Isolation and screening of *Actinobacteria* with antagonistic activity against *Botrytis cinerea* and identification of the active compound

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Botrytis cinerea is a necrotrophic phytopathogen able to attack more than 200 different host species worldwide, including crop plants, causing strong yield losses. Many synthetic fungicides have been developed to control this pathogen and nowadays agriculture relies heavily on these. Unfortunately, these products have harmful side effects on ecosystems and can lead to increase pathogen resistance to pesticide. Even if this is an effective mean to fill the gap between supply and demand, there is therefore an urgent and critical need for bio-based agriculture. There is a large body of literature describing potential uses of bacteria as agents managing plant health.

The aim of my master project was to identify *Actinobacteria* strains showing antagonistic activity against *Botrytis cinerea* to contribute to plant protection in an environmental-friendly way. *Actinobacteria* are promising candidates because they are well-known for their secondary metabolites with antimicrobial and/or antifungal activities. Indeed, they are recognized as the most prolific group in antibiotic production. Thus, a large portion of marketed antibiotics originates from these eubacteria. Because of these features, using *Actinobacteria* as biocontrol agents could be an efficient mean to replace synthetic fungicides.

We have isolated 17 *Actinobacteria* strains from 9 different Swiss soils and selected the isolates showing antagonist activity against *Botrytis cinerea*. Three *Actinobacteria* isolates were then selected, all belonging to the *Streptomyces* genus. We tested the activity of these three eubacteria against different stages of *Botrytis cinerea* development, and noticed that their culture filtrates were able to inhibit spore germination and delay mycelial growth *in vitro.* When testing this antagonistic activity on *Arabidoposis thaliana*, we observed that plants were more resistant to *Botrytis cinerea* once treated with the filtrates of *Actinobacteria*. Similar results were observed when the soil of plants was previously inoculated with *Actinobacteria* spores. This project also aimed to identify the active compound produced by *Actinobacteria.* For this part we choose the strain conferring the best protection of the plants. The screening of the organic and aqueous phases of the culture filtrate shows that the antifungal compound was in the organic one. The molecules of this phase were separated on a thin-layer chromatography (TLC) plate. This plate was then placed face down on an agar plate previously inoculated with pathogen spore suspension. An inhibition zone corresponding to a single band of the TLC plate was visible and the compounds corresponding to this band were isolated and analyzed by mass spectrometry. The masses of the obtained peaks correspond to germicidin A and B and this was confirmed by HPLC. Until now, these polyketide compounds were known to inhibit the spore germination of *Actinobacteria*, but their activity against pathogen has not been reported.

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