microbiome compositions and several diseases [9]. An outcome of these investigations may allow new clinical treatment relying on the modulation of the human microbiome. For example, intake of probiotics (lactic-acid bacteria in yogurt, for example) is now an accepted habit for health maintenance. Other examples of innovating clinical treatment were also described, such as modulating the gut microbiome via prebiotics or transplanting the fecal microbiota [10]. It is now essential to understand the molecular basis of these properties. To get answers, it is also important to address the modes of action of the compounds produced in human-beneficial bacteria against various diseases.

State of the art

A) Plant microbiota

A.1 Definition of plant holobiont and related terms

In nature, plants are not living in a sterile environment and are daily in interactions with many microorganisms such as Archaea, bacteria, fungi, oomycetes, and viruses. These interactions occur in the close vicinity of the plant as well as inside the plant tissues. The relationship between plants and microorganisms is categorized as beneficial, pathogenic, or commensal. They rely on carbon resources from plants, such as sugars or amino acids, for example [11]. In turn, interactions with microorganisms can provide benefits to the plants and contribute to their adaptation to a changing environment. These plant-associated microbial communities can, for example, improve plant nutrient acquisition. Because of these tight interactions, plants and their associated microbes can be considered as a meta-organism named the plant holobiont [12]. The microbes associated with the host plant constitute the microbiota, and their collective genomes constitute the microbiome [13].

A.2 Compartments of plant microbiota

Plants interact with microbes belowground and aboveground, and microbes can colonize plant surfaces and/or inner tissues (Fig. 2). In the belowground compartment, roots interact with soil microbial communities from the rhizosphere that is the soil under the influence of living roots [11,14]. Plants also interact with microbes colonizing the plant surface called epiphytes (Fig. 2).

Some of the microbes can also enter and develop into the plant and are called endophytes. Among endophytic microbes, some mutualistic microbes such as Rhizobia or arbuscular mycorrhizal fungi are present intracellularly by forming particular structures such as nodules or arbuscules, respectively (Fig. 3).

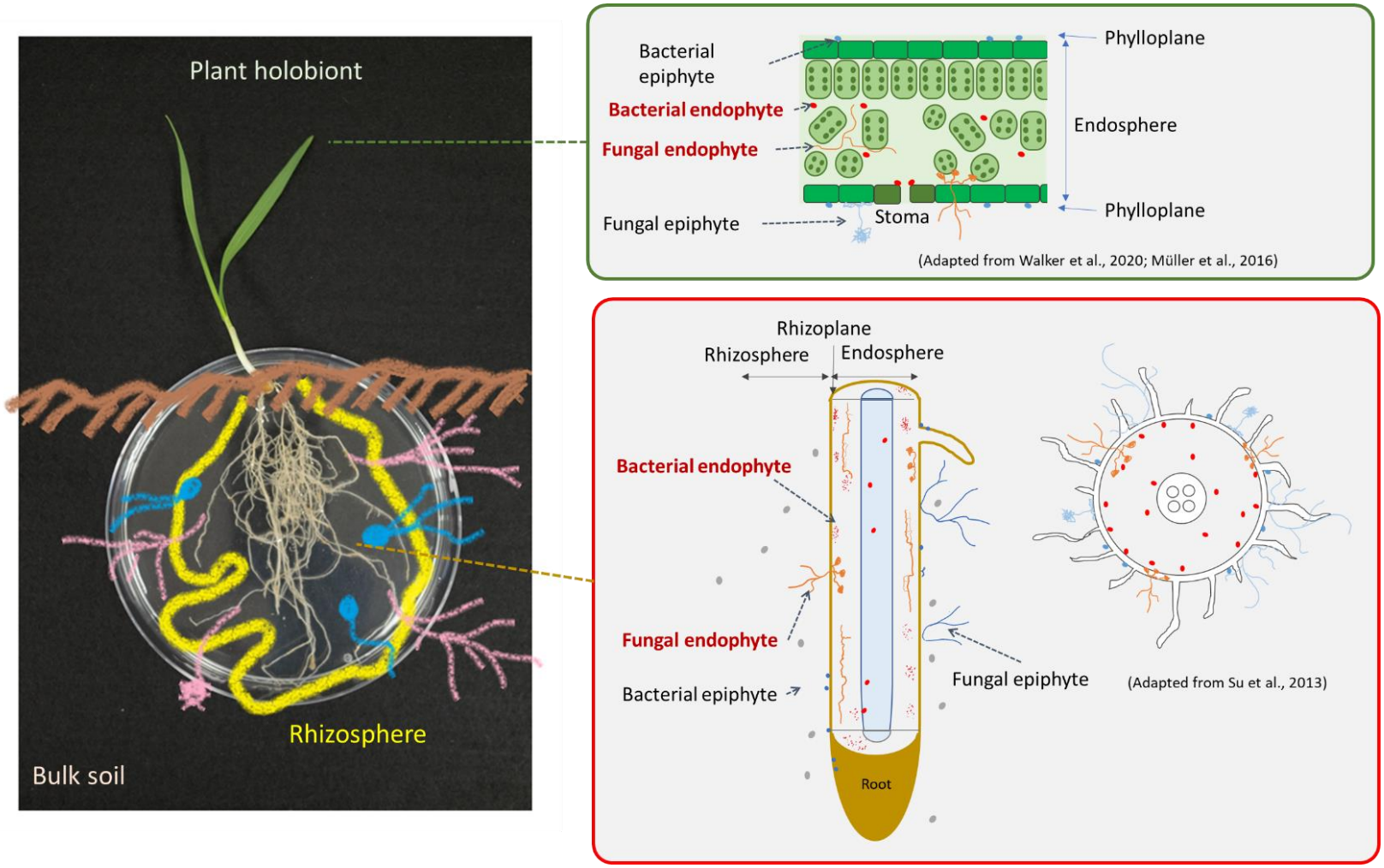

Fig. 2 | Plant microbiota compartments
Anatomic illustrations were based on Müller et al., 2016; Su et al., 2013; Walker et al., 2020.

Fig. 3 |Specific organ (nodules) for symbiosis of *Lotus japonicus* with rhizobia (indicated with yellow arrows)

B) Definition of endophytes

The word "endophyte" is derived from endon (within) and phyte (plants) [15]. These microbes were found in all the plant organs (roots, stems, leaves, flowers, fruits, and seeds). As stated in the previous paragraph, endophytic microorganisms include fungi, bacteria, oomycetes, archae, protists, and viruses. However, most studies concerning endophytes focused on fungal and bacterial communities since they account for the major part of the endophytic microbiota [16]. Endophytic microbes have received various definitions through the past 50 years. The most frequently used one is the one given by Orlando Petrini in 1991 [17], which defined endophytes as microbes colonizing plant tissues without any visible symptoms during part or whole life cycles. This definition has been revised many times, and the most recent definition has been established by Hardoim and collaborators in 2015 [18]. For these authors endophytes "correspond to all organisms habiting inside plant tissues, regardless of their outcome on host plants [18]. In the present work, we will adopt the definition of Petrini.

C) Evolution of the research field related to Endophytes.

Plant-microbe interactions have been intensively studied for many decades. A text mining [19] done in the Web of Science database using "endophyte" as a key word led to the identification of 9,913 articles in a range from the 1950s to 2021 (Fig. 4). The main studies concerning endophytes first focused on mutualistic interactions, such as the nitrogen-fixing symbiosis between Rhizobia and leguminous plants or the arbuscular-mycorrhizal (AM) symbiosis (Fig. 3 and 4).

The analysis showed a transition in endophyte research close to the 1990s. Words such as tall fescue, ryegrass, pasture, and grazing, appeared in the bibliography. A number of studies were related to fungi affiliated with *Neotrophodium* sp. that colonize perennial grasses and have been

shown to produce specialized metabolites, in particular alkaloids [20]. This discovery shed light on this particular reservoir of microbes and led to a significant increase in studies on this topic. Since then, this new field of research has attracted many new scientists raising a wide diversity of concerns such as the ecology and diversity of these microbial communities. Therefore, the research trend shifted towards the analyses of the diversity of endophytic communities, their potential to protect plants against bioagressors (biocontrol), to improve plant growth (biostimulant), or to produce bioactive compounds such as antimicrobial compounds (Fig. 4). Finally, since the 2010s, this research field is mainly associated with the word "microbiome" (Fig. 4), which shows that scientists do not consider only microbes as individuals but are starting to investigate communities and interactions that occur between them.

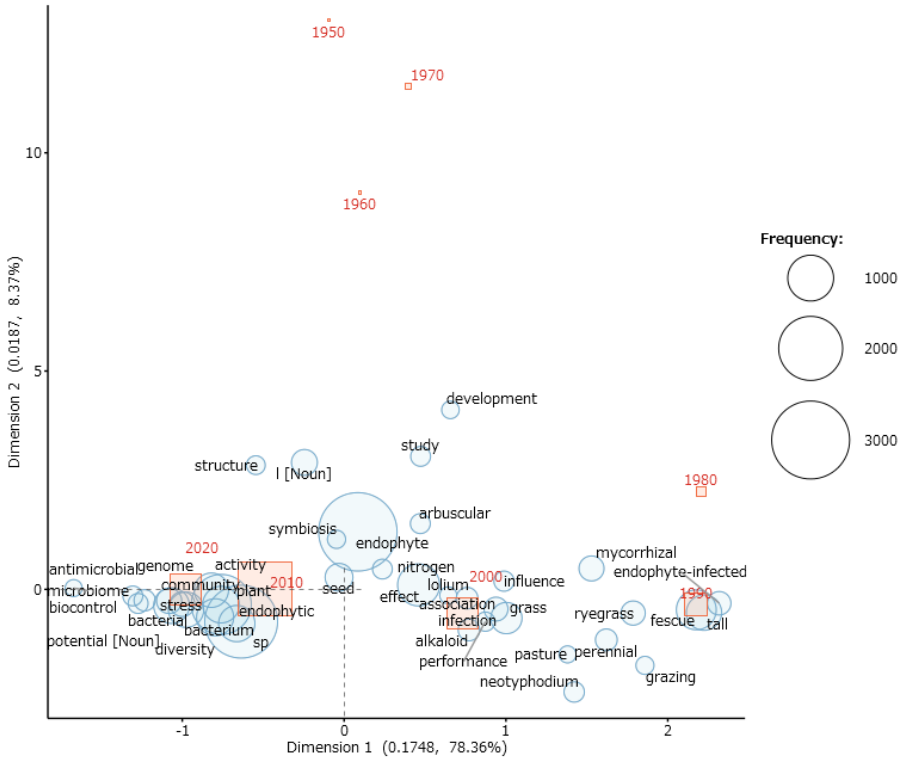

Fig. 4 | Multi-dimensional analysis on 40 top differentiated words (except verbs) in the text mining of 9,913 research article titles regarding "endophyte" in Web of Science from 1953 to 2021 (August), analyzed by KH coder (Higuchi, Koichi, 2017)

D) Ecology of endophytes

D.1 Origin of endophytic microbes

Endophytic microbes can colonize plants both from the air and the soil that is surrounding roots (rhizospheric soil, horizontal colonization). However, the soil is probably the most important reservoir for plant colonization and could explain more than 40% of the diversity of bacterial communities [21]. From the rhizospheric soil, bacteria enter plant at root emergence zones and injured points. They can also invade intact roots by producing cell-wall-degrading enzymes [15].

Another possibility relies on the vertical transmission of endophytes by seeds. The importance of seed endophytes in the final community composition after germination is still unclear. In any case, early-colonizers have a large advantage regarding nutrient and space acquisition in the plant endosphere [22,23]. Bacterial endophytes are thought to be brought by water flow from roots to leaves [16]. Bai and collaborators showed that root and leaf shared some endophytic compositions [24].

D.2 Major components of bacterial endophytes

The majority of bacterial endophytes are affiliated to the Proteobacteria phylum, followed by Actinobacteria, Firmicutes, and Bacteridetes [16]. Acidobacteria phylum is dominant in the soil and in the rhizosphere but this phylum is not well represented in the endophytic compartment [16,25]. Proteobacteria endophytes are represented by different genera such as *Gluconobacter*, *Azospirillum*, *Gluconacetobacter*, *Burkholderia*, *Azoarcus*, *Herbaspirillum*, *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Serratia*, *Stenotrophomonas* [26,27]. Among them, *Burkholderia phytofirmans* and *Pseudomonas fluorescens* are well known for their benefits in host plant growth and protection. In the Actinobacteria phylum, *Streptomyces* is reported as a representative genus, already known to produce a number of antibiotics [3]. Nevertheless, minor Actinobacterial genera were also found as endophytes [28]. In Firmicutes phylum, *Bacillus* genus is well studied and these bacteria are mainly affiliated with *B. amyloliquefaciens*, *B. subtilis*, *B. thuringiensis* species [29]. Finally, Bacteroidetes include members from Chitinophagaceae and Flavobacteriaceae families [27,30,31].

D.3 Factors influencing the composition of the endophytic communities

The development of the next generation sequencing (NGS) technologies paved the way to analyze the community structure of plant microbiomes and to network analyses between microorganisms and plant. Many aspects were investigated in order to identify the main factors influencing the assemblage of endophytic microbes, such as abiotic factors (soil types, climates, geography, nutrient conditions, and soil pollution …), host plant (genotypes, plant tissues, plant developmental stages, plant metabolites…). Other factors have also been analyzed such as biotic stresses (plant diseases, herbivory) or some agricultural practices (fertilization, pesticides, crop successions, tillage, organic farming…) [16,26].

Depending of the scale of analysis, geography and plant compartment seem to be the most important factors influencing the structure of endophytic microbial communities [32]. For example, the structure of the root-associated bacteriota of rice differed depending on geographical origin, followed by the tissue compartments, the developmental stages of the plant and the agronomical practices (organic vs. conservative) [33]. In another study, it has been shown that variation in the barley root-associated bacteriota was explained by the root-microbiota compartment (bulk soil, rhizosphere, root) (with 22% of the variance explained) but also bythe

host genotype (domesticated vs. wild, 5.7% of the variance explained) [30]. These insights are consistent with another study considering maize [34]. In this study it was demonstrated that soil origin and the plant compartment have a significant effect on the composition of the endophytic bacterial communities whereas little effect was observed with the host cultivar [34].

Although the influence of the host genotype is much lower than that of the soil and the plant compartment, some root morphological traits such as the cell wall or the root diameter were reported to alter the root-associated bacterial communities [35,36]. Since many endophytes originate from soil, it also sounds reasonable to imagine that different root exudates, such as sugars, amino acids, and phenolics, could affect the endophytic communities through attraction processes [37]. Several plant molecules were described as modulators of microbial compositions in the rhizosphere and in the root compartment. For example, salicylic acid [38], flavonoids [39], triterpenes [40], glucosinolates [41], coumarins [42] and alkaloids [43,44] have been described as plant molecules influencing the composition of the root associated microbiota .

E) Approaches to characterize endophytic bacteria

The study of endophytic microbial populations requires access to the internal tissues of plants and, in parallel, getting rid of surface microbes. This sounds evident but is obviously not as easy to be implemented. Several aspects have to be addressed, as I will show in the following part of this manuscript.

E.1 Culture dependent approaches

The first significant concern to isolate endophytes is to make sure that no contamination by surface microbes occurs [45]. This crucial part of the work is highly dependent on the plant material, such as the tissues but also the plant species. Different protocols (Fig. 5) for surface sterilization have been described in the scientific literature. These protocols were set up not only for isolating microbes but also to develop *in vitro* culture of many different plant models. Generally, after the disinfection procedure (mostly done with ethanol and/or hypochlorite), the plant material is extensively washed with sterile water. The sterility is verified by plating the last rinsing water on a solid microbiological medium or rolling the surface of the treated plant pieces on the medium, and these plates are observed after a few days of incubation. Although the absence of colonies is not a definitive proof of the sterility, it provides a piece of good information about it. Once these preliminary treatments of the tissues are established, the second step aims to extract the endophytes from the different plant organs. This is mainly done by crushing the tissues using sterilized pestles and spreading the extract on a medium. Different media such as Nutrient Agar (NA), Luria – Bertani (LB) media, Tryptic Soy Agar (TSA) can be used for this purpose. However, this approach does not give a “complete picture” of the

diversity of endophytes. Indeed, it is well-known that more than 85% of the microbes present in the environment cannot be cultured on such artificial media [46]. In order to set up an optimized media to improve the isolation of culturable endophytes, some scientists include plant extracts in their recipes to mimic the microbe native habitat [47,48]. Although the idea is interesting, the scientist had to face some problems such as the concentration and nature of plant extracts to be added to the culture medium. Another approach is the limiting dilution technique which can help to isolate slow growers. This strategy is used to incubate a single bacterium in small wells to avoid masking slow-growing strains by colonies of rapid-growing strains [49]. Finally, in 2018, Kwak and his collaborators explored another way to improve the medium. By using information obtained from the whole genome sequencing of the microbiome, they optimized the composition of the culture medium for bacterial target features [50].

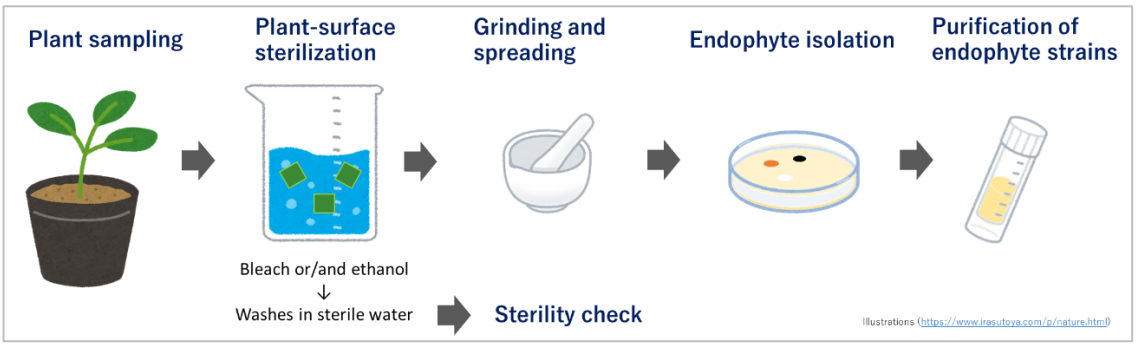

Fig. 5 | Work flow of isolation and characterization of endophytic strains in the culture dependent way

E.2 Culture-independent way

Compared to culture-dependent methods, approaches based on the analysis of DNA help to feed the knowledge about the composition of endophytic communities. A general strategy consists to analyze some specific regions of the bacterial genome containing conserved genes but with sufficient variations through evolution to be used as taxonomical markers. These specific regions are amplified by PCR using crude extracts containing the plant DNA and all the microbial DNA as template. The resulting mix of amplicons is finally digested and analyzed on high-resolution gel or sequenced using Next Generation Sequencing technologies.

In general, the target gene is the 16S ribosome RNA gene (16S rDNA). This gene consists of conserved regions and nine hypervariable regions (V1 to V9) (Fig. 6). This gene has been set up for a long time to characterize rhizospheric bacterial communities. The target regions were generally V3-V4 or V1-V3 [33,44,50]. Concerning the analyses of endophytic communities, the template contains mainly plant DNA and only a few percentages of microbial DNA. When

targeting the conventionally used regions with such DNA mixture, the sequences are often accounted for by amplifications of chloroplastic and mitochondrial DNA of the host plant which makes the analysis difficult to be done. Therefore, new targets were defined within V5-V7 regions, which enables the removal of the amplification of the organellar DNA (Fig. 6) [51]. These regions are now generally recommended for the analysis of bacterial endophytic communities [49].

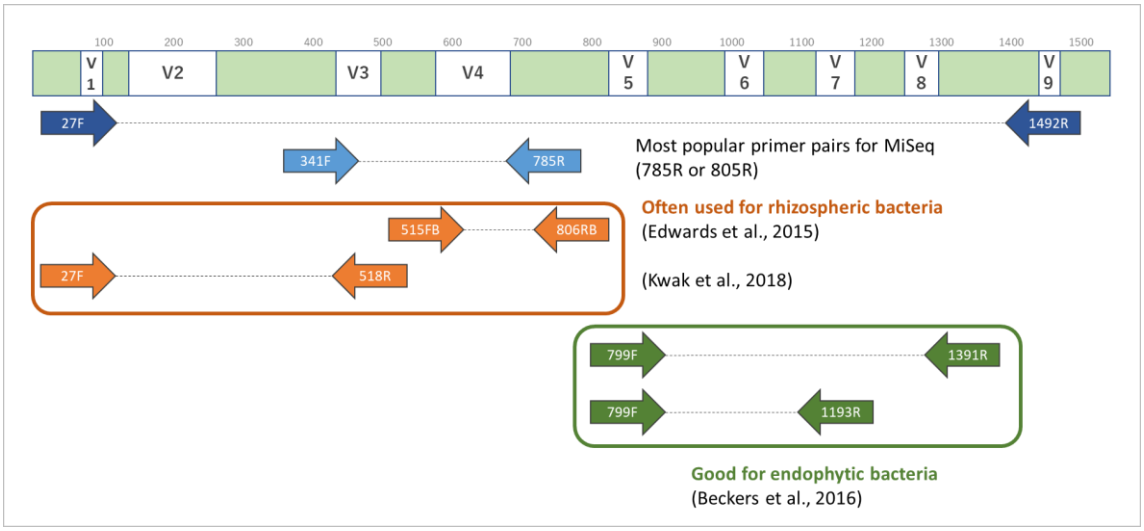

Fig. 6 | Conserved and hypervariable regions of 16S rRNA genes and primer positions for amplicon sequencing analysis of bacterial communities
Arrows represent universal primers for 16S rDNA.

F) Endophyte functions: goodwill practices

Endophytes are known to receive benefits from host plants, such as nutrient supply (mainly C, N …) and protection from UV. In turn, the endophytes can enhance plant fitness to various environments involving abiotic and biotic stresses by promoting growth and maintaining health (Fig. 7). In this section, I will overview the beneficial traits of endophytic bacteria.

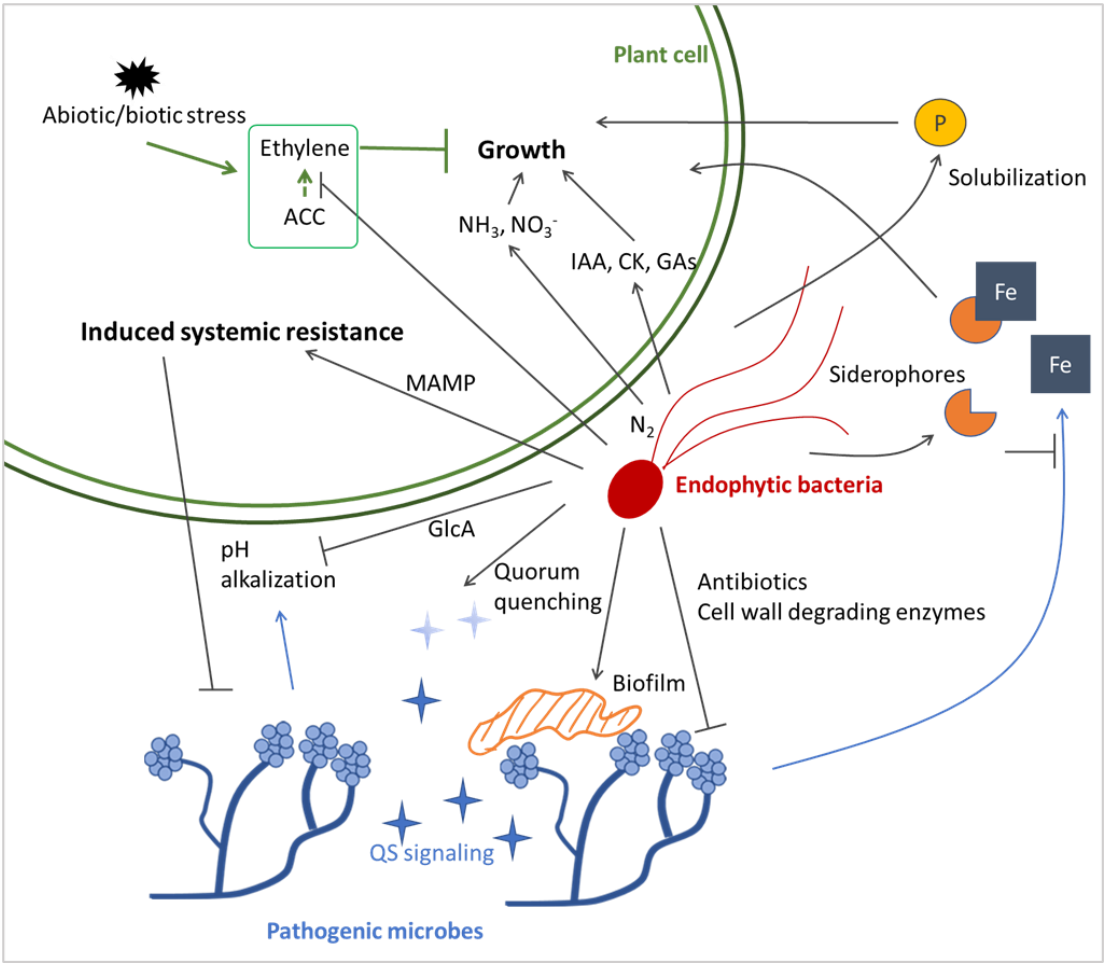

Fig. 7 | Beneficial traits of endophytic bacteria regarding plant growth promotion and plant protection from plant pathogens
In this figure, arrows indicate plant-bacterium interactions and '⊥'s indicate inhibition effects. Black lines show approaches of bacterial endophytes and blue lines show those of plant pathogens. ACC, 1 aminocyclopropane-1-carboxylic acid; IAA, indoleacetic acid; CK, cytokinin; GAs, gibberellins; MAMP, microbe-associated molecular pattern; GlcA, gluconic acid; QS, quorum sensing; Endophytic bacteria also can supply plants with nutrients such as phosphate (P), iron (Fe), and nitrogen (NH_3 or NO_3^-).

G) Plant growth promotion

Endophytes have often been described as plant growth enhancers. Different mechanisms were reported, such as improving nutrient acquisition and modulating plant hormones, even under stressful conditions. These features may suggest the use of endophytic microbes as biofertilizers and biostimulants instead of chemical ones.

G.1 Nitrogen, Phosphorus and Iron

Nitrogen (N) is one of the essential elements for all organisms, including plants. It is involved in the composition of many molecules such as nucleic acid, proteins, chlorophyll, specialized metabolites. Diazotrophic bacteria can convert N_2 into ammonium which is catalyzed by nitrogenase. This process is called biological nitrogen fixation [52]. Many endophytic bacteria, in addition to Rhizobia, can fix N_2. Several bacterial genera were studied for their nitrogen-fixation capabilities, such as *Herbaspirillum*, *Azospirillum*, *Burkholderia*, *Pantoea*, *Bacillus*, *Klebsiella*, and *Gluconacetobacter*, *Novosphingobium* or *Ochrobactrum* [52–54]. Isolates from those genera were also used in bioengineering strategies to improve nitrogen-fixation activities in the field [55].

Phosphorus (P) is also an important element for organisms since it is a component of nucleic acids or ATP, for example. Plants can absorb phosphate ions such as $H_2PO_4^-$ and HPO_4^{2-}. However, in soils, P often exists in insoluble forms. In its inorganic form, P forms complexes with metal ions such as Al or Fe. Some endophytes, like those affiliated with *Pantoea* sp., could solubilize these complex by secreting organic acids [52,56]. Although some plants can also acquire phosphorus by chelating minerals with organic acids from roots [57], such microbial help improvement of nutrient acquisition in plants.

Iron (Fe) is an essential element for plants, especially for chlorophyll biosynthesis. Iron deficiency, therefore, causes leaf yellowing, especially in alkaline soils, in which Fe is in insoluble forms (ferric iron, Fe^{3+})[58]. In limiting conditions, bacteria produce siderophores that can chelate Fe^{3+}, which is then delivered in microbial cells in its soluble form Fe^{2+} (ferrous iron) [52]. Such siderophore production was observed in endophytic bacteria [56]. For example, serobactins (Fig. 10) were discovered in the culture of *Herbaspirillum* strain (Table 1) [59]. As this bacterium can fix N_2, it has been suggested that the efficiency of the nitrogenase could depend on the siderophore, because N fixation requires iron [59]. Another endophyte, *Streptomyces* sp. GMKU3100, producing desferrioxamine as a siderophore, exhibited an improvement of wheat and mungbean growth in an iron depletion medium, while its mutant failed to promote plant growth [60].

G.2 Modulation of plant hormone signaling

Endophytes are involved in plant growth promotion (PGP) by producing hormones or by modulating the homeostasis of plant hormones [29].

Auxin is a well-known class of plant hormones influencing growth by stimulating cell elongation in stems and cell division and differentiation in roots. Indole-3-acetic acid (IAA) (Fig. 8) is one of the most famous molecules in this group. Its production by bacteria can be considered as an indicator of their plant growth promotion effect. Interestingly, many endophytes are able to produce IAA, for example, *Lysinibacillus* and *Paenibacillus* strains [61] or one *Luteibacter* strain isolated from apple plants and used to promote the biomass of barley seedlings [62].

Gibberellins (Fig. 8) are plant hormones facilitating germination and cell elongation. One strain affiliated with *Sphingomonas* has been reported to produce active and inactive gibberellins [63]. These authors made evidence that the inoculation of tomatoes with this strain led to an improvement of the plant biomass and the chlorophyll content in leaves. In another study, a rice endophyte, *B. amyloliquefaciens* RWL-1, was also able to produce various gibberellins. The inoculation of rice with this strain brought about a biomass increase, which was more effective than the chemical treatment with an synthetic gibberellin [64].

Cytokinin activates cell divisions and suppresses leaf aging. The production of cytokinin-like compounds was detected in *Pseudomonas* and *Paenibacillus* endophytic strains [65]. Another plant-associated bacterium, *Methylobacterium oryzae*, was shown to produce various cytokinins, such as cis- and trans-zeatins (Fig. 8), and increased the drought tolerance of lentils [66].

Abscisic acid (ABA) (Fig. 8) is increased when plants face hydric and/or salinity stresses, in order to reduce transpiration by closing stomata. A rice seed endophyte, *B. amyloliquefaciens* RWL-1, producing ABA, improved rice growth under a salinity stress [67]. Interestingly, following rice inoculation with *B. amyloliquefaciens* RWL-1, the endogenous ABA decreased whereas the salicylic acid content increased.

Jasmonic acid (JA) and salicylic acid (SA) (Fig. 8) are involved in the induction of plant defenses in response to pest attacks. SA has been shown to be produced by endophytic bacteria isolated from various plants. A high-SA-producing endophyte, *P. tremae*, showed an excellent protectiven effect on *N. benthaminana* against a complex of pathogens [68].

G.3 Ethylene

Ethylene is a multi-functional gas hormone influencing plant growth and senescence. In particular, ethylene governs the growth and development of leaves, flowers, and fruits and is

involved in fruit ripening. Ethylene also regulates plant responses to biotic and abiotic stresses. Increasing the level of endogenous ethylene in plants leads to growth inhibition and induces senescence, so bacterial strains producing ethylene cannot be appreciated regarding plant growth promotion [29]. Beneficial endophytes often possess 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Fig. 8) [69]. An interesting study showed the importance of this ACC deaminase. When tomato seeds are coated with *Pseudomonas migulae* 8R6 or *P. fluorescens* YsS6, the resulting plants displayed tolerance to saline media. Interestingly, when the seeds were coated with the bacterial mutant strains lacking ACC deaminase, the resulting plants were more sensitive to salt stress [70].

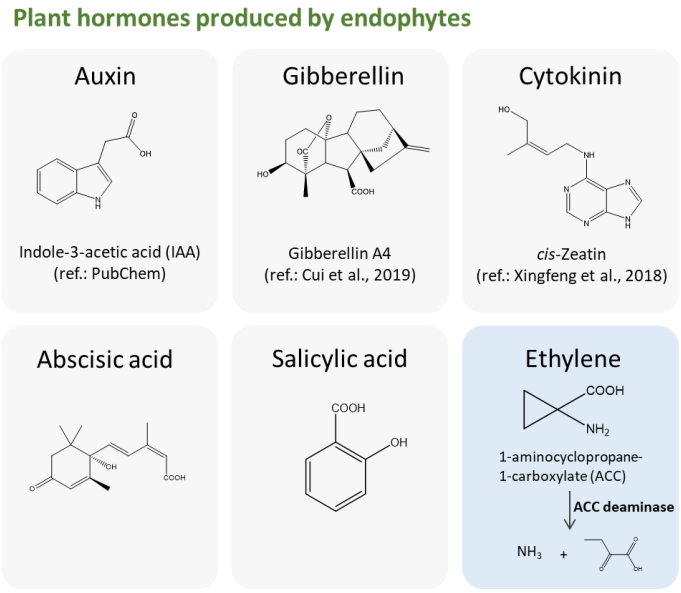

Fig. 8 | Plant hormones that can be produced and modulated by endophytic bacteria
Production of plant hormones and hormone-like compounds of endophytes are thought to conducive to mitigation of biotic/abiotic stresses of host plants. Regarding ethylene, endophytic microbes reducing ethylene can be beneficial for plants, by breaking down a precursor of ethylene, 1-aminocyclopropane-10carboxylate (ACC), into ammonium and α-ketobutyric acid.

H) Plant bioprotection

In addition to their effects on plant growth promotion, some endophytes are known to be beneficial for plant health. These microbes can increase plant tolerance against bioagressors in two ways: 1) indirectly, through the induction of systemic resistance and specialized metabolite production in host plants, and 2) directly through antagonistic interactions with the pathogens.

I) Plant-microbe interactions

I.1 Plant systemic resistance

Endophytes, as rhizospheric microbes, are known to Induce Systemic Resistance (ISR). This mechanism primes the whole plant to enhance its defense mechanisms and finally prepare the plant to better fight against different bioagressors. ISR is characterized by triggering an oxidative burst, the induction of the production of a large panel of specialized metabolites, and the accumulation of pathogen-related proteins. The pathway leading to the ISR by plant beneficial microbes has been investigated, especially with the rhizobacteria *Pseudomonas fluorescens* WCS417r. In this case, it was shown that jasmonate and ethylene play a central role in the regulation of ISR [71]. However, although ISR is often regulated through SA-independent mechanisms, some authors showed that some beneficial microbes could trigger an SA-dependent ISR [72,73]. Despite these interesting insights, the molecular mechanisms involved in ISR by endophytes is still far from being elucidated. Their investigation is, however, of major interest since ISR can be considered as having less risk for the development of resistant pathogens [74].

I.2 Specialized metabolites

Because plants cannot move, they had to find original ways to adapt themselves to various abiotic/biotic stresses. One strategy is to produce a huge panel of molecules displaying outstanding properties. These specialized metabolites are molecules that are extremely diverse but are essentially divided into three major chemical families: terpenes, alkaloids, and polyphenols. They are generally produced in very small quantities in plants (in the order of mg/g DW). These molecules can be very complex and, therefore, cannot always be produced efficiently by synthetic chemistry approaches (e.g., paclitaxel). So far, more than 300,000 metabolites have been identified in the plant biodiversity [75]. These molecules are produced specifically by plants, and each plant species has its own metabolomic fingerprint.

Endophytes were described to have an impact on this plant's specialized metabolism. According to Etalo et al. (2018), the modulation of plant chemistry by endophytic or root-associated bacteria was observed in 53 species from 28 plant families [76]. The metabolic profile modification concerns many compounds belonging to all the metabolite families (quinones, alkaloids, terpenoids, phenolic acids, saponins, coumarins, glucosinolates, benzoxazinoids, flavonoids, isoflavonoids) [76]. These interactions and the resulting modification of the metabolic signature have been highlighted when inoculating bacteria to plants producing terpenoids (such as withanolide in *Withania somnifera*) or alkaloids (such as morphine in *Papaver somniferum*, galantamine in *Lycoris radiata*, nicotine in *Nicotiana tabacum*) [68,77,78]. The molecular mechanisms involved in these changes were only partially revealed so far. The increased

synthesis of these molecules may result from the upregulation of the expression of the genes involved in the corresponding biosynthesis pathways. These reports make evidence that the inoculation of plants with endophytes might be a tool to preserve plants against bioagressors and, therefore, for agronomical purposes. Using these microbes could also be useful to stimulate plants for producing interesting molecules that might be used in the pharmaceutical field.

Plant specialized metabolites sometimes can be synthesized by endophytic fungi independently from their host plants. A famous and promising example concerns paclitaxel that is used as an anticancer drug. It has been shown for a long time that this molecule is produced in the yew tree but at a low level, which raises a real supply problem. Recently, it has been shown that several endophytic fungi are able to produce this molecule *in vitro* [79,80]. Other examples have also been reported, such as the production in other fungal endophytes of camptothecin and vinblastine, which are well-known antibiotics or anticancer drugs [81]. This ability of endophytes to synthesize molecules mainly found in plants might be explained by horizontal gene transfer between plants and fungi. However, some studies also revealed that fungal genes appeared independently from host plants, at least during the recent period [80].

Finally, it is also interesting to notice that some bacterial endophytes can mimic compounds detected in their host plants. It is the case for camptothecin, for example (Table 1). Interestingly, all the bacterial strains producing camptothecin derivatives that are identified so far belong to *Bacillus* genus [82,83]. A study done in 2017 suggest that the production of this molecule in at least one Bacillus strain relies on the presence of a specific plasmid [83].

I.3 Direct antagonisms against pathogenic microbes

The modes of actions of endophytes against pathogenic microbes are quite diverse. For example, antibiotics are responsible for specific antagonism. However, in many cases, different mechanisms that act synergistically participate in the antagonistic effect of endophytes against pathogens. In this section, I am going to highlight some major mechanisms of their antagonistic activities.

I.4 Niche competition

Beneficial endophytes can compete with pathogenic microbes for nutrient resources. For example, it is reported that *Pantoea vagans* C9-1 (an apple endophyte) competes with *Erwinia amylovora* (a causal bacterium of fire blight), for carbon and for iron, through the production of siderophores [7,84]. Beneficial endophytes can also compete with pathogenic microbes for infection sites.

I.5 Secretion of cell-wall degrading enzymes

Some bacterial endophytes secrete lytic enzymes, such as glucanases, chitinases, chitosanases, or proteases, targeting the cell wall of pathogenic microbes, and that will help to limit the growth of the plant pathogen [16,85,86].

I.6 Biofilm and pH

Some endophytic strains required motility and biofilm formation to be effective against fungal pathogens. It was exemplified for some endophytes such as a maize endophyte, *Burkholderia gladioli* strain 3A12 against *Sclerotinia homoeocarpa*, and a finger-millet endophyte, *Enterobacter* sp. M6, against *Fusarium graminearum* [87,88]. Another endophytic bacterium, *Rahnella aquatilis* Ra36, is able to inhibit the invasion of *F. oxysporum* in tomatoes [89]. This study showed that the endophytic strain produced gluconic acid, leading to the acidification of the external pH. This led to increased protection against *F. oxysporum* that needs alkaline conditions for an efficient penetration in the plant tissues. Interestingly, this study also showed that *R. aquatilis* used the hyphae of *F. oxysporum* as shuttles to colonize the roots.

I.7 Quorum quenching

Quorum sensing is a communication system to sense population density between microbes in order to induce specific phenotypes for biofilm formation, cell-cell communications, reproduction, competition and pathogenicity [16]. Chemicals involved in quorum sensing can be classified into three types: 1) autoinducer-2 system, 2) diffusible signal factor system, 3) N-acyl homoserine lactone (AHL) system [90]. Degradation or interference with these signal molecules, known as quorum quenching, could be a promising mechanism through which endophytes could antagonize plant pathogens [91]. For example, *Bacillus* and *Brevibacillus* strains, isolated from *Cannabis* plants, showed a quorum quenching against AHL system (Kusari et al., 2014).

I.8 Some important antibiotic molecules

As stated above, microbes are well-known for producing active biomolecules. Isolating endophytes from plants might be an original way to identify some new compounds for application as biological control agents against plant disease in agriculture and new bioactive drugs for human health [93].

A number of investigations concerning antimicrobial compounds have been done mainly for fungal endophytes. The bioactive compounds found in fungal endophytes are well summarized in several review articles [45,94] and will not be considered here since they are out of my scope. Fewer studies have been devoted to bioactive compounds produced by bacterial endophytes [95]. Recent studies described that endophytic bacteria also could produce antibiotics that were mainly found in *Streptomyces* and *Bacillus* species, two gram-positive bacterial genera [96]. These

metabolites include various compounds, such as hydrogen cyanide, alkaloids, lipopeptides, polyketides, terpenoids, volatile organic compounds (VOCs) [95]. These compounds possessed not only antifungal and antibacterial activities but also antiviral activities and might have other interests as anticancer, antioxidants, etc. [79,97].

J) Alkaloids

Alkaloids, which are molecules containing N atom included most of the time in a heterocyclic system, are biosynthetically formed from an amino acid. These compounds are attractive, especially for medicinal applications since they have significant pharmacological activity. I already mentioned camptothecin and its derivatives (Fig. 9) isolated from bark of *Camptotheca acuminate.* These highly cytotoxic plant specialized metabolites were also isolated found in *Bacillus* endophytes [82,83]. Camptothecin is known to inhibit topoisomerases, but its toxicity and very low solubility doesn't allow its use in anticancer therapy. Other alkaloids displaying antivirus, antifungal, or antitrypanosomal properties were isolated from endophytic Actinomycete bacteria (Table 1) [98–101]. As the last example, maytansine, which was identified in extracts of *Celastraceous* plants, has a strong antitumor activity, targeting microtubules (Fig. 9) [102]. Interestingly, Kusari and collaborators made evidence that the actual synthesis of these compounds was carried out by root-endophytic bacterial communities [103].

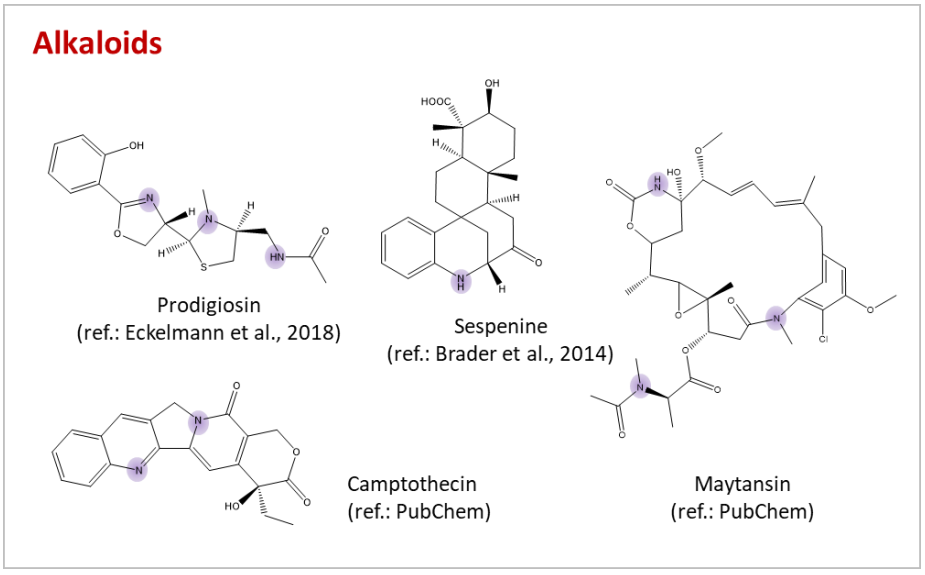

Fig. 9 | Alkaloids produced in endophytic bacteria

K) Lipopeptides and polyketides

Ribosomal synthesized antimicrobial peptides, non-ribosomal synthesized peptides (NRPs),

and polyketides (PKs) are well-known families of antibiotics produced by bacteria, especially *Bacillus* species. Among NRPs, lipopeptides (LPs) are studied for their various biological activities, *i.e.,* antimicrobial activities, ISR triggering, chelating iron, or facilitating plant colonization [104]. LPs are amphiphilic molecules composed of a linear or cyclic oligopeptide with a fatty acid chain. They are classified into different families depending on the length of the amino acid chain. Among each family, LPs varied in their composition in amino acids (L and D amino acids, modified amino acids) and in the length of the lipid tail.

Among bacteria, *Pseudomonas* and *Bacillus* species were well investigated for their ability to produce such molecules [104]. LPs of *Bacillus subtilis* can be categorized into three families depending on their structures: iturin, surfactin, and fengycin (plipastatin) (Fig. 10) [105]. When these bacteria are inoculated in maize plants, upregulation of two genes of pathogenesis-related proteins was observed [106]. As a second example, ecomycin is a LP identified in *Pseudomonas viridiflava* and displays antifungal activities against various pathogens (Miller et al., 1998). Serrawettin W1 is another LPs found in *Serratia marcescens* MSRBB2 (Fig. 10) [107]. This compound has antimicrobial but also antitumoral properties [108]. Finally, serobactin A, B, and C were isolated from a rice endophyte, *Herbaspirillum seropedicae* Z67, and are considered siderophores [59].

Bacterial PKs also have a large diversity in their structures, such as macrolide, ansamycin, tetracyclines, etc. [105]. They are synthesized with acyl CoA precursors, receiving various modifications after polyketide chain synthesis. Actinoallolides, divergolides, maytansine isolated from endophytic bacteria were shown to have pharmaceutical properties of interest [103,109,110].

Bacterial NRPs and PKs are both biologically synthesized with modular enzymes, NRP synthase (NRPS) and mainly type I PK synthase (PKS), respectively. The one-dimensional structures of the enzymes are composed of a series of elongation modules sandwiched between the initiation and the termination modules [105]. In the elongation module, one module corresponds to one component of NRP or PK compounds. Such regular structures and substrate specificity are well conserved in the biosynthesis gene cluster, which encourages genome mining and gene engineering [111,112]. Therefore, some bioinformatic tools were set up to help find gene clusters of NRPS/PKS, for instance, antiSMASH [113] and NaPDoS [114]. Some open databases and tools are available for genome mining of NRPS and PKS. These NRPS and PKS genes are used to evaluate endophytes' functions [31,95].

Sometimes, hybrid enzymes between NRPS and PKS can be found, and amicoumacin is an example of this [115]. This compound was also found in an endophytic *Bacillus subtilis* (Table 1).

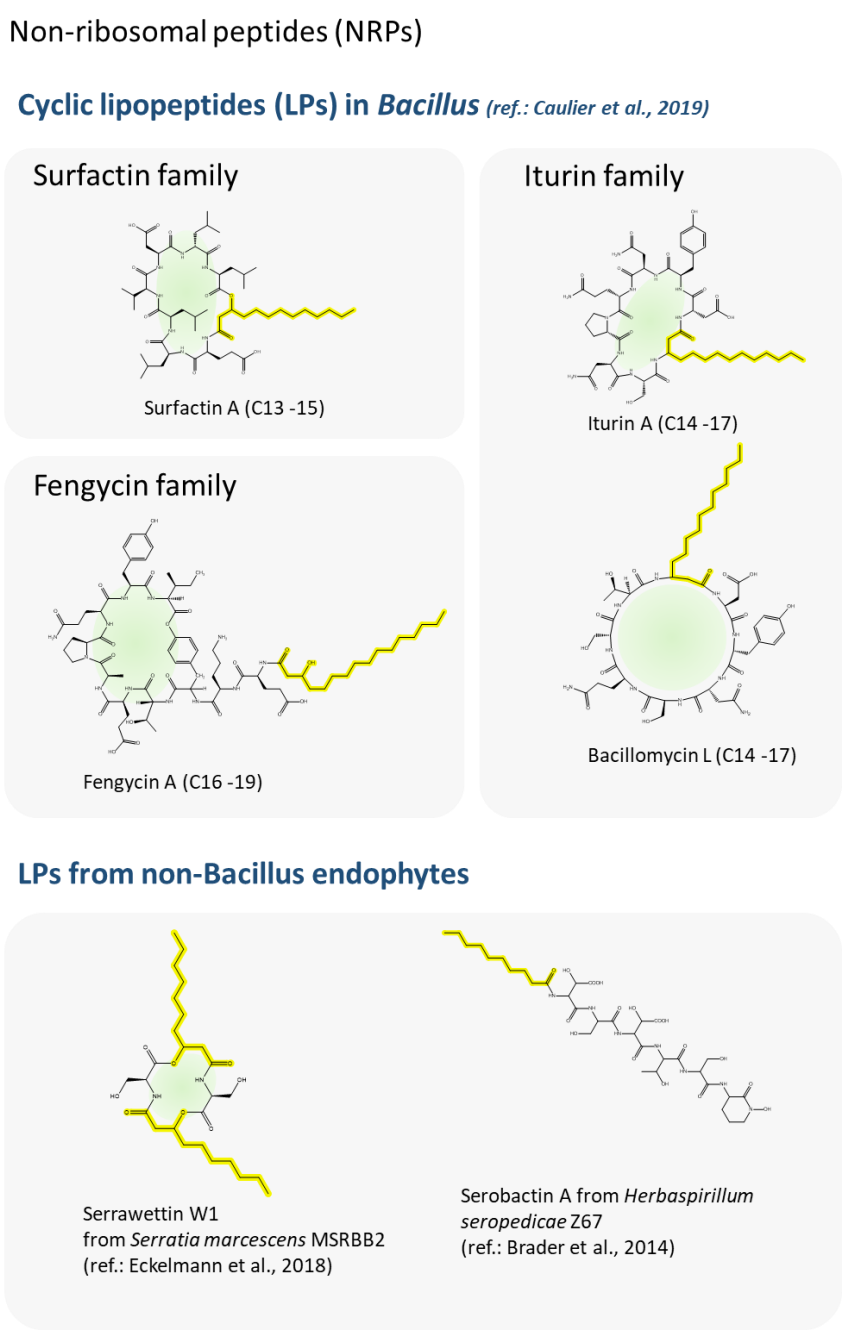

Fig. 10 | Lipopeptides produced in endophytic bacteria

L) VOCs

Plant-associated bacteria produce various organic and inorganic volatile compounds in order to interact with plants and other microbes [105]. Volatile organic compounds (VOCs) are small compounds emitted into the air by plants and microorganisms. Recently, microbial VOCs have gained attention due to their antifungal activities and their beneficial effect on plants by eliciting ISR and inducing growth promotion [13]. These compounds were collected from the air

and identified by GC-MS approaches [116]. In endophytic bacteria, antifungal VOCs were found from *Pseudomonas*, *Stenotrophomonas*, *Acinetobacter*, and *Burkholderia*, which all belong to the Proteobacteria phylum [117–122].

These compounds were revealed as compounds including sulfur, represented by dimethyl disulfide and dimethyl trisulphide, pyrazines, indole, fatty acids (Table 1, Fig. 11). The profile of VOCs in different taxa still needs to be investigated, but their bioactivities are expected to be used for assessing the role of endophyte (volatilomics) and possibly as post-harvest crop protection tools [13].

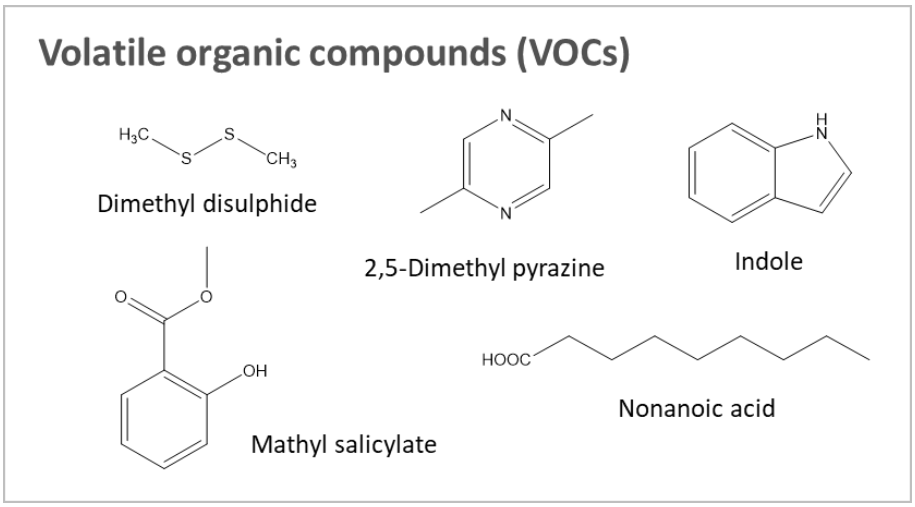

Fig. 11 | VOCs in endophytic bacteria, with antifungal activities

M) Other compounds

Prodiginine is a red pigment produced by endophytic *Serratia marcescens* MSRBB2 [123]. This compound has various biological activities, such as antifungal activities. The production of this molecule is strongly induced when it is co-cultivated with fungal endophytes isolated from the same host. Interestingly, no induction could be noticed when the co-culture was done in the presence of non-endophytic fungi [123]. A similar observation was done for serratamolides [123]. This study strongly suggests that such bioactive molecules play some role in microbe-microbe interactions.

Table 1 | Bioactive compounds isolated in bacterial endophytes

Phylum	Bacteria	Host plants	Bioactive compounds	Chemical type	Activity	Reference
Actinobacteria	*Jishengella endophytica* 161111	*Xylocarpus granatum*	perlolyrine 1-hydroxy-β-carboline lumichrome 1H-indole-3-carboxaldehyde	alkaloid	anti-influenza-A virus	Wang et al., 2014
Actinobacteria	*Streptomyces* sp. GT20021503	*Bruguiera gymnorrhiza*	xiamycin and methyl ester derivative	alkaloid	Anti HIV activity	Ding et al et al., 2010
Actinobacteria	*Streptomyces* sp. KIB-H033	*Camellia sinensis*	rubrolone B	alkaloid	cardioprotection	Yan 2016; Gao 2018
Actinobacteria	*Streptomyces* sp. TP-A0595	*Allium tuberosum*	6-prenylindole	alkaloid	antifungal	Sasaki et al., 2002
Actinobacteria	*Streptosporangium oxazolinicum* K07-0450T	orchids	spoxazomicins A-C	alkaloid	antitrypanosomal (sponxazomycin A and B)	Inahashi et al., 2011
Firmicutes	*Bacillus amyloliquefaciens* KY741854	*Pyrenacantha volubilis* Hook.	camptothecine	alkaloid	anticancer	Soujanya et al., 2017
Firmicutes	*Bacillus cereus*	*Miquelia dentata* Bedd.	camptothecine	alkaloid	anticancer	Shweta et al., 2013
Firmicutes	*Bacillus* sp.	*Miquelia dentata* Bedd.	camptothecine	alkaloid	anticancer	Shweta et al., 2013
Firmicutes	*Bacillus* sp. KP125955	*Pyrenacantha volubilis*	camptothecine	alkaloid	anticancer	Soujanya et al., 2017
Firmicutes	*Bacillus* sp. KP125956	*Pyrenacantha volubilis*	camptothecine	alkaloid	anticancer	Soujanya et al., 2017
Firmicutes	*Bacillus subtilis*	*Miquelia dentata* Bedd.	camptothecine, 9-methoxy camptothecine	alkaloid	anticancer	Shweta et al., 2013
Firmicutes	*Bacillus subtilis* KY741853	*Pyrenacantha volubilis*	camptothecine	alkaloid	anticancer	Soujanya et al., 2017
Firmicutes	*Lysinibacillus* sp.	*Miquelia dentata* Bedd.	camptothecine	alkaloid	anticancer	Shweta et al., 2013
Proteobacteria	*Serratia marcescens* MSRBB2	*Maytenus serrata*	prodiginines	alkaloid	antifungal	Eckelmann et al., 2018
Actinobacteria	*Streptomyces* sp. TP-A0556	*Aucuba japonica* Thumb	7'-demethylnovobiocin and 5"-demethylnovobiocin	coumarins	antimicrobial	Sasaki et al., 2001
Firmicutes	*Bacillus amyloliquefaciens* strain Ar10	potato		glycolipids	antimicrobial	Azaiez et al., 2018
Actinobacteria	*Streptomyces* sp. HKI0595	*Kandelia candel*	xiamycin A and B, indosespene, sespenine	indolosesquiterpenes	antibacterial (xiamycin A and indosespene)	Ding et al., 2011a
Firmicutes	*Bacillus amyloliquefaciens* strain TEB1	*Citrus lemon*		lipopeptides	antifungal	Kalai et al., 2013
Firmicutes	*Bacillus subtilis* (SG JW.03)	*Zea mays* indurata	Iturin A, Bacillomycin, Fengycin	lipopeptides	antifungal	Gond et al., 2015
Proteobacteria	*Herbaspirillum seropedicae* Z67	rice	serobactin A, B and C	lipopeptides	siderophore	Baldani and Baldani et al., 1986 Rosconi et al., 2013
Proteobacteria	*Pseudomonas viridiflava*	grass	ecomycin	lipopeptides	Anti-fungal	Miller et al., 1998
Proteobacteria	*Serratia marcescens* MSRBB2	*Maytenus serrata*	serrawettin W1 serratamolides	lipopeptides	antibacterial	Eckelmann et al., 2018; Marques-Pereira et al., 2020
Actinobacteria	*Actinoallomurus fulvus* MK10-036	*Capsicum frutescens*	actinoallolides A–E	macrolides (PK)	anti-trypanosomal (actinoallolide A)	Inahashi et al., 2015
Actinobacteria	*Streptomyces* sp. HKI0576	*Bruguiera gymnorrhiza*	divergolides A–D	ansamycins (PK)	antibacteria	Ding et al., 2011b

Table 1 | Bioactive compounds isolated in bacterial endophytes

Phylum	Bacteria	Host plants	Bioactive compounds	Chemical type	Activity	Reference
	Endophytic bacterial communities	*Putterlickia verrucosa*	maytansine	ansamycins (PK) alkaloid	cytotoxicity	Kusari et al., 2014
	Endophytic bacterial communities	*Putterlickia retrospinosa*	maytansine	ansamycins (PK) alkaloid	cytotoxicity	Kusari et al., 2014
Actinobacteria	*Streptomyces* NRRL 3052	*Kennedia nigriscans*	munumbicins A, B, C and D	peptide	antibacteria	Castillo et al., 2002
Actinobacteria	*Streptomyces* NRRL 30562	*Kennedia nigriscans*	munumbicins E-4 and E-5	peptide	antimicrobial, antimaratial	Castillo et al., 2006
Actinobacteria	*Streptomyces* sp. MSU-2110	*Monstera* sp.	coronamycins	peptide	antimicrobial, antimaratial	Ezra et al., 2004
Actinobacteria	*Streptomyces* sp. NRRL 30566	*Grevillea pteridifolia*	kakadumycin A	peptide	antibacteria, antimararia	Castillo et al., 2003
Proteobacteria	*Pseudomonas brassicacearum* YC5480	*Artemisia* sp.	2,4-diacetylphloroglucinol	phenolic compounds	antifungal, phytotoxic	Chung et al., 2008
Actinobacteria	*Streptomyces hygroscopicus* TP-A0326	*Clethra barbinervis*	clethramycin	macrolides (PK)	antifungal	Furumai et al., 2003
Firmicutes	*Bacillus subtilis*	unknown	amicoumacin	PK/NRP	Antibacterial, anti-inflammatory	Pinchuk et al., 2002
Proteobacteria	*Acinetobacter* sp. Ys	tomato		VOCs	antifungal	Lopez et al., 2021
Proteobacteria	*Burkholderia cenocepacia*	*Sophora tonkinensis* Gagnep.	dimethyl trisulfide, indole, methyl anthranilate, methyl salicylate, methyl benzoate, benzyl propionate, benzyl acetate, 3,5-di-tert-butylphenol, allyl benzyl ether and nonanoic acid	VOCs	antifungal	Chen et al., 2020
Proteobacteria	*Pseudomonas fluorescens* ALEB	*Atractylodes lancea*	dimethyl disulphide	VOCs	antifungal	Zhou et al., 2014
Proteobacteria	*Pseudomonas putida* BP25	black pepper	dimethyl trisulphide 2, 5-dimethyl pyrazine 2-methyl pyrazine 2-ethyl-5-methyl pyrazine 2-ethyl-3, 6-dimethyl pyrazine	VOCs	antifungal, antibacterial, nematicidal	Sheoran et al., 2015 Agisha et al., 2019
Proteobacteria	*Pseudomonas stutzeri* E25 *Stenotrophomonas maltophilia* CR71	*Physalis ixocarpa*		VOCs	antifungal	Rojas-Solís et al., 2018
Proteobacteria	*Stenotrophomonas* sp. Us	tomato		VOCs	antifungal	Lopez et al., 2021
Actinobacteria	*Polymorphospora rubra* K07-0510	orchids	trehangelins A-C		"inhibitory activity against hemolysis of red blood cells induced by light-activated pheophorbide a"	Nakashima et al., 2013
Actinobacteria	*Streptomyces albidoflavus*	*Bruguiera gymnorrhiza*	antimycin A18		moderate antifungal	Yan et al., 2010
Actinobacteria	*Streptomyces hygroscopicus* TP-A0451	*Pteridium aquilinum*	pteridic acids A and B		root growth protmotion (pteridic acid A)	Igarashi et al., 2002

Table 1 | Bioactive compounds isolated in bacterial endophytes

Phylum	Bacteria	Host plants	Bioactive compounds	Chemical type	Activity	Reference
Actinobacteria	*Streptomyces hygroscopicus* TP-A0451		pterocidin		antitumor	Igarashi et al., 2006
Actinobacteria	*Streptomyces* sp. TP-A0456	*Cryptomeria japonica*	cedarmycins A and B		antifungal (cedamycin A)	Sasaki et al., 2001
Actinobacteria	*Streptomyces* sp. TP-A0569	*Allium fistulosum*	fistupyrone		Inhibition of spore germination of Alternaria brassicicola	Igarashi et al., 2000
Actinobacteria	*Streptomyces* sp. YIM66403	*Isodon eriocalyx*	misamycin		Antitumor	Wei et al., 2015
Actinobacteria	*Streptomyces thermoviolaceus* TP-A0648	*Aucuba japonica*	anicemycin		tumor cell growth inhibitor	Igarashi et al., 2005
Firmicutes	*Bacillus cereus* MZ269485	strawberry	N-(3-oxo-hexanoyl)- homoserine lactone		antifungal	Moura et al., 2021
Firmicutes	*Bacillus cereus* MZ269486	strawberry	Maculosin, Baptifoline, 8 3-Butylidene-7- hydroxyphthalide		antifungal	Moura et al., 2021
Firmicutes	*Bacillus subtilis* MZ269482	strawberry	salicylamide, maculosin, herniarin		antifungal	Moura et al., 2021
Firmicutes	*Bacillus subtilis* MZ269483	strawberry	baptifoline, undecanedioic acid, botrydial		antifungal	Moura et al., 2021
Proteobacteria	*Burkholderia gladioli* HDXY-02	*Lycoris aurea*	toxoflavin		antifungal	Li et al., 2019
Proteobacteria	*Burkholderia stabilis* EB159 (PG159)	ginseng	pyrrolnitrin		antifungal	Kim et al., 2020
Proteobacteria	*Enterobacter* sp. M6	finger millet	phenazine		antifungal	Mousa et al., 2016
Proteobacteria	*Pantoea* sp. MZ269487	strawberry	salicylamide, Maculosin, Herniarin, Baptifoline, Botrydial		antifungal	Moura et al., 2021
Proteobacteria	*Sphingomonas* sp. ZJ26	rice	anthranilic acid			Matsumoto et al., 2021
Actinobacteria	*Streptomyces* sp. GMKU3100	Thai jasmine rice (*Oryza sativa* L. cv. KDML105)	desferrioxamine		siderophore	Rungin 2012

N) How to modulate the function of endophyte communities

N.1 Building an adapted community to answer to an agronomical concern: Setting up a suppressive soil

A soil in which a crop is continuously grown can develop a certain degree of tolerance against plant pathogens. This phenomenon is known as "disease-suppressive soil," where pathogens cannot manifest visible symptoms [124]. Such soil has been reported for the pathosystem wheat and *Gaeumannomyces graminis* var Tritici. This pathogen is responsible for the "Take all" disease. It leads to seedling stuns and affects roots, crowns, and stems [125]. A suppressive soil against this disease has been reported for decades. The observation made on such a system revealed the major conditions necessary for developing suppressive soil. The method requires 1) a monoculture of sensitive cultivars, 2) the presence of the causal pathogen, 3) at least one outbreak of this disease in the field history [126]. Generally speaking, to get rid of soil-borne diseases and reduce the population of pathogenic microorganisms, it is necessary to perform crop rotation, destroy harvest residues, and disinfect soil [14]. In the case of the Take-all disease, the strategy set up was significantly different. Actually, monocropping for years led to the modification of soil microbiomes leading to an increase of the concentration of antagonistic microbes against the pathogen after several outbreaks of the disease. The suppressiveness of soil can be described through two components: general suppression and specific suppression. The general suppression is derived from the function of inherent soil, whereas the specific suppression is attributed to the accumulation of antagonistic microbes against specific pathogens [126].

This phenomenon can be of great interest in terms of disease management with a viewpoint of plant-microbiome control. The number of studies dealing with disease-suppressive soil from a microbiological point of view is increasing since 2010 [127]. Examples of the disease-suppressive soil are, however, very limited to a few combinations of plants and diseases. Although the theory of the disease suppressive soil seems suitable for sustainable agriculture, the application to other soil-borne diseases is still controversial, and it is essential to figure out the mechanisms of formulation of antagonistic soil microbiomes through microbe-microbe and microbe-plant interactions [124]. During the past few years, there was some important insight about plant-associated microbiome suppressing microbial diseases. Berendsen et al. showed that the soil inoculated with *Hyaloperonospora arabidopsidis*, causing downy mildew of leaves in *Arabidopsis thaliana*, at the first generation, could decrease the abundance of the pathogen at the 2nd generation [128]. More recently, Carrión et al. investigated endophytic bacterial communities isolated from sugar beets grown in conducive soil and suppressive soil for *Rhizoctonia solani*. This study revealed that *Flavobacterium* and *Chitinophaga* bacteria, isolated from plants in suppressive soil with *R. solani* inoculation, seemed to play important roles for

suppression of the disease on host plants, containing genes related to NRPS and PKS [31]. They also showed that the inoculation of sugar beet seedlings with the consortium of those bacteria showed a more stable protection effect against the pathogen than those with the single isolated bacteria. There are other examples of the consortium application of endophytes other than other plant-associated microbes [129]. The above studies exemplified that the presence of pathogenic microbes can lead to the accumulation of antagonistic bacteria in soil and roots, and also that the suppressive soil can provide an antagonistic consortium of endophytic bacteria against disease development.

N.2 Host mediated plant microbiome engineering to accumulate plant-phenotype-related microbiomes in the soil

Like the disease-suppressive soil development, some attempts were made for forming a soil or rhizosphere microbiome relating to a phenotype of host plants. The procedure generally consists of selections of soil lines in which plants showed better target traits, collection of microbes from those soil, transfer the microbial community to axenic soil of next generation of plants. This cycle can be repeated several times. Panke-Buisse et al. succeeded in the acquisition of soil lines strongly related to early-flowering phenotype and late-flowering phenotype of four varieties of *Arabidopsis thaliana* after ten generations [130]. In this study, the microbial communities isolated from soil-related to late-flowering traits showed reproducibility of the same trait in novel *A. thaliana* plants. In another study, a soil microbial community related to the dry resistance of wheat was developed after six cycles of selection and cultivation [131]. These studies showed that artificial developments of soil microbiomes associated with host plant phenotypes are possible by enrichment after several soil selections.

Nowadays, many articles suggest a need to assess the resistance of plant varieties against disease, considering the function of the plant-associated microbiota [132]. For example, in a study of Kwak et al., the heritage of antagonism of soil with two tomato cultivars having different resistance against *Ralstonia solanacearum*, Hawaii7996 (resistance) and Moneymaker (sensitive), were investigated. As a result, the soil planted with the resistant cultivar at the first cultivation showed an increase of the disease resistance of the sensitive one at second cultivation. On the opposite, the soil hosting the sensitive cultivar at the first round did not change the resistance of the sensitive one at the second round [50]. The authors concluded that this resistance improvement resulted from rhizospheric microbes but not from the root exudate of Hawaii7996 (resistance) because its root exudates did not have antagonism against *R. solanacearum* growth. This led to the assumption that known resistant crop cultivars may modify or organize the soil microbial communities to antagonize the disease development.

Moreover, an evolution of *Pseudomonas protegens* from pathogenic to mutualistic for *A. thaliana* was obtained through six cycles of monocultures [133]. It suggested a possibility that

plants can establish a win-win relationship even with pathogenic microbes through taking time and several generations.

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Objectives

Endophytes are microorganisms colonizing inner plant tissues during their partial or entire life cycles [18]. These microorganisms can sometimes establish mutualistic relationships with their host plants and promote plant growth and health. The enhancement of plant defenses by endophytes is achieved via the combination of several mechanisms such as plant immunity induction, niche competition, and the production of biomolecules such as specialized metabolites and antibiotics. These biomolecules are involved in the communication with the host plants and with other microorganisms. They are also involved in antagonistic effects against some plant pests and pathogens. Therefore, it is assumed that microbes living inside plants can produce a higher diversity of specialized metabolites than free-living microorganisms that can be observed in soil [95].

Our hypothesis is that endophytes and the specialized metabolites they are able to produce could constitute an outstanding reservoir for the development of new bioactive solutions that could be used for agriculture and pharmacy applications. The identification of new biomolecules of interest is part of the objectives of the work package 1 of the biomolecules impact project (https://lue.univ-lorraine.fr/fr/ressources-naturelles-et-environnement/projet-lue-impact-biomolecules). In the framework of my thesis, we wanted to explore the production of such molecules for two different applications.

1) In the first chapter, we investigated the endophytic bacterial communities of *Leucojum aestivum* 'Gravity giant', an Amaryllidaceae species. This plant is well-known for its ability to produce alkaloids that are widely used as drugs against Alzheimer's disease. The general idea developed in this chapter is to isolate, screen, and identify bacterial strains producing alkaloids with new biological activities.

2) In the second chapter, I focused on *Chrysopogon zizanioides* (*i.e.,* vetiver), which is known for its essential oil used for perfume production that displays anti-microbial properties. This plant species, due to its specific characteristics, might host endophytic bacteria with original bioactivities.

In both chapters, the taxonomical compositions of the microbial communities were assessed using culture-dependent and/or independent approaches. The isolated bacterial endophytes were further studied for their metabolic characteristics and for their antifungal activities against some wheat phytopathogens, especially *Fusarium graminearum*.

3) The work done in the two first parts of this Ph.D. project led to the isolation of a high number of bacteria strains and, therefore, to the construction of big libraries. However, the number of interesting candidates was not so high in spite of taking a long time for isolation and purification of the endophyte isolates. In chapter 3, we set up an innovative way to engineer a soil in order to increase the concentration of interesting bacteria that might be involved in the resistance mechanisms against plant diseases. To realize the proof of concept, we focused on *Triticum aestivum* (wheat) and *F. graminearum* in order to create a suppressive soil in which the concentration of bacteria with bioprotective properties is increased.

Chapter 1

Isolation and characterization of endophytic bacteria from *Leucojum aestivum* 'Gravity giant'

Amaryllidaceae is a bulbous and perennial plant family that is famous for its alkaloid productions (Fig. 0-1) [1]. Amaryllidaceae alkaloid profiles differ in plant organs, and it was reported that they are especially concentrated in bulbs [2].

Among these molecules, Lycorine is known for its allelopathic activity, and Galantamine is being used for commercial drugs against Alzheimer's disease [2]. The Laboratoire Lorrain de Chimie Moléculaire (L2CM) has been working with this plant family to control Amaryllidaceous alkaloid production in *in vitro* plants [3,4]. A few reports made evidence that these plants can host several endophytes [5,6]. Recently, Spina and collaborators isolated a new endophytic bacterium from *in vitro* bulblets of *Leucojum aestivum* [7].

The objective of Chapter 1 is to extend the investigation of the diversity of endophytic culturable bacteria collected from three organs of *L. aestivum* 'Gravity giant' that were not cultured in sterile conditions (Fig. 0-2). Different candidates were selected in order to evaluate their capabilities for producing alkaloids and display antagonistic effects against plant fungal pathogens.

