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

4

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9

10 Author contribution

11 **YM**: Writing - original draft preparation, investigation, visualization. **CG**: Investigation. **JG**:
12 Investigation. **FB**: Conceptualization and resources. **AH**: Writing – review & editing, funding
13 acquisition. **SSD**: Conceptualization, methodology, investigation, writing – review & editing,
14 funding acquisition

15

16 Abstract

17 Given the current trend towards reducing the use of chemical controls in agriculture,
18 microbial resources such as plant endophytes are being intensively investigated for traits
19 that are conducive to plant protection. Among the various important target pathogens,
20 *Fusarium graminearum* is a fungal pathogen of cereal crops that is responsible for severe
21 yield losses and mycotoxin contamination in grains. In the present study, we investigated
22 the bacterial endophytic communities from vetiver (*Chrysopogon zizanioides* (L.) Roberty)
23 roots originating from 5 different geographic locations across Europe and Africa. This study
24 relies on a global 16S metabarcoding approach and the isolation/functional characterization

25 of bacterial isolates. The results we obtained showed that geographical location is a factor
26 that influences the composition and relative abundance of root endophyte communities in
27 vetiver. Three hundred eighty-one bacterial endophytes were isolated and assessed for
28 their *in vitro* antagonistic activities towards *F. graminearum* mycelium growth. In total, 46%
29 of the isolates showed at least 50% inhibitory activity against *F. graminearum*. The
30 taxonomic identification of the bioactive isolates revealed that the composition of these
31 functional culturable endophytic communities was influenced by the geographic origins of
32 the roots. The selected communities consisted of 15 genera. Some endophytes in *Bacillus*,
33 *Janthinobacterium*, *Kosakonia*, *Microbacterium*, *Pseudomonas*, and *Serratia* showed strong
34 growth inhibition activity ($\geq 70\%$) against *F. graminearum* and could be candidates for
35 further development as biocontrol agents.

36

37 Keywords

38 Bacterial endophytes, *Fusarium graminearum*, antagonistic activity, vetiver root microbiome,
39 geographic location, 16S rDNA high-throughput sequencing

40

41 **1. Introduction**

42 *Fusarium graminearum* is a causal agent of important diseases in small-grain cereals
43 such as wheat (Dean et al., 2012; Taheri, 2018). This pathogen can induce seedling blight,
44 root and stem-based rots and ear blight, which can lead to significant yield losses (Bottalico
45 and Perrone, 2002). In addition, as this pathogenic fungus is able to contaminate cereals
46 with mycotoxins, it can threaten human and animal health (Xu and Nicholson, 2009).
47 Agricultural practices such as crop rotation, soil tillage, the application of chemical
48 fungicides and the use of resistant cultivars are considered as the most effective strategies

49 for controlling *F. graminearum*. However, these strategies are not always fully effective in
50 controlling disease symptoms and preventing the accumulation of mycotoxins (Lamichhane
51 et al., 2015; van Lenteren et al., 2018; Wegulo et al., 2015). The use of chemical fungicides
52 may also cause concern for human health and the environment (Lamichhane et al., 2015).
53 Therefore, reducing the use of these chemicals has become a major concern in European
54 regulations in recent years. In this context, biocontrol agents, within the framework of
55 integrated pest management strategies, are promising solutions that protect plants from
56 biotic stresses and considered relatively environmentally friendly (Köhl et al., 2019; van
57 Lenteren et al., 2018; Wegulo et al., 2015).

58 Many bacterial and fungal microorganisms isolated from different environments, such as
59 the rhizosphere or the surface of wheat anthers, were investigated in both *in vitro* assays
60 and field experiments for their antagonistic activity towards *F. graminearum* (Shahzad et al.,
61 2018; Tian et al., 2016). Recently, microbial endophytes that colonize inner plant tissues
62 during at least part of their life cycle have attracted attention. It is assumed that they might
63 protect plants more efficiently than rhizospheric and epiphytic microorganisms (Backman
64 and Sikora, 2008; Brader et al., 2014). These beneficial effects on plant health might be
65 specifically related to the ability of endophytic microorganisms to produce antimicrobial
66 substances (Ek-Ramos et al., 2019; Gouda et al., 2016; Singh et al., 2017). Since
67 endophytes have to survive in very different environmental conditions, they might be able to
68 produce a greater diversity of antimicrobial metabolites than free-living microorganisms
69 (Brader et al., 2014; Hardoim et al., 2015). Endophytes represent, therefore, pathways for
70 identifying new sources of biocontrol agents, and their diversity needs to be further
71 investigated (Hardoim et al., 2015; Lebeis et al., 2015; Thiergart et al., 2020; Toju et al.,
72 2019).

