SUMMARY

Key words: Fusarium, Bacillus, compost tea, molecular identification, chemotyping, biocontrol, stimulatory, in vivo antagonism

The infection caused by *Fusarium*, a phytopathogen frequently encountered with cereals, tends to become a worrying problem at a global scale, causing important crop yields and economical losses, the damage being amplified due to the capability of this genus to synthesize mycotoxins. At the moment approximately 19 *Fusarium* species have been identified, each species having its own mycotoxin profile and its own preference regarding the host plant.

Identifying the pathogen strains of *Fusarium* that are present in the contaminated plant samples is extremely important for the diagnosing of induced diseases in order to prevent and control the mycotoxin contamination.

Regarding the control of these fungi many strategies were proposed, from which crop rotation, growing resistant breeds and fungicide treatment represent the main practical approaches. Over the last years, a special interest was taken into the methods of biological control of fungal infections, which could significantly reduce the negative environment impact of chemical pesticides.

For these reasons, the primary goal of the project is to identify new biological control agents for fungi belonging to the *Fusarium* genus: *F. graminearum* and *F. culmorum*, the usual suspects of cereal infections.

The PhD thesis entitled "RESEARCH REGARDING THE BIOCONTROL METHODS OF FUNGI BELONGING TO *FUSARIUM* GENUS" includes 5 chapters conducted on 155 pages including 25 tables, 67 figures out of which 48 original contributions and 190 bibliographical references.

Chapter I provides information regarding the importance of *Fusarium* genus, its taxonomy, life cycle, pathogenicity, identification methods and methods of prevention and control. Apprehension of these aspects contribute to a better understanding of the *Fusarium* genus fungi and of the methods used in differentiation between species, being a vital step for elaborating experiments that aim to rapidly identify the mycotoxigenic fungi in order to prevent and control them.

In **Chapter II** are presented the biological control methods as alternative to classical control methods by using biological control agents. Also it reveals the compost as suppressor for pathogenic fungi and as a source to isolate microorganisms with potential antifungal effect.

This chapter contributes to the better understanding of the strategies of biocontrol and the knowhow that was acquired was used in the biocontrol experiments presented in this study.

The primary goal of the thesis is to identify new agents for biological control of fungi from *Fusarium* genus: F. graminearum and F. culmorum, the ones responsible for cereal grain infections.

The research objectives were:

- Isolation and characterization of some new strains of indigenous microorganisms capable to inhibit the growth of fusaria strains;

- Characterization of some fungal isolates with concern to their mycotoxigenic capabilities and their species identification;

- Identification of some mechanisms of action of antagonistic microorganisms manifested against fusaria.

In **Chapter III** is presented the biological material and the methodology used in the study.

The **biological material** is represented by 34 *Fusarium* strains, new isolates from the surface of wheat grains from 2013 and 2014. Two reference strains belonging to *F. graminearum* (Fg183) and *F. culmorum* (Fc46) species were obtained from diverse collections of European microorganisms.

Likewise in the study were used diverse species of fungal phytopathogens belonging to the Faculty of Biotechnology, Genetic Laboratory collection of microorganisms. As for antagonistic microorganisms, 6 bacterial strains newly isolated from compost and 2 reference bacterial strains belonging to the Faculty of Biotechnology, Genetic Laboratory collection were tested.

The research methods used pertain to: the isolation and cultivation of microorganisms used in the experiments (bacteria and filamentous fungi); genomic DNA isolation from the selected bacterial and fungal strains; the identification with the help of the BIOLOG system and of molecular techniques of the species to whom the microbial isolates used belong to; the molecular chemotyping in the case of the *Fusarium* genus fungi; the pathogenicity assay of the fungi and the antagonistic capability assay and the characterization of the bacterial strains.

Chapter IV contains the results of own research grouped mainly on the objectives proposed in the thesis. Thereby, in the first subchapter are presented the results concerning the isolation from compost extract (tea) of some new bacterial strains with antagonistic properties towards a wide range of phytopathogenic fungi. These results have proven, by testing many experimental variants that the compost extract presents inhibitory effects on the growth of

phytopatogenic fungi, this activity being largely associated with the presence of the benefic microorganisms at its level.

The second subchapter includes the results related to the species identification to which the 8 bacterial strains that showed clear antifungal effects belong to. For the identification of the new bacterial isolates (6 originating from compost and 2 from soil) two methods were coupled: the BIOLOG system and molecular analysis. The BIOLOG system was efficient for the identification, at a genus level of all the isolates and at a species level of 5 of them, whereas by using the species-specific primers (multiplex PCR) it was possible to identify all the 8 bacterial strains used.

The RAPD analyses in the case of the strains that belong to the same species have shown a light intraspecific polymorphism which proves that, although the isolates come from the same sources, the bacterial strains are different.