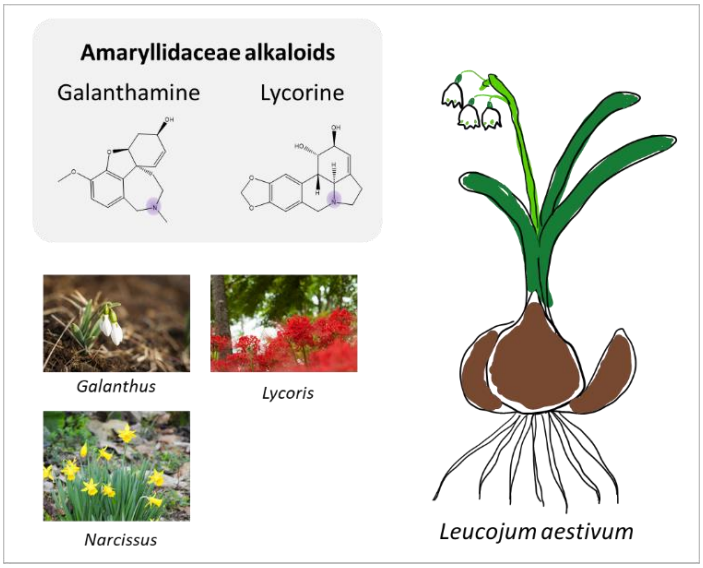


Fig. 0-1 | Representative Amaryllidaceae alkaloids and Amaryllidaceae plant family
(photos were provided from www.pakutaso.com)

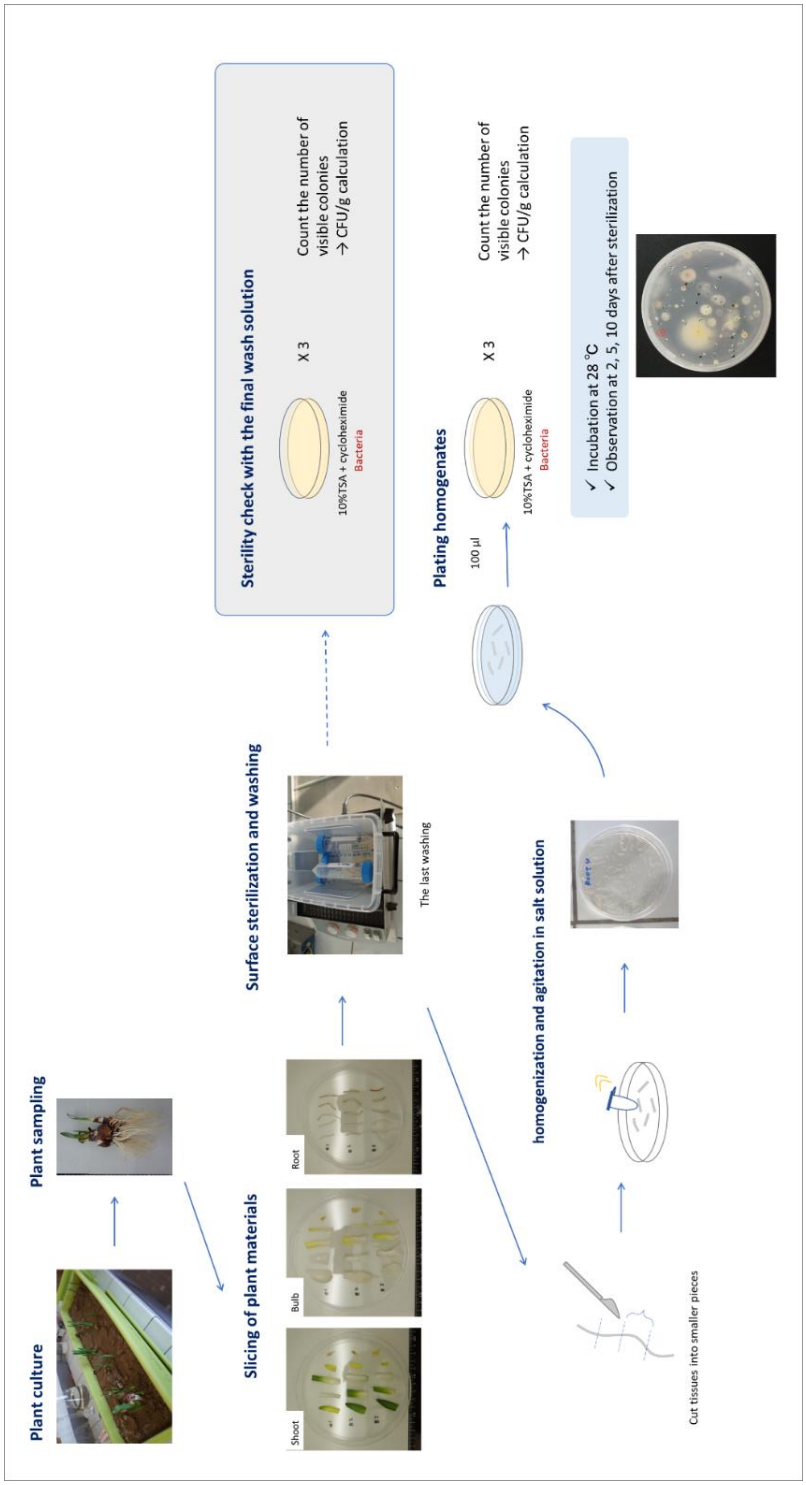


Fig. 0-2 | Overview of the isolation of endophytic bacteria from *L. aestivum* plant tissues
The isolation of endophytic bacteria was carried out through several steps: plant-material preparation, surface sterilization, extraction of endophytes from the treated plant materials, and appearance of colonies of culturable endophytes on microbiological media. The efficiency of the sterilization of the host plant material was confirmed by spreading the water used for washing the disinfected plant materials. As host plants, three individual plants of L. aestivum ' Gravity giant' just after seedlings appeared above ground were used.

Screening of endophytic bacteria of *Leucojum aestivum* 'Gravity giant' as a potential source of alkaloids and as antagonist to some plant fungal pathogens

Yuka Munakata[1,2], Rosella Spina[2], Julie Genestier[1], François Dupire[2], Alain Hehn[1], Sophie Slezack-Deschaumes[1], Dominique Laurain-Mattar[2]

1 Université de Lorraine – INRAE ; LAE, F-54000 Nancy, France

2 Université de Lorraine – CNRS ; L2CM, F-54000 Nancy, France

1. Introduction

Amaryllidaceae is a family of bulbous plants famous for the synthesis of isoquinoleic alkaloids also known as Amaryllidaceae alkaloids like lycorine and galantamine. Since the isolation of lycorine from *Narcissus pseudonarcissus* in 1877 [8], more than 500 Amaryllidaceae alkaloids have been identified and characterized [9]. These alkaloids exhibit a wide range of biological activities such as antiviral, antibacterial, and antitumor for lycorine [10] and acetylcholinesterase inhibition for galantamine. This plant family has been used in traditional medicines. For example, in the middle of the 20th century, the populations of the Caucasian mountains applied bulbs of *Galanthus woronowii* for treatment of young children displaying symptoms of poliomyelitis [3]. Nowadays, galantamine is commercially used for the palliative treatment of Alzheimer's disease. The production of galantamine relies on plant extracts from Amaryllidaceae bulbs such as *Narcissus* spp. and *L. aestivum*. Full chemical synthesis of galantamine has also been attempted in the 1990s [1]. However, the structure of this molecule contains three asymmetric carbons, and the respect of the configuration leads to a tedious and complex process [11].

In Eastern Europe, cultures of *Leucojum aestivum* have been established for galantamine extraction for industrial application. However, the alkaloid yield from the bulbs varies depending on environmental conditions, with galantamine concentrations ranging from 0.1% to 0.52% (FW) [1]. This variation of alkaloid extraction from crop plants is dependent on climate fluctuations, pathogens infections and also relies on the political situation in producing countries [12]. A biotechnological approach to secure the production of this molecule is therefore considered as an interesting way for its production [3]. In order to optimize the production, various factors need to be considered, such as the degree of tissue and organ differentiation, the chemical composition of the medium, the addition of precursors, and elicitors [4,13,14]. These different tracks were investigated and revealed to interesting to improve the accumulation of galantamine in *in vitro* cultures. However, to date, this approach is not compatible with large-scale production.

Endophytic microbes have been identified in all plant species that have been studied so far. These microorganisms are able to colonize different plant tissues, such as roots, stems, leaves, flowers, fruits, and seeds. Although endophytes involve archaea, protists, and viruses, most of them are represented by bacteria and fungi. The structure of the endophytic community is influenced by many factors (geographical locations, plant tissues, pedoclimatic conditions, biotic and abiotic stresses, plant genotypes, and agricultural practices) [15,16]. Compared to the microbial communities in the rhizosphere, the endophytic root microbiome is less diversified, which led some authors to assume that plants selectively recruit microbes from the environment [15,17,18]. Moreover, alkaloid profiles in different tissues of a medicinal plant, *Macleaya cordata*, affected the compositions of endophytic bacteria [19]. This latter example might reveal that the phytochemical environment may impact the recruitment of endophytes.

The microbes isolated from inner plant tissues are attractive and might constitute original factories for producing new bioactive molecules. It is well-known that bacteria possessing benefic effects for plants often produce phytohormone and phytohormone-like molecules, such as 3-indole acetic acid, gibberellin, cytokinin, or salicylic acid [20]. These bacteria are also able to produce bioactive compounds with antifungal, antibacterial, antivirus, anticancer, anti-inflammatory properties, like non-ribosomally synthesized peptides, polyketides, volatile organic compounds, alkaloids, sesquiterpenes, flavonoids, saponins, lactones, organic acids [21,22]. Actually, bioactive molecules such as leucinostatin A, ecomycins, and maytansine, were discovered and isolated from fungal and bacterial endophytes [23–26]. Such new molecules may be further used to develop new lead compounds for agrochemical and pharmaceutical applications. Interestingly, some endophytes isolated from plants have also been reported to synthesize compounds that the host plants are supposed to produce. This is the case for paclitaxel, vinblastine, and quercetin for example [27,28]. Although such plant specialized metabolites were found in fungal endophytes it was demonstrated that a *Bacillus* strain isolated from an Icacinaceae was also able to produce camptothecin, a quinoline alkaloid, independently from the host plant [29,30]. These examples clearly establish that endophytes might be used as bioreactors or as genomic resources to create cell factories for producing phytochemicals whose production relies, nowadays, on harvesting plants or on complex industrial synthesis.

In this study, we isolate endophytic bacteria and create a bacterial library from bulbs, roots and shoots of *L. aestivum* 'Gravity giant'. This plant is a horticultural variety that grows quite fast and has large flowers [31]. Because Amaryllidaceae alkaloids differently accumulated depending on plant tissues [1] and the plant tissue type is also known as one of influencers on the composition of endophytes [32], we assumed that the different tissues of *L. aestivum* 'Gravity giant' may host different endophytic communities in terms of taxonomy and metabolites. The alkaloid production might be an indicator to evaluate it. Although, in the past, several studies isolated endophytic bacteria and fungi from Amaryllidaceae plants [5,6,33], the endophytic microbes were not investigated in *L. aestivum*. Recently, we succeeded in the isolation of endophytic *Bacillus*

strains from *in vitro* bulblets of *L. aestivum* 'Gravity giant' [7]. Here we deeper investigate the endophytic composition of the tissues of the plant. We screened bacterial isolates producing alkaloids and performed fine LC-MS analysis of methanol extracts from bacterial pellets. The isolates were taxonomically identified using 16S RNA sequencing and their potential use as biocontrol agent was assessed through antagonistic test towards some plant fungal pathogens.

2. Materials and methods

2.1. Source of Plant samples

Soil was sampled in an experimental field in la Bouzule, France, in October 2018. Since December 2018, seven plants of *Leucojum aestivum* 'Gravity giant' were cultured in this soil, which was placed indoor with watering once a week until bacterial isolation. Three entire plants were harvested on March 4th to 8th in 2019, being taken out from the soil carefully. The plant materials were washed with running tap water and the different tissues were separated using blades, such as aerial part (shoot), bulbs, and root. Shoot and root parts were washed again with tap water and then dried on paper towels. For bulbus parts, the outer tunic was pealed by blade and hands, and their basal plate was removed by blade because of their difficulty for disinfection. The treated bulb was cut into half and individual scales were separated by hands. Each tissue material was weighed as fresh material weight, just after the sampling. The fresh materials were then cut into certain size of pieces: for shoot and bulbus materials, 1 to 1.5 cm in horizontal length and 2 to 3 cm in vertical length; for root materials, around 3 cm in length. The trimmed materials were randomly distributed by around 1.1 g in a sterile 50 mL tube and stored at 4 °C. They were used for bacterial isolation within 48 hours after collection.

2.2. Bacterial isolation and cultivation

The plant materials were processed as described by Munakata et al. 2021[16], with a minor modification depending on the plant tissue. Briefly, for the surface sterilization, the shoot materials were treated in 70% ethanol for 1 min and in 2.6% bleach solution for 2.5 min; the bulb materials were treated in 70% ethanol for 1 min and in 2.6% bleach solution for 5 min; the root materials were treated in 70% ethanol for 1 min 3 times and in 2.6% bleach solution for 5 min. At last, all the materials were rinsed in sterile ultrapure water for 1 min and 3 times. All the treatments and the rinses were performed in 20 mL solution with agitation. The final rinse solution was used for testing the efficiency of the surface sterilization by spreading 100 µL on 10% Tryptic soy agar (10%TSA) media (Sigma) containing cycloheximide at 100 mg/L (10%TSAC). The treated plant materials were cut into smaller pieces using a sterile blade and then grinded by sterile tubes on sterile Petri dishes. They were then incubated in 10 mL of 0.85% (w/v) sodium chloride for 1 hour with agitation at 60 rpm at 28 °C. One hundred µL of

the incubated suspension was spread on three plates of 10%TSAC, with a dilution series of 1 time and 10 times. The plates were incubated at 28 ℃ for 20 days. For the calculation of Colony Forming Unit, the average number of colonies among 3 plates was used. The obtained colonies were purified by several subcultures on 10%TSAC plates, followed by on 10%TSA plates. The pure isolates were cultured in 2 mL Nutrient broth (NB) (Sigma) conserved in 15% glycerol solution at -80 ℃.

2.3. Molecular identification

The bacteria were taxonomically identified by PCR amplification of the 16S rDNA regions, performed with a method described in Munakata et al. 2021. For some isolated bacteria that had a difficulty in the amplification of the gene region with this method, DNA extraction was done using the DNeasy® UltraClean® Microbial Kit a (QIAGEN). The extracted genomic DNA solution was used as the template for PCR amplification with the primer set of 27F (5' – GAGAGTTTGATCCTGGCTCAG – 3') and 1492R (5' - CTACGGCTA CCTTGTTACGA – 3'). The PCR 50 µL reaction mix contained 1 µL of PrimeSTAR® GXL DNA Polymerase （Takara）, 10 µL of 5 × PrimeStar GXL buffer, 4 µL of dNTPs (2.5 mM for each), 1 µL of each primer at 10 µM, 1 µL of genomic DNA. PCR amplification was performed using the following program: 10 s 98 ℃, followed by 30 cycles of 10 s 98 ℃, 15 s 55 ℃, 2 min 68 ° C, with the final elongation at 68 ° C for 2 min. The result of the PCR was confirmed by gel electrophoresis and then purified as described by Munakata et al., 2021.

2.4. Crude extract preparation of bacterial pellet

Crude extracts for alkaloid detection were prepared from bacterial pellets. A preculture of the bacteria was done in 2 mL NB at 28 ℃ and 180 rpm for 24 hours. Hundred µL of the precultures were added to 50 mL NB in 250 mL Erlenmeyer flasks. After 72-hours cultivation at 28 ℃ 180 rpm, the bacterial pellets were collected by a centrifugation at 4000 xg, at room temperature for 40 min. The pellets were suspended in 1.5 mL methanol and treated with ultrasonication for 30 min, followed by maceration for 30 min at room temperature. The supernatant was then obtained by a centrifugation at 12,800 xg, at room temperature, for 10 min.

2.5. Thin layer chromatography (TLC) analysis and alkaloid detection with Dragendorff's reagent

Twenty µL of the extracts and 5 µL of a standard mixture of lycorine and galantamine at 1 mg/mL for each were sprayed with LINOMAT 5 (Camag®) onto HPTLC plates 60 F_{254} (Merck), 20 x 10 cm. The plates were developed in the mobile phase consisting of 10 mL of ethyl acetate,

1.1 mL of acetic acid, 1.1 mL of formic acid, and 2.5 mL of ultrapure water. After the development, the plates were air dried. To screen the presence of alkaloids, the plate was sprayed with Dragendorff's reagent containing a mixture of solution A, solution B, acetic acid and water (1: 1: 4: 20). Herein solution A was 0.85 g basic bismuth nitrate in 10 mL acetic acid and 40 mL ultrapure water, solution B was 8 g of potassium iodide in 30 mL ultrapure water. The colorized plates were observed successively just after spraying, after 30 min and after one day. The orange coloration of a spot indicated the potential detection of an alkaloid.

2.6. Dual culture assay to test the antagonistic activity of endophytic bacteria against two cereal pathogens

The antagonistic activities of 99 bacterial isolates were analyzed using an in vitro dual-inoculation method. *Fusarium graminearum* strain was kindly provided by Institute Français De La Brasserie Et De La Malterie (IFBM), and *Microdochium nivale* strain (MUCL 18523) was purchased from BCCL/MUCL. They were cultivated on Potato Dextrose Agar (PDA) (Sigma-Aldrich®) at 28 ℃ for *F. graminearum* and at 20 ° C for *M. nivale*, in darkness. Eight-mm-diameter mycelial plugs of these fungi were prepared from an active growing zone of the fresh cultures. The plugs were inoculated at the center of a plate containing 25 mL of PDA. Fifty µL of a bacterial suspension obtained from a liquid culture of an isolated bacterium in 2 mL of NB at 28 ℃ during 24 hours or 72 hours was dropped on 4 spots at 3 cm far from the center of plates. Three replicates were made for each bacterial isolate. The plates were incubated at 28 ℃ for *F. graminearum*, and at 25 ℃ for *M. nivale*, until the fungal colonies reached to the edge of the plates. The diameter of the fungal colonies was measured and averaged among replicates. Plates inoculated with the fungi without bacterial cultures were used as controls. Antagonistic activities were expressed as the inhibitory rate (%), calculated as follows: $((d_{control} - d_{assay})/d_{control} \times 100)$, wherein d means diameter of the colony of the fungi.

2.7. Diffusion assay of cell-free culture broth and methanolic extracts of the selected strains against *F. graminearum* mycelia

The crude extracts of LaBFB3301, LaBFS1102, LaBFS2103 were prepared as above described. The 24-hours-cultured bacterial broth in 2 mL NB were centrifuged at 15,000 rpm for 10 min and filtrated with Acrodisc® Syringe Filter 0.2 µm Supor® Membrane. For the diffusion assay, ϕ 5.3-mm paper discs (Whatman) were autoclaved and dried at 70 ℃. Twenty-five µL of each sample were applied to each paper disc and the discs were dried under laminar flow hood. Four discs per 20 mL PDA plate were placed diagonally at 1 cm from the center. Fungal plugs were prepared and placed at the center as above described. The plates were incubated at 28 ℃ for *F. graminearum* and at 20 ℃ for *M. nivale*. The diameters of the fungal mycelial colonies were

measured every single day. However, when the fungal colonies overlapped the discs at Day 1 and 2, the edge of some colonies was hard to observe. At Day 3, all the fungal colonies developed passing the discs. So, as growth inhibition rate, the values from Day 3 were adapted for comparison. Growth inhibition rate was calculated as mentioned in 2.6.

2.8. LC-MS Equipment and Analysis

Untargeted metabolomics was done with UHPLC-Q-Exactive-Orbitrap (Thermo Fisher Scientific, San Jose, CA, USA). The column was Kinetex XB-C18 (150*2.1 mm × 2.6μm) (Phenomenex, Torrance,CA, USA). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B) with 200 μL/min. Gradient elution was performed with a program: 0-1 min, 10 – 10 % B; 1-15 min, 10 – 60% B; 15 – 30 min, 60 – 95 % B; 30 – 35 min, 95 – 95% B. MS data were obtained from Orbitrap IDX mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) using positive ionization mode. The ionization was achieved with the heated electrospray ionization (HESI) source and the scan range was 120 to 1200 m/z. The spectrometer worked with the data-dependent scan. MS2 fragments were obtained with collision-induced dissociation (HCD) at 30% energy. The resolution was 60,000 for MS1 and 30,000 for MS2.

The results were analyzed by Compound Discoverer 3.1 (Thermo Fisher Scientific, San Jose, CA, USA). The peaks were extracted with the threshold at minimum intensity = 50,000 m/z and then aligned and identified for metabolites comparing with the public databases, such as mzCloud. The peaks having full match in mzCloud were used for the further analysis, and categorized as “enriched” in test samples when they satisfied log2 fold change to medium control >1 and $p < 0.05$.

To identify tryptophol and confirm the absence of lycorine, galantamine, and indole-3-acetic acid (IAA), the bacterial methanolic extracts were analyzed by LC-MS/MS system using positive ionization mode. It was realized by ESI- High Resolution Mass Spectrometry (HRMS) with micrOTOF$_Q^{TM}$ (Bruker Daltonics, Bruker, Bremen, Germany) apparatus. The column was an Hypersil gold (100 mm×3 μm) (Thermo Scientific, Bellefonte, PA, USA) and the flow rate was at 200 μL/min using a program: 5% solvent B for 5 min, a gradient from 5% solvent B at 5 min to 99% of solvent B at 40 min, followed by 99% solvent B for 5 min. Solvent A composed with 1% formic acid in water with LC/MS grade and solvent B composed with pure acetonitrile. The calibration was done with a peak of sodium formate in each analysis. The spectrometer worked with the auto MS/MS mode. Each detected mass peak was isolated and fragmented with the collision energy at 20 eV. The results were analyzed by Bruker Compass® software.

2.9. Data analysis

R studio with R version 3.6.3. was used for statistical test and analysis. The Bray-Curtis distance was calculated with the R package "vegan." This package was also used for performing PERMANOVA (permutations = 10,000) with adonis. Principal Coordinate Analysis (PCoA) and scatter plots were illustrated using the ggplot2 package. Tukey-Kramer test was realized with the R package "multcomp."

3. Results

3.1. Bacterial collection isolated from L. aestivum 'Gravity giant'

In 2019, bacterial colonies were isolated from 3 different tissues of 3 plants of *L. aestivum* 'Gravity giant' at the vegetative stage (Fig. 1, Supplemental figure 1). The results we obtained from 1 g of fresh weight materials (g FW) made evident that the number of Colony-forming units (CFU) was different depending on the original organs. For roots we got 8,569 CFU/g FW), while shoot and bulb parts led to a smaller number of colonies (1,210 CFU/FW and 169 CFU/g FW, respectively) (Supplemental figure 1). In total, 985 colonies appeared in 10% TSAC media, containing 848 colonies from root, 115 colonies from the shoot and 22 from the bulbs. After the isolation step, several subculturing on 10% TSAC and 10% TSA media, only 138 remaining colonies were successfully purified (Table 1, Supplemental Table 1). This bacterial collection consisted of 67 from strains isolated form roots, 64 from the shoot material and only 7 seven from the bulbs.

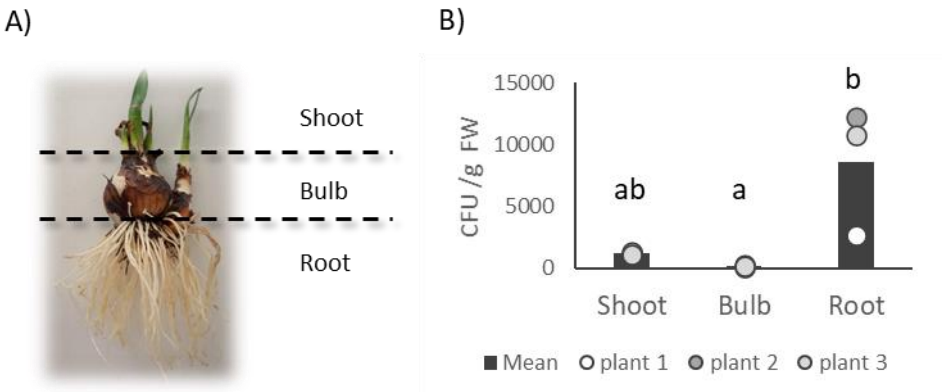


Fig. 1 | A sampled plant (plant 2) and the definition of different tissues in this study and CFU per 1 g of each fresh material 20 days after isolation

Table 1 | Taxonomical compositions of the bacterial collection isolated from *L. aestivum* Gravity giant

	Plant tissues			
Phylum / Genus	**Shoot**	**Bulb**	**Root**	**Total per taxon**
Actinobacteria	**8**	**4**	**21**	**33**
Arthrobacter	1			1
Curtobacterium	1			1
Herbiconiux	1			1
Leifsonia	1		1	2
Microbacterium	2		4	6
Microlunatus			1	1
Mycobacterium		1	1	2
Mycolicibacterium	1			1
Nocardia			2	2
Nocardioides			1	1
Paenarthrobacter			1	1
Patulibacter	1	2		3
Promicromonospora			2	2
Rathayibacter			1	1
Streptomyces		1	7	8
Bacteroidetes	**2**		**1**	**3**
Chitinophaga	2			2
Pedobacter			1	1
Firmicutes	**1**	**1**	**29**	**31**
Bacillus			12	12
Paenibacillus		1	11	12
Peribacillus			3	3
Rossellomorea			1	1
Staphylococcus	1		2	3
Proteobacteria	**53**	**2**	**16**	**71**
Achromobacter	2			2
Agrobacterium		1	2	3
Caulobacter			2	2
Comamonas	1			1
Dyella	1			1
Ensifer			2	2
Erwinia	1			1
Herbaspirillum	2			2
Inquilinus			1	1
Labrys			1	1
Luteibacter	5	1		6
Moraxella			1	1
Novosphingobium	1			1
Paraburkholderia	2		3	5
Phyllobacterium			2	2
Pseudomonas	1			1
Rahnella	27			27
Roseateles	1			1
Sphingopyxis			2	2
Variovorax	9			9
Total per tissue	**64**	**7**	**67**	**138**

3.2. Compositional analysis on the L. aestivum 'Gravity gian't bacterial collection

The strains were taxonomically identified by sequencing a 16S rDNA region amplified by PCR and sequence comparison Basic Local Alignment Search Tool (BLAST) in GenBank. These analyses showed that 42 genera belonged to 4 phyla. To assess the relative abundance of the compositions of the isolates from the three tissues at the genus level, we realized a principal coordinate analysis (PCoA) using Bray-Curtis distance (Fig. 2). The analysis showed that root and shoot groups were well distinguished by the 1st axis, while the bulb group was not separated from the other two groups. This observation was validated by Permutation ANOVA (permutations = 10000); tissue origins gave distinguishment with a p-value of 0.0351.

The composition of the bacterial collection at the phylum level differed depending on the source of plant tissues: Proteobacteria were dominant in the shoot bacterial collection (83%), Actinobacteria were the most abundant in the bulb one (57%), and Firmicutes were mainly detected in the root (43%) (Fig. 3). At the genus level, 21 genera, 25 genera, and 6 genera were found in the shoot, the root, the bulb, respectively. However, no genus was common among all the three tissues (Fig. 4). The isolates from the bulb were affiliated to 6 genera, which were shared with the shoot (2 genera) and root (4 genera) parts, and no genera were unique in bulbs. The most abundant genera were *Rahnella*, *Patulibacter*, and *Bacillus*, in the shoot, bulb, root, respectively.

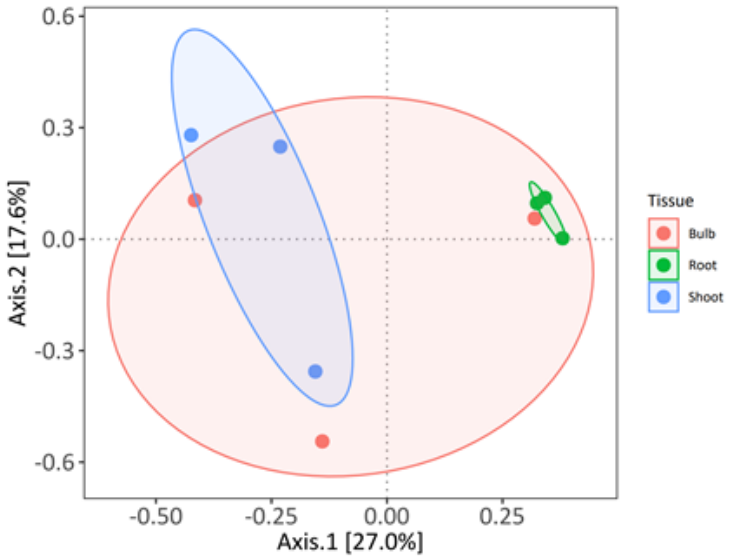


Fig. 2 | Principal coordinate analysis (PCoA) with Bray-Curtis distance of the *L.aestivum* bacterial collections at the genus level

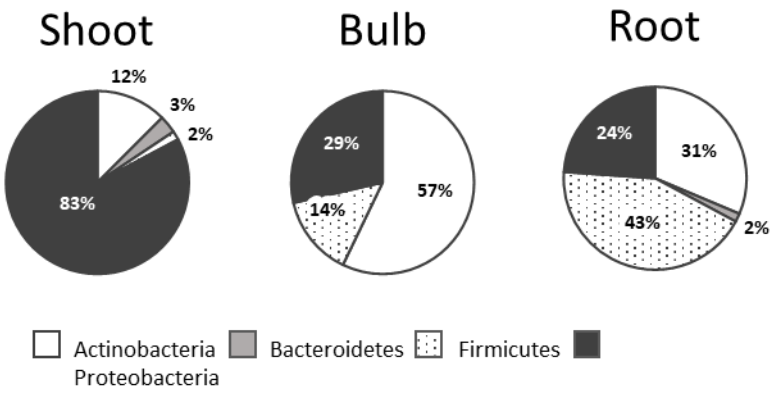


Fig. 3 | Relative abundance of each phylum in the bacterial collection of the different plant organs

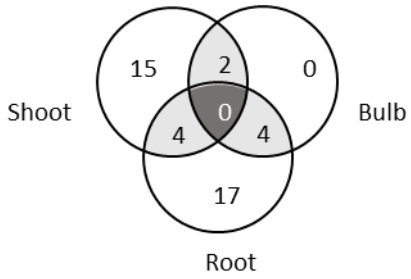


Fig. 4 | Venn diagram of Shared and unique bacterial genera in *L. aestivum* 'Gravity giant' plant organs

3.3. Alkaloid screening using HPTLC

Amaryllidaceae plants, especially *L. aestivum*, are famous for producing Amaryllidaceae alkaloid in bulbs. As it is known that endophytes are known to produce bioactive compounds, we performed a phytochemical analysis of endophytic bacteria extracts. For this purpose, the methanolic extracts obtained from bacterial pellets were screened for any alkaloid detection by spraying Dragendorff's reagent after the development of HPTLC plates. Over the time after the Dragendorff spray, some orange or pink signals were observed. Among 138 tested strains, 28 showed some coloring reaction for Dragendorff's reagent (Table 2), but none of these molecules had the same Rf value of the standard compounds of lycorine and galantamine. These molecules should now be deeper investigated especially through LC-MS analyses.

Table 2 | List of strains of which extract (possibly) reacted to Dragendorff's reagent
The growth inhibition rates against *F. graminearum* and *M. nivale* are also shown in the right columns (mean +/- SE).

Source tissues	Strain	Dragendorff	Blast top hit	Accession	E value	Inhibition rate vs Fg (mean +/- SE)	Inhibition rate vs Mn (mean +/- SE)
Shoot	**LaBFS1102**	Yes	*Paraburkholderia fungorum* strain LMG 16225	NR_025058.1	0	**41.91 ± 0.19**	**56.69 ± 0.12**
	LaBFS1106	Yes	*Patulibacter ginsengiterrae* strain P4-5	NR_108221.1	0	-	-
	LaBFS1107	Yes	*Chitinophaga ginsengisegetis* strain Gsoil 040	NR_041486.1	0	**26.57 ± 0.67**	14.54 ± 2.3
	LaBFS1112	Yes	*Variovorax ginsengisoli* strain Gsoil 3165	NR_112562.1	0	0 ± 0.2	9.15 ± 0.76
	LaBFS1201	Yes	*Staphylococcus warneri* strain AW 25	NR_025922.1	0	**37.71 ± 0.57**	**29.73 ± 3.06**
	LaBFS1208	Yes	*Mycolicibacterium setense* strain CIP 109395	NR_116291.1	1E-151	2.81 ± 0.74	8.87 ± 0.82
	LaBFS1307	Yes	*Microbacterium luteolum* strain IFO 15074	NR_024636.1	0	4.79 ± 0.95	4.17 ± 1.21
	LaBFS2103	Yes	*Comamonas denitrificans* strain 123	NR_025080.1	0	**43.51 ± 0.09**	**58.82 ± 1.19**
	LaBFS2201	Yes	*Microbacterium arthrosphaerae* strain CC-VM-Y	NR_117046.1	7E-55	-0.8 ± 0.06	6.17 ± 0.16
	LaBFS3105	Yes	*Variovorax paradoxus* NBRC 15149	NR_113736.1	0	3.4 ± 0.23	7.54 ± 2.97
	LaBFS3313	Not done	*Arthrobacter oryzae* strain KV-651	NR_041545.1	0	**30.73 ± 0.96**	18.46 ± 3.11
Bulb	LaBFB1102	Yes	*Paenibacillus amylolyticus* strain JCM 9906	NR_112163.1	0	-0.4 ± 0.12	0 ± 0
	LaBFB1103	Not done	*Streptomyces canescens* strain DSM 40001	NR_119346.1	0	**49.59 ± 0.79**	**59.85 ± 0.82**
	LaBFB1301	Yes	*Patulibacter ginsengiterrae* strain P4-5	NR_108221.1	0	2.99 ± 1.04	**32.61 ± 0.36**
	LaBFB1302	Yes	*Patulibacter ginsengiterrae* strain P4-5	NR_108221.1	0	1.6 ± 0.35	**32.62 ± 0.91**
	LaBFB3301	Yes	*Luteibacter rhizovicinus* strain LJ96	NR_042197.1	0	**37.32 ± 0.2**	**48.06 ± 1.01**
Root	LaBFR1204a	To be validated	*Bacillus licheniformis* strain DSM 13	NR_118996.1	9E-69	**26.43 ± 0.13**	**45.43 ± 0.23**
	LaBFR1205b	To be validated	*Bacillus licheniformis* strain DSM 13	NR_118996.1	3E-69	**43.33 ± 0.42**	**40.76 ± 0.52**
	LaBFR1305a	To be validated	*Staphylococcus warneri* strain AW 25	NR_025922.1	0	18.45 ± 0.17	**29.54 ± 0.84**
	LaBFR2101	To be validated	*Microbacterium maritypicum* strain DSM 12512	NR_114986.1	0	3.77 ± 0.65	14.58 ± 1.82
	LaBFR2204a	To be validated	*Bacillus subtilis* strain IAM 12118	NR_112116.2	0	**34.49 ± 1.19**	**52.85 ± 1.47**
	LaBFR3101b	To be validated	*Paenarthrobacter nitroguajacolicus* strain G2-1	NR_027199.1	0	15.18 ± 1.85	**28.13 ± 0.32**
	LaBFR3103	To be validated	*Streptomyces canescens* strain DSM 40001	NR_119346.1	0	**45.97 ± 0.3**	**58.11 ± 0.89**
	LaBFR3106	To be validated	*Microbacterium maritypicum* strain DSM 12512	NR_114986.1	0	1.59 ± 0.25	0.94 ± 2.01
	LaBFR3107a	To be validated	*Promicromonospora alba* strain 1C-HV12	NR_148779.1	0	-2.51 ± 0.56	20.02 ± 2.65
	LaBFR3107b	To be validated	*Promicromonospora alba* strain 1C-HV12	NR_148779.1	0	-1.71 ± 0.67	21.15 ± 1.21
	LaBFR3212	To be validated	*Staphylococcus epidermidis* strain Fussel	NR_036904.1	0	**35.96 ± 0.51**	12.84 ± 1.46
	LaBFR3305b	To be validated	*Streptomyces canescens* strain DSM 40001	NR_119346.1	2E-147	**48.81 ± 0.36**	**57.26 ± 0.61**
	LaBFR3308a	To be validated	*Streptomyces canescens* strain DSM 40001	NR_119346.1	0	-2.31 ± 0.6	**21.36 ± 1.33**
	LaBFR3308b	To be validated	*Streptomyces canescens* strain DSM 40001	NR_119346.1	0	**44.07 ± 0.34**	**59.25 ± 0.61**

3.4. Antifungal activities of the isolates

To screen the potential of the isolates to be antagonistic to plant pathogens, we performed a dual-culture test of 99 bacterial strains against two fungi: *F. graminearum* and *M. nivale* (Fig. 5). Since many strains shared the same taxonomical affiliation, like *Rahnella*, we chose to test only a few strains from the same taxon (Supplemental Table 1). Moreover, the isolates which required longer than three days for sufficient growth in the liquid culture were removed from this test. The antagonistic activities of the isolates against *F. graminearum* varied from 0 to 49.6% and differed between the bacterial group from shoot and root (Tukey-Kramer method, $p = 0.00817$) (Supplemental figure 2). On the other hand, those against *M. nivale* had a variation from 10.6% to 60.4%, but no significant differences were related to the source of plant tissues. The antifungal activities for both fungi showed a good correlation (Fig. 6). Among them, 24 isolates, indicated in a red square in Fig. 6, showed more than 30% inhibition against *F. graminearum* and more than 40% against *M. nivale*. This group contained 16 Proteobacteria strains (5 *Paraburkholderia*, 1 *Comamonas*, 8 *Rahnella*, 1 *Ensifer*, 1 *Luteibacter*, 1 *Erwinia*), five Actinobacteria strains (5 *Streptomyces*), and two Firmicutes strains (2 *Bacillus*).

The bacterial genera with the highest antifungal activities were different depending on the origin of plant tissues. In the shoot group, three Proteobacteria genera, such as *Comamonas*, *Rahnella*, and *Paraburkholderia*, had a higher inhibition rate than the others, while it was

Streptomyces in the bulb, and *Paraburkholderia*, *Streptomyces*, and *Bacillus* had better antifungal activities in the root group (Fig. 7). Interestingly, *Paraburkholderia*, which appeared from the shoot and root parts, showed higher antagonisms with smaller variation among the isolates.

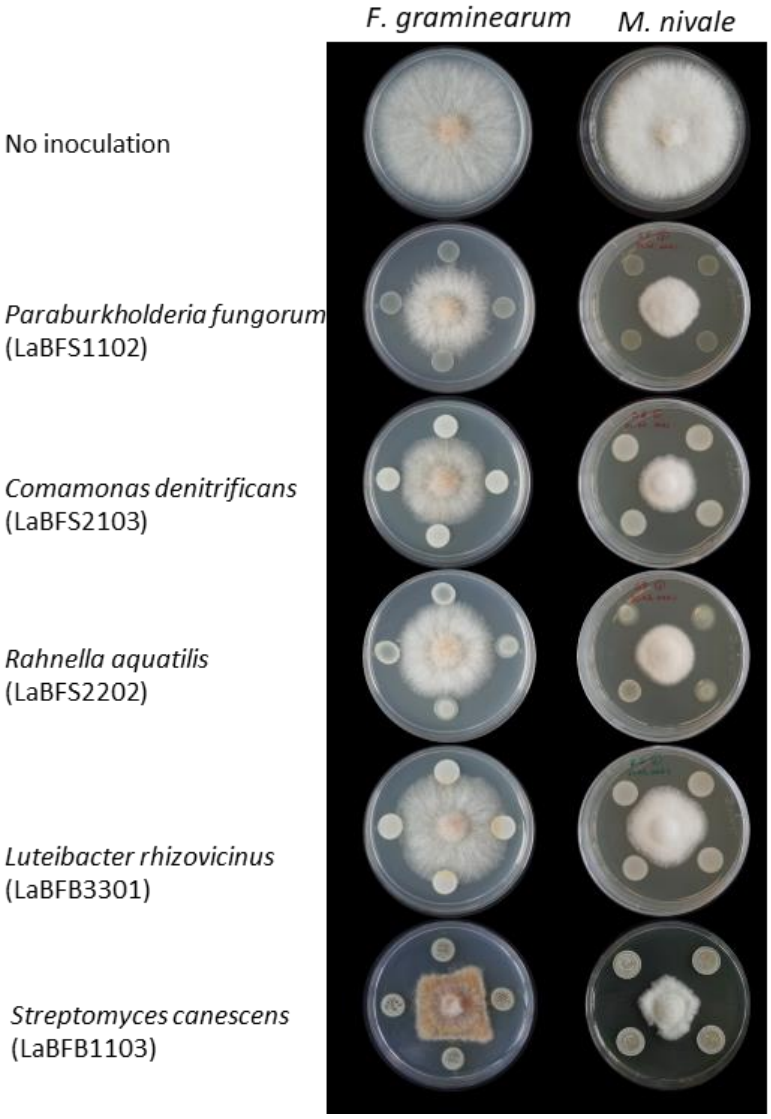


Fig. 5 | Dual-culture test of representative strains tested.

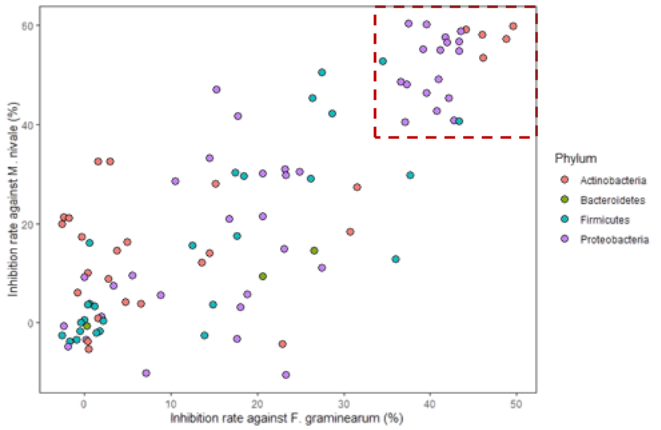


Fig. 6 | Correlations between antifungal activities against *F. graminearum* and *M. nivale*, colored by taxonomical affiliation at the phylum level

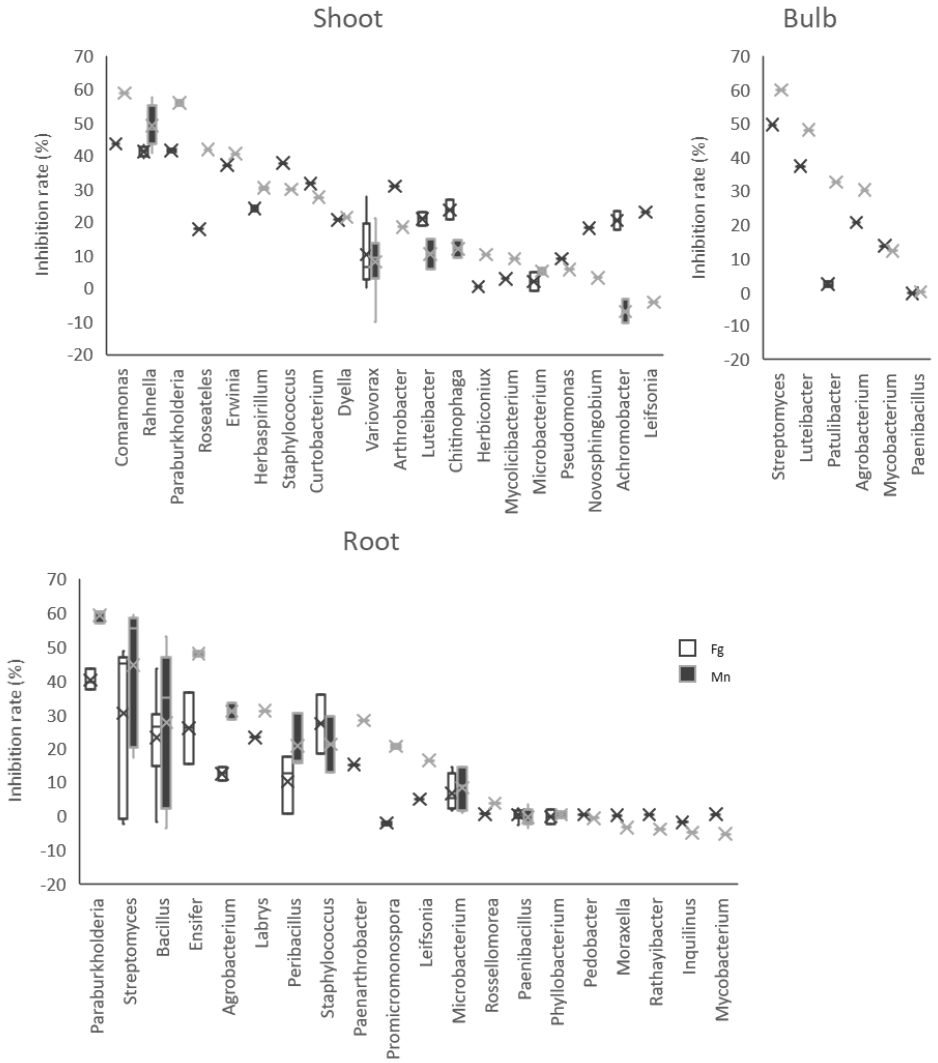


Fig. 7 | Growth inhibition rate against *F. graminearum* (Fg) and *M. nivale* (Mn) by genus of bacterial isolates

3.5. Antagonism activities of cell-free supernatant and methanol extract against F. graminearum

Among all the candidates, we selected three isolates, LaBFB3301 (*Luteibacter rhizovicinus*), LaBFS1102 (*Paraburkholderia fungorum*), LaBFS2103 (*Commamonas denitrificans*), that rapidly grew in NB liquid culture, and who produce original alkaloids (Table 2). The results we obtained In diffusion assays of the cell-free supernatant of the culture broth or the methanol extracts, showed that the methanol extracts from LaBFB3301 and LaBFS2103 delayed the growth of *F. graminearum* colonies on PDA, at 15.5% and 19.9% as mean inhibition rate, respectively (Fig. 8). All the samples from LaBFS1102 had no or slight effect on the growth of the fungus (Fig. 8 (a)). The responsible compounds of antagonism of LaBFS1102 are anticipated to be poorly produced and present in too low concentration in the samples. It is also possible that these molecules are induced in the presence of the fungi.

3.6. Untargeted LC-MS-Based Metabolomics

Liquid Chromatography coupled with high-resolution Mass Spectrometry (LC-MS) analysis was realized on methanolic extracts of the three endophytic bacteria strains displaying signals on TLC for Dragendorff's reagent (LaBFS1102, LaBFB3301, LaBFS2103) using the same analytical conditions as previously published (Spina et al., 2020). LC-MS analysis performed in positive-ion mode confirmed the absence of lycorine and galantamine in the methanolic extracts as previously observed with the HPTLC analysis (Supplemental figure 3).

LC-MS profiling generated an extensive mass list corresponding to a wide variety of metabolites in the endophytic extracts from *L. aestivum* 'Gravity gianst' bulb (LaBFB3301) and from *L. aestivum* 'Gravity giant' shoot (LaBFS1102, LaBFS2103), using HPLC-Q-Exactive-Orbitrap®. Using the Compound discoverer software led to the identification of 159 peaks in total thanks to MS/MS spectra comparison with online databases such as Mzcloud. 60 peaks of metabolites were enriched in the methanolic extracts of the bacterial pellets (Fig. 9). Ten compounds were commonly observed in the three strains, and 11, 13, and 0 compounds were specific in LaBFS1102, LaBFB3301, LaBFS2103, respectively. 25 additional compounds were shared commonly between LaBFB3301 and LaBFS2103, and only 1 compound was shared between LaBFB3301 and LaBFS1102.

In the shared compounds between LaBFB3301 and LaBFS2103, we identified the indolic alkaloid tryptophol,3-(2-hydroxyethyl) indole both by MS analysis ($C_{10}H_{11}NO$, $[M + H]^+$ 162.0913) and by its retention time (12.4 min) (Fig. 10). This result was confirmed using the commercial standard tryptophol (Supplementary Figure 4). The same peaks and fragments were highlighted in all the triplicates of LaBFB3301 and LaBFS2103 samples (Supplemental figure 4). Tryptophol is a precursor of various indole alkaloids in plants and microorganisms

(Palmieri and Petrini, 2019). The origin of tryptophol is closely related to the tryptophan metabolic pathway. However, the amino acid tryptophan ($C_{11}H_{12}N_2O_2$, $[M + H]^+$ 205.0972) was not identified in the three endophytic bacteria strains. The peak of tryptophol was observed at 13.8 – 14.0 min with m/z 162, which had MS2 fragments with m/z 143, 130,115. Also, IAA, which shares precursors of tryptophol and takes part in plant growth promotion effect, was not found in any samples, even compared with the standard compound.

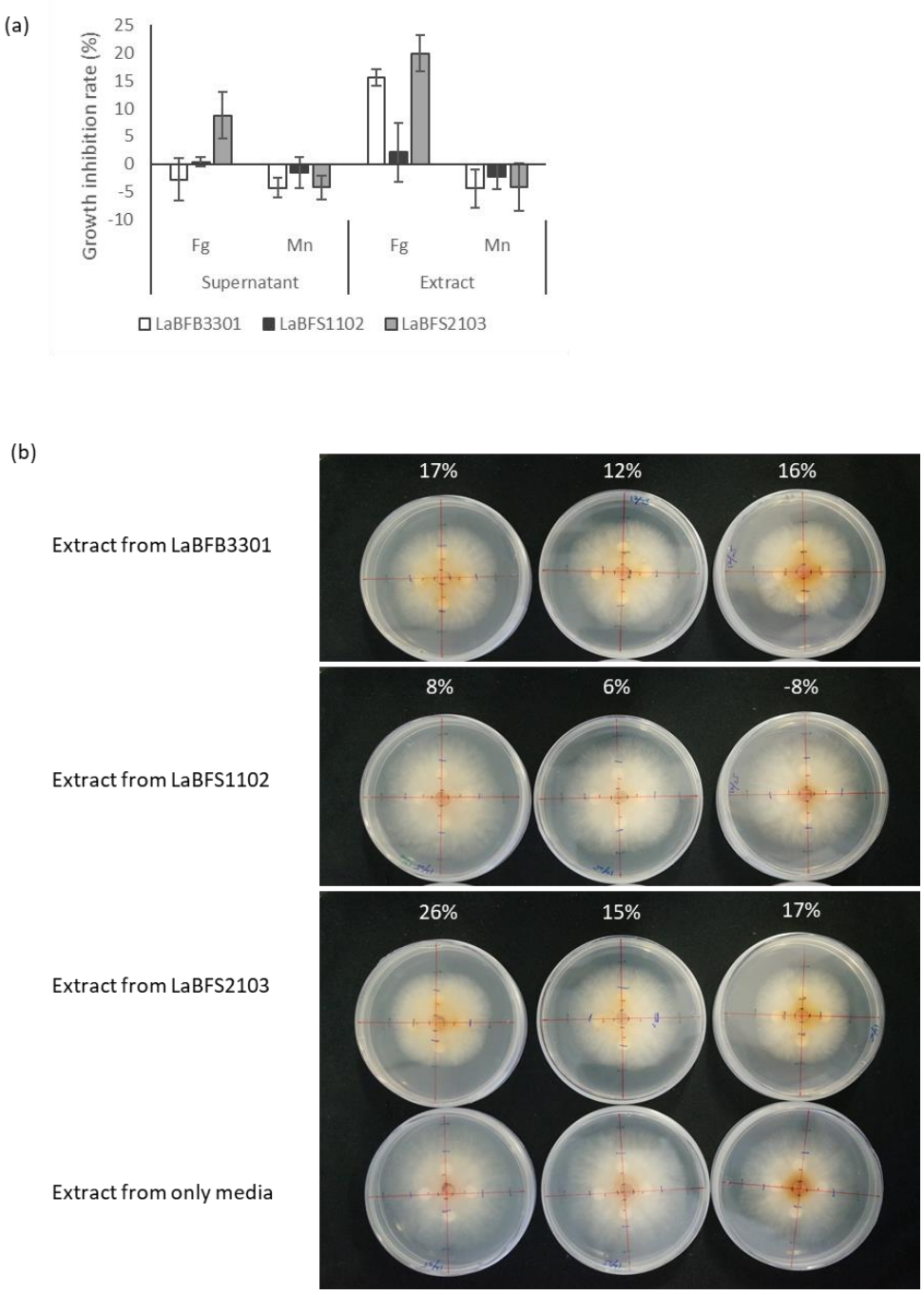


Fig. 8 | Growth inhibition test of cell-free supernatant and methanol extracts of 3 endophytic bacteria, LaBFB3301, LaBFS1102, LaBFS2103, against mycelia of *F. graminearum* (Fg) and *M. nivale* (Mn) after 3 day co-culturing

(a) Growth inhibition rate; (b) Growth inhibition of the methanol extracts for Fg after 3 days. The above number represents the growth inhibition rate.

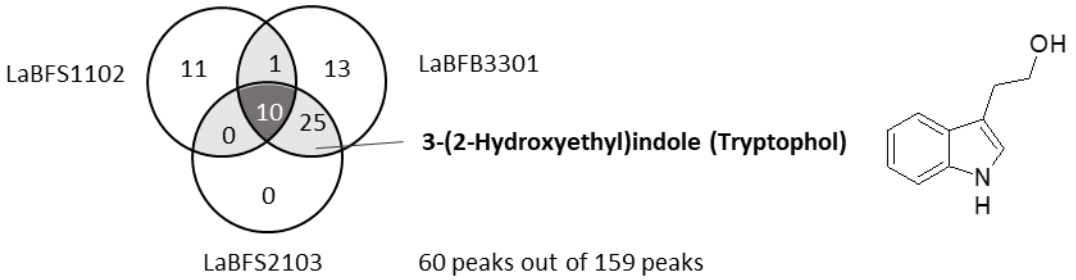


Fig. 9 | Venn diagram of shared and unique peaks of enriched metabolites

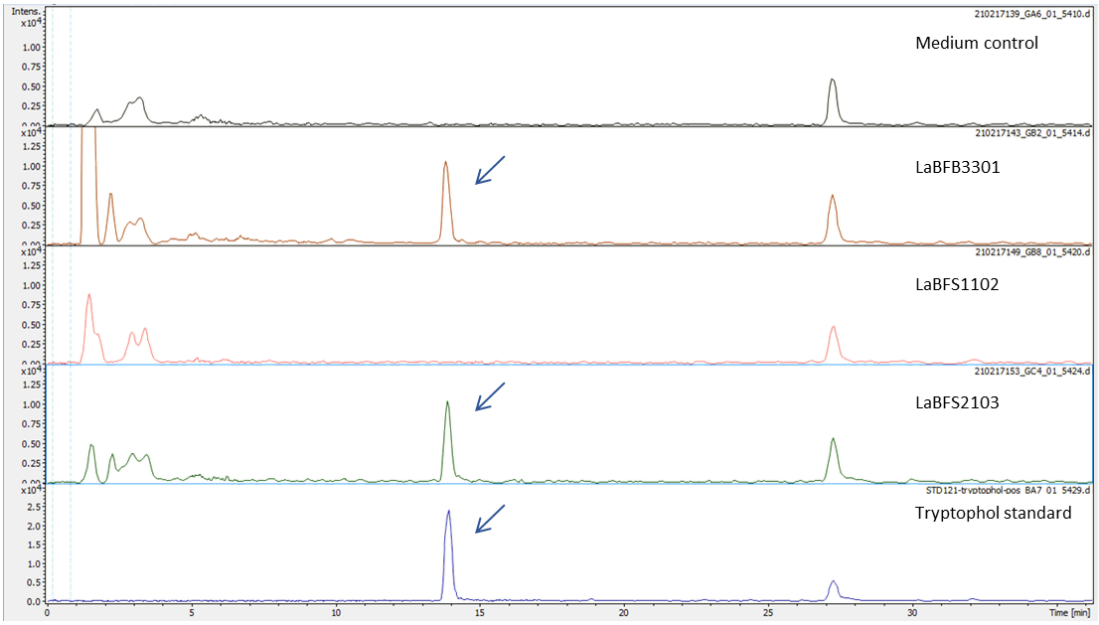


Fig. 10 | Ion chromatogram of m/z 162.0900 ± 0.02 for tryptophol found in LaBFB3301 and LaBFS2103

4. Discussion

It is known that Amaryllidaceae alkaloids showed many biological activities, including antibacterial activity [34]. A recent study showed that alkaloid profiles in different plant tissues are related to the endophytic bacterial community [19]. It is therefore of great interest to investigate whether the Amaryllidaceae alkaloid profile in various tissues or plant species modulates endophytic bacteria compositions. Endophytic bacteria and fungi were isolated from many plant species, but only a few Amaryllidaceae plants have been studied for this point so far. The plants that have been investigated so far are *Narcissus tazetta* [35], *Crinum macowanii* [33,36,37], and *Lycoris radiata* [6]. The latest example was reported by Spina et al. in 2021, with *in vitro* bulblets of *L. aestivum*. In our study, bulbs harbor a low number of cultivable bacterial strains, which is consistent with the observation of the few endophytic bacteria isolated from *in vitro* bulblets of *L. aestivum* [7]. This result could be associated with the amount of alkaloids like galantamine which is the highest in the bulbs of Leucojum plants [38].In contrast, the roots were found to contain the highest number of bacterial endophytes, probably explained by the contact of the roots with a large reservoir of bacteria in the soil [39].

The data collected in our study showed that the dominant bacterial phyla in *L. aestivum* 'Gravity giant' among culturable endophytes are Proteobacteria (51.4%), Actinobacteria (23.9%), and Firmicutes (22.5%) Bacteroidetes (2.2%). A similar distribution was reported for *Lycoris radiata* [6] or *Zea mays* [40]. When focusing on the taxonomical distribution of endophytic bacteria throughout plant tissue, no genus was shared among the three different tissues we analyzed in *Leucojum aestivum* 'Gravity giant'. This seems to be common, at least with in *Lycoris radiata*, for which the analysis was done among five different organs (flower, scape, leaf, bulb, and root) (Liu et al., 2020). This is an important point for further studies that would like to identify bacterial strains with some specific properties, and a relationship between metabolomic profiles and bacterial diversity might be established.

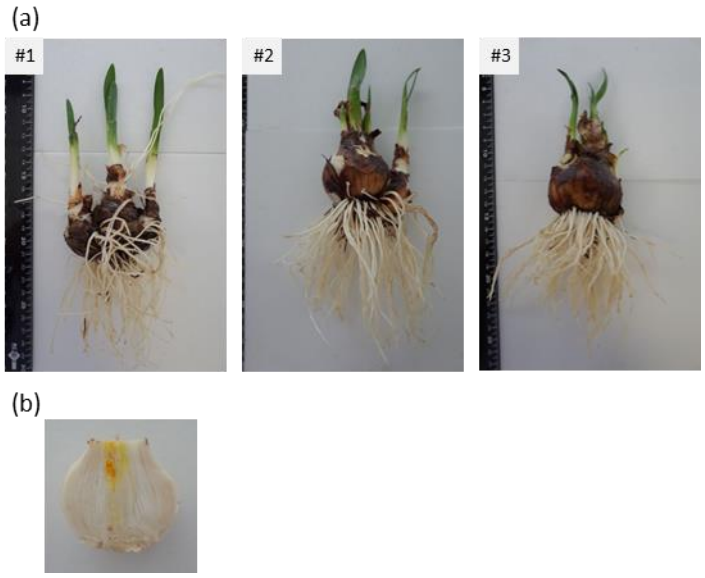
Amaryllidaceae plants seem to share a similar pattern of endophytic bacterial diversity. A survey realized in 7 different studies (including our study) showed that the most frequently isolated genera were *Bacillus* (5/7), followed by *Acinetobacter*, *Novosphingobium*, and *Pseudomonas* (3/7) (Supplemental Table 2). When going deeper in detail and when focusing on different plant tissues, we could highlight similarities but also some interesting differences. The leaf endophytic bacteria, *Arthrobacter, Luteibacter*, *Pseudomonas,* were common to other Amaryllidaceae plant leaf tissues [6,36]. Concerning roots, *Bacillus* and *Paenibacillus* genera were predominant in *Leucojum aestivum* as well as *Lycoris radiata* [6]. But most interesting is that *Bacillus,* which were mostly isolated from bulbs in previous studies, could not be highlighted in our *Leucojum aestivum* 'Gravity giant' bulb.

In this study, we obtained taxonomical information for 138 isolated bacterial strains. Many of their affiliations are already known as endophytes in some plants. Endophytic bacteria

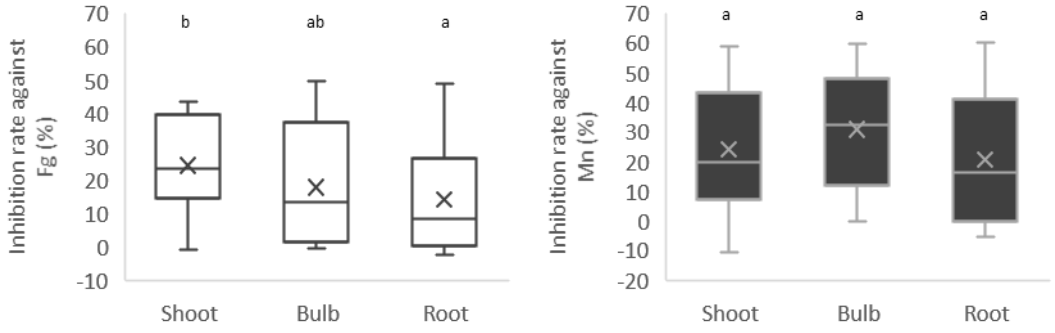
belonging to *Bacillus* and *Streptomyces* genera are well known for their metabolic capability to produce various bioactive compounds as well as their function of plant protection [41]. An endophytic strain of *Paenibacillus amylolyticus* was reported to produce dipicolinic acid with antibacterial activity [42], and *Paraburkholderia fungorum* was reported as an endophyte of rice, and it showed plant growth promotion in strawberry [43,44]. *Chitinophaga ginsengisegetis* was reported as root endophytes in grapevine [45]. *Rahnella aquatilis* was also isolated from *Crinum macowanii* Baker [37]. *Luteibacter rhizovicinus* was found in apple shoots, and displays plant growth promotion properties in barley [46]. The genus *Variovorax* is famous for its metabolic diversity and is also known for its plant growth-promoting function [47]. *Staphylococcus* is a well-known genus since some of their species are human pathogens, but this genus is also isolated as endophytes. Rebotiloe et al. isolated bacterial endophytes from *Crinum macowanii* Baker, an Amaryllidaceae plant, and three isolates among the total of five isolates were *Staphylococcus* species [33]. They also observed moderate antibacterial activities from those strains. *Mycolicibacterium* species were also isolated as endophytes, and some of them showed enhancement of plant growth promotion of beneficial fungal endophytes [48].

The results obtained in this study showed some isolates displaying nice antagonism against wheat fungal pathogens. Interestingly all the isolates belonging to *Paraburkholderia fungorum* that were isolated from the shoot and root tissues showed better and less various mycelial growth inhibition. In the genus *Paraburkholderia*, there is a famous and beneficial endophytic strain named *P. phytofirmans* PsJN. This strain has the capability to promote plant growth and mitigate biotic/abiotic stress in a wide range of host plants by producing various compounds [49]. However, because the methanolic extracts and filtrate of the culture broth of LaBFS1102 didn't show antagonism for the same pathogens, it might be possible that the amounts of bioactive compounds was too low in those samples or that their production relies on induction by the presence of target microorganisms. LaBFS2103, *Comamonas denitrificans*, showed higher growth inhibition against *F. graminearum* in the dual culture test and in the diffusion assay of the methanolic extract. It also displayed an antagonistic activity against *M. nivale* in the dual culture test, but the supernatant sample and the methanolic extract didn't have such activity, which suggested different modes of action against fungi. LaBFB3301, *Luteibacter rhizobicinus*, showed a similar trend to LaBFS2103. In fact, we found a common compound in the methanolic extract of both strains, tryptophol. This molecule is a precursor of different indole alkaloids in plants and microorganisms [50]. However, indole-3-acetic acid, which shared precursors of tryptophol and took part in the plant growth promotion effect, was not found in any samples, even compared with several standard compound. Tryptophol itself has been found in plants and microorganisms and also discovered as one of the quorum-sensing molecules in fungi. It might be possible that it is involved in the induction of cell apoptosis in specific fungi [51]. Tryptophol also has a growth promotion effect in roots and leaves of plants [50]. It is of interest to confirm if this compound relates to the observed antifungal activity and also to search its derivatives in

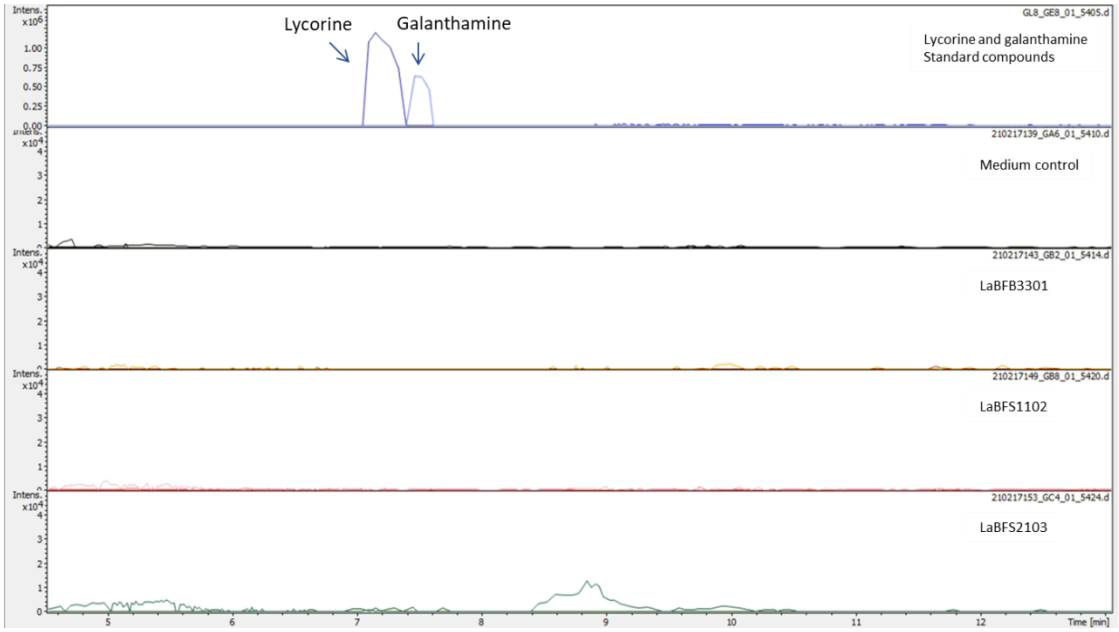
the methanolic extracts. Other candidate compounds representing antifungal activities of LaBFS2103, *Comamonas testosteroni*, isolated from nematode, produced diketopiperazines having antimicrobial activities [52]. On the other hand, endophytic *Luteibacter rhizobicinus* was reported to produce IAA, and the strain did not have harmful effects on plant growth [46]. However, we were not able to find IAA in the methanolic extracts. Nevertheless, it might be interesting to characterize the plant protection effect and the plant growth promotion effect of LaBFB3301 and LaBFS2103 as the next step.



Supplemental figure 1
(a) Sampled plants after washing with running tap water; (b) A segment of the bulb of plant 2.

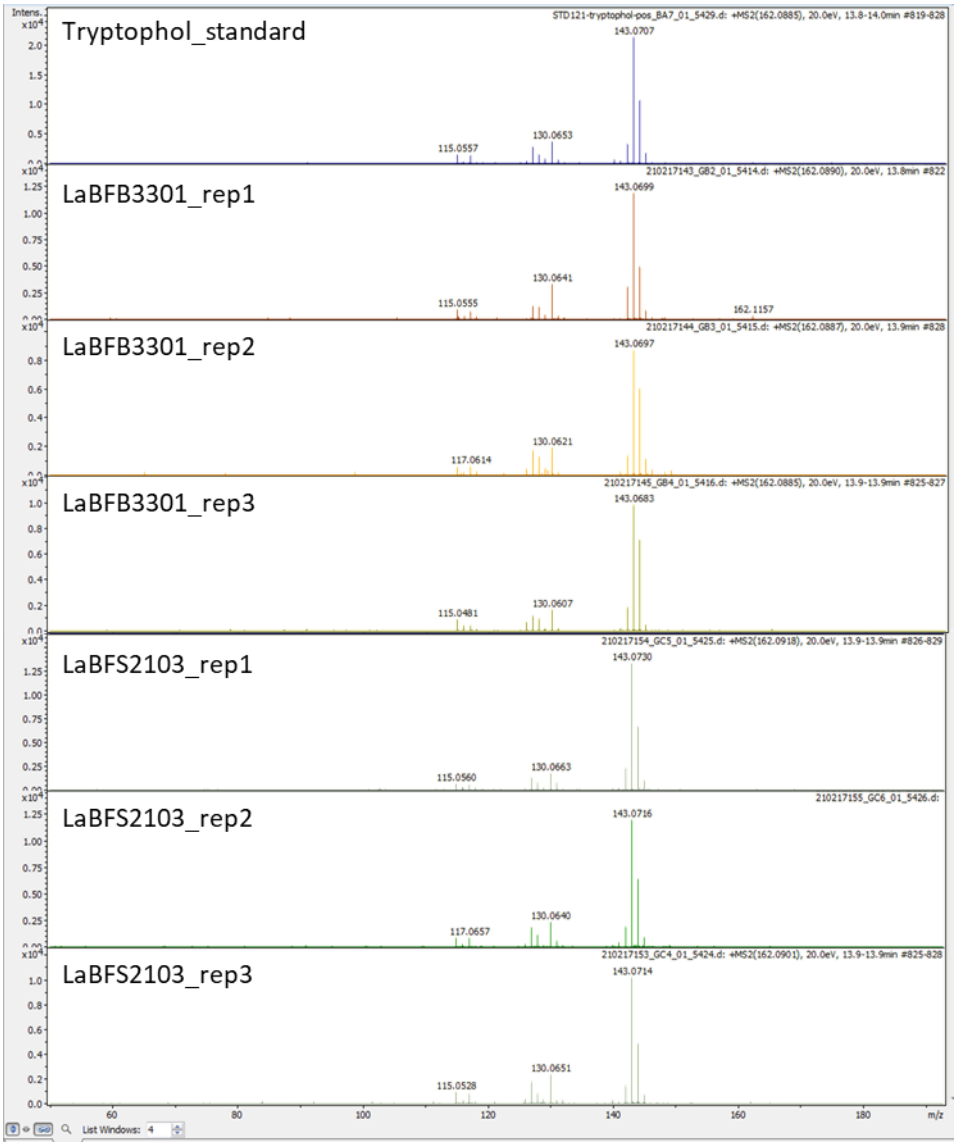


Supplemental figure 2
Antifungal activities against *F. graminearum* (Fg) were differed between the groups of shoot and root bacterial collections, but no difference in the antifungal activities for *M. nivale* (Mn). Different letters showed significant difference with $p < 0.05$ by Tukey-Kramer method.



Supplemental figure 3

Ion chromatograms of m/z 288.1266 ± 0.01 for lycorine and 288.1564 ± 0.01 for galanthamine



Supplemental figure 4
Identification of tryptophol in the extract of LaBFB3301 and LaBFS2103

Supplemental Table 1
Bacterial isolates *of L. aestivum* endophytes
The growth inhibition rates against *F. graminearum* and *M. nivale* are also shown in the right columns (mean +/- SE).