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

80 Vetiver essential oil is composed of highly diverse sesquiterpene derivatives (Belhassen
81 et al., 2015; Champagnat et al., 2006). Its composition is known to depend on different
82 factors, including environmental conditions and root-associated bacteria (Adams et al., 2004,
83 2003). Del Giudice and coworkers (Del Giudice et al., 2008) showed that some bacteria
84 isolated from vetiver roots could metabolize sesquiterpenes into the compounds that are
85 present in vetiver essential oil. These results therefore suggest that the endophytic bacterial
86 community from vetiver roots may contribute to the overall metabolic composition of the
87 essential oil, which has been reported to have antifungal activities (Del Giudice et al., 2008;
88 Hammer et al., 1999; Monteiro et al., 2011).

89 In this report, we assumed that the endophytic bacterial community of vetiver roots
90 contributes to bioactivity against *F. graminearum*. Since bacterial communities are
91 influenced by the surrounding environmental conditions, we investigated vetiver roots
92 originating from different geographical locations assuming that the endophytic bacterial
93 communities would differ in their taxonomical composition and bioactivity against *F.*
94 *graminearum*. The results described here are related to Sunshine vetiver roots collected in
95 four different geographical regions *i.e.*, Madagascar, Portugal, Reunion Island, and Senegal.
96 To thoroughly describe endophytic bacterial community of vetiver roots, culture dependent

97 and independent approaches were used. Using the high resolutive meta-barcoding
98 approach, we assessed the structure and diversity of the bacterial endophytes. Through a
99 complementary culture-dependent approach, we functionally characterized the bacterial
100 endophytes with antagonistic activity towards *F. graminearum*. This allowed us to better
101 understand the role that these microorganisms can have and it also revealed which
102 microorganisms could be used in subsequent studies to develop biocontrol solutions with
103 respect to *F. graminearum*.

104

105 **2. Materials and methods**

106

107 *2.1. Sampling of vetiver roots*

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

118

119 *2.2. Preparation of root samples*

120 The vetiver roots were first rinsed in running tap water to eliminate soil and surface

121 debris prior to surface sterilization under sterile conditions. For this purpose, they were cut
122 into small pieces of approximately 3 to 4 cm in length. The root pieces were treated with
123 70% ethanol for 1 min 5 times, followed by immersion in 2.6% sodium hypochlorite solution
124 for 15 min. The samples were then rinsed three times with sterile water for 1 min. After root
125 surface sterilization, four grams of plant material were stored at -80°C until DNA extraction,
126 and 1 g of root tissues was used for the isolation of bacterial endophytes.

127

128 *2.3. Isolation of bacteria*

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

142

143 *2.4. DNA extraction from surface-sterilized vetiver roots*

144 Total genomic DNA was extracted from 3 g of surface-sterilized vetiver roots according

145 to Ranjard et al. (Ranjard et al., 2003) with minor modifications. Briefly, frozen root materials
146 were ground in liquid nitrogen with a mortar and a pestle, both of which were autoclaved in
147 advance. Three grams of the ground material was distributed into 5 microtubes, and the
148 aliquots were mixed in 1 ml of lysis buffer (Ranjard et al., 2003) using a Retsch® MM400
149 agitator at 30 per sec for 2.5 min. After incubation at 70°C for 30 min, supernatants were
150 collected after centrifugation at 7000 g for 5 min at 15 - 20°C. The supernatants from the
151 same original root material were combined as a crude DNA solution. The obtained crude
152 extracts were purified using the GENE CLEAN® Turbo Nucleic Acid Purification kit (MP
153 Biomedicals, France).

154

155 *2.5. Metabarcoding Amplicon libraries*

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

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

169 Although the Genome Taxonomy Database recently proposed the repositioning of
170 Betaproteobacteria as an order (Burkholderiales) in Gammaproteobacteria (Parks et al.,
171 2018), we decided to conserve the old classification to maintain consistency with previous
172 studies. OTUs were further filtered with a threshold of at least 99% BLAST identity and
173 100% coverage. The number of remaining reads after the 2nd filtration was in the range of
174 10,440 to 23,950 reads, with an average of 14,805 reads per plant. To compare the data
175 coming from the different vetiver samples, the sequencing results were normalized to the
176 same number of reads as that in the sample with the lowest total count (10,440 reads).
177 Rarefaction curves were depicted in parallel with the R packages vegan and phyloseq
178 (McMurdie and Holmes, 2013) (Figure S2). Phylogenetic tree data were prepared with
179 FastTree, following a multiple alignment by PyNAST in FROGS.

180

181 *2.6. Dual inoculation test with F. graminearum*

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

193 where d means the diameter of the *F. graminearum* mycelium.

194

195 *2.7. Sequencing of the 16S rRNA gene of cultured bacterial endophytes*

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

203

204 *2.8. Diversity and compositional analysis of the bacterial communities*

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

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

216 The composition of the functionally selected endophyte communities was converted into a

217 table that includes the relative abundance at the genus level. These data were visualized
218 using the ggplot2 R package. The table was used to measure the beta diversity among
219 samples with a distance metric, Bray-Curtis dissimilarity, using vegdist in the vegan R
220 package. The result was visualized by PCoA with ggplot2 and ggpubr R packages.