The following subchapter relates to the identification and characterization of the newly *Fusarium* isolates obtained from wheat seeds. Identification of *Fusarium* strains was done using classical methods, microbiological ones for the genus classification. For the identification of the species to which they belong molecular methods were used. In the experiments it was optimized a method of fungal genomic DNA isolation which lead to obtaining of quantitative and qualitative superior DNA for use in the future experiments. In order to identify the species of fusaria, a multiplex PCR was used with species-specific primers, which led to the identification of 34 *Fusarium* strains: 5 were identified as belonging to the *F. culmorum* species and 29 belong to *F. graminearum* species, these results confirming the dominance of this species.

The RAPD analysis of the *F. graminearum* and *F. culmorum* isolates highlighted, in general, a certain degree of molecular polymorphism between the strains examined (intraspecific) but did not permit the association between the obtained polymorphism bands and any specific character of said fungi. In this subchapter are also presented the results obtained through chemotyping of the fungal isolates. Using specific primers for the *Tri 5* gene permitted the detection of this gene in the majority of the fungal strains analyzed suggesting their capabilities to synthesize trichothecenes.

Using characteristic sets of primers for other geners involved in the biosynthesis of trichothecenes allowed for the identification of 2 distinct chemotypes: 15-ADON and 3-ADON. It is worth noting that all the *F. culmorum* isolates belong to the 3-ADON chemotype. The results obtained following the research confirms the usefulness of the molecular techniques for the characterization of new strains of *Fusarium*.

Subchapter 4.4. contains the pathogenicity assay of the selected *Fusarium* isolates, assessing both the fungal influence over the germination process and growth of wheat plantlets

as well as the possibility to associate a trait with mycotoxin production. With the help of the experiments it was shown that the pathogenicity of fungal strains is clearly associated with the level of mycotoxins produced, demonstrated through the usage of TLC technique that showed the clear presence of DON (and of other potential toxic compounds) at some of the fungi.

The last 3 subchapters are dedicated to the antagonistic properties of selected bacteria, the mechanisms of action behind them and to the evaluation of their protecting/stimulatory effects over the targeted plants. The obtained results demonstrated that the selected bacterial isolates have the potential to significantly inhibit the growth of *Fusarium graminearum* and *Fusarium culmorum* species, and the inhibitory efficiency is influenced by specific interactions between bacterial strains and targeted fungi, as shown by the microscopy analysis.

The antifungal compounds produced by bacteria are affected both by temperature as well as proteolytic enzymes, which leads to the conclusion that the nature of these compounds is protein. Specific mechanisms of action of the selected bacteria are linked to the production of hydrolytic enzymes (chitinase and protease) as well as to biosurfactants (genes for biosynthesis of bacillomycin, fengycin, bacilysin and surfactin being detected at the majority of the tested bacteria).

Regarding the tested hydrolytic enzymes, the results suggest that although extracellular, the majority of them remains localized at a cell wall level, most lileky at the level of complex macromolecules associated with it. The strain *Bacillus amyloliquefaciens* BIR (B8) showed the most powerful antifungal effects, as well as the highest enzymatic activity and containing genes for all the biosurfactants analyzed. Assays done with different bacterial strains highlighted that some of them are capable of influencing the level of DON produced by target filamentous fungi, either by the inhibition of DON synthesis or by mycotoxin degradation. Likewise, it was proven that the selected bacteria have the capability to solubilize inorganic phosphorus, which shows their usefulness not only toward plant protection but also to stimulate plant growth, by ensuring the availability of important nutrients. In the case of antagonistic properties evaluation of bacterial isolates on seed or plant treatment, the conducted analysis haven shown that the seed germination process was not negatively affected by the bacterial treatment, on the contrary, it prevented the seed infection with fungi and assured a high germination rate of 2-3.5 times higher comparatively to the variants where the treatment was lacking. Two of the bacterial strains, B3 and B8 (BIR) stimulated both wheat seed germination and plantlet growth.

The thesis ends with a chapter of conclusions in which are concentrated the main results obtained, followed by a bibliographical reference chapter that contains a cited number of 190 scientific papers.

The obtained results during the PhD studies were disseminated by publishing 2 articles in BDI indexed journals; a published article in an ISI indexed journal and another article published in a volume of ISI Proceedings. Likewise the great majority of results were presented at diverse national and international scientific proceedings.

In conclusion, the experiments conducted in this PhD thesis resulted in the selection of at least two bacterial strains, one from compost and another one from soil level. Through their identification and complex characterization, these strains could be considered as important candidates for the production of some biopreparates usable as is or as components in compost for the biological control of *Fusarium* species, thereby preventing the plant contamination. The results have a novel character on a national and even international level through the selected and characterized biological material used, the methods optimized during the research effort and through emphasizing the effect of reduction of DON quantity accumulated in the culture media in the case of using the selected bacterial strains.