				Blast search result							Average growth inhibition rate			
Plant	Tissue	Strain	Genbank Accession	Blast top hit	E Value	Tax Id	Genus	Order	Phylum		*F. gramin*	*M. nivale*	Fg/SE	Mn/SE
1	Bulb	LaBFB1102	OL307013	Paenibacillus amylolyticus strain JCM 9906	0	1451	Paenibacillus	Bacillales	Firmicutes		-0.40	0.00	0.12	0.00
1	Bulb	LaBFB1103	OL307014	Streptomyces canescens strain DSM 40001	0	53445	Streptomyces	Streptomycetales	Actinobacteria		49.59	59.85	0.79	0.82
1	Bulb	LaBFB1301	OL307015	Patulibacter ginsengiterrae strain P4-5	0	556890	Patulibacter	Solirubrobacterales	Actinobacteria		2.99	32.61	1.04	0.36
1	Bulb	LaBFB1302	OL307016	Patulibacter ginsengiterrae strain P4-5	0	556890	Patulibacter	Solirubrobacterales	Actinobacteria		1.60	32.62	0.35	0.91
1	Bulb	LaBFB1601	OL307017	Mycobacterium hackensackense strain 147-0552	0	228909	Mycobacterium	Corynebacteriales	Actinobacteria		13.60	12.14	0.24	1.29
1	Root	LaBFR1102a	OL305761	Bacillus mycoides strain 273	0	1405	Bacillus	Bacillales	Firmicutes		27.50	50.52	1.38	0.20
1	Root	LaBFR1103	OL305762	Paraburkholderia fungorum strain LMG 16225	0	134537	Paraburkholderia	Burkholderiales	Proteobacteria		39.59	60.18	0.36	0.56
1	Root	LaBFR1104	OL305763	Paenibacillus populi strain LAM0705	0	1473572	Paenibacillus	Bacillales	Firmicutes		-2.51	-2.59	0.56	0.31
1	Root	LaBFR1105	OL305764	[Brevibacterium] frigoritolerans strain DSM 8801	0	450367	Bacillus	Bacillales	Firmicutes		17.66	17.51	0.77	1.03
1	Root	LaBFR1107	OL305765	Streptomyces canescens strain DSM 40001	0	53445	Streptomyces	Streptomycetales	Actinobacteria		46.14	53.50	0.40	0.51
1	Root	LaBFR1108c	OL305766	Bacillus simplex NBRC 15720 = DSM 1321	0	1349754	Peribacillus	Bacillales	Firmicutes		17.52	30.38	0.50	0.77
1	Root	LaBFR1109	OL305767	Paraburkholderia fungorum strain LMG 16225	0	134537	Paraburkholderia	Burkholderiales	Proteobacteria		43.34	56.87	0.09	0.24
1	Root	LaBFR1113a	OL305768	Bacillus simplex NBRC 15720 = DSM 1321	0	1349754	Peribacillus	Bacillales	Firmicutes		12.48	15.66	1.00	1.01
1	Root	LaBFR1113b	OL305769	Bacillus idriensis strain SMC 4352-2	0	324768	Bacillus	Bacillales	Firmicutes		-1.69	-3.69	0.53	0.62
1	Root	LaBFR1114	OL305770	Bacillus idriensis strain SMC 4352-2	0	324768	Bacillus	Bacillales	Firmicutes		N.D.	N.D.		
1	Root	LaBFR1201	OL305771	Bacillus idriensis strain SMC 4352-2	0	324768	Bacillus	Bacillales	Firmicutes		N.D.	N.D.		
1	Root	LaBFR1204a	-	Bacillus licheniformis strain DSM 13	9.00E-69	1402	Bacillus	Bacillales	Firmicutes		26.43	45.43	0.13	0.23
1	Root	LaBFR1204b	OL305772	Moraxella osloensis strain DSM 6998	0	34062	Moraxella	Pseudomonadales	Proteobacteria		0.18	-3.40	0.32	1.07
1	Root	LaBFR1205a	OL305773	Nocardioides cavernae strain YIM A1136	0	1921566	Nocardioides	Propionibacteriales	Actinobacteria		N.D.	N.D.		
1	Root	LaBFR1205b	-	Bacillus licheniformis strain DSM 13	3.00E-69	1402	Bacillus	Bacillales	Firmicutes		43.33	40.76	0.42	0.52
1	Root	LaBFR1206	OL305774	Streptomyces bottropensis ATCC 25435	0	1054862	Streptomyces	Streptomycetales	Actinobacteria		-0.29	17.35	0.67	2.49
1	Root	LaBFR1207	OL305775	Microbacterium natoriense strain TNJL143-2	0	284570	Microbacterium	Micrococcales	Actinobacteria		14.52	13.97	0.10	0.80
1	Root	LaBFR1301b	OL305776	Rathayibacter festucae strain VKM Ac-1390	0	110937	Rathayibacter	Micrococcales	Actinobacteria		0.40	-3.85	0.06	0.39
1	Root	LaBFR1303a	-	[Brevibacterium] frigoritolerans strain DSM 8801	0	450367	Bacillus	Bacillales	Firmicutes		14.90	3.69	1.01	0.20
1	Root	LaBFR1303b	OL305777	[Brevibacterium] frigoritolerans strain DSM 8801	0	450367	Bacillus	Bacillales	Firmicutes		13.86	-2.50	0.45	0.11
1	Root	LaBFR1304b	OL305778	Paenibacillus endophyticus strain PECAE04	0	1294268	Paenibacillus	Bacillales	Firmicutes		N.D.	N.D.		
1	Root	LaBFR1305a	OL305779	Staphylococcus warneri strain AW 25	0	1292	Staphylococcus	Bacillales	Firmicutes		18.45	29.54	0.17	0.84
1	Root	LaBFR1305b	OL305780	Paenibacillus endophyticus strain PECAE04	0	1294268	Paenibacillus	Bacillales	Firmicutes		0.00	0.50	0.00	0.90
1	Root	LaBFR1307a	OL305781	Caulobacter rhizosphaerae strain 7F14	0	2010972	Caulobacter	Caulobacterales	Proteobacteria		N.D.	N.D.		
1	Root	LaBFR1308	OL305782	Labrys methylaminiphilus JLW10	0	1096091	Labrys	Hyphomicrobiales	Proteobacteria		23.21	31.09	2.41	0.37
1	Root	LaBFR1309	OL305783	Paraburkholderia fungorum strain LMG 16225	0	134537	Paraburkholderia	Burkholderiales	Proteobacteria		37.49	60.37	0.28	0.37
1	Root	LaBFR1311	OL305784	Agrobacterium rhizogenes strain ATCC 11325	0	359	Agrobacterium	Hyphomicrobiales	Proteobacteria		10.52	28.57	0.87	1.33
1	Shoot	LaBFS1102	OL307020	Paraburkholderia fungorum strain LMG 16225	0	134537	Paraburkholderia	Burkholderiales	Proteobacteria		41.91	56.69	0.19	0.12
1	Shoot	LaBFS1106	OL307021	Patulibacter ginsengiterrae strain P4-5	0	556890	Patulibacter	Solirubrobacterales	Actinobacteria		N.D.	N.D.		
1	Shoot	LaBFS1107	OL307022	Chitinophaga ginsengisegetis strain Gsoil 040	0	393003	Chitinophaga	Chitinophagales	Bacteroidetes		26.57	14.54	0.67	2.30
1	Shoot	LaBFS1112	OL307023	Variovorax ginsengisoli strain Gsoil 3165	0	363844	Variovorax	Burkholderiales	Proteobacteria		0.00	9.15	0.20	0.76
1	Shoot	LaBFS1201	OL307024	Staphylococcus warneri strain AW 25	0	1292	Staphylococcus	Bacillales	Firmicutes		37.71	29.73	0.57	3.06
1	Shoot	LaBFS1206	OL307025	Achromobacter marplatensis strain B2	0	470868	Achromobacter	Burkholderiales	Proteobacteria		17.63	-3.33	1.63	1.05
1	Shoot	LaBFS1208	-	Mycolicibacterium setense strain CIP 109395	1.00E-151	431269	Mycolicibacterium	Corynebacteriales	Actinobacteria		2.81	8.87	0.74	0.82
1	Shoot	LaBFS1303	OL307026	Luteibacter jiangsuensis strain JW-64-1	0	637577	Luteibacter	Xanthomonadales	Proteobacteria		N.D.	N.D.		
1	Shoot	LaBFS1306	-	Dyella terrae strain JS14-6	0	522259	Dyella	Xanthomonadales	Proteobacteria		20.60	21.42	0.14	0.08
1	Shoot	LaBFS1307	OL307027	Microbacterium luteolum strain IFO 15074	0	69367	Microbacterium	Micrococcales	Actinobacteria		4.79	4.17	0.95	1.21
1	Shoot	LaBFS1311	OL307028	Roseateles depolymerans strain 61A	9.00E-157	76731	Roseateles	Burkholderiales	Proteobacteria		17.80	41.75	1.87	0.42
2	Bulb	LaBFB2301	OL307018	Agrobacterium rhizogenes strain ATCC 11325	0	359	Agrobacterium	Hyphomicrobiales	Proteobacteria		20.61	30.23	0.86	0.93
2	Root	LaBFR2101	OL305785	Microbacterium maritypicum strain DSM 12512	0	300020	Microbacterium	Micrococcales	Actinobacteria		3.77	14.58	0.65	1.82
2	Root	LaBFR2103	OL305786	Streptomyces phaeochromogenes strain NBRC 12898	0	1923	Streptomyces	Streptomycetales	Actinobacteria		N.D.	N.D.		
2	Root	LaBFR2105a	OL305787	Phyllobacterium ifriqiyense strain STM 370	0	314238	Phyllobacterium	Hyphomicrobiales	Proteobacteria		1.98	1.27	0.21	0.45
2	Root	LaBFR2105b	OL305788	[Brevibacterium] frigoritolerans strain DSM 8801	0	450367	Bacillus	Bacillales	Firmicutes		26.20	29.14	0.28	0.46
2	Root	LaBFR2105c	OL305789	Bacillus simplex NBRC 15720 = DSM 1321	0	1349754	Peribacillus	Bacillales	Firmicutes		0.60	16.17	0.17	2.29
2	Root	LaBFR2107	OL305790	Paenibacillus harenae strain B519	0	306543	Paenibacillus	Bacillales	Firmicutes		N.D.	N.D.		
2	Root	LaBFR2108	OL305791	Microlunatus nigridraconis strain CPCC 203993	0	1710544	Microlunatus	Propionibacteriales	Actinobacteria		N.D.	N.D.		
2	Root	LaBFR2201	OL305792	Leifsonia naganoensis strain DB103	0	150025	Leifsonia	Micrococcales	Actinobacteria		4.96	16.37	0.35	2.65
2	Root	LaBFR2202	OL305793	Mycobacterium hackensackense strain 147-0552	0	228909	Mycobacterium	Corynebacteriales	Actinobacteria		0.53	-5.29	0.61	1.09

				Blast search result							Average growth inhibition rate			
Plant	**Tissue**	**Strain**	**Genbank Accession**	**Blast top hit**	**E Value**	**Tax Id**	**Genus**	**Order**	**Phylum**		***F. gramin***	***M. nivale***	**Fg/SE**	**Mn/SE**
2	Root	LaBFR2204a	OL305794	Bacillus subtilis strain IAM 12118	0	1423	Bacillus	Bacillales	Firmicutes		34.49	52.85	1.19	1.47
2	Root	LaBFR2301	OL305795	Microbacterium natoriense strain TNJL143-2	0	284570	Microbacterium	Micrococcales	Actinobacteria		6.55	3.79	0.77	1.09
2	Root	LaBFR2303	-	Pedobacter ginsengisoli strain Gsoil 104	0	363852	Pedobacter	Sphingobacteriales	Bacteroidetes		0.37	-0.60	0.31	1.71
2	Shoot	LaBFS2101	OL307029	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		40.92	49.11	0.15	2.00
2	Shoot	LaBFS2102	OL307030	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2103	OL307031	Comamonas denitrificans strain 123	0	117506	Comamonas	Burkholderiales	Proteobacteria		43.51	58.82	0.09	1.19
2	Shoot	LaBFS2104	OL307032	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2105	OL307033	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2109	OL307034	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2110	OL307035	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2111	OL307036	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		41.71	57.71	0.25	1.10
2	Shoot	LaBFS2112	OL307037	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2113	OL307038	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2201	-	Microbacterium arthrosphaerae strain CC-VM-Y	7.00E-55	792652	Microbacterium	Micrococcales	Actinobacteria		-0.80	6.17	0.06	0.16
2	Shoot	LaBFS2202	OL307039	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		43.29	54.83	0.65	0.50
2	Shoot	LaBFS2203	OL307040	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2204	OL307041	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2205	OL307042	Erwinia persicina strain NBRC 102418	0	55211	Erwinia	Enterobacterales	Proteobacteria		37.11	40.48	0.83	0.62
2	Shoot	LaBFS2208	OL307043	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		39.11	55.15	0.30	1.14
2	Shoot	LaBFS2301	OL307044	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		42.12	45.43	0.16	1.17
2	Shoot	LaBFS2302	OL307045	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2303	OL307046	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		39.52	46.42	0.25	0.97
2	Shoot	LaBFS2304	OL307047	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		42.71	40.86	0.50	0.88
2	Shoot	LaBFS2305	OL307048	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2306	OL307049	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2308	OL307050	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		40.71	42.74	0.41	1.36
2	Shoot	LaBFS2309	OL307051	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	8.00E-127	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2310	OL307052	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2313	OL307053	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2314	OL307054	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2315	OL307055	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2316	OL307056	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
2	Shoot	LaBFS2317	OL307057	Achromobacter marplatensis strain R-46660	0	470868	Achromobacter	Burkholderiales	Proteobacteria		23.36	-10.60	0.14	1.81
2	Shoot	LaBFS2318	OL307058	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 45	0	745277	Rahnella	Enterobacterales	Proteobacteria		N.D.	N.D.		
3	Bulb	LaBFB3301	OL307019	Luteibacter rhizovicinus strain LJ96	0	242606	Luteibacter	Xanthomonadales	Proteobacteria		37.32	48.06	0.20	1.01
3	Root	LaBFR3101a	OL305796	Paenibacillus lautus strain AB236d	0	1401	Paenibacillus	Bacillales	Firmicutes		1.81	-1.64	0.36	0.16
3	Root	LaBFR3101b	OL305797	Paenarthrobacter nitroguajacolicus strain G2-1	0	211146	Paenarthrobacter	Micrococcales	Actinobacteria		15.18	28.13	1.85	0.32
3	Root	LaBFR3102b	OL305798	Paenibacillus lautus strain AB236d	0	1401	Paenibacillus	Bacillales	Firmicutes		-0.42	-1.63	0.29	0.33
3	Root	LaBFR3103	OL305799	Streptomyces canescens strain DSM 40001	0	53445	Streptomyces	Streptomycetales	Actinobacteria		45.97	58.11	0.30	0.89
3	Root	LaBFR3104	OL305800	Paenibacillus lautus strain AB236d	0	1401	Paenibacillus	Bacillales	Firmicutes		-0.82	-3.50	0.33	0.16
3	Root	LaBFR3105b	OL305801	Paenibacillus lautus strain AB236d	0	1401	Paenibacillus	Bacillales	Firmicutes		1.42	-2.06	0.41	0.12
3	Root	LaBFR3106	OL305802	Microbacterium maritypicum strain DSM 12512	0	300020	Microbacterium	Micrococcales	Actinobacteria		1.59	0.94	0.25	2.01
3	Root	LaBFR3107a	OL305803	Promicromonospora alba strain 1C-HV12	0	1616110	Promicromonospora	Micrococcales	Actinobacteria		-2.51	20.02	0.56	2.65
3	Root	LaBFR3107b	OL305804	Promicromonospora alba strain 1C-HV12	0	1616110	Promicromonospora	Micrococcales	Actinobacteria		-1.71	21.15	0.67	1.21
3	Root	LaBFR3108	OL305805	Paenibacillus lautus strain AB236d	0	1401	Paenibacillus	Bacillales	Firmicutes		0.40	3.59	0.11	1.04
3	Root	LaBFR3109	OL305806	Paenibacillus lautus strain AB236d	0	1401	Paenibacillus	Bacillales	Firmicutes		2.18	0.30	0.32	0.11
3	Root	LaBFR3110	OL305807	Paenibacillus lautus strain AB236d	0	1401	Paenibacillus	Bacillales	Firmicutes		1.22	3.30	0.27	0.93
3	Root	LaBFR3202	OL305808	Bacillus marisflavi strain TF-11	0	189381	Rossellomorea	Bacillales	Firmicutes		0.61	3.79	0.20	0.25
3	Root	LaBFR3203	OL305809	Agrobacterium tumefaciens strain IAM 12048	0	358	Agrobacterium	Hyphomicrobiales	Proteobacteria		14.52	33.33	0.51	0.21
3	Root	LaBFR3207	OL305810	Inquilinus limosus strain AU476	0	171674	Inquilinus	Rhodospirillales	Proteobacteria		-1.89	-4.89	0.48	1.15
3	Root	LaBFR3209	OL305811	Caulobacter segnis strain MBIC 2835	0	88688	Caulobacter	Caulobacterales	Proteobacteria		N.D.	N.D.		
3	Root	LaBFR3210	OL305812	Phyllobacterium trifolii strain PETP02	0	300193	Phyllobacterium	Hyphomicrobiales	Proteobacteria		-2.30	-0.61	0.50	0.10
3	Root	LaBFR3211	OL305813	Nocardia rhamnosiphila NBRC 108938 strain 202GMO	0	1223547	Nocardia	Corynebacteriales	Actinobacteria		N.D.	N.D.		

				Blast search result							Average growth inhibition rate			
Plant	**Tissue**	**Strain**	**Genbank Accession**	**Blast top hit**	**E Value**	**Tax Id**	**Genus**	**Order**	**Phylum**		***F. gramin***	***M. nivale***	**Fg/SE**	**Mn/SE**
3	Root	LaBFR3212	OL305814	Staphylococcus epidermidis strain Fussel	0	1282	Staphylococcus	Bacillales	Firmicutes		35.96	12.84	0.51	1.46
3	Root	LaBFR3301b	OL305815	Sphingopyxis chilensis strain S37	0	180400	Sphingopyxis	Sphingomonadales	Proteobacteria		N.D.	N.D.		
3	Root	LaBFR3303	OL305816	Sphingopyxis chilensis strain S37	0	180400	Sphingopyxis	Sphingomonadales	Proteobacteria		N.D.	N.D.		
3	Root	LaBFR3305a	OL305817	Ensifer adhaerens strain LMG 20216	0	106592	Ensifer	Hyphomicrobiales	Proteobacteria		15.28	47.11	0.60	0.35
3	Root	LaBFR3305b	-	Streptomyces canescens strain DSM 40001	2.00E-147	53445	Streptomyces	Streptomycetales	Actinobacteria		48.81	57.26	0.36	0.61
3	Root	LaBFR3307	OL305818	Bacillus cereus strain IAM 12605	0	1396	Bacillus	Bacillales	Firmicutes		28.67	42.32	0.88	0.55
3	Root	LaBFR3308a	OL305819	Streptomyces canescens strain DSM 40001	0	53445	Streptomyces	Streptomycetales	Actinobacteria		-2.31	21.36	0.60	1.33
3	Root	LaBFR3308b	OL305820	Streptomyces canescens strain DSM 40001	0	53445	Streptomyces	Streptomycetales	Actinobacteria		44.07	59.25	0.34	0.61
3	Root	LaBFR3311	OL305821	Nocardia jinanensis strain 04-5195	0	382504	Nocardia	Corynebacteriales	Actinobacteria		N.D.	N.D.		
3	Root	LaBFR3312	OL305822	Ensifer adhaerens strain LMG 20216	0	106592	Ensifer	Hyphomicrobiales	Proteobacteria		36.59	48.70	2.86	0.15
3	Shoot	LaBFS3102	OL307059	Variovorax guangxiensis strain GXGD002	0	1775474	Variovorax	Burkholderiales	Proteobacteria		16.79	20.95	0.88	1.10
3	Shoot	LaBFS3103	OL307060	Luteibacter jiangsuensis strain JW-64-1	0	637577	Luteibacter	Xanthomonadales	Proteobacteria		N.D.	N.D.		
3	Shoot	LaBFS3104	OL307061	Variovorax paradoxus NBRC 15149	0	1321610	Variovorax	Burkholderiales	Proteobacteria		7.19	-10.26	0.98	2.07
3	Shoot	LaBFS3105	OL307062	Variovorax paradoxus NBRC 15149	0	1321610	Variovorax	Burkholderiales	Proteobacteria		3.40	7.54	0.23	2.97
3	Shoot	LaBFS3108	OL307063	Herbiconiux ginsengi strain wged11	0	381665	Herbiconiux	Micrococcales	Actinobacteria		0.40	10.12	0.21	1.95
3	Shoot	LaBFS3109	OL307064	Paraburkholderia fungorum strain LMG 16225	0	134537	Paraburkholderia	Burkholderiales	Proteobacteria		41.16	55.01	0.24	0.79
3	Shoot	LaBFS3201	OL307065	Variovorax boronicumulans NBRC 103145	0	1321609	Variovorax	Burkholderiales	Proteobacteria		27.50	11.19	0.27	1.63
3	Shoot	LaBFS3203	OL307066	Variovorax boronicumulans NBRC 103145	0	1321609	Variovorax	Burkholderiales	Proteobacteria		N.D.	N.D.		
3	Shoot	LaBFS3205	OL307067	Pseudomonas brassicacearum strain DBK11	0	930166	Pseudomonas	Pseudomonadales	Proteobacteria		8.82	5.65	0.44	1.48
3	Shoot	LaBFS3206	OL307068	Variovorax boronicumulans NBRC 103145	0	1321609	Variovorax	Burkholderiales	Proteobacteria		N.D.	N.D.		
3	Shoot	LaBFS3208	OL307069	Novosphingobium lindaniclasticum LE124	0	1096930	Novosphingobium	Sphingomonadales	Proteobacteria		18.10	3.14	0.74	2.31
3	Shoot	LaBFS3209	OL307070	Variovorax boronicumulans NBRC 103145	0	1321609	Variovorax	Burkholderiales	Proteobacteria		N.D.	N.D.		
3	Shoot	LaBFS3301	OL307071	Herbaspirillum hiltneri N3	0	1262470	Herbaspirillum	Burkholderiales	Proteobacteria		23.29	29.85	0.19	0.95
3	Shoot	LaBFS3302	OL307072	Herbaspirillum hiltneri N3	0	1262470	Herbaspirillum	Burkholderiales	Proteobacteria		24.91	30.55	0.20	1.33
3	Shoot	LaBFS3303	OL307073	Leifsonia naganoensis strain DB103	0	150025	Leifsonia	Micrococcales	Actinobacteria		22.89	-4.27	0.20	0.71
3	Shoot	LaBFS3304	OL307074	Chitinophaga ginsengisegetis strain Gsoil 040	0	393003	Chitinophaga	Chitinophagales	Bacteroidetes		20.67	9.30	0.73	2.07
3	Shoot	LaBFS3305	OL307075	Variovorax ginsengisoli strain Gsoil 3165	0	363844	Variovorax	Burkholderiales	Proteobacteria		5.60	9.54	0.91	0.95
3	Shoot	LaBFS3306	OL307080	Luteibacter jiangsuensis strain JW-64-1	0	637577	Luteibacter	Xanthomonadales	Proteobacteria		23.07	14.92	1.08	2.15
3	Shoot	LaBFS3307	OL307076	Luteibacter jiangsuensis strain JW-64-1	0	637577	Luteibacter	Xanthomonadales	Proteobacteria		N.D.	N.D.		
3	Shoot	LaBFS3309	OL307077	Curtobacterium citreum strain DSM 20528	0	2036	Curtobacterium	Micrococcales	Actinobacteria		31.54	27.43	0.40	0.58
3	Shoot	LaBFS3313	OL307078	Arthrobacter oryzae strain KV-651	0	409290	Arthrobacter	Micrococcales	Actinobacteria		30.73	18.46	0.96	3.11
3	Shoot	LaBFS3315	OL307079	Luteibacter jiangsuensis strain JW-64-1	0	637577	Luteibacter	Xanthomonadales	Proteobacteria		18.86	5.74	0.57	0.74

Supplemental Table 2

Bacterial genera found as endophytes in Amaryllidaceae plants

Bacterial genus	*Lycoris radiata* (Liu et al., 2020)	*Crinum macowanii* Baker (Rebotile et al., 2018)	*Crinum macowanii* Baker (Sebola et al., 2019)	*Leucojum aestivum* (this study)	*Narcissus tazetta* (Wang et al., 2015b)	*Leucojum aestivum* (Spina et al., 2021)	*Lycoris radiata* (Liu et al., 2020)	*Crinum macowanii* Baker (Sebola et al., 2020)	*Leucojum aestivum* (this study)	*Lycoris radiata* (Liu et al., 2020)	*Leucojum aestivum* (this study)
Achromobacter	0	0	0	0	1	0	0	0	1	0	0
Acinetobacter	0	1	1	0	0	0	1	1	0	1	0
Agrobacterium	0	0	0	1	0	0	0	0	0	0	1
Arthrobacter	0	0	0	0	0	0	0	1	1	0	0
Azoarcus	1	0	0	0	0	0	0	0	0	0	0
Bacillus	1	1	1	0	0	1	1	1	0	1	1
Brachybacterium	0	0	0	0	0	0	0	0	0	0	0
Brevibacterium	0	0	0	0	0	0	1	0	0	0	1
Brevundimonas	0	0	0	0	0	0	0	0	0	1	0
Burkholderia	1	0	1	0	0	0	0	0	0	1	0
Caulobacter	0	0	0	0	0	0	0	0	0	0	1
Chitinophaga	0	0	0	0	0	0	0	0	1	0	0
Cohnella	0	0	0	0	0	0	0	0	0	1	0
Comamonas	0	0	0	0	0	0	0	0	1	0	0
Curtobacterium	0	0	0	0	0	0	0	0	1	0	0
Dyella	0	0	0	0	0	0	0	0	1	0	0
Ensifer	0	0	0	0	0	0	0	0	0	0	1
Enterobacter	1	0	0	0	0	0	0	1	0	1	0
Erwinia	0	0	0	0	0	0	0	0	1	0	0
Fictibacillus	0	0	0	0	0	0	0	0	0	1	0
Herbaspirillum	0	0	0	0	0	0	0	0	1	1	0
Herbiconiux	0	0	0	0	0	0	0	0	1	0	0
Inquilinus	0	0	0	0	0	0	0	0	0	0	1
Labrys	0	0	0	0	0	0	0	0	0	0	1
Leifsonia	0	0	0	0	0	0	0	0	1	0	1
Luteibacter	0	0	0	1	0	0	1	0	1	0	0
Lysobacter	0	0	0	0	0	0	0	0	0	1	0
Methylobacterium	0	0	0	0	0	0	1	0	0	0	0
Microbacterium	1	0	0	0	0	0	0	0	1	0	1
Microlunatus	0	0	0	0	0	0	0	0	0	0	1
Moraxella	0	0	0	0	0	0	0	0	0	0	1
Mycobacterium	0	0	0	1	0	0	0	0	0	0	1
Mycolicibacterium	0	0	0	0	0	0	0	0	1	0	0
Nocardia	0	0	0	0	0	0	0	0	0	0	1
Nocardioides	0	0	0	0	0	0	1	0	0	0	1
Novosphingobium	0	0	1	0	0	0	0	0	1	1	0
Paenarthrobacter	0	0	0	0	0	0	0	0	0	0	1
Paenibacillus	0	0	0	1	0	0	0	0	0	1	1
Pantoea	1	0	0	0	0	0	1	0	0	0	0
Paraburkholderia	0	0	0	0	0	0	0	0	1	0	1
Patulibacter	0	0	0	1	0	0	0	0	1	0	0
Pedobacter	0	0	0	0	0	0	0	0	0	0	1
Phyllobacterium	0	0	0	0	0	0	0	0	0	0	1
Promicromonospora	0	0	0	0	0	0	0	0	0	0	1
Pseudacidovorax	0	0	0	0	0	0	1	0	0	0	0
Pseudomonas	0	0	1	0	0	0	1	1	1	1	0
Rahnella	0	0	1	0	0	0	0	0	1	0	0
Raoultella	0	0	1	0	0	0	0	1	0	0	0
Rathayibacter	0	0	0	0	0	0	0	0	0	0	1
Rhizobium	1	0	0	0	0	0	0	0	0	0	0
Roseateles	0	0	0	0	0	0	0	0	1	1	0
Rosenbergiella	0	0	0	0	0	0	0	0	0	0	0
Sphingobacterium	0	0	0	0	0	0	1	0	0	0	0
Sphingomonas	0	0	0	0	0	0	1	0	0	0	0
Sphingopyxis	0	0	0	0	0	0	0	0	0	0	1
Sporosarcina	0	0	0	0	0	0	1	0	0	0	0
Staphylococcus	0	1	0	0	0	0	0	0	1	0	1
Stenotrophomonas	0	0	0	0	0	0	0	0	0	0	0
Streptomyces	0	0	0	1	0	0	0	0	0	0	1
Variovorax	0	0	0	0	0	0	0	0	1	1	0
Total number of genera	7	3	7	6	1	1	12	6	21	14	24

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Chapter 2

Isolation and characterization of endophytes from *Chryzopogon zizanioides* (L.) Roberty roots

Vetiver (*Chryzopogon zizanioides* (L.) Roberty) is a perennial species belonging to Poaceae. Originally from India, vetiver is now cultivated all over the world, but mainly in tropical and subtropical areas [1]. for its essential oil extracted from the roots. This essential oil contains various sesquiterpenes and is mainly used for the production of perfumes. It has also been reported that this essential oil has antifungal and antibacterial activities against human and plant pathogens, notably *F. graminearum* [2].

Previous studies have shown that endophytic microorganisms in vetiver roots influence the biosynthesis and composition of essential oils [3]. Interestingly, its composition also depends on the environmental conditions in which the plants were grown. Overall, these results suggest that the endophytic bacterial community from vetiver roots may contribute to the metabolic composition of the essential oil (Fig. 0).

Therefore, we assumed that some endophytic bacteria from vetiver roots could exhibit antimicrobial activities against pathogens such as the plant pathogen *F. graminearum*. In order to identify these bacterial candidates that could be used in the future as biocontrol agents in agriculture, my objective was to characterize the endophyte bacterial communities in vetiver roots collected in different geographical origins and to assess their antifungal activities (Munakata et al., 2021, chapter 2_part 1). Then, we tried to characterize the specialized metabolites that might be involved in the antimicrobial activities of the most interesting bacterial strains (Chapter 2_part 2).

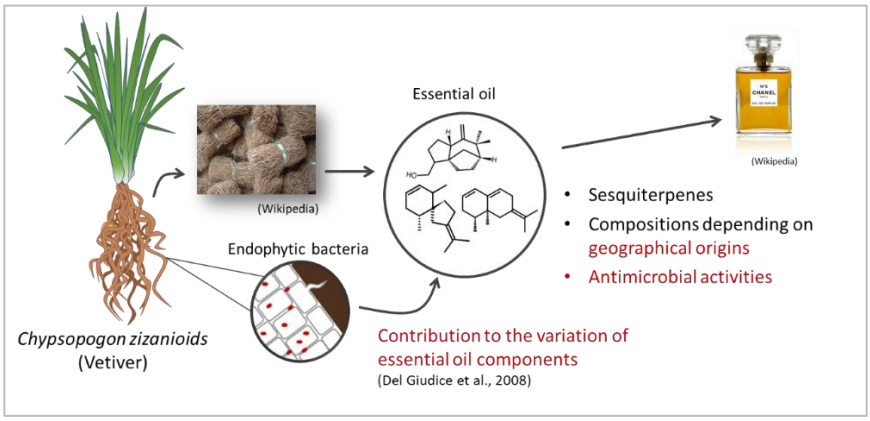

Fig. 0 | Overview of relationships between Vetiver root essential oil and endophytic bacteria

Part 1

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Composition and functional comparison of vetiver root endophytic microbiota originating from different geographic locations that show antagonistic activity towards *Fusarium graminearum*

Yuka Munakata [a], Carole Gavira [b], Julie Genestier [a], Frédéric Bourgaud [b], Alain Hehn [a], Sophie Slezack-Deschaumes [a,*]

[a] Université de Lorraine – INRAE, LAE, F-54000, Nancy, France
[b] Plant Advanced Technologies, F54500, Vandoeuvre-lès-Nancy, France

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ABSTRACT

Given the current trend towards reducing the use of chemical controls in agriculture, microbial resources such as plant endophytes are being intensively investigated for traits that are conducive to plant protection. Among the various important target pathogens, *Fusarium graminearum* is a fungal pathogen of cereal crops that is responsible for severe yield losses and mycotoxin contamination in grains. In the present study, we investigated the bacterial endophytic communities from vetiver (*Chrysopogon zizanioides* (L.) Roberty) roots originating from 5 different geographic locations across Europe and Africa. This study relies on a global 16S metabarcoding approach and the isolation/functional characterization of bacterial isolates. The results we obtained showed that geographical location is a factor that influences the composition and relative abundance of root endophyte communities in vetiver. Three hundred eighty-one bacterial endophytes were isolated and assessed for their *in vitro* antagonistic activities towards *F. graminearum* mycelium growth. In total, 46 % of the isolates showed at least 50 % inhibitory activity against *F. graminearum*. The taxonomic identification of the bioactive isolates revealed that the composition of these functional culturable endophytic communities was influenced by the geographic origins of the roots. The selected communities consisted of 15 genera. Some endophytes in *Bacillus*, *Janthinobacterium*, *Kosakonia*, *Microbacterium*, *Pseudomonas*, and *Serratia* showed strong growth inhibition activity (≥70 %) against *F. graminearum* and could be candidates for further development as biocontrol agents.

1. Introduction

Fusarium graminearum is a causal agent of important diseases in small-grain cereals such as wheat (Dean et al., 2012; Taheri, 2018). This pathogen can induce seedling blight, root and stem-based rots and ear blight, which can lead to significant yield losses (Bottalico and Perrone, 2002). In addition, as this pathogenic fungus is able to contaminate cereals with mycotoxins, it can threaten human and animal health (Xu and Nicholson, 2009). Agricultural practices such as crop rotation, soil tillage, the application of chemical fungicides and the use of resistant cultivars are considered as the most effective strategies for controlling *F. graminearum*. However, these strategies are not always fully effective in controlling disease symptoms and preventing the accumulation of mycotoxins (Lamichhane et al., 2015; Wegulo et al., 2015). The use of chemical fungicides may also cause concern for human health and the environment (). Therefore, reducing the use of these chemicals has become a major concern in European regulations in recent years. In this context, biocontrol agents, within the framework of integrated pest management strategies, are promising solutions that protect plants from biotic stresses and considered relatively environmentally friendly (Köhl et al., 2019; van Lenteren et al., 2018; Wegulo et al., 2015).

Many bacterial and fungal microorganisms isolated from different environments, such as the rhizosphere or the surface of wheat anthers, were investigated in both *in vitro* assays and field experiments for their antagonistic activity towards *F. graminearum* (Tian et al., 2016). Recently, microbial endophytes that colonize inner plant tissues during at least part of their life cycle have attracted attention. It is assumed that they might protect plants more efficiently than rhizospheric and epiphytic microorganisms (Brader et al., 2014). These beneficial effects on plant health might be specifically related to the ability of endophytic

* Corresponding author.
E-mail address: sophie.deschaumes@univ-lorraine.fr (S. Slezack-Deschaumes).

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microorganisms to produce antimicrobial substances (Ek-Ramos et al., 2019; Gouda et al., 2016; Singh et al., 2017). Since endophytes have to survive in very different environmental conditions, they might be able to produce a greater diversity of antimicrobial metabolites than free-living microorganisms (Brader et al., 2014; Hardoim et al., 2015). Endophytes represent, therefore, pathways for identifying new sources of biocontrol agents, and their diversity needs to be further investigated (Hardoim et al., 2015; Lebeis et al., 2015; Thiergart et al., 2020; Toju et al., 2019).

Vetiver (*Chryzopogon zizanioides* (L.) Roberty) is a perennial poaceous plant that is native to India. This plant was introduced in tropical and subtropical regions such as Haiti and Reunion Island by traders and has spread worldwide (Belhassen et al., 2015). Vetiver is cultivated mainly for its essential oil, extracted from its roots, which is used for perfume production, cosmetics and aromatherapy (Burger et al., 2017). A study showed that vetiver essential oil also displays antimicrobial activities against several plant fungal pathogens (Sharma et al., 2009).

Vetiver essential oil is composed of highly diverse sesquiterpene derivatives (Belhassen et al., 2015; Champagnat et al., 2006). Its composition is known to depend on different factors, including environmental conditions and root-associated bacteria (Adams et al., 2004, 2003). Del Guidice and coworkers (Del Giudice et al., 2008) showed that some bacteria isolated from vetiver roots could metabolize sesquiterpenes into the compounds that are present in vetiver essential oil. These results therefore suggest that the endophytic bacterial community from vetiver roots may contribute to the overall metabolic composition of the essential oil, which has been reported to have antifungal activities (Del Giudice et al., 2008; Hammer et al., 1999; Monteiro et al., 2011).

In this report, we assumed that the endophytic bacterial community of vetiver roots contributes to bioactivity against *F. graminearum*. Since bacterial communities are influenced by the surrounding environmental conditions, we investigated vetiver roots originating from different geographical locations assuming that the endophytic bacterial communities would differ in their taxonomical composition and bioactivity against *F. graminearum*. The results described here are related to Sunshine vetiver roots (Adams et al., 1998) collected in four different geographical regions *i.e.*, Madagascar, Portugal, Reunion Island, and Senegal. To thoroughly describe endophytic bacterial community of vetiver roots, culture dependent and independent approaches were used. Using the high resolutive meta-barcoding approach, we assessed the structure and diversity of the bacterial endophytes. Through a complementary culture-dependent approach, we functionally characterized the bacterial endophytes with antagonistic activity towards *F. graminearum*. This allowed us to better understand the role that these microorganisms can have and it also revealed which microorganisms could be used in subsequent studies to develop biocontrol solutions with respect to *F. graminearum*.

2. Materials and methods

2.1. Sampling of vetiver roots

Roots of *Chrysopogon zizanioides* (L.) Roberty were collected in summer 2013 in Portugal in Sao Bartolomeu de Messines, in Madagascar on the Bemasoandro plantation in Antananarivo, in Senegal at the Naac Baal nursery in Sebikotane, and on Reunion Island in the areas Pierrefonds (Reunion1) and Domaine Vidot (Reunion2) (Fig. S1). Three individual plants were obtained from each geographical location. The collected fresh plants were shipped at cold temperatures and processed immediately after being received. The map of vetiver origins (Fig. S1) was based on Global Map Version 1(2)-Elevation published by the Geospatial Information Authority of Japan (https://maps.gsi.go.jp/development/ichiran.html); this map was edited and points representing the sampling locations were added.

2.2. Preparation of root samples

The vetiver roots were first rinsed in running tap water to eliminate soil and surface debris prior to surface sterilization under sterile conditions. For this purpose, they were cut into small pieces of approximately 3–4 cm in length. The root pieces were treated with 70 % ethanol for 1 min 5 times, followed by immersion in 2.6 % sodium hypochlorite solution for 15 min. The samples were then rinsed three times with sterile water for 1 min. After root surface sterilization, four grams of plant material were stored at −80 °C until DNA extraction, and 1 g of root tissues was used for the isolation of bacterial endophytes.

2.3. Isolation of bacteria

The root samples were cut into smaller pieces (0.5 mm to 1 cm in length) and ground using a Potter homogenizer. The samples were macerated in 10 ml of M9 medium without a carbon source containing 100 mg L^{-1} cycloheximide (Sigma-Aldrich™, USA) for 1 h at 28 °C under gentle agitation. A serial dilution of the homogenate from 10^{-1} to 10^{-6} was prepared in phosphate buffer PBS, and 100 µl of each dilution was plated on 10 % tryptic soy agar (TSA, Sigma-Aldrich™, USA) media containing 100 mg L^{-1} cycloheximide. The plates were incubated at 28 °C for 10 days. Three replicates per dilution were prepared. Similarly, the efficacy of the root surface sterilization was evaluated by plating 100 µl of the last root washing water on 10 % TSA media containing 100 mg L^{-1} cycloheximide for 10 days at 28 °C. The number of microbial colonies was counted 10 days after isolation and expressed as log CFU g^{-1} of root dry matter. Bacterial colonies were randomly removed from the plates and purified by repeated subculturing. The purified bacterial isolates were stored frozen at −80 °C in 15 % glycerol.

2.4. DNA extraction from surface-sterilized vetiver roots

Total genomic DNA was extracted from 3 g of surface-sterilized vetiver roots according to Ranjard et al. (Ranjard et al., 2003) with minor modifications. Briefly, frozen root materials were ground in liquid nitrogen with a mortar and a pestle, both of which were autoclaved in advance. Three grams of the ground material was distributed into 5 microtubes, and the aliquots were mixed in 1 ml of lysis buffer (Ranjard et al., 2003) using a Retsch® MM400 agitator at 30 per sec for 2.5 min. After incubation at 70 °C for 30 min, supernatants were collected after centrifugation at 7000 *g* for 5 min at 15–20 °C. The supernatants from the same original root material were combined as a crude DNA solution. The obtained crude extracts were purified using the GENECLEAN® Turbo Nucleic Acid Purification kit (MP Biomedicals, France).

2.5. Metabarcoding amplicon libraries

Target V5-V7 regions of 16S rRNA were prepared using the Metabiote® v 2.0 procedure (Genoscreen, Lille, France) and were paired-end sequenced (2 × 250 bp) using an Illumina MiSeq instrument.

Demultiplexed sequence reads were obtained using the CASAVA v 1.0 tool (Illumina) by Genoscreen. Sequence reads were processed with the FROGS (Find Rapidly OTUs with Galaxy Solution) pipeline (Escudié et al., 2018). Reads 1 and 2 were merged under conditions of 10 bp minimum overlap and 10 % mismatch allowance using the VSEARCH algorithm (Rognes et al., 2016). Contigs were selected in the range of 380 bp to 500 bp, and those including ambiguous bases were eliminated. These sequences were clustered with Swarm (Mahé et al., 2014). Chimaeras were removed using a filtering tool with the following criteria: OTUs needed to contain sequences shared by at least three samples and with a read abundance higher than 0.005 % of the total number of reads. The OTUs were taxonomically associated using the Silva138 16S rRNA gene database (Quast et al., 2013). Although the Genome Taxonomy Database recently proposed the repositioning of Betaproteobacteria as an order (Burkholderiales) in Gammaproteobacteria (Parks et al., 2018),

we decided to conserve the old classification to maintain consistency with previous studies. OTUs were further filtered with a threshold of at least 99 % BLAST identity and 100 % coverage. The number of remaining reads after the 2nd filtration was in the range of 10,440 to 23,950 reads, with an average of 14,805 reads per plant. To compare the data coming from the different vetiver samples, the sequencing results were normalized to the same number of reads as that in the sample with the lowest total count (10,440 reads). Rarefaction curves were depicted in parallel with the R packages vegan and phyloseq (McMurdie and Holmes, 2013) in FROGS (Fig. S2). Phylogenetic tree data were prepared with FastTree, following a multiple alignment by PyNAST in FROGS.

2.6. Dual inoculation test with F. graminearum

The antagonistic activities of bacterial isolates were analysed using an *in vitro* dual-inoculation method. *F. graminearum* was cultivated on potato dextrose agar medium (PDA) (Sigma-Aldrich™, USA) for 4 days. A 5-mm-diameter mycelial plug of fresh *F. graminearum* cultures was inoculated at the centre of a 9 cm diameter plate containing 12 mL of PDA. Fifty microlitres of a bacterial suspension obtained from a liquid culture of an isolated bacterium incubated in 2 ml of nutrient broth (Sigma-Aldrich™, USA) at 28 °C for 12 h was placed at 4 points 3 cm from the centre of the Petri dish. Three independent replicates were performed for each bacterial isolate. The plates were incubated at 28 °C for 5 days. The diameter of the fungus was measured and averaged among the replicates. Plates inoculated with only *F. graminearum* were used as controls. Antagonistic activity was expressed as the inhibition rate (%), calculated as follows: $((d_{control} - d_{assay})/d_{control}) \times 100$, where d means the diameter of the *F. graminearum* mycelium.

2.7. Sequencing of the 16S rRNA gene of cultured bacterial endophytes

Selected bacterial strains were cultured on 10 % TSA medium for 1–4 days. 16S rDNA amplification was performed on one single colony, as described in Cregut et al. (2009). PCR products were purified with the NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Germany) according to the manufacturer's recommendations. The clean amplicons were sequenced by Eurofins (Luxembourg). The sequences were compared to the GenBank database using BLASTn (http://www.ncbi.nlm.nih.gov/), and their taxonomic associations were determined using the Silva138 16S rRNA gene database.

2.8. Diversity and compositional analysis of the bacterial communities

All data treatments herein were performed using R (version 3.6.3) unless otherwise noted.

For the calculation and illustration of the number of shared OTUs and genera across locations, we used the Venn diagram R package. The number of observed OTUs and the Chao1, Shannon, and inverse-Simpson indices were used to describe richness and evenness within a location and were calculated with phyloseq. To evaluate the recovery rate of richness in our samples, the coverage ratio (%) was calculated by dividing the number of observed OTUs by the estimated richness, *i.e.*, the Chao1 values (Marzano et al., 2016). The beta diversity among vetiver bacterial communities by weighted UniFrac distance was visualized through principal coordinate analysis (PCoA) using the phyloseq and ggplot2 R packages.

The composition of the functionally selected endophyte communities was converted into a table that includes the relative abundance at the genus level. These data were visualized using the ggplot2 R package. The table was used to measure the beta diversity among samples with a distance metric, Bray-Curtis dissimilarity, using vegdist in the vegan R package. The result was visualized by PCoA with ggplot2 and ggpubr R packages.

2.9. Statistical analyses

The inhibition rate was visualized with ggplot2 and tested by the Tukey-Kramer method with the multcomp R package. Multiple comparisons of alpha diversity indices and the relative abundance of taxa were performed with the Tukey-Kramer method using the multcomp R package. For the meta-barcoding analysis, the influence of the location factor was tested by PERMANOVA (permutations = 9999) with Adonis available in the vegan R package equipped in FROGS. The influence of the location factor on the variation in the Bray-Curtis dissimilarity metric among the culturable endophyte communities was tested by PERMANOVA (permutations = 10,000) with adonis in the vegan R package.

3. Results

3.1. Biodiversity analysis of endophytic bacterial microbiota in vetiver

Vetiver roots were sampled from different places located in Madagascar, Portugal, Senegal and Reunion Island (Fig. S1). Illumina sequencing performed on the DNA extracted from each sample generated a total of 366,169 raw sequences, and after a FROGS process, 222,081 sequences were retained. These sequences were clustered into 894 different OTUs (between 233 and 500 OTUs per sample) (Table S1). Among them, 55 OTUs were shared by all samples, whereas 32, 38, 100, 115 and 64 specific OTUs were detected only in the Madagascar, Portugal, Reunion1, Reunion2 and Senegal vetiver root samples, respectively (Fig. S3). The observed number of OTUs differed according to the geographic origin of the vetiver roots and was significantly higher in the samples from Reunion Island than in the samples from the other geographic locations (Table 1). The Chao1, Shannon, and inverse-Simpson indices were calculated to characterize the alpha diversity of the bacterial communities associated with vetiver roots (Table 1). Overall, although the differences were not always significant, our results show that the values of all diversity indices were higher in the Reunion Island samples. This could indicate more diverse bacterial communities in vetiver root samples from Reunion Island than in samples from the other geographic locations (Table 1). To evaluate how our data reflect the diversity, we calculated the ratio of observed OTUs to the Chao1 index values for each location. The analysis showed that the Madagascar samples had significantly lower diversity coverage than the Reunion samples (Table 1).

Principal coordinate analysis (PCoA) based on weighted UniFrac distances among samples showed that the endophytic root bacterial communities from the 5 vetiver geographic locations were clearly separated from each other (Fig. 1). The first two axes explained 78.4 % of the variation. The samples from Reunion Island and Madagascar were separated along the first axis (PCoA 1 = 51.6 %), whereas the samples from Senegal and Portugal were separated along the second axis (PCoA 2 = 26.8 %). These results, which were further validated statistically by PERMANOVA (Df = 4, R^2 = 85 %, P = 0.0001), indicated that the endophytic bacterial communities from vetiver roots are structured by geographic location.

3.2. Taxonomic composition of endophytic bacterial communities in vetiver roots from different geographic locations

The detected vetiver root bacterial OTUs were affiliated with 13 bacterial phyla (Fig. 2A). However, almost all OTUs were distributed in 4 phyla that represented 98.7 % of the total abundance. These phyla were *Proteobacteria* (70.3 % +/− 12.1 %), *Bacteroidota* (13.7 % +/− 8.2 %), *Firmicutes* (7.8 % +/− 9.7 %) and *Actinobacteria* (5.5 % +/− 8.5 %). Proteobacteria were common to all samples in relatively equal abundance, with three main subphyla, Gammaproteobacteria (67.6 % +/− 13.7 %), Alphaproteobacteria (29.7 % +/− 13.2 %) and Betaproteobacteria (2.6 % +/− 2.5 %). In contrast, the other dominant phyla were

Table 1
Alpha diversity of Vetiver bacterial communities in five locations using the indices of richness (the number of observed OTUs and Chao 1), coverage (the ratio of observed OTUs and Chao 1), and the indices of evenness (Shannon and InvSimpson), with ± standard deviation. Different letters besides the values indicate differences with p value <0.05 within an index by location group.

Location	Richness		Coverage (%)	Evenness	
	Observed	Chao1		Shannon	InvSimpson
Madagascar	275.3±56.9b	349.1±64.8c	78.7±1.6b	3.5±0.4c	13.2±3.3b
Portugal	354.0±19.2b	417.6±43.6bc	85.1±4.7ab	3.8±0.3bc	12.9±5.4b
Reunion1	471.7±25.1a	530.6±35.5ab	89.0±3.0a	4.8±0.1a	38.1±8.8a
Reunion2	487.0±11.8a	559.9±29.0a	87.1±3.2a	4.6±0.3ab	33.3±13.6ab
Senegal	316.3±32.3b	382.1±39.8c	82.8±1.6ab	3.7±0.5bc	16.9±9.6ab

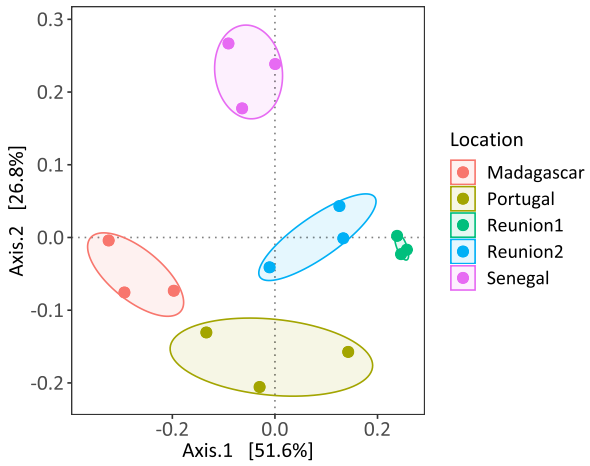

Fig. 1. PCoA plot based on weighted UniFrac distance of Vetiver samples. Points represent individual plants, Points represent individual plants, and ellipses show a 95 % confidential zone of each locational group.

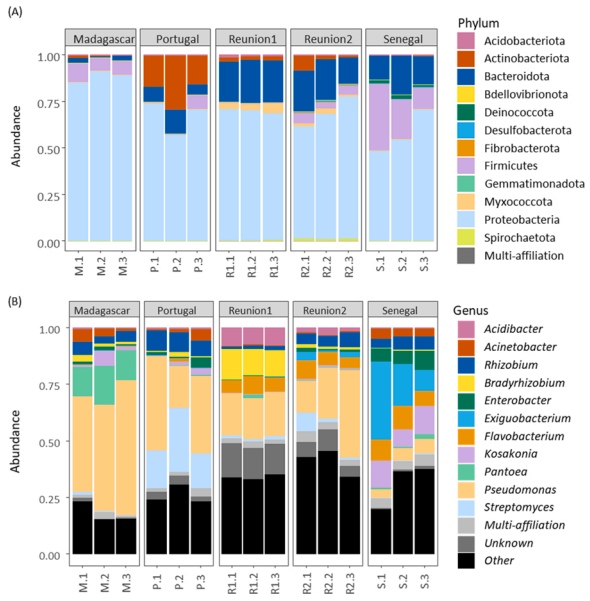

Fig. 2. Taxonomical compositions of 13 phyla (A) and dominant 12 genera (B) in bacterial endophyte communities of Vetiver roots in 5 locations.

more specifically enriched the certain vetiver locations (Fig. 2A). Bacteroidota were more abundant in root samples from Reunion and Senegal (from 13.1% to 23.1%) than in the samples from the other locations (Fig. 2A). The abundances of Firmicutes and Actinobacteriota also varied greatly among vetiver locations. The Firmicutes abundance in vetiver roots originating from Senegal was 20.6 % (4.0 % on average in the other samples), whereas Actinobacteria was mostly represented in samples from Portugal (20.6 %) (1.7 % on average in the other samples) (Fig. 2A).

Similar patterns were observed at lower levels of taxonomical classification. At the family level, Pseudomonadaceae were dominant (from 19 % to almost 50 % in abundance), with the exception of the samples from Senegal in which Enterobacteriaceae dominated (Fig. S4). The abundance of other dominant families differed depending on the geographic origin of the vetiver. In particular, Streptomycetaceae and Exiguobacteraceae were only abundant in the Portugal and Senegal samples, respectively, whereas Flavobacteriaceae was shown to be more abundant in the Senegal and Reunion samples.

At the genus level, a total of 258 genera were found in our samples. Among them, 53 genera were shared across geographic sites; these genera accounted for 20.5 % of the total genera (Fig. 3). The shared genera belonged to 6 phyla (Proteobacteria, Myxococcota, Firmicutes, Bdellovibrionota, Bacteroidota, and Actinobacteriota) and made up an average relative abundance of 72.5 % +/− 12.7 % of the total abundance at each location.

Except in the Senegal samples, in which *Exigobacterium* dominated,

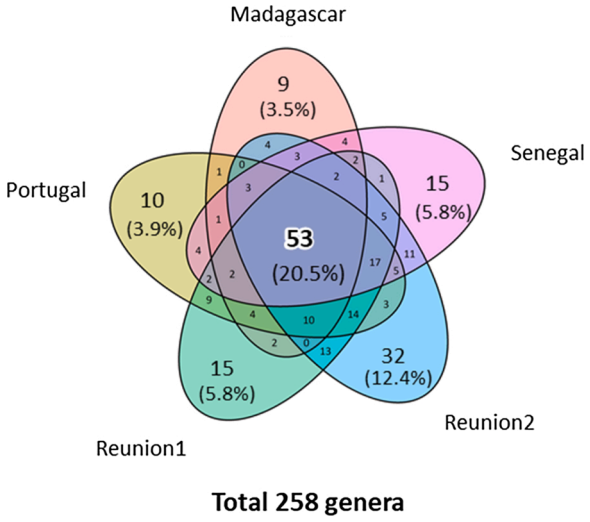

Fig. 3. Venn diagram of the number of shared genera over locations and specific genera for each location. The shown percentages are percentage of shared or specific genera against total number of genera found through all samples.

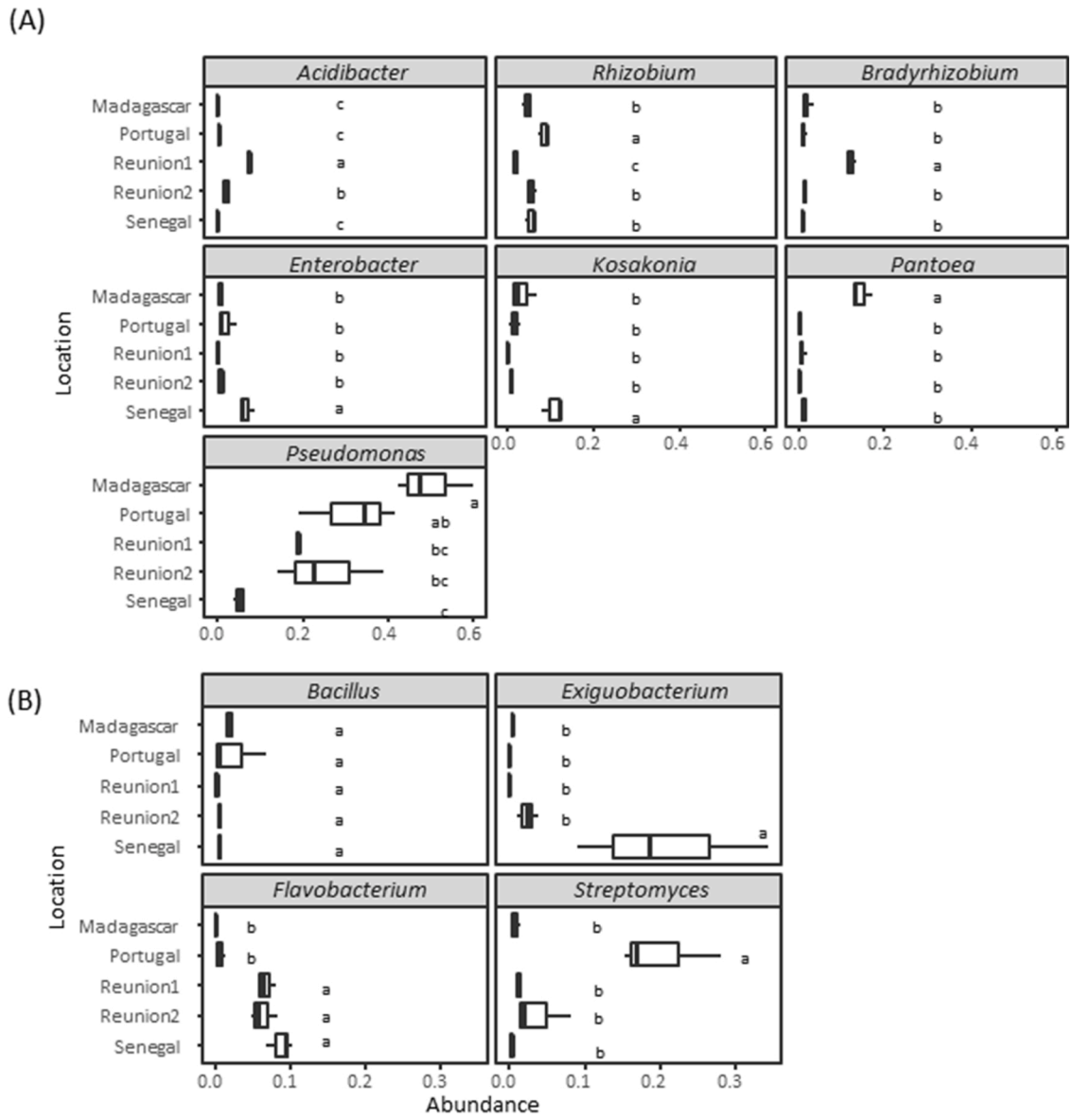

Fig. 4. Relative abundance of dominant genera in 4 phyla, in Vetiver root endophyte communities. (A) Proteobacteria, (B) *Bacillus* and *Exiguobacterium* (Firmicutes), *Flavobacterium* (Bacteroidota), *Streptomyces* (Actinobacteriota). The same letters within a genus mean no differences with p value <0.05 (Tukey-Kramer test).

the most abundant genus was *Pseudomonas* (3.7–59.7% of the total abundance) (Figs. 2B, 4 A). The abundance of other dominant genera differed significantly depending on the vetiver geographic location (p value < 0.05, Fig. 4A). Some genera, such as *Pantoea, Rhizobium, Bradyrhizobium, Acidibacter*, *Enterobacter* and *Kosakonia*, were specifically enriched in samples originating from specific geographic locations, whereas *Flavobacterium* was shown to be enriched in samples from Senegal and Reunion Island (Fig. 5).

3.3. Bacterial endophyte isolation and antagonistic activity towards *F. graminearum*

We isolated bacteria from the same vetiver roots that were used for the metagenomic approach. First, the density of cultivable endophytic bacteria from the vetiver root samples was estimated. We observed that the average density of cultivable endophytic bacteria in vetiver roots did not differ significantly between each geographic location (5.2–6.2 log10 CFU g^{-1} root dry weight) (Table 2). A total of 381 endophytes were recovered from vetiver root samples and were tested for their ability to inhibit *F. graminearum* mycelial growth in *in vitro* culture. The inhibition rate varied greatly among bacterial isolates (from −3.3 to 93.3), but nearly all isolates (99.7 %, Table S2) presented this functional trait. The mean inhibition rate of the bacterial isolates was significantly lower in the isolates from Reunion1 ($p < 0.05$, 31.7 % +/− 14.9 %) than in the isolates from the other samples (Table 2). The endophytic bacterial isolates with an inhibitory rate of at least 50 % were selected for further analyses. They represented 49 % (176 isolates) of the total isolates, and 20 % (36 isolates) of them had an inhibition rate of at least 70 % against *F. graminearum* (Table S2). The relative frequency of these functional isolates differed significantly among the vetiver samples (p-value<0.05, Table 2). In particular, only 14 % of isolates from Reunion1 showed an inhibition rate ≥50 %, while 77 % of the isolates from Madagascar showed an inhibition rate ≥ 50 %. The isolates with inhibition rate ≥70 % were isolated from vetiver originating from Madagascar (2 isolates), Portugal (20 isolates), Reunion2 (11 isolates) and Senegal (3 isolates) (Table S4).

Based on partial 16S rDNA sequencing, 153 of these bacterial isolates were taxonomically characterized (Table S3). They included Proteobacteria (86.2 % +/− 22.0), Firmicutes (12.2 % +/− 22.4) and Actinobacteriota (1.4 % +/− 3.0) (Fig. S5). A total of 15 genera were

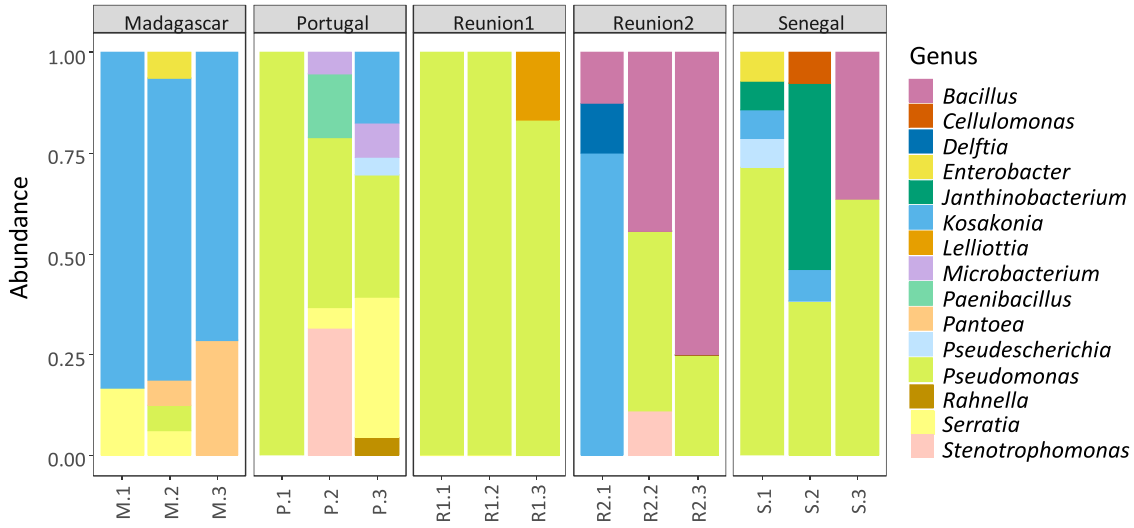

Fig. 5. Taxonomical compositions of 15 genera in functionally selected bacterial endophyte communities of Vetiver roots in 5 sites.

Table 2
| Summary of isolation and antagonistic activities of endophytic isolates of Vetiver roots from 5 different locations. Colony forming unit (CFU) of Vetiver root endophytes per 1 g of dried root materials (dry weight, DW). The same letters within a column indicate no significant difference among locations with p value <0.05 (Tukey-Kramer test).

Site	Log 10 CFU/g of DW	Total number of isolates	Inhibition rate against *F. graminearum* (%)			Isolates with ≥50 % antagonism against *F. graminearum*	
			Min.	Max.	Average ± SD	Number of isolates	Population within a site
Madagascar	6.1±0.3a	48	0.5	83.5	55.1±11.6a	37	77 %a
Portugal	5.7±0.5a	89	28.7	79.9	54.2±14.9a	46	52%ab
Reunion 1	5.6±0.2a	86	−3.3	65.0	31.7±14.9b	12	14 %b
Reunion 2	5.6±0.3a	76	18.3	93.3	52.0±17.2a	43	57%ab
Senegal	6.2±0.3a	82	22.9	82.8	52.9±12.6a	38	46 %ab
total		381				176	

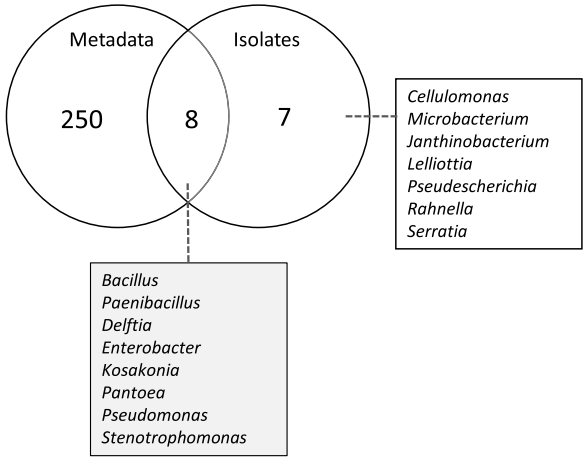

Fig. 6. Venn diagram of the number of common or unique genera in the metabarcoding data and the culturable communities.

identified (Fig. 5). Among them, 8 genera had already been described using 16S rDNA high-throughput sequencing (Fig. 6).

Pseudomonas was the dominant genus (47.0 % +/− 36.3 %) and was observed in all samples except those from Madagascar (Fig. 5). *Bacillus* was mostly identified in samples from Reunion 2, whereas *Kosakonia* was mostly observed in Madagascar samples (Fig. 5). The presence and/or proportion of each genus varied according to the samples. It should be noted that the bacterial isolates possessing the highest inhibition rates (≥70 %) against *F. graminearum* were mainly affiliated with the *Pseudomonas*, *Serratia* and *Bacillus* genera (Table 3). The *Pseudomonas* isolates seemed to be most closely related to 3 species, *P. koreensis*, *P. vancouverensis* and *P. nitroreducens,* whereas the *Serratia* isolates belonged to 2 species, *S. marcescens* and *S. grimesii*, and the *Bacillus* isolates were *B. subtilis* and *B. cereus*.

The table of the relative abundance of genera was used to generate a Bray-Curtis distance matrix that was subjected to PCoA (Fig. 7). This analysis showed that the functional microbial communities from Reunion2 and Madagascar were distinct from each other and from a cluster composed of the microbial communities from the Reunion1, Portugal and Senegal samples. These results were validated with PERMANOVA showing that the geographic location factor significantly altered the composition of the cultivable endophytic communities with antagonistic activity towards *F. graminearum* in vetiver roots (Df = 4, R^2 = 66.4 %, $p < 0.001$).

4. Discussion

Diseases caused by *F. graminearum* are among the most important diseases in grain cereals. Chemical fungicides are still widely used to control this fungus, although their detrimental impacts on the environment and human health are now well known. To reduce the use of such chemicals while maintaining crop yields, it is now necessary to find and develop alternative crop protection solutions. A number of previous studies revealed that bacterial endophytes may help plants cope with pathogens (Card et al., 2015; Eljounaidi et al., 2016; Liu et al., 2009; Mousa et al., 2016). In particular, bacterial endophytes are able to produce a range of specialized metabolites that have antimicrobial activity (Brader et al., 2014). In addition, coevolution processes with their hosts led endophytes to mimic natural plant compounds (Baltrus, 2017).

Table 3
Bacterial endophyte isolates that exhibited more than 70 % of inhibition activity against *F. graminearum* mycelia.

Site	Accession	Inhibition rate (%)	Phylum	Genus	Top hit	Identity (%)
Madagascar	M1_08	71.3	Proteobacteria	*Serratia*	*Serratia marcescens* subsp. marcescens ATCC 13880 strain DSM 30121	100
Portugal	P2_28	70.6	Actinobacteriota	*Microbacterium*	*Microbacterium hominis* strain IFO 15708	100
	P2_02	73.5			*Pseudomonas koreensis* strain Ps 9-14	100
	P2_16	72.1			*Pseudomonas vancouverensis* strain DhA-51	100
	P2_06	78.5	Proteobacteria	*Pseudomonas*	*Pseudomonas vancouverensis* strain DhA-51	99.61
	P2_25	79.2			*Pseudomonas vancouverensis* strain DhA-51	100
	P2_15	73.1		*Serratia*	*Serratia grimesii* strain DSM 30063	99.67
	P3_18	70.6			*Pseudomonas koreensis* strain Ps 9-14	99.44
	P3_08	71.0			*Pseudomonas koreensis* strain Ps 9-14	100
	P3_19	71.7			*Pseudomonas koreensis* strain Ps 9-14	99.42
	P3_26	72.1		*Pseudomonas*	*Pseudomonas koreensis* strain Ps 9-14	99.79
	P3_27	73.8			*Pseudomonas koreensis* strain Ps 9-14	99.51
	P3_24	74.2			*Pseudomonas koreensis* strain Ps 9-14	99.45
	P3_01	72.1			*Serratia grimesii* strain DSM 30063	99.69
	P3_07	73.1	Proteobacteria		*Serratia grimesii* strain DSM 30063	99.68
	P3_25	73.1			*Serratia grimesii* strain DSM 30063	99.65
	P3_30	73.5			*Serratia grimesii* strain DSM 30063	99.7
	P3_17	73.8		*Serratia*	*Serratia grimesii* strain DSM 30063	99.67
	P3_13	74.9			*Serratia grimesii* strain DSM 30063	99.67
	P3_28	78.1			*Serratia grimesii* strain DSM 30063	99.67
	P3_29	79.9			*Serratia grimesii* strain DSM 30063	99.71
Reunion2	R22_05	81.7			*Bacillus subtilis* strain IAM 12118	99.83
	R22_06	81.7	Firmicutes	*Bacillus*	*Bacillus subtilis* strain IAM 12118	100
	R22_17	93.3			*Bacillus subtilis* strain IAM 12118	97.95
	R22_08	70.0	Proteobacteria	*Pseudomonas*	*Pseudomonas nitroreducens* strain NBRC 12694	100
	R23_17	78.3			*Bacillus cereus* ATCC 14579	100
	R23_12	70.0	Firmicutes	*Bacillus*	*Bacillus subtilis* strain IAM 12118	99.86
	R23_28	73.3			*Bacillus subtilis* strain IAM 12118	100
	R23_08	75.0			*Bacillus subtilis* strain IAM 12118	100
Senegal	S1_29	82.8	Proteobacteria	*Kosakonia*	*Kosakonia oryzendophytica* strain REICA_082	100
	S2_18	76.0	Proteobacteria	*Janthinobacterium*	*Janthinobacterium agaricidamnosum* strain NBRC 102515	100
	S2_11	82.5			*Janthinobacterium lividum* strain DSM 1522	99.71

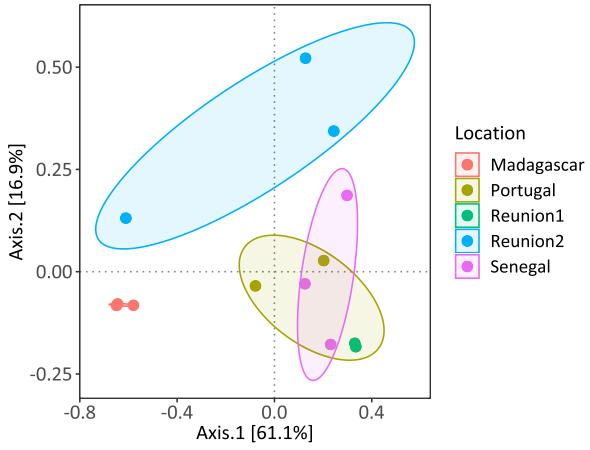

Fig. 7. PCoA ordination of isolated endophyte communities with bioactivities against *F. graminearum* Points represent individual plants, and ellipses show a 95 % confidential zone of each locational group.

