POTENTIALITIES OF ACTINOMYCETES FROM SOILS FOR THE DEVELOPMENT OF NOVEL BIOCONTROL PRODUCTS TO MANAGE CEREALS FUNGAL DISEASES AND MYCOTOXINS RISK

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INTRODUCTION

Cereal crops, a primary source of nourishment around the world, are mostly attacked by fungi of the genus *Fusarium*, *Aspergillus* and *Penicillium*. In addition to potential yield losses, this fungi can produce mycotoxins which represent a major health concern because of their severe toxic effects.
Nowadays, the main strategy to control fungal contamination is the use of chemical fungicides, leading to environmental pollution and potentially harmful effects on animal and human health. Alternative strategies are growingly evaluated, such as the inhibition of pathogenic fungi by antagonistic microorganisms. Actinobacteria are a promising class of microorganisms because of their ability to produce a large amount of metabolites and enzymes that confer them strong antagonist properties. Until now, a lot of studies described their potential for fungal growth inhibition *in vitro,* but studies dealing with their direct effect on mycotoxin production are scarce. Moreover *in planta* validation is often left aside.

AIMS OF THE PROJECT

- ✔ Identify actinobacteria able to reduce fungal growth and/or mycotoxins production.
- ✔ Evaluate the plant growth promoting activities (PGPR) and enzymatic activity of most promising strains.
- ⧗ Confirm the *in vitro* observations by *in planta* assays.
- ⧗ Optimize the production of selected strains

MATERIALS AND METHODS

PGPR: Siderophores, ammonia, indole-acetic-acid
Chitinolytic activity

59 actinobacteria strains

- *F. graminearum* (INRA 149) → DON / 15-ADON
- *F. Verticillioides* (INRA 63) → FB1 / FB2
- *A. Flavus* (E73 TOXALIM) → AFB1/B2/G1/G2
- *P. Verrucosum* (NRRL 5571) → OTA

Mycotoxins

HPLC

$$\text{Growth ratio (\%)} = \frac{\text{Assay area}}{\text{Control area}} \times 100$$

Control = fungi alone *Assay = fungi in confrontation with actinobacteria*

$$\text{Specific production (Psp)(\%)} = \frac{\text{Amount mycotoxins produced (ng)}}{\text{Colony area (cm}^2\text{)}}$$

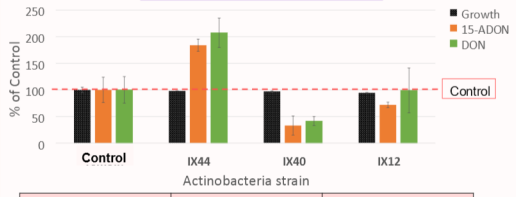
$$P_{sp}\,\text{ratio(\%)} = \frac{P_{sp}\,\text{assay}}{P_{sp}\,\text{control}} \times 100$$

RESULTS AND DISCUSSION

In vitro interactions observed between actinobacteria and fungi

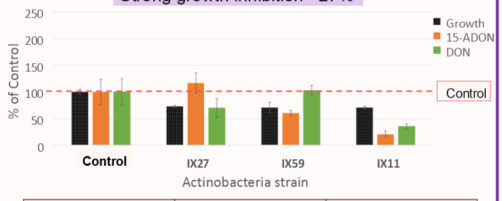
Fusarium graminearum

Weak growth inhibition <16%



Mycotoxin impact	Number of Strains	Example
P_{sp} ↗	14	IX44
P_{sp} ↘	7	IX40
P_{sp} ~ C	9	IX12

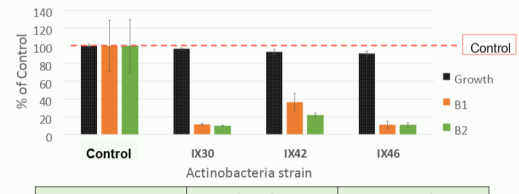
Strong growth inhibition >27%



Mycotoxin impact	Number of Strains	Example
P_{sp} ↗	4	IX27
P_{sp} ↘	19	IX11
P_{sp} ~ C	6	IX59