221

222 *2.9. Statistical analyses*

223 The inhibition rate was visualized with ggplot2 and tested by the Tukey-Kramer method with
224 the multcomp R package. Multiple comparisons of alpha diversity indices and the relative
225 abundance of taxa were performed with the Tukey-Kramer method using the multcomp R
226 package. For the meta-barcoding analysis, the influence of the location factor was tested by
227 PERMANOVA (permutations = 9999) with Adonis available in the vegan R package
228 equipped in FROGS. The influence of the location factor on the variation in the Bray-Curtis
229 dissimilarity metric among the culturable endophyte communities was tested by
230 PERMANOVA (permutations = 10000) with adonis in the vegan R package.

231

232 **3. Results**

233

234 *3.1. Biodiversity analysis of endophytic bacterial microbiota in vetiver*

235

236 Vetiver roots were sampled from different places located in Madagascar, Portugal,
237 Senegal and Reunion Island (Figure S1). Illumina sequencing performed on the DNA
238 extracted from each sample generated a total of 366,169 raw sequences, and after a
239 FROGS process (29), 222,081 sequences were retained. These sequences were clustered
240 into 894 different OTUs (between 233 and 500 OTUs per sample) (Table S1). Among them,

241 55 OTUs were shared by all samples, whereas 32, 38, 100, 115 and 64 specific OTUs were
242 detected only in the Madagascar, Portugal, Reunion1, Reunion2 and Senegal vetiver root
243 samples, respectively (Figure S3). The observed number of OTUs differed according to the
244 geographic origin of the vetiver roots and was significantly higher in the samples from
245 Reunion Island than in the samples from the other geographic locations (Table 1). The
246 Chao1, Shannon, and inverse-Simpson indices were calculated to characterize the alpha
247 diversity of the bacterial communities associated with vetiver roots (Table 1). Overall,
248 although the differences were not always significant, our results show that the values of all
249 diversity indices were higher in the Reunion Island samples. This could indicate more
250 diverse bacterial communities in vetiver root samples from Reunion Island than in samples
251 from the other geographic locations (Table 1). To evaluate how our data reflect the diversity,
252 we calculated the ratio of observed OTUs to the Chao1 index values for each location. The
253 analysis showed that the Madagascar samples had significantly lower diversity coverage
254 than the Reunion samples (Table 1).

255 Principal coordinate analysis (PCoA) based on weighted UniFrac distances among samples
256 showed that the endophytic root bacterial communities from the 5 vetiver geographic
257 locations were clearly separated from each other (Figure 1). The first two axes explained
258 78.4% of the variation. The samples from Reunion Island and Madagascar were separated
259 along the first axis (PCoA 1 = 51.6%), whereas the samples from Senegal and Portugal
260 were separated along the second axis (PCoA 2 = 26.8%). These results, which were further
261 validated statistically by PERMANOVA (Df=4, R²= 85%, P=0.0001), indicated that the
262 endophytic bacterial communities from vetiver roots are structured by geographic location.

263

264 *3.2. Taxonomic composition of endophytic bacterial communities in vetiver roots from*

265 *different geographic locations*

266

267 The detected vetiver root bacterial OTUs were affiliated with 13 bacterial phyla (Figure
268 2A). However, almost all OTUs were distributed in 4 phyla that represented 98.7% of the
269 total abundance. These phyla were *Proteobacteria* (70.3% +/- 12.1), *Bacteroidota* (13.7%
270 +/- 8.2), *Firmicutes* (7.8% +/- 9.7) and *Actinobacteria* (5.5% +/- 8.5). Proteobacteria were
271 common to all samples in relatively equal abundance, with three main subphyla,
272 Gammaproteobacteria (67.6% +/- 13.7), Alphaproteobacteria (29.7% +/- 13.2) and
273 Betaproteobacteria (2.6% +/- 2.5). In contrast, the other dominant phyla were more
274 specifically enriched the certain vetiver locations (Figure 2A). Bacteroidota were more
275 abundant in root samples from Reunion and Senegal (from 13.1% to 23.1%) than in the
276 samples from the other locations (Figure 2A). The abundances of Firmicutes and
277 Actinobacteriota also varied greatly among vetiver locations. The Firmicutes abundance in
278 vetiver roots originating from Senegal was 20.6% (4.0% on average in the other samples),
279 whereas Actinobacteria was mostly represented in samples from Portugal (20.6%) (1.7% on
280 average in the other samples) (Figure 2A).

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

288 At the genus level, a total of 258 genera were found in our samples. Among them, 53

289 genera were shared across geographic sites; these genera accounted for 20.5% of the total
290 genera (Figure 3). The shared genera belonged to 6 phyla (Proteobacteria, Myxococcota,
291 Firmicutes, Bdellovibrionota, Bacteroidota, and Actinobacteriota) and made up an average
292 relative abundance of 72.5% +/- 12.7 of the total abundance at each location.