Because of these findings, it is now accepted that bacterial endophytes contribute to the biological activity of plant extracts, including their antimicrobial activity (Miller et al., 2012; Strobel et al., 2004). The metabolic potential of bacterial endophytes suggests the possibility of exploiting these microorganisms for biocontrol applications. In this study, we focused on the bacterial endophytic community of vetiver roots since it has been shown that some members of this community are involved in the synthesis of vetiver essential oil, which has been reported to display antimicrobial activity (Chahal et al., 2015; Del Giudice et al., 2008; Hammer et al., 1999; Powers et al., 2018).

The vetiver roots we investigated mainly harbour endophytic bacteria belonging to four major phyla, *i.e.*, Proteobacteria, Bacteroidota, Firmicutes and Actinobacteriota. These results are consistent with those of several previous studies showing that these phyla are preferentially enriched in plant roots compared to their levels in the soil and rhizosphere (Bulgarelli et al., 2013; Hardoim et al., 2015; Lundberg et al., 2012). Additionally, among them, Proteobacteria were overrepresented in all vetiver roots sampled, which is similar to the results obtained by Monteiro et al. (2011). This predominance might be related to the fact that such copiotrophic microorganisms prefer a C-rich environment (Fierer et al., 2007; Yang et al., 2017). They may therefore benefit from the flow of photoassimilates from shoots to roots, which creates a nutrient-rich environment inside the roots. We showed that the Proteobacteria were mostly *Gammaproteobacteria* and were dominated by a few families and genera. Among them, the genus *Pseudomonas* is a good root colonizer and was prevalent. However, other genera, such as *Kosakonia*, *Enterobacter* and *Pantoea,* were also observed. Most of these genera can benefit plants by promoting plant growth and/or protection, although some of them have been described as pathogens (Bulgarelli et al., 2013). Bacteroidetes were represented by the dominant *Flavobacterium* genus, which is often reported as an important member of root microbiomes, and some strains have traits that are beneficial to plants (Kolton et al., 2016). Finally, Actinobacteria and Firmicutes were represented by the *Streptomyces* and *Exiguobacterium* genera, respectively, which are known to produce antimicrobial compounds (Hardoim et al., 2015; Kasana and Pandey, 2018). Our study also provides additional insights since we showed that the geographic location of the vetiver samples seemed to influence the composition and the relative abundance of some of the taxa in the roots. Recently, Thiergart et al. (2020) provided evidence that soil is the primary factor that influences root bacterial communities, regardless of the spatial scale of the analysis or the host genotype. These data support the findings of previous studies and led us to suggest that the variations we observed within the vetiver root bacterial communities might be related to the differences in soil properties across a geographic gradient from Europe to Africa (Bulgarelli et al., 2013; Chen et al., 2019; Fierer et al., 2007; Lundberg et al., 2012).

As some of the microbial groups we identified in vetiver roots have been described as potential biocontrol agents against plant pathogens, we evaluated the ability of this microbiota to suppress the growth of *F. graminearum*. For that purpose, the bacteria were isolated and profiled in *in vitro* dual-culture assays with *F. graminearum*. Among the 381 isolates, most displayed antagonistic activity towards *F. graminearum*, with almost 50 % producing an inhibition rate of more than 50 %. These bioactivities might be related to the ability of the isolates to synthesize diverse antimicrobial compounds (specialized metabolites) (Saleh et al., 2019; Strobel et al., 2004). Furthermore, these results reinforce the hypothesis that microbial endophytes can contribute to the bioactivity of plant extracts and, in our case, to the antimicrobial properties of the vetiver essential oil (Chahal et al., 2015; Del Giudice et al., 2008; Egamberdieva et al., 2017; Miller et al., 2012).

The 16S rDNA sequence analysis showed that the bacterial isolates mostly belong to Proteobacteria, followed by Firmicutes and Actinobacteriota, and that their distribution is related to their geographic location. Our results also showed, at lower taxonomic levels, that the geographic location shapes the cultivable bacteria in vetiver roots. As previously suggested by Monteiro et al. (2011), soil could be an important factor influencing bacterial communities from vetiver. Interestingly, the culture-based and molecular-based datasets do not completely overlap. For instance, in the metagenomic data, *Pseudomonas* was highly represented in the Madagascar samples, whereas most of the bacterial isolates in these samples were affiliated with *Kosakonia* and *Pantoea*. This relative discrepancy could result from the fact that most of the bacteria present in a sample cannot grow on the traditionally used culture media (Eevers et al., 2015; Pascual et al., 2016). Bacteria with the capacity to inhibit the *in vitro* growth of *F. graminearum* were affiliated with 15 genera. Some of these genera, such as *Pseudomonas*, *Enterobacter*, and *Serratia*, have already been identified in vetiver roots (Del Giudice et al., 2008).

This study performed on vetiver roots resulted in the isolation and identification of 32 highly bioactive isolates displaying at least 70 % inhibition rates against *F. graminearum*. These isolates were mostly from the genera *Pseudomonas*, *Serratia*, and *Bacillus*. Only 4 isolates were affiliated with different genera, *i.e.*, *Kosakonia*, *Janthinobacterium* and *Microbacterium*. Various reports have described bacteria affiliated with these dominant genera that were isolated from different cultivated plants and display *in vitro* inhibition activity against *Fusarium* spp. including *F. graminearum* (Comby et al., 2017; Haas and Défago, 2005; Khalaf and Raizada, 2018; Ju et al., 2013). Furthermore, *Serratia*, *Pseudomonas*, *Bacillus*, and *Microbacterium* have been reported to produce antimicrobial compounds. *Bacillus*, which is one of the most investigated and commercially utilized genera worldwide (Lopes et al., 2018), is known to produce antimicrobial compounds such as lipopeptides, exopolysaccharides, siderophores and polyketides (Ek-Ramos et al., 2019). In addition to *Bacillus*, *Pseudomonas* strains, including those affiliated with *P. koreensis*, are also known to produce structurally diverse specialized metabolites with antimicrobial properties (Kaur et al., 2019; Raaijmakers and Mazzola, 2012). Likewise, some strains in the *Serratia* genus can produce antibiotics whose synthesis is regulated by carbon sources (M Mai, 2018). Another study performed on *Janthinobacterium* strains revealed that one of the modes of action of this genus against *F. graminearum* occurs through biofilm formation (Haack et al., 2016). More recently, new antifungal compounds, such as janthinopolyenemycins, were identified through the co-culturing of two *Janthinobacterium* strains isolated from marine sediments (Anjum et al., 2018).

5. Conclusions

By combining culture-based and molecular-based approaches, we provided new insights into the composition of the endophytic bacterial communities of vetiver roots. We showed that the taxonomic composition as well as the ability of the members of the endophytic community to exhibit *in vitro* antagonistic activity towards *F. graminearum* were influenced by the geographic location of the vetiver roots. These results open new pathways for accessing a wider diversity of bacteria and therefore identifying new functional bacteria. The most effective bacteria for inhibiting the growth of *F. graminearum in vitro* might be further studied as promising biological control solutions. The next step will now consist of confirming the bioactivity of these isolates in plant inoculation assays and/or characterizing the antimicrobial compounds produced.

CRediT authorship contribution statement

Yuka Munakata: Writing - original draft, Investigation, Visualization. **Carole Gavira:** Investigation. **Julie Genestier:** Investigation. **Frédéric Bourgaud:** Conceptualization, Resources. **Alain Hehn:** Writing - review & editing, Funding acquisition. **Sophie Slezack-Deschaumes:** Conceptualization, Methodology, Investigation, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.micres.2020.126650.

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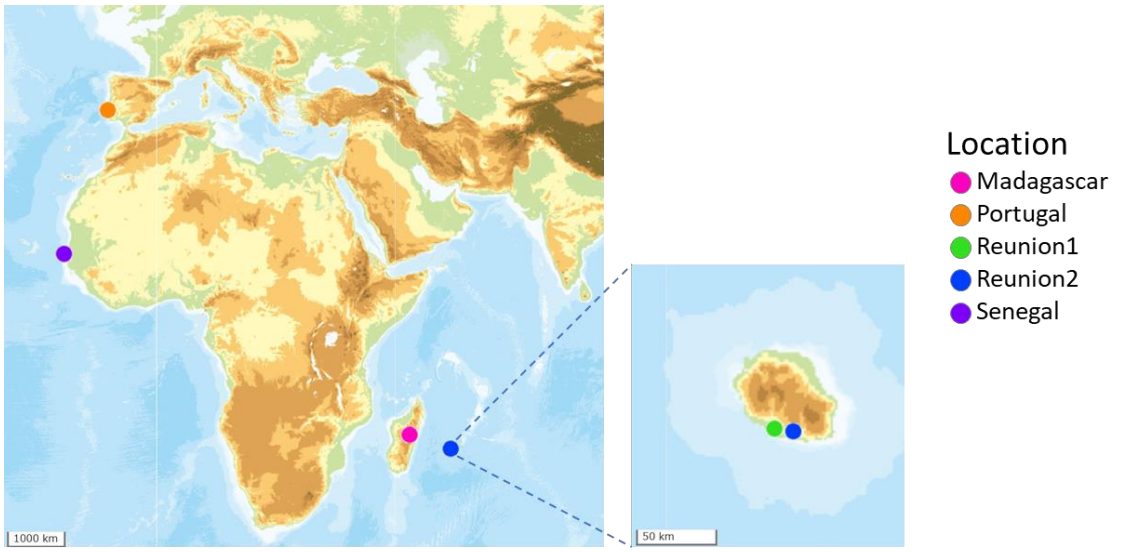


Figure S1| Geographical origins of Vetiver roots used for the present study.
The map was based on Global Map Version1(2) -Elevation- published by Geospatial Information Authority of Japan (https://maps.gsi.go.jp/development/ichiran.html), with editing and processing.

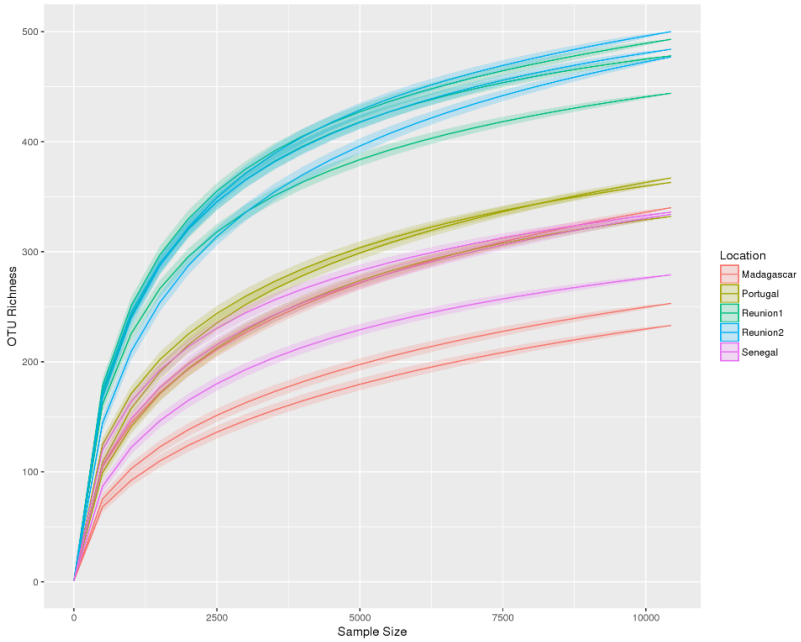


Figure S2| Rarefaction curves of the bacterial community of Vetiver root samples from Madagascar (red), Portugal (olive), Reunion1 (green), Reunion2 (blue), Senegal (pink). Colored regions indicate ±standard error.

Table S1| OTU table of bacterial communities of Vetiver root samples from five different

locations

→ ANNEX 2

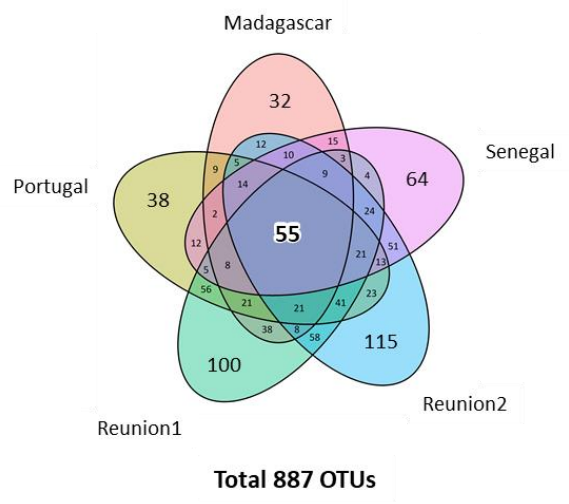


Figure S3| Venn diagram of the number of shared or specific OTUs for each location, herein OTUs that were found in at least two specimens within a set of biological replicates in a location were counted (887 OTUs out of 894 OTUs).

Seven OTUs did not meet this criterium, which were removed.

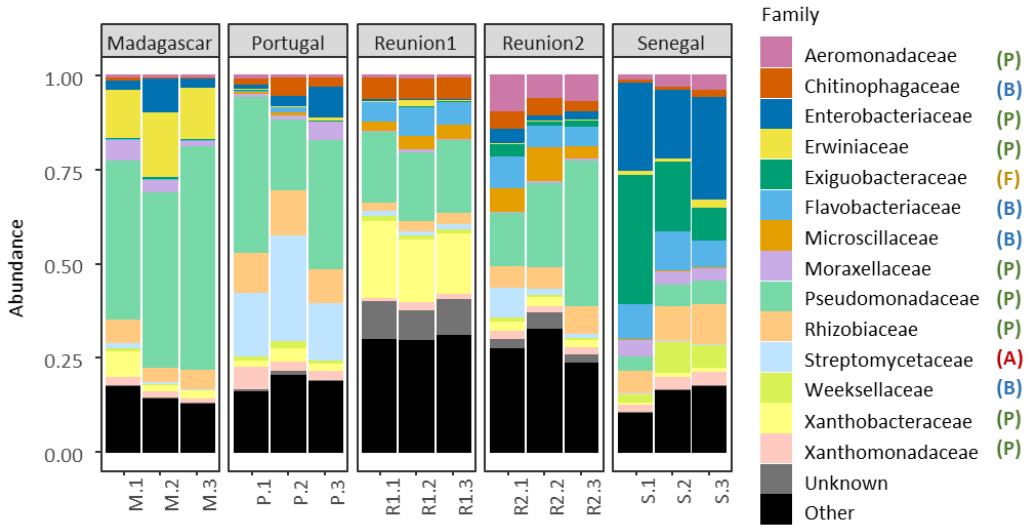


Figure S4| Taxonomical compositions of dominant 14 families in bacterial endophyte communities of Vetiver roots in 5 locations.

A, Actinobacteriota; B, Bacteroidota; F, Firmicutes; P, Proteobacteria.

Table S2| Population of antagonistic Vetiver endophytic isolates against *F. graminearum*.

The same letters within a column indicate no significant difference among locations with p value <0.05 (Tukey-Kramer test).

Site	Isolates with >0% antagonism against *F. graminearum*		Isolates with ≥70% antagonism against *F. graminearum*	
	Number of isolates	Population within a site	Number of isolates	Population within a site
Madagascar	48	100%	2	5%
Portugal	89	100%	20	23%
Reunion 1	85	99%	0	0%
Reunion 2	76	100%	11	14%
Senegal	82	100%	3	3%
total	380		36	

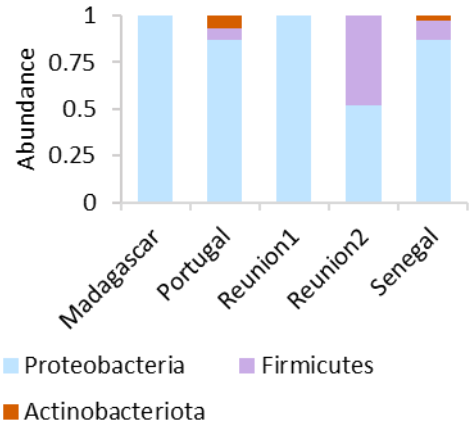


Figure S5| Taxonomical compositions of 3 phyla in functionally selected bacterial endophyte communities of Vetiver roots in 5 sites

Table S3| A list of bacterial isolates exhibiting more than 50% of inhibition activity against *F. graminearum* mycelial growth → ANNEX 3

Part 2

Screening of antimicrobial activities and lipopeptide production of endophytic bacteria isolated from vetiver roots

Yuka Munakata[1], Egon Heuson[2], Théo Daboudet[3], Barbara Deracinois[3], Matthieu Duban[3], Alain Hehn[1], François Coutte[3], Sophie Slezack-Deschaumes[1]

Author affiliations

1 Université de Lorraine – INRAE, LAE, F-54000 Nancy, France

2 Univ. Lille, CNRS, Centrale Lille, Univ. Artois, UMR 8181 - UCCS - Unité de Catalyse et Chimie du Solide, F-59000 Lille, France

3 Université de Lille, UMRt BioEcoAgro 1158-INRAE, équipe Métabolites secondaires d'origine microbienne, Institut Charles Viollette, F-59000 Lille, France

1. Introduction

Microorganisms are an important source for the mining of bioactive ingredients, which can then be used as drugs or leading compounds for pharmaceutical and agrochemical industries [4,5]. For example, a number of bioactive compounds have been characterized by soil-derived microorganisms such as Streptomyces [6]. Nowadays, discovering new bioactive compounds and/or new modes of action is needed to better control multi-drug-resistant microbes that threaten sustainable developmental goals set by the United Nations regarding human health and food security (https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance). In addition, due to societal expectations for more sustainable agriculture in the world and in particular in Europe, the characterization of bio-alternatives to chemical inputs that are assumed to be less toxic for the environment and human health is really expected [7,8]. Hence, exploring microbial diversity from less investigated environments could offer opportunities to discover new bioactive compounds and/or new modes of action

In this context, endophytic microorganisms could be an attractive reservoir [9]. Compared to free-living microorganisms, endophytic microorganisms are deeply interacting with their host plants. Since they are living in various ecological niches, they are supposed to produce a higher diversity of bioactive compounds. For example, they were reported to produce antimicrobial compounds such as ecomycin, kakadumycin, and munubicins [10–12].

Among these antimicrobial biomolecules, biosurfactants such as lipopeptides have been already described [13]. For example, lipopeptides produced by *Bacillus* species, such as fengycins (plipastatins), iturins, and bacillomycins were reported as their high antagonists against various fungal phytopathogens [14–17]. In addition to such direct effect, the presence of lipopeptides

sometimes enhances the efficacy of chemical fungicides due to the alteration of the cell membrane permeability [18]. Some lipopeptides are also known to induce systemic resistance in plants, which is promising for the development of biocontrol solutions with a lower risk of resistance emergence [19,20].

Since the development of sequencing technologies, genome mining has become a powerful tool to investigate the synthesis of new natural compounds [9]. Especially, NRPS genes involved in the biosynthesis of lipopeptides are considered to be one indicator for the prediction of biological activities or functions of certain bacteria [21,22]. NRPSs often consist of regularly arranged modules, where one module is responsible for adenylation, condensation, and thiolation of one amino acid, leading *to* an extension of peptide chains. Due to the modular structure of NRPS, homology analysis of predicted NRPS genes identified thanks to bioinformatic tools, can help to predict peptide sequences [23]. A database dedicated to non-ribosomal peptides, named NORINE [24], was developed to help to identify such peptides. So far, lipopeptides were characterized in only limited endophytic bacteria [11,12,14,25].

Vetiver plants (*Chrysopogon zizanioides* (L.) is a plant species used for its essential oil for the production of perfume. In our previous work, we studied the composition of endophytic bacterial communities of vetiver roots sampled in five different geographical regions (Madagascar, Portugal, Senegal, Reunion Island) [26]. Using a 16S metabarcoding approach, we revealed that the composition of root endophyte communities differed according to the geographic origin of the plants. After isolating and evaluating their antagonistic activities *in vitro* towards the mycelial growth of *F. graminearum*, we also observed that the composition of the functional communities is influenced by the origin of the vetiver. We identified 31 strains affiliated with *Bacillus*, *Janthinobacterium*, *Kosakonia*, *Microbacterium*, *Pseudomonas*, and *Serratia* showing strong growth inhibition activity (≥70 %) against *F. graminearum* and that could be candidates for further development as biocontrol agents. Here we further characterized these strains for their spectrum of antagonistic activities against other fungal pathogens and microorganisms. In addition, we performed a screening for their ability to produce lipopeptides using cell-free culture broth that was analyzed by Matrix-Assisted Laser Desorption/Ionization-Time of Flight-mass spectrometry (MALDI-ToF). The genome of *Bacillus subtilis* strains that produced different classes of lipopeptides with strong antifungal activities was also investigated to identify NRPS genomic clusters implicated in lipopeptide biosynthesis.

2. Materials and methods

2.1. Bacterial and fungal strains

A collection of 31 bacterial strains previously isolated from surface-sterilized roots of vetiver was used [26]. These strains were selected for their growth inhibition activity against Fusarium

graminearum in in vitro dual cultures and characterized taxonomically (Table 1). They are stored in a 15% glycerol solution at -80 ° C.

The fungal strains used in this study were *Fusarium graminearum*, *F. oxysporum,* and *F. culmorum*. *The* strain of *F. graminearum* was kindly provided by IFBM (Institut Français de la Brasserie et de la Malterie), and the others strains were purchased from BCCM/MUCL: *F. oxysporum* f. sp. radicis-lycopersici Jarvis & Shoemaker (MUCL 039790) and *F. culmorum* (W.G. smith) Saccardo (MUCL 042823). The fungal strains were regularly subcultured on Potato Dextrose Agar (PDA) (OXOID) plates at 28 ° C for *F. graminearum* and 25 ° C for *F. oxysporum* and *F. culmorum*.

One strain of *Escherichia coli* K12 and one strain of *Saccharomyces cerevisiae* were also used. Both were stored at -80 ° C.

2.2. Dual culture test of selected vetiver bacterial endophytes against *F. oxysporum* and *F. culmorum*

Bacterial strains were cultured in 2 mL of Nutrient Broth (NB) (Sigma Aldrich) inoculated with a single colony for about 24 h at 28° C. 50 µL of the liquid culture adjusted to an Optical Density at 600 nm of 1.0 were placed at 2 points 3 cm from an eight mm-diameter mycelial plug of fresh *F. graminearum* cultures placed at the center of a Petri dish (9 cm in diameter) containing 20 mL of Potato Dextrose Agar (PDA) (Sigma-Aldrich, USA). Three replicates were prepared for each bacterial strain. The plates were incubated at 25 ° C. The diameters of fungal colonies were measured at six and/or 11 days, according to the radial growth rate of the fungal species. The inhibition rate (%) was calculated with the following formula: 100 x (1- Dt/Dc), where Dt is the mean diameter of Fusarium strains in test plates and Dc in the control plates.

2.3. Preparation of supernatant samples of endophytes

For the evaluation of the inhibitory activity of bacterial supernatants, the bacterial cultures, incubated at 28 ° C 180 rpm for 24 h in 2 mL of King's B and/or NB, were centrifuged at 11,000 xg 4 ° C for 10 min. Then, the supernatant was filtered through 0.2 µm polyethersulfone membrane (Acrobatic® Syringe Filter with Supor Membrane). Supernatants from a bacterial culture on King B and NB were tested against *F. graminearum* while only culture supernatants from KB were tested against E. coli and S. cerevisiae.

For MALDI-TOF analysis, a single colony of each bacterial strain was cultured in 2 mL of King's B medium at 28 ° C 180 rpm for around 24 h, and then 200 µL of the preculture was transferred into 10 mL of King's B in 50 mL sterile tubes and cultured at the same conditions. After 48 and 72 h, 1 mL of each culture was taken in 2 mL sterile tubes and their pH was adjusted to around

7 by dropping 1 M KOH. The tubes were then centrifuged at 10,000 xg at 4 ° C for 10 min, and 500 µL of the clear supernatant was collected in new sterile tubes. As a control sample, we used treated medium with the same way for the other samples was also prepared. The samples were kept at 4 ° C for 4 to 5 days until the analysis by MALDI-TOF MS.

2.4. Antagonism test of cell-free supernatant of the selected vetiver bacterial endophytes against *F. graminearum*

The inhibitory activity of bacterial supernatants against *F. graminearum* mycelial growth was tested in 9 cm-diameter dishes containing 20 mL PDA. 100 µL of the supernatant samples were placed in ϕ 8 mm wells, made at 2 cm distant from the centre of the plate. A ϕ 8 mm fungal plug was inoculated at the centre of the plate. The Petri dish was incubated at 28 ° C in darkness. One plate had three different samples and one control sample. Control samples were non-inoculated medium filtered through 0.2 µm membrane. Three independent replicates were prepared. The radiuses of *F. graminearum* colonies were measured after 4 days the incubation. The inhibition rate was calculated with the following formula: Inhibition rate (%) = 100 x (1-r_t/r_c). Herein, r_t is the radius of test samples and r_c is the radius of control sample in the same plate of the test samples.

2.5. Antagonism test of cell-free supernatant of the endophytes against E. coli and S. cerevisiae

E. coli and S. cerevisiae were precultured in Mueller-Hinton (MH) and Yeast Extract-Peptone-Glycerol (YPG) media, respectively. The Optical Density (OD) at 600 nm was adjusted to 0.1 before inoculation. The supernatants (1 mL) were transferred manually into a 2 mL DeepWell plate for further use. The supernatant was then mixed with the target strains using a Biomek FXp liquid handler (Beckman Coulter, USA) in a polystyrene 96-well microtiter plated under sterile condition to achieve the following plate design (Supplemental Fig. 1).

The plates were lidded and then incubated at 37 ° C for *E. coli* and 30 ° C for *S. cerevisae* for 24 hours. The growth inhibition (GI) was calculated with the following formula: 100×(1-(ODt/ODg), herein ODt is the mean OD600 value of a test samples, ODg is the mean OD600 value of the growth control samples (MH or YPG + inoculum). For yeast, because only filtered King's B medium had also a certain reduction of yeast growth, the inhibition values were normalized with the values of control samples (King's B + YPG + inoculum).

2.6. Lipopeptide detection with MALDI-TOF

For lipopeptides detection in supernatants, MALDI mass spectrometry analyses were

performed on a Autoflex Speed (Bruker Daltonics, USA). All analyses were performed in reflectron positive mode using an in-house method for lipopeptide detection (RP_700-3500_Da_Lipopetides.par), created from the manufacturer's automatic method RP_700-3500.par. Equipment parameters were as follow: voltage values of ion sources #1 and #2 set as 19.00 and 16.50 keV respectively; voltage values of reflectron #1 and #2 set as 21.00 and 9.50 keV respectively; lens tension 8.00 keV; pulsed extraction 120 ns; laser intensity between 60 and 70 %; laser Global attenuator Offset set to 41 %; Attenuator Offset set to 16 %, Attenuator Range set to 32 %; Detector Gain set to 1880 V; Smartbeam Parameter set to ultra and Sample Rate and Digitizer Settings set to 4.00 GS/s. The MS signals were acquired by summing 10000 laser shots per spectrum. Prior to each analysis, the spectrometer was calibrated using the monoisotopic values of the manufacturer's PeptideCalibrationStandard II calibrant (Bruker Daltonics, USA), containing Bradykinin Fragment 1-7, Angiotensin II, Angiotensin I, Substance P, Bombesin, Renin Substrate, ACTH clip 1-17, ACTH clip 18-39, Somatostatin 28. Calibrant was prepared by mixing 5 µL of calibrant diluted mixture prepared according to manufacturer's specification with 5 µL of a 10 $mg{\cdot}mL^{-1}$ HCCA matrix in 50/47.5/2.5: acetonitrile/MS grade water/trifluoroacetic acid, and 2 µL were then spotted on a Polished Steel 384 MALDI target (Bruker Daltonics, USA). Samples were prepared by mixing 5 µL of supernatant with 5 µL of a 10 $mg{\cdot}mL^{-1}$ HCCA matrix in 50/47.5/2.5: acetonitrile/MS grade water/trifluoroacetic acid, and 2 µL were then spotted on a Polished Steel 384 MALDI target (Bruker Daltonics, USA). The plate was dried prior to analysis under a laminar airflow hood at room temperature. Mass spectra were visualized using FlexAnalysis software (version 3.4; Bruker Daltonics).

2.7. Data analysis

All the figures and statistical tests were realized with R studio with R version 3.6.3. Box plots were all prepared using ggplot2.

In all the inhibition assays against plant pathogens, *E. coli*, and yeast, the negative inhibition values were replaced with 0%.

3. Results

3.1. Antifungal activities of the vetiver endophytic bacterial strains against plant pathogens

In this study, 31 selected endophytic bacteria from vetiver roots were investigated (Table 1). These bacteria affiliated with the *Bacillus*, *Enterobacter*, *Janthinobacterium*, *Pseudomonas*, *Serratia*, *Microbacterium*, and *Yokenella* genera have previously shown high inhibition rates against *F. graminearum* in *in vitro* cultures (Table 1) [26]. In this study, we evaluated their ability to inhibit the mycelial growth of two other well-known Fusarium plant pathogenic species, *F. culmorum* and *F. oxysporum* (Fig. 1).

Depending on the bacterial genus, the inhibitory rate varied from 1.1 to 53.4 % and 0 to 55.3 % against *F. culmorum* and *F. oxysporum*, respectively. Bacteria affiliated to *Serratia* and *Bacillus* genera showed the highest inhibitory rates against *F. culmorum* and *F. oxysporum* (Fig. 2). *Serratia* strains, closely related to *S. grimesii* species, showed an inhibitory rate around 46% against *F. culmorum* and 48% against *F. oxysporum*. *Bacillus* strains belonged to *B. subtilis*, *B. tequilensis*, and *B. cereus* and showed a higher inhibitory rate against *F. oxysporum* (around 40%) compared to *F. culmorum* (around 38%). It should be noted that although bacterial strains affiliated to *Serratia* or *Bacillus* genus showed similar capabilities to inhibit the mycelial growth of *F. culmorum*, the appearance of the fungal colonies differs, depending on the bacterial strains with which the fungus interacts (Fig. 1).

For other bacterial strains, the one affiliated with *Enterobacter* (strain, number 29) had a good inhibitory rate against *F. culmorum* (47%) while bacteria affiliated to *Janthinobacterium*, *Pseudomonas*, and *Microbacterium* genera showed low inhibitory rates against *F. culmorum* and *F. oxysporum*.

Table 1 | Summary of antimicrobial activities of the Vetiver endophytes

			Dual-culture test [*1]			Diffusion assay with cell-free supernatant [*1]		Growth inhibition assay with cell-free supernatant [*2]	
Strain	**Accession**	**BLAST top hit**	***F. graminearum*** (Munakata et al., 2021)	***F. culmorum***	***F. oxysporum***	*F. graminearum* **King's B**	*F. graminearum* **NB**	*E. coli*	*S. cerevisiae*
1	M1_08	*Yokenella regensburgei* strain CIP 105435	71.3	26.8 ± 5	27.7 ± 8.1	8.5 ± 7.8	6.8 ± 5.3	-	-
2	P2_02	*Pseudomonas koreensis* strain Ps 9-14	73.5	11.4 ± 12.1	1.7 ± 1.9	6 ± 3.9	6.8 ± 3.9	-	-
3	P2_06	*Pseudomonas vancouverensis* strain DhA-51	78.5	8.8 ± 8.4	18.9 ± 17.4	8.5 ± 1.5	7.7 ± 5.1	-	-
4	P2_15	*Serratia grimesii* strain DSM 30063	73.1	46.2 ± 3.3	48.7 ± 7.7	1.9 ± 1.6	0 ± 0	-	-
5	P2_16	*Pseudomonas vancouverensis* strain DhA-51	72.1	1.1 ± 1.9	17.4 ± 3.5	3.7 ± 1.6	0 ± 0	-	-
6	P2_25	*Pseudomonas vancouverensis* strain DhA-51	79.2	3.2 ± 5.6	6.1 ± 10.5	3.7 ± 4.2	1.9 ± 3.2	-	-
7	P2_28	*Microbacterium hominis* strain DSM 12509	70.6	10.9 ± 6.2	0 ± 0	1.8 ± 3.1	0 ± 0	-	-
8	P3_01	*Serratia grimesii* strain DSM 30063	72.1	45.3 ± 2.7	48.1 ± 9.6	6.3 ± 1.6	0 ± 0	-	-
9	P3_07	*Serratia grimesii* strain DSM 30063	73.1	46.1 ± 4	37.2 ± 27.5	6.3 ± 1.6	0 ± 0	-	-
10	P3_08	*Pseudomonas koreensis* strain Ps 9-14	71.0	16.5 ± 11.7	0.2 ± 0.4	2.8 ± 4.8	0.9 ± 1.6	-	-
11	P3_13	*Serratia grimesii* strain DSM 30063	74.9	45.7 ± 4.6	48.3 ± 4.2	9.3 ± 4.2	3.7 ± 1.6	-	-
12	P3_17	*Serratia grimesii* strain DSM 30063	73.8	39.9 ± 5.2	46.6 ± 3	6.5 ± 1.6	0.9 ± 1.6	-	-
13	P3_18	*Pseudomonas koreensis* strain Ps 9-14	70.6	8.6 ± 11.9	6.7 ± 2.4	0 ± 0	0 ± 0	-	-
14	P3_19	*Pseudomonas koreensis* strain Ps 9-14	71.7	12.4 ± 6.7	11.6 ± 1.5	1 ± 1.6	0 ± 0	-	-
15	P3_24	*Pseudomonas koreensis* strain Ps 9-14	74.2	15.3 ± 5.6	1.9 ± 3.3	1 ± 1.6	1.9 ± 1.6	-	-
16	P3_25	*Serratia grimesii* strain DSM 30063	73.1	43.1 ± 5.1	47 ± 6.3	3.7 ± 3.2	1.9 ± 1.6	-	-
17	P3_26	*Pseudomonas koreensis* strain Ps 9-14	72.1	23.9 ± 6.8	4.8 ± 4.3	2.8 ± 4.8	1.9 ± 1.6	-	-
18	P3_27	*Pseudomonas koreensis* strain Ps 9-14	73.8	11.2 ± 10	4.6 ± 7.9	1.9 ± 1.6	4.6 ± 1.6	-	-
19	P3_28	*Serratia grimesii* strain DSM 30063	78.1	49.9 ± 5.2	46 ± 3.1	1 ± 1.7	0 ± 0	-	-
20	P3_29	*Serratia grimesii* strain DSM 30063	79.9	53.4 ± 7	55.3 ± 9.6	0 ± 0	0 ± 0	-	-
21	P3_30	*Serratia grimesii* strain DSM 30063	73.5	51.2 ± 3.5	55.2 ± 5.8	0 ± 0	0 ± 0	-	-
22	R22_05	*Bacillus subtilis* strain IAM 12118	81.7	45.8 ± 2.9	33 ± 5.6	46.5 ± 4	24.6 ± 1.5	+/-	-
23	R22_06	*Bacillus subtilis* strain IAM 12118	81.7	46.5 ± 5.2	39.2 ± 2.8	43 ± 6.6	22.8 ± 1.5	+/-	-
24	R22_08	*Pseudomonas nitroreducens* strain NBRC 12694	70.0	26.4 ± 9.7	27.7 ± 6.5	6.1 ± 1.5	6.1 ± 1.5	-	-
25	R23_08	*Bacillus tequilensis* strain 10b	75.0	37.7 ± 7.9	41 ± 0.2	18.1 ± 6.6	11.4 ± 2.9	-	-
26	R23_12	*Bacillus subtilis* strain IAM 12118	70.0	47.4 ± 2.2	41.1 ± 4.3	34.3 ± 2.9	32.4 ± 3.3	+	-
27	R23_17	*Bacillus cereus* ATCC 14579	78.3	19.6 ± 3.5	36.2 ± 4	1 ± 1.6	1 ± 1.6	-	-
28	R23_28	*Bacillus subtilis* strain IAM 12118	73.3	47 ± 2.8	40.5 ± 4	46.3 ± 4.2	30.6 ± 10	+++	-
29	S1_29	*Enterobacter cloacae* subsp. dissolvens strain ATCC 23373	82.8	47.4 ± 10	N.D.	2.8 ± 0	1.9 ± 1.6	+++	-
30	S2_11	*Janthinobacterium lividum* strain DSM 1522	82.5	7.3 ± 6.8	30 ± 15.8	0 ± 0	3.7 ± 3.2	-	-
31	S2_18	*Janthinobacterium lividum* strain DSM 1522	76.0	34 ± 11	0 ± 0	0 ± 0	0 ± 0	-	-

*1 Growth inhibition rate is shown as mean value ± SD. More than 30% growth inhibition is shown in gray. (n = 3)

*2 Growth inhibition activities are shown in 5 clesses from "-" to "+++". +++, all the dilution series had more than 10% inhibition;
+, only one dilution had more than 10% inhibition; +/-, only one dilution had more than 0% but less than 10% inhibition;-, no dilution series had more than 10% inhibition

(a) Against *F. culmorum*

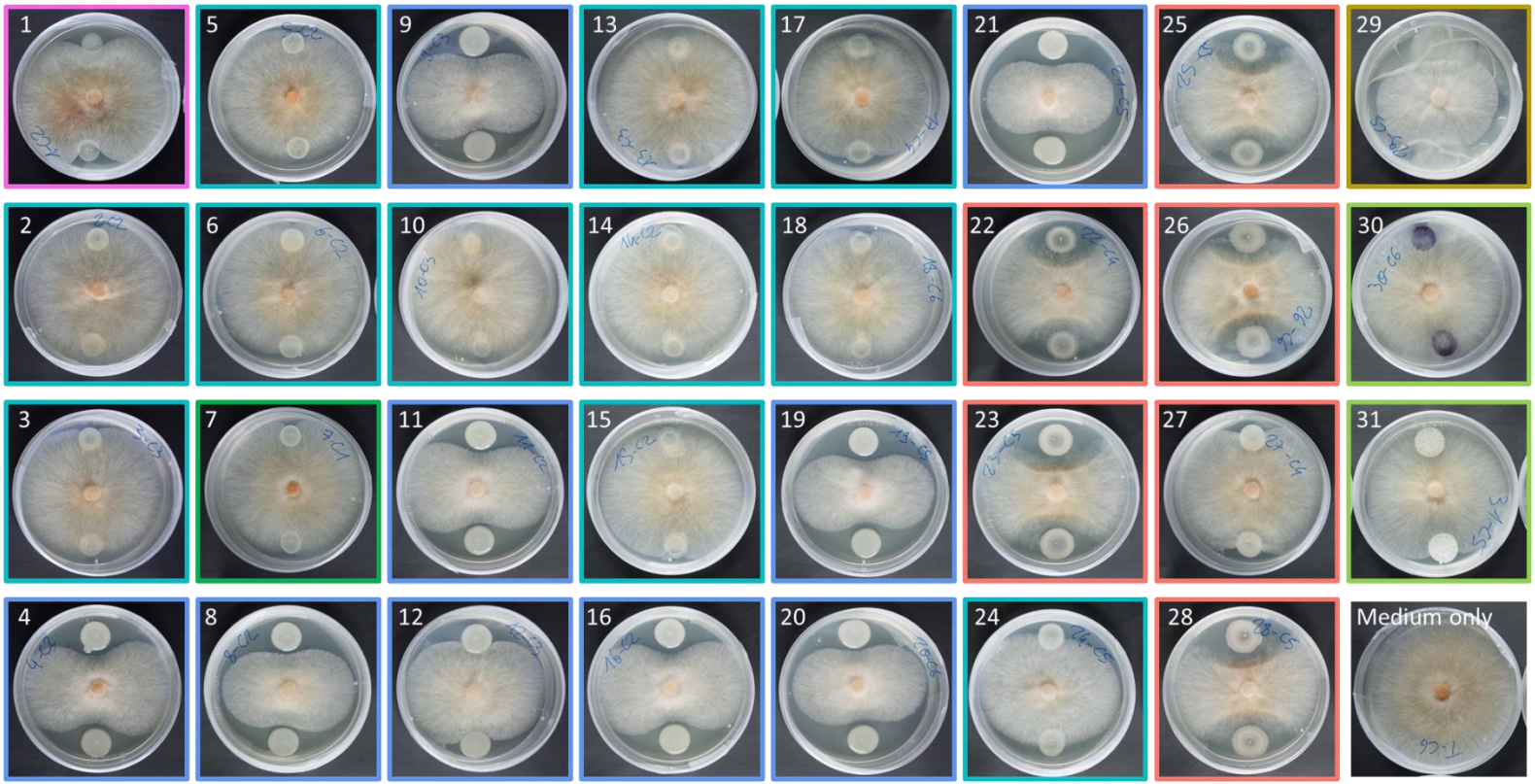
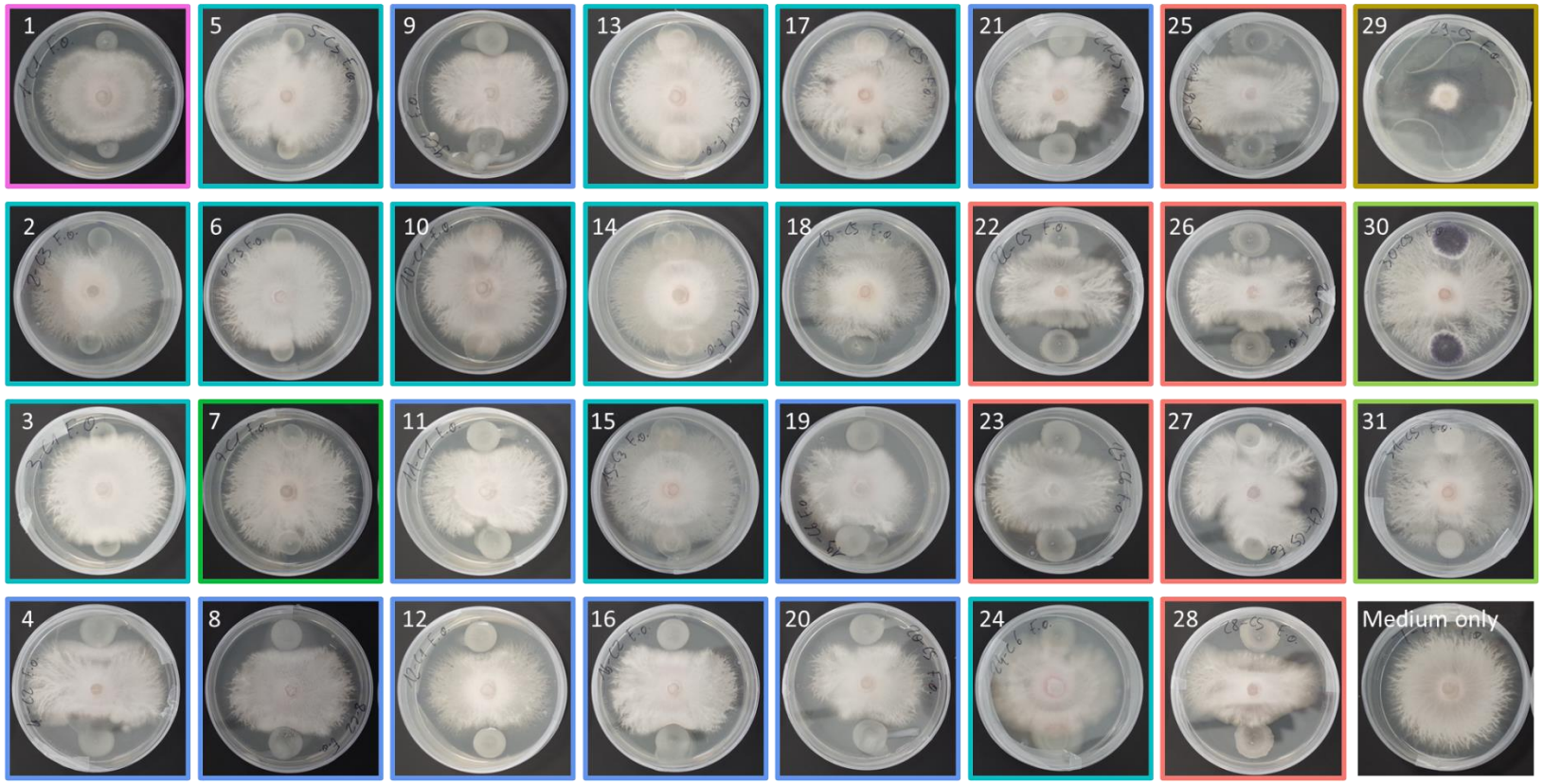
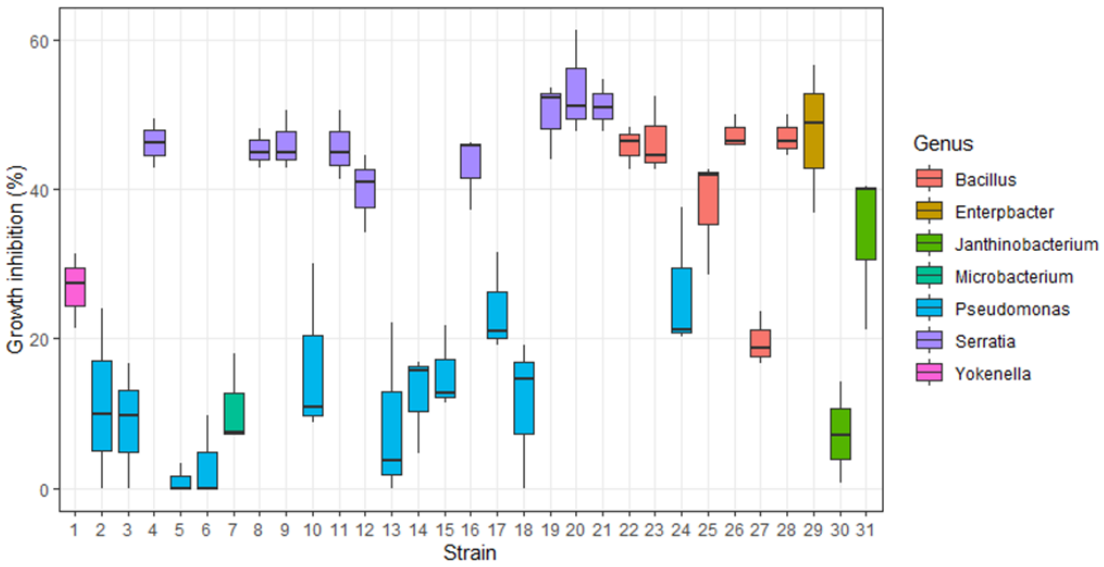


Fig. 1 | Dual culture test of Vetiver endophytes and *F. culmorum* (a) and *F. oxysporum* (b)
The frame color corresponds to the color of each genus, used in the graphs of Fig. 2, 3, and 4.

(b) Against *F. oxysporum*



(a) *F. culmorum*



(b) *F. oxysporum*

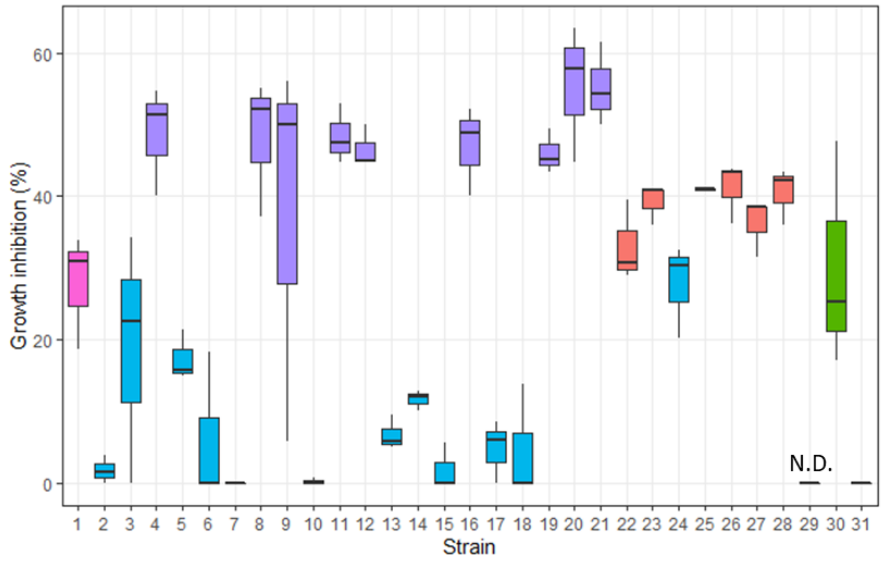


Fig. 2 | Growth inhibition rate of Vetiver endophytic strains against (a) *F. culmorum* (Day 6) and (b) *F. oxysporum* (Day 11)

3.2. Antimicrobial activities of the cell-free crude supernatants of the vetiver bacterial endophytes

We screened the antimicrobial activities (antifungal and antibacterial) of filtered culture broths of the endophytic bacteria. For that purpose, bacterial supernatants were obtained from bacterial cultures in King B and NB media.

The inhibitory activities of *the* bacterial supernatants against *F. graminearum* were tested by

diffusion assays (Fig. 3). The results that we obtained showed an inhibition rate that varied from 0% to 50% (Fig. 3b). Only supernatants *from* strains affiliated to *B. subtilis* (strains number 22, 23, 26 and 28) and *B. tequilensis* (strain number 25) displayed a significant inhibitory rate against *F. graminearum*, as indicated by the clear zones in the diffusion test (Fig. 3a.).

The inhibitory activities of supernatants of the bacterial cultures in King's B medium were also tested against *E. coli* and *S. cerevisiae* in 96-well plates. Supernatants were serially diluted 2 to 8 times. For *E. coli*, only 3 strains affiliated to *Enterobacter* (strain number 29) and *Bacillus* (strains number 26 and 28), showed little to moderate antibacterial activities (Fig. 4a). The *Enterobacter*-affiliated strain (strain number 29) showed the highest inhibition rate (37.6%, observed in the 2-times dilution), followed by the *Bacillus subtilis*-affiliated strains (strains 26 and 28), which showed inhibition rates of 16% and 10%, in the 8-times dilution, respectively. Regarding the test with *S. cerevisiae*, no strain showed a growth inhibition effect (Fig. 4b).

3.3. Screening for lipopeptides in supernatant samples of vetiver endophytes by MALDI-TOF

In a preliminary approach, to screen lipopeptide production by endophytic bacteria, cell-free supernatants from 24-hour cultures were analyzed using MALDI-TOF MS. King B medium was used as a control and subjected to MALDI-TOF MS analysis, in order to remove molecules deriving from the medium (table 2). The peaks at m/z = 746, 926, 1020, 1036, 1173, 1189, 1190, were, therefore, eliminated (Table 2). Four strains affiliated with *B. subtilis* (strains number 22, 23, 26, 28) and whose supernatants showed significant antifungal activities against *F. graminearum*, had similar peak profiles within the mass ranges of m/z = 1033 to 1516. The mass spectra of these strains showed three distinct groups of peaks within the mass ranges of m/z = 1033 to 1075, m/z = 1096 to 1118 and m/z = 1486 to 1516. The masses of all these peaks detected appear to be similar to those of lipopeptide. Furthermore, we observed that strains affiliated with *B. subtilis* (strains number 23, 26, 28) and the one affiliated with *B. tequilensis* (strain number 25) also shared a group of peaks in the mass range of m/z 3400 to 3469.

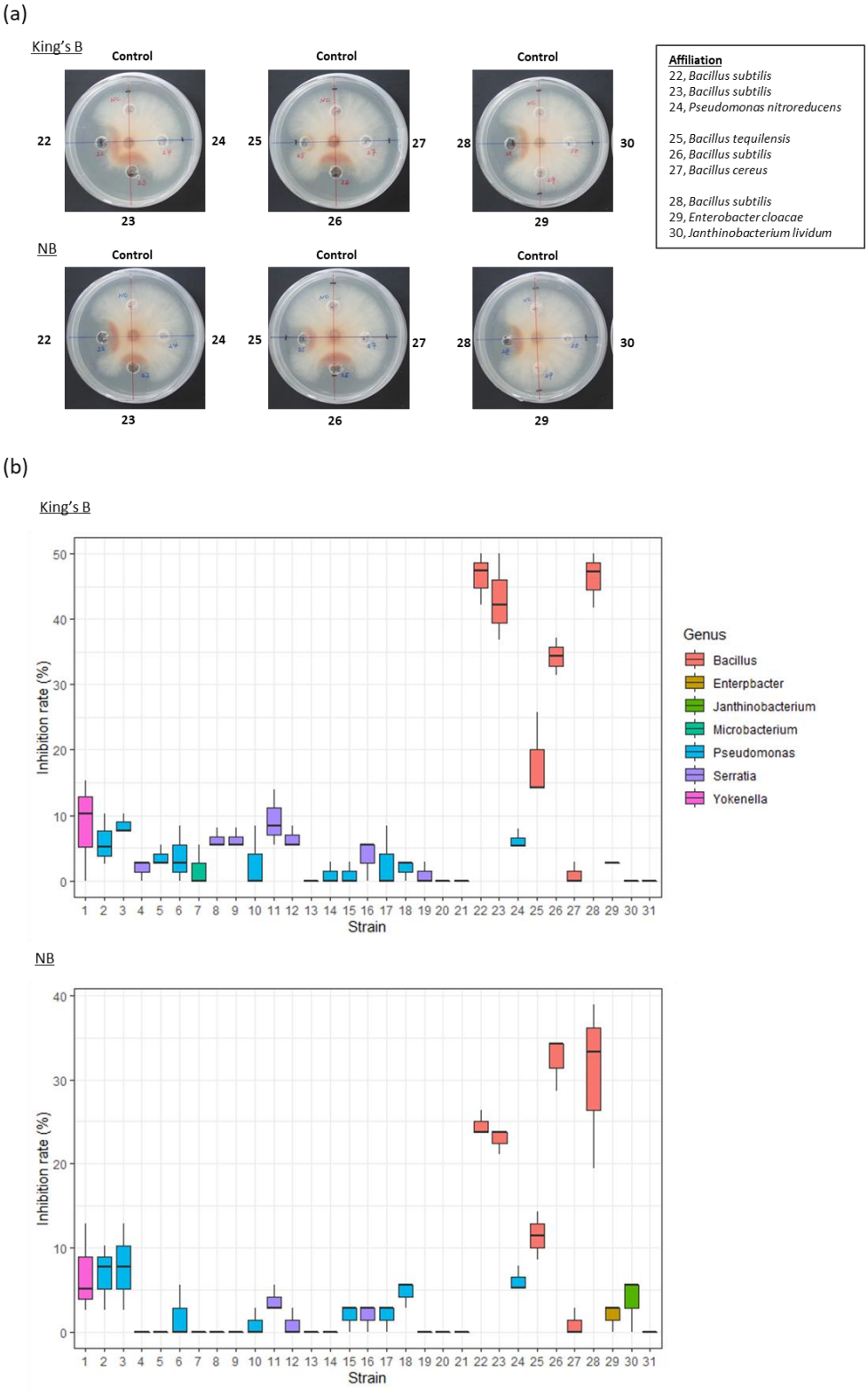
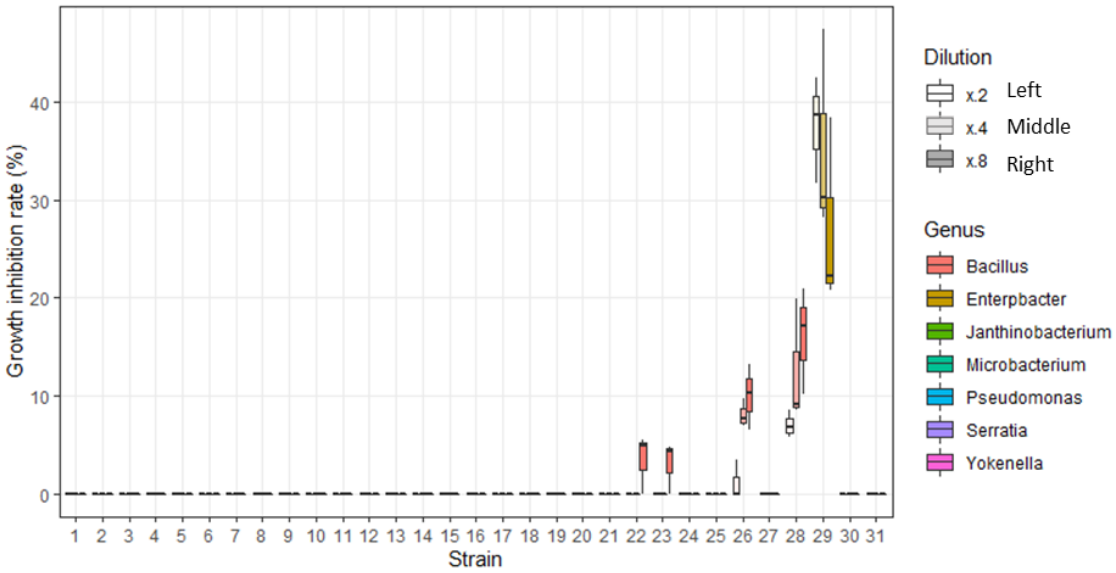


Fig. 3 | Growth inhibition rate of the cell-free supernatant of Vetiver endophytic strains against *F. graminearum*

(a) diffusion assay for strain 22 to 30; (b) the growth inhibition rate of the samples in King's B and NB

(a) *E. coli*



(b) *S. cerevisiae*

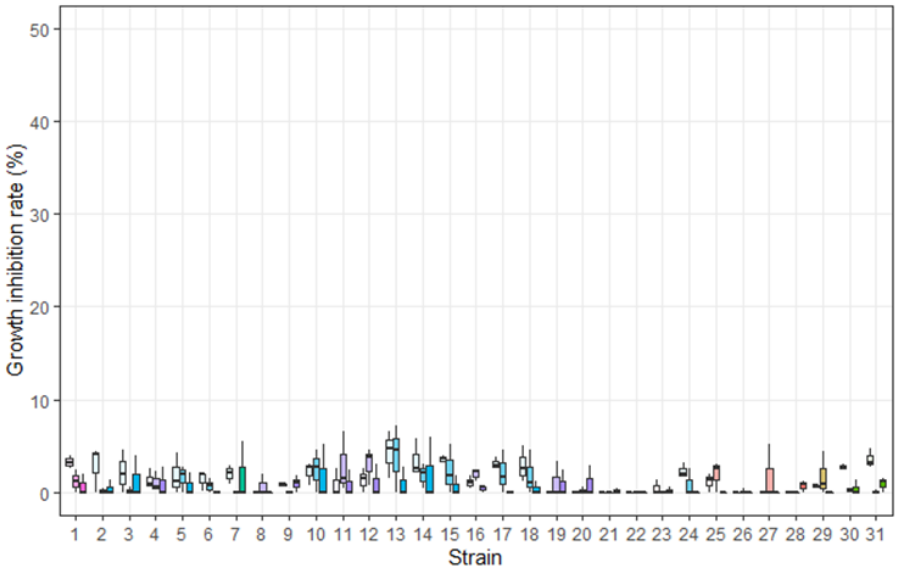


Fig. 4 | Growth inhibition rate of the cell-free supernatant of Vetiver endophytic strains against *E.coli* (a) and *S. cerevisiae* (b)

Table 2 | Profile of peaks detected by MALDI-ToF

m/z values were rounded by removing after the decimal point, and the peaks appeared in the control samples (King's B) were shown in blue and pale blue. Unique peaks in strain 22, 23, 26, 28 were highlighted in yellow, and unique peaks presenting in strain 23, 25, 26, 28 were shown in orange.

Average of 3 replicates

Strain	Peaks m/z 724	725	746	747	762	763	828	909	910	926	1019	1020	1021	1033	1035	1036
1	0	0	0	0	0	288.7914	0	0	0	315.4112	0	0	0	0	0	788.6964
2	0	0	625.6287	0	0	1426.664	0	547.3568	0	2053.515	0	0	1761.472	0	0	2588.286
3	0	0	0	0	0	0	0	0	0	1230.2	0	0	0	0	0	655.7114
4	486.4879	0	0	0	743.6579	0	0	0	0	1664.89	0	0	0	0	0	0
5	639.8877	0	0	0	0	0	0	0	0	367.7683	0	0	0	0	0	0
6	0	0	0	0	643.1126	443.6828	0	0	0	0	0	668.6666	0	0	0	2052.023
7	0	410.9113	360.6141	183.6162	201.2062	320.2261	0	0	350.0512	1185.493	0	1000.75	0	0	0	2041.887
8	361.5211	442.292	0	0	0	0	0	0	0	1924.208	0	0	0	0	0	0
9	567.325	622.2198	0	0	0	0	0	0	0	2259.606	0	0	0	0	0	0
10	0	464.0156	0	0	0	404.4868	0	0	0	1536.365	0	0	0	0	0	510.5885
11	1993.188	918.168	0	0	0	0	0	0	0	1634.993	0	0	0	0	0	0
12	1803.563	0	0	0	0	426.7381	0	0	0	0	0	0	0	0	0	0
13	233.118	0	0	0	0	0	0	0	0	1446.311	0	0	0	0	0	0
14	0	834.6719	0	424.3016	0	0	0	0	247.1541	1254.196	0	0	0	0	0	0
15	0	706.127	0	0	0	0	0	0	0	635.104	0	0	0	0	0	0
16	2304.784	0	0	0	0	0	0	0	0	1678.803	0	0	0	0	0	0
17	469.7151	0	579.8388	0	0	0	0	0	0	1269.869	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	3557.074	0	0	0	0	0	0
19	0	0	0	0	0	0	610.3777	0	0	3860.323	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	1281.871	0	0	0	0	0	0
21	766.7865	0	0	0	0	911.7894	0	0	0	3818.32	0	0	0	0	0	0
22	757.783	0	0	0	0	0	0	0	0	0	2171.946	0	0	1856.934	0	0
23	776.3131	0	0	0	0	280.655	0	0	0	0	0	264.4096	0	1549.571	0	0
24	0	0	0	0	412.7307	0	0	0	0	494.642	0	0	0	0	0	0
25	770.629	0	186.312	0	806.7692	0	0	0	0	0	0	443.8181	462.4251	0	0	2393.716
26	401.9108	0	0	0	132.599	0	0	0	0	0	0	0	0	294.5706	0	0
27	2978.624	0	0	0	0	0	0	0	0	0	0	1494.004	0	0	0	6076.44
28	538.3363	0	0	0	0	0	0	0	0	0	0	0	0	499.7487	0	0
29	0	0	0	0	0	1085.531	0	0	0	476.1907	0	0	0	0	919.4918	480.6253
30	0	0	0	0	0	337.2448	0	0	0	0	0	479.4274	0	0	0	953.3198
31	0	0	0	0	0	310.9347	0	0	0	0	1151.336	0	0	0	338.584	2038.453
KB	0	0	722.7692	0	0	0	0	0	0	695.8743	0	1445.347	0	0	0	3480.633

Table 2 (Continued)

Strain	1046	1057	1059	1073	1075	1096	1097	1105	1106	1118	1173	1189	1190	1486	1487	1501	1502
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	781.2822	783.7166	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	1114.873	861.5696	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	444.2593	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	403.8567	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	858.3587	0	0	0	0	0
14	0	0	0	0	0	0	0	390.3016	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	547.7221	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	403.2433	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	1107.533	883.1588	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	8406.401	0	18067.86	8.322064	23806.17	0	0	3501.892	0	1152.833	1629.879	0	3982.011	1045.487	1137.906	1640.432	3485.639
23	6904.506	17.25067	16499.35	27.47302	20051.38	0	0	2749.386	0	0	251.6632	0	1100.419	502.8827	1527.534	0	4034.329
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	3019.354	0	8234.966	0	10378.65	292.8684	292.8684	0	0	406.5179	0	0	0	1628.365	0	2406.915	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	2960.741	0	8078.91	0	10495.15	0	0	0	0	0	0	0	243.3752	292.7213	0	2095.275	0
29	0	0	0	0	0	0	0	0	0	0	0	629.4976	0	0	0	0	0
30	0	0	0	0	0	0	0	446.6118	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	762.7676	0	0	0	0	0	0	0	0	0
KB	0	0	0	0	0	0	0	0	0	0	1227.316	1044.741	1133.92	0	0	0	0

Table 2 (Continued)

Strain	1515	1516	1562	1808	1885	2002	2160	2514	3400	3421	3422	3432	3438	3453	3454	3458	3469
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	2310.348	0	0	2123.93	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	2865.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	3355.367	0	0	0	0	0	0	1936.263	0	3105.22	0	4911.566	0	0	0	0
24	0	0	1067.135	0	1161.687	1557.538	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	2157.202	0	669.2994	2215.721	0	6534.7
26	965.7737	1053.306	0	0	0	0	0	0	0	721.9474	1725.932	0	3847.346	0	0	1235.018	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	1763.694	0	0	0	0	0	0	0	443.6048	1566.967	0	2967.973	0	0	0	0
29	0	0	0	0	0	0	0	670.815	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

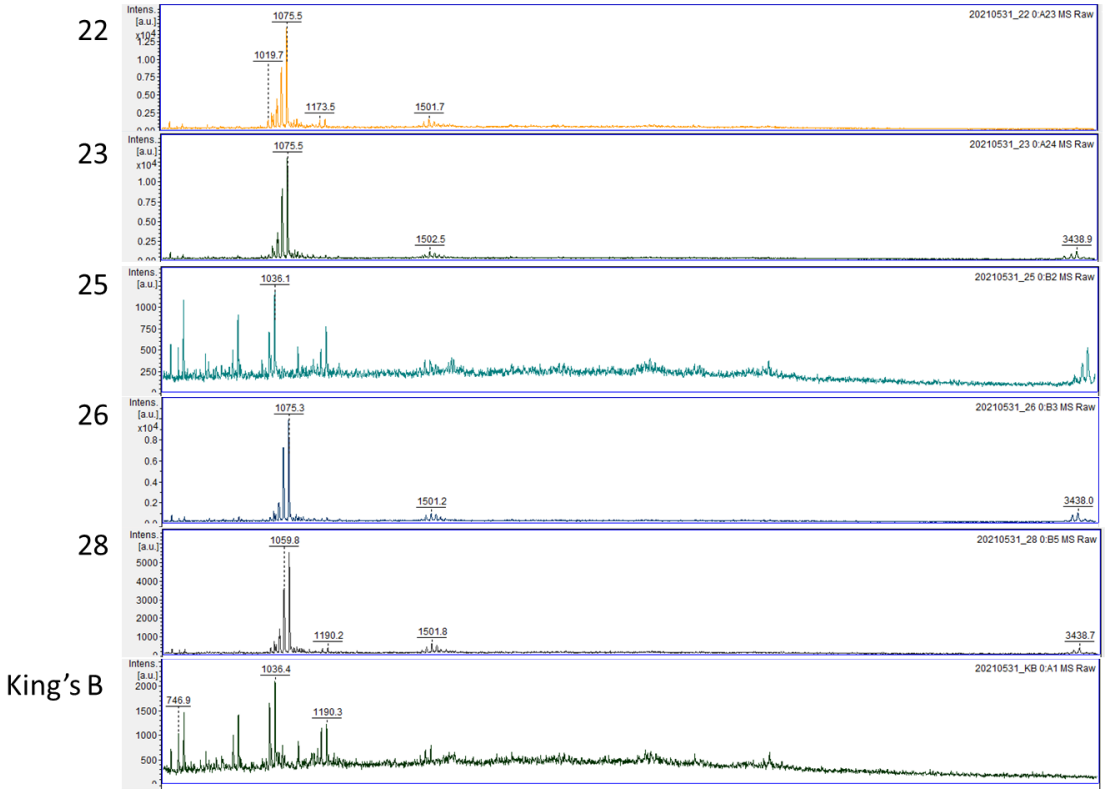


Fig. 5 | MALDI-TOF mass spectra of supernatant samples of *B. subtilis* (strain 22, 23 26, 28) and *B. tequilensis* (strain 25) , and KB medium after 24 h cultures. Mass spectra represent ion intensity in the mass range of 800 to 3500 m/z.

To increase the concentration of lipopeptides, bacteria were grown for 48-72 h in liquid culture in King's B medium. The culture supernatants were then subjected to MALDI-TOF-MS analysis. As there was a risk of lipopeptide retention on the filter used to remove the bacterial cells, the culture supernatants were simply centrifuged before MALDI-TOF-MS analysis. As described previously, King's B medium was used as a control, and displayed relatively high intensity peaks at m/z = 1019, 1035, 1046, 1105, 1162. These peaks were not considered further. In this analysis, only strains belonging to *Bacillus* genus (number 22, 23, 25, 26, 28), showed a spectrum with unique and high intensity peaks within the mass range of m/z 1030 - 1900 (Table 3). Some peaks at m/z = 1030 to 1122 were found in the spectra of 48h cultivation supernatants of strains number 22, 23, 26, and 28 closely related to *B. subtilis*, whereas those peaks were only detected in the spectrum of the strain number 25 belonging to *B. tequilensis*, after 72-hours of cultivation. In contrast, the pattern of peaks in the mass range of m/z =1134 to 1571 was dependent on the strain considered. The detected peaks were considered to belong to two lipopeptide families, surfactins and fengycins (plipastatins).

Table 3 | Presence and absence of lipopeptide signals isolated by MALDI-ToF-MS for the supernatant of five *Bacillus* strains after 48- or 72-hour cultivation

	22		23		25		26		28	
m/z	48h	72h	48h	72h	48h	72h	48h	72h	48h	72h
1030.719	+	+	+	+	-	-	+	+	+	+
1044.738	+	+	+	+	-	+	+	+	+	+
1058.757	+	+	+	+	-	+	+	+	+	+
1060.716	+	+	+	+	-	+	+	+	+	+
1074.738	+	+	+	+	-	+	+	+	+	+
1088.735	+	+	+	+	-	-	+	+	+	+
1096.715	+	+	+	+	-	-	+	+	+	+
1122.626	+	+	+	+	-	+	+	+	+	+
1134.521	-	-	+	-	-	-	+	-	+	+
1151.683	+	+	+	+	-	+	+	+	+	+
1484.640	-	-	+	+	-	+	+	-	+	-
1485.860	-	+	-	-	-	-	-	+	-	+
1500.000	-	+	+	+	+	+	+	+	+	+
1515.889	+	+	+	+	-	-	-	-	+	+
1571.628	-	-	+	-	+	-	+	+	+	-

The peaks observed in all supernatants of the 5 strains after 72 hours of culture at m/z = 1030, 1044, 1058 differ by 14 Da, suggesting a group of compounds with different fatty chain lengths. These peaks were attributed to sodium adducts of surfactins, with fatty acid chain of C13, C14, C15, respectively (Table 4). Moreover, the peaks at m/z = 1060, 1074, 1088 also differed by 14 Da and were attributed to potassium adducts of surfactins C14, C15, C16, respectively. These compounds were found in the supernatants of strains 22, 23, 26, and 28, related to *B. subtilis* after 48 h of culture, whereas they were only detected after 72h of culture in the strain 25 belonging to *B. tequilensis*. The observed peaks at m/z = 1485, 1500, 1515 and 1571 might be assigned to potassium or sodium adducts of plipastatins A or B (Table 4). The peak at m/z 1485 was only detected after 72 h of culture in the supernatants of strains number 22, 26, and 28 (*B. subtilis*) whereas the peak at m/z 1500 was identified in almost all samples, with the exception of the 48-hour supernatant of the strain number 22. The peak at m/z 1515 appeared only in the supernatants of strains number 22, 23 and 28. Finally, the peak at m/z 1571, attributed to a potassium adduct of plipastatin B C19, was only detected in the supernatants of strains number 23, 25, 28 after 48h of culture and after both 48 and 72 h of culture, in the supernatant of strain number 26.

Table 4 | Possible assignment of lipopeptides detected in *Bacillus subtilis* (strain 22, 23 26, 28) and *B. tequilensis* (strain 25)
Bold letters mean having references from Koumoutis et al., 2004 and Gong et al., 2015.

