



HAL
open science

Bacteria/fungi Interactions (BFI): a way to awake secondary metabolite gene clusters

Matthieu Nicault, Cyril Bontemps, Éric Gelhaye, Pierre Leblond

► To cite this version:

Matthieu Nicault, Cyril Bontemps, Éric Gelhaye, Pierre Leblond. Bacteria/fungi Interactions (BFI): a way to awake secondary metabolite gene clusters. Lorraine Université d'Excellence (LUE) Séminaire Impact Biomolécules, May 2018, Nancy, France. hal-03216622

HAL Id: hal-03216622

<https://hal.univ-lorraine.fr/hal-03216622v1>

Submitted on 4 May 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Bacteria/fungi interactions: a way to awake secondary metabolite gene clusters

Matthieu NICAULT^{1,2}, Cyril BONTEMPS¹, Eric GELHAYE² and Pierre LEBLOND¹

¹Université de Lorraine, INRA, DynAMic, F-54000 Nancy, France

²Université de Lorraine, INRA, IAM, F-54000 Nancy, France

Introduction

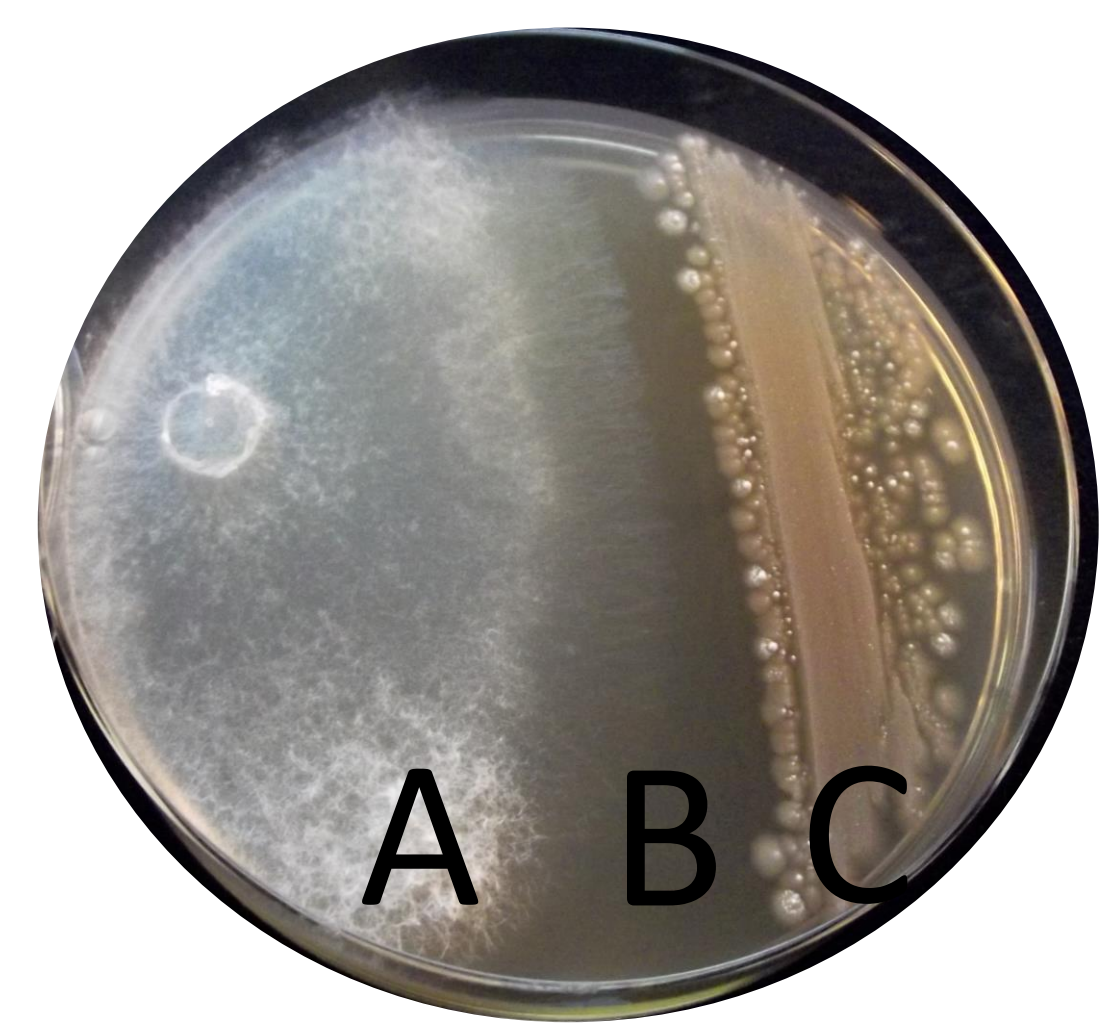
- The emergence of new multiresistant bacteria is nowadays a public health issue.
- The discovery of new antibiotics became crucial to overcome this problem.
- Bacteria (mostly actinomycetes like *Streptomyces*) and fungi are a major sources of antibiotics.
- However many gene clusters encoding for secondary metabolites production remain not expressed in lab conditions.
- Co-cultures, that mimic the interactions occurring in nature that induce production of specialized metabolites, is a way to activate these silent gene clusters.
- Objectives of this work are :
 - To awake these silent gene clusters through co-cultures between microorganisms.
 - To characterize the induced metabolites.
 - To decipher the basis of the interactions leading to secondary metabolite biosynthesis.