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

300

301 3.3. Bacterial endophyte isolation and antagonistic activity towards *F. graminearum*

302 We isolated bacteria from the same vetiver roots that were used for the metagenomic
303 approach. First, the density of cultivable endophytic bacteria from the vetiver root samples
304 was estimated. We observed that the average density of cultivable endophytic bacteria in
305 vetiver roots did not differ significantly between each geographic location (5.2 to 6.2 log₁₀
306 CFU g⁻¹ root dry weight) (Table 2). A total of 381 endophytes were recovered from vetiver
307 root samples and were tested for their ability to inhibit *F. graminearum* mycelial growth in *in*
308 *vitro* culture. The inhibition rate varied greatly among bacterial isolates (from -3.3 to 93.3),
309 but nearly all isolates (99.7%, Table S2) presented this functional trait. The mean inhibition
310 rate of the bacterial isolates was significantly lower in the isolates from Reunion1 (p<0.05,
311 31.7% +/- 14.9) than in the isolates from the other samples (Table 2). The endophytic
312 bacterial isolates with an inhibitory rate of at least 50% were selected for further analyses.

313 They represented 49% (176 isolates) of the total isolates, and 20% (36 isolates) of them had
314 an inhibition rate of at least 70% against *F. graminearum* (Table S2). The relative frequency
315 of these functional isolates differed significantly among the vetiver samples (p-value<0.05,
316 Table 2). In particular, only 14% of isolates from Reunion1 showed an inhibition rate \geq 50%,
317 while 77% of the isolates from Madagascar showed an inhibition rate \geq 50%. The isolates
318 with inhibition rate \geq 70% were isolated from vetiver originating from Madagascar (2
319 isolates), Portugal (20 isolates), Reunion2 (11 isolates) and Senegal (3 isolates) (Table S4).

320 Based on partial 16S rDNA sequencing, 153 of these bacterial isolates were
321 taxonomically characterized (Table S3). They included Proteobacteria (86.2% +/- 22.0),
322 Firmicutes (12.2% +/- 22.4) and Actinobacteriota (1.4% +/- 3.0) (Figure S5). A total of 15
323 genera were identified (Figure 5). Among them, 8 genera had already been described using
324 16S rDNA high-throughput sequencing (Figure 6).

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

335 The table of the relative abundance of genera was used to generate a Bray-Curtis
336 distance matrix that was subjected to PCoA (Figure 7). This analysis showed that the

337 functional microbial communities from Reunion2 and Madagascar were distinct from each
338 other and from a cluster composed of the microbial communities from the Reunion1,
339 Portugal and Senegal samples. These results were validated with PERMANOVA showing
340 that the geographic location factor significantly altered the composition of the cultivable
341 endophytic communities with antagonistic activity towards *F. graminearum* in vetiver roots
342 (Df =4, R²= 66.4%, p< 0.001).

343

344 **4. Discussion**

345 Diseases caused by *F. graminearum* are among the most important diseases in grain
346 cereals. Chemical fungicides are still widely used to control this fungus, although their
347 detrimental impacts on the environment and human health are now well known. To reduce
348 the use of such chemicals while maintaining crop yields, it is now necessary to find and
349 develop alternative crop protection solutions. A number of previous studies revealed that
350 bacterial endophytes may help plants cope with pathogens (Berg et al., 2013; Card et al.,
351 2015; Eljounaidi et al., 2016; Liu et al., 2009; Mancini and Romanazzi, 2014; Mousa et al.,
352 2016). In particular, bacterial endophytes are able to produce a range of specialized
353 metabolites that have antimicrobial activity (Brader et al., 2014). In addition, coevolution
354 processes with their hosts led endophytes to mimic natural plant compounds (Baltrus, 2017).
355 Because of these findings, it is now accepted that bacterial endophytes contribute to the
356 biological activity of plant extracts, including their antimicrobial activity (Miller et al., 2012;
357 Strobel et al., 2004). The metabolic potential of bacterial endophytes suggests the possibility
358 of exploiting these microorganisms for biocontrol applications. In this study, we focused on
359 the bacterial endophytic community of vetiver roots since it has been shown that some
360 members of this community are involved in the synthesis of vetiver essential oil, which has

361 been reported to display antimicrobial activity (Chahal et al., 2015; Del Giudice et al., 2008;
362 Hammer et al., 1999; Powers et al., 2018).