Compounds	Mass peaks (m/z)	Possible assignments	
Surfactin	1030.719	**Surfactin A C13 $[M+Na]^+$**	
	1044.738	**Surfactin A C14 $[M+Na]^+$**	
	1058.757	**Surfactin A C15 $[M+Na]^+$**	
	1060.716	**Surfactin A C14 $[M+K]^+$**	
	1074.738	**Surfactin A C15 $[M+K]^+$**	
	1088.735	Surfactin A C16 $[M+K]^+$	
Plipastatin	1485.860	**Plipastatin A C16 $[M+Na]^+$**	Plipastatin B C14 $[M+Na]^+$
	1500.000	Plipastatin A C16 $[M+K]^+$	Plipastatin B C15 $[M+Na]^+$
		Plipastatin A C17 $[M+Na]^+$	Plipastatin B C14 $[N+K]^+$
	1515.889	**Plipastatin A C17 $[M+K]^+$**	Plipastatin B C15 $[M+K]^+$
		Plipastatin A C18 $[M+Na]^+$	Plipastatin B C16 $[M+Na]^+$
	1571.628		Plipastatin B C19 $[M+K]^+$

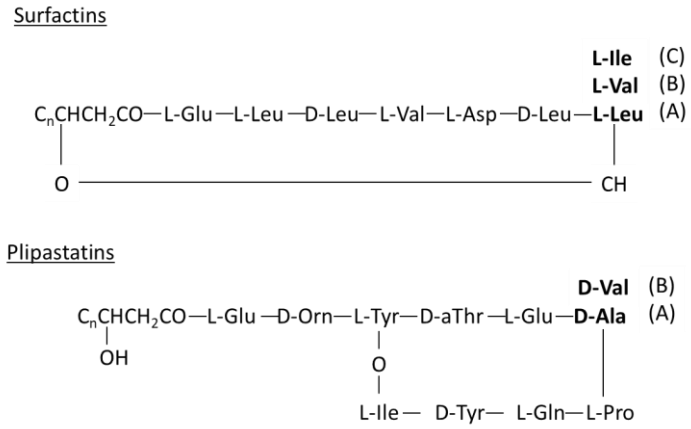


Fig. 6 | Primary structures of surfactins and plipastatins (Caulier et al., 2019)

3.5. Genome sequencing and NRPS clusters characterization

Strains affiliated to *Bacillus* and closely related to *B. subtilis* were sequenced and potential specialized metabolites clusters were screened using the genome mining tool antiSMASH [27]. Very preliminary results showed that the genomes of the four strains might harbor four NRPS gene clusters. These clusters might include genes encoding lipopeptides, surfactin A and plipastatin B, already detected in the supernatants. The other clusters might encode the antimicrobial dipeptide bacilysin, the siderophore bacillibactin and the bacteriocin subtilosin A. The analysis of the results is now under progress.

4. Discussion

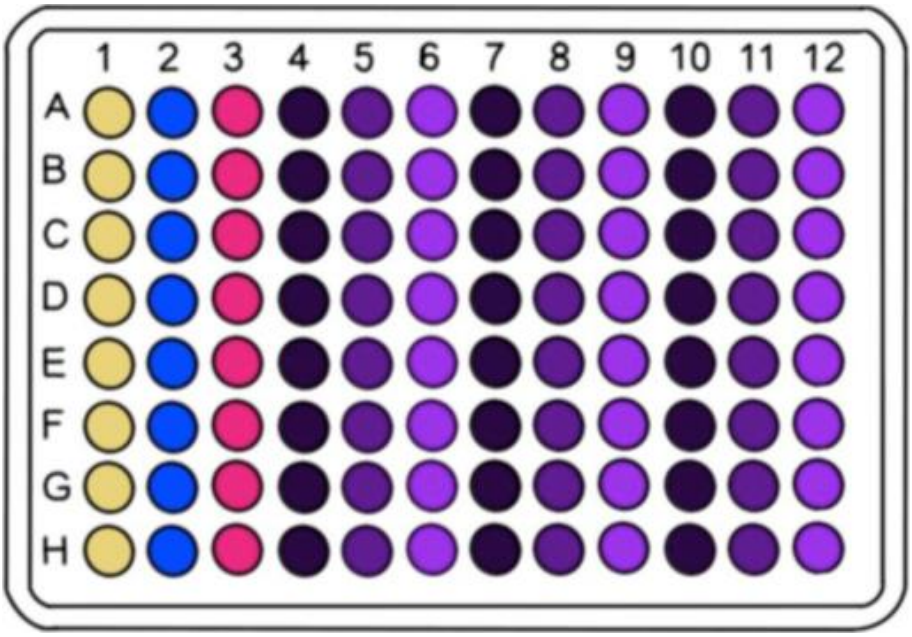
There is an increasing need to develop original biocontrol solutions in agriculture solutions or to identify new drugs in pharmaceuticals to answer to some society concerns. Microorganisms and/or the associated bioactive compounds that they can synthesize might be a promising reservoir for this purpose.

In the present study, we investigated in deeper details, the potential use 31 endophytic bacterial strains isolated from vetiver, which showed strong growth inhibitory activity against *F. graminearum* [26]. These different bacteria were characterized for their antimicrobial activities, against microorganisms, including phytopathogenic fungi affiliated to the genus *Fusarium*, yeasts and bacteria. They were also analyzed for their ability to produce lipopeptides that might be involved in any antagonistic activities.

The results we obtained showed that strains affiliated to *Serratia grimosii* and *Bacillus subtilis* displayed the strongest growth inhibition in *in vitro* dual culture against *F. culmorum* and *F. oxysporum*. However, analysis of the antimicrobial activities of the cell-free culture supernatants of all the bacteria strains showed that only the supernatants of *B. subtilis* (strains number 22, 23, 26, 28) and *B. tequilensis* (strain number 25) retained antagonistic activity towards the target microorganisms. The mechanisms involved in these activities are therefore different. In the latter case, these activities are obviously related to the production of some molecules that are excreted in the culture medium. *Bacillus* species are well known as biocontrol agents, especially against plant pathogens such as *F. graminearum* or *F. oxysporum* [28]. Their bioactivity is related to the production of various antimicrobial compounds.

By using MALDI-TOF analyses we highlighted several molecules that have molecular weights closed to those of lipopeptides. Surfactins and plipastatins were detected in supernatants of four strains belonging *to B. subtilis* and one to *B. tequilensis*. These results are consistent with the presence of NRPS gene clusters detected in the genomes of *Bacillus* sp. Both molecules were already described to be produced by *B. subtilis* species [29]. But interestingly, surfactins are mostly reported as antibacterial molecules rather than antifungal molecules [15,17,18,30–32]. *B. tequilensis* which is phylogenetically closely related to *B. subtilis* [33], has also been reported for the production of bacteriocins and lipopeptides (surfactins, iturins, and fengycins) [34,35]. In this study, this species exhibited antifungal activities in the dual culture but also through the molecules they produce and that are detected in cell-free supernatant. In our experiments, the putative surfactins need to be more clearly identified both in *B. subtilis* and *B. tequilensis* strains.

Finally, to complete this analysis, it is noteworthy to mention that the *Bacillus* strains we isolated, excepting strain number 22, had similar profiles of MS peaks in more than m/z 3000. Some bacteriocins, such as subtilocins, have molecular weight of around 3000 (for example subtilosin A that has a mw 3015). But this needs to be more deeply investigated.



Supplemental figure 1

The layout of 96 well plates for growth inhibition test against E. coli and S. cerevisiae

Plate design for liquid antimicrobial activity testing. Each plate line is used to test a supernatant. Column 1(●) is the sterility control (200 µL of culture medium), column 2 (●) is the supernatant sterility control (50 µL of supernatant + 150 µL of culture medium), column 3 (●) corresponds to the growth control (100 µL of culture medium + 100 µL of target strain). Columns 4, 7 and 10 (●) correspond to growth in the culture medium in the presence of the tested supernatant diluted by 1/2 (100 µL of supernatant + 100 µL of inoculum). Columns 5, 8 and 11 (●) correspond to growth in culture medium in the presence of 1/4 diluted test supernatant (100 µL of mixture from columns 4, 7 and 10 respectively + 100 µL of culture medium – 100µL of mixture + 100 µL of inoculum). Columns 6, 9 and 12 (●) correspond to growth in culture medium in the presence of the 1/8 diluted test supernatant (100 µL of mixture from columns 5, 8 and 10 respectively + 100 µL of culture medium - 100 µL of mixture + 100 µL of inoculum).

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Article

Screening of Antimicrobial Activities and Lipopeptide Production of Endophytic Bacteria Isolated from Vetiver Roots

Yuka Munakata [1], Egon Heuson [2], Théo Daboudet [3], Barbara Deracinois [3], Matthieu Duban [3], Alain Hehn [1], François Coutte [3] and Sophie Slezack-Deschaumes [1,*]

[1] UMR 1121, Laboratoire Agronomie et Environnement (LAE), Université de Lorraine—INRAE, F-54000 Nancy, France; yuka.munakata@univ-lorraine.fr (Y.M.); alain.hehn@univ-lorraine.fr (A.H.)
[2] Univ. Lille, CNRS, Centrale Lille, Univ. Artois, UMR 8181—UCCS—Unité de Catalyse et Chimie du Solide, F-59000 Lille, France; egon.heuson@centralelille.fr
[3] Université de Lille, UMRt BioEcoAgro 1158-INRAE, Équipe Métabolites Secondaires d'Origine Microbienne, Institut Charles Viollette, F-59000 Lille, France; theo.daboudet@univ-lille.fr (T.D.); barbara.deracinois@univ-lille.fr (B.D.); matthieu.duban@univ-lille.fr (M.D.); francois.coutte@univ-lille.fr (F.C.)
* Correspondence: sophie.deschaumes@univ-lorraine.fr; Tel.: +33-3-72-74-40-63

Abstract: The exploration of certain microbial resources such as beneficial endophytic microorganisms is considered a promising strategy for the discovery of new antimicrobial compounds for the pharmaceutical industries and agriculture. Thirty-one endophytic bacterial strains affiliated with *Bacillus*, *Janthinobacterium*, *Yokenella*, *Enterobacter*, *Pseudomonas*, *Serratia*, and *Microbacterium* were previously isolated from vetiver (*Chrysopogon zizanioides* (L.) Roberty) roots. These endophytes showed antifungal activity against *Fusarium graminearum* and could be a source of antimicrobial metabolites. In this study, in particular, using high-throughput screening, we analyzed their antagonistic activities and those of their cell-free culture supernatants against three species of *Fusarium* plant pathogens, a bacterial strain of *Escherichia coli*, and a yeast strain of *Saccharomyces cerevisiae*, as well as their capacity to produce lipopeptides. The results showed that the culture supernatants of four strains close to *B. subtilis* species exhibited antimicrobial activities against *Fusarium* species and *E. coli*. Using mass spectrometry analyses, we identified two groups of lipopeptides (surfactins and plipastatins) in their culture supernatants. Whole-genome sequencing confirmed that these bacteria possess NRPS gene clusters for surfactin and plipastatin. In vitro tests confirmed the inhibitory effect of plipastatin alone or in combination with surfactin against the three *Fusarium* species.

Keywords: bacterial endophytes; lipopeptides; antifungal activities; high-throughput screening; *Bacillus subtilis*; *Fusarium* sp.

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1. Introduction

Microorganisms are an important resource for the mining of bioactive ingredients, which can then be used as drugs or leading compounds for the pharmaceutical and agrochemical industries [1,2]. For example, a number of bioactive compounds have been characterized in soil-derived microorganisms such as *Streptomyces* sp. [3]. Currently, discovering new bioactive compounds and/or new modes of action appears crucial to control multidrug-resistant microbes, which threaten the sustainable developmental goals set by the United Nations regarding human health and food security. In addition, due to societal expectations for more sustainable agriculture around the world and in Europe in particular, the characterization of bioalternatives to chemical inputs that are assumed to be less toxic to the environment and human health is vital [4,5]. Hence, exploring microbial diversity in less investigated environments could offer opportunities to discover new bioactive compounds and/or new modes of action.

In this context, endophytic microorganisms could be an attractive reservoir [6]. Indeed, endophytic microorganisms that occupy different ecological niches in the internal tissues of the plant are known to produce a high diversity of secondary metabolites, which can have pharmaceutical and agricultural applications [7,8]. In particular, these microorganisms produce several chemical classes of biomolecules with antimicrobial properties, such as ecomycin, kakadumycin, and munubicins [7,9,10].

Among the antimicrobial biomolecules, lipopeptides can be distinguished [11,12]. These biosurfactants can be produced by different microorganisms. For example, bacterial strains affiliated with the *Bacillus* genus were reported to produce a mixture of lipopeptides of the fengycin (plipastatin), iturin, and surfactin families [12]. These antimicrobial compounds are thought to play a key role in the antagonistic activities of these *Bacillus* strains against various fungal phytopathogens or foodborne pathogens [11,13–16]. In addition to such direct effects, lipopeptides could aid in plant tissue colonization. Finally, some lipopeptides are also known to induce systemic resistance in plants, which is promising for the development of biocontrol solutions that carry a lower risk of resistance emergence [17,18]. To date, lipopeptides have been characterized in only limited numbers of endophytic bacteria [9–11,19].

Since the development of whole-genome sequencing, genome mining has become a powerful tool with which to investigate the potential of a strain for the synthesis of new natural compounds [6]. In particular, non-ribosomal peptide synthetase (NRPS) genes involved in the biosynthesis of lipopeptides are considered to be an indicator for the prediction of the biological activities or functions of certain bacteria [20,21]. NRPS operons often consist of regularly arranged modules, where one module is responsible for the adenylation, condensation, and thiolation of one amino acid, leading to an extension of peptide chains. Due to the modular structure of NRPS, homology analysis of predicted NRPS genes identified thanks to bioinformatic tools can help to predict peptide sequences [22]. A database dedicated to non-ribosomal peptides, named NORINE [23], was developed to help to identify such peptides and could therefore be used to assess the potential of endophytic bacteria to produce lipopeptides.

Vetiver (*Chrysopogon zizanioides* (L.) Roberty) is a perennial grass species used for its essential oil, mainly in the production of perfume. In our previous work, we identified 31 endophytic bacterial isolates derived from vetiver roots, showing strong inhibitory activity toward the phytopathogenic fungus *Fusarium graminearum* in in vitro culture [24]. These strains affiliated with *Bacillus, Janthinobacterium, Yokenella, Microbacterium, Pseudomonas*, *Enterobacter*, and *Serratia* genera could be a source of bioactive molecules with antimicrobial activities such as lipopeptides.

In this work, given the large number of bacterial candidates, we developed a new high-throughput workflow for the evaluation of the spectrum of antagonistic activities of these strains against reference yeast, bacterial strains, and phytopathogenic *Fusarium* species, as well as for the detection of lipopeptides that might be responsible for these activities. This workflow involves an automatic liquid handler for antimicrobial activity characterization and sample preparation and two mass spectrometry analysis techniques for molecule identification. Finally, to confirm the accuracy of the mass analysis, the genomes of the best lipopeptide-producing bacterial strains were sequenced and analyzed by bioinformatics, revealing the presence of NRPS gene clusters responsible for the production of antifungal lipopeptides.

2. Materials and Methods

2.1. Bacterial and Fungal Strains

A collection of 31 endophytic bacterial strains previously isolated from vetiver roots was used [24]. These strains were selected for their growth inhibition activity against *F. graminearum* in in vitro dual cultures and characterized taxonomically (Table 1). They were stored in a 15% glycerol solution at −80 °C. The 16S rDNA sequences of the 31 strains were deposited in GenBank, and the accession numbers are shown in Table 1.

Table 1. Summary of the *in vitro* growth inhibition activities of the cell-free supernatants of the 31 vetiver bacterial endophytes against *Escherichia coli* and *Saccharomyces cerevisiae.*

				Cell-Free Supernatant *1	
Strain	**Genbank Accession**	**Accession (Munakata et al., 2021)**	**BLAST Top Hit Species**	***E. coli***	***S. cerevisiae***
1	OK662633	M1_08	*Yokenella regensburgei*	−	−
2	OK662634	P2_02	*Pseudomonas koreensis*	−	−
3	OK662635	P2_06	*Pseudomonas vancouverensis*	−	−
4	OK662636	P2_15	*Serratia grimesii*	−	−
5	OK662637	P2_16	*Pseudomonas vancouverensis*	−	−
6	OK662638	P2_25	*Pseudomonas vancouverensis*	−	−
7	OK662639	P2_28	*Microbacterium hominis*	−	−
8	OK662640	P3_01	*Serratia grimesii*	−	−
9	OK662641	P3_07	*Serratia grimesii*	−	−
10	OK662959	P3_08	*Pseudomonas koreensis*	−	−
11	OK662642	P3_13	*Serratia grimesii*	−	−
12	OK662643	P3_17	*Serratia grimesii*	−	−
13	OK662644	P3_18	*Pseudomonas koreensis*	−	−
14	OK662645	P3_19	*Pseudomonas koreensis*	−	−
15	OK662646	P3_24	*Pseudomonas koreensis*	−	−
16	OK662647	P3_25	*Serratia grimesii strain*	−	−
17	OK662648	P3_26	*Pseudomonas koreensis*	−	−
18	OK662649	P3_27	*Pseudomonas koreensis*	−	−
19	OK662650	P3_28	*Serratia grimesii*	−	−
20	OK662651	P3_29	*Serratia grimesii*	−	−
21	OK662652	P3_30	*Serratia grimesii*	−	−
22	OK662653	R22_05	*Bacillus subtilis*	+/−	−
23	OK662654	R22_06	*Bacillus subtilis*	+/−	−
24	OK662655	R22_08	*Pseudomonas nitroreducens*	−	−
25	OK662656	R23_08	*Bacillus tequilensis*	−	−
26	OK662657	R23_12	*Bacillus subtilis*	+	−
27	OK662658	R23_17	*Bacillus cereus*	−	−
28	OK662659	R23_28	*Bacillus subtilis*	+++	−
29	OK662660	S1_29	*Enterobacter cloacae subsp. dissolvens*	+++	−
30	OK662661	S2_11	*Janthinobacterium lividum*	−	−
31	OK662662	S2_18	*Janthinobacterium lividum*	−	−

*1 Growth inhibition activities are shown in 5 classes from "−" to "+++". +++, all the dilution series have more than 10% inhibition; +, only one dilution has more than 10% inhibition; +/−, only one dilution has more than 0% but less than 10% inhibition; −, no dilution series have more than 10% inhibition.

The phytopathogenic fungal strains used in this study were *F. graminearum*, *F. oxysporum*, and *F. culmorum*. The *F. graminearum* strain was kindly provided by IFBM (Institut Français de la Brasserie et de la Malterie), while the other 2 strains were purchased from BCCM/MUCL: *F. oxysporum* f. sp. *radicis-lycopersici* Jarvis & Shoemaker (MUCL 039790) and *F. culmorum* (W.G. smith) Saccardo (MUCL 042823). The fungal strains were sub-cultured on potato dextrose agar (PDA) (OXOID) plates at 28 °C for *F. graminearum* and 25 °C for *F. oxysporum* and *F. culmorum*.

One strain of *Escherichia coli* K12 (ATCC, Manassas, MA, USA) and one strain of *Saccharomyces cerevisiae* DSM1333 (DSMZ, Braunschweig, Germany) were also used. Both were stored at −80 °C.

2.2. Dual Culture Test of Selected Vetiver Bacterial Endophytes against F. oxysporum and F. culmorum

Bacterial strains were cultured in 2 mL of nutrient broth (NB) (Sigma Aldrich, St. Louis, MO, USA) inoculated with a single colony for approximately 24 h at 28 °C. Fifty µL of the liquid culture adjusted to an optical density at 600 nm of 1.0 was placed at 2 points 3 cm from an eight-mm-diameter mycelial plug from a 5–10-day-old culture of *F. oxysporum* or *F. culmorum* on PDA at 25 °C, placed at the center of a Petri dish (9 cm in diameter) containing 20 mL of PDA (Sigma-Aldrich, St. Louis, MO, USA). Three replicates were prepared for each bacterial strain. The plates were incubated at 25 °C. The diameters of the fungal colonies were measured at 6 and/or 11 days, according to the radial growth of the fungal species. The inhibition rate (%) was calculated with the following formula: 100 × (1 − dt/dc), where dt is the mean diameter of the *Fusarium* strains in the test plates and dc in the control plates.

2.3. Preparation of Supernatant Samples of Bacterial Endophytes

For evaluation of the inhibitory activity of cell-free bacterial supernatants in diffusion assays, bacteria were cultured at 28 °C 180 rpm for 24 h in 2 mL of King's B (KB, proteose peptone 20 g/L, KH_2PO_4 1.5 g/L, $MgSO_4$, $(H_2O)_7$ 1.5 g/L, glycerol 10 mL/L, pH 7.2) or NB (Sigma Aldrich, St. Louis, MO, USA) medium. Bacterial cultures were then centrifuged at 11,000× *g* and 4 °C for 10 min and the supernatant was filtered through a 0.2 µm polyethersulfone membrane (Acrobatic® Syringe Filter with Supor Membrane). KB and NB media were chosen because they are often used for the growth of heterotrophic bacteria such as *Pseudomonas* and *Bacillus* [25,26]. Moreover, KB medium was also studied for the production of antifungal compounds by *Serratia* strains [27].

For mass spectrometry analysis, a single colony of each endophytic bacterial strain was cultured in 2 mL of KB medium at 28 °C 180 rpm for approximately 24 h. Then, 200 µL of the preculture was transferred into 10 mL of KB in 50 mL sterile tubes and cultured under the same conditions. After 24, 48, and 72 h, 1 mL of each culture was placed in 2 mL sterile tubes and the pH was adjusted to approximately 7 by dropping 1 M KOH. The tubes were then centrifuged at 10,000× *g* at 4 °C for 10 min, and 500 µL of the supernatant was collected in new sterile tubes. As a control sample, we used medium treated in the same way. The samples were kept at 4 °C for 4 to 5 days until analysis by MALDI-TOF.

*2.4. Antagonism Test of Cell-Free Supernatants of the Selected Vetiver Bacterial Endophytes against Fusarium S*pecies

Cell-free supernatants from a bacterial culture on KB or NB were tested against *F. graminearum*, *F. oxysporum*, and *F. culmorum* in 9-cm-diameter dishes containing 20 mL PDA. One hundred µL of the supernatant sample was placed in 8-mm-diameter wells 2 cm distant from the center of the plate. An 8-mm-diameter fungal plug from a 5–10-day-old culture on PDA at 25 °C of one of the 3 *Fusarium* species was inoculated at the center of the plate. The Petri dish was incubated at 28 °C in darkness. Each plate had three different samples and one control sample. The control sample corresponded to a non-

inoculated medium filtered through a 0.2 µm membrane. Three independent replicates were prepared. The radii of the fungal colonies were measured after 3 days (*F. graminearum* and *F. culmorum*) and 8 or 11 days (*F. oxysporum*) of incubation at 28 °C. The inhibition rate was calculated with the following formula: Inhibition rate (%) = 100 × (1 − rt/rc). Herein, rt is the radius of test samples, and rc is the radius of the control sample in the same plate as the test samples.

2.5. Fully Automated Workflow for Cell-Free Supernatant Screening for Antimicrobial Metabolites

The workflow used in this study is presented in Figure 1. This workflow requires a liquid handler (Biomek FXP, Beckman Coulter, Brea, CA, USA). This robot is used first for the realization of antimicrobial activity in the plates. For this, the cell-free supernatants are placed in contact with the target strains at different dilutions, including the required control experiments, following the plate map described in Figure S1.

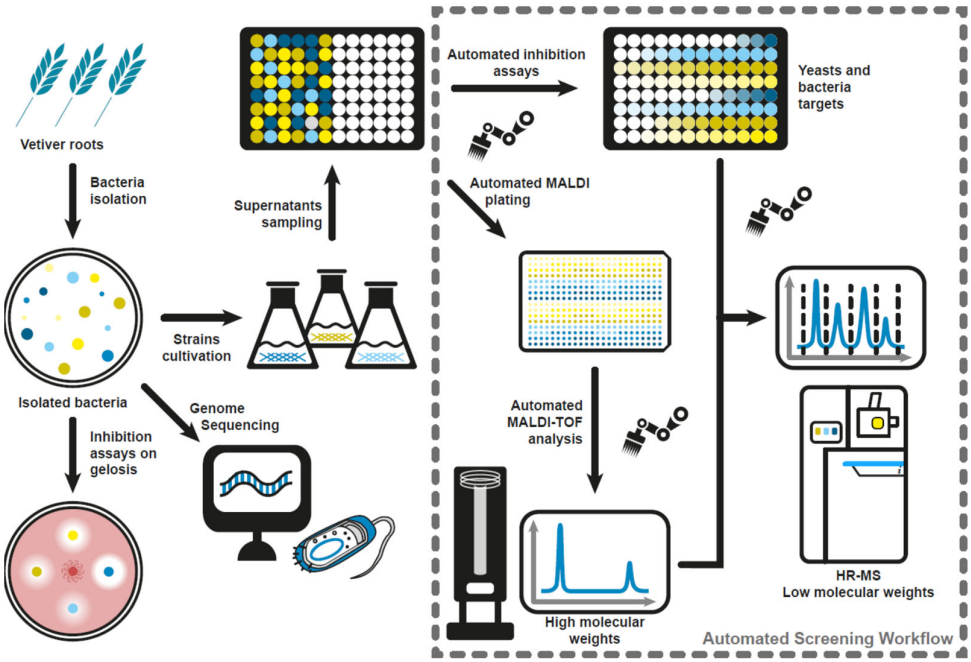

Figure 1. Diagram showing the screening workflow used in this work. This is composed of a first step of screening of antifungal activities on Petri dish and a second robotized step of high-throughput screening of antimicrobial activities and identification of secondary lipopeptide metabolites by high-resolution mass spectrometry.

The liquid handler was used to first distribute the samples into the different plates required for the liquid assays and MS analysis. This included the direct deposition of the samples onto the MALDI target, after their mixing with the matrix, again using the liquid handler. The plates were then incubated inside an off-robot incubation chamber, and then placed back on the liquid handler for mixing and reading of the plate after the incubation time. Finally, the liquid handler was used to prepare the samples that were analyzed by HPLC-TOF. After incubation at the proper temperature, the prepared plates were read for their OD using a spectrophotometric plate reader (Filtermax F5, Beckman Coulter, 600 nm) present on the robot deck. The liquid handler was also used in parallel for the conditioning of the cell-free supernatants and their automated deposition onto the MALDI target for the first step of mass spectrometry analyses. For this, 15 µL of sample was mixed with 15 µL of matrix (10 mg/mL HCCA matrix in 50/47.5/2.5: acetonitrile/MS-grade water/trifluoroacetic acid) and 2 µL of the mixture was precisely deposited onto each spot of

the MALDI target. These analyses were then performed with a matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF, Autoflex Speed, Bruker) to rapidly detect high-molecular-weight secondary metabolites, with a special focus on the lipopeptides that are released by the strains into the culture supernatant. Finally, the samples were gathered into 96-well microtiter plates and then dried using a rotative vacuum concentrator, before being taken in half a volume of 100% methanol. The most promising samples were then analyzed by UPLC-Q-TOF mass spectrometry (Synapt G2Si, Waters) to detect the other potentially produced metabolites and perform high-resolution analyses of the lipopeptides detected with MALDI-TOF. The protocols used for the mass spectrometry analyses are detailed below in their respective paragraphs.

2.6. Antagonism Test of Cell-Free Supernatant of the Endophytes against E. coli and S. cerevisiae

E. coli and *S. cerevisiae* were precultured in Mueller–Hinton (MH, Sigma-Aldrich, St. Louis, MO, USA) and YPG (yeast extract 10 g/L, bacto-peptone 20 g/L, glycerol 20 mL/L, pH 7.2) media, respectively. The optical density at 600 nm was adjusted to 0.1 before inoculation. The cell-free supernatants (1 mL) from the bacterial cultures in KB medium were transferred manually into a 2 mL DeepWell plate for further use. The supernatant was then mixed with the target strains using a Biomek FXp liquid handler (Beckman Coulter, Pasadena, CA, USA) in a polystyrene 96-well microtiter plate under sterile conditions to achieve the following plate design (Figure S1).

The plates were lidded and then incubated at 37 °C for *E. coli* and 30 °C for *S. cerevisiae* for 24 h. The growth inhibition (GI) was calculated with the following formula: 100 × (1 − (ODt/ODg), where ODt is the mean OD600 value of the test samples and ODg is the mean OD600 value of the growth control samples (MH or YPG + inoculum). For yeast, because filtered KB medium also caused a certain reduction in yeast growth, the inhibition values were normalized to the values of the control samples (KB + YPG + inoculum).

2.7. Lipopeptide Detection with MALDI-TOF

For lipopeptide detection in the supernatants, MALDI-TOF mass spectrometry analyses were performed on an Autoflex Speed™ (Bruker Daltonics, Billerica, MA, USA). The molecular mass measurements were performed in the range of 700 to 3500 m/z and in automatic reflectron mode using FlexControl™ 3.4 software. The equipment parameters were as follows: voltage values of ion sources #1 and #2 set as 19.00 and 16.50 keV, respectively; voltage values of reflectrons #1 and #2 set as 21.00 and 9.50 keV, respectively; lens tension 8.00 keV; pulsed extraction 120 ns; laser intensity between 60 and 70%; smartbeam parameters set to ultra and sample rate; and digitizer settings set to 4.00 GS/s. The MS signals were acquired by summing 10,000 laser shots per spectrum. Prior to each analysis, the spectrometer was calibrated using the monoisotopic values of the manufacturer's Peptide Calibration Standard II calibrant (Bruker Daltonics, Billerica, MA, USA), containing Bradykinin Fragment 1–7, Angiotensin II, Angiotensin I, Substance P, Bombesin, Renin Substrate, ACTH clip 1–17, ACTH clip 18–39, Somatostatin 28. The calibrant was prepared by mixing 5 µL of calibrant-diluted mixture according to the manufacturer's specification with 5 µL of a 10 mg/mL HCCA matrix in 50/47.5/2.5: acetonitrile/MS-grade water/trifluoroacetic acid, and 2 µL was then spotted on a Polished Steel 384 MALDI target (Bruker Daltonics, Billerica, MA, USA). Samples were prepared by mixing 5 µL of supernatant with 5 µL of a 10 mg/mL HCCA matrix in 50/47.5/2.5: acetonitrile/MS-grade water/trifluoroacetic acid, and 2 µL was then spotted on a Polished Steel 384 MALDI target (Bruker Daltonics, Billerica, MA, USA). The plate was dried prior to analysis under a laminar airflow hood at room temperature. Mass spectra were visualized using FlexAnalysis software (version 3.4; Bruker Daltonics, Billerica, MA, USA).

2.8. Accurate Mass Measurement of Lipopeptides by Liquid Chromatography Coupled to High-Resolution Mass Spectrometry (LC-HRMS)

Ten µL of the collected supernatant was chromatographically separated at 30 °C in reversed-phase ultrahigh-performance liquid chromatography (RP-HPLC) using an Uptisphere Strategy C18-RP column (250 × 3.0 mm, 5 µm particles, Interchim, Montluçon, France) on a biocompatible ACQUITY UPLC system (Waters, Manchester, UK) and an acetonitrile gradient (flow rate 0.6 mL/min) with solvent A (0.1% formic acid/99.9% H_2O (*v*/*v*)) and solvent B (0.1% formic acid/99.9% acetonitrile (*v*/*v*)) as follows: 5% solvent B for 5 min, then 5 to 100% solvent B for 35 min, followed by washing and equilibrating procedures with 100 and 5% solvent B for 5 and 10 min, respectively. The HPLC eluent was then ionized into the electrospray source of the SYNAPT-G2-Si mass spectrometer (Waters) at a voltage of 3 kV, using a desolvation gas (N_2) at a flow of 900 L.h-1, a nebulizer gas flow of 6.5 bar, and source and desolvatation temperatures of 120 and 500 °C, respectively. The mass spectrometer was previously calibrated using a sodium formate solution and measurements were performed in sensitivity, positive, and data-dependent modes using the proprietary MassLynx software (version 4.1, Waters). A maximum of 5 precursor ions with an intensity threshold of 1000 were selected for the fragmentation (MSMS) by collision-induced dissociation (CID) with specified voltages ranging from 10 to 15 V and from 20 to 100 V for the lower- and higher-molecular-mass ions, respectively. Mass data were collected in the measurement range of 200 to 2000 m/z for MS and of 10 to 2000 m/z for MSMS using lock mass correction with 556.632 m/z, corresponding to simply charged leucine enkephalin.

2.9. DNA Extraction and Whole-Genome Sequencing

The genomic DNA of four endophytic strains affiliated with *B. subtilis* (strains 22, 23, 26, and 28) was extracted using a DNeasy® UltraClean® Microbial Kit (Qiagen, Hilden, Germany).

Genomic DNA libraries were prepared using the Nextera XT Library Prep Kit (Illumina, San Diego, CA, USA) following the manufacturer's protocol, with the following modifications: input DNA was increased 2-fold, and PCR elongation time was increased to 45 s. DNA quantification and library preparation were carried out on a Hamilton Microlab STAR automated liquid handling system (Hamilton Bonaduz AG, Rapperswil-Jona, Switzerland). Pooled libraries were quantified using the Kapa Biosystems Library Quantification Kit for Illumina. Libraries were sequenced with Illumina sequencers (HiSeq/NovaSeq) using a 250 bp paired-end protocol. Reads were trimmed using Trimmomatic 0.30 with a sliding window quality cutoff of Q15 [28]. De novo assembly was performed on the samples using SPAdes version 3.7 [29], and the contigs were annotated using Prokka 1.11 [30].

The genomes of four *B. subtilis* strains were submitted to antiSMASH (version 6.0.1) [31] to predict the biosynthesis gene clusters of the secondary metabolites.

2.10. Effects of Surfactin and Plipastatin on the In Vitro Growth of Fusarium Species

Surfactin and plipastatin (synonym fengycin) were purchased from Sigma-Aldrich (ref. S3523, SMB00292, respectively). Surfactin was dissolved in dimethyl sulfoxide (DMSO) at 100 mg/mL and then diluted in sterile water to 1 mg/mL. Plipastatin was dissolved in 0.1% DMSO at 1 mg/mL and then filtered through a 0.2 µm pore membrane. A mixture of surfactin and plipastatin was prepared at a ratio = 1:1 (*w*/*w*). The lipopeptides were then diluted to their final concentrations of 5, 10, 25, 50, 100, 200, and 400 µg/mL. As a control, 1, 0.1, and 0.55% DMSO were used for the surfactin, plipastatin, and mixed solutions, respectively. An in vitro assay was performed with the plate layout described in Section 2.4., applying 40 µL lipopeptide solution per well (diameter 6 mm).

2.11. Data Analysis

The figures and statistical tests were performed with R studio with R version 3.6.3. To compare the growth inhibition rate of surfactins, plipastatins, and mixed solutions, the Tukey–Kramer test was performed for each concentration, using the multcomp package.

In all the inhibition assays against plant pathogens, *E. coli*, and yeast, the negative inhibition values were replaced with 0%.

3. Results

3.1. Antifungal Activities of Vetiver Endophytic Bacterial Strains against Plant Pathogens

In this study, 31 selected endophytic bacteria from vetiver roots were investigated (Table S1). These bacteria, affiliated with the *Bacillus*, *Enterobacter*, *Janthinobacterium*, *Pseudomonas*, *Serratia*, *Microbacterium*, and *Yokenella* genera, have previously shown high inhibition rates against *F. graminearum* in *in vitro* cultures (Table S1, [24]). In this study, we evaluated their ability to inhibit the mycelial growth of two other well-known *Fusarium* plant pathogenic species, *F. culmorum* and *F. oxysporum* (Figures 2 and S2). Depending on the bacterial genus, the inhibitory rate varied from 1.1 to 53.4% and 0 to 55.3% against *F. culmorum* and *F. oxysporum*, respectively. Bacteria affiliated with the genera *Serratia* and *Bacillus* showed the highest inhibitory rates against *F. culmorum* and *F. oxysporum* (Figure 2). *Serratia* strains, closely related to *S. grimesii* species, showed similar inhibition rates against *F. culmorum* (approximately 47%) and *F. oxysporum* (approximately 48%). On the other hand, except for the strain affiliated with *B. cereus*, *Bacillus* strains belonging to *B. subtilis* and *B. tequilensis* showed a higher inhibitory rate against *F. culmorum* (approximately 45%) than *F. oxysporum* (approximately 39%). It should be noted that although bacterial strains affiliated with the *Serratia* or *Bacillus* genus showed similar capabilities to inhibit the mycelial growth of *F. culmorum*, the appearance of the fungal colonies differed, depending on the bacterial strains with which the fungus interacted (Figure S2).

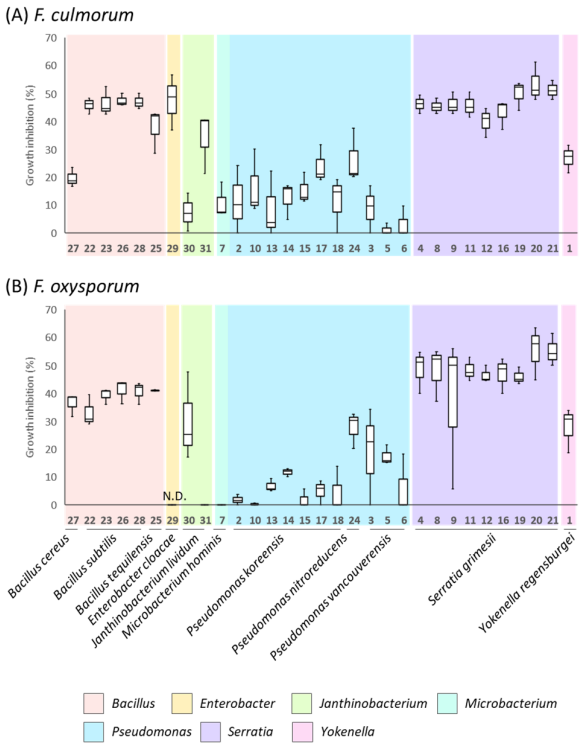

Figure 2. Growth inhibition rate of 31 vetiver bacterial endophytes against *F. culmorum* (Day 6) (**A**) and *F. oxysporum* (Day 11) (**B**).

For other bacterial strains, the strain affiliated with genus *Enterobacter* had a good inhibitory rate against *F. culmorum* (47%), while bacteria affiliated with *Janthinobacterium, Pseudomonas, Yokenella,* and *Microbacterium* genera showed overall lower inhibitory rates against *F. culmorum* and *F. oxysporum*.

3.2. Antimicrobial Activities of the Cell-Free Crude Supernatants of the Vetiver Bacterial Endophytes

The antimicrobial (antifungal and antibacterial) activities of filtered culture supernatants from the endophytic bacteria were then evaluated. For this purpose, KB and NB media were used to grow the bacteria.

The inhibitory activities of the bacterial supernatants against *Fusarium* species were tested by diffusion assays (Figure 3). The results obtained showed contrasting inhibitory effects of the culture supernatants depending on the bacterial strains, with inhibition rates ranging from 0% to almost 43% (Figure 3, Table S1). Only supernatants from strains affiliated with *B. subtilis* (strain numbers 22, 23, 26, and 28) and, specifically, those from liquid culture in KB medium displayed a significant inhibitory rate against the three *Fusarium* pathogenic species (Figure 3, Table S1). The inhibitory effects of the supernatants from bacterial culture in NB medium varied depending on the *B. subtilis* strain and the *Fusarium* species (Figure S3).

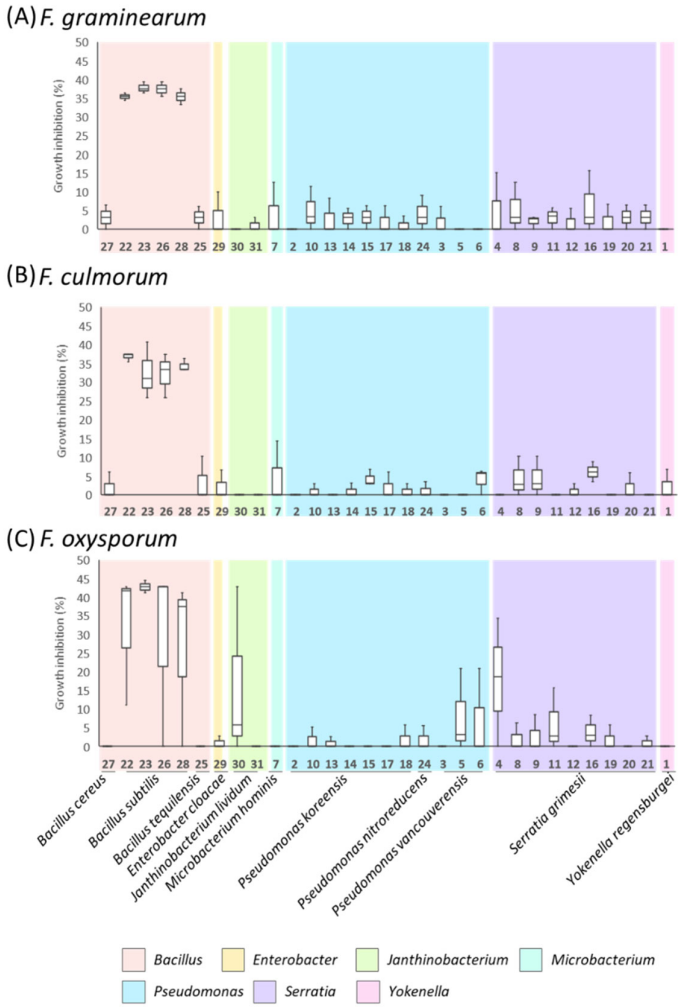

Figure 3. Growth inhibition rates of the cell-free supernatants of vetiver endophytic bacteria grown in KB medium against *F. graminearum* (**A**), *F. culmorum* (**B**), and *F. oxysporum* (**C**).

The inhibitory activities of supernatants of the bacterial cultures in KB medium were also tested against *E. coli* and *S. cerevisiae* in 96-well plates. The supernatants were serially diluted 2 to 8 times. For *E. coli*, only three strains affiliated with *Enterobacter* (strain number 29) and *Bacillus* (strains number 26 and 28) showed little to moderate antibacterial activity (Table 1, Figure S4). The *Enterobacter*-affiliated strain showed the highest inhibition rate (37.6%, observed at the 2-fold dilution), followed by the two *B. subtilis*-affiliated strains, which showed inhibition rates of 16 and 10% at the 8-fold dilution, respectively (Figure S4). Regarding the test with *S. cerevisiae*, no strain showed a growth inhibition effect (Figure S4, Table 1).

3.3. Screening for Lipopeptides in Supernatant Samples of Vetiver Endophytes by MALDI-TOF

In a preliminary approach, to screen lipopeptide production by endophytic bacteria, cell-free supernatants from 24 h cultures of the 31 endophytic strains were analyzed using MALDI-TOF, and the main results are presented in Figure 4. KB medium was used as a control and subjected to MALDI-TOF analysis to remove molecules derived from the medium. The peaks at m/z = 909.0, 925.0, 1019.0, 1020.8, 1034.9, 1036.4, 1173.1, 1188.9, and 1190.3 were, therefore, eliminated. Of the 31 strains analyzed, five belonging to *the Bacillus* genus yielded interesting profiles, as shown in Figure 4. Four strains affiliated *with B. subtilis* (strain numbers 22, 23, 26, and 28) and whose supernatants showed significant antifungal activities against *Fusarium* species had similar peak profiles within the mass ranges of m/z = 1020 to 1550 (Figure 4). The mass spectra of these strains showed two distinct groups of peaks within the mass ranges of m/z = 1020 to 1080 and m/z = 1450 to 1550. The appearance of this group of peaks appears to be similar to that of lipopeptides. Furthermore, we observed that 3 of the 4 strains affiliated with *B. subtilis* (strain numbers 23, 26, 28) and the strain affiliated with *B. tequilensis* (strain number 25) also shared a group of peaks in the mass range of m/z 3400 to 3480. The latter mass seems to correspond to the production of subtilosin A, a bacteriocin produced by certain strains of *Bacillus*.

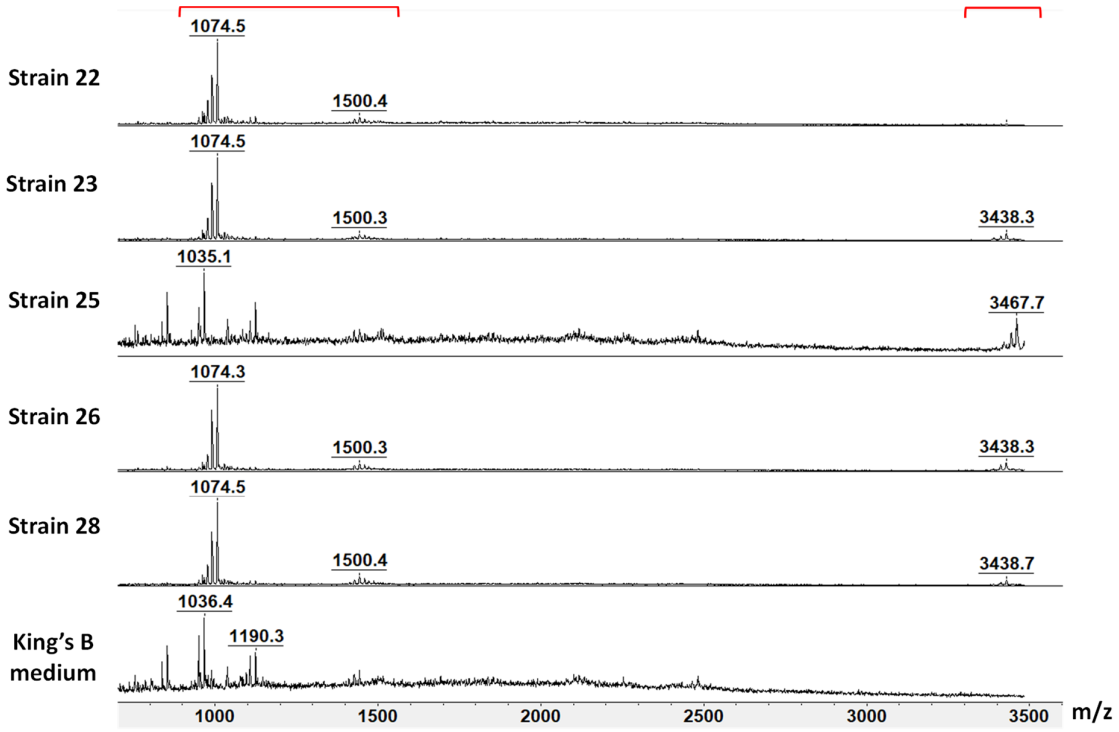

Figure 4. MALDI-TOF mass spectra of supernatant samples of *B. subtilis* (strain 22, 23, 26, 28), *B. tequilensis* (strain 25), and KB medium after 24 h cultures. Mass spectra represent ion intensity in the mass range of 800 to 3500 m/z. Brackets highlight the two groups of peaks detected.

In-depth analysis of the culture supernatants at 48 and 72 h was therefore undertaken for these five strains. Compared to strains affiliated with *B. subtilis* (strain numbers 22, 23, 26, and 28), some peaks (such as the ones at m/z = 1030, 1088, 1485, and 1515) were not detected or were found only after 72 h of cultivation (peaks at m/z =1044 to 1074) in the supernatant of the strain belonging to *B. tequilensis* (strain number 25) (Table 2). The detected peaks were considered to potentially correspond to two lipopeptide families, surfactins and plipastatins.

Table 2. Identification of lipopeptide signals present in the analysis by MALDI-TOF of the supernatant of samples of *B. subtilis* (strain 22, 23 26, 28) and *B. tequilensis* (strain 25) after 48 or 72 h cultivation.

m/z	Identification	Strain 22		Strain 23		Strain 25		Strain 26		Strain 28	
		48 h	72 h	48 h	72 h	48 h	72 h	48 h	72 h	48 h	72 h
724.44	Plipastatin B C13 $[M + H_2]^{2+}$ Plipastatin A C15 $[M+H_2]^{2+}$	+	+	+	+	+	+	+	+	+	+
746.43	Plipastatin B C13 $[M + Na_2]^{2+}$ Plipastatin A C15 $[M + Na_2]^{2+}$	+	+	+	+	+	+	+	+	+	+
762.42	Plipastatin B C13 $[M + K_2]^{2+}$ Plipastatin A C15 $[M + K_2]^{2+}$	+	+	+	+	+	+	+	+	+	+
781.35	Plipastatin B C18 $[M + Na_2]^{2+}$	-	-	+	-	-	+	-	-	-	-
1030.72	Surfactin C13 $[M + Na]^+$ [Val7] Surfactin C14 $[M + Na]^+$	+	+	+	+	-	-	+	+	+	+
1044.74	[Ala4] Surfactin C15 $[M + Na]^+$ Surfactin C14 $[M + Na]^+$ [Val7] Surfactin C15 $[M + Na]^+$	+	+	+	+	-	+	+	+	+	+
1058.75	Surfactin C15 $[M + Na]^+$ [Val7] Surfactin C16 $[M + Na]^+$	+	+	+	+	-	+	+	+	+	+
1060.71	Surfactin C14 $[M + K]^+$ [Val7] Surfactin C15 $[M + K]^+$	+	+	+	+	-	+	+	+	+	+
1074.74	Surfactin C15 $[M+K]^+$ [Val7] Surfactin C16 $[M + K]^+$	+	+	+	+	-	+	+	+	+	+
1088.73	Surfactin C16 $[M + K]^+$	+	+	+	+	-	-	+	+	+	+

1485.86	[Val7] Surfactin C17 $[M + K]^+$ Plipastatin B C14 $[M + Na]^+$ Plipastatin A C16 [M + Na]+	-	+	-	-	-	-	-	+	-	+
1515.89	Plipastatin B C15 $[M + K]^+$ Plipastatin A C17 $[M + K]^+$	+	+	+	+	-	-	-	-	+	+
1571.63	Plipastatin B C19 $[M + K]^+$	-	-	+	-	+	-	+	+	+	-

The peaks observed in the supernatants of all *Bacillus* strains after 72 h of culture at m/z = 1030.72, 1044.74, and 1058.75 differed by 14 Da, suggesting a group of compounds with different fatty chain lengths. These peaks were attributed to sodium adducts of surfactin, with fatty acid chains of C13, C14, and C15, respectively (Table 2). As this analysis did not allow us to identify the amino acid at position 7 of the peptidic cycle, it is also possible that these masses correspond to the C14, C15, and C16 isoforms of surfactin A with a valine at position 7. Moreover, the peaks at m/z = 1060.71, 1074.74, and 1088.73 also differed by 14 Da and were attributed to potassium adducts of surfactin C14, C15, and C16 or to the C14, C15, and C16 isoforms of surfactin A with a valine at position 7. These compounds were found in the supernatants of strains 22, 23, 26, and 28 related to *B. subtilis* after 48 h of culture, whereas they were only detected after 72 h of culture in strain 25 belonging to *B. tequilensis*.

The peaks observed at m/z = 724.44, 746.43, and 762.42 were identified as the dicharged form of plipastatin A C15 or plipastatin B C13 with hydrogen, sodium, and potassium adducts, respectively. The peaks at m/z 781.35 were identified as the dicharged form of the plipastatin A C16 or plipastatin B C18 isoform with unsaturated fatty acid chains and sodium adducts. All plipastatin isoforms were observed in the supernatants of the five different strains at 48 and 72 h of culture time. The observed peaks at m/z = 1485.86, 1515.89, and 1571.63 might be assigned to potassium or sodium adducts of plipastatin A or B (Table 2). The peak at m/z 1485.86 was only detected after 72 h of culture in the supernatants of strain numbers 22, 26, and 28 (*B. subtilis*). The peak at m/z 1515.89 appeared only in the supernatants of strains 22, 23, and 28. Finally, the peak at m/z 1571.63, attributed to a potassium adduct of plipastatin B C19, was only detected in the supernatants of strain numbers 23, 25, and 28 after 48 h of culture and after both 48 and 72 h of culture in the supernatant of strain number 26.

3.4. Accurate Mass Measurement by LC-HRMS

The culture supernatants of the five *Bacillus* strains (22, 23, 25, 26, and 28) were subjected to LC-HRMS analysis to obtain more resolutive mass signals and to assign the correct masses to the lipopeptides. Different masses of surfactins and plipastatins were clearly detected in the five *Bacillus* strains, confirming the assignments proposed in Table 2. Figure 5 represents, as an example of the results, the isotopic clusters of surfactin A C13 (Figure 5A) and C15 (Figure 5B) and plipastatin A C17 or B C15 and A C18 or B C16 (Figure 5C) and A C19 or B C17 (Figure 5D) extracted from the analysis of supernatant of the *B. subtilis* strain 23. MSMS analysis of the different forms of surfactin confirmed the predominant presence of surfactin A C15 with leucine at position 7 (Figure 6, fragmentation pattern in blue). However, MSMS spectra revealed the presence, with a lower intensity, of surfactin B C16 with valine at position 7 (Figure 6, fragmentation pattern in red). Regarding plipastatin, MSMS analysis revealed no informative spectra due to the poor ionization of the different forms of this lipopeptide.

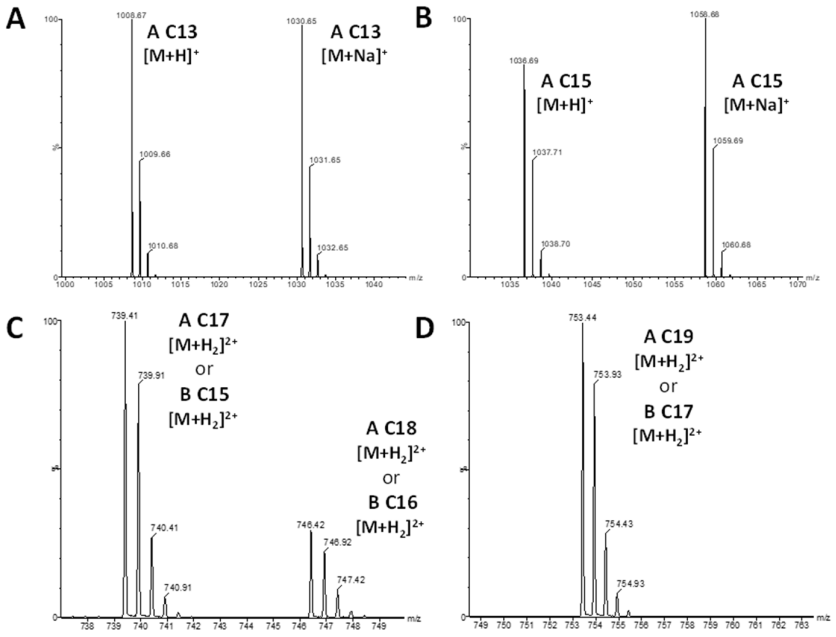

Figure 5. Mass spectra of surfactin (**A**,**B**) and plipastatin (**C**,**D**) from LC-HRMS analysis of supernatant sample of *B. subtilis* (strain 23) after 48 h culture in KB medium.

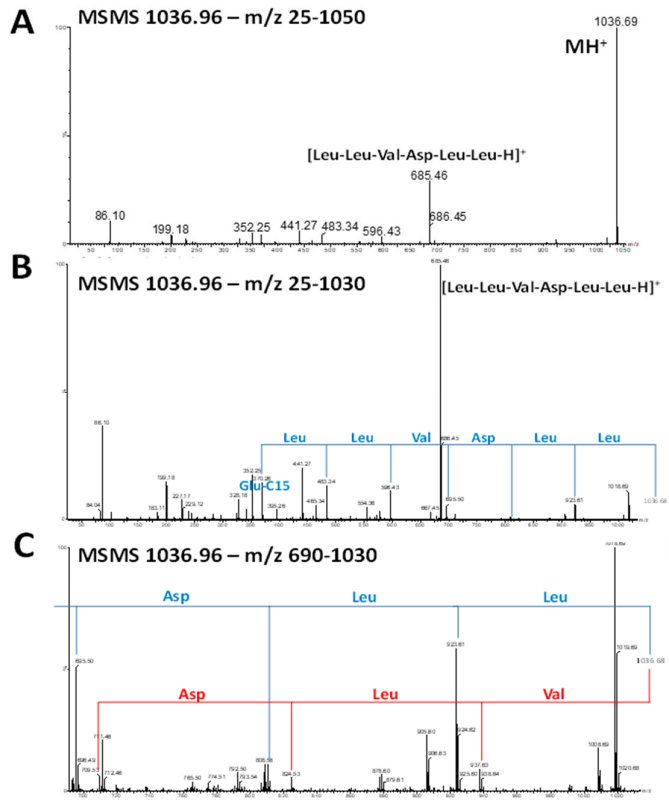

Figure 6. Fragmentation spectra of ion m/z 1036.96 from LC-HRMS analysis of supernatant sample of *B. subtilis* (strain 23) after 48 h culture. (**A**) corresponds to the full MSMS spectrum, (**B**) to the MSMS spectrum enlarged to the m/z range 25–1030, and (**C**) to the MSMS spectrum enlarged to the m/z range 690–1030. The fragmentation proposals for surfactin A C15 and B C16 are presented in blue and red, respectively.

3.5. Genome Sequencing and NRPS Cluster Characterization

Strains affiliated with *Bacillus* and closely related to *B. subtilis* were sequenced. Analysis of draft genomes of the four sequenced *Bacillus* strains showed that these genomes are composed of 4,042,990 to 4,269,420 bp, included in 14 to 26 contigs, with L50 ranging from 377,686 to 2,301,381 bp. GC content ranged from 43.2 to 43.7%. Draft genome annotation identified 4036 to 4338 coding sequences and 83 to 84 tRNA genes. Potential secondary metabolite clusters were screened using the genome mining tool antiSMASH. The *Bacillus* strain close to the *B. tequilensis* species (strain 25) was excluded from this analysis as the culture supernatant of this strain did not show any antifungal activity against *Fusarium* sp. The results showed that the genomes of the four strains might harbor four NRPS gene clusters (Table 3). These predicted clusters included genes encoding lipopeptides, surfactin, and plipastatin (Figure 7), already detected in the supernatants. Notably, the known peptide sequences of plipastatins have glutamine at the eighth position, while antiSMASH affiliated glutamic acid at this position of the putative plipastatin B (Table 3). The difference between glutamine and glutamic acid gives an additional one to the monoisotopic mass of known plipastatin B. The masses obtained during the HRMS analysis are precise enough to confirm the presence of glutamine at position 8 of the plipastatin molecule. Concerning the surfactin, both isoforms with leucine and valine at position 7 are produced by *Bacillus* strains belonging to *B. subtilis* species.

Table 3. Predicted gene clusters of NRP and other secondary metabolites by antiSMASH in the genomes of *B. subtilis* strains (number 22, 23 26, and 28). The percentage refers to the similarity of a gene cluster to a reference gene cluster.

		***B. subtilis* Strains**				
Cluster	**Category**	**22**	**23**	**26**	**28**	**Remarks**
Bacilysin	Other	100%	100%	100%	100%	
Subtilosin A	Thiopeptide	100%	100%	100%	100%	
Bacillibactin	NRP	100%	100%	100%	100%	
Bacillaene	Polyketide + NRP	100%	100%	100%	100%	
Plipastatin	NRP	100%	80% 46%, 23%	100%	100%	possible peptide chain: Glu-D-Orn-Tyr-D-Thr-Glu-D-Val-Pro-Glu-D-Tyr-Ile
Surfactin	NRP	82%	82%	82%	43%, 43%, 8%	possible peptide chain: Glu-Leu-D-Leu-Val-Asp-D-Leu-Leu
Icosalide A/B	NRP				100%	
Sporulation killing factor	RiPP: Head-to-tail cyclized peptide	100%				

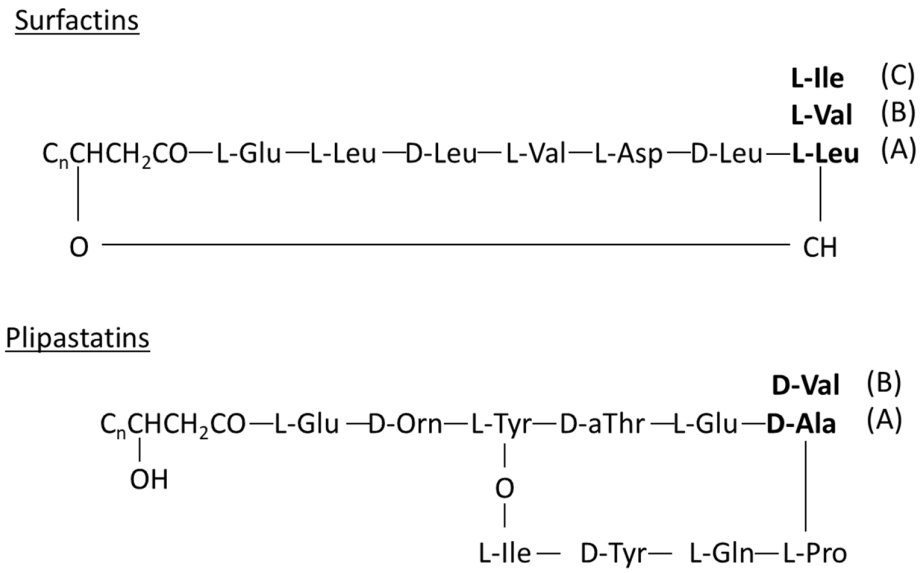

Figure 7. Primary structures of surfactins and plipastatins [11,32].

The other clusters might encode the antimicrobial dipeptide bacilysin, the siderophore bacillibactin, and the bacteriocin subtilosin A.

3.6. Antagonistic Activities of Surfactins and Plipastatins against Fusarium Species

We analyzed the effects of surfactin and plipastatin identified in the supernatant samples of *B. subtilis* (strain numbers 22, 23, 26, and 28) and *B. tequilensis* strains (strain number 25) alone or combined against *F. graminearum*, *F. culmorum*, and *F. oxysporum.* Surfactin had no clear inhibitory activity against the *in vitro* mycelial growth of *F. graminearum* and *F. culmorum* at any concentration (Figure S5). In contrast, plipastatin exhibited antifungal activities from the 25 μg/mL concentrations for the two *Fusarium* species (Figure S5). Thus, for *F. graminearum*, plipastatin showed an inhibition rate of approximately 15% at a concentration of 25 μg/mL, which increased to approximately 30% for higher concentrations. For *F. culmorum*, plipastatin displayed better antifungal activity, with an inhibition rate of approximately 40% from the concentration of 25 μg/mL. For *F. oxysporum*, inhibition effects were not clear up to concentrations of 100 μg/mL for all solutions. In contrast, plipastatin showed an inhibition rate of approximately 40% from the concentration of 200 μg/mL (Figure S5).

Higher antifungal activities were recorded when plipastatin was tested in combination with surfactin, with growth inhibition of *F. graminearum and F. culmorum* observed from 10 μg/mL. These effects were not observed with *F. oxysporum* (Figure S5).

4. Discussion

There is an increasing need to develop original biocontrol solutions in agriculture and to identify new drugs for use as pharmaceuticals to address various social concerns. Microorganisms and/or the associated bioactive compounds that they can synthesize might constitute a promising reservoir for this purpose.

In the present study, we investigated in greater detail the potential of 31 endophytic bacterial strains isolated from vetiver, which showed strong growth inhibitory activity against *F. graminearum* [24]. To better investigate the potential of these strains, we set up an automated high-throughput screening workflow combining antimicrobial testing on microtiter plates and mass spectrometry analysis using MALDI-TOF and LC-HRMS. Thus, these various bacteria were characterized for their antimicrobial activities against microorganisms, including phytopathogenic fungi affiliated with the genus *Fusarium*,

yeast, and bacteria. They were also analyzed for their ability to produce lipopeptides that might be involved in any antagonistic activities.

The results that we obtained showed that strains affiliated with *S. grimosii*, *B. subtilis,* and *B. tequilensis* displayed the strongest growth inhibition in an in vitro dual culture against *F. culmorum* and *F. oxysporum*. These results confirm that *S. grimosii*, *B. subtilis,* and *B. tequilensis* strains are useful antagonists of plant fungal pathogens [27,33]. Their antagonistic activities are known to be related to the synthesis and release of a broad range of secondary metabolites with antimicrobial activities. Analysis of the antimicrobial activities of the cell-free culture supernatants of these bacterial strains showed that only the supernatants of *B. subtilis* (strain numbers 22, 23, 26, 28) retained antagonistic activity toward *Fusarium* species but also against *E. coli*. These results may suggest different antibiosis mechanisms between *Serratia* and *Bacillus* strains, whose metabolism is highly variable, especially with regard to the type of antimicrobial compounds produced (soluble and/or volatile compounds) [34]. It should also be noted that the culture supernatants were obtained from mono-cultivated bacteria under non-limiting resource conditions. As a result, some of the compounds involved in the antagonistic activities observed in dual cultures may not have been produced or produced only weakly. Indeed, it has been shown that resource competition and inter- and intraspecific microbial interactions are often implicated in the induction of the biosynthesis of antimicrobial compounds [35–37].

By using MALDI-TOF analyses, we characterized inhibitory compounds in the cell-free supernatants of *Bacillus* strains. We highlighted the very probable mass of two lipopeptide families. Surfactins and plipastatins were detected in supernatants of the four strains belonging to *B. subtilis* (strain numbers 22, 23, 26, 28) and the strain belonging to *B. tequilensis* (strain number 25). These results are consistent with the presence of NRPS gene clusters detected in the genomes of *Bacillus subtilis* strains. Both molecules are already described to be produced by *B. subtilis* species and the closely related species *B. tequilensis* [38]. Although surfactins are rarely reported as antibacterial molecules except against *Legionella* species [39,40], they could also have antifungal activities and act synergistically with plipastatins (synonyms of fengycins) to potentiate the antagonistic activities of some *Bacillus* strains against phytopathogenic fungi such as *F. graminearum* or *F. oxysporum* [41,42]. In our study, these molecules seem to explain the antagonistic effects of the *B. subtilis* strains toward the three phytopathogenic *Fusarium* species. Other lipopeptides, such as iturins and bacillomycin, have been thought to play a role in the antagonistic activities of some *Bacillus* strains against *Fusarium* species [11,14,43]. In our case, these compounds were not detected in the supernatant samples of *B. subtilis* strains (strain numbers 22, 23, 26, 28) by MALDI-TOF, LC-HRMS, or by whole-genome analysis. This reflects the metabolic variations between bacterial strains belonging to the same species that may result from the different habitats in which these microorganisms have grown before being isolated.

Finally, it is interesting to mention that our *B. subtilis* and *B. tequilensis* strains, except for *B. subtilis* strain number 22, showed similar MS peak profiles at m/z over 3000. These compounds could correspond to some bacteriocins, such as a subtilosin A or a variant of subtilosin, which have a molecular weight at m/z of approximately 3400. Our chromatogram showed minor species at m/z 3438 that correspond to potassium adducts, as previously described [44,45]. These compounds could explain the activity of *B. subtilis* strains against *E. coli*.

5. Conclusions

This paper revealed that four *B. subtilis* strains isolated from vetiver roots originating from Reunion Island have interesting antagonistic activities against several *Fusarium* plant pathogenic species in *in vitro* cultures. These inhibitory activities could partly be due to the production of lipopeptides, surfactins, and plipastatins. These strains are promising for the protection of plants against *Fusarium* diseases. Further studies are now needed to evaluate the crop protection activity of the four *B. subtilis* strains and to study their mode

of action *in planta*. It would also be interesting to analyze, for the other strains in this study, such as those affiliated with *Serratia* or *Pseudomonas*, the metabolic traits that may participate in their antagonistic activities in in vitro dual cultures.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/article/10.3390/microorganisms10020209/s1, Figure S1: The layout of 96-well plates for growth inhibition test against *E. coli* and *S. cerevisiae*; Figure S2: Dual culture test of vetiver endophytes and *F. culmorum* (a) and *F. oxysporum* (b); Figure S3: Growth inhibition rate of the cell-free supernatants of vetiver endophytic bacterial strains in NB against *F. graminearum*, *F. culmorum*, and *F. oxysporum*; Figure S4: Growth inhibition rate of the cell-free supernatant of vetiver endophytic strains against *E.coli* (a) and *S. cerevisiae* (b); Figure S5: Growth inhibition rate of the solutions of commercial surfactins, plipastatins, and mixtures of the two lipopeptides against *F. graminearum*, *F. culmorum*, and *F. oxysporum*; Figure S6: Liquid handler protocol for the high-throughput screening assays; Figure S7: Liquid handler protocols for the MALDI-TOF target preparation; Table S1: Summary of the in vitro growth inhibition activities of the 31 vetiver endophytes against *Fusarium* plant pathogens with the bacterial cells or the cell-free supernatants.

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Chapter 3

Toward an innovative way to improve the selection of biocontrol agent candidates of endophytes: microbiome engineering

The results described in the first two chapters of this thesis have allowed me to identify interesting microorganisms that could be used for biocontrol purposes in agriculture or as a tool for the production of molecules of interest. However, the strategies that we have implemented show a limitation regarding time and labor. Whether on Leucojum or Chrysopogon, we isolated a large number of bacterial strains, and only a very limited number proved to be interesting. In order to increase the yield of interesting candidates,we explored a new approach allowing to enrich a substrate (the soil) in the microorganism of interest, using host plant phenotypes. To realize this work, we kept F. graminearum as a model, but we focused this time on a plant of agronomic interest. The general idea was to find the best bacteria, or even the best communities of bacteria allowing to protect the plants in natural conditions, by developing soil microbiota related to host-plant resistance.

A soil in which a crop is continuously grown can develop a certain degree of tolerance against certain plant pathogens. This phenomenon is known as "disease-suppressive soil," which is caused by an enrichment of the soil with antagonistic microbes [1,2]. These microbial populations then constitute the first line of defense against these pathogens. Recently, Carrion and collaborators showed that root endophytic microbial communities could also provide a second line against the pathogen. These results illustrate that plants can modulate the composition of their root microbiota in the rhizosphere and the endosphere by selecting beneficial microorganisms that increase their tolerance to plant pathogens. In addition, it has recently been shown that some pathogen-tolerant cultivars can modify the rhizosphere microbiome to generate a more protective microbiome, compared to a pathogen-sensitive cultivar [3]. Overall, these results suggest that it is possible to use host plants to select in the root microbiota microorganisms with specific characteristics of interest, such as those enhancing plant tolerance to pathogens [4,5].

Isolation of microorganisms able to produce interesting molecules may result in a new crop protection product. However, as mentioned in the state of the arts, the introduction of exogenous microbial biocontrol agents (BCAs) into soil or as a seed treatment sometimes fails because these microorganisms cannot colonize the plant due to competition with native microbial communities. To avoid such a problem, it might be more interesting to use native

microbial endophytes of a defined plant of interest and a defined soil.

All these elements led me to investigate the possibility to engineer a microbial community displaying antagonistic properties against plant pathogens by using disease-suppressive soil and plant varieties differing in their tolerance to these plant pathogens.

To assess this hypothesis, I chose wheat as host plant. Wheat is one of the most cultivated crops, which accounted for 15% of harvest area among primary crops over the world in 2019 (FAOSTAT 2019). France is the leading European producer of wheat and this crop occupies the main part of the French agricultural area (around 40% in 2019). I focused on root rot diseases (FRR) caused by *Fusarium* sp., using *F. graminearum* as a model strain. Until now, these diseases which can have an impact on crop yields have been controlled by seed treatments using chemical fungicides. However, in the framework of agri-environmental measures, the replacement of these seed treatments by biocontrol solutions can be a way to achieve public policy objectives regarding the use of chemical inputs (Ecophyto 2+ plan).

1. Principles of the methodology

My goal is to improve the efficiency of functional endophyte isolation from wheat using host-mediated microbiome engineering [6] and host plant genotypes differing in their tolerance profile towards FRR plants. For that purpose, a series of experiments was designed (Figure 0), consisting of several cycles of wheat cultivation, as described below. Different wheat varieties, inoculated or not at sowing with *F. graminearum*, are grown in the soil until the 1-2 leaf stage. At each cycle, the soils of the least symptomatic inoculated plants are collected and used for a new crop cycle. Crop cycles are repeated until a significant decrease in disease symptoms is observed. Root bacterial endophytes are then isolated and taxonomically and functionally characterized. At each cycle, the root microbiota, including rhizosphere and endophytic microbiota, should be analyzed by a metabarcoding approach in order to observe the evolution of the root microbiota composition during the selection process.