Methods

- 144 co-cultures between 8 *Streptomyces* and 9 fungal strains were performed on two media.
- The antibiosis activities of the ethyl acetate extracts of single cultures (A and C in fig1) and of their interaction zones (B in fig 1) are then tested and compared against indicator microorganisms (*E. coli*, *Bacillus*, yeast)

Figure 1 : Example of a co-culture between a *Streptomyces* and a fungus

On MA medium, a fungus (A) and a *Streptomyces* (C) are incubated at the same time. The zone (B) represents the interaction zone between the two microorganisms.



Results

1) All the tested *Streptomyces* exhibited direct antifungal capacities during the co-cultures (1 to 9 out of 9 fungi were inhibited depending of *Streptomyces* strains) and gave interaction zones (B in fig1).

2) 12 out of 144 interaction zones showed an induced activity in comparison to single cultures.

3) A differential metabolite production was observed between single and co-cultures.

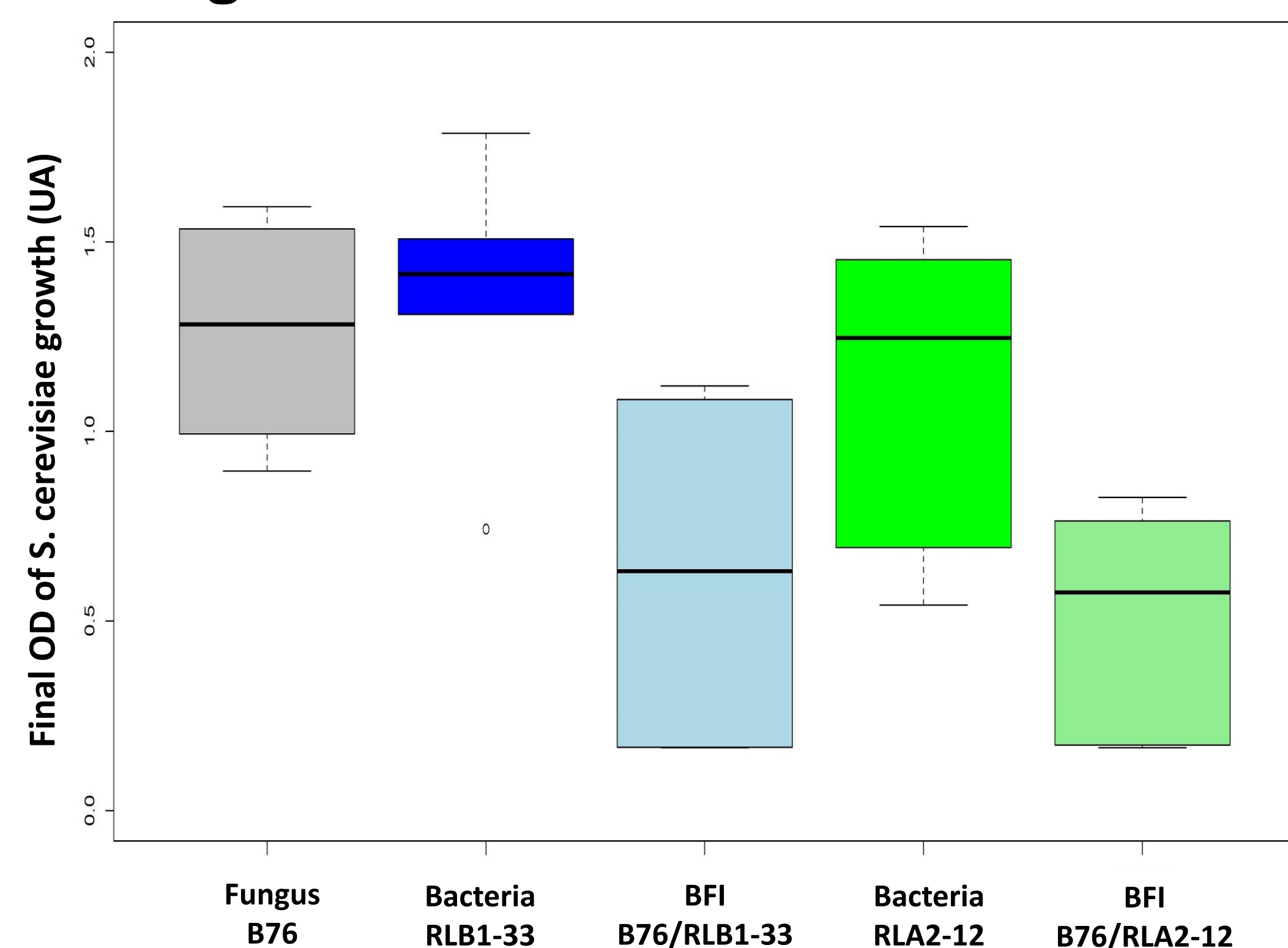


Figure 2: Example of enhanced inhibitory activities in co-culture interaction zones compared to single cultures

Yeast growth was measured by spectrophotometry in media supplemented with extracts from single strain cultures of the fungus B76 (grey), the bacteria RLB1-33 (blue) or RLA2-12 (green). Comparisons with the interaction zone extracts of the fungus/bacteria co-cultures B76/RLB1-33 (lightblue) and B76/RLA2-12 (lightgreen) showed greater inhibitory activities.

