EXECUTIVE SUMMARY

of PhD thesis entitled:

RESEARCH ON THE BIODEGRADATIVE POTENTIAL OF SOME MICROORGANISMS USED IN BIOREMEDIATION

Written by drd. Andreea DOBRE under the scientific coordination of

Professor Petruța Călina CORNEA.

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The environmental pollution reached alarming rated in the past century due to the agressive antropic development against the ecosystems which they inhabit. The abusive and irational exploitation of natural resources, the development of unsustainable industrial practices and the lack of focusing on research and implementation of sustainable ways to handle Terra's resources led to a future under negative auspices. In order to reduce the environmental pollution it have been developed bioremediation practices by the correlation of life sciences and technology, in order to result specific products and services which are optimum for bioremediation of polluted sites. The main used agents in bioremediation are the microorganisms which in their turn can be subjected to biotechnological practices in order to promote the characteristics usable in contaminated sites decontamination.

The informations from scientific literature being the starting point, the purpose of the present thesis is the study of the involved microorganisms in bioremediation of polluted soils with organic and anorganic compounds of phosphorus and sinc which are also capable to promote the plant development and protection.

The conducted experiments over the two years of study have been realized in the laboratories of Biotechnologies Faculty from University of Agronomic Sciences and Veterinary Medicine from Bucharest and in the National Institute of Development and Research for Biological Sciences, Bucharest. The PhD thesis named "RESEARCH ON THE BIODEGRADATIVE POTENTIAL OF SOME MICROORGANISMS USED IN BIOREMEDIATION" comprise 7 chapters, deployed in 137 pages, with 17 tables, 51 figures and 156 bibliographical references.

The first chapter, THE USE OF BIOTECHNOLOGY IN BIOREMEDIATION PROCESSES, targeted the scientific literature study regarding the involvement of biotechnologies in soil and water bioremediation by microorganisms utilisation, with reference to rizoremediation benefits, as alternative to the clasical remediation technologies of pollute

sites. The second chapter, BIOREMEDIATION OF SOILS POLLUTED BY PHOSPHORUS, describes the state of the art of research regarding the thesis subject, namely the phosphorus pollution impact and the specific bioremediation techniques developed until now. Moreover they were summarized the existent problems regarding this aspects. PHYTASES, the third chapter describes the main enzymes involved in organic phosphorus bioleaching, used as bioremediation agents. It is realized a complex study regarding their classification, the phytases sources, the conditions and the action mode of these. The forth chapter, BIOREMEDIATION OF SITES POLLUTED WITH HEAVY METALS, develops the heavy metals impact over the environment, focusing on the zinc compounds pollution. Also targets the microorganisms involved in bioremediation of contaminated sites with this kind of contaminants. The chapter five, IMPACT, PURPOSE, OBJECTIVES AND INNOVATIVE CHARACTER OF THE RESEARCH WORK, describes the importance of this reseearch paper and also the purpose, objectives and the innovative aspect of the thesis. The innovative character consists in the global view approached regarding the study of the microorganisms usable in bioremediation. The research started from the idea that microorganisms which are growing in natural stress conditions have already developed a resistance system. So the soil prelevation from various areas eventual polluted with phosphorus and other pollutants such as heavy metals is a logical step in microorganisms isolation which are able to bioremediate the polluted sites. Besides this fact it was studied the ability to promote the development of cultivated plants. Soils with waste deposits produced by swine, the rizosphere of some cultivated plants, polluted soils with petroleum hydrocarbons or farmlands, compost, were the isolation sources chosen regarding the indigenous microorganisms identification. Starting from these afirmations, the purpose of this Phd thesis was the study of some isolated microorganisms from various sources which have the capacity to bioremediate the polluted sites with organic and anorganic compounds of phosphorus and zinc and which also have the capacity to promote/protect the developement of plants.

The objectives suggested within the thesis refers to:

- Isolation from various natural sources of some microorganisms able to leach the phosphorus and/or zinc insoluble compounds;
- Characterisation of new microbial isolats regarding the bioleaching particularities and examination of the mechanisms involved in this process (phytase biosynthesis, organic acids production);
- Antifungal activity evaluation of bacterial isolats with phosphorus leaching properties, usable as biocontrol agents against various fungal phytopatogens (*Pythium debaryanum, Phytophthora infestans* etc);

- Phytasic activity cuantification of the selected microbial isolats and kinetic parameters optimisation.