In order to set up this experiment, I first had to: 1) establish the conditions for inoculating plants with *F. graminearum*; 2) evaluate the symptoms of FRR/FCR on wheat plants; 3) select wheat varieties differing in their tolerance to FRR/FCR. For that purpose, I selected four commercial wheat varieties with different levels of tolerance to *Fusarium* head blight (FHB), as this is the only *Fusarium* disease that is assessed during variety registration in France. We hypothesized that tolerance to FHB is correlated with FRR.

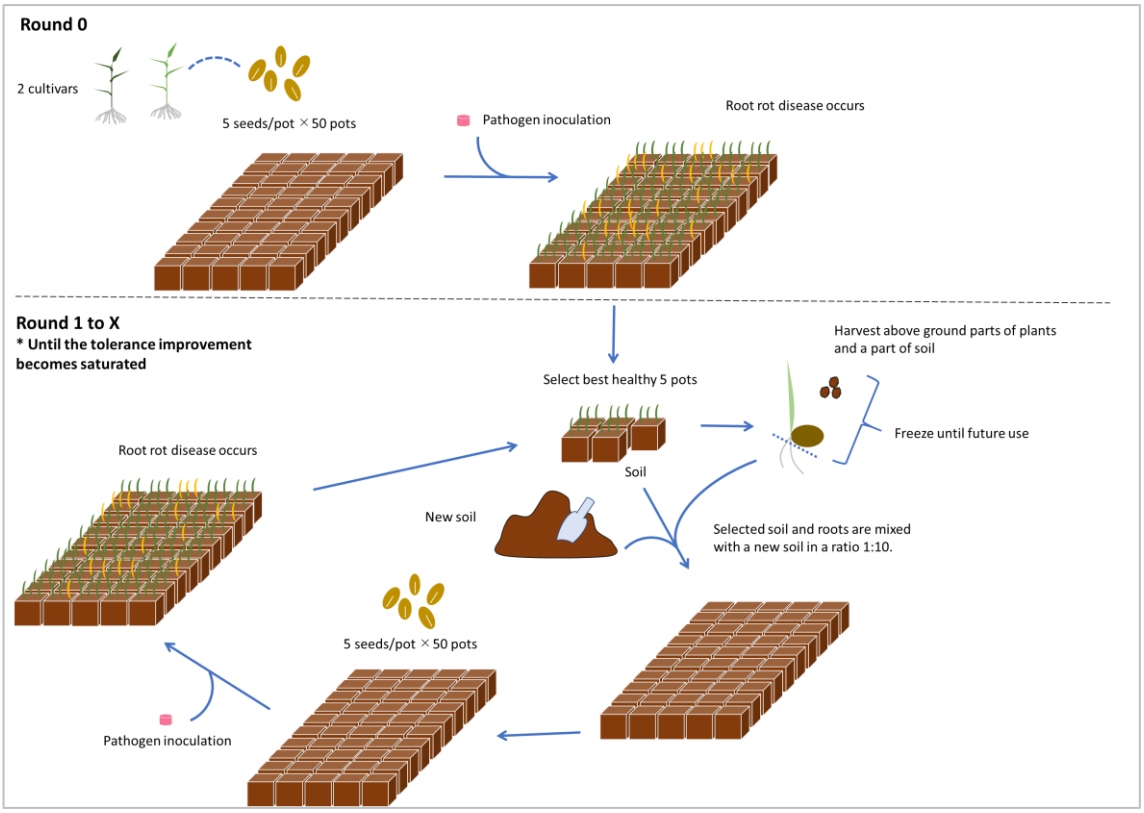


Fig. 0 | Global image of the wheat experiment

2. Materials and methods

2.1. Plant materials

Four soft wheat (*Triticum aestivum*) varieties with different susceptibilities to *Fusarium* were selected from commercially available varieties and on the basis of ARVALIS data (Table 3-1): Oregrain and Pilier are considered resistant to FHB while Mutic and Concret are susceptible to FHB. Seeds free of any treatment were purchased by Florimond Desprez (France) in 2020.

Table 1 | Chosen varieties of soft wheat and their resistant level against fungal diseases, summarized based on the database of ARVALIS (http://www.fiches.arvalis-infos.fr/liste_fiches.php?fiche=var&type=512)

			Disease resistance					
Variety	Year of the registration	Latest year of disease sensitivity test	Eyespot	Powdery mildew	Wheat yellow rust	Septoria leaf blotch	Wheat leaf rust	Risk of DON
Oregrain	2012	2020	-	-	-	--	-	++
Pilier	2018	2020	-	+/-	--	--	+/-	+/-
Mutic	2017	2019	--	++	++	+/-	--	-
Concret	2018	2019	-	+/-	++	+/-	--	-

2.2. Seedling preparation

Seeds were surface-sterilized before sowing, as follows: seeds were treated in 4 times diluted bleach (9.6% active chlorine, ORAPI EUROPE) for 10 min, then rinsed twice in sterile deionized water for 3 min. The seeds were then dried on sterile Petri dishes for 3 hours in a laminar flow hood. Ten seeds were then placed on autoclaved paper filters (Whatman No. 42) with 4 ml sterile deionized water in sterile 9 cm diameter Petri dishes and incubated at 20 ° C in the dark until germination.

2.3. Soil conditions

The soil used in this experiment was sampled in November 2018 in the experimental field of ENSAIA in la Bouzule, France. After sieving with a 5 mm sieve, the soil was conserved at 4 ° C until the experiment. This soil was mixed with sand at a ratio of soil/sand = 3/7 (v/v). Around 200 g of this substrate was put in a pot (7 cm × 7 cm × 8 cm) used to cultivate wheat. Before transplanting plants, the pots having the soil were kept at 15 ° C with aluminum cover and watered every 7 or 10 days, keeping water content to around 50% of Water Holding Capacity (WHC), in order to activate the soil microbiota.

2.4. Fungal strain

A strain of *F. graminearum*, kindly provided by the Institute Français De La Brasserie Et De La Malterie (IFBM), was cultured on PDA in 9-cm Petri dishes at 28 ° C in the dark. To prepare agar-plug inoculum, the active growing edge of fungal colonies were cut into ϕ 8-mm plugs, using sterile tips.

The conidial inoculum was prepared by transferring two fungal plugs in 100 mL flasks containing 25 mL of Mung Bean Broth (MBB) and cultured at 26 ° C and on a shaker at 90 rpm in darkness for five days (Fig. 1 a). MBB was made by diffusing 40 g of Mung beans (ALNATURA, Germany, organic agriculture) in 1 L of boiling ultrapure water for 30 sec, followed by filtering beans with Miracloth and autoclave. The spore production cultures were collected in 50 mL sterile tubes, passing a Falcon® sterile cell strainer with 40 µm mesh. The conidia were collected by centrifugation at 4,200 rpm and room temperature for 10 min, followed by removing the supernatant gently with sterile pipettes. The pellets were washed in autoclaved tap water by repeating centrifugation at 4,200 rpm for 10 min and removal of the supernatant two times. Finally, all the conidia were integrated into one tube. The concentration of the conidia was adjusted to 1×10^5 conidia/mL with autoclaved tap water after counting the number of the spores with Malassez cell numeration (Fig. 1 b). To verify the viability of the spores, 100 µL of dilution series of the conidia from 10 to 1000 times were spread on PDA media and incubated at 28 ° C in darkness for 2 days. The colony-forming unit (CFU) was calculated with 1000-time dilution and it was confirmed almost 100% viability of spores.

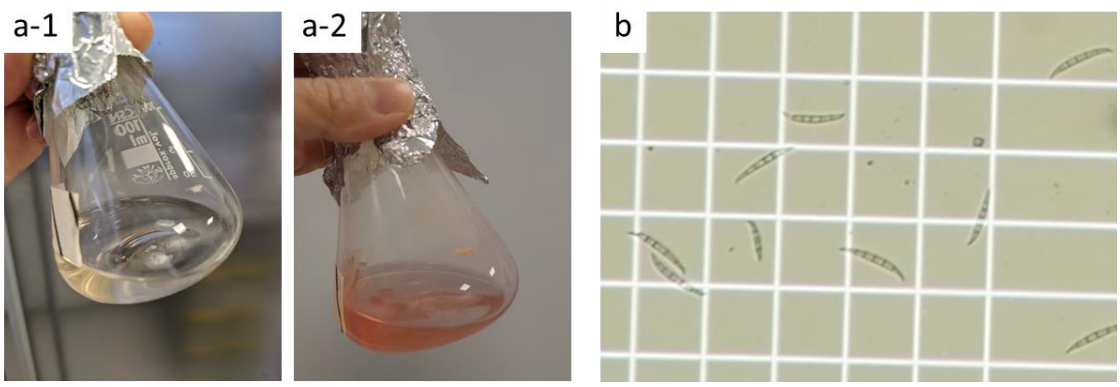


Fig. 1 | The spore production of *F. graminearum* a-1, before cultivation in MBB
(a) 5-day-after cultivation in MBB, (b) spores of *F. graminearum* on Malassez cell (a gap between white lines is 50 µm)

2.5. *In vitro* inoculation test

Seedlings of the four wheat varieties at 3-day old were axenically transferred to 9-cm Petri dishes containing 25 mL of 0.8% water agar medium, with three plantlets/plate. A ϕ8-mm agar plug of *F. graminearum* was prepared from the active growing edge of the 8-day-cultured mycelia. PDA plugs were used as control. Fungal and PDA plugs were placed upside-down on a root of each plantlet. Nine replicates per treatment were made for each of the wheat varieties Oregrain, Pilier, and Mutic, while only six replicates were made for Concret due to its low germination rate. Plates were sealed with parafilm and incubated in the same chamber, raising wheat plants as mentioned below. Observation of the plates was done every single day after the inoculation.

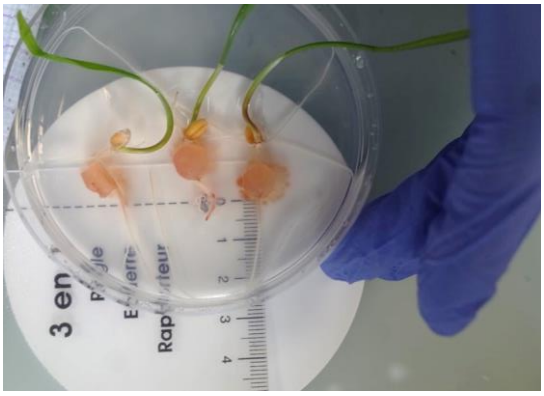


Fig. 2 | Measurement of the colored region of the primary root of the inoculated seedlings

The assessment of Fusarium root rot symptoms was performed referring to the protocol described in Ali et al., 2021 [7]. Five days after inoculation, plantlets were sampled. Symptoms were evaluated by measuring the extension of the necrosis of the primary root of the seedlings (Fig. 2).

2.6. Inoculation of wheat seedlings with *F. graminearum* spores and culture conditions

Two methods for inoculating wheat seedlings with *F. graminearum* were tested.

For the first method, inoculation of wheat seedlings with *F. graminearum* conidia was achieved using the protocol described in chapter 2. Briefly, three-day-old germinated seeds (9 / plate) of the four wheat varieties, with root primordia larger than 1 cm, were incubated in 5.2-cm Petri dishes in a suspension of *F. graminearum* macroconidia at 1×10^5 conidia/mL at 20 ° C and 60 rpm for 2 hours (10^5 spores/seedling). As a control, another set of seedlings was incubated with autoclaved tap water. Three replicates/treatment was done.

For the second method, seedlings (9 seedlings/pot) were inoculated just after transplanting with a suspension of *F. graminearum* macroconidia at 1×10^5 conidia/mL (10^5 /seedling), applied to the soil surface. As control treatment (mock inoculation), autoclaved tap water was applied to the soil surface. The pots were covered with aluminum foil during the first night after

transplanting. Plants were grown in a climatic room under a controlled environment with 16h-day photoperiod, 250 µmol/m/s, and 70% humidity under a controlled temperature (15 ° C day / 10 ° C). The soil was maintained at 70% of its WHC during the experiment by weighting the pots and watering them every three days. The position of the pots was regularly randomized.

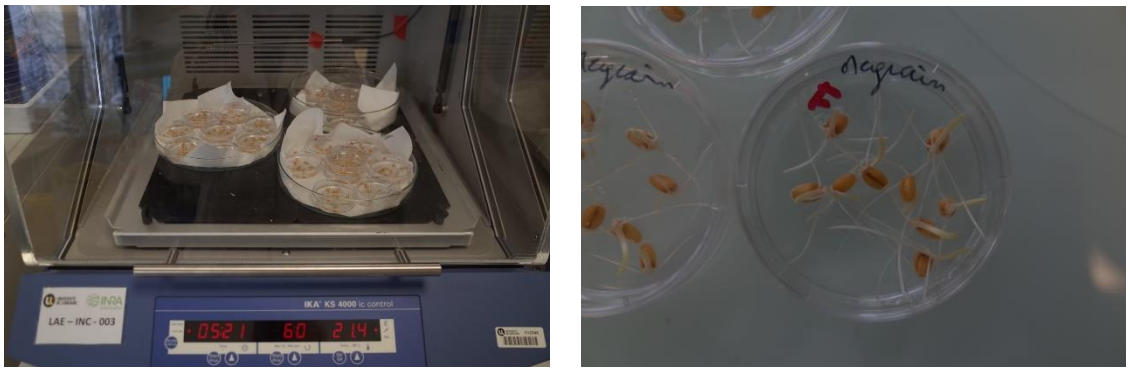


Fig. 3 | Inoculation of wheat seedlings with *F. graminearum* macroconidia
Seedlings in the suspension of F.g. spores (left) and incubation (right)

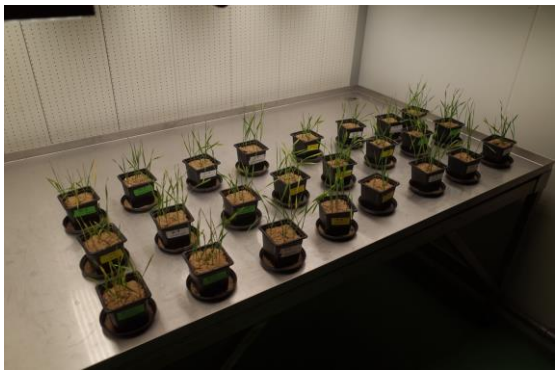


Fig. 4 | Growing wheat plants in the artificial climate chamber

After 21 days, young plants (3 unfolded leaves) were harvested. The root systems were carefully removed from the pots, gently cleaned with tap water two times, and dried in paper towels. Plants were stored at four ° C until processing (within one week).

2.7. Measurement of plant characteristics

The length of the root system and of the aerial parts were measured. Leaves and fresh root masses (mg) were determined. Roots were then cut into pieces of about 4 cm. An aliquot (about 10% of total fresh weight) was used to measure the dry mass. Fresh material (leaves and root aliquots) was incubated at 70 ° C for 48 hours and weighted to measure the dry masses (mg).

The remaining root material was treated in 20 mL of 70% ethanol for 2 min, 29 mL of 1.2% active chlorine bleach solution for 5 min, 20 mL of sterile deionized water for 1 min 3 times. The treated materials were frozen in liquid nitrogen and stored at -80 ° C for future use in metabarcoding and metabolomics (not done in this study).

2.8. FRR visual assessment

FRR symptoms were assessed using a rating index described by Wang et al. (2015). Symptoms on stem bases (SbSI) and roots (RSI) were observed for each individual plant. Stem-based and root browning were estimated using the following scale: 0 = no symptom, 1 = slightly necrotic (pale yellow), 2 = moderately necrotic (pale brown), 3 = severely necrotic (brown), 4 = completely necrotic (completely brown or black) (Fig. 5 a and b). The extension of stem and root browning was defined as the percentage of the total area that is rotten, using the following scale: 0 = no symptom, 1 = < 25%, 2 = 25% ≤ but <50%, 3 = 50% ≤ and <75%, 4 = 75% ≤. SbSI or RSI was defined as the sum of the browning score and the extension rate, which can give the maximum score of 8. Then, mean SbSI and RSI were calculated per pot. In the case that some plants disappeared because of the hard disease at the beginning of the cultivation, the number of such plants was not taken into account for these indices.

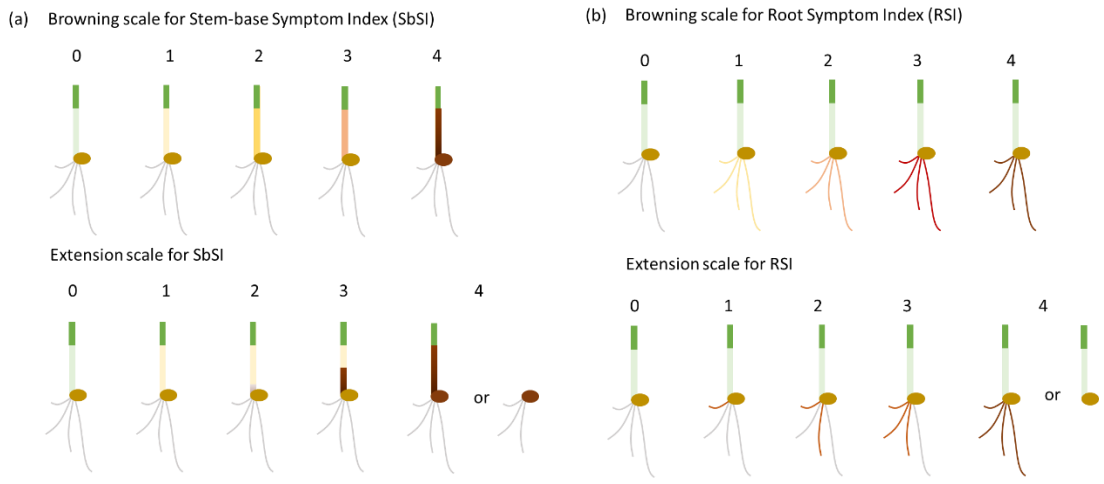


Fig. 5 | Visual indicators of SbSI and RSI

2.9. Data analyses

All the figures and statistical tests were realized with Excel 2016 or R studio with R version 3.6.3. Tukey-Kramer test was performed with the R package "multcomp." Welch's t-test was done using the statistical tool of Microsoft Excel 2016. Principal component analysis was done with the R package "Factoshiny."

3. Results

3.1. In vitro sensitivity test of wheat seedlings against F. graminearum

In a first experiment, I evaluated the pathogenicity of the *F. graminearum* strain available in the laboratory against the four wheat varieties that had been selected. This evaluation was done in *in vitro* conditions. A mycelial plug of *F. graminearum* was carefully placed on the primary root of 3-days old seedlings. Regardless of the varieties considered, symptoms were observed five days after inoculation. Compared to the controls, the roots have turned orange and red in the area of contact with the fungal plugs (Figure 6 b). Regarding the extension of these necrotic zones, no significant differences were observed under these conditions between wheat varieties (Fig. 6 a). This first experiment allowed us to confirm the pathogenicity of the *F. graminearum* strain against wheat.

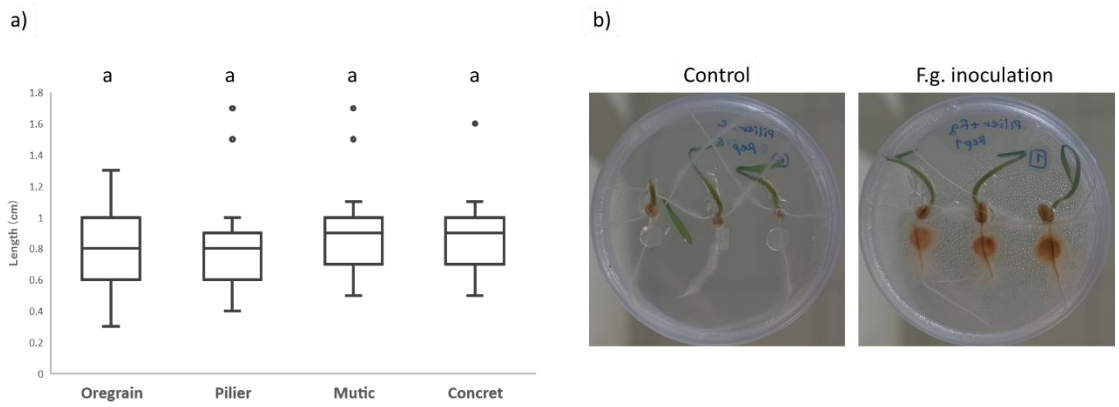


Fig. 6 | In vitro inoculation test of the primary root of wheat seedlings against *F. graminearum* (a) length of necrosis in the primary root of seedlings after 5 days incubation. The same letter above the graph shows no significant difference with $p<0.05$ (Tukey-Kramer test). (b) Photos of control plants and inoculated plants of Pilier after 5 days incubation.

3.2. Comparison of two methods of infection of wheat roots with F. graminearum

To establish the conditions for inoculating wheat roots with a conidial suspension of *F. graminearum*, I tested two strategies. In the first approach, the primary roots of the germinated seeds were inoculated with the conidial suspension of *F. graminearum* (then called root treatment). In the second approach, the infection by the pathogen was carried out by spraying the conidial suspension at the time of sowing (then called soil treatment).

After 21 days, the young plants (3 unfolded stages) were harvested (Fig. 7), the disease symptoms were quantified, and several morphological traits were estimated (Fig. 8 and 9).

Overall, plants from the soil treatment were less affected by *F. graminearum* than the plants from the root treatment (Fig. 8). Among the plants in the root treatment, leaf and root length was reduced by 20% in the Oregrain and Pilier varieties inoculated with *F. graminearum* compared to the control (Fig. 8 c). Among the plants in the soil treatment, leaf length was reduced by 16% and root length by 11% only in Pilier after inoculation with *F. graminearum*, whereas this pathogen relatively did not seem to affect the variety Concret. FRR symptoms in the stem base and roots were more clearly visible in the root treatment plants compared to the soil treatment plants, regardless of the wheat variety considered (Fig. 7 and 9).

These preliminary results led me to select the root treatment for the following experiments.

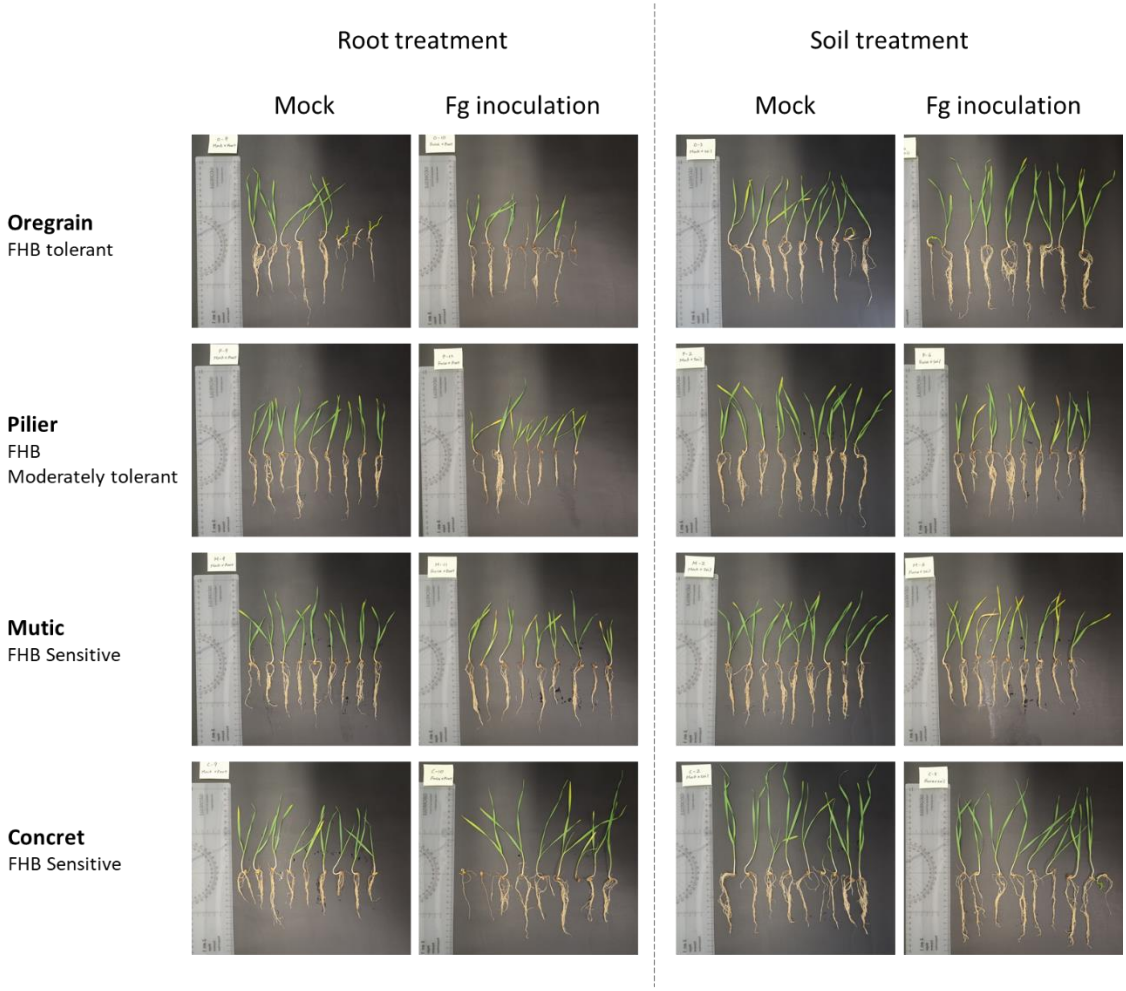


Fig. 7 | The sampled plants inoculated with *F. graminearum* spore suspension (Fg inoculation) via root or soil

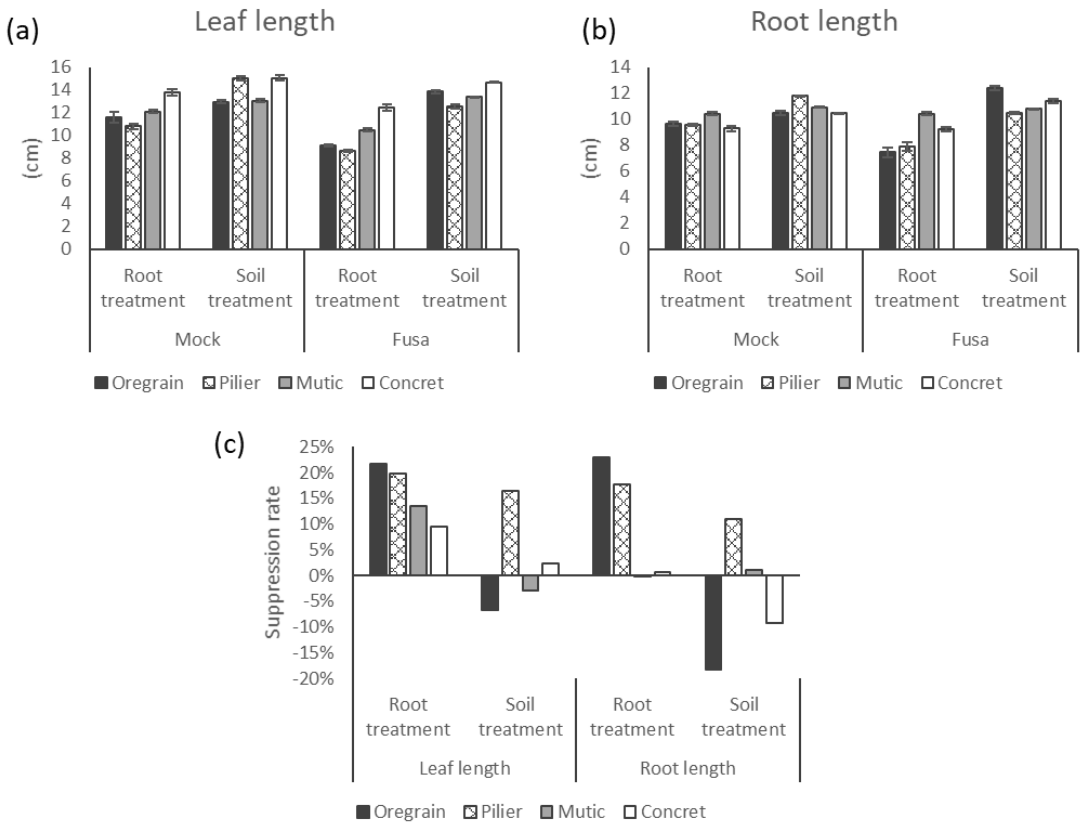


Fig. 8 | Physical traits and FRR symptom indices of the samples plants
(a) longest leaf length, (b) longest root length, (n = 3 for each condition, the error bars = SE), (c) Suppression rate comparing to the mean length of leaves and roots in Mock treatment plants

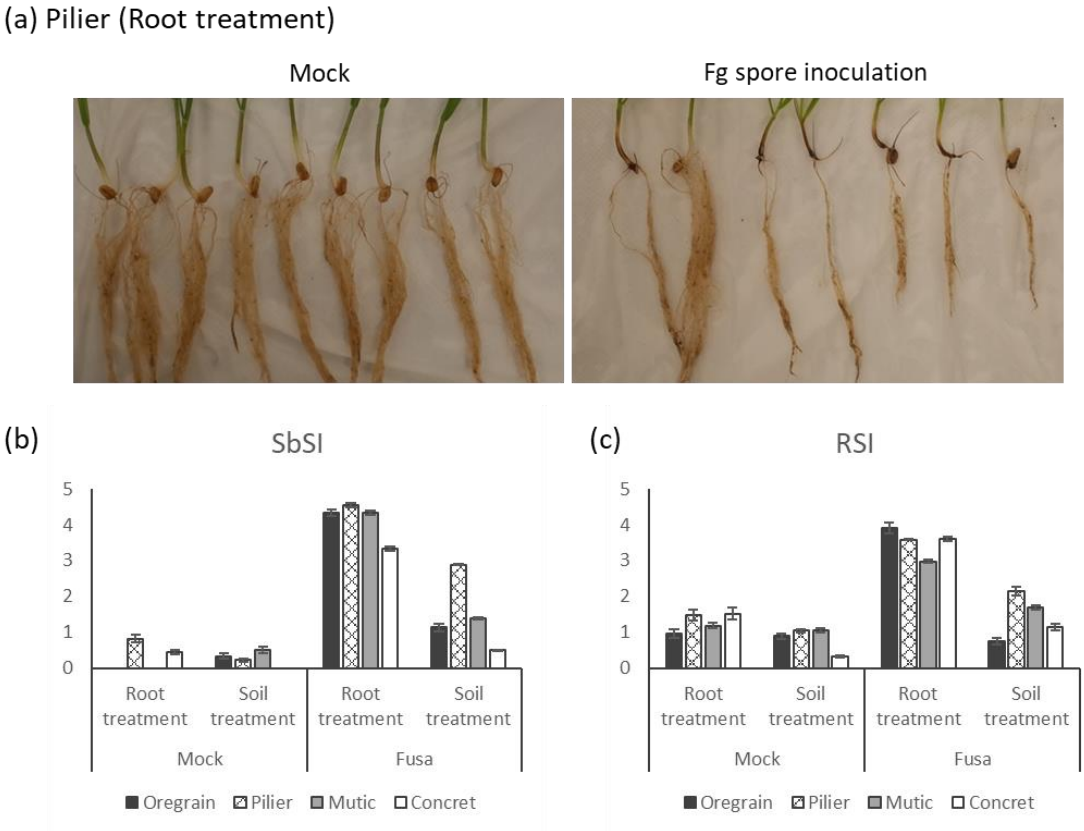


Fig. 9 | Symptoms on stem and root
(a) representative appearance of stem and root in mock and Fg-spore-inoculated plants of Pilier, (b) Stem base symptom index (Maximum value = 8), (c) Root symptom index (Max. value = 8). (n = 3 for each condition, the error bars = SE)

3.3. Comparison of the sensitivity to FRR of the four wheat varieties

The plants for which roots were infected were further analyzed in order to estimate their sensitivity to *Fusarium*. The dry weight of shoot and root parts was significantly reduced in the three varieties, Oregrain and Mutic ($p < 0.05$, Welch's t-test), and Pilier ($p < 0.1$) by root-inoculation with *F. graminearum* (Fig. 11 a). For the Concret variety, no significant differences in dry weight of shoot and root parts could be highlighted in inoculated plants compared to non-inoculated plants. On the other hand, no significant difference was observed in the root biomass between inoculated and mock plants overall wheat varieties (Fig. 11 a). Nevertheless, the infection of Oregrain and Pilier with *F. graminearum* reduced the average dry weight of shoots and roots by 50% and 40%, respectively (Fig. 11 b). Concerning Mutic, the average dry weight of shoots and roots was reduced by 29% and 12%, respectively. Finally, the Concret variety was only slightly affected by *F. graminearum*, with a reduction in average shoot and dry root weight of only about 5%. This variety, therefore, appeared to be the most tolerant to FRR.

In order to establish relationship and go deeper in details, I realized principal component analysis (PCA) done on the four wheat varieties, using morphological traits (length, dry weight), the number of survival plants and the symptom indices (SbSI and RSI) as parameter (Fig. 12). The four varieties were not distinguished in mock treatment. By contrast, when plants were inoculated with *F. graminearum*, the four varieties were clustered into two groups along PC1. The first group included the varieties Concret and Mutic, whereas the second group corresponded to the varieties Oregrain and Pilier. The construction of the first axis was strongly contributed by leaf length and dry weight of shoot and root. With this PCA, Concret was characterized as a relatively resistant variety with substantial growth of seedlings and fewer symptoms in the stem base; in contrast, Pilier was thought of as a relatively sensitive variety with growth suppression in leaves and more severe symptoms in the stem base.

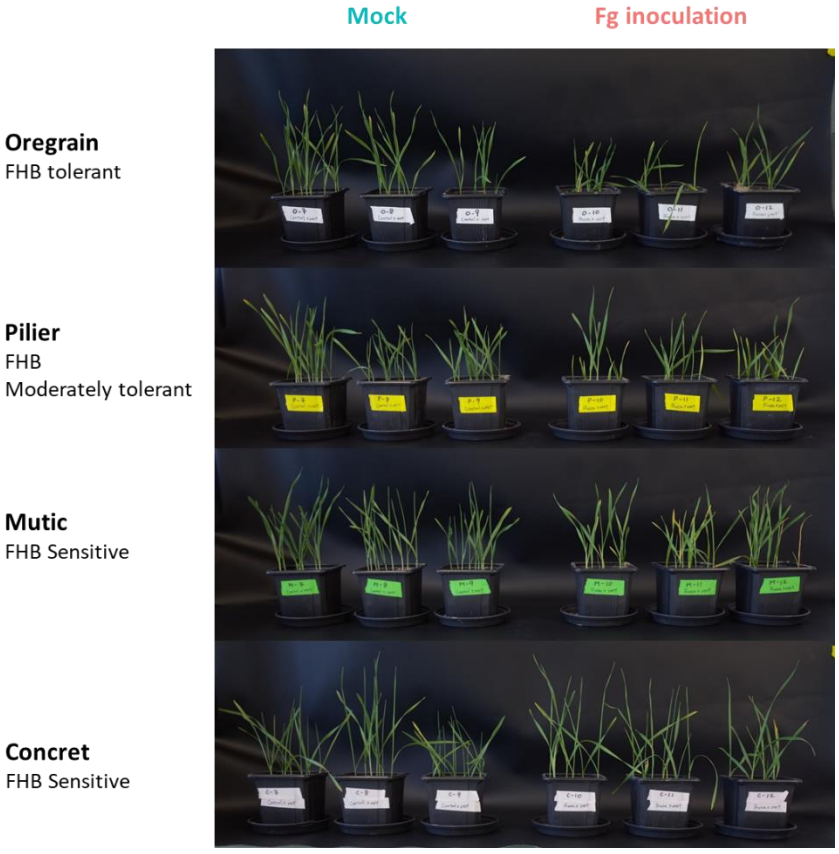


Fig. 10 | Appearance of the plants inoculated with *F. graminearum* spore suspension via root

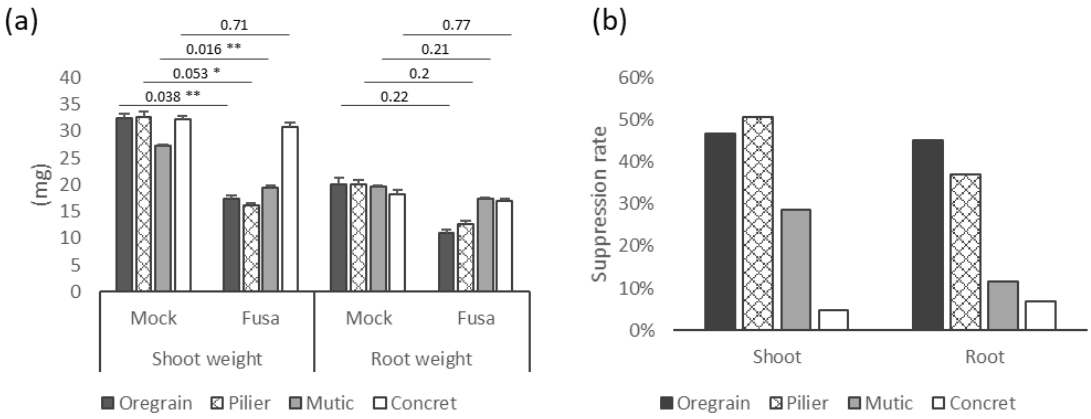
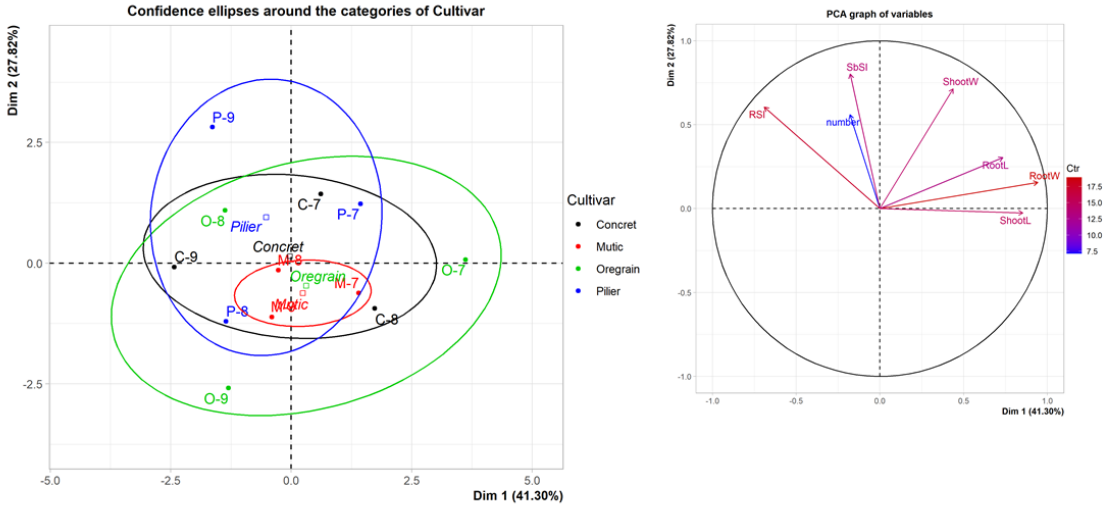


Fig. 11 | Comparison of shoot and root dry weight between non inoculation (Mock) and Fg inoculation (Fusa)
(a) dry weight of shoot and root tissues of mock and Fusa treatment plants. "**" indicates $p<0.05$, "*" does $p<0.1$ (Welch's t test) (error bars = SE). (b) The suppression rate of Fusa treated plants compared to Mock treatment

(a) Mock



(b) Fg inoculation

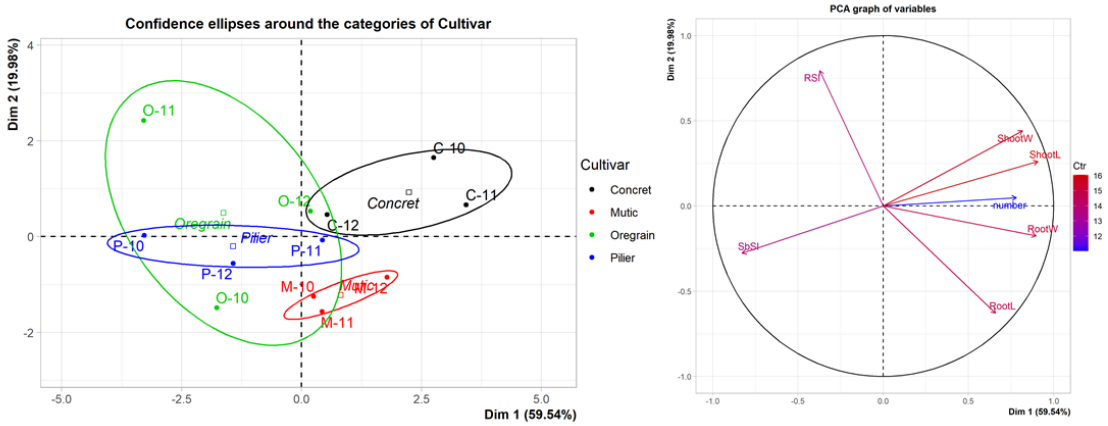


Fig. 12 | PCA of the measurement of Mock and Fg-spore inoculated plants
Left, graph by wheat varieties; Right, loading plot of variables, consisting of stem base symptom index (SbSI), root symptom index (RSI), shoot dry weight (ShootW), shoot length (ShootL), root dry weight (RootW), root length (RootL), the number of germinated plants/pot (number).

4. Discussion

The preliminary results obtained in this last part of my work are, of course, not robust enough to raise a conclusion about the possibility of using suppressive soils as a strategy for the sustainable agriculture. However, they pave the road toward a strategy that might be interesting for a future strategy. These preliminary experiments with the four selected commercial wheat varieties have shown that they differ in their susceptibility to FRR.

To prevent *Fusarium* diseases, different management strategies have been identified. Among these strategies, we can distinguish between the use of seed treatments and resistant cultivars (mainly against FHB) [8]. With regard to resistance, comparisons of the level of resistance of wheat cultivars to FRR and FHB have been made in several studies. To date, there is no evidence of clear correlations between resistance to FHB and to FRR [9,10]. It may be explained by some differences in the development of the fungus after infection. One study showed that the gene expression profile of *F. graminearum* was different in FHB compared to FRR/FCR, with the exception of genes implicated in the biosynthesis of DON that was up-regulated in both cases [11].

Even though the infection machinery between FHB and FRR/FCR is different, FRR/FCR seems a good model for the selection of beneficial wheat microbiomes against disease development, because 1) the symptoms are visible, making the selection of plant pots easy, and 2) the symptoms appear at the early stage of plant development, allowing us to have a short cycle of plant cultivation. We therefore consider that the FRR is a good model to establish the proof of concept that we can improve the selection of bacterial endophytes with promising properties for the biocontrol of plant pathogens using host-mediated microbiome engineering.

A series of preliminary experiments were carried out to establish the conditions for inoculating wheat roots with *F. graminearum* and to compare the sensitivity of different wheat varieties to FRR. The FRR caused by *F. graminearum* starts with the infection at the stem base or *via* contaminated seeds. The infected seedlings show necrosis of the roots and stem, resulting in growth failures of healthy seedlings. A study on 12 wheat cultivars showed that the development of FRR leads to a significant reduction in root biomass (up to 69.4%), root length (up to 34.4%) and aerial parts (up to 22%), 21 days after root inoculation (Wang et al., 2015). In this study, it was also reported that colonization of roots by *F. graminearum* starts as early as 1 day after inoculation. We also observed that the first necrosis appeared 3 days post-inoculation on the roots and 5 to 7 days PI at the base of the stem. By comparing the two methods, inoculation of root primordia from germinated wheat seeds seemed the most efficient method in terms of symptom development. This method will be used in future works.

I chose four wheat varieties, commercially available, which present different level of tolerance to FHB: Oregrain (low sensitivity), Pilier (moderate sensitivity), Mutic, and Concret (high sensitivity). Under *in vitro* conditions, after inoculation of young seedlings with a mycelium

plug of *F. graminearum*, no difference in symptoms could be observed between the four selected varieties. However, in the pot test, inoculation of the roots with *F. graminearum* caused a strong decrease in the length of the aerial parts and roots as well as in the biomass of the aerial parts of the varieties Pilier and Oregrain, while inoculation of the plants of the variety Concret had no significant effect on the biomass, despite the presence of symptoms. Using Principal Component Analysis, we showed that Concret was the most different from the other three varieties. These observations suggest that Concret can be considered the most tolerant variety to FRR/FCR and that Pilier is the most susceptible.

It should be noted that the variety Concret is considered to be susceptible to FHB. This variety therefore behaves the opposite way in the case of FRR. There are no elements in the literature to explain these differences in terms of sensitivity to FHB or FRR. Wang et al. (2018) observed that the rate of colonization of the root cortex by *F. graminearum* differed significantly between susceptible and tolerant varieties [12]. Monitoring the kinetics of colonization of FRR-susceptible and FRR-tolerant varieties using molecular tools could provide insights into these differences in susceptibility.

5. Reference

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General discussion and perspectives

In 2015, the member states of the United Nations signed a charter for sustainable development to be implemented by 2030. This plan sets out 17 different goals for a better future for all humanity. These 17 goals include 'zero hunger' (goal number 2), 'good health and well-being (goal number 3), 'sustainable consumption and production' (goal number 12), and 'life on earth' (goal number 15). In order to meet these different goals, it is necessary to think in new ways and to find new solutions to address the problems that have emerged during the millennium that has just ended. My thesis project is part of one of the societal challenges set out in the framework of the Science Innovation Territory Economy Initiative (ISITE) Lorraine University of Excellence and, more specifically, in the framework of the IMPACT Biomolecules project. The overall objective of this project is to identify new molecules to meet the essential needs of society. The first need concerns the implementation of a renewed and more environmentally friendly agronomy. This requires an agroecological transition and the implementation of agricultural practices that reduce the use of chemical inputs, which have been massively used since the green revolution after the Second World War. The second need concerns human health and the identification of new molecular models to treat diseases that have become major in an aging society but also to circumvent the problems of antibiotic resistance that have arisen as a result of the massive use of molecules that have been available for almost a century.

Within the framework of the Biomolecules project, we have initiated work on the study of interactions between plants and microorganisms with the aim of identifying innovative molecules for both pharmaceutical and/or agricultural applications. Since the pioneering articles on the identification of salicylate in 1826 and the study of secondary metabolism by Fraenkel in 1959, many advances have been made in the characterization of molecules of interest by plants. In parallel, the bibliography contains countless works concerning the identification of molecules of microbial origin, including, for example, the production of penicillin, the first and most famous antibiotic discovered by Flemming in 1928. This work has been carried out between two different laboratories at the University of Lorraine: the L2CM and the LAE.

A) Taxonomic composition of culturable endophytes and their functional analysis

In this thesis, we studied the variation of the taxonomical composition of endophytic bacterial communities and the variability of the metabolic capacity of some culturable endophytes isolated from two different plants, *Leucojum aestivum* and *Chrysopogon zizanioides* (vetiver). The taxonomic compositions of cultivable endophytic bacterial communities of *L. aestivum*

were differentiated according to plant tissues. Regarding vetiver, we focused on roots that were sampled in five different geographical worldwide areas. Using both culture-dependent and culture-independent approaches, my results clearly demonstrated that the taxonomic compositions of endophytic bacterial communities differ according to the geographical origins of the vetiver roots. By exploring this diversity, we have identified different bacterial genera with antagonistic activities against fungal pathogens. We showed that some isolated endophytes display efficient growth inhibition activities against *Fusarium graminearum*, *F. oxysporum*, *F. culmorum*, and *Microdochium nivale*. These results were obtained in *in vitro* co-culture tests and therefore in standardized and sterile conditions. The results of these high potential strains correspond to genera well documented in the literature for their antagonistic activities (*Bacillus*, *Paenibacillus*, *Pseudomonas*, *Serratia*, *Streptomyces*) but also to less investigated ones (*Patulibacter*, *Variovorax*, *Chitinophaga*, *Paraburkholderia*, *Luteibacter*).

Further experiments allowed us to reveal clues to explain the mechanisms involved in the antifungal activity of the bacterial strains of interest. For two endophyte strains isolated from *L. aestivum*, we anticipate that tryptophol might be involved in the antifungal activity. For five *Bacillus* strains isolated from vetiver roots, we showed the production of lipopeptides (surfactin A and plipastatin) which could participate in the growth inhibition activities of different pathogenic species, including *F. graminearum*. Preliminary results from the sequencing of the genome of these strains suggest that they may produce other antimicrobial compounds. These bacterial strains are therefore promising biocontrol agents. They need to be more deeply studied in order to better understand their modes of action.

During this Ph.D. project, we focused on a restricted (but promising) number of isolated culturable endophytes. It will be interesting to extend our analysis to the other endophytic bacteria displaying antagonistic activities in our *in vitro* dual culture tests. For this purpose, we might perform bioactivity-guided fractionation using extracts done on the microorganisms. Other tracks also need to be deeper investigated, like the identification of volatile compounds, cell-wall degradation enzymes, and siderophore production. All of them can be considered involved in the mode of action (Fig. 1). The production of such bioactive molecules are sometimes induced only when co-culture with the target microbes or the presence of endophytic microbes from the same plants [1–3].

Finally, microorganisms that show no growth inhibition of pathogenic fungi under *in vitro* co-culture conditions should be evaluated *in planta* for their bioprotective effects. The gap between *in vitro* conditions and *in planta* tests is a reality. It was already exemplified that bacterial strains without significant growth inhibition against *F. graminearum in vitro* showed a nice efficacy on plants against the disease [4]. This study gave an alert for ignorance of microbial strains that cannot show *in vitro* antagonisms.

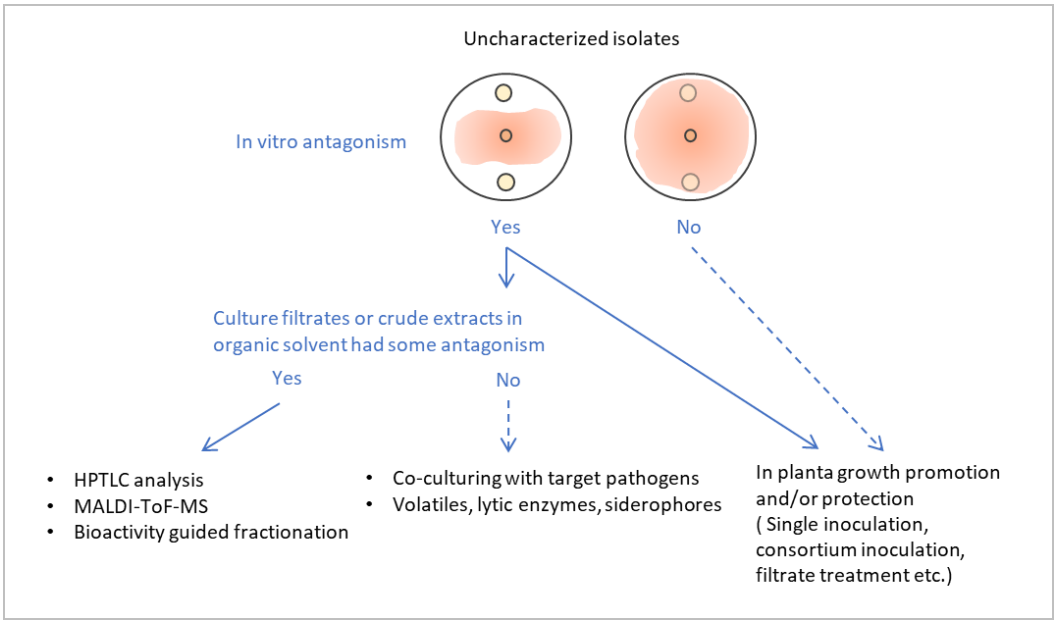

Fig. 1 | Strategies to identify modes of actions and antagonistic molecules from remaining isolates.

B) Investigation in the function of endophytic microbiota

It is widely described and accepted that the analysis of the microbiota using a culturable way is very restrictive. Using a DNA-based approach allowed a better understanding of the microbial community available in plants that can be considered as meta-organisms. The information obtained using such approaches is of greatest interest since it might help to synthesize new communities adapted for different applications.

In this thesis, amplicon sequence analysis on vetiver root endophyte communities was carried out using 16S rRNA gene regions. Such a metabarcoding strategy can provide some information about the taxonomical composition of endophytic communities at the genus level. A quantitative PCR (qPCR) targeting more specific genes could additionally help to access a deeper resolution and accessing the taxonomical identity. This qPCR strategy can also be used to estimate certain functions of the endophyte community by targeting functional genes involved in the biosynthesis of antimicrobial compounds such as the NRPS and PKS cluster genes [5].

In order to have a broader view, an alternative approach can be considered. Instead of targeting and sequencing some specific genes, it is now possible to perform metagenome sequencing (shotgun genomics). This strategy provides information about all the genomes present in the DNA template. Such an approach has been set up for research on endophytes [6,7]. In addition to

the knowledge of the composition of the microbial community in plants, this technique may also help to improve the efficiency of culturing endophyte microbes [8].

Finally, to more deeply understand plant-microbe interactions, single-cell trans-omics analysis has been proposed [9]. Plant tissues are composed of different cell bases, with several cell types. By taking this heterogeneity into account and by using single-cell technologies, we could gain a more accurate understanding of the mechanisms of plant-microbe interaction. Recently, it was revealed that the root zones in which antimicrobial compounds were synthesized, differed depending on whether the roots were colonized by a pathogen or a beneficial microorganism [10]. These techniques are also interesting to elucidate relationships between the microbiota of different plant genotypes. Especially, they may allow us to observe local interactions between microbes and specialized metabolites in specific tissues of medicinal plants, such as bulbs of *L. aestivum* and essential oil in vetiver root.

C) Microbiome engineering

The knowledge gained on the composition of the microbiota of several plants and on the factors influencing this microbiota opens up new perspectives for identifying solutions to combat various pathogens. In particular, it is possible, through engineering approaches, to modulate the composition of the microbiota and to enrich it with microorganisms that are potentially more effective in protecting the plant against a target pathogen. I started to explore these possibilities during my thesis and this work is still ongoing.

We hypothesized that the composition of the endophytic microbiota of wheat could be influenced according to the resistance profile of the variety used and by performing several successive cycles of cultivation on the same soil in the presence of the target pathogen.

Analysis of microbial networks in several host plant species have uncovered the presence of hub and core microbes, which play an important role in the composition of microbial communities [11,12]. Thus, the use of these core microbes can help plants recruit functional microbial species from the environment that enhance their defense against abiotic/biotic stresses. These data highlight that it may be difficult to exploit a single microorganism to enhance plant protection as these beneficial effects are rather the result of a set of interacting microorganisms within the plant microbiota. For example, Santhanam et al. (2015) showed that some endophytic bacteria do not provide significant protection to plants when used alone but rather when used in consortia [13]. Although networking analysis still presents some challenges as it often relies on the relative abundance of microbial taxa [14], the increased knowledge on microbe-plant relationships should be useful for monitoring the native plant microbiota. This means that the presence of core microbes, the level of diversity of the microbiota could be indices of the plant's

tolerance/sensitivity to stresses. Some researchers have already proposed to use plant traits related to the formulation of beneficial microbiota for selection [15–17]. Furthermore, as the composition of plant microbiota can be captured very quickly and easily through sequencing, it may be possible to prescribe chemicals or microbial isolates for field crops on demand, leading to a reduction in agrochemicals and chemical fertilizers [12]. In the experiment with wheat varieties, we hope to observe changes in the composition of the microbiota and interactions within this microbiota. With the techniques described above, it may then be possible to identify the key microbes that are linked to the development of plant tolerance to the pathogen. In addition, the combination with other omics techniques could allow progress in the knowledge of the molecular mechanisms by which the endophytic microbiota influences the plant tolerance phenotype. A second approach to designing a functional microbiota with beneficial effects on the plant is to create synthetic communities, by exploiting collections of strains and analyzing their effects, alone or in consortia on the plant phenotype. This allows the relative contribution of each microbial taxon to the expected function to be assessed and the most relevant microbial assemblages to be identified [18]. This method is called "SynCom" [19]. This strategy has the advantage of improving the efficiency and reproducibility of the effects of microorganisms on the plant [20]. However, this synthetic community method requires sufficient coverage of the diversity of the plant-associated microbiota in order not to neglect the effect of rare taxa. This methodology is also interesting since the endophytic microbe communities from different disease-resistant wheat plants can be re-organized in other axenic plants. In this case, it might be possible to evaluate the acquired resistance of the inoculated plants. Another application might be the possibility to engineer the specialized metabolites of plants. There were already some examples about the inoculation of a single strain of some endophytic fungus or bacterium increased the contents and altered the composition of plant specialized metabolites as well as photosynthesis [21,22]. The review article of Etalo et al. proposed to use the alteration of biosynthesis gene expression of plant metabolisms by microbial inoculation for identifying new biosynthesis genes without preparing mutant lines [23].

General conclusion

My thesis focused on hundreds of endophytic bacteria isolated from *Leucojum aestivum* 'Gravity giant' and *Chrysopogon zizanioides* (L.) Roberty (vetiver) roots. We analyzed the composition of cultivable endophytes from different tissues of *L. aestivum*, as well as the composition of endophytic bacterial communities from vetiver roots, by both a metabarcoding approach and a cultivable-based approach. The taxonomic analysis revealed that the composition of the endophytic communities differed between plant organs or the geographical origins of plants. We characterized 42 bacterial genera within the endophytic microbial community of *L. aestivum* and 16 within vetiver roots. Some of these microbial genera had already been described in the endophytic communities of different plant species. Other genera are still poorly characterized, such as *Luteibacter*, *Comamonas*, *Patulibacter,* or *Chitinophaga*, for example. The isolated bacteria obtained were tested for their antifungal activities against different plant pathogens affiliated with *Fusarium*. Some of these bacteria showed significant inhibition of the growth of fungal colonies *in vitro*. They could be promising candidates for the development of biocontrol solutions in agriculture. In addition, some isolates were characterized metabolically for their ability to produce specialized metabolites such as alkaloids (*L. aestivum* endophytes) and lipopeptides (vetiver root endophytes). We identified two endophytic strains of *L. aestivum* that produced tryptophol, an indole alkaloid precursor, and five strains of *Bacillus* from vetiver that produced lipopeptides (surfactins and plipastatins). The remaining strains still need to be tested for their ability to produce bioactive molecules involved in their antifungal activities. Furthermore, although many endophyte strains do not seem to have a direct antagonistic effect *in vitro* towards target pathogens, it seems interesting to evaluate their effects *in planta*. Finally, it is also necessary to characterize other bioactive compounds (solubles and volatiles) that could be produced by our bacterial endophytes, such as compounds with antitumoral, antiviral, insecticidal, antioxidant activities. The last part of my thesis was devoted to the development of a new strategy to select microorganisms of interest for biocontrol. Only a series of preliminary tests could be performed, but this work is still ongoing.

My thesis marked the beginning of research projects around endophytes in the host laboratory, but it gave the basis for this work and gives really interesting perspectives for the future.

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Résumé en Français

Au cours de leur croissance, les plantes interagissent avec un ensemble de microorganismes qui constituent le microbiote de la plante [1]. Au sein de ce microbiote, il est possible de distinguer les microorganismes endophytes, majoritairement des bactéries et champignons, qui effectuent tout ou partie de leur cycle de développement dans les tissus internes de la plante [2]. Ces microorganismes, qui ont été identifiés dans presque toutes les espèces végétales étudiées jusqu'à présent, peuvent coloniser l'ensemble des organes de la plante, des racines aux parties aériennes. Au niveau du compartiment racinaire, il a été mis en évidence que la structure taxonomique de la communauté bactérienne endophyte diffère de celle de la communauté rhizosphérique, suggérant que la plante peut filtrer les microorganismes colonisant les racines [3–5]. Comme cela a été très largement décrit dans le cas d'interactions symbiotiques tels que l'interaction *Rhizobium*-Légumineuses, les plantes, par l'intermédiaire de certains métabolites spécialisés, établissent un dialogue moléculaire complexe avec des microorganismes présents dans leur environnement. Des composés issus du métabolisme spécialisé des plantes pourraient donc influencer la composition de la communauté endophyte. D'autres molécules comme l'acide salicylique, une phytohormone qui participe à la régulation des mécanismes de défense de la plante pourraient également avoir un effet sur la structure de ces communautés microbiennes [6,7].

Les microorganismes endophytes peuvent moduler la croissance de la plante mais aussi ses capacités d'adaptation à des stress biotiques [3,8,9]. En effet, ces microorganismes, en produisant notamment une grande diversité de métabolites à activité antimicrobienne et en influençant la synthèse de métabolites spécialisés par la plante et plus largement ses défenses, sont susceptibles d'améliorer la tolérance de cette plante à des bioagresseurs et notamment à des agents pathogènes [2,8,10,11]. Le microbiote de la plante peut donc être considéré comme un réservoir de microorganismes bénéfiques qu'il semble pertinent d'explorer pour développer de nouvelles solutions antimicrobiennes, valorisables en agriculture et en santé humaine et susceptibles de répondre à certains des enjeux dans ces deux secteurs d'activité. Concernant l'agriculture, l'utilisation de microorganismes bénéfiques et/ou de leurs métabolites comme solution de biocontrôle est ainsi considérée comme l'une des stratégies les plus prometteuses pour atteindre les objectifs du plan « Ecophyto » (https://agriculture.gouv.fr/le-plan-ecophyto-quest-ce-que-cest), qui sont de réduire l'utilisation de produits phytosanitaires de 50 % d'ici 2025 et ce, afin de limiter les impacts négatifs de ces produits sur l'environnement et la santé humaine [12–17]. Concernant le domaine de la santé, la valorisation des microorganismes du microbiote de la plante pourrait permettre d'identifier de nouveaux composés bioactifs, avec des modes d'action originaux pour le développement de nouveaux médicaments [18–21]. Il a ainsi été mis en évidence que certains champignons et bactéries endophytes isolés de plantes

médicinales produisent des métabolites spécialisés doués de propriétés pharmacologiques identiques à ceux de la plante hôte [22–24].

En conséquence, les microorganismes endophytes constitue un réservoir intéressant à explorer pour identifier de nouvelles « substances » bioactives valorisables dans les secteurs de la santé et/ou de la protection des plantes.

Objectifs de la thèse

En 2015, les États membres des Nations unies ont signé une charte pour le développement durable à mettre en œuvre d'ici 2030. Ce plan définit 17 objectifs différents pour un avenir meilleur pour toute l'humanité. Ces 17 objectifs comprennent " la faim zéro " (objectif numéro 2), " une bonne santé et le bien-être " (objectif numéro 3), " une consommation et une production durables " (objectif numéro 12) et " la vie sur terre " (objectif numéro 15). Afin d'atteindre ces différents objectifs, il est nécessaire de penser de manière nouvelle et de trouver de nouvelles solutions pour faire face aux problèmes qui sont apparus au cours du millénaire qui vient de s'achever.

Mon projet de thèse s'inscrit dans l'un des défis sociétaux énoncés dans le cadre de l'Initiative Science Innovation Territoire Economie (ISITE) Université d'Excellence de Lorraine et plus particulièrement dans le cadre du projet IMPACT Biomolécules. L'objectif général de ce projet est d'identifier de nouvelles molécules pour répondre aux besoins essentiels de la société. Le premier besoin concerne la mise en place d'une agronomie renouvelée et plus respectueuse de l'environnement. Cela passe par une transition agro-écologique et la mise en œuvre de pratiques agricoles réduisant l'utilisation d'intrants chimiques, massivement utilisés depuis la révolution verte après la Seconde Guerre mondiale. Le second besoin concerne la santé humaine et l'identification de nouveaux modèles moléculaires pour traiter des maladies devenues majeures dans une société vieillissante, mais aussi pour contourner les problèmes de résistance aux antibiotiques apparus suite à l'utilisation massive et irraisonnée de molécules disponibles depuis près d'un siècle. Depuis les articles pionniers sur l'étude du métabolisme secondaire de Fraenkel en 1959 ou l'identification de la salicyline en 1826, de nombreuses avancées ont été réalisées dans l'étude de la production de molécules d'intérêt par les plantes. En parallèle, la bibliographie contient d'innombrables travaux concernant l'identification de molécules d'origine microbienne, dont, par exemple, la production de pénicilline, le premier et le plus célèbre antibiotique découvert par Flemming en 1928.

Dans le cadre du projet Biomolécules, nous avons initié un travail sur l'étude des interactions entre plantes et microorganismes dans le but d'identifier des molécules innovantes pour des applications pharmaceutiques et/ou agricoles. Ce travail a été réalisé entre deux laboratoires différents de l'Université de Lorraine : le L2CM et le LAE.

L'objectif général de mon travail de thèse a donc été d'analyser la diversité taxonomique et fonctionnelle des microorganismes endophytes de différentes espèces végétales afin de caractériser des micro-organismes qui pourraient être de bons candidats pour le développement *i)* de solutions biocontrôle en agriculture et/ou ii) pour la production de composés bioactifs pour la pharmacie. Concernant la recherche de solutions microbiennes pour le biocontrôle, le principal modèle de bioagresseur qui a été retenu est le champignon pathogène *Fusarium graminearum*. Il s'agit en effet de l'un des principaux agents pathogènes d'une culture majeure, le blé et vis-à-vis duquel les moyens de lutte restent encore relativement limités, notamment vis-à-vis des pourritures racinaires provoquées par cet agent pathogène. Concernant la recherche de candidats pour la production de bioactifs, c'est le modèle de plantes qui a piloté la réflexion. Le travail a consisté à isoler les microorganismes sans a priori et sélectionner ceux qui étaient capable de produire des alcaloïdes connus ou moins étudiés.

Pour identifier les microorganismes candidats intéressants, j'ai exploré la diversité taxonomique et fonctionnelle des communautés microbiennes endophytes chez des espèces végétales d'intérêt agronomique ou pharmaceutique, en me concentrant sur les communautés bactériennes qui sont relativement moins étudiées que les communautés fongiques, notamment par rapport à leurs potentialités en termes de production de composés bioactifs [25].

Dans une première approche, je me suis intéressée à des espèces végétales présentant des propriétés particulières, susceptibles d'héberger des microorganismes présentant des potentialités originales [26], à savoir *Leucojum aestivum*, une plante de la famille des Amaryllidacées qui produit des alcaloïdes isoquinoléiques d'intérêt thérapeutique et le Vétiver (*Chryzopogon zizianoides*), une plante aromatique d'origine tropicale de la famille des Poacées. Dans une seconde approche, encore préliminaire et dédiée à la caractérisation de microorganismes antagonistes de *F. graminearum*, j'ai établi les conditions permettant d'améliorer la sélection de microorganismes d'intérêt, en utilisant des variétés de blé qui diffèrent dans leur tolérance vis-à-vis de l'agent pathogène cible et en m'appuyant sur le concept de suppressivité [27,28].

Chapitre 1 : Criblage des bactéries endophytes de *Leucojum aestivum* Gravety Giant comme source potentielle d'alcaloïdes et comme source d'antagonistes de certains agents pathogènes fongiques de blé

La famille des Amaryllidaceae renferme des plantes à bulbes qui produisent des alcaloïdes isoquinoléiques, appelés alcaloïdes d'Amaryllidaceae [29]. Parmi ces alcaloïdes, nous pouvons citer la lycorine qui est connue pour son activité allélopathique en réprimant les mauvaises herbes dans les champs de riz et la galantamine qui est utilisée en thérapeutique pour ses propriétés inhibitrices de l'acétylcholinestérase contre la maladie d'Alzheimer [30]. Le Laboratoire Lorrain de Chimie Moléculaire (L2CM) travaille sur cette famille de plantes en vue de contrôler

la production d'alcaloïdes d'Amaryllidaceae à partir de plantes *in vitro* [31,32]. L'équipe a découvert une bactérie endophyte isolée à partir de bulbes initiés en conditions *in vitro* de *Leucojum aestivum* capable de synthétiser des alcaloïdes d'Amaryllidaceae comme la lycorine et la tazettine par exemple [33]. Il n'y a eu que quelques articles sur les endophytes isolés de plantes de la famille des Amaryllidaceae [34,35], mais aucune étude n'a été réalisée sur la diversité taxonomique et fonctionnelle de ces endophytes chez *L. aestivum*.

Les alcaloïdes des Amaryllidaceae sont particulièrement concentrés dans les bulbes mais les profils alcaloïdiques diffèrent selon les organes végétaux [30]. La nature des alcaloïdes d'Amaryllidaceae est également influencée par les origines géographiques de la plante, son stade de développement et la présence d'agents pathogènes [36,37]. Notre objectif a été d'étudier la composition taxonomique des communautés bactériennes endophytes dans les différentes parties de la plante de *L. aestivum* et d'étudier les capacités métaboliques des membres de cette communauté.

Environ 140 souches bactériennes endophytes ont été isolées à partir de racines, de bulbes et de des parties aériennes de *L. aestivum* au stade végétatif. Ces souches ont été caractérisées taxonomiquement par séquençage de l'ARNr 16S. Nous avons mis en évidence que ces souches sont affiliées à 42 genres bactériens différents, au sein de 4 phyla i.e. les Protéobactéries, Firmicutes, Actinobactéries et Bacteroidetes. Une analyse NMDS a mis en évidence que la structure de la communauté d'endophytes bactériens cultivables diffère selon l'organe de la plante considéré.

La capacité des souches bactériennes endophytiques à produire des alcaloïdes a été analysée par chromatographie sur couche mince à haute performance (HPTLC) et en utilisant le réactif de Dragendorff comme révélateur. Ces analyses ont permis d'identifier 28 souches au niveau des bulbes, des racines et des tiges productrices d'alcaloïdes.

Les activités antagonistes des souches bactériennes endophytes vis-à-vis de *F. graminearum* et d'un autre champignon impliqué dans les symptômes de fusariose, *Microdochium nivale* ont également été évaluées en conditions de co-culture *in vitro*. Vingt-quatre souches ont présenté des activités antagonistes intéressantes vis-à-vis des 2 agents pathogènes, avec des taux d'inhibition (Ti%) d'au moins 30%. Ces souches sont affiliées i) aux Protéobactéries et aux genres *Parabulkholderia*, *Comamonas*, *Rahnella*, *Ensifer*, *Luteibacter* et *Erwinia*, ii) aux Actinobactéries, genre *Streptomyces* et iii) aux Firmicutes, genre *Bacillus*. Parmi ces souches, certaines d'entre elles ont été identifiées comme susceptibles de produire des alcaloïdes. Trois souches proches des espèces *P. fungorum*, *L. rhizovicinus* et *C. denitrificans* ont été sélectionnées afin d'identifier par LC-MS les alcaloïdes présents dans les extraits méthanoliques de ces souches. La présence d'alcaloïdes d'Amaryllidaceae n'a pu être mise en évidence dans ces extraits. Cependant un autre alcaloïde, le tryptophol, précurseur de divers alcaloïdes indoliques dans les plantes et les micro-organismes, a été identifié dans 2 des 3 souches (*L.*

rhizovicinus et *C. denitrificans)*. Le tryptophol pourrait participer aux effets antagonistes de ces souches vis-à-vis de *F. graminearum* et *M. nivale* [38]. Il serait intéressant de confirmer l'activité antifongique de cet alcaloïde mais aussi de rechercher d'autres alcaloïdes indoliques dans les extraits méthanoliques des bactéries endophytes isolées de *L. aestivum*.