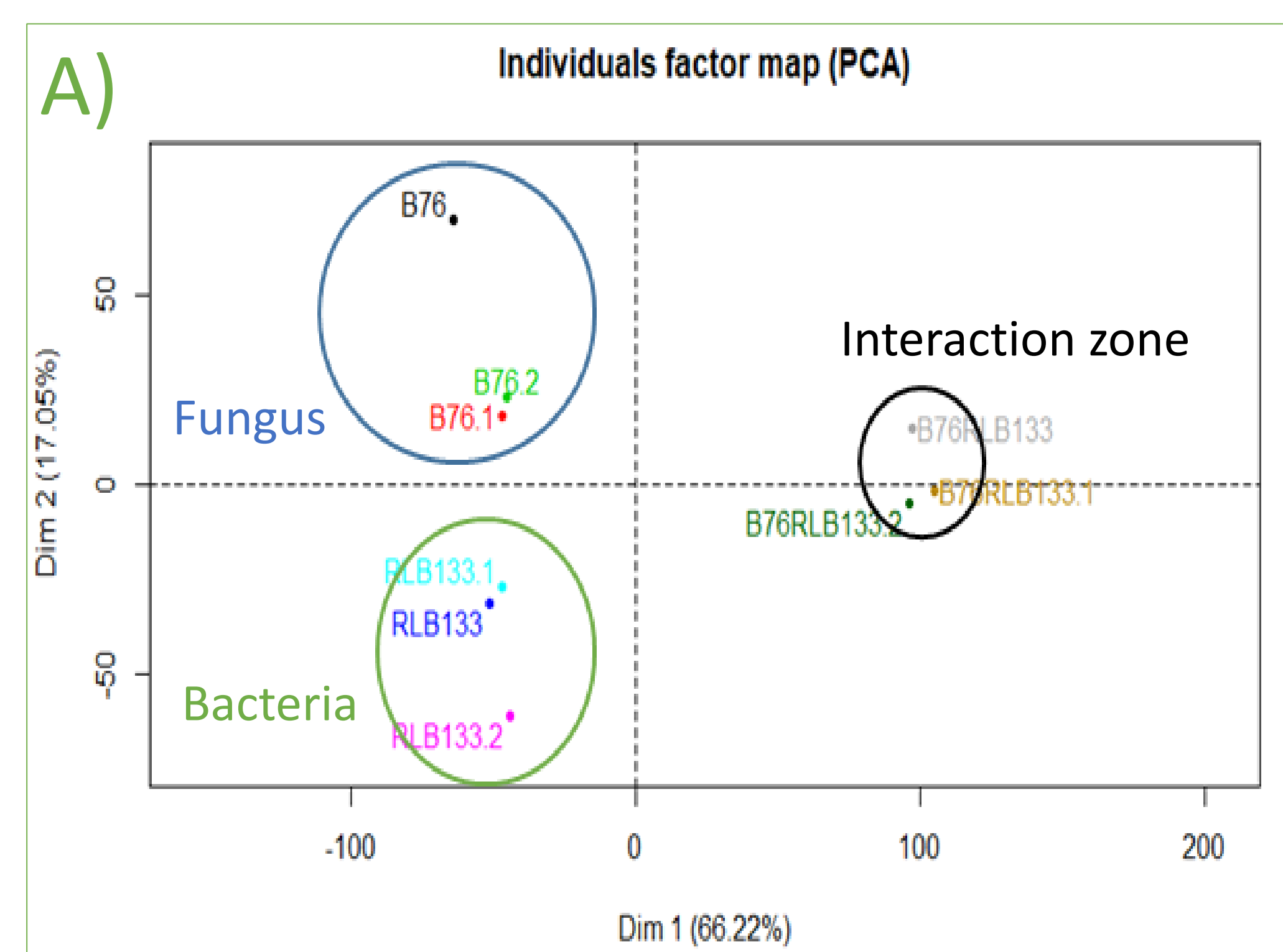


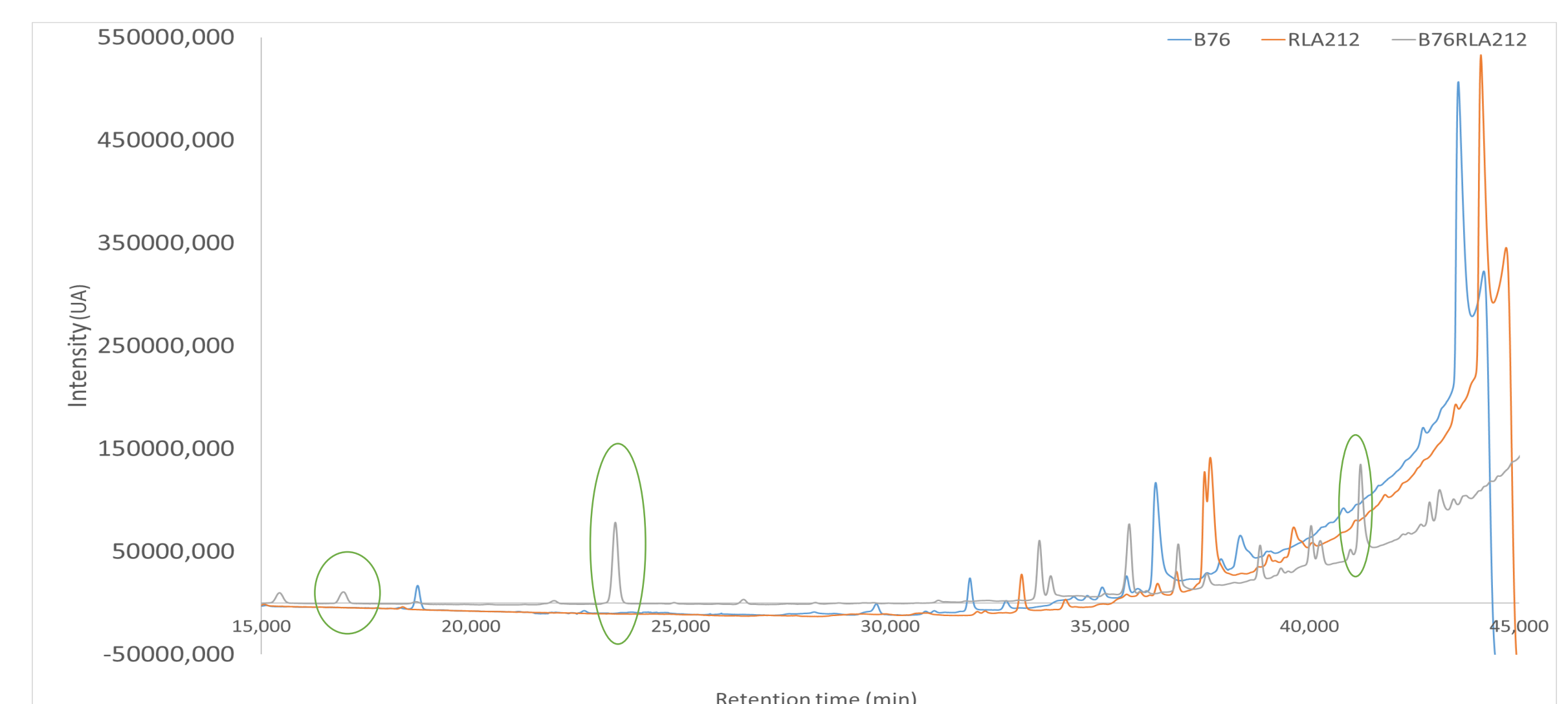
Figure 3: Observations of differential metabolite production

A) The global metabolite production of single cultures of the fungus B76 and of the bacterium RLB1-33 are statistically different from their interaction zones (PCA analysis). B) A blue pigment is present in the interaction zone of the co-culture between the fungus 6601-A and the bacterium S1D4-11, but not the single cultures, indicating a difference in metabolite production.

4) HPLC chromatograms of single and co-cultures indicated the presence of new metabolites

Figure 4: Chromatograms of single and co-cultures between the fungus B76 and the *Streptomyces* sp. RLA2-12

The chromatograms were recorded at 254 nm with MeOH(+ 0,1% acid formic)/Water(+ 0,1% acid formic) gradient. The orange chromatogram represents the metabolite profile of *Streptomyces* RLA2-12. The blue chromatogram represents the metabolite profile of the fungus B76. The grey chromatogram represents the metabolite profile of interaction zone. Interaction between the fungus B76 and *Streptomyces* RLA2-12 could induce the production of 3 new metabolites (green circles)



Conclusion/Perspectives

- The screening method of the interaction is set up.
- In some cases, co-cultures showed an enhancement of the inhibitory activity that can rely on new metabolite production.
- Further experiments :
 - To extend the screening to others couples and indicator strains (multiresistant and phytopathogenic microorganisms).
 - To identify and to characterize the induced metabolites (new peaks on chromatograms) and their biosynthetic pathways.
 - To decipher the molecular mechanisms involved during the interactions

