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

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Introduction

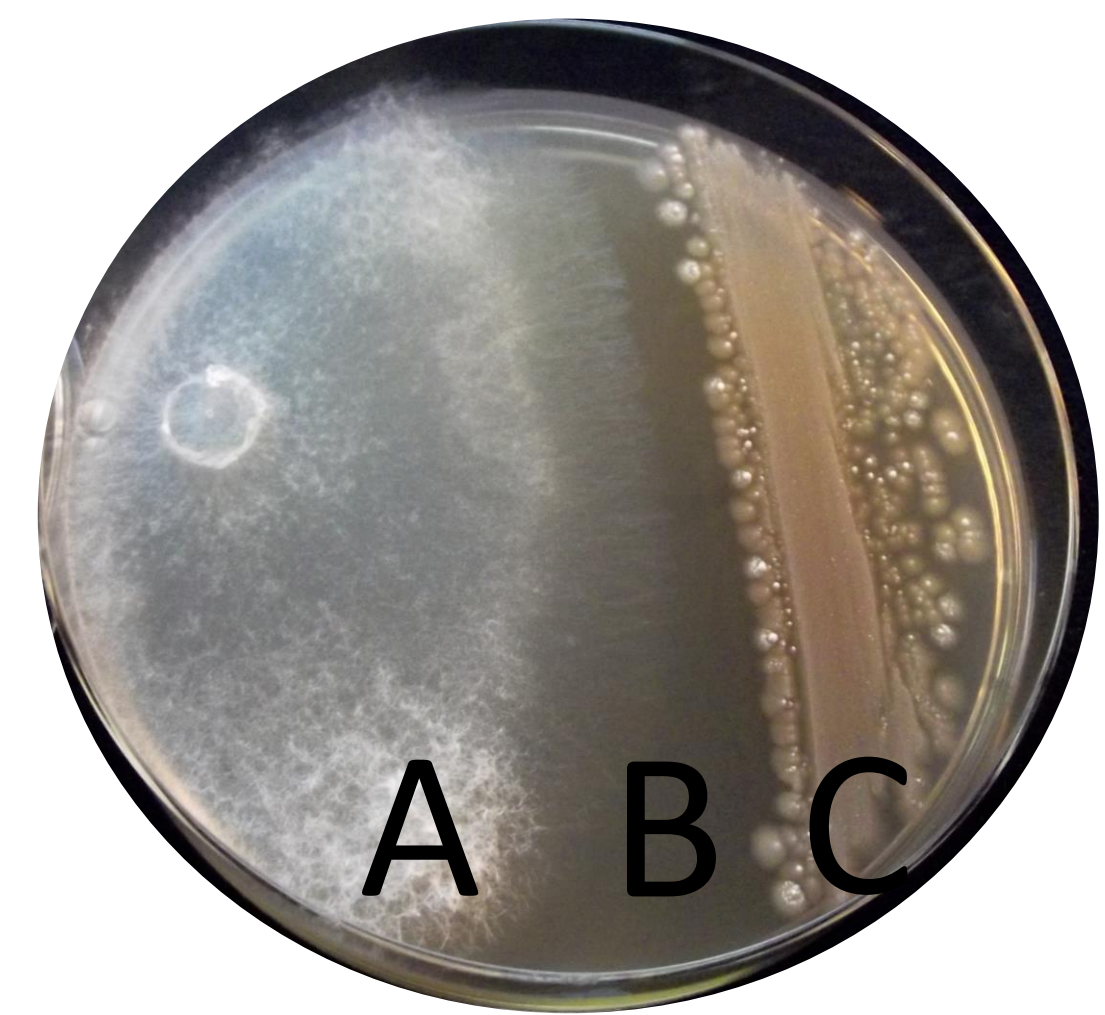
- The emergence of new multiresistant bacteria is nowadays a public health issue.
- The discovery of new antibiotics became crucial to overcome this problem.
- Bacteria (mostly actinomycetes like *Streptomyces*) and fungi are a major sources of antibiotics.
- However many gene clusters encoding for secondary 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.