Chapitre 2 : Caractérisation taxonomique des communautés bactériennes endophytes des racines de Vétiver et analyse des activités antagonistes vis-à-vis d'agents phytopathogènes

Le vétiver (*Chryzopogon zizanioides* (L.) Roberty) est une espèce végétale pérenne, de la famille des Poacées principalement valorisée en parfumerie et cosmétique pour son huile essentielle (HE) extraite des rhizomes. En plus de ses propriétés olfactives, l'HE de vétiver possède également des propriétés antimicrobiennes, notamment vis-à-vis de *F. graminearum* [39] (Slezack-Deschaumes, communication personnelle), ces propriétés dépendant de la qualité de l'HE considérée. Les travaux d'Adams et al. (2003) puis de Del Guidice et al. (2008) ont montré que les microorganismes endophytes des rhizomes de vétiver et en particulier certaines bactéries, influencent la synthèse de l'HE ainsi que sa composition phytochimique [40,41]. L'ensemble de ces données nous ont conduit à émettre l'hypothèse que certaines bactéries du microbiote endophyte du vétiver pourraient contribuer aux activités inhibitrices de l'HE vis-à-vis de *F. graminearum*, via la production de métabolites antimicrobiens. Ces communautés microbiennes pouvaient donc constituer un réservoir de solutions de biocontrôle vis-à-vis d'agents pathogènes tel que *F. graminearum.* Nous avons donc entrepris de caractériser taxonomiquement les communautés bactériennes endophytes associées aux racines de vétiver et nous avons évalué leurs activités antifongiques. Sachant que la diversité des communautés microbiennes endophytes peut être influencée par le contexte pédoclimatique dans lequel les plantes se développent, nous avons, pour maximiser les ressources microbiennes disponibles, entrepris d'échantillonner des vétivers dans plusieurs zones géographiques en Europe et en Afrique (Réunion, Madagascar, Sénégal et Portugal). Pour analyser la composition des communautés bactériennes endophytes globales dans les racines de vétiver, nous avons utilisé une approche de métabarcoding en ciblant les régions V5-V7 des gènes codant l'ARNr 16S

Nous avons montré que les principaux phyla bactériens des racines de vétiver sont Proteobacteria, Firmicutes, Bacteroidota, and Actinobacteriota (figure 1a). De plus, nous avons montré que les structures de ces communautés bactériennes étaient significativement différentes, l'origine géographique des vétivers (figure 1b). Une banque de souches bactériennes endophytes a également été constituée. Ainsi, 381 bactéries endophytes ont été isolés à partir de racines de vétiver prélevées sur les différents sites géographiques. Les activités antagonistes de ces isolats vis à vis de *F. graminearum* ont été analysés en co-culture *in vitro*. Cette analyse nous a permis de mettre en évidence que près de 50% des souches en collection

(153 souches) présentaient Ti% *in vitro* de la croissance mycélienne de *F. graminearum* d'au moins 50% et que 8% de ces souches (soit 31 souches) avaient un Ti% d'au moins 70% vis-à-vis de *F. graminearum*. La caractérisation taxonomique des 153 souches bactériennes ayant un Ti% d'au moins 50% a montré que ces microorganismes sont affiliés à 15 genres différents et notamment aux genres *Bacillus*, *Janthinobacterium*, *Kosakonia*, *Microbacterium*, *Pseudomonas* et *Serratia* pour les souches ayant un Ti% d'au moins 70% (Figure 1c). Ces analyses nous ont également permis de montrer que la structure de ces communautés bactériennes fonctionnelles cultivables est influencée par l'origine géographique des vétivers (Figure 1d).

Nous avons poursuivi le travail de caractérisation des 32 souches bactériennes les plus prometteuses en regard de leurs activités antagonistes vis-à-vis de *F. graminearum*. Nous avons tout d'abord analysé leur spectre d'activité vis-à-vis de 2 autres champignons pathogènes affiliés au genre *Fusarium* i.e. *F. oxysporum* et *F. culmorum*, une souche de l'espèce bactérienne *Escherichia coli* et une souche de levure *Saccharomyces cerevisiae*. Nous avons observé que les souches de bactéries endophytes affiliées aux genres *Serratia* et *Bacillus* sont celles qui présentent les activités antagonistes *in vitro* les plus importantes vis-à-vis de *F. oxysporum* et *F. culmorum*. Nous avons alors tenté de caractériser les métabolites sécrétés par ces souches bactériennes et qui pourraient participer à l'activité antagoniste. Pour cela, nous avons préparé des surnageants de culture de ces souches qui ont été filtrés afin d'éliminer les cellules bactériennes. Seuls les filtrats de culture des 5 souches proches de l'espèce *B. subtilis* ont montré une inhibition de la croissance mycélienne de *F. graminearum in vitro*. En collaboration avec les Dr. F. Coutte et E. Heuson de l'Université de Lille, nous avons entrepris de caractériser les lipopeptides produits par ces souches dans la mesure où ces métabolites secondaires microbiens sont considérés comme les principaux composés intervenant dans les effets d'inhibiteurs des espèces de *Bacillus* vis-à-vis de *F. graminearum* (REF). Ces analyses ont été complétées par des analyses *in silico* du génome des souches bactériennes affiliées à *B. subtilis* à l'aide de l'outil antiSmach, afin d'identifier les clusters de gènes NRPS pouvant participer à la synthèse des différentes classes de lipopeptides. Nous avons ainsi identifié 5 clusters NRPS dans le génome des souches de *B. subtilis*, avec notamment 2 clusters impliqués dans la synthèse de surfactine A et de plipastatine B/fengycine. La production de ces métabolites par nos souches candidates a été confirmée par les analyses en MALDI-TOF. Comme cela a été préalablement observé par Gong et al. (2015)[42], ces métabolites pourraient être impliqués dans les activités antifongiques des souches de *B. subtilis* endophytes des racines de vétiver. Il sera intéressant de poursuivre le travail de caractérisation des métabolites à activité antimicrobienne produits par d'autres souches candidates identifiées dans ce travail comme celles affiliées au genre *Serratia*. Au-delà des composés solubles, il serait intéressant également d'étudier les composés volatils produits par les souches candidates et qui pourraient également contribuer à l'effet antimicrobien observé.

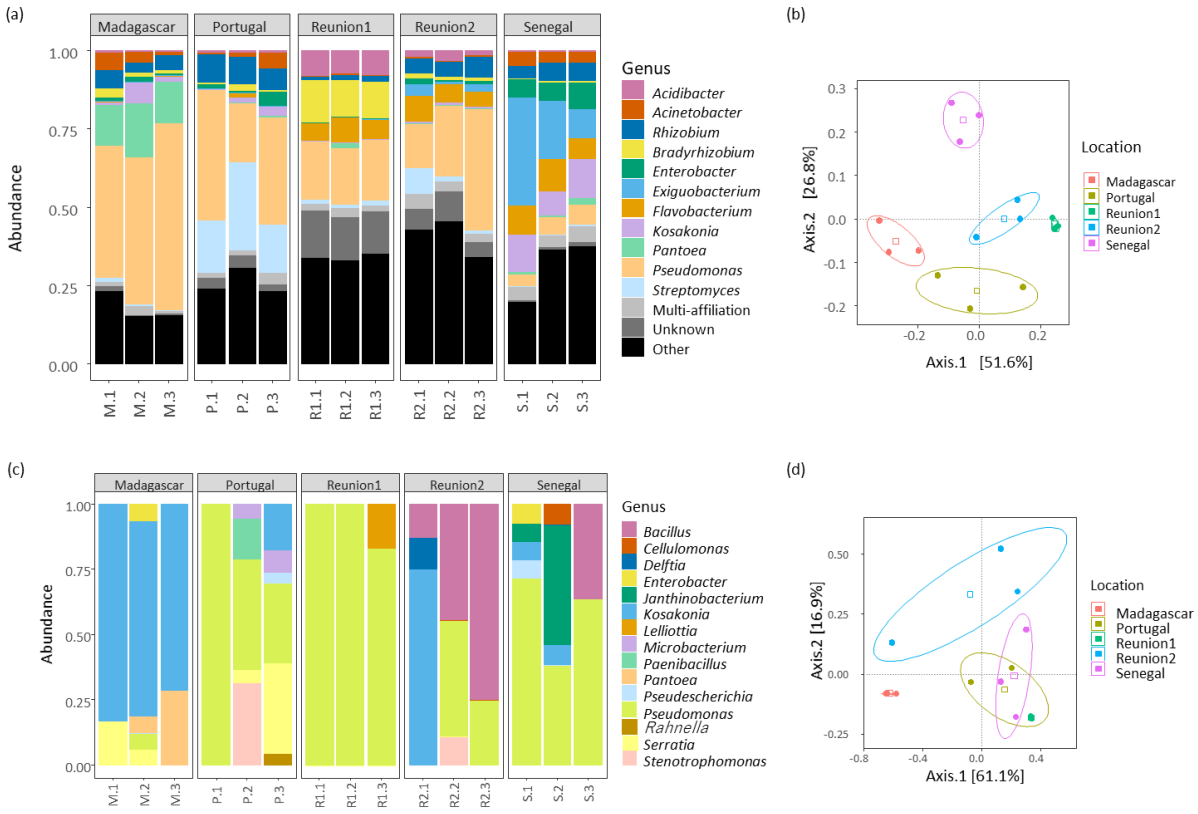

Figure 1 Analyse de la composition taxonomique et de la structure des communautés bactériennes endophytes de vétiver provenant de différentes zones géographiques (Munakata et al., 2021). *(a) Relative abundance of dominant genera of bacterial endophytes; (b) PCoA with weighted UniFrac distances, colored by location; (c) Relative abundance of bacterial genera presenting in the functionally selected communities; (d) PCoA with Bray-Curtis dissimilarity, colored by location. In PCoAs, round points indicate individual plants, and squares mean the center of gravity within a location group.*

Chapitre 3: Tests préliminaires pour créer artificiellement le microbiome du sol suppressif contre *Fusarium*, la pourriture des racines chez le blé

La dernière partie de mon travail a été consacrée à la mise au point d'une méthode d'ingénierie du microbiote de la plante et ce, afin d'améliorer l'efficacité de sélection de microorganismes ayant une activité de biocontrôle vis-à-vis de *F. graminearum*. Cette approche définie par Sachs et Mueller (2015) sous le terme « Host Mediated Microbiome Engineering – HMME » s'appuie sur une hypothèse selon laquelle la plante peut recruter, dans des conditions de stress, des microorganismes lui permettant d'améliorer sa tolérance à ces conditions [28,43]. Pour mettre en place l'approche HMME, nous avons envisagé d'utiliser différents génotypes de la plante hôte de *F. graminearum* (i.e. le blé), différant dans leur profil de tolérance vis-à-vis de la fusariose des épis. En effet, il a été montré récemment que certains cultivars tolérants à des agents

pathogènes peuvent héberger dans leur microbiote racinaire et comparativement à des cultivars sensibles, des taxons ayant un effet bioprotecteur vis-à-vis de ces agents pathogènes [44,45]. L'utilisation de génotypes de blé plus ou moins tolérants à *F. graminearum* pourraient permettre d'identifier au sein du microbiote endophyte, des micro-organismes candidats améliorant la tolérance du blé à *F. graminearum* [46,47]. Pour renforcer la probabilité de sélection de ces microorganismes d'intérêt, nous avons également envisagé d'exploiter le phénomène de suppressivité, initialement décrit pour les sols. Un sol suppressif est en effet un sol sur lequel l'incidence d'une maladie décroit avec le temps et ce, même si la plante hôte de l'agent pathogène responsable de cette maladie est cultivée sur ce sol. Ce phénomène résulte majoritairement du développement, dans la rhizosphère de la plante hôte et sous l'effet de la pression de sélection (agent pathogène), de populations microbiennes qui vont protéger la plante vis-à-vis de l'agent pathogène [48]. Récemment, Carrion et al. (2019) ont montré que les communautés microbiennes endophytes des racines pouvaient également contribuer à ce phénomène de suppressivité [27]. Ces résultats permettaient donc d'exploiter ce phénomène de suppressivité pour la sélection de microorganismes endophytes antagonistes de *F. graminearum*.

Quatre variétés de blé (Pilier, Concret, Oregrain et Mutic, Desprez semences) ont été sélectionnées pour ces expérimentations. Ces variétés présentent une tolérance différente vis à vis de la fusariose de l'épi (FHB). Afin d'utiliser ces variétés pour sélectionner des microorganismes qui pourraient permettre de protéger le blé vis à vis des pourritures racinaires provoquées par *F. graminearum* (FRR), nous avons établi leur profil de sensibilité vis-à-vis de cette maladie. Les plantes ont été cultivées jusqu'au stade 1-2 feuilles dans un sol agricole. Avant semis, semences pré-germées ont été traitées avec une suspension de conidies de *F. graminearum*. Environ 21 jours après semis (stade 1-2 feuilles), les symptômes de maladie, la biomasse des jeunes plantules et la longueur des racines ont été estimés. Ces premières analyses nous ont permis de mettre en évidence que la variété Concret est la variété la plus tolérante à la FRR, tandis que la variété Pilier est-elle la plus sensible. Les deux autres variétés présentent des tolérances intermédiaires. Le gradient de tolérance des variétés de blé vis-à-vis de la FRR pourrait être associé une composition différente du microbiote endophyte, avec notamment la présence ou des différences d'abondance relative de taxons ayant des effets antagonistes vis-à-vis de *F. graminearum* variant selon la variété considérée. Ces premiers résultats permettent de mettre en œuvre ces variétés pour l'approche de sélection des microorganismes d'intérêt décrite précédemment.

Conclusion générale et perspectives

Dans le cadre de ce travail de thèse, nous avons exploré la diversité taxonomique et fonctionnelle de communautés bactériennes d'endophytes afin d'identifier des microorganismes candidats intéressants pour le développement de solutions de biocontrôle (à

base du microorganisme ou de ses métabolites) vis-à-vis d'un agent pathogène majeur du blé *F. graminearum* et/ou pour la production de composés d'intérêt pharmaceutique. Pour le sourcing de ces microorganismes, nous avons principalement étudié deux espèces végétales ayant des propriétés originales, le vétiver et *L. aestivum*. Afin d'accroitre la diversité microbienne disponible pour la caractérisation des microorganismes d'intérêt, nous avons considéré des plantes provenant de différentes zones géographiques (cas du vétiver) ou différents organes de la plante (cas de *L. aestivum*). Nous avons montré tout d'abord que la structure taxonomique des communautés bactériennes d'endophytes variait selon l'origine géographique des plantes ou selon les organes considérés. Nous avons ensuite constitué, pour chacune des espèces végétales considérées, des banques de souches bactériennes qui ont été caractérisées taxonomiquement et fonctionnellement pour leurs capacités i) à inhiber la croissance mycélienne en conditions *in vitro* de *F. graminearum* et/ou ii) à produire des métabolites spécialisés à activité antimicrobienne ou d'intérêt thérapeutique.

Concernant les souches isolées des parties aériennes et souterraines (racines et bulbes) de *L. aestivum*, nous avons montré que sur les 140 souches analysées, 28 d'entre elles produisent des alcaloïdes et ce, même si aucune de ces souches ne produit les alcaloïdes d'Amaryllidacées. Nous avons également mis en évidence 24 souches qui inhibent la croissance mycélienne de *F. graminearum* mais aussi d'un autre agent pathogène impliqué dans les fusarioses, *M. nivale*. Parmi ces souches, certaines d'entre elles produisent des alcaloïdes et notamment 2 souches affiliées à *C. denitrificans* et *L. rhizovicinus* qui produisent du tryptophol, un précurseur des alcaloïdes indoliques, qui possède des propriétés antifongiques mais aussi phytostimulantes. Ces souches constituent les premiers candidats prometteurs pour le biocontrôle des agents pathogènes des fusarioses et sont aussi une source prometteuse de biomolécules à intérêt pharmaceutique, comme par exemple des molécules antimicrobiennes mais aussi des composés antitumoraux, antiviraux, antioxydants… [26]. Il serait intéressant de poursuivre l'étude phytochimique des souches bactériennes d'endophytes obtenues dans cette thèse afin d'évaluer leurs potentialités en termes de production de molécules bioactives.

Concernant les souches de vétiver, sur les près de 400 souches caractérisées, 32 d'entre elles présentent des activités antagonistes prometteuses vis-à-vis de *F. graminearum* en tests de co-culture *in vitro*. Ces souches sont affiliées à 6 genres de bactéries dont le genre *Bacillus* dont certains membres sont connus pour leurs activités antagonistes vis-à-vis de *F. graminearum*. L'analyse des métabolites à activité antimicrobienne produits par les 5 souches affiliées à l'espèce *B. subtilis* isolées montre qu'elles produisent des lipopeptides et notamment de la surfactine A et de la plipastatine qui sont des composés qui pourraient intervenir dans leur mode d'action vis-à-vis de *F. graminearum*. Ces souches constituent, là aussi, des premiers candidats prometteurs en tant qu'agents de biocontrôle vis-à-vis de *F. graminearum* dont il conviendra *i)* de mieux comprendre le mode d'action (effet sur la croissance des plantes, induction des défenses de la plante…) et *ii)* d'évaluer l'efficacité réelle *in planta*. Il sera

intéressant également de poursuivre la caractérisation des métabolites produits par les autres souches candidates identifiées dans ce travail et notamment celles affiliées au genre *Serratia* qui montrent, en plus de leurs effets sur la croissance mycélienne de *F. graminearum,* une activité intéressante d'inhibition de la germination des spores de cet agent.

L'ensemble de ce travail a permis, en explorant la diversité microbienne associée à des plantes ayant des propriétés particulières de disposer d'un matériel biologique très riche à valoriser en tant que solutions de biocontrôle ou pour la production de composés bioactifs.

En ouverture à ces travaux, nous avons envisagé une autre approche pour sélectionner des microorganismes d'intérêt. Pour cela nous avons établi les conditions pour mettre en place une approche innovante d'ingénierie du microbiote de la plante pour la sélection de souches bactériennes d'endophytes efficientes dans la protection du blé vis-à-vis de *F. graminearum*. Ce travail, en cours, vise à apporter une première preuve de concept de l'intérêt de s'appuyer sur le génotype de l'hôte et la suppressivité des sols pour orienter le recrutement par la plante de microorganismes bénéfiques. Il pourrait ouvrir de nouvelles perspectives pour la sélection de solutions microbiennes de biocontrôle.

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Annex

Chapter 2 _ Part 1 Table S1 185
Chapter 2 _ Part 1 Table S3 201

Chapter 2 _ Part 1

Table S1| OUT table of bacterial communities of vetiver root samples from five different locations

#comment	blast_taxo	Phylum	Class	Order	Family	Genus	Species	blast_subj	blast_perc	blast_perc	blast_eval	blast_aln_	seed_id	observatic	observatic	M.1	M.2	M.3	P.1	P.2	P.3	R1.1	R1.2	R1.3	R2.1	R2.2	R2.3	S.1	S.2	S.3	
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Spirosoma	Spirosoma	Spirosoma	LC221821.	99.76	100	0	423	M05317:1	Cluster_16	101	0	0	0	74	27	0	0	0	0	0	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Mucilagini	Multi-affili	multi-subj	99.52	100	0	420	M05317:1	Cluster_16	288	1	1	5	36	4	2	49	67	70	16	21	8	3	3	2	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Labraceae	Labrys	Labrys ne	DQ417335	100	100	0	427	M05317:1	Cluster_16	132	8	4	1	28	29	47	4	5	4	0	0	0	2	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_16	125	0	0	0	0	1	0	0	0	0	9	5	1	30	50	29	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Neorhizob	AM237359	100	100	0	423	M05317:1	Cluster_16	149	16	17	100	6	0	0	0	4	1	1	0	3	1	0	0	
na	Bacteria	Proteobac	Gammapr	Alteromor	Alteromor	Rheinheim	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_16	111	0	0	0	10	63	0	0	0	0	8	16	10	2	2	0	
na	Bacteria	Proteobac	Gammapr	Salinispha	Solimonac	Nevskia	Nevskia sp	MH69868	100	100	0	423	M05317:1	Cluster_51	50	0	0	0	0	0	0	13	10	27	0	0	0	0	0	0	
na	Bacteria	Desulfoba	Desulfovit	Desulfovib	Desulfovit	Desulfovit	unknown :	AB678351	100	100	0	425	M05317:1	Cluster_51	39	0	0	0	0	0	0	0	0	0	13	1	25	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Pseudorhc	unknown :	KJ590689.	100	100	0	427	M05317:1	Cluster_51	45	0	0	0	3	0	0	1	1	3	10	13	4	2	8	0	
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Chitinoph	Multi-affili	multi-subj	99.76	100	0	423	M05317:1	Cluster_20	18	0	0	0	0	1	0	0	2	0	0	0	0	2	6	7	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	unknown :	multi-subj	100	100	0	426	M05317:1	Cluster_23	122	7	2	2	0	0	0	46	37	28	0	0	0	0	0	0	
na	Bacteria	Myxococc	Polyangia	Haliangial	Haliangiac	Haliangiur	unknown :	HQ119152	99.53	100	0	425	M05317:1	Cluster_69	35	0	2	0	0	0	0	2	18	13	0	0	0	0	0	0	
na	Bacteria	Myxococc	Myxococci	Myxococc	Myxococc	P3OB-42	metageno	FPLS01020	99.53	100	0	422	M05317:1	Cluster_51	23	0	0	0	7	5	1	1	3	2	4	0	0	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	FR872468.	100	100	0	423	M05317:1	Cluster_16	141	105	3	0	3	8	0	6	4	10	0	1	1	0	0	0	
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Rhizobact	Multi-affili	multi-subj	100	100	0	419	M05317:1	Cluster_13	17	0	0	0	0	0	1	3	6	3	1	0	3	0	0	0	
na	Bacteria	Proteobac	Gammapr	Cellvibrior	Cellvibrior	Cellvibrio	unknown :	JN408903.	100	100	0	425	M05317:1	Cluster_13	21	0	0	0	2	0	0	0	0	0	7	6	6	0	0	0	
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Pseudomo	LKBM0100	100	100	0	425	M05317:1	Cluster_25	1092	58	144	147	63	19	63	35	30	38	38	255	202	0	0	0	
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_24	953	129	88	52	4	0	4	155	322	185	4	3	7	0	0	0	
na	Bacteria	Proteobac	Gammapr	Enterobac	Erwiniace	Pantoea	Pantoea sp	KJ607243.	100	100	0	424	M05317:1	Cluster_27	508	137	260	111	0	0	0	0	0	0	0	0	0	0	0	0	
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Pseudomo	multi-subj	100	100	0	425	M05317:1	Cluster_26	481	167	170	144	0	0	0	0	0	0	0	0	0	0	0	0	
na	Bacteria	Proteobac	Gammapr	Enterobac	Erwiniace	Pantoea	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_21	806	255	255	157	5	8	2	2	0	7	0	11	1	18	15	70	
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	432	M05317:1	Cluster_20	1549	14	6	3	11	349	121	24	13	27	761	131	86	1	1	1	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_23	779	186	87	281	12	14	18	48	44	66	5	6	5	3	2	2	
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Enterobac	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_22	1674	66	67	36	92	47	462	0	8	8	186	16	81	149	188	268	
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Acidovora	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_19	125	0	0	0	23	16	1	0	1	0	9	3	10	8	25	29	
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	99.07	100	0	432	M05317:1	Cluster_19	156	0	0	0	35	79	42	0	0	0	0	0	0	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Mucilagini	Multi-affili	multi-subj	99.76	100	0	420	M05317:1	Cluster_19	76	7	1	61	0	0	0	0	1	6	0	0	0	0	0	0	
na	Bacteria	Proteobac	Gammapr	Gammapr	unknown	Acidibacte	unknown :	KC331225.	100	100	0	423	M05317:1	Cluster_29	836	5	2	0	25	31	26	43	41	46	182	291	131	2	3	8	
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Enterobac	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_28	1704	31	109	2	0	1	16	0	0	0	2	20	19	457	416	631	
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Multi-affili	multi-subj	100	100	0	418	M05317:1	Cluster_19	79	45	12	7	0	0	0	0	0	0	2	10	2	0	1	0	
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Sphingopy	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_14	11	2	1	0	0	0	0	0	0	0	4	3	1	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	NS9 marin	unknown	unknown :	FJ947136.	99.52	100	0	419	M05317:1	Cluster_13	15	1	0	0	0	0	0	0	0	0	5	9	0	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown	unknown :	multi-subj	99.05	100	0	420	M05317:1	Cluster_48	52	0	0	0	4	0	1	6	17	18	2	1	0	0	3	0	
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Puia	unknown :	KC172308.	100	100	0	423	M05317:1	Cluster_14	13	7	0	0	0	0	0	2	0	4	0	0	0	0	0	0	
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	431	M05317:1	Cluster_58	74	14	1	1	1	4	1	0	0	0	20	14	15	0	2	1	
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	99.29	100	0	425	M05317:1	Cluster_76	13	0	3	4	3	0	0	1	0	1	0	0	1	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	multi-subj	100	100	0	423	M05317:1	Cluster_76	22	0	0	0	0	0	0	0	0	0	0	0	1	3	12	6	
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Mucilagini	Multi-affili	multi-subj	100	100	0	419	M05317:1	Cluster_76	20	3	1	0	0	4	0	0	0	0	0	0	12	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhodobac	Rhodobac	Defluviimc	unknown :	KF697580.	99.06	100	0	424	M05317:1	Cluster_76	27	0	0	0	0	0	0	0	0	0	9	16	2	0	0	0	
na	Bacteria	Fibrobacte	Fibrobacte	Fibrobacte	Fibrobacte	possible g	unknown :	JQ798485.	100	100	0	427	M05317:1	Cluster_76	32	0	0	0	0	0	0	1	11	18	0	0	2	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Chitinoph	unknown :	EU133723	100	100	0	423	M05317:1	Cluster_69	37	0	0	0	0	0	0	1	0	0	35	0	0	0	1	0	
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Multi-affil	Multi-affili	multi-subj	99.76	100	0	419	M05317:1	Cluster_56	34	0	0	0	0	0	0	0	0	0	11	15	8	0	0	0	
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	432	M05317:1	Cluster_56	35	2	0	1	0	0	0	6	8	17	0	0	0	0	0	1	
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	100	100	0	417	M05317:1	Cluster_60	70	0	0	0	0	0	0	8	10	4	3	41	4	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Pseudolab	unknown :	LN567255.	100	100	0	426	M05317:1	Cluster_60	29	0	0	0	5	11	6	0	0	0	1	1	1	2	1	1	
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	432	M05317:1	Cluster_60	32	12	4	4	0	0	0	4	1	6	1	0	0	0	0	0	
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	431	M05317:1	Cluster_56	55	6	2	2	3	0	1	0	0	0	12	17	10	0	0	2	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	A0839	unknown	unknown :	multi-subj	100	100	0	422	M05317:1	Cluster_56	81	0	0	0	0	0	0	2	22	9	16	22	10	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiale	Nordella	unknown :	JN643000.	100	100	0	427	M05317:1	Cluster_56	46	0	0	0	0	0	0	18	7	20	0	1	0	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Sphingom	unknown :	FJ432345.	99.53	100	0	427	M05317:1	Cluster_18	11	0	0	0	4	4	1	0	0	1	1	0	0	0	0	0	
na	Bacteria	Multi-affil	Multi-affili	Multi-affil	Multi-affili	Multi-affil	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_56	43	5	3	1	3	4	4	11	8	2	1	1	0	0	0	0	
na	Bacteria	Firmicutes	Clostridia	unknown	Hungateic	Ruminiclo	Multi-affili	multi-subj	99.76	100	0	423	M05317:1	Cluster_83	25	1	0	0	0	0	0	0	0	0	0	1	23	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	99.76	100	0	418	M05317:1	Cluster_69	29	0	0	0	0	0	0	3	10	3	3	9	1	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Afipia	unknown :	AF382111.	99.77	100	0	427	M05317:1	Cluster_36	45	38	3	0	0	1	0	2	0	0	0	0	0	0	0	1	
na	Bacteria	Firmicutes	Bacilli	Bacillales	Planococc	Solibacillu	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_51	31	17	1	4	0	0	6	0	0	0	0	0	0	0	0	3	
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Chitinoph	Multi-affili	multi-subj	99.76	100	0	423	M05317:1	Cluster_52	36	0	0	0	0	0	0	0	0	0	2	0	0	5	12	17	
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	multi-iden	multi-cove	0	multi-aligr	M05317:1	Cluster_10	8	0	2	6	0	0	0	0	0	0	0	0	0	0	0	0	
na	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillacea	Bacillus	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_11	18	2	1	0	0	0	2	0	1	0	2	9	1	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	unknown :	multi-subj	99.3	100	0	427	M05317:1	Cluster_10	16	3	0	1	2	4	4	0	1	1	0	0	0	0	0	0	
na	Bacteria	Acidobact	Acidobacti	Acidobact	unknown	unknown	unknown :	JF428965.	99.53	100	0	425	M05317:1	Cluster_10	24	0	0	0	1	1	0	3	11	8	0	0	0	0	0	0	
na	Bacteria	Myxococc	Polyangia	Haliangial	Haliangiac	Haliangiur	unknown :	KF836277.	99.53	100	0	426	M05317:1	Cluster_94	20	0	0	0	0	0	0	0	0	0	4	12	4	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	KR051039.	99.76	100	0	423	M05317:1	Cluster_94	18	6	0	0	0	0	0	2	0	0	1	5	3	0	1	0	

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na	Bacteria	Actinobac	Thermolec	Solirubrot	67-14	unknown	unknown	multi-subj	99.76	100	0	423	M05317:1	Cluster_19	123	16	4	3	1	9	6	26	35	23	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Puia	Multi-affili	multi-subj	99.05	100	0	423	M05317:1	Cluster_12	15	0	0	0	0	4	2	0	7	2	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	R7C24	unknown	unknown	unknown	multi-subj	100	100	0	423	M05317:1	Cluster_12	35	0	0	0	0	0	0	1	2	0	4	20	8	0	0	0
na	Bacteria	Acidobact	Acidobacti	Bryobacte	Bryobacte	Bryobacte	unknown	multi-subj	100	100	0	424	M05317:1	Cluster_12	14	0	0	0	0	0	1	0	0	0	4	5	4	0	0	0
na	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillacea	Bacillus	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_12	12	4	5	2	0	0	0	0	0	1	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	Chryseolin	Multi-affili	multi-subj	99.76	100	0	420	M05317:1	Cluster_13	173	3	0	0	15	34	10	1	3	8	56	36	6	0	1	0
na	Bacteria	Acidobact	Acidobacti	Subgroup	unknown	unknown	unknown	multi-subj	100	100	0	423	M05317:1	Cluster_18	19	1	0	0	0	0	0	9	4	5	0	0	0	0	0	0
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Comamon	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_64	39	0	0	0	0	0	0	1	5	4	5	13	6	1	1	3
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Rhodopse	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_64	54	7	3	7	2	3	4	0	6	6	7	5	3	1	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown	unknown	EU133746	99.76	100	0	419	M05317:1	Cluster_64	29	0	0	0	0	0	0	0	0	0	16	10	3	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhodobac	Rhodobac	Falsirhodc	Falsirhodo	HE662814	99.53	100	0	424	M05317:1	Cluster_13	108	53	21	34	0	0	0	0	0	0	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Tardiphag	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_13	219	159	9	41	2	6	0	0	1	1	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	HQ220107	99.77	100	0	427	M05317:1	Cluster_13	178	0	0	0	0	0	0	0	0	0	22	14	6	24	73	39
na	Bacteria	Proteobac	Gammapr	Aeromona	Aeromona	Aeromona	unknown	JN697753.	100	100	0	425	M05317:1	Cluster_13	192	0	0	0	0	0	1	0	0	0	14	5	6	19	55	92
na	Bacteria	Proteobac	Gammapr	Enterobac	Erwiniace	Pantoea	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_13	125	29	5	89	1	0	0	0	0	1	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Novosphir	Multi-affili	multi-subj	99.76	100	0	423	M05317:1	Cluster_52	84	0	0	0	5	3	6	0	0	0	19	18	12	3	7	11
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Tahibacte	Multi-affili	multi-subj	99.53	100	0	426	M05317:1	Cluster_52	49	0	0	0	5	1	0	0	4	1	26	2	2	1	4	3
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	99.06	100	0	multi-aligr	M05317:1	Cluster_52	48	0	0	0	1	1	2	14	13	14	0	0	3	0	0	0
na	Bacteria	Multi-affil	Multi-affili	Multi-affil	Multi-affili	Multi-affil	Multi-affili	multi-subj	99.53	100	0	423	M05317:1	Cluster_20	8	0	0	0	4	0	0	0	0	0	0	2	2	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhodobac	Rhodobac	Rhodobac	unknown	multi-subj	99.76	100	0	424	M05317:1	Cluster_52	41	0	0	0	0	0	0	0	0	0	3	28	10	0	0	0
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Dokdonell	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_11	180	4	9	0	27	58	19	10	23	22	1	0	0	0	1	6
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	unknown	KF963650.	100	100	0	424	M05317:1	Cluster_48	59	1	0	0	5	2	2	7	4	6	15	11	5	0	1	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	JF424606.	100	100	0	423	M05317:1	Cluster_48	45	0	0	0	0	0	0	0	2	1	21	6	14	0	0	1
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	99.52	100	0	417	M05317:1	Cluster_48	48	0	0	0	0	0	0	3	1	0	39	4	0	0	1	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Rhodopse	unknown	multi-subj	99.53	100	0	425	M05317:1	Cluster_52	35	29	0	1	0	2	0	1	1	1	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Flavobacte	MF538728	100	100	0	419	M05317:1	Cluster_48	92	1	0	0	0	0	0	9	13	11	16	18	9	5	7	3
na	Bacteria	Myxococc	Polyangia	Polyangial	Sandaracii	unknown	unknown	HQ120302	99.3	100	0	427	M05317:1	Cluster_30	72	0	0	0	9	2	0	0	0	0	18	36	7	0	0	0
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Multi-affil	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_58	387	19	50	17	1	80	71	0	0	0	3	3	1	84	22	36
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Klebsiella	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_59	572	1	21	0	0	0	0	0	0	0	1	3	5	154	193	194
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	A0839	unknown	unknown	EU644201	99.53	100	0	422	M05317:1	Cluster_70	31	0	0	0	0	0	0	8	12	11	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	FJ025129.	100	100	0	423	M05317:1	Cluster_54	348	52	89	17	43	54	21	3	15	13	2	1	37	0	0	1
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Mesorhizc	Mesorhizo	AF514800.	99.77	100	0	427	M05317:1	Cluster_55	811	42	21	26	99	198	134	91	70	54	11	13	8	9	7	28
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	Ohtaekwa	metageno	FPLS01029	99.29	100	0	420	M05317:1	Cluster_57	588	0	0	0	0	0	0	1	1	0	107	395	84	0	0	0
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	431	M05317:1	Cluster_50	414	0	1	0	162	183	66	0	0	0	1	1	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Niastella	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_51	502	4	3	12	2	8	2	28	29	55	144	67	45	25	29	49
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Caulobact	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_52	364	2	3	0	121	110	55	8	37	7	8	6	7	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	unknown	LN568159.	100	100	0	427	M05317:1	Cluster_53	587	4	9	1	58	61	13	3	13	9	121	92	45	34	64	60
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	unknown	multi-subj	100	100	0	417	M05317:1	Cluster_70	31	0	0	0	3	6	0	4	6	10	0	0	2	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Hyphomic	Hyphomic	unknown	KJ548893.	99.76	100	0	424	M05317:1	Cluster_28	21	2	0	0	2	1	2	1	1	0	2	6	4	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Chryseoba	HG738133	99.76	100	0	419	M05317:1	Cluster_81	21	0	0	0	0	0	0	0	0	0	0	0	0	5	14	2
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Pedobacte	Pedobacte	FJ517612.	99.52	100	0	420	M05317:1	Cluster_23	13	0	0	0	0	0	0	1	4	7	0	1	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Multi-affili	Multi-affil	Multi-affili	multi-subj	99.06	100	0	427	M05317:1	Cluster_15	21	0	0	0	0	0	0	0	0	0	4	0	1	2	8	6
na	Bacteria	Acidobact	Acidobacti	Solibacter	Solibacter	Candidatu	unknown	HM06203	100	100	0	426	M05317:1	Cluster_15	22	0	0	0	0	1	0	8	4	9	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Altereryth	Multi-affili	multi-subj	99.77	100	0	427	M05317:1	Cluster_95	21	0	0	0	7	4	4	0	0	0	3	2	0	0	1	0
na	Bacteria	Myxococc	Polyangia	Haliangial	Haliangiac	Haliangiur	unknown	EU134563	99.77	100	0	426	M05317:1	Cluster_10	38	4	5	0	0	0	0	7	10	12	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Pseudofla	unknown	GQ263710	99.76	100	0	423	M05317:1	Cluster_11	12	0	0	0	1	3	3	0	0	0	0	0	1	0	2	2
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Edaphoba	unknown	EU133689	99.53	100	0	424	M05317:1	Cluster_73	23	0	0	0	0	10	1	0	3	0	2	1	6	0	0	0
na	Bacteria	Fibrobacte	Fibrobacte	Fibrobacte	Fibrobacte	possible g	unknown	KC432198.	100	100	0	427	M05317:1	Cluster_63	53	0	0	0	0	1	0	13	10	16	3	7	3	0	0	0
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Phenyloba	Multi-affili	multi-subj	100	100	0	422	M05317:1	Cluster_82	52	1	0	0	2	7	1	4	5	3	10	11	3	2	3	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	99.06	100	0	425	M05317:1	Cluster_27	17	0	2	4	0	0	6	0	0	2	2	0	1	0	0	0
na	Bacteria	Proteobac	Gammapr	Enterobac	Erwiniace	Pantoea	Mixta thei	AB907776	100	100	0	424	M05317:1	Cluster_82	24	0	0	0	0	0	0	0	0	0	0	0	0	3	6	15
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Caulobact	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_16	19	0	0	0	0	0	0	4	10	5	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	metageno	FPLS01013	100	100	0	424	M05317:1	Cluster_63	30	0	0	0	0	0	0	1	3	0	4	1	2	5	1	13
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Cyclobacte	unknown	unknown	FM866274	99.29	100	0	421	M05317:1	Cluster_73	25	0	0	0	19	3	1	0	0	0	0	0	0	1	0	1
na	Bacteria	Proteobac	Gammapr	Enterobac	Erwiniace	Pantoea	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_9	1676	448	755	438	6	3	4	3	1	7	0	0	0	2	3	6
na	Bacteria	Proteobac	Alphaprot	Rhodobac	Rhodobac	Gemmoba	Gemmoba	multi-subj	99.76	100	0	425	M05317:1	Cluster_63	33	0	0	0	0	0	0	0	0	0	1	23	8	0	1	0
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Dokdonell	unknown	JF833464.	99.77	100	0	426	M05317:1	Cluster_63	78	0	0	0	7	21	3	19	14	14	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Taibaiella	Multi-affili	multi-subj	99.52	100	0	420	M05317:1	Cluster_63	51	1	16	0	0	0	0	3	6	23	0	0	2	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiale	Alsobacte	Alsobacte	multi-subj	100	100	0	425	M05317:1	Cluster_16	10	0	0	0	3	5	2	0	0	0	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	unknown	unknown	EU202832	99.53	100	0	424	M05317:1	Cluster_13	30	0	0	0	0	2	0	14	6	8	0	0	0	0	0	0
na	Bacteria	Myxococc	Polyangia	Haliangial	Haliangiac	Haliangiur	unknown	multi-subj	100	100	0	428	M05317:1	Cluster_44	43	6	2	3	2	0	0	15	8	7	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown	unknown	AB240511	100	100	0	420	M05317:1	Cluster_23	23	0	0	0	0	0	0	2	4	1	3	5	8	0	0	0
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	432	M05317:1	Cluster_4	3265	2	0	0	566	1756	931	0	0	0	7	0	1	1	0	1

na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	unknown	HQ190331	100	100	0	425	M05317:1	Cluster_45	84	2	0	0	2	1	2	16	15	16	8	2	2	0	9	9
na	Bacteria	Gemmatin	Gemmatin	Gemmatin	Gemmatin	unknown	unknown	JF265839.	100	100	0	428	M05317:1	Cluster_25	11	0	0	0	0	0	0	0	7	4	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Moraxella	Acinetoba	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_10	15	5	9	1	0	0	0	0	0	0	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Chryseoba	KX228235.	100	100	0	418	M05317:1	Cluster_10	20	0	0	0	0	0	0	0	0	0	0	0	0	6	10	4
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Niastella	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_22	113	0	1	0	7	3	3	39	15	45	0	0	0	0	0	0
na	Bacteria	Firmicutes	Bacilli	Paenibacil	Paenibacil	Paenibacil	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_10	14	1	0	0	0	0	12	0	0	0	0	1	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	multi-subj	100	100	0	423	M05317:1	Cluster_22	85	0	0	1	25	24	10	2	3	4	5	0	9	0	0	2
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Ferruginib	Multi-affili	multi-subj	99.53	100	0	423	M05317:1	Cluster_22	101	0	0	0	11	56	21	0	2	1	1	6	3	0	0	0
na	Bacteria	Deinococc	Deinococc	Deinococc	Deinococc	Deinococc	Multi-affili	multi-subj	99.76	100	0	421	M05317:1	Cluster_10	18	0	0	0	0	0	0	0	0	0	0	0	0	4	4	10
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown	unknown	multi-subj	100	100	0	420	M05317:1	Cluster_22	132	0	0	0	0	0	0	67	25	40	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown	Multi-affili	multi-subj	99.76	100	0	420	M05317:1	Cluster_22	160	2	0	0	22	3	3	16	49	54	0	1	10	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	unknown	KC331556.	100	100	0	417	M05317:1	Cluster_22	215	0	0	0	0	0	0	16	70	32	75	2	2	0	16	2
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Ochrobact	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_73	19	0	0	9	3	4	2	0	0	0	0	0	0	0	1	0
na	Bacteria	Proteobac	Gammapr	Enterobac	Erwiniacea	Erwinia	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_13	12	2	4	0	0	0	0	1	1	0	0	0	0	0	0	4
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	unknown	KJ191963.	99.77	100	0	427	M05317:1	Cluster_28	113	7	2	2	0	0	0	59	20	23	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_1	19663	2058	1814	1920	3705	1505	2306	1474	1062	1366	187	1139	1123	0	1	3
na	Bacteria	Proteobac	Gammapr	Gammapr	unknown	Acidibacte	unknown	LN567553.	100	100	0	423	M05317:1	Cluster_90	460	5	5	2	5	3	6	140	138	134	3	2	4	5	5	3
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Multi-affil	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_91	269	5	3	1	54	8	10	20	24	19	54	37	21	2	9	2
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	Ohtaekwa	unknown	LN570710.	99.29	100	0	420	M05317:1	Cluster_91	25	0	0	0	0	0	0	0	2	0	5	6	11	0	1	0
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Novosphir	unknown	AY792290.	99.29	100	0	424	M05317:1	Cluster_91	34	0	0	0	0	0	0	0	0	0	0	0	0	5	24	5
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Edaphoba	metageno	multi-subj	99.53	100	0	423	M05317:1	Cluster_15	21	0	0	0	0	2	0	5	4	8	1	0	1	0	0	0
na	Bacteria	Proteobac	Alphaprot	Reyranella	Reyranella	Reyranella	unknown	KU713259	99.53	100	0	427	M05317:1	Cluster_91	30	0	0	0	1	0	0	13	4	12	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Gammapr	unknown	Acidibacte	unknown	multi-subj	99.05	100	0	423	M05317:1	Cluster_15	12	0	0	0	0	0	0	4	6	2	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Altereryth	unknown	FJ889313.	100	100	0	427	M05317:1	Cluster_91	23	0	0	0	1	15	4	0	1	1	0	1	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Spirosoma	Dyadobac	Dyadobac	HQ231938	99.76	100	0	421	M05317:1	Cluster_91	38	0	0	0	8	0	3	2	16	8	1	0	0	0	0	0
na	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillacea	Bacillus	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_11	29	1	1	0	0	0	0	0	0	0	8	10	3	1	2	3
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Asticcacau	unknown	LN571254.	99.53	100	0	422	M05317:1	Cluster_63	51	0	0	0	14	0	1	1	0	2	11	13	9	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Ferruginib	unknown	multi-subj	99.53	100	0	multi-aligr	M05317:1	Cluster_85	31	0	0	0	5	8	1	4	4	8	0	0	1	0	0	0
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	unknown	unknown	EU202832	99.06	100	0	424	M05317:1	Cluster_11	15	1	0	0	0	0	0	9	1	4	0	0	0	0	0	0
na	Bacteria	Myxococc	Polyangia	Nannocys	Nannocyst	Nannocyst	metageno	FPLS01003	100	100	0	424	M05317:1	Cluster_85	29	0	0	0	0	0	0	6	5	16	2	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Azospirilla	Inquilinac	Inquilinus	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_27	18	0	1	0	0	0	0	6	2	3	0	0	0	0	5	1
na	Bacteria	Spirochae	Spirochae	Spirochae	Spirochae	Spirochae	unknown	JQ311881.	99.76	100	0	420	M05317:1	Cluster_85	28	0	0	0	0	0	0	8	9	10	0	0	1	0	0	0
na	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillacea	Bacillus	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_94	151	33	17	66	0	0	0	0	1	3	0	3	0	0	11	17
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Taibaiella	Taibaiella	KC571459.	99.76	100	0	420	M05317:1	Cluster_85	21	0	0	0	0	9	0	7	1	4	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Nubsella	unknown	multi-subj	100	100	0	420	M05317:1	Cluster_63	37	0	0	0	3	14	6	3	1	3	1	0	1	0	3	2
na	Bacteria	Proteobac	Alphaprot	Rhodobac	Rhodobac	Pseudorhc	metageno	FPLS01059	99.53	100	0	424	M05317:1	Cluster_11	21	0	0	0	13	2	4	0	0	0	1	1	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Spirosoma	Emticicia	Emticicia f	multi-subj	99.52	100	0	418	M05317:1	Cluster_11	33	2	1	0	0	10	2	1	4	1	4	3	2	1	0	2
na	Bacteria	Proteobac	Alphaprot	Reyranella	Reyranella	Reyranella	Multi-affili	multi-subj	99.77	100	0	427	M05317:1	Cluster_12	19	0	0	0	1	0	0	5	9	3	0	1	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	unknown	HM27071	99.52	100	0	417	M05317:1	Cluster_59	75	0	0	0	0	0	0	1	23	14	4	31	2	0	0	0
na	Bacteria	Proteobac	Gammapr	211ds20	unknown	unknown	unknown	HQ190333	100	100	0	426	M05317:1	Cluster_59	34	0	0	0	1	0	0	0	0	0	6	16	10	0	1	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Moraxella	unknown	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_12	16	0	1	0	1	0	0	1	8	2	2	0	1	0	0	0
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Diaphorot	Multi-affili	multi-subj	100	100	0	419	M05317:1	Cluster_13	14	0	0	0	0	0	0	0	0	0	0	0	0	2	5	7
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Streptomy	GQ258685	100	100	0	432	M05317:1	Cluster_13	18	0	0	0	0	0	0	2	8	8	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhodobac	Rhodobac	Gemmoba	Tabrizicol	KP731978.	99.06	100	0	424	M05317:1	Cluster_13	12	0	0	0	4	4	4	0	0	0	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	CCD24	unknown	unknown	Multi-affili	multi-subj	99.53	100	0	424	M05317:1	Cluster_45	94	3	0	0	0	1	1	23	22	30	7	6	0	0	1	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	env.OPS 1	unknown	metageno	FPLS01011	100	100	0	419	M05317:1	Cluster_13	15	0	0	0	0	0	0	6	2	7	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Flavobacte	KC306428.	99.76	100	0	417	M05317:1	Cluster_13	23	0	0	0	0	0	0	9	12	2	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Reyranella	Reyranella	Reyranella	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_13	16	2	0	0	0	0	0	2	5	4	0	2	1	0	0	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	multi-iden	multi-cove	0	multi-aligr	M05317:1	Cluster_45	42	7	2	2	9	4	11	2	1	2	0	0	2	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Pseudorhc	unknown	multi-subj	100	100	0	427	M05317:1	Cluster_59	39	0	0	3	9	0	0	0	1	0	9	12	3	1	1	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Hyphomic	Pedomicr	unknown	multi-subj	100	100	0	424	M05317:1	Cluster_29	89	3	2	0	2	3	4	14	24	17	8	5	3	0	4	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Multi-affili	multi-subj	99.28	100	0	419	M05317:1	Cluster_29	131	0	0	0	0	0	0	0	2	0	0	0	1	67	18	43
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	100	100	0	417	M05317:1	Cluster_35	77	0	0	0	0	0	0	4	59	11	0	2	1	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	unknown	multi-subj	100	100	0	424	M05317:1	Cluster_35	60	2	4	0	4	0	2	5	7	7	5	4	0	5	5	10
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	NS11-12 m	unknown	unknown	GQ264293	100	100	0	420	M05317:1	Cluster_35	72	0	0	0	0	1	0	10	29	28	1	2	0	0	1	0
na	Bacteria	Firmicutes	Clostridia	Clostridial	Clostridiac	Clostridiur	Multi-affili	multi-subj	100	100	0	422	M05317:1	Cluster_35	60	0	0	0	0	0	0	0	0	0	2	0	56	2	0	0
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	unknown	unknown	JQ427253.	100	100	0	427	M05317:1	Cluster_19	19	0	0	0	2	4	2	1	5	2	0	1	1	1	0	0
na	Bacteria	Myxococc	Polyangia	Haliangial	Haliangiac	Haliangiur	unknown	JX505170.	100	100	0	428	M05317:1	Cluster_35	82	9	4	3	0	0	0	35	11	19	1	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_29	62	11	0	36	0	0	0	2	3	6	3	0	0	1	0	0
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Rhodanob	unknown	HQ440080	99.77	100	0	426	M05317:1	Cluster_63	73	19	2	8	2	1	2	22	4	13	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	Multi-affil	Multi-affili	multi-subj	99.05	100	0	420	M05317:1	Cluster_29	82	0	0	0	0	0	0	19	33	30	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown	unknown	AB614529	99.29	100	0	421	M05317:1	Cluster_29	93	0	0	0	0	0	0	0	0	0	36	34	23	0	0	0
na	Bacteria	Proteobac	Alphaprot	Micropeps	Micropeps	unknown	unknown	EF018850.	100	100	0	422	M05317:1	Cluster_31	92	7	0	0	0	2	2	40	22	19	0	0	0	0	0	0

na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Chitinoph	unknown :	EU133718	100	100	0	423	M05317:1	Cluster_3€	59	46	0	0	0	0	0	0	0	0	1	0	0	3	7	2
na	Bacteria	Proteobac	Gammapr	Gammapr	unknown	Acidibacte	Multi-affili	multi-subj	99.53	100	0	multi-aligr	M05317:1	Cluster_1(	44	0	0	0	8	2	3	3	14	12	0	2	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Phyllobact	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_1€	12	0	2	0	0	0	0	3	2	5	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Flavobacte	KJ589516.	99.28	100	0	417	M05317:1	Cluster_1€	15	0	0	0	0	0	0	3	5	7	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	unknown :	EU589260	99.53	100	0	424	M05317:1	Cluster_6€	30	4	1	1	2	6	3	3	7	2	0	0	0	0	0	1
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Brevundin	Multi-affili	multi-subj	100	100	0	422	M05317:1	Cluster_7:	26	3	2	3	0	2	2	0	0	2	8	2	0	0	0	2
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Beijerincki	Methylobi	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_6€	20	9	8	2	0	1	0	0	0	0	0	0	0	0	0	0
na	Bacteria	Myxococc	Polyangia	Polyangia	BIrii41	unknown	unknown :	LN570777.	99.77	100	0	427	M05317:1	Cluster_3:	149	0	11	0	1	0	3	14	64	30	8	12	6	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Multi-affili	multi-subj	99.52	100	0	418	M05317:1	Cluster_6€	46	0	0	0	0	0	0	0	0	0	0	0	0	17	11	18
na	Bacteria	Proteobac	Alphaprot	Reyranell	Reyranella	Reyranell	unknown :	multi-subj	100	100	0	427	M05317:1	Cluster_6€	29	1	1	0	0	0	0	16	6	4	0	0	0	1	0	0
na	Bacteria	Proteobac	Alphaprot	Ferrovibri	Ferrovibric	Ferrovibri	Ferrovibric	KY117477.	100	100	0	428	M05317:1	Cluster_6€	47	0	0	1	0	0	0	0	1	0	16	11	6	4	4	4
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Beijerincki	unknown	metageno	FPLL01006	99.76	100	0	424	M05317:1	Cluster_1(	17	4	0	1	2	4	1	4	1	0	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	99.76	100	0	417	M05317:1	Cluster_17	275	0	0	0	0	1	0	80	84	63	6	8	33	0	0	0
na	Bacteria	Proteobac	Gammapr	Cellvibrior	Cellvibrior	Cellvibrio	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_17	140	0	0	0	2	14	0	0	3	0	19	30	7	30	14	21
na	Bacteria	Proteobac	Gammapr	Enterobac	Multi-affili	Multi-affil	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_17	178	0	1	4	0	0	0	0	0	0	4	5	0	73	19	72
na	Bacteria	Proteobac	Gammapr	Xanthomo	Xanthomo	Lysobacte	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_17	94	51	23	10	0	0	0	0	0	0	0	0	3	2	4	1
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Novosphir	Multi-affili	multi-subj	99.53	100	0	423	M05317:1	Cluster_17	168	2	15	2	2	6	6	3	7	3	11	80	12	3	6	10
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Beijerincki	Methylobi	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_17	128	23	7	10	3	61	10	2	4	4	0	0	1	0	2	1
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_17	136	0	0	0	0	0	0	0	4	0	11	33	13	15	21	39
na	Bacteria	Bacteroidc	Bacteroidi	Cytophag	Spirosoma	Arcicella	Multi-affili	multi-subj	100	100	0	419	M05317:1	Cluster_17	114	5	0	0	0	0	0	0	0	0	14	51	44	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Puia	unknown :	GU940802	99.76	100	0	423	M05317:1	Cluster_17	289	4	0	3	4	21	2	88	81	85	1	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Xanthomo	Xanthomo	Stenotrop	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_18	1155	16	87	67	16	27	90	39	102	99	108	77	82	110	98	137
na	Bacteria	Proteobac	Gammapr	Enterobac	Erwiniace	Pantoea	Pantoea e	KY038312.	100	100	0	425	M05317:1	Cluster_19	880	174	274	424	0	0	0	0	0	0	0	0	2	0	6	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Bradyrhizc	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_1(	2244	188	75	68	51	176	49	614	438	497	8	7	17	8	29	19
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_11	1705	40	16	12	304	94	274	0	3	1	49	6	30	256	281	339
na	Bacteria	Proteobac	Gammapr	Pseudomc	Moraxella	Acinetoba	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_12	1390	219	110	13	54	130	466	10	48	11	2	1	3	132	97	94
na	Bacteria	Proteobac	Gammapr	Xanthomo	Xanthomo	Xanthomo	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_13	1506	146	34	22	584	231	150	4	13	11	28	58	43	28	86	68
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	432	M05317:1	Cluster_14	1602	54	25	8	787	306	287	38	20	53	11	8	1	1	2	1
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Kosakonia	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_15	4868	116	687	214	17	70	239	4	0	0	51	90	55	1228	809	1288
na	Bacteria	Firmicutes	Bacilli	Staphyloc	Staphyloc	Staphyloc	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_16	1082	366	270	438	0	0	0	0	0	0	8	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Aeromona	Aeromona	Aeromona	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_17	2662	2	0	0	26	6	12	2	21	6	930	594	667	42	137	217
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Flavobacte	GU564236	100	100	0	417	M05317:1	Cluster_12	189	0	0	0	22	148	12	0	0	0	0	7	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Parasegeti	unknown :	KJ600837.	100	100	0	424	M05317:1	Cluster_6(	38	0	0	0	0	0	0	0	0	0	6	20	8	1	2	1
na	Bacteria	Proteobac	Gammapr	Enterobac	Erwiniace	Pantoea	Pantoea sp	multi-subj	100	100	0	424	M05317:1	Cluster_12	188	5	7	9	0	0	0	0	155	1	3	2	0	3	1	2
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Chitinoph	Chitinoph	MH73490	100	100	0	423	M05317:1	Cluster_77	26	0	0	0	4	0	0	2	0	0	13	1	3	1	2	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	unknown :	JQ769558.	100	100	0	424	M05317:1	Cluster_77	40	0	0	0	0	0	0	0	2	1	3	2	4	5	7	16
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Pseudolab	unknown :	EF020007.	99.77	100	0	427	M05317:1	Cluster_1(	18	2	3	0	1	0	6	0	0	0	3	0	0	1	0	2
na	Bacteria	Proteobac	Gammapr	Pseudomc	Moraxella	Acinetoba	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_12	160	33	7	4	2	0	5	2	1	0	0	6	0	31	29	40
na	Bacteria	Bacteroidc	Bacteroidi	Cytophag	Microscill	Ohtaekwa	unknown :	GU444094	99.76	100	0	420	M05317:1	Cluster_77	28	0	0	0	0	0	0	0	0	1	10	14	1	0	2	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiale	Nordella	unknown :	multi-subj	100	100	0	426	M05317:1	Cluster_77	23	1	2	0	1	2	2	1	4	0	3	6	1	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Pseudolab	unknown :	KJ877455.	100	100	0	426	M05317:1	Cluster_77	39	0	0	1	6	15	4	1	1	1	1	2	3	0	0	4
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Rhodopla	metageno	multi-subj	99.53	100	0	427	M05317:1	Cluster_77	28	0	0	0	0	0	0	10	6	12	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Edaphoba	unknown :	HQ118574	99.29	100	0	424	M05317:1	Cluster_21	15	0	0	0	0	3	0	1	7	3	0	0	1	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	unknown :	multi-subj	100	100	0	427	M05317:1	Cluster_57	32	7	1	2	0	0	0	18	2	2	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	100	100	0	417	M05317:1	Cluster_6:	30	0	0	0	0	0	0	0	0	0	5	2	1	4	12	6
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Beijerincki	Roseiarcus	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_21	16	3	2	1	0	0	0	9	1	0	0	0	0	0	0	0
na	Bacteria	Firmicutes	Bacilli	Paenibacil	Paenibacil	Paenibacil	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_18	14	0	0	0	0	0	0	0	0	0	0	0	0	10	3	1
na	Bacteria	Bacteroidc	Bacteroidi	Cytophag	Microscill	Ohtaekwa	Bacteroide	AB539998	99.29	100	0	420	M05317:1	Cluster_67	31	0	0	1	0	0	0	0	20	6	0	4	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhodobac	Rhodobac	Paracoccu	Multi-affili	multi-subj	100	100	0	422	M05317:1	Cluster_57	34	6	0	1	0	2	0	0	0	0	11	11	0	2	0	1
na	Bacteria	Proteobac	Alphaprot	Rhodobac	Rhodobac	Multi-affil	Multi-affili	multi-subj	100	100	0	422	M05317:1	Cluster_57	32	1	0	0	0	0	0	0	0	0	0	2	0	7	8	14
na	Bacteria	Acidobact	Thermoan	Thermoan	Thermoan	Subgroup	unknown :	KC329607.	99.77	100	0	427	M05317:1	Cluster_57	34	0	0	0	13	17	4	0	0	0	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	KT387299.	100	100	0	423	M05317:1	Cluster_57	28	5	5	2	1	0	1	0	5	3	6	0	0	0	0	0
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Azohydror	Azohydror	AB681804	100	100	0	419	M05317:1	Cluster_57	21	0	0	0	2	13	6	0	0	0	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Rhodanob	unknown :	EU015100	99.77	100	0	427	M05317:1	Cluster_67	23	1	0	0	8	10	4	0	0	0	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophag	Spirosoma	Dyadobac	unknown :	GU444105	99.52	100	0	421	M05317:1	Cluster_57	58	4	1	0	12	1	0	0	1	0	7	2	14	6	4	6
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_5	2887	956	266	887	30	140	38	11	15	18	95	127	138	35	74	57
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Asticcacau	unknown :	multi-subj	99.76	100	0	423	M05317:1	Cluster_12	170	3	0	0	30	73	28	10	6	11	6	1	2	0	0	0
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Novosphir	unknown :	LN568288.	100	100	0	427	M05317:1	Cluster_8:	38	0	0	0	0	0	0	0	1	0	10	1	13	5	3	5
na	Bacteria	Bacteroidc	Bacteroidi	Cytophag	Microscill	OLB12	unknown :	JN397978.	99.76	100	0	420	M05317:1	Cluster_8:	23	0	0	0	0	0	0	7	10	6	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	Devosia ri	multi-subj	100	100	0	424	M05317:1	Cluster_22	119	0	0	0	1	2	1	0	0	2	14	24	11	12	29	23
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiale	Nordella	unknown :	multi-subj	100	100	0	426	M05317:1	Cluster_8:	44	2	1	0	0	0	0	6	7	7	2	1	0	7	5	6
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	100	100	0	417	M05317:1	Cluster_8:	26	0	0	0	0	0	0	8	14	4	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Multi-affili	multi-subj	100	100	0	418	M05317:1	Cluster_8:	20	0	0	0	0	0	0	0	0	0	11	0	0	0	0	9

| | | |

na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Streptomy	MG38820(	100	100	0	431	M05317:1	Cluster_35	47	0	0	0	26	14	4	1	0	1	1	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Steroidob	Steroidob	Steroidob	metageno	FPLS01046	99.53	100	0	423	M05317:1	Cluster_15	18	0	0	0	0	2	0	1	8	2	1	2	2	0	0	0	0
na	Bacteria	Firmicutes	Bacilli	Bacillales	Planococc	Lysinibacil	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_21	10	4	0	1	0	0	2	0	0	0	0	0	1	0	1	1	0
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Ahniella	unknown	JN868991.	99.53	100	0	426	M05317:1	Cluster_21	13	0	0	0	0	0	0	0	0	0	5	6	2	0	0	0	0
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	unknown	unknown	KR110071.	99.05	100	0	419	M05317:1	Cluster_10	222	0	0	0	0	0	0	0	1	1	68	99	53	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Gammapr	unknown	Acidibacte	unknown	HM18631!	99.53	100	0	423	M05317:1	Cluster_10	245	0	0	0	0	0	3	65	92	80	1	1	3	0	0	0	0
na	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillacea	Bacillus	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_10	389	8	1	5	37	3	210	0	0	0	5	21	7	19	15	58	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Methylolig	unknown	unknown	EF018354.	99.76	100	0	419	M05317:1	Cluster_20	23	2	3	1	5	0	0	3	1	1	3	3	1	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Novosphir	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_10	149	1	42	7	21	30	15	0	2	2	15	2	3	3	2	4	
na	Bacteria	Proteobac	Gammapr	Pseudomc	Moraxella	Acinetoba	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_10	271	1	0	0	0	0	0	0	0	0	8	5	73	63	121		
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Ensifer	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_10	490	2	2	0	3	0	1	2	13	14	29	11	28	71	167	147	
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Multi-affil	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_10	262	1	0	1	0	0	0	0	0	0	17	11	23	74	80	55	
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Chryseoba	multi-subj	99.28	100	0	418	M05317:1	Cluster_10	200	0	0	0	80	73	22	1	0	0	0	2	0	9	3	10	
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	NS9 marin	unknown	Multi-affili	multi-subj	99.05	100	0	419	M05317:1	Cluster_18	27	0	0	0	0	0	0	1	0	0	14	7	4	0	0	1	
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	99.06	100	0	425	M05317:1	Cluster_85	16	0	0	0	10	4	2	0	0	0	0	0	0	0	0	0	
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Franconib	Cronobact	KF531638.	99.76	100	0	424	M05317:1	Cluster_53	51	0	0	0	0	0	0	0	0	0	0	0	9	21	21		
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Salmonell	Salmonell	multi-subj	99.29	100	0	424	M05317:1	Cluster_53	41	0	0	0	0	0	0	0	0	0	0	0	23	3	15		
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiale	Bauldia	unknown	JQ798401.	100	100	0	428	M05317:1	Cluster_53	43	0	0	0	0	3	3	13	12	12	0	0	0	0	0	0	
na	Bacteria	Myxococc	Polyangia	Polyangial	Blrii41	unknown	unknown	JF185760.	100	100	0	427	M05317:1	Cluster_53	54	0	0	0	9	11	1	0	0	0	4	19	10	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Pseudolab	unknown	GU983358	99.06	100	0	426	M05317:1	Cluster_53	40	1	1	0	0	0	0	21	7	10	0	0	0	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Chitinoph	Multi-affili	multi-subj	99.76	100	0	423	M05317:1	Cluster_47	45	0	0	0	0	0	0	38	3	4	0	0	0	0	0	0	
na	Bacteria	Proteobac	Gammapr	Pseudomc	Moraxella	Multi-affil	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_11	13	0	0	0	0	0	0	0	0	0	1	0	0	7	3	2	
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Cloacibact	Multi-affili	multi-subj	100	100	0	418	M05317:1	Cluster_43	554	0	0	0	0	0	0	0	0	0	1	0	0	61	252	240	
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Novosphir	metageno	FPLS01058	100	100	0	427	M05317:1	Cluster_42	453	26	129	124	12	8	13	2	8	16	17	51	6	14	16	11	
na	Bacteria	Proteobac	Gammapr	Enterobac	Multi-affil	Multi-affil	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_41	1711	74	231	58	32	22	249	20	60	20	157	24	101	186	168	309	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Rhodopse	Multi-affili	multi-subj	99.53	100	0	425	M05317:1	Cluster_40	619	91	11	29	0	0	0	272	96	120	0	0	0	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Multi-affili	multi-subj	100	100	0	418	M05317:1	Cluster_47	494	0	0	0	0	0	0	0	0	0	0	0	0	20	463	11	
na	Bacteria	Proteobac	Gammapr	Pseudomc	Moraxella	Acinetoba	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_46	722	147	186	31	0	0	0	0	0	0	4	2	2	139	145	66	
na	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillacea	Bacillus	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_45	408	125	112	86	1	0	1	2	0	3	32	24	4	6	6	6	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Beijerincki	Bosea	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_44	566	25	19	8	94	118	57	1	5	2	48	29	35	27	32	66	
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Acidovora	Multi-affili	multi-subj	100	100	0	419	M05317:1	Cluster_48	667	1	0	0	5	2	0	2	7	6	19	33	23	100	277	192	
na	Bacteria	Firmicutes	Bacilli	Staphyloc	Staphyloc	Staphyloc	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_33	414	156	168	86	1	0	0	0	0	0	2	0	1	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	unknown	CU919466	99.29	100	0	424	M05317:1	Cluster_10	29	0	4	0	0	0	0	0	19	4	0	2	0	0	0	0	
na	Bacteria	Myxococc	Polyangia	Polyangial	Phaselicys	Phaselicys	unknown	EF516177.	99.53	100	0	428	M05317:1	Cluster_86	24	0	0	0	0	0	0	8	4	12	0	0	0	0	0	0	
na	Bacteria	Firmicutes	Clostridia	unknown	Hungateic	Ruminiclo	Multi-affili	multi-subj	99.53	100	0	423	M05317:1	Cluster_86	24	0	0	0	0	0	0	0	0	0	6	11	7	0	0	0	
na	Bacteria	Proteobac	Gammapr	Gammapr	unknown	Acidibacte	unknown	AB672209	99.05	100	0	423	M05317:1	Cluster_23	21	0	0	0	0	0	0	10	4	6	0	0	0	0	0	1	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	unknown	GQ093827	99.76	100	0	425	M05317:1	Cluster_75	46	0	0	0	1	1	0	1	1	0	8	20	9	0	1	4	
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Niastella	Multi-affili	multi-subj	99.05	100	0	423	M05317:1	Cluster_23	19	0	0	1	0	0	0	2	2	2	7	2	0	1	0	2	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Beijerincki	Microvirga	Microvirga	FR733708.	100	100	0	427	M05317:1	Cluster_16	18	0	0	0	0	0	3	0	0	0	1	1	0	3	1	9	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Mesorhizc	metageno	FPLL01003	99.77	100	0	427	M05317:1	Cluster_62	31	2	1	0	0	1	0	10	6	4	1	0	0	2	1	3	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	unknown	EF018832.	100	100	0	427	M05317:1	Cluster_74	33	0	2	0	7	4	7	4	1	2	1	3	2	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Bacteroida	Paludibact	Paludibact	Paratrima	GAFH0100	99.53	100	0	422	M05317:1	Cluster_74	27	0	0	0	0	0	0	0	0	0	1	5	21	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Sediminib	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_62	39	0	0	1	0	0	0	0	0	0	12	19	7	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	unknown	unknown	JF188955.	99.53	100	0	424	M05317:1	Cluster_62	62	0	1	0	1	3	1	4	7	14	5	16	10	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	unknown	metageno	FPLS01050	99.76	100	0	423	M05317:1	Cluster_62	43	1	0	0	0	8	1	10	11	11	1	0	0	0	0	0	
na	Bacteria	Firmicutes	Multi-affil	Multi-affil	Multi-affil	Multi-affil	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_10	10	3	1	1	0	0	0	0	2	0	0	0	0	2	0	1	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Multi-affil	Multi-affil	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_62	42	0	0	0	0	0	0	0	2	1	9	9	5	1	7	8	
na	Bacteria	Proteobac	Gammapr	Xanthomo	Xanthomo	Lysobacte	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_23	110	0	0	0	0	0	0	31	30	22	6	1	0	3	0	17	
na	Bacteria	Acidobact	Acidobacti	Solibacter	Solibacter	Candidatu	unknown	multi-subj	100	100	0	424	M05317:1	Cluster_94	27	0	0	0	0	1	1	13	6	6	0	0	0	0	0	0	
na	Bacteria	Actinobac	Thermoleo	Solirubrob	67-14	unknown	unknown	JF236076.	100	100	0	423	M05317:1	Cluster_65	43	1	1	0	5	2	6	9	10	5	0	2	0	0	2	0	
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	env.OPS 1	unknown	unknown	LN570735.	100	100	0	420	M05317:1	Cluster_20	12	0	0	0	0	3	0	4	0	0	2	2	1	0	0	0	
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Hydrogen	unknown	KP663399.	99.52	100	0	419	M05317:1	Cluster_10	35	0	0	0	0	0	0	0	0	0	13	13	8	0	1	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Beijerincki	Methylobi	Methylobi	HQ009873	99.76	100	0	424	M05317:1	Cluster_86	27	14	7	3	0	0	0	1	0	0	0	0	1	0	1	0	
na	Bacteria	Proteobac	Alphaprot	Rhodobac	Rhodobac	Gemmoba	Gemmoba	KU973508	100	100	0	424	M05317:1	Cluster_21	121	0	0	0	0	0	0	0	0	0	0	33	41	4	6	37	
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Escherichi	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_21	128	2	9	2	0	0	2	2	1	6	4	1	6	31	11	51	
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Chryseoba	JN196134.	100	100	0	418	M05317:1	Cluster_21	215	0	0	16	1	1	2	54	37	33	17	20	7	10	6	11	
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	100	100	0	417	M05317:1	Cluster_21	116	0	0	0	0	0	0	0	1	0	9	0	0	11	41	54	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	metageno	FPLS01034	99.06	100	0	427	M05317:1	Cluster_21	105	0	0	0	0	0	0	0	0	0	0	0	0	23	28	54	
na	Bacteria	Proteobac	Gammapr	Xanthomo	Xanthomo	Thermom	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_21	109	0	0	0	1	1	1	0	1	0	4	10	90	0	1	0	
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Taibaiella	unknown	JF176697.	99.29	100	0	420	M05317:1	Cluster_14	11	0	0	0	3	1	2	0	1	0	1	3	0	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Caulobact	unknown	FJ432432.	99.53	100	0	422	M05317:1	Cluster_14	24	0	0	0	0	0	0	3	12	6	1	1	1	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	OLB12	unknown	multi-subj	99.52	100	0	multi-aligr	M05317:1	Cluster_59	50	0	0	0	0	0	0	0	0	0	9	35	6	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	env.OPS 1	unknown	metageno	FPLS01027	99.52	100	0	418	M05317:1	Cluster_96	31	0	0	0	0	0	0	19	6	5	0	0	0	1	0	0	

na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Labraceae	Labrys	Labrys sp.	KY117513.	100	100	0	427	M05317:1	Cluster_9€	18	7	2	1	0	0	0	2	2	1	0	2	0	0	1	0
na	Bacteria	Proteobac	Alphaprot	Rhodobac	Rhodobac	Paracoccu	Multi-affili	multi-subj	100	100	0	422	M05317:1	Cluster_9€	20	0	0	0	0	0	0	0	0	0	5	3	12	0	0	0
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Citrobacte	unknown :	GQ154924	99.06	100	0	424	M05317:1	Cluster_1!	13	0	0	0	0	0	0	0	0	0	0	0	0	8	2	3
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Multi-affili	multi-subj	99.52	100	0	418	M05317:1	Cluster_5!	40	3	0	1	0	0	0	14	16	4	0	0	2	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Pedobacte	unknown :	HM27807(	100	100	0	420	M05317:1	Cluster_2€	16	0	0	0	0	0	0	2	7	1	0	6	0	0	0	0
na	Bacteria	Firmicutes	Bacilli	Paenibacil	Paenibacil	Paenibacil	Paenibacil	MG30941(	100	100	0	428	M05317:1	Cluster_2!	11	0	0	0	0	0	0	0	0	4	0	0	0	3	0	4
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Sphingoba	Multi-affili	multi-subj	99.76	100	0	420	M05317:1	Cluster_8€	31	0	0	0	0	0	0	0	0	0	0	0	3	18	4	6
na	Bacteria	Desulfoba	Desulfovit	Desulfovit	Desulfovit	Desulfovit	Desulfovit	AUBQ010(	99.06	100	0	424	M05317:1	Cluster_8€	37	0	0	0	0	0	0	0	0	0	15	10	12	0	0	0
na	Bacteria	Proteobac	Alphaprot	Caulobact	Hyphomor	unknown	unknown :	FM201202	99.77	100	0	428	M05317:1	Cluster_8€	19	0	3	0	1	1	0	0	0	0	6	7	1	0	0	0
na	Bacteria	Deinococc	Deinococc	Deinococc	Deinococc	Deinococc	Multi-affili	multi-subj	100	100	0	421	M05317:1	Cluster_8€	29	0	0	0	0	0	0	0	0	0	0	0	0	11	15	3
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown	unknown :	EU133748	100	100	0	420	M05317:1	Cluster_8€	25	0	0	0	1	0	0	1	3	14	3	1	2	0	0	0
na	Bacteria	Acidobact	Acidobacti	Bryobacte	Bryobacte	Bryobacte	unknown :	multi-subj	99.76	100	0	424	M05317:1	Cluster_1:	13	0	0	0	0	0	0	4	5	4	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Flavisoliba	unknown :	JN409282.	99.76	100	0	423	M05317:1	Cluster_1:	23	0	0	0	4	8	3	0	0	0	0	0	1	2	1	4
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Edaphoba	unknown :	HQ681985	99.53	100	0	423	M05317:1	Cluster_1:	15	0	0	0	0	2	0	0	0	0	0	1	1	1	2	8
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Ferruginib	unknown :	multi-subj	100	100	0	423	M05317:1	Cluster_1:	24	0	0	0	0	0	0	1	0	0	7	7	9	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Sphingoba	Multi-affili	multi-subj	99.52	100	0	419	M05317:1	Cluster_1:	42	11	4	0	0	0	0	1	0	0	19	2	4	0	1	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Moraxella	Acinetoba	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_1:	8	7	1	0	0	0	0	0	0	0	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Xanthomo	Xanthomo	Luteimon	unknown :	multi-subj	99.77	100	0	426	M05317:1	Cluster_4-	119	1	0	0	8	13	8	6	21	7	3	0	0	6	11	35
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Bradyrhizc	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_8	2454	118	57	45	20	22	15	636	544	518	151	124	91	32	34	47
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	37-13	unknown	metageno	FPLS01037	100	100	0	423	M05317:1	Cluster_1:	39	0	0	0	7	2	3	8	9	9	0	1	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_6	2530	91	206	681	174	58	567	145	192	213	30	48	125	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	100	100	0	417	M05317:1	Cluster_7	2400	0	1	0	0	0	0	2	0	0	50	4	12	879	918	534
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Sinorhizob	AJ879127.	100	100	0	427	M05317:1	Cluster_1:	13	0	1	0	0	0	0	0	0	0	8	3	0	1	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Ochrobact	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_1:	11	1	0	0	0	0	9	0	0	1	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_2	10484	570	1980	2081	308	163	507	85	173	113	692	544	1966	327	452	523
na	Bacteria	Firmicutes	Bacilli	Exiguobac	Exiguobac	Exiguobac	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_3	7137	31	22	40	4	4	0	3	2	4	361	105	238	3537	1884	902
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	DQ100062	99.53	100	0	423	M05317:1	Cluster_4-	44	0	0	0	3	24	7	1	1	0	2	1	5	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	unknown :	multi-subj	100	100	0	417	M05317:1	Cluster_1:	17	0	0	0	0	0	0	0	0	0	8	7	2	0	0	0
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Novosphir	unknown :	multi-subj	100	100	0	423	M05317:1	Cluster_3€	61	1	0	0	2	13	5	7	7	15	3	2	2	0	4	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Chitinoph	Multi-affili	multi-subj	99.76	100	0	423	M05317:1	Cluster_3€	63	12	0	0	0	28	18	0	0	0	3	2	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Reyranella	Reyranella	Reyranella	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_9-	438	2	10	1	0	0	1	178	125	105	5	3	7	1	0	0
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Asticcacau	Asticcacau	KR338952.	99.53	100	0	422	M05317:1	Cluster_3€	48	0	0	0	30	5	13	0	0	0	0	0	0	0	0	0
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	432	M05317:1	Cluster_9!	205	0	0	0	43	91	51	6	11	3	0	0	0	0	0	0
na	Bacteria	Myxococc	Polyangia	Haliangial	Haliangiac	Haliangiur	unknown :	multi-subj	99.53	100	0	425	M05317:1	Cluster_9€	272	0	0	0	0	0	0	50	31	190	0	0	0	0	1	0
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Multi-affil	Multi-affili	multi-subj	100	100	0	419	M05317:1	Cluster_9-	218	0	0	0	0	0	0	0	0	0	142	54	20	0	1	1
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	432	M05317:1	Cluster_9!	186	1	0	0	31	77	54	6	2	11	2	0	0	0	1	1
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Multi-affili	multi-subj	100	100	0	418	M05317:1	Cluster_9!	173	0	0	1	27	61	84	0	0	0	0	0	0	0	0	0
na	Bacteria	Myxococc	Polyangia	Haliangial	Haliangiac	Haliangiur	unknown :	KJ191901.:	100	100	0	426	M05317:1	Cluster_1:	14	4	2	0	0	0	0	7	0	1	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	100	100	0	418	M05317:1	Cluster_2€	100	0	0	0	0	0	0	0	0	0	7	23	55	5	4	6
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	432	M05317:1	Cluster_2€	148	2	0	1	11	25	7	12	3	1	19	6	2	7	16	36
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Flavobacte	KX263321.	100	100	0	417	M05317:1	Cluster_8:	38	0	0	0	0	0	0	5	10	8	0	0	1	3	11	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Nitrobacte	Nitrobacte	KY117526.	100	100	0	425	M05317:1	Cluster_3!	51	1	0	0	6	41	3	0	0	0	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Sphingoba	Multi-affili	multi-subj	99.52	100	0	420	M05317:1	Cluster_1:	19	17	0	0	0	0	0	1	0	0	0	0	1	0	0	0
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Altereryth	unknown :	multi-subj	99.77	100	0	427	M05317:1	Cluster_2:	12	0	0	0	0	1	0	0	0	0	5	5	0	0	1	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Hyphomic	Hyphomic	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_1-	9	2	0	0	2	2	3	0	0	0	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Parvibacul	Parvibacul	unknown	unknown :	DQ840467	99.06	100	0	426	M05317:1	Cluster_1€	35	0	0	0	0	0	0	11	15	9	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Beijerincki	unknown	unknown :	DQ129631	99.76	100	0	422	M05317:1	Cluster_1-	10	0	0	0	2	7	0	0	0	0	0	0	1	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Ferruginib	unknown :	multi-subj	100	100	0	423	M05317:1	Cluster_1-	20	0	0	0	0	0	0	3	10	4	1	2	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	unknown :	AY922001.	100	100	0	427	M05317:1	Cluster_1(	18	0	0	0	0	2	0	0	0	0	5	8	2	0	0	1
na	Bacteria	Myxococc	Myxococci	Myxococci	Myxococci	P3OB-42	unknown :	KT122372.	100	100	0	422	M05317:1	Cluster_1(	36	0	0	0	0	0	0	2	2	0	9	22	1	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Mucilagini	Mucilagini	KY171988.	100	100	0	420	M05317:1	Cluster_1(	26	0	0	0	8	8	0	2	6	2	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Cellvibrior	Cellvibrior	Cellvibrio	unknown :	GQ263640	99.06	100	0	425	M05317:1	Cluster_1(	16	0	0	0	0	0	0	0	0	0	9	5	2	0	0	0
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Asticcacau	unknown :	FJ602413.:	100	100	0	422	M05317:1	Cluster_1-	125	0	0	0	19	27	32	3	7	9	7	5	4	5	5	2
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Terrimona	unknown :	EF612370.	100	100	0	423	M05317:1	Cluster_1-	159	2	0	1	9	2	2	15	36	22	25	10	30	1	2	2
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Multi-affil	Multi-affili	multi-subj	100	100	0	419	M05317:1	Cluster_4:	33	16	2	8	0	0	0	0	0	0	0	0	0	3	1	3
na	Bacteria	Proteobac	Alphaprot	Dongiales	Dongiacea	Dongia	unknown :	KC432240.	100	100	0	426	M05317:1	Cluster_4€	58	0	0	0	0	0	0	11	24	17	1	2	3	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Chitinoph	Chitinoph	FM865977	99.76	100	0	423	M05317:1	Cluster_1-	135	0	11	2	16	26	18	11	27	20	0	4	0	0	0	0
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	unknown	Aquincola	LN794224.	100	100	0	419	M05317:1	Cluster_1-	163	0	0	0	11	23	6	19	18	15	18	25	14	0	9	5
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	unknown :	JQ738853.	99.77	100	0	427	M05317:1	Cluster_1-	175	0	0	0	0	0	0	44	63	45	21	1	1	0	0	0
na	Bacteria	Proteobac	Gammapr	Xanthomo	Xanthomo	Lysobacte	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_1-	253	1	1	0	1	0	0	19	24	24	38	43	16	18	36	32
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Shinella	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_1-	138	0	0	0	0	0	0	0	0	0	21	17	13	12	33	42
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Niastella	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_1-	153	1	0	1	18	37	18	27	14	14	1	0	0	5	0	17
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Pedobacte	Multi-affili	multi-subj	99.76	100	0	420	M05317:1	Cluster_2!	103	1	0	15	0	0	0	22	34	31	0	0	0	0	0	0