In order to reach these objectives, in chapter six, MATERIALS AND METHODS, they were detailed the materials and methods used in present research aproach. In chapter seven were revealed the RESULTS AND DISCUSSIONS related to the purpose and objectives of the current research thesis, targeting the correlation between the isolation sources, the identified species and their properties which are useful in the bioremediation of the contaminated soils with phosphorus organic and anorganic compounds and also with zinc anorganic compounds. In order to achieve the first objective they were selected microorganisms according to the isolation source. Thus, in this paper we have been used previously identified microorganisms that are part of Collection of Microorganisms of the Department of Genetics, Faculty of Biotechnology, UASVMB. To this series of microorganisms it was added a number of isolated bacteria in the thesis development, from the soil contaminated with swine landfill mentioned earlier. The next step was to identify the microorganisms ability to leach both phytic acid and inorganic forms of phosphorus. Thus they were selected a number of bacteria which have both properties or one of them. Several specific media, among which are mentioned PSM, NBRIY, NBRIP, PVK, etc were used at this stage. Subsequently, it was focused on the ability of the bacteria to secrete organic acids involved in the solubilization of inorganic phosphorus or by lowering the pH of the medium so as to allow the activation of enzymes synthesized by microorganisms. It should be noted that most of the microorganisms studied within this thesis belong to the genus *Bacillus* which is recognized for its ability to synthesize phytases as well as the ability to potentiate the growth of plants. After this stage were identified bacteria capable of inhibiting the development of microorganisms that are known as extremely aggressive phytopathogenic. They are *Pythium debaryanum* and *Phytophthora infestans*. It was also identified the ability of a microorganism to synthesize proteases and to leach zinc oxide. For zinc oxide has been used a number of specific media that included dyes targeted specifically to highlight bioleaching halo. In the study of microorganisms identification which are capable of synthesizing phytase were included also lactic acid bacteria because of the well-known controversy over this ability. Following the conducted studies were identified bacteria strains that have shown a high enzymatic activity, enabling the theory consolidation about the fact that LAB can degrade phytic acid and its salts. To determine the enzymatic activity of bacteria were initially selected microorganisms isolated from soil with swine landfills who also have achieved good results. Finally, to come into effect a comparative study between 5 isolation sources, two species of *Bacillus*, *B. amyloliquefaciens*, *B. subtilis* and bacteria isolated from compost belonging to *Bacillus* genus. To determine the catalytic activity the standard method was used, namely molybdate-blue method, but after an

extensive study of literature, were compiled eight variations thereof identify the optimal reaction parameters. The reaction conditions in the study were neutral pH, 37°C, 30 min, 50°C neutral pH, 20 min, acid pH (5.5), 37°C, 30 min, acid pH (5.5), 50°C, 20 min. All four of these variants was carried out in the presence or absence of the inducer.

Finally, the paper describes the GENERAL CONCLUSIONS revealed by the related research approach. Putting the initial focus on isolation source as a determinant of the bacteria capacity to hydrolyse phosphorus, it has been shown that bacterial isolates that had swine manure polluted soils as a isolation source had this property. However, evaluation of strains isolated from other isolation sources (rhizosphere of crop plants, heavily polluted soils with petroleum hydrocarbons) have demonstrated their ability to solubilize both organic and inorganic phosphorus compounds. The most notable results were obtained by the strains 7.1, BW, BIR, BPA, OS15, OS17, B3, B4, B5, B6, all belonging to the genus *Bacillus* and by the strain An (*Aspergillus niger*). Regarding the characterization of these strains, their ability to solubilize inorganic phosphorus B3, B4, OS15, BPA, BIR, BW, OS17, ATCC 6633, 35, 10 gave the best results in experiments on PVK and NBRIP. The theory according to which the organic acids are involved in the solubilization of inorganic phosphorus has been validated by the use of a pH indicator dyes. However, after the analysis on PSM medium it was highlighted the strains (BIR, BPA, ICPC, OS15, OS17, BW, 10, 35) enzymatic capacity to hydrolyse the organic phosphorus. Naturally, this observation does not preclude the phosphorus hydrolysis by phosphatases potential synthesized by bacteria, the organic acids involvement in this process being reduced. But the production of organic acids may be involved in another feature of strains namely solubilization of inorganic zinc, the use of pH indicators resulting in clear halos highlighting in case of some strains like BPA, B4, ATCC 6633, 32 and 35. Also, most strains involved in the study have demonstrated the ability to synthesize proteases. The strains characterized in this scientific demarche have been tested regarding their utility as biocontrol agents against phytopathogens. Strains B3, B4, 9, 10, 12, 30, 32 and 35 showed inhibitory effect against *Phytophthora infestans* clear by dual culture method *in vitro* conditions. These bacteria also had effect and under controlled conditions in the climatic chamber as preventive treatment applied to tomatoes. Compared to *P. debaryanum*, the culture filtrate from strains 9, 10 and B3 showed a slight reduction in diameter of fungal culture with 24%, 12% and 10%. Clear inhibitory effect against phytopathogens were evident in this case of bacterial strains noted B5, B6, BPA and BW (direct confrontation). Regarding the quantification of enzyme activity and optimization of reaction parameters was tested a number of bacterial strains studied in this thesis, to which were added lactic acid bacterial strains. Of the four strains of lactic bacteria, *Lactobacillus plantartum* Lpl recorded the highest enzyme activity. This will determine their

applicability in terms of reducing phytic acid or salts thereof in foods of plant origin, which is known as anti-nutritive agent. Among isolates from soils with swine manure deposits, the strains 10, 9, 35 and 12 had the highest values of enzymatic activity. Subsequent the optimization studies for phytase kinetic parameters were determined by studying the pH, temperature, reaction time and the presence of inducers, demonstrating variation both depending on the species and also intraspecific. The highest enzymatic activity was highlighted by the strain *B. subtilis* ATCC 6633, followed by *Bacillus sp*. B3, then *B. subtilis* 10 and *B. amyloliquefaciens* BW. The last positions in terms of phytase activity were occupied by strain *Bacillus* sp. B4 and *B. amyloliquefaciens* OS15.

The results may contribute to the development of knowledge in the field and can contribute to the achievement of effective bioremediation technologies based on the use of natural microorganisms with various beneficial properties. So after the study of indigenous bacteria within the present thesis it was concluded that they have a potential beneficial impact on the environment, finance and human, animals and plants health.

The results obtained from the scientific demarche of this thesis were capitalized by one ISI publication on the subject of the thesis and two in the field, 3 BDI articles and three in the field, plus participation in numerous international conferences.