363

364 The vetiver roots we investigated mainly harbour endophytic bacteria belonging to four
365 major phyla, i.e., Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteriota. These
366 results are consistent with those of several previous studies showing that these phyla are
367 preferentially enriched in plant roots compared to their levels in the soil and rhizosphere
368 (Bulgarelli et al., 2013; Hardoim et al., 2015; Lundberg et al., 2012). Additionally, among
369 them, Proteobacteria were overrepresented in all vetiver roots sampled, which is similar to
370 the results obtained by Monteiro et al. (2011). This predominance might be related to the
371 fact that such copiotrophic microorganisms prefer a C-rich environment (Fierer et al., 2007;
372 Yang et al., 2017). They may therefore benefit from the flow of photoassimilates from shoots
373 to roots, which creates a nutrient-rich environment inside the roots. We showed that the
374 Proteobacteria were mostly *Gammaproteobacteria* and were dominated by a few families
375 and genera. Among them, the genus *Pseudomonas* is a good root colonizer and was
376 prevalent. However, other genera, such as *Kosakonia*, *Enterobacter* and *Pantoea*, were
377 also observed. Most of these genera can benefit plants by promoting plant growth and/or
378 protection, although some of them have been described as pathogens (Bulgarelli et al.,
379 2013). Bacteroidetes were represented by the dominant *Flavobacterium* genus, which is
380 often reported as an important member of root microbiomes, and some strains have traits
381 that are beneficial to plants (Kolton et al., 2016). Finally, Actinobacteria and Firmicutes were
382 represented by the *Streptomyces* and *Exiguobacterium* genera, respectively, which are
383 known to produce antimicrobial compounds (Hardoim et al., 2015; Kasana and Pandey,
384 2018). Our study also provides additional insights since we showed that the geographic

385 location of the vetiver samples seemed to influence the composition and the relative
386 abundance of some of the taxa in the roots. Recently, Thiergart et al. (2020) provided
387 evidence that soil is the primary factor that influences root endophytic bacterial communities,
388 regardless of the spatial scale of the analysis or the host genotype. These data support the
389 findings of previous studies and led us to suggest that the variations we observed within the
390 vetiver root bacterial communities might be related to the differences in soil properties
391 across a geographic gradient from Europe to Africa (Bulgarelli et al., 2013; Chen et al.,
392 2019; Fierer et al., 2007; Lundberg et al., 2012).

393

394 As some of the microbial groups we identified in vetiver roots have been described as
395 potential biocontrol agents against plant pathogens, we evaluated the ability of this
396 microbiota to suppress the growth of *F. graminearum*. For that purpose, the bacteria were
397 isolated and profiled in *in vitro* dual-culture assays with *F. graminearum*. Among the 381
398 isolates, most displayed antagonistic activity towards *F. graminearum*, with almost 50%
399 producing an inhibition rate of more than 50%. These bioactivities might be related to the
400 ability of the isolates to synthesize diverse antimicrobial compounds (specialized
401 metabolites) (Saleh et al., 2019; Strobel et al., 2004). Furthermore, these results reinforce
402 the hypothesis that microbial endophytes can contribute to the bioactivity of plant extracts
403 and, in our case, to the antimicrobial properties of the vetiver essential oil (Chahal et al.,
404 2015; Del Giudice et al., 2008; Egamberdieva et al., 2017; Miller et al., 2012).

405

406 The metagenomic data showed that the bacterial isolates mostly belong to
407 Proteobacteria, followed by Firmicutes and Actinobacteriota, and that their distribution is
408 related to their geographic location. Our results also showed, at lower taxonomic levels, that

409 the geographic location shapes the cultivable bacteria in vetiver roots. As previously
410 suggested by Monteiro et al. (2011), soil could be an important factor influencing bacterial
411 communities from vetiver. Interestingly, the culture-based and molecular-based datasets do
412 not completely overlap. For instance, in the metagenomic data, *Pseudomonas* was highly
413 represented in the Madagascar samples, whereas most of the bacterial isolates in these
414 samples were affiliated with *Kosakonia* and *Pantoea*. This relative discrepancy could result
415 from the fact that most of the bacteria present in a sample cannot grow on the traditionally
416 used culture media (Eevers et al., 2015; Pascual et al., 2016). Bacteria with the capacity to
417 inhibit the *in vitro* growth of *F. graminearum* were affiliated with 15 genera. Some of these
418 genera, such as *Pseudomonas*, *Enterobacter*, and *Serratia*, have already been identified in
419 vetiver roots (Del Giudice et al., 2008).

420

421 This study performed on vetiver roots resulted in the isolation and identification of
422 32 highly bioactive isolates displaying at least 70% inhibition rates against *F. graminearum*.
423 These isolates were mostly from the genera *Pseudomonas*, *Serratia*, and *Bacillus*. Only 4
424 isolates were affiliated with different genera, i.e., *Kosakonia*, *Janthinobacterium* and
425 *Microbacterium*. Various reports have described bacteria affiliated with these dominant
426 genera that were isolated from different cultivated plants and display *in vitro* inhibition
427 activity against *Fusarium* spp. including *F. graminearum* (Bredow et al., 2015; Haas and
428 Défago, 2005; Ju et al., 2013). Furthermore, *Serratia*, *Pseudomonas*, *Bacillus*, and
429 *Microbacterium* have been reported to produce antimicrobial compounds. *Bacillus*, which is
430 one of the most investigated and commercially utilized genera worldwide (Lopes et al.,
431 2018), is known to produce antimicrobial compounds such as lipopeptides,
432 exopolysaccharides, siderophores and polyketides (Ek-Ramos et al., 2019). In addition to