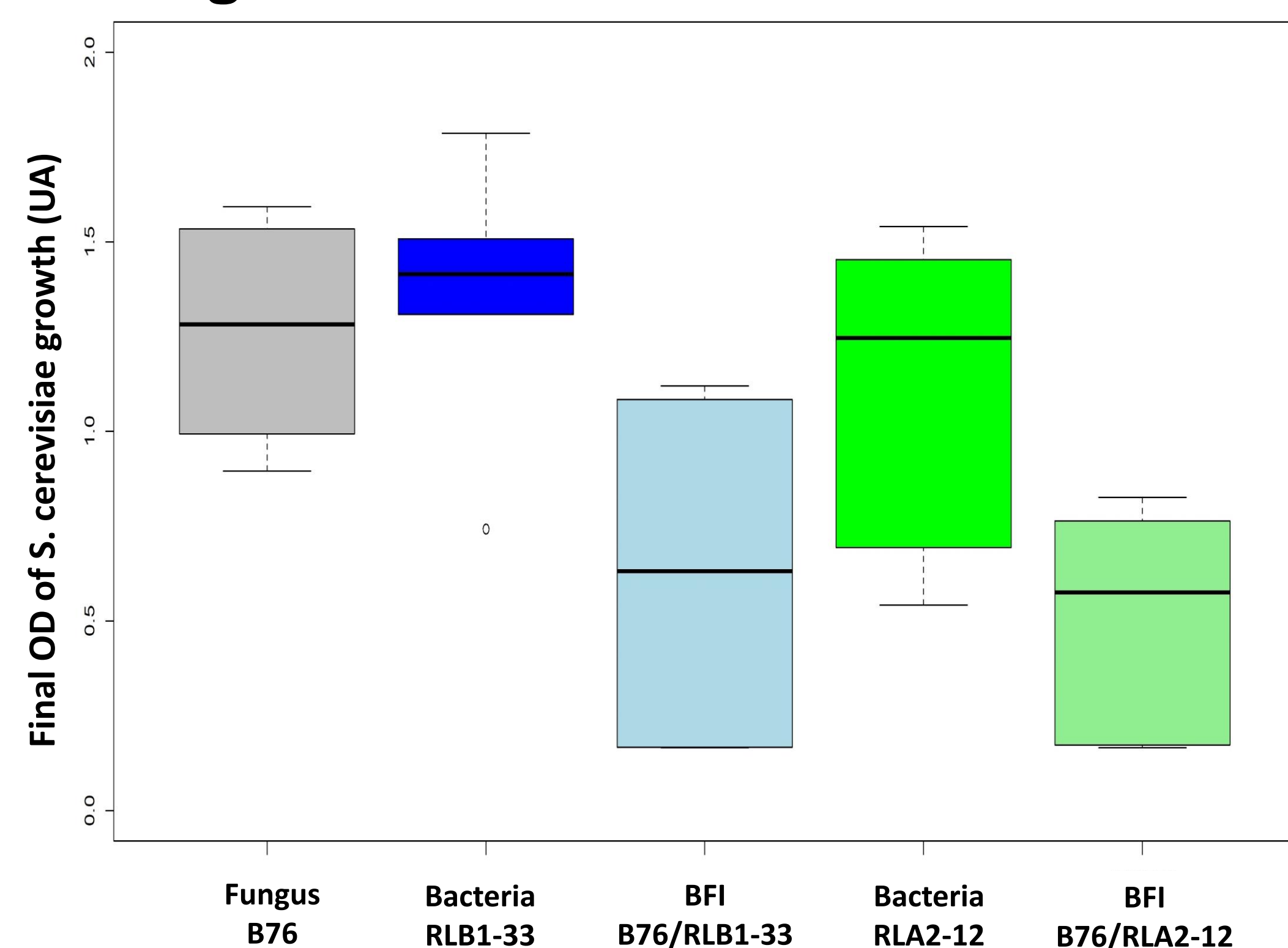


Figure 2: Example of enhanced inhibitory activities in co-culture interaction zones compared to single cultures

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.