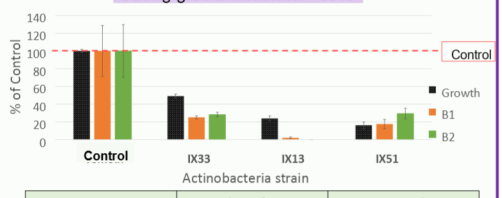
Aspergillus flavus

Weak growth inhibition <47%



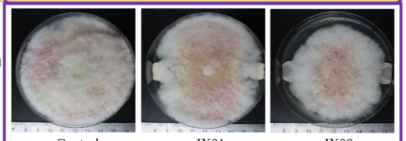
Mycotoxin impact	Number of Strains	Example
P_{sp} ↗	0	-
P_{sp} ↘	30	IX30, IX42, IX46
P_{sp} ~ C	0	-

Strong growth inhibition >50%

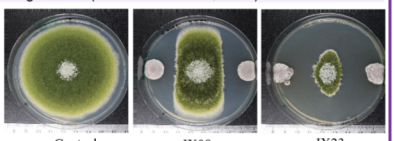


Mycotoxin impact	Number of Strains	Example
P_{sp} ↗	0	-
P_{sp} ↘	30	IX33, IX13, IX51
P_{sp} ~ C	0	-

Discussion



F. Graminearum growth was hardly inhibited by actinobacteria strains (<35%). Different behaviors were observed regarding mycotoxin production. The mechanisms leading to an increase or a decrease of mycotoxin production remain to be elucidated but could result from an activation or inhibition of the trichotecenes pathway, or from biodegradation by actinobacteria or fungus itself (Wachowska et al., 2017).



A. Flavus growth and mycotoxin production were easily inhibited by actinobacteria strains. Almost all the strains diminished mycotoxin production whether the growth was inhibited or not. This observation suggests a more universal process than for *F. graminearum*. It might result from microbial degradation (Harkai et al., 2015) or inhibition of AF pathway by well known molecules produced by *Streptomyces* sp. such as blasticidin or aflastatin (Sakuda, 2010).

PGPR and enzymatic activity *in vitro* assays

Strains able to reduce mycotoxin production/content showing chitinase activity and/or PGPR (Plant Growth Promoting Rhizobacteria) traits:

	Chitinolytic activity	Siderophore production	Ammonia production	Indole-acetic-acid production	Phosphate solubilization
Strains with antagonistic activity showing PGPR and/or enzymatic traits	Total:41 Antagonistic:2 IX12 (*A. flavus*) IX46 (*A. flavus*)	Total: 8 Antagonistic: None	Total: 7 Antagonistic: None	Total: Antagonistic: 3 IX02 (*F. graminearum*) IX11 (*F. graminearum*) IX43 (*A. flavus*)	Total: 20 Antagonistic: 1 IX46 (*A. flavus*)

The PGPR trait might provide an additional advantage during *in planta* validation experiment. Nevertheless, strains with negative results could also have those properties but do not express them *in vitro*. Strains exhibiting the most promising antagonistic activity were not the ones combining the most PGPR traits

CONCLUSION AND PERSPECTIVES

We were able to identify three main profiles regarding mycotoxin production/content during the antagonistic experiments against actinobacteria: strains that provoked its reduction, those that did not affect the specific production, and finally those who enhanced it. Furthermore, degradation experiments will be performed, as well as active metabolites identification produced by the most promising strains in order to elucidate the mechanisms involved. This study highlights the importance of taking into account mycotoxin production when selecting bacterial strains for biocontrol against mycotoxinogenic fungi. As demonstrated, strains that inhibit growth often induce mycotoxin overproduction. An *in planta* screening of the 59 strains will be implemented to verify our *in vitro* findings. The results of this screening will allow us to select the most promising actinobacteria from the collection. The technological properties of the selected candidates such as biomass production yield, sporulation yield and resistance to conservation process will be studied.

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