433 *Bacillus*, *Pseudomonas* strains, including those affiliated with *P. koreensis*, are also known
434 to produce structurally diverse specialized metabolites with antimicrobial properties (Kaur et
435 al., 2019; Raaijmakers and Mazzola, 2012). Likewise, some strains in the *Serratia* genus
436 can produce antibiotics whose synthesis is regulated by carbon sources (M Mai, 2018).
437 Another study performed on *Janthinobacterium* strains revealed that one of the modes of
438 action of this genus against *F. graminearum* occurs through biofilm formation following
439 quorum sensing (Haack et al., 2016). More recently, new antifungal compounds, such as
440 janthinopolyenemycins, were identified through the co-culturing of two *Janthinobacterium*
441 strains isolated from marine sediments (Anjum et al., 2018).

442

443 **5. Conclusions**

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

454

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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 \pm standard deviation. Different letters besides the values indicate differences with p value < 0.05 within an index by location group.

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

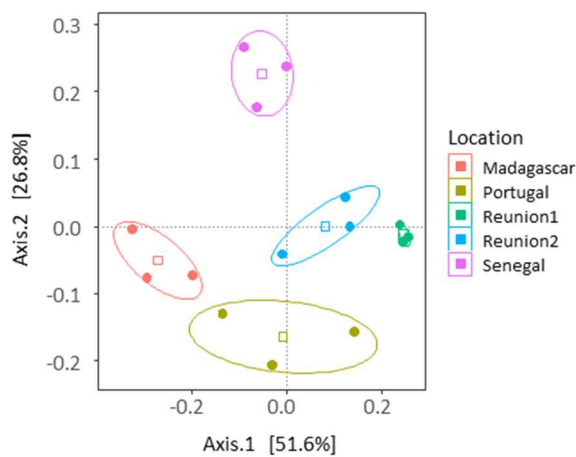


Figure 1| PCoA plot based on weighted UniFrac distance of Vetiver samples. Points represent individual plants, Points represent individual plants, and squares and ellipses show the center of gravity and a 95% confidential zone of each locational group, respectively.