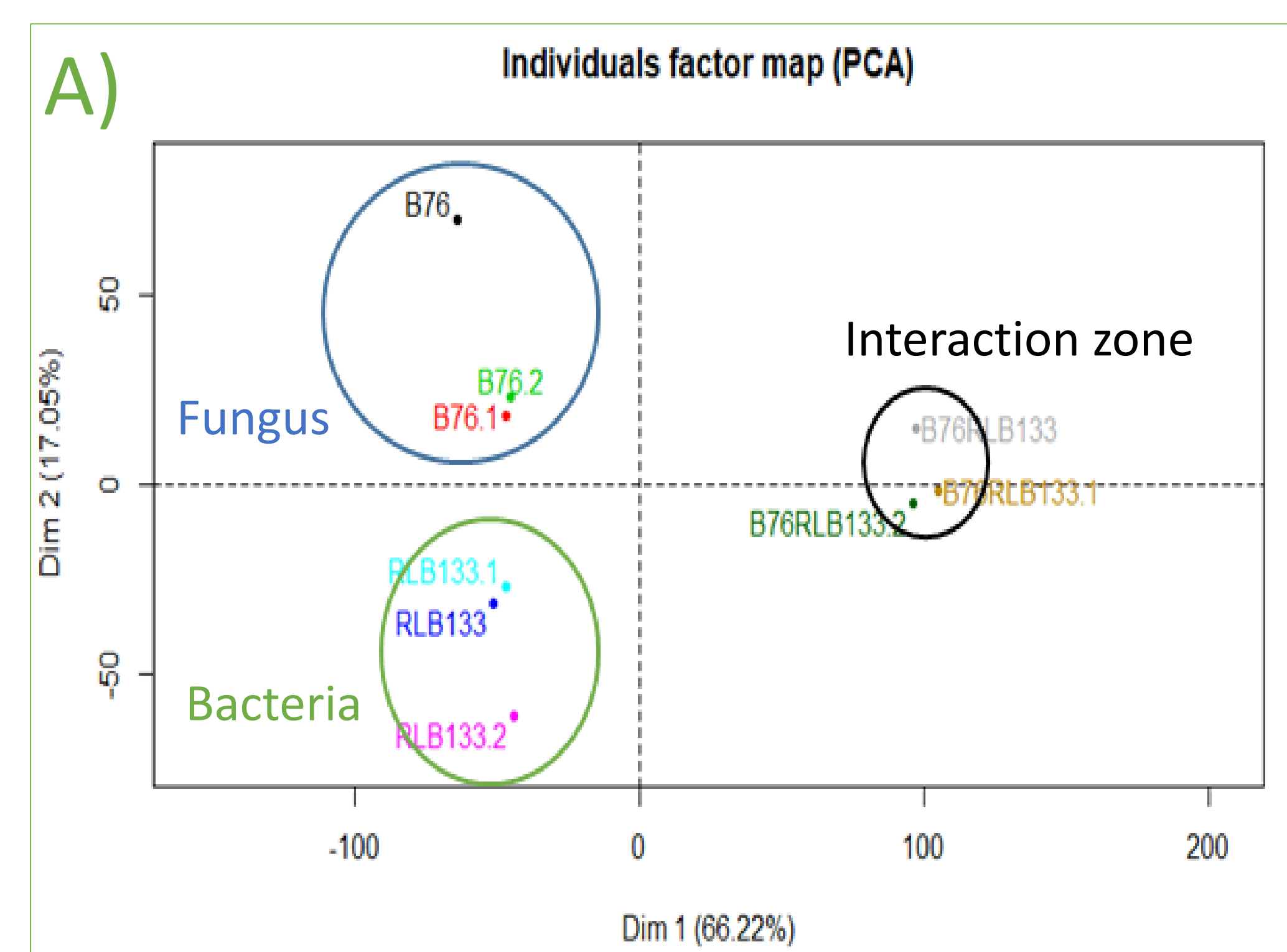


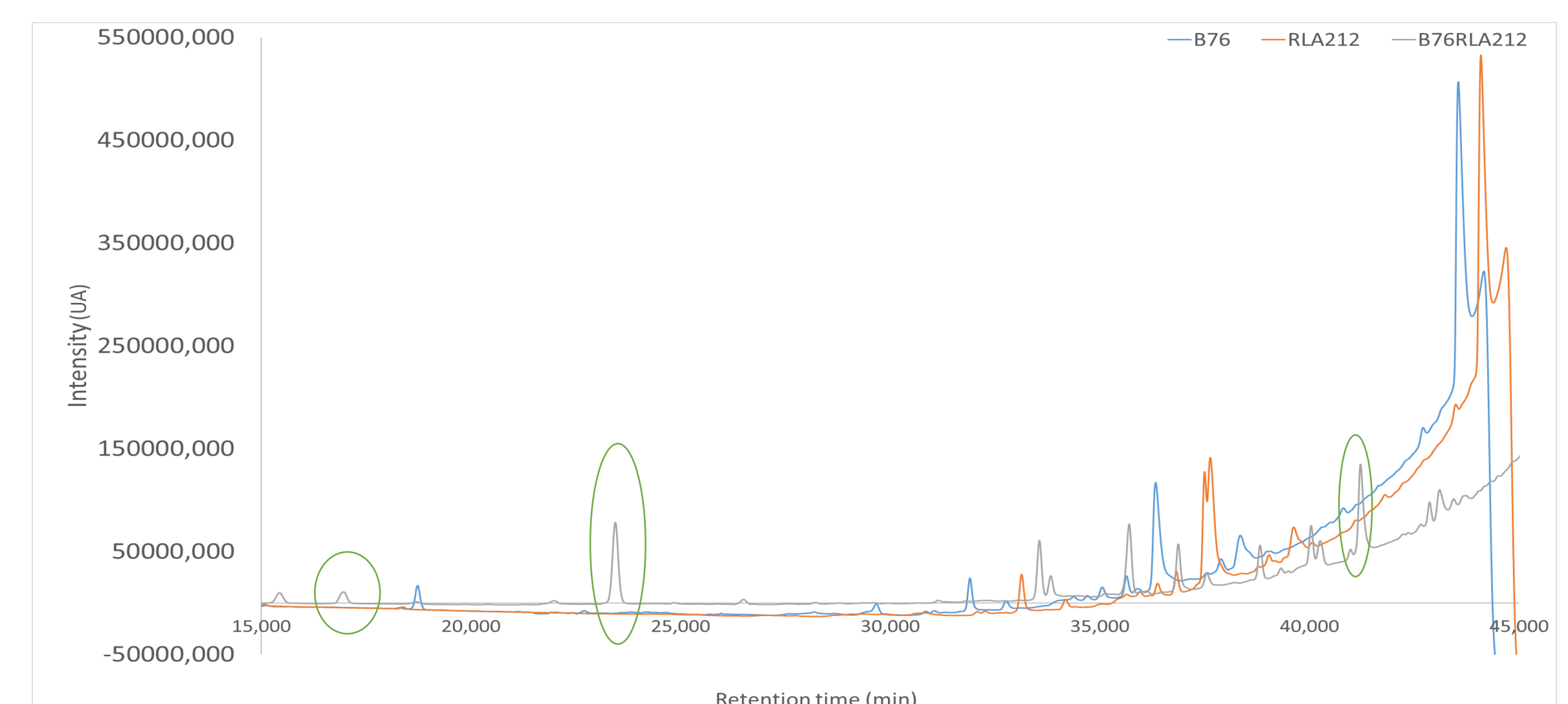
Figure 3: Observations of differential metabolite production

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