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na	Bacteria	Proteobac	Gammapr	Xanthomo	Xanthomo	Pseudoxar	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_24	115	0	0	0	2	1	0	0	3	1	4	2	8	20	28	46
na	Bacteria	Firmicutes	Bacilli	Brevibacill	Brevibacill	Brevibacill	Brevibacill	JN378519.	100	100	0	424	M05317:1	Cluster_11	9	6	0	0	0	0	2	0	0	0	0	1	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Rhodanob	unknown :	LC093156.	99.53	100	0	426	M05317:1	Cluster_24	88	0	0	0	7	26	16	4	22	13	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Multi-affil	Multi-affili	multi-subj	99.77	100	0	426	M05317:1	Cluster_24	136	11	5	4	0	2	2	47	44	21	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Tistrellale	Tistrellace	Tistrella	Rhodobiur	EU982843	99.53	100	0	424	M05317:1	Cluster_10	18	0	0	0	0	0	0	0	0	0	1	12	5	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown f	unknown :	JN559190.	99.52	100	0	420	M05317:1	Cluster_24	97	0	0	0	6	7	2	4	7	0	0	45	26	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Labraceae	Labrys	Labrys sp.	HM14953	99.77	100	0	427	M05317:1	Cluster_24	83	1	0	0	62	7	6	2	0	1	0	0	0	1	2	1
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Spirosoma	Dyadobac	Multi-affili	multi-subj	99.29	100	0	421	M05317:1	Cluster_33	68	2	0	0	0	0	0	0	0	0	18	6	3	13	9	17
na	Bacteria	Proteobac	Gammapr	Aeromona	Aeromona	Aeromona	unknown :	multi-subj	100	100	0	424	M05317:1	Cluster_69	24	0	0	0	0	0	0	0	0	0	0	0	0	2	3	19
na	Bacteria	Acidobact	Holophaga	Holophaga	Holophaga	Geothrix	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_66	41	0	0	0	0	0	0	0	0	0	8	10	23	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	99.28	100	0	419	M05317:1	Cluster_11	59	1	0	0	0	0	0	4	15	7	6	9	4	4	1	8
na	Bacteria	Firmicutes	Negativicu	Veillonella	Sporomus	Pelosinus	unknown :	AB487569	99.77	100	0	426	M05317:1	Cluster_14	13	0	0	0	0	0	0	0	0	0	4	1	8	0	0	0
na	Bacteria	Myxococc	Polyangia	Haliangial	Haliangiac	Haliangiur	unknown :	HQ119531	99.77	100	0	426	M05317:1	Cluster_37	99	0	0	0	0	0	0	37	21	41	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Pseudomo	multi-subj	100	100	0	424	M05317:1	Cluster_20	18	0	0	0	1	1	0	0	0	0	0	1	0	4	4	7
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Ahniella	metageno	FPLS01042	99.3	100	0	426	M05317:1	Cluster_81	33	0	0	0	0	0	0	0	1	3	4	24	1	0	0	0
na	Bacteria	Spirochae	Spirochae	Spirochae	Spirochae	Spirochae	Spirochae	FR749896.	99.76	100	0	420	M05317:1	Cluster_93	40	0	0	0	0	0	0	0	0	0	3	22	15	0	0	0
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Brevundin	Multi-affili	multi-subj	100	100	0	422	M05317:1	Cluster_93	48	1	0	2	0	0	0	0	0	2	16	6	5	5	3	8
na	Bacteria	Firmicutes	Bacilli	Bacillales	Planococc	Psychroba	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_93	34	11	1	1	0	0	21	0	0	0	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	unknown :	JF185803.	99.76	100	0	419	M05317:1	Cluster_93	20	1	0	0	0	0	0	0	2	0	0	2	0	6	1	8
na	Bacteria	Desulfoba	Desulfovit	Desulfovib	Desulfovib	Desulfovib	Multi-affili	multi-subj	99.06	100	0	424	M05317:1	Cluster_93	20	0	0	0	0	0	0	0	0	0	4	1	15	0	0	0
na	Bacteria	Proteobac	Alphaprot	Dongiales	Dongiacea	Dongia	Dongia rig	HQ436504	99.53	100	0	427	M05317:1	Cluster_93	36	0	0	0	11	1	1	0	0	1	5	4	13	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown f	metageno	FPLS01007	99.29	100	0	420	M05317:1	Cluster_93	17	0	0	0	0	0	0	11	3	3	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhodobac	Rhodobac	Rhodobac	unknown :	CU919138	99.76	100	0	424	M05317:1	Cluster_18	176	0	0	0	0	0	0	0	0	0	49	62	65	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Bradyrhizc	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_18	118	20	0	5	0	0	0	5	60	28	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Ensifer	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_18	192	0	0	0	1	0	0	0	0	0	0	0	1	40	46	104
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Tahibacte	unknown :	HQ120445	100	100	0	426	M05317:1	Cluster_18	188	4	4	3	0	0	0	4	8	1	90	51	22	0	1	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	multi-subj	100	100	0	423	M05317:1	Cluster_18	103	5	1	2	0	66	9	1	0	1	0	0	0	3	10	5
na	Bacteria	Proteobac	Gammapr	Enterobac	Erwiniace	Erwinia	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_18	77	1	5	48	0	0	0	0	1	0	0	0	0	9	0	13
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Chitinoph	Multi-affili	multi-subj	99.29	100	0	423	M05317:1	Cluster_18	97	0	0	0	6	33	58	0	0	0	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	99.52	100	0	417	M05317:1	Cluster_12	15	0	0	0	0	0	0	0	0	0	13	2	0	0	0	0
na	Bacteria	Myxococc	Polyangia	Haliangial	Haliangiac	Haliangiur	unknown :	JF833916.	99.76	100	0	425	M05317:1	Cluster_18	190	0	2	4	0	0	0	36	72	76	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Enterobac	Erwiniace	Pantoea	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_18	117	1	0	0	0	0	0	0	0	1	0	0	3	38	23	51
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Arcticbact	unknown :	multi-subj	100	100	0	419	M05317:1	Cluster_12	13	0	0	0	0	0	0	0	0	0	0	0	0	2	8	3
na	Bacteria	Actinobac	Thermolec	Solirubrot	67-14	unknown f	unknown :	JF167851.	99.53	100	0	423	M05317:1	Cluster_12	40	2	0	2	7	4	3	12	6	4	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	multi-iden	100	0	multi-aligr	M05317:1	Cluster_43	46	2	0	0	11	14	14	0	0	2	0	1	2	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	unknown :	JF776892.	99.76	100	0	424	M05317:1	Cluster_43	44	1	0	1	10	10	3	11	2	6	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Rhodanob	unknown :	AY218596.	99.77	100	0	426	M05317:1	Cluster_43	51	1	1	0	5	3	0	20	5	5	10	1	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Saprospira	Haliscome	Multi-affili	multi-subj	100	100	0	418	M05317:1	Cluster_64	51	0	0	0	0	0	0	0	0	0	11	27	13	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Puia	metageno	FPLS01058	99.53	100	0	423	M05317:1	Cluster_43	55	2	0	1	0	0	0	16	4	32	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Hyphomic	unknown f	unknown :	multi-subj	100	100	0	424	M05317:1	Cluster_43	91	3	1	1	6	5	1	3	4	1	15	23	7	4	12	5
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Hydrogen	Multi-affili	multi-subj	100	100	0	419	M05317:1	Cluster_10	230	0	0	0	0	0	0	0	0	0	80	86	53	0	6	5
na	Bacteria	Actinobac	Thermolec	Solirubrot	Solirubrob	Conexibac	Multi-affili	multi-subj	99.76	100	0	423	M05317:1	Cluster_98	12	1	0	0	1	7	2	0	0	0	0	0	0	0	0	1
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	unknown f	metageno	FPLS01052	99.53	100	0	424	M05317:1	Cluster_19	12	0	0	0	0	0	0	3	3	5	0	1	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	env.OPS 1	unknown f	Multi-affili	multi-subj	99.05	100	0	419	M05317:1	Cluster_17	15	0	0	0	0	0	0	2	3	2	3	1	4	0	0	0
na	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillacea	Bacillus	Bacillus ha	MK156167	99.29	100	0	424	M05317:1	Cluster_12	12	2	1	7	0	0	0	0	0	0	2	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Oceanospi	Pseudoho	Blyi10	unknown :	AY212747.	100	100	0	426	M05317:1	Cluster_12	13	0	0	0	0	0	0	0	0	0	4	8	1	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Flavitalea	unknown :	HM18648	100	100	0	423	M05317:1	Cluster_12	14	0	1	0	3	0	2	0	1	0	0	0	0	2	3	2
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown f	unknown :	AB821098	100	100	0	427	M05317:1	Cluster_14	18	0	0	1	0	0	0	12	3	2	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Dongiales	Dongiacea	Dongia	unknown :	multi-subj	99.3	100	0	426	M05317:1	Cluster_34	15	1	0	0	1	0	0	0	0	0	3	8	1	0	1	0
na	Bacteria	Bdellovibr	Bdellovibr	Bdellovibr	Bdellovibr	Bdellovibr	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_14	21	0	0	0	3	0	0	0	2	3	3	2	1	4	2	1
na	Bacteria	Firmicutes	Bacilli	Paenibacil	Paenibacil	Saccharib	Saccharib	KY412984.	100	100	0	425	M05317:1	Cluster_11	385	0	0	0	0	0	0	0	0	0	5	0	0	75	236	69
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Flavobacte	FN547415	99.76	100	0	417	M05317:1	Cluster_11	223	1	0	0	0	0	0	0	0	1	52	25	140	1	3	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	100	100	0	417	M05317:1	Cluster_11	251	0	0	0	0	0	0	118	55	78	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Cellvibrior	Cellvibrio	Cellvibrio	Multi-affili	multi-subj	99.53	100	0	425	M05317:1	Cluster_11	255	0	0	0	0	0	0	0	5	4	27	151	68	0	0	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	unknown :	JX221879.	100	100	0	425	M05317:1	Cluster_11	129	44	15	69	0	0	0	0	0	0	0	1	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Multi-affili	multi-subj	99.76	100	0	418	M05317:1	Cluster_11	299	1	0	2	2	57	10	0	0	0	54	5	5	39	15	109
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	KM25307	99.76	100	0	423	M05317:1	Cluster_11	209	0	2	0	82	41	62	7	1	10	2	0	2	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown f	unknown :	FJ936955.	99.53	100	0	426	M05317:1	Cluster_15	22	0	0	1	9	7	4	0	0	0	0	0	1	0	0	0
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Salmonell	Salmonell	multi-subj	99.53	100	0	424	M05317:1	Cluster_50	87	0	0	0	0	0	0	0	0	0	0	0	0	39	8	40
na	Bacteria	Firmicutes	Clostridia	Lachnospi	Lachnospi	Multi-affil	Multi-affili	multi-subj	99.76	100	0	424	M05317:1	Cluster_50	82	0	0	0	0	0	0	0	0	0	26	3	39	1	0	13
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Spirosoma	Emticicia	Emticicia s	AB636297	100	100	0	417	M05317:1	Cluster_50	35	0	1	0	9	4	0	0	0	0	2	9	2	1	2	5
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Comamon	unknown :	multi-subj	99.76	100	0	419	M05317:1	Cluster_20	14	0	0	0	0	0	0	0	0	0	9	3	2	0	0	0
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	unknown f	Escherichi	KX279980.	100	100	0	424	M05317:1	Cluster_50	114	0	0	0	0	0	15	0	0	0	1	9	4	32	18	35

na	Bacteria	Proteobac	Gammapr	Gammapr	unknown	Acidibacte	unknown	multi-subj	99.76	100	0	423	M05317:1	Cluster_3	881	29	3	4	2	0	0	276	254	313	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_3	501	183	79	166	0	0	0	14	1	2	8	15	5	5	8	15
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Dyella	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_3	784	40	25	30	25	94	19	126	129	243	8	42	3	0	0	0
na	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillacea	Bacillus	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_3	581	51	10	5	2	0	461	0	0	1	5	27	6	0	5	8
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_3	701	0	0	0	0	0	0	0	0	0	54	217	406	4	18	2
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	multi-subj	100	100	0	423	M05317:1	Cluster_7	244	22	6	5	14	171	26	0	0	0	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Mucilagini	Multi-affili	multi-subj	100	100	0	419	M05317:1	Cluster_3	656	10	9	6	113	252	76	89	29	70	0	0	2	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	KT590042.	100	100	0	423	M05317:1	Cluster_3	946	82	27	11	153	174	119	42	45	34	95	33	54	10	41	26
na	Bacteria	Proteobac	Alphaprot	Micropep	Micropeps	unknown	unknown	LN567328.	100	100	0	422	M05317:1	Cluster_7	603	21	1	3	10	36	17	157	159	182	4	7	5	0	1	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Moraxella	Acinetoba	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_3	459	169	36	89	3	2	44	0	7	5	22	11	25	31	13	2
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	99.76	100	0	425	M05317:1	Cluster_3	640	50	16	16	0	0	0	24	86	70	30	17	328	1	0	2
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	99.52	100	0	417	M05317:1	Cluster_4	76	0	0	0	0	0	0	0	0	0	5	64	7	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Multi-affil	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_2	140	0	1	0	1	5	6	9	19	15	32	43	9	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	A0839	unknown	unknown	multi-subj	99.53	100	0	422	M05317:1	Cluster_1	28	2	0	0	0	0	0	5	12	9	0	0	0	0	0	0
na	Bacteria	Deinococc	Deinococc	Deinococc	Deinococc	Deinococc	Deinococc	multi-subj	100	100	0	421	M05317:1	Cluster_2	94	0	0	0	0	1	0	0	0	0	1	2	0	15	42	33
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Methyloli	unknown	unknown	multi-subj	100	100	0	419	M05317:1	Cluster_7	36	2	0	0	4	4	5	5	1	4	1	9	1	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Aurantisol	unknown	JF168389.	99.76	100	0	422	M05317:1	Cluster_7	21	0	0	0	0	0	0	0	0	0	1	8	12	0	0	0
na	Bacteria	Firmicutes	Clostridia	Oscillospir	Oscillospir	Sporobact	unknown	multi-subj	100	100	0	421	M05317:1	Cluster_1	20	0	0	1	0	0	0	0	0	0	5	4	10	0	0	0
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Streptomy	AB840588	99.54	100	0	431	M05317:1	Cluster_6	41	4	0	0	6	21	5	0	0	0	5	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Cytophaga	Siphonoba	Siphonoba	FJ177421.	99.05	100	0	420	M05317:1	Cluster_2	11	0	2	3	0	0	0	0	0	0	2	3	0	0	1	0
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Caulobact	Caulobact	KX792139.	99.53	100	0	424	M05317:1	Cluster_9	49	0	1	0	2	0	0	10	12	8	12	0	4	0	0	0
na	Bacteria	Proteobac	Gammapr	Alteromor	Alteromor	Rheinheim	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_6	69	0	0	0	0	0	0	0	0	0	13	25	30	0	1	0
na	Bacteria	Spirochae	Spirochae	Spirochae	Spirochae	Spirochae	unknown	LN571115.	99.29	100	0	420	M05317:1	Cluster_6	42	0	0	0	0	0	0	6	4	31	0	1	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Lacibacter	Lacibacter	KC759435.	100	100	0	424	M05317:1	Cluster_6	35	0	0	0	0	0	0	0	0	0	4	26	5	0	0	0
na	Bacteria	Firmicutes	Clostridia	unknown	Gracilibac	Lutispora	unknown	AB488221	100	100	0	422	M05317:1	Cluster_1	15	0	0	0	0	0	0	0	0	0	10	3	2	0	0	0
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_1	13	0	0	0	0	0	0	0	0	0	0	1	2	1	7	2
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Hyphomic	Hyphomic	Multi-affili	multi-subj	99.76	100	0	424	M05317:1	Cluster_6	29	0	0	0	0	0	0	1	0	0	8	9	8	2	0	1
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Parablastc	unknown	KF712699.	99.77	100	0	427	M05317:1	Cluster_2	14	0	0	0	1	0	0	2	0	1	5	3	2	0	0	0
na	Bacteria	Myxococc	Polyangia	Polyangial	Phaselicys	Phaselicys	unknown	EF516177.	99.53	100	0	428	M05317:1	Cluster_1	16	2	1	0	0	0	0	5	5	3	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Flavobacte	KF532125.	99.28	100	0	417	M05317:1	Cluster_7	28	0	0	0	0	0	0	1	17	10	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Micropep	Micropeps	unknown	unknown	multi-subj	100	100	0	422	M05317:1	Cluster_2	254	2	0	1	4	12	8	109	48	59	1	5	5	0	0	0
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Novosphir	unknown	HM43856	99.06	100	0	427	M05317:1	Cluster_2	105	0	19	0	7	37	10	6	9	14	0	1	0	0	1	1
na	Bacteria	Proteobac	Gammapr	Alteromor	Shewanell	Shewanell	gamma pr	GU594658	99.53	100	0	425	M05317:1	Cluster_2	136	9	45	31	0	1	6	1	0	0	7	7	15	3	2	9
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscill	unknown	metageno	FPLS01025	99.05	100	0	420	M05317:1	Cluster_2	127	0	0	0	3	1	1	16	47	58	1	0	0	0	0	0
na	Bacteria	Firmicutes	Bacilli	Exiguobac	Exiguobac	Exiguobac	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_2	160	1	0	1	0	0	0	0	0	0	28	0	0	54	51	25
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	KD3-93	unknown	unknown	JN391972.	99.76	100	0	420	M05317:1	Cluster_2	126	0	0	0	0	0	0	81	13	32	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Mucilagini	unknown	multi-subj	99.76	100	0	420	M05317:1	Cluster_1	32	0	0	0	0	0	0	18	3	11	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Filimonas	eubacteriu	EU571161	99.53	100	0	423	M05317:1	Cluster_1	18	0	0	0	2	3	0	5	2	3	0	0	0	0	3	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	100	100	0	417	M05317:1	Cluster_2	126	0	0	0	0	0	0	0	0	1	3	85	37	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Flavobacte	EF424395.	100	100	0	417	M05317:1	Cluster_2	118	0	0	0	0	0	0	0	0	0	1	1	0	10	32	74
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	unknown	JQ855616.	100	100	0	417	M05317:1	Cluster_1	16	0	0	0	0	0	0	5	8	3	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Pedobacte	Multi-affili	multi-subj	100	100	0	420	M05317:1	Cluster_8	425	2	0	107	1	0	0	110	78	91	1	11	24	0	0	0
na	Bacteria	Proteobac	Gammapr	Cellvibrior	Cellvibrior	Cellvibrio	unknown	multi-subj	100	100	0	425	M05317:1	Cluster_1	28	0	0	0	0	0	0	1	1	3	3	10	9	1	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Cytophaga	Cytophaga	unknown	LN565004.	99.76	100	0	420	M05317:1	Cluster_1	14	0	0	0	1	5	3	0	0	2	0	3	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Cnuella	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_1	14	0	0	0	0	0	0	0	0	0	0	0	0	1	8	5
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	metageno	FPLS01019	99.29	100	0	424	M05317:1	Cluster_9	28	0	0	0	0	1	1	1	6	4	5	5	4	1	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Methyloli	unknown	unknown	JF168849.	100	100	0	419	M05317:1	Cluster_1	16	1	0	0	3	0	0	5	4	2	1	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Multi-affili	multi-subj	100	100	0	418	M05317:1	Cluster_1	17	0	0	0	0	0	0	0	4	0	6	0	7	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Kaistiacea	Kaistia	Kaistia ge	AM409362	99.77	100	0	427	M05317:1	Cluster_1	16	9	5	0	0	0	0	0	0	0	0	2	0	0	0	0
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	432	M05317:1	Cluster_2	13	1	0	0	0	0	0	5	0	7	0	0	0	0	0	0
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	431	M05317:1	Cluster_1	22	7	5	1	0	0	3	0	0	0	1	0	0	0	2	3
na	Bacteria	Proteobac	Gammapr	Xanthomo	Xanthomo	Arenimon	unknown	HQ592545	99.77	100	0	426	M05317:1	Cluster_1	23	0	0	0	0	0	0	0	0	0	5	16	2	0	0	0
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Novosphir	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_8	29	0	0	0	0	0	0	7	7	7	3	2	2	0	1	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Aminobac	Multi-affili	multi-subj	99.06	100	0	427	M05317:1	Cluster_9	40	0	0	0	4	0	0	0	0	0	1	0	1	7	6	21
na	Bacteria	Deinococc	Deinococc	Deinococc	Deinococc	Deinococc	Deinococc	KP128919.	99.76	100	0	421	M05317:1	Cluster_8	18	0	0	0	2	9	3	0	0	0	0	0	0	2	2	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Multi-affil	Multi-affili	multi-subj	100	100	0	427	M05317:1	Cluster_8	57	0	0	0	0	0	0	0	0	0	5	8	7	0	24	13
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Rudaea	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_8	27	0	0	0	3	3	1	9	6	5	0	0	0	0	0	0
na	Bacteria	Actinobac	Thermolec	Gaiellales	unknown	unknown	unknown	JX501984.	99.76	100	0	425	M05317:1	Cluster_1	12	1	1	0	3	2	2	1	1	1	0	0	0	0	0	0
na	Bacteria	Firmicutes	Bacilli	Lactobacil	Leuconost	Weissella	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_8	16	8	5	1	0	0	0	0	0	0	0	0	0	1	0	1
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Beijerincki	Methyloba	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_8	25	5	3	0	0	3	3	0	0	8	1	0	1	0	0	1
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Puia	Multi-affili	multi-subj	99.29	100	0	multi-aligr	M05317:1	Cluster_8	25	1	3	0	0	0	0	10	3	8	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	A0839	unknown	unknown	FJ936900.	99.76	100	0	422	M05317:1	Cluster_4	44	0	0	0	0	0	0	7	22	13	1	0	1	0	0	0
na	Bacteria	Proteobac	Gammapr	Gammapr	unknown	Acidibacte	unknown	KC331225.	99.05	100	0	423	M05317:1	Cluster_4	51	1	0	0	1	3	2	0	5	2	11	14	12	0	0	0

na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Pseudomo	MH99841	100	100	0	425	M05317:1	Cluster_37	54	10	27	16	1	0	0	0	0	0	0	0	0	0	0	0	0
na	Bacteria	Actinobac	Thermolec	Solirubrot	Solirubrob	Solirubrot	unknown	AJ863193.	99.53	100	0	423	M05317:1	Cluster_47	35	0	0	0	2	19	14	0	0	0	0	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Pedobacte	Multi-affil	multi-subj	99.29	100	0	420	M05317:1	Cluster_47	48	0	0	0	0	1	0	30	10	7	0	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Multi-affil	multi-subj	100	100	0	418	M05317:1	Cluster_10	17	0	0	0	0	0	0	0	0	0	0	0	1	6	4	6	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Hyphomic	Multi-affil	Multi-affil	multi-subj	99.76	100	0	424	M05317:1	Cluster_47	43	1	1	1	3	0	1	13	9	11	0	2	1	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Reyranella	Reyranella	Reyranella	Multi-affil	multi-subj	100	100	0	427	M05317:1	Cluster_58	82	10	1	0	7	10	2	16	11	9	1	4	5	1	2	3	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Pseudamii	Multi-affil	multi-subj	100	100	0	427	M05317:1	Cluster_10	13	0	0	0	4	7	1	0	0	0	0	0	0	1	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Cloacibact	Multi-affil	multi-subj	99.76	100	0	418	M05317:1	Cluster_37	58	0	0	0	0	0	0	0	0	0	0	0	0	3	12	43	
na	Bacteria	Myxococc	Polyangia	Haliangial	Haliangiac	Haliangiur	unknown	JQ427231.	99.3	100	0	426	M05317:1	Cluster_37	50	0	0	0	22	11	3	0	4	10	0	0	0	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Hyphomic	Pedomicrc	Multi-affil	multi-subj	100	100	0	424	M05317:1	Cluster_10	26	1	0	0	1	0	0	3	1	2	11	1	6	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Pedobacte	Pedobacte	AM27921	100	100	0	420	M05317:1	Cluster_13	14	0	0	0	0	0	0	0	1	2	4	1	6	0	0	0	
na	Bacteria	Spirochae	Spirochae	Spirochae	Spirochae	Salinispira	metageno	FPLS01028	99.05	100	0	420	M05317:1	Cluster_42	58	0	0	0	0	0	0	4	2	4	34	6	8	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	unknown	KX753123.	100	100	0	426	M05317:1	Cluster_10	22	0	1	0	1	0	3	4	9	1	0	1	1	1	0	0	
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Altereryth	unknown	LN561674.	99.77	100	0	427	M05317:1	Cluster_10	16	0	0	0	6	2	0	0	2	2	1	0	3	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affil	multi-subj	99.28	100	0	417	M05317:1	Cluster_87	466	0	0	0	0	0	0	28	54	62	125	97	45	36	8	11	
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Luteibacte	Multi-affil	multi-subj	100	100	0	426	M05317:1	Cluster_86	238	6	1	2	26	88	26	0	10	4	13	55	1	0	3	3	
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affil	multi-subj	100	100	0	432	M05317:1	Cluster_27	82	0	0	0	3	7	9	24	29	7	2	0	0	0	1	0	
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Mucilagini	Multi-affil	multi-subj	100	100	0	420	M05317:1	Cluster_27	183	1	1	0	6	18	0	39	71	44	0	0	0	0	2	1	
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affil	multi-subj	100	100	0	425	M05317:1	Cluster_83	569	19	53	1	10	21	9	5	11	7	228	118	67	3	8	9	
na	Bacteria	Proteobac	Alphaprot	Caulobact	Hyphomon	Hirschia	Multi-affil	multi-subj	100	100	0	420	M05317:1	Cluster_27	100	0	1	0	1	2	5	10	22	22	5	24	8	0	0	0	
na	Bacteria	Firmicutes	Bacilli	Bacillales	Planococc	Kurthia	Multi-affil	multi-subj	100	100	0	426	M05317:1	Cluster_81	203	115	82	6	0	0	0	0	0	0	0	0	0	0	0	0	
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Cronobact	Multi-affil	multi-subj	100	100	0	425	M05317:1	Cluster_80	311	0	0	0	0	0	0	0	0	0	0	0	0	98	86	127	
na	Bacteria	Proteobac	Gammapr	Gammapr	unknown	Acidibacte	unknown	HQ674840	99.76	100	0	423	M05317:1	Cluster_27	98	4	1	1	0	1	0	65	12	14	0	0	0	0	0	0	
na	Bacteria	Firmicutes	Bacilli	Paenibacil	Paenibacil	Sacchariba	Sacchariba	KX523193.	99.76	100	0	425	M05317:1	Cluster_11	29	0	0	0	0	0	0	0	0	0	0	0	0	6	16	7	
na	Bacteria	Proteobac	Gammapr	Pseudomc	Pseudomo	Pseudomc	Multi-affil	multi-subj	100	100	0	425	M05317:1	Cluster_89	228	18	0	3	2	45	0	0	6	0	84	31	30	0	5	4	
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Altereryth	unknown	FQ658507	99.77	100	0	427	M05317:1	Cluster_13	20	0	1	0	0	0	0	0	0	0	4	12	3	0	0	0	
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Multi-affil	Multi-affil	multi-subj	100	100	0	419	M05317:1	Cluster_97	58	0	0	0	0	0	0	6	5	3	2	12	7	1	12	10	
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Spirosoma	Spirosoma	Spirosoma	LC221823.	99.29	100	0	423	M05317:1	Cluster_11	15	0	0	0	5	4	6	0	0	0	0	0	0	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown	unknown	multi-subj	99.29	100	0	420	M05317:1	Cluster_51	67	0	0	2	0	0	0	0	0	0	28	25	7	1	1	3	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	Multi-affil	multi-subj	99.06	100	0	424	M05317:1	Cluster_14	14	0	0	0	0	0	0	0	0	0	6	3	1	0	3	1	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	unknown	JN023338.	99.77	100	0	427	M05317:1	Cluster_14	16	0	0	0	0	0	0	13	1	1	0	0	1	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Filimonas	Filimonas	multi-subj	99.29	100	0	423	M05317:1	Cluster_13	27	0	2	2	5	5	1	0	0	1	1	1	1	0	7	1	
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Multi-affil	Multi-affil	multi-subj	99.76	100	0	424	M05317:1	Cluster_13	14	0	0	0	0	0	0	0	0	0	0	0	4	1	9		
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Pedobacte	Pedobacte	KM073965	99.52	100	0	421	M05317:1	Cluster_14	15	0	0	0	0	1	0	2	1	11	0	0	0	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Mucilagini	unknown	JQ919782.	99.52	100	0	420	M05317:1	Cluster_13	18	0	0	0	0	0	0	13	2	3	0	0	0	0	0	0	
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Multi-affil	Multi-affil	multi-subj	99.29	100	0	423	M05317:1	Cluster_15	54	0	0	0	8	0	0	8	5	0	14	11	8	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Phenyloba	Phenyloba	AM41191	100	100	0	422	M05317:1	Cluster_36	12	1	0	0	1	2	0	1	1	0	3	1	1	0	0	1	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiale	Bauldia	unknown	LN570376.	100	100	0	427	M05317:1	Cluster_10	11	1	0	1	4	4	1	0	0	0	0	0	0	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	unknown	KC211858.	99.06	100	0	424	M05317:1	Cluster_10	19	0	0	0	0	0	0	0	0	0	0	0	0	6	2	11	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Multi-affil	Multi-affil	multi-subj	100	100	0	425	M05317:1	Cluster_42	55	2	1	3	5	12	8	8	3	8	2	2	0	1	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Multi-affil	multi-subj	100	100	0	427	M05317:1	Cluster_15	250	0	0	0	58	22	15	1	23	8	32	74	6	3	3	5	
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Niastella	unknown	LN555077.	99.76	100	0	423	M05317:1	Cluster_15	272	0	0	0	0	0	0	77	91	80	4	2	2	5	0	11	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Multi-affil	multi-subj	100	100	0	423	M05317:1	Cluster_15	146	3	14	0	21	12	0	0	2	0	7	4	7	15	27	34	
na	Bacteria	Myxococc	Polyangia	Haliangial	Haliangiac	Haliangiur	unknown	KC432172.	99.77	100	0	426	M05317:1	Cluster_15	157	0	0	0	0	0	3	2	1	1	38	79	31	0	1	1	
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Sphingopy	Multi-affil	multi-subj	100	100	0	427	M05317:1	Cluster_15	170	4	25	0	13	8	8	5	3	4	32	15	13	12	18	10	
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	37-13	unknown	unknown	EF111096.	100	100	0	423	M05317:1	Cluster_15	139	0	0	0	16	52	5	20	23	23	0	0	0	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Multi-affil	multi-subj	99.29	100	0	423	M05317:1	Cluster_15	118	0	0	0	27	46	27	0	0	0	16	2	0	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Rhodopla	unknown	multi-subj	100	100	0	427	M05317:1	Cluster_15	357	7	3	3	11	17	7	83	105	87	12	13	6	0	1	2	
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Multi-affil	Multi-affil	multi-subj	99.76	100	0	419	M05317:1	Cluster_11	21	0	0	0	0	0	0	0	0	1	5	14	1	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Methyloli	unknown	metageno	FPLS01049	100	100	0	419	M05317:1	Cluster_13	11	2	0	0	4	1	1	0	0	1	1	1	0	0	0	0	
na	Bacteria	Bdellovibr	Bdellovibr	Bdellovibr	Bdellovibr	Bdellovibr	Bdellovibr	LUKE0100	99.06	100	0	424	M05317:1	Cluster_13	15	0	0	0	0	0	0	0	0	0	1	0	1	8	5		
na	Bacteria	Proteobac	Alphaprot	Rickettsial	Mitochon	unknown	Syzygium	multi-subj	99.53	100	0	425	M05317:1	Cluster_13	11	0	0	0	0	5	4	0	1	0	0	1	0	0	0	0	
na	Bacteria	Spirochae	Spirochae	Spirochae	Spirochae	Spirochae	Spirochae	FR749896.	99.29	100	0	420	M05317:1	Cluster_38	69	0	0	0	1	0	0	17	26	20	0	1	4	0	0	0	
na	Bacteria	Deinococc	Deinococc	Deinococc	Deinococc	Deinococc	Deinococc	JF806527.	100	100	0	421	M05317:1	Cluster_38	57	0	0	0	0	0	0	0	0	0	0	0	0	23	17	17	
na	Bacteria	Myxococc	Polyangia	Polyangial	Blrii41	unknown	unknown	multi-subj	99.3	100	0	427	M05317:1	Cluster_38	98	0	0	0	0	1	0	39	26	32	0	0	0	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Labraceae	Labrys	Multi-affil	multi-subj	99.3	100	0	427	M05317:1	Cluster_38	46	27	0	1	1	8	3	2	1	3	0	0	0	0	0	0	
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Escherichi	Multi-affil	multi-subj	99.29	100	0	424	M05317:1	Cluster_19	18	3	0	0	0	0	0	0	0	0	0	0	1	9	4	1	
na	Bacteria	Firmicutes	Clostridia	unknown	Hungateic	Ruminiclo	Ruminiclo	multi-subj	99.29	100	0	423	M05317:1	Cluster_19	15	0	0	0	0	0	0	0	0	0	6	7	2	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	unknown	JF181187.	99.53	100	0	423	M05317:1	Cluster_19	15	1	0	0	6	2	0	1	1	1	3	0	0	0	0	0	
na	Bacteria	Proteobac	Gammapr	Gammapr	unknown	Acidibacte	unknown	FJ478892.	99.53	100	0	423	M05317:1	Cluster_38	67	0	0	0	1	1	3	12	23	26	0	0	0	0	0	1	
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	unknown	metageno	FPLS01022	99.76	100	0	422	M05317:1	Cluster_12	21	0	0	0	1	0	0	7	8	5	0	0	0	0	0	0	
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Hyphomic	Hyphomic	Multi-affil	multi-subj	99.53	100	0	424	M05317:1	Cluster_30	9	2	0	1	0	0	0	0	0	0	3	1	2	0	0	0	
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Edaphoba	Multi-affil	multi-subj	99.29	100	0	423	M05317:1	Cluster_12	39	0	0	0	0	3	0	8	12	14	0	0	2	0	0	0	

| | | |

na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	99.52	100	0	417	M05317:1	Cluster_83	26	0	0	0	0	0	0	0	0	0	1	22	3	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Parafilimo	Multi-affili	multi-subj	99.53	100	0	423	M05317:1	Cluster_72	53	0	0	0	3	32	8	4	3	2	1	0	0	0	0	0
na	Bacteria	Firmicutes	Clostridia	unknown	Hungateic	Ruminiclo	unknown :	JX843556.	99.53	100	0	423	M05317:1	Cluster_72	19	0	0	0	0	0	0	0	0	0	14	1	4	0	0	0
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	100	100	0	431	M05317:1	Cluster_72	20	11	3	0	1	1	0	0	0	0	0	1	3	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown	unknown :	KR110072.	99.76	100	0	420	M05317:1	Cluster_16	16	0	0	0	0	0	0	4	8	4	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	Cellvibrior	Cellvibrior	Cellvibrio	Multi-affili	multi-subj	100	100	0	425	M05317:1	Cluster_72	45	0	0	0	0	0	0	3	11	6	6	6	13	0	0	0
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Blastomor	Multi-affili	multi-subj	99.77	100	0	427	M05317:1	Cluster_11	18	0	0	0	0	0	0	0	0	0	0	0	0	4	6	8
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	Multi-affili	multi-subj	99.77	100	0	427	M05317:1	Cluster_17	11	0	0	0	0	0	0	2	3	0	1	2	0	0	1	2
na	Bacteria	Spirochae	Spirochae	Spirochae	Spirochae	Spirochae	Spirochae	AB540020	100	100	0	420	M05317:1	Cluster_84	327	0	0	0	0	0	0	0	0	0	120	73	134	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Beijerincki	Microvirga	unknown :	AY725254.	99.53	100	0	427	M05317:1	Cluster_17	22	0	0	1	0	0	0	0	0	0	2	3	2	4	3	7
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Hyphomic	Pedomicro	unknown :	LN562377.	99.76	100	0	425	M05317:1	Cluster_25	13	2	0	1	0	0	0	0	1	0	2	2	1	1	2	1
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Asticcacau	Multi-affili	multi-subj	99.76	100	0	422	M05317:1	Cluster_10	20	0	0	0	0	0	0	0	0	0	1	9	7	0	2	1
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Beijerincki	Microvirga	unknown :	DQ29834(	99.3	100	0	427	M05317:1	Cluster_99	32	0	0	0	0	0	0	0	0	0	0	0	0	5	9	18
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown	metageno	FPLS01013	99.29	100	0	420	M05317:1	Cluster_99	17	0	0	0	0	0	0	0	0	1	14	1	1	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	unknown :	multi-subj	100	100	0	426	M05317:1	Cluster_10	32	1	0	1	1	3	4	1	2	0	6	4	3	0	3	3
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Terrimona	unknown :	JX047141.	99.05	100	0	423	M05317:1	Cluster_99	23	0	0	0	0	0	0	0	21	0	1	0	1	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Neorhizob	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_11	17	0	0	0	9	2	1	0	2	2	0	0	1	0	0	0
na	Bacteria	Actinobac	Actinobac	Streptomy	Streptomy	Streptomy	Multi-affili	multi-subj	99.07	100	0	432	M05317:1	Cluster_33	56	0	1	2	39	4	9	0	0	0	1	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Rurimicrol	unknown :	multi-subj	99.53	100	0	multi-align	M05317:1	Cluster_11	16	0	0	0	0	0	0	0	0	0	4	11	1	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	unknown :	HM270719	99.52	100	0	417	M05317:1	Cluster_10	22	0	0	0	0	0	0	0	1	0	15	6	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	unknown	unknown :	multi-subj	99.3	100	0	427	M05317:1	Cluster_12	11	2	0	0	4	3	0	1	1	0	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Hyphomic	Pedomicro	metageno	FPLS01045	99.76	100	0	424	M05317:1	Cluster_99	18	0	0	1	2	1	0	2	8	3	1	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_23	106	3	0	0	1	1	2	1	4	2	35	35	22	0	0	0
na	Bacteria	Proteobac	Gammapr	Enterobac	Enterobac	Citrobacte	unknown :	JQ918039.	99.76	100	0	424	M05317:1	Cluster_23	113	0	0	0	0	0	0	0	0	0	106	2	5	0	0	0
na	Bacteria	Actinobac	Thermolec	Gaiellales	Gaiellacea	Gaiella	unknown :	JX133301.	100	100	0	425	M05317:1	Cluster_11	26	0	1	0	1	7	3	1	10	1	0	2	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	99.76	100	0	417	M05317:1	Cluster_24	98	0	0	0	0	0	0	74	1	13	0	2	8	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Flavobacte	Flavobacte	Multi-affili	multi-subj	99.76	100	0	417	M05317:1	Cluster_77	47	0	0	0	0	0	0	5	22	13	0	6	1	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Flavobacte	Weeksella	Chryseoba	Multi-affili	multi-subj	100	100	0	418	M05317:1	Cluster_27	259	2	0	1	0	0	0	82	48	58	2	20	45	0	1	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Niastella	unknown :	multi-subj	100	100	0	423	M05317:1	Cluster_10	18	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5	8
na	Bacteria	Firmicutes	Bacilli	Bacillales	Planococc	Lysinibacil	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_24	17	0	0	2	0	0	5	0	0	0	0	0	0	2	2	6
na	Bacteria	Proteobac	Alphaprot	unknown	unknown	unknown	unknown :	GQ203642	99.06	100	0	427	M05317:1	Cluster_15	22	0	0	0	0	0	0	0	0	0	7	11	4	0	0	0
na	Bacteria	Myxococc	Polyangia	Haliangial	Haliangiac	Haliangiur	unknown :	GU208498	99.29	100	0	425	M05317:1	Cluster_15	18	0	0	0	2	2	1	3	6	4	0	0	0	0	0	0
na	Bacteria	Actinobac	Thermolec	Gaiellales	unknown	unknown	unknown :	KT342857.	100	100	0	424	M05317:1	Cluster_90	27	3	0	0	1	4	4	7	3	5	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Caulobact	Caulobact	Caulobact	unknown :	GQ389044	99.05	100	0	422	M05317:1	Cluster_90	32	0	0	0	2	2	1	0	0	2	5	10	0	3	1	6
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Beijerincki	Microvirga	Multi-affili	multi-subj	99.3	100	0	427	M05317:1	Cluster_11	64	0	0	1	2	0	7	0	0	0	0	0	0	2	17	35
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Mesorhizc	unknown :	HM30855.	100	100	0	426	M05317:1	Cluster_11	28	5	6	0	1	0	0	0	3	1	2	6	0	1	0	3
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Arcticibact	Arcticibact	JX949238.	99.29	100	0	420	M05317:1	Cluster_90	29	0	0	0	0	0	0	0	0	0	0	0	5	13	4	7
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown	unknown :	EU133763	100	100	0	420	M05317:1	Cluster_32	148	0	0	0	0	0	0	0	3	0	27	103	15	0	0	0
na	Bacteria	Actinobac	Thermolec	Solirubrob	67-14	unknown	Solirubrob	MKST0100	100	100	0	423	M05317:1	Cluster_15	11	0	0	1	1	2	1	0	1	2	0	3	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Edaphoba	unknown :	LN570644.	99.05	100	0	423	M05317:1	Cluster_15	10	0	0	0	2	3	3	0	0	1	0	0	0	1	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Microscilla	unknown	unknown :	HQ119896	99.76	100	0	420	M05317:1	Cluster_90	29	0	0	0	0	0	0	0	0	0	5	11	8	0	4	1
na	Bacteria	Proteobac	Alphaprot	Dongiales	Dongiacea	Dongia	unknown :	JN024013.	100	100	0	427	M05317:1	Cluster_24	132	0	0	0	0	3	0	43	56	30	0	0	0	0	0	0
na	Bacteria	Myxococc	Polyangia	Haliangial	Haliangiac	Haliangiur	unknown :	AM08488	99.53	100	0	425	M05317:1	Cluster_67	29	0	0	0	0	0	0	3	0	7	0	4	15	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	unknown	Chitinoph	JF922313.	99.29	100	0	423	M05317:1	Cluster_10	25	0	0	0	1	10	7	1	0	0	1	1	3	0	1	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Hyphomic	Hyphomic	Multi-affili	multi-subj	100	100	0	424	M05317:1	Cluster_42	81	39	3	5	14	6	4	2	2	2	2	1	0	0	0	1
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	unknown	Multi-affili	multi-subj	100	100	0	419	M05317:1	Cluster_67	39	0	0	0	0	0	0	23	4	12	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Pedobacte	Pedobacte	multi-subj	99.52	100	0	420	M05317:1	Cluster_12	15	0	0	0	1	1	8	0	1	4	0	0	0	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Cytophaga	Spirosoma	Emticicia	Emticicia s	JN712179.	99.28	100	0	417	M05317:1	Cluster_58	42	0	0	0	0	0	0	0	0	0	1	39	2	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Beijerincki	Methyloba	Multi-affili	multi-subj	100	100	0	423	M05317:1	Cluster_12	16	13	2	1	0	0	0	0	0	0	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Sphingom	Sphingom	Sphingom	metageno	FPLS01038	99.76	100	0	425	M05317:1	Cluster_13	21	0	0	0	1	1	0	6	0	4	2	6	1	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Rhizobium	FWXU0100	99.77	100	0	427	M05317:1	Cluster_98	22	0	0	0	0	0	0	0	0	0	7	11	2	0	0	2
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	env.OPS 1	unknown	unknown :	multi-subj	99.76	100	0	421	M05317:1	Cluster_42	49	0	0	0	1	0	0	3	3	4	1	3	34	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Xanthobac	Pseudolab	unknown :	JN409198.	100	100	0	426	M05317:1	Cluster_13	11	0	0	0	1	2	2	0	0	0	0	0	4	2	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Pedobacte	Pedobacte	AM27921	99.05	100	0	420	M05317:1	Cluster_13	14	1	0	2	0	0	0	0	0	0	2	1	8	0	0	0
na	Bacteria	Proteobac	Gammapr	Xanthomo	Rhodanob	Rhodanob	unknown :	multi-subj	100	100	0	426	M05317:1	Cluster_42	67	0	0	1	0	0	0	21	12	33	0	0	0	0	0	0
na	Bacteria	Bdellovibr	Bdellovibr	Bdellovibr	Bdellovibr	Bdellovibr	Bdellovibr	LUKE0100	99.53	100	0	424	M05317:1	Cluster_13	18	1	0	1	4	6	3	0	0	0	1	0	1	0	1	0
na	Bacteria	Proteobac	Alphaprot	Dongiales	Dongiacea	Dongia	unknown :	multi-subj	99.53	100	0	426	M05317:1	Cluster_11	16	0	0	0	0	3	0	0	0	0	6	2	5	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Puia	unknown :	FJ466157.	99.53	100	0	423	M05317:1	Cluster_34	72	17	0	0	0	1	0	32	3	19	0	0	0	0	0	0
na	Bacteria	Proteobac	Gammapr	R7C24	unknown	unknown	unknown :	JF198689.	99.76	100	0	423	M05317:1	Cluster_34	85	0	0	0	1	5	3	2	2	2	27	21	22	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Devosia	unknown :	HQ119475	100	100	0	424	M05317:1	Cluster_34	130	1	1	0	20	10	14	8	25	12	6	23	8	0	2	0
na	Bacteria	Firmicutes	Bacilli	Bacillales	Planococc	Psychroba	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_34	56	5	0	1	0	0	47	0	0	0	0	2	1	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Mucilagini	unknown :	HM27879	99.52	100	0	420	M05317:1	Cluster_98	20	0	0	0	0	0	0	2	10	8	0	0	0	0	0	0
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhodomic	Rhodomic	unknown :	multi-subj	99.53	100	0	425	M05317:1	Cluster_34	76	1	0	0	0	0	0	11	39	24	1	0	0	0	0	0

na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	Allorhizob	Multi-affili	multi-subj	99.77	100	0	427	M05317:1	Cluster_34	60	0	0	0	1	0	0	0	0	0	4	0	47	0	4	4
na	Bacteria	Proteobac	Gammapr	Xanthomo	Xanthomo	Lysobacte	Multi-affili	multi-subj	100	100	0	426	M05317:1	Cluster_45	45	2	11	0	0	0	0	1	17	9	1	0	1	1	2	0
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	Sphingoba	Pedobacte	Pedobacte	MG71999	100	100	0	420	M05317:1	Cluster_13	24	0	0	0	0	1	1	0	0	0	0	0	0	1	10	11
na	Bacteria	Bacteroidc	Bacteroidi	Sphingoba	NS11-12 m	unknown	Multi-affili	multi-subj	100	100	0	420	M05317:1	Cluster_19	14	0	0	0	0	0	0	0	1	0	5	6	2	0	0	0
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Niastella	unknown	LN563474	100	100	0	423	M05317:1	Cluster_19	18	0	0	0	0	0	0	0	7	0	2	0	1	1	4	3
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Heliimona	unknown	JQ684312	99.05	100	0	423	M05317:1	Cluster_30	10	1	0	0	0	5	0	0	0	2	1	0	1	0	0	0
na	Bacteria	Proteobac	Gammapr	Steroidob	Steroidob	Multi-affil	Multi-affili	multi-subj	99.53	100	0	423	M05317:1	Cluster_30	15	0	0	0	0	0	0	0	1	0	4	1	1	2	4	2
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Rhizobiace	unknown	unknown	EF123312	99.3	100	0	427	M05317:1	Cluster_13	17	1	0	0	1	3	2	0	0	0	0	0	1	0	6	3
na	Bacteria	Bacteroidc	Bacteroidi	Chitinoph	Chitinoph	Dinghuiba	unknown	MH04099	99.76	100	0	423	M05317:1	Cluster_14	12	0	0	0	0	0	0	0	0	0	5	4	3	0	0	0
na	Bacteria	Proteobac	Betaprote	Burkholde	Comamon	Multi-affil	Multi-affili	multi-subj	100	100	0	419	M05317:1	Cluster_13	38	1	0	0	4	7	1	0	0	1	8	8	6	1	1	0
na	Bacteria	Proteobac	Gammapr	Aeromona	Aeromona	Aeromona	Aeromona	multi-subj	100	100	0	425	M05317:1	Cluster_36	61	0	0	0	0	0	0	0	0	0	0	0	0	2	19	40
na	Bacteria	Proteobac	Alphaprot	Rhizobiale	Devosiace	Multi-affil	Multi-affili	multi-subj	99.06	100	0	multi-aligr	M05317:1	Cluster_14	14	0	0	0	0	0	0	7	6	1	0	0	0	0	0	0
na	Bacteria	Firmicutes	Clostridia	Peptostrep	Anaerovor	Anaerovor	unknown	JX222367.	99.53	100	0	423	M05317:1	Cluster_14	25	0	0	0	0	0	0	0	0	0	11	5	9	0	0	0

Chapter 2 _ Part 1

Table S3| A list of bacterial isolates exhibiting higher than 50% of inhibition activity against *Fusarium graminearum* mycelial growth

Region	Location	Replicate	Accession	Inhibition rate (%)	Phylum	Family	Genus	Top hit	identity
			M1_03	50.9	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			M1_07	54.5	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia radicincitans DSM 16656	99.67
		M.1	M1_08	71.3	Proteobacteria	Yersiniaceae	Serratia	Serratia marcescens subsp. marcescens ATCC 13880 str	100
			M1_10	65.2	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia sacchari strain SP1	100
			M1_11	58.1	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia radicincitans DSM 16656	99.45
			M1_16	60.2	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			M2_01	59.9	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			M2_03	61.7	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			M2_04	57.4	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	99.65
			M2_05	59.9	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			M2_06	52.7	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			M2_08	62.4	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	99.51
			M2_09	51.6	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	99.7
		M.2	M2_10	56.6	Proteobacteria	Erwiniaceae	Pantoea	Pantoea anthophila strain LMG 2558	100
	Madagascar		M2_11	52.7	Proteobacteria	Enterobacteriaceae	Enterobacter	Enterobacter kobei strain CIP 105566	100
			M2_12	62.4	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			M2_13	55.2	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			M2_14	50.2	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas nitroreducens strain NBRC 12694	100
			M2_19	62.4	Proteobacteria	Yersiniaceae	Serratia	Rouxiella chamberiensis strain 130333	99.58
			M2_23	57.7	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	99.39
			M2_24	52.3	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			M2_25	52.3	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			M3_01	54.5	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			M3_06	57.7	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			M3_11	52.3	Proteobacteria	Erwiniaceae	Pantoea	Pantoea ananatis strain 1846	99.71
		M.3	M3_12	66.7	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia oryziphila strain REICA_142	99.35
			M3_13	66.3	Proteobacteria	Erwiniaceae	Pantoea	Pantoea ananatis strain 1846	99.84
			M3_14	53.1	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia oryzendophytica strain REICA_082	100
			M3_17	56.6	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			P1_03	51.3	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas baetica strain a390	99.74
		P.1	P1_11	64.9	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas mediterranea CFBP 5447	99.67
			P1_18	63.1	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas mediterranea CFBP 5447	99.34
			P2_01	64.9	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			P2_02	73.5	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			P2_03	67.0	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			P2_06	78.5	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas vancouverensis strain DhA-51	99.61
			P2_07	59.2	Proteobacteria	Xanthomonadaceae	Stenotrophomona	Stenotrophomonas maltophilia strain NBRC 14161	100
			P2_08	55.9	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			P2_09	68.1	Firmicutes	Paenibacillaceae	Paenibacillus	Paenibacillus pabuli strain JCM 9074	99.38
			P2_10	54.8	Firmicutes	Paenibacillaceae	Paenibacillus	Paenibacillus pabuli strain JCM 9074	96.6
			P2_12	65.6	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
		P.2	P2_15	73.1	Proteobacteria	Yersiniaceae	Serratia	Serratia grimesii strain DSM 30063	99.67
			P2_16	72.1	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas vancouverensis strain DhA-51	100
			P2_21	67.7	Proteobacteria	Xanthomonadaceae	Stenotrophomona	Stenotrophomonas maltophilia strain ATCC 13637	98.53
			P2_22	55.2	Proteobacteria	Xanthomonadaceae	Stenotrophomona	Stenotrophomonas maltophilia strain ATCC 13637	98.51
			P2_24	68.8	Proteobacteria	Xanthomonadaceae	Stenotrophomona	Stenotrophomonas maltophilia strain ATCC 13637	98.86
			P2_25	79.2	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas vancouverensis strain DhA-51	100
			P2_26	59.1	Proteobacteria	Xanthomonadaceae	Stenotrophomona	Stenotrophomonas maltophilia strain ATCC 13637	98.51
			P2_28	70.6	Actinobacteriota	Microbacteriaceae	Microbacterium	Microbacterium hominis strain IFO 15708	100
			P2_29	56.6	Proteobacteria	Xanthomonadaceae	Stenotrophomona	Stenotrophomonas maltophilia strain ATCC 13637	99.03
			P2_30	55.9	Firmicutes	Paenibacillaceae	Paenibacillus	Paenibacillus mobilis strain S8	99.75
	Portugal		P3_01	72.1	Proteobacteria	Yersiniaceae	Serratia	Serratia grimesii strain DSM 30063	99.69
			P3_03	61.3	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia cowanii JCM 10956 = DSM 18146 strain 888	99.71
			P3_04	51.6	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia cowanii JCM 10956 = DSM 18146 strain 888	99.86
			P3_05	62.0	Proteobacteria	Yersiniaceae	Rahnella1	Rahnella aquatilis CIP 78.65 = ATCC 33071 strain DSM 4	98.87
			P3_06	67.8	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia cowanii JCM 10956 = DSM 18146 strain 888	99.84
			P3_07	73.1	Proteobacteria	Yersiniaceae	Serratia	Serratia grimesii strain DSM 30063	99.68
			P3_08	71.0	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			P3_10	67.4	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			P3_13	74.9	Proteobacteria	Yersiniaceae	Serratia	Serratia grimesii strain DSM 30063	99.67
			P3_15	53.8	Actinobacteriota	Microbacteriaceae	Microbacterium	Microbacterium maritypicum strain DSM 12512	100
			P3_16	66.3	Proteobacteria	Enterobacteriaceae	Pseudescherichia	Pseudescherichia vulneris strain NBRC 102420	99.74
		P.3	P3_17	73.8	Proteobacteria	Yersiniaceae	Serratia	Serratia grimesii strain DSM 30063	99.67
			P3_18	70.6	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	99.44
			P3_19	71.7	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	99.42
			P3_20	64.9	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia oryzendophytica strain REICA_082	100
			P3_22	67.4	Actinobacteriota	Microbacteriaceae	Microbacterium	Microbacterium maritypicum strain DSM 12512	100
			P3_24	74.2	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	99.45
			P3_25	73.1	Proteobacteria	Yersiniaceae	Serratia	Serratia grimesii strain DSM 30063	99.65
			P3_26	72.1	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	99.79
			P3_27	73.8	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	99.51

Region	Location	Replicate	Accession	Inhibition rate (%)	Phylum	Family	Genus	Top hit	identity
Reunion island	Reunion1	R1.1	P3_28	78.1	Proteobacteria	Yersiniaceae	Serratia	Serratia grimesii strain DSM 30063	99.67
			P3_29	79.9	Proteobacteria	Yersiniaceae	Serratia	Serratia grimesii strain DSM 30063	99.71
			P3_30	73.5	Proteobacteria	Yersiniaceae	Serratia	Serratia grimesii strain DSM 30063	99.7
			R11_01	65.0	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	98.84
			R11_02	55.0	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			R11_03	50.8	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
		R1.2	R11_10	50.0	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas baetica strain a390	99.05
			R12_27	63.3	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas helmanticensis strain OHA11	99.41
			R12_28	51.7	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			R13_10	60.0	Proteobacteria	Enterobacteriaceae	Lelliottia	Lelliottia amnigena strain JCM1237	99.42
		R1.3	R13_17	65.0	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas baetica strain a390	99.29
			R13_23	51.7	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			R13_28	61.7	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas helmanticensis strain OHA11	99.16
			R13_29	60.0	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas umsongensis strain Ps 3-10	98.87
			R13_30	63.3	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas baetica strain a390	99.43
		R2.1	R21_01	56.7	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia radicincitans DSM 16656	100
			R21_03	66.7	Proteobacteria	Comamonadaceae	Delftia	Delftia lacustris strain 332	100
			R21_04	65.0	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia arachidis strain Ah-143	100
			R21_05	50.0	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia radicincitans strain DSM 16656	99.4
			R21_07	50.0	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia oryzendophytica strain REICA_082	100
			R21_11	55.0	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia radicincitans DSM 16656	100
			R21_29	65.0	Firmicutes	Bacillaceae	Bacillus	Bacillus subtilis strain IAM 12118	100
			R21_30	65.0	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia radicincitans strain DSM 16656	99
		R2.2	R22_05	81.7	Firmicutes	Bacillaceae	Bacillus	Bacillus subtilis strain IAM 12118	99.83
			R22_06	81.7	Firmicutes	Bacillaceae	Bacillus	Bacillus subtilis strain IAM 12118	100
			R22_08	70.0	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas nitroreducens strain NBRC 12694	100
			R22_12	58.3	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas nitroreducens strain NBRC 12694	99.8
			R22_13	53.3	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas nitroreducens strain NBRC 12694	100
			R22_15	56.7	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas nitroreducens strain NBRC 12694	99.26
	Reunion2		R22_17	93.3	Firmicutes	Bacillaceae	Bacillus	Bacillus subtilis strain IAM 12118	97.95
			R22_19	65.0	Firmicutes	Bacillaceae	Bacillus	Bacillus subtilis strain IAM 12118	97.99
			R22_24	50.0	Proteobacteria	Xanthomonadaceae	Stenotrophomona	Stenotrophomonas tumulicola strain T5916-2-1b	98.09
		R2.3	R23_01	60.0	Firmicutes	Bacillaceae	Bacillus	Bacillus subtilis strain IAM 12118	100
			R23_05	68.3	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas plecoglossicida strain NBRC103162	99.81
			R23_08	75.0	Firmicutes	Bacillaceae	Bacillus	Bacillus subtilis strain IAM 12118	100
			R23_10	50.0	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas plecoglossicida strain NBRC 103162	99.5
			R23_12	70.0	Firmicutes	Bacillaceae	Bacillus	Bacillus subtilis strain IAM 12118	99.86
			R23_13	58.3	Firmicutes	Bacillaceae	Bacillus	Bacillus subtilis strain IAM 12118	100
			R23_17	78.3	Firmicutes	Bacillaceae	Bacillus	Bacillus cereus ATCC 14579	100
			R23_21	61.7	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas plecoglossicida strain NBRC 103162	100
			R23_22	61.7	Firmicutes	Bacillaceae	Bacillus	Bacillus megaterium strain ATCC 14581	100
			R23_27	68.3	Firmicutes	Bacillaceae	Bacillus	Bacillus subtilis strain IAM 12118	100
			R23_28	73.3	Firmicutes	Bacillaceae	Bacillus	Bacillus subtilis strain IAM 12118	100
			R23_30	50.0	Firmicutes	Bacillaceae	Bacillus	Bacillus aryabhattai B8W22	100
Senegal		S.1	S1_01	54.1	Proteobacteria	Enterobacteriaceae	Pseudescherichia	Pseudescherichia vulneris strain ATCC 33821	99.87
			S1_02	65.2	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	99.51
			S1_10	54.8	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	99.5
			S1_12	69.9	Proteobacteria	Oxalobacteraceae	Janthinobacterium	Janthinobacterium lividum strain DSM 1522	99.71
			S1_13	62.0	Proteobacteria	Enterobacteriaceae	Enterobacter	Enterobacter cloacae strain ATCC 13047	100
			S1_15	62.7	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	99.47
			S1_19	64.5	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	99.47
			S1_20	64.5	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	99.23
			S1_21	68.8	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			S1_26	64.2	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	99.32
			S1_27	63.1	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	99.33
			S1_28	64.5	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			S1_29	82.8	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia oryzendophytica strain REICA_082	100
			S1_30	59.1	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
		S.2	S2_02	64.5	Proteobacteria	Oxalobacteraceae	Janthinobacterium	Janthinobacterium agaricidamnosum strain NBRC 1025	100
			S2_03	66.0	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			S2_07	67.8	Proteobacteria	Oxalobacteraceae	Janthinobacterium	Janthinobacterium lividum strain DSM 1522	99.74
			S2_08	67.0	Actinobacteriota	Cellulomonadaceae	Cellulomonas	Cellulomonas persica strain I	100
			S2_09	52.3	Proteobacteria	Oxalobacteraceae	Janthinobacterium	Janthinobacterium agaricidamnosum strain NBRC 1025	100
			S2_11	82.5	Proteobacteria	Oxalobacteraceae	Janthinobacterium	Janthinobacterium lividum strain DSM 1522	99.71
			S2_14	62.7	Proteobacteria	Oxalobacteraceae	Janthinobacterium	Janthinobacterium lividum strain DSM 1522	99.86
			S2_15	65.6	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			S2_18	76.0	Proteobacteria	Oxalobacteraceae	Janthinobacterium	Janthinobacterium agaricidamnosum strain NBRC 1025	100
			S2_20	65.6	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			S2_21	62.4	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			S2_27	58.8	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas koreensis strain Ps 9-14	100
			S2_28	65.2	Proteobacteria	Enterobacteriaceae	Kosakonia	Kosakonia oryzendophytica strain REICA_082	100
			S3_05	64.5	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas lurida strain P 513/18	100
			S3_11	54.1	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas lurida strain P 513/18	100
			S3_15	69.5	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas otitidis strain MCC10330	99.57

Region	Location	Replicate	Accession	Inhibition rate (%)	Phylum	Family	Genus	Top hit	identity
			S3_18	63.4	Firmicutes	Bacillaceae	Bacillus	Bacillus pumilus strain NBRC 12092	100
			S3_19	68.8	Firmicutes	Bacillaceae	Bacillus	Bacillus pumilus strain NBRC 12092	100
		S.3	S3_21	67.4	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas endophytica strain BSTT44	100
			S3_22	62.4	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas endophytica strain BSTT44	100
			S3_23	57.4	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas lurida strain P 513/18	100
			S3_24	67.7	Proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas lurida strain P 513/18	100
			S3_28	67.0	Firmicutes	Bacillaceae	Bacillus	Bacillus stratosphericus strain 41KF2a	100
			S3_30	69.9	Firmicutes	Bacillaceae	Bacillus	Bacillus pumilus strain NBRC 12092	100