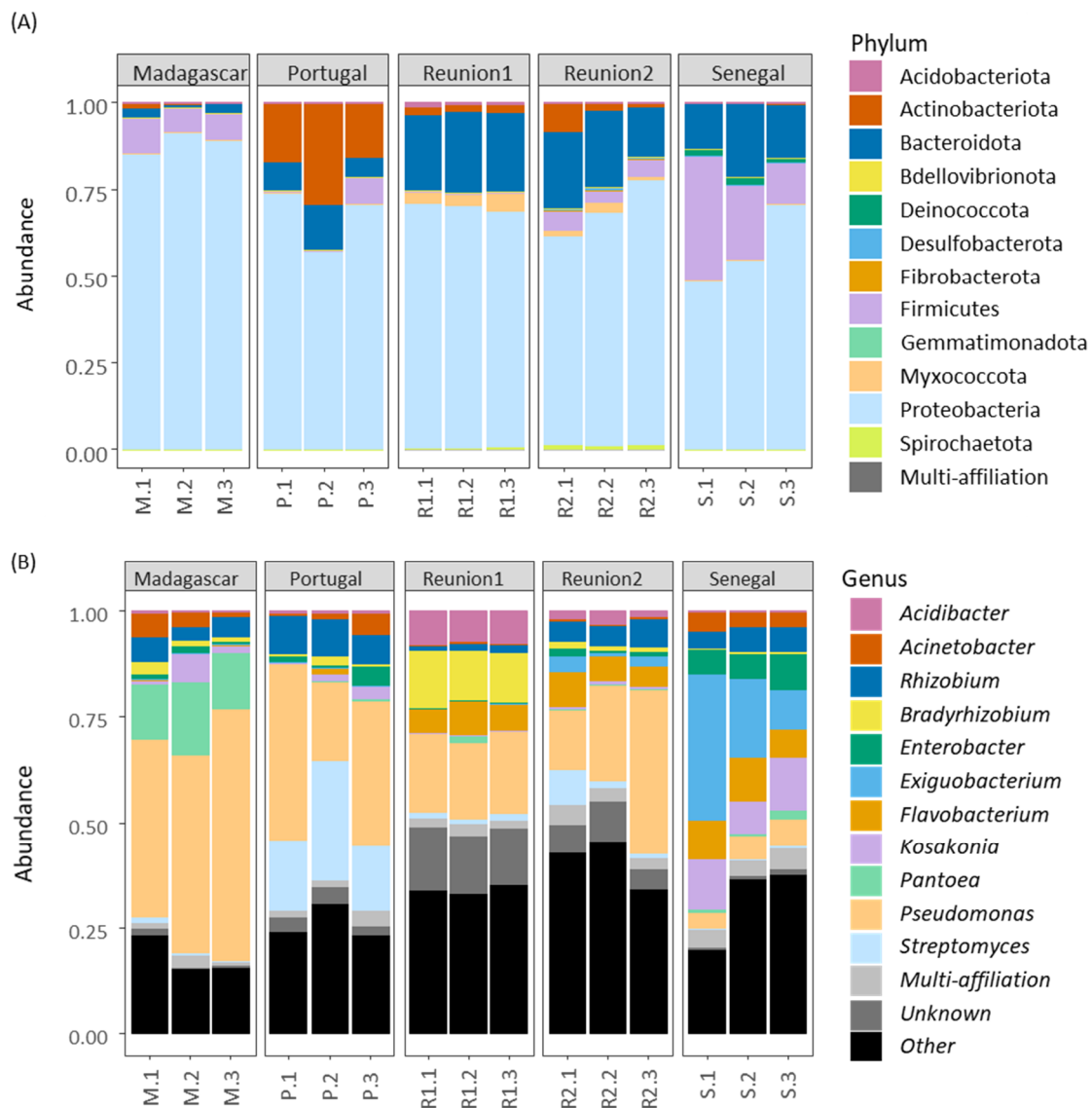


Figure 2| Taxonomical compositions of 13 phyla (A) and dominant 12 genera (B) in bacterial endophyte communities of Vetiver roots in 5 locations.

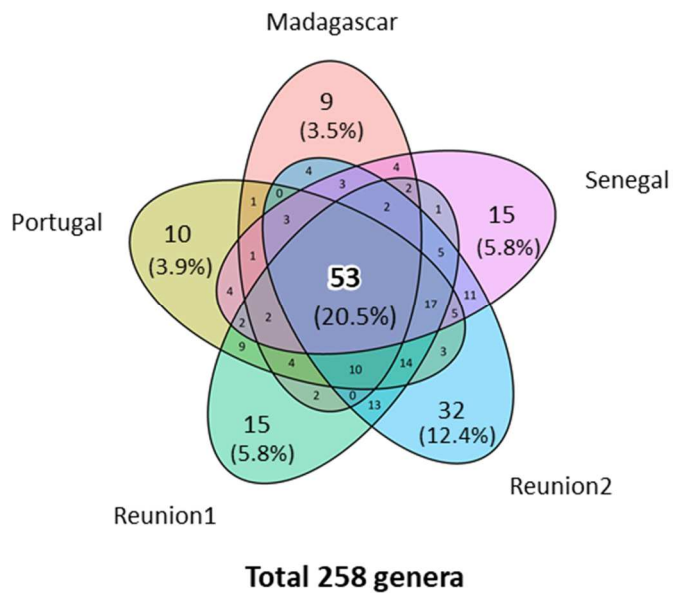
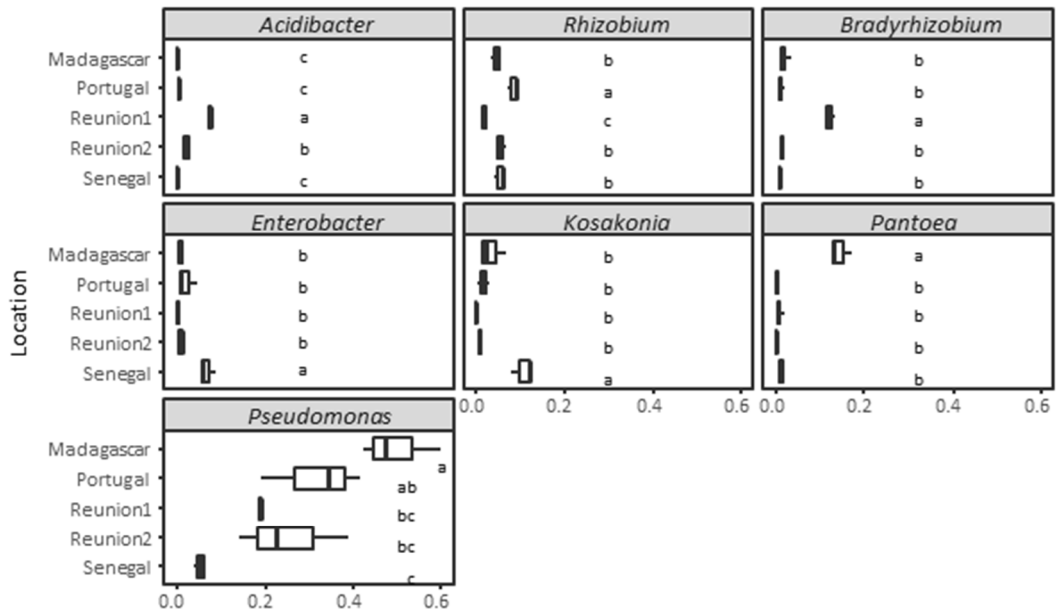


Figure 3| Venn diagram of the number of shared genera over locations and specific genera for each location. The shown percentages are percentage of shared or specific genera against total number of genera found through all samples.

(A)



(B)

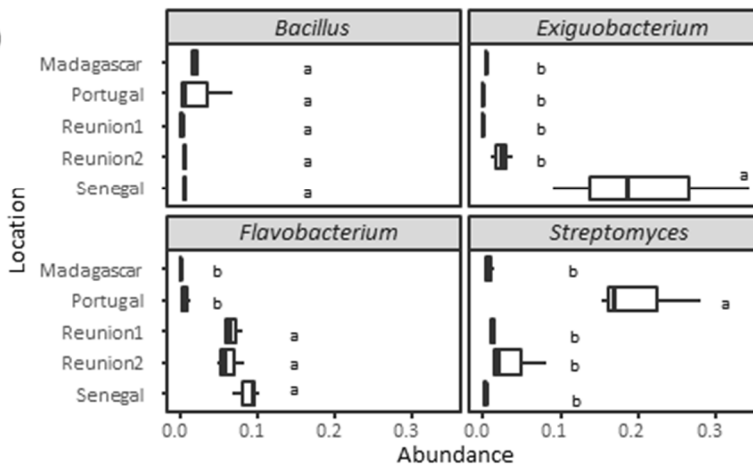


Figure 4| Relative abundance of dominant genera in 4 phyla, in Vetiver root endophyte communities. (A) Proteobacteria, (B) *Bacillus* and *Exiguobacterium* (Firmicutes), *Flavobacterium* (Bacteroidota), *Streptomyces* (Actinobacteriota). The same letters within a genus mean no differences with p value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

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 <i>F. graminearum</i> (%)			Isolates with $\geq 50\%$ antagonism against <i>F. graminearum</i>	
			Min.	Max.	Average \pm SD	Number of isolates	Population within a site
Madagascar	6.1 \pm 0.3 a	48	0.5	83.5	55.1 \pm 11.6 a	37	77% a
Portugal	5.7 \pm 0.5 a	89	28.7	79.9	54.2 \pm 14.9 a	46	52% ab
Reunion 1	5.6 \pm 0.2 a	86	-3.3	65.0	31.7 \pm 14.9 b	12	14% b
Reunion 2	5.6 \pm 0.3 a	76	18.3	93.3	52.0 \pm 17.2 a	43	57% ab
Senegal	6.2 \pm 0.3 a	82	22.9	82.8	52.9 \pm 12.6 a	38	46% ab
total		381				176	



Figure 5| Taxonomical compositions of 15 genera in functionally selected bacterial endophyte communities of Vetiver roots in 5 sites

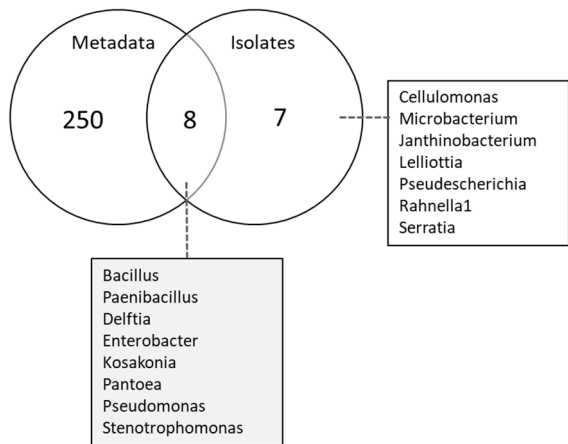


Figure 6 | Venn diagram of the number of common or unique genera in the metabarcoding data and the culturable communities.

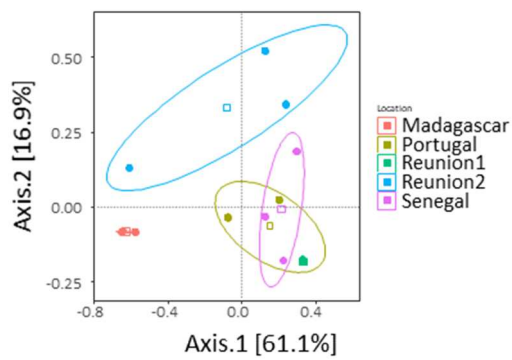


Figure 7 | PCoA ordination of isolated endophyte communities with bioactivities against *F. graminearum* using Bray-Curtis distance. Points represent individual plants, and squares and ellipses show the center of gravity and a 95% confidential zone of each locational group, respectively.

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	Proteobacteria	Pseudomonas	Pseudomonas vancouverensis strain DhA-51	100	
	P2_06	78.5			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			Proteobacteria	Pseudomonas	Pseudomonas koreensis strain Ps 9-14
	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 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	Serratia grimesii strain DSM 30063	99.68			
	P3_25	73.1	Serratia grimesii strain DSM 30063	99.65			
	P3_30	73.5	Serratia	Serratia grimesii strain DSM 30063			99.7
	P3_17	73.8		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	Firmicutes	Bacillus	Bacillus subtilis strain IAM 12118	99.83	
	R22_06	81.7			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	Firmicutes	Bacillus	Bacillus cereus ATCC 14579	100	
	R23_12	70.0			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	