The collection of beneficial soil microorganisms held in the SYMBIO BANK

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Abstract

*R **Results of studies to date have shown that there are large differences in the occurrence of mycorrhizal fungi depending on the species and plant cultivation method. At present, the material collected in the bank of isolated spores of mycorrhizal fungi and the bank of PGPR bacteria comes from the nearby ecological orchards and plantations and from ecological orchards and plantations in the Bieszczady and Białowieża region. Trap cultures have been used to isolate and identify spores of the following species of arbuscular mycorrhizal fungi:* Ambispora fennica, A. gerdemannii, Gigaspora margarita, Glomus aggregatum, G. caledonium, G. claroideum, G. constrictum, G. drummondii, G. fasciculatum G. macrocarpum, G. microaggregatum, G. mosseae, G. pallidum, G. rubiforme, Scutellospora dipurpurescens*. The collection in the SYMBIO BANK contains (approximately): Spores isolated from the soil of the following plant species: strawberry 16.0 thousand, apple 6.5 thousand, sour cherry 1.1 thousand pear 8.1 thousand. Isolates of bacteria:* Pseudomonas fluorescens *-170, dissolving phosphorus compounds - 40, digesting cellulose - 40, producing spores - 110, fixing atmospheric nitrogen - 10, Actinomycetes - 40. Isolates of microscopic fungi - 50, including Trichoderma sp. - 30. The work of isolating and identifying species and strains of AM fungi and PGPR bacteria is continued. They are collected, catalogued and stored in a Bank of Symbiotic Microorganisms, called SYMBIO BANK, specially established for this purpose. The collected strains and species are identified, characterized and stored in a cryoprotectant (glycerol) at the temp. of -80°C. In the near future, a website of the SYMBIO BANK will be launched, which will contain a list of the isolates held in the collection and their descriptions, which will serve as a source of key information for the identification of the species of AM fungi and PGPR bacteria in Poland. This will contribute to the knowledge of the biodiversity of these symbionts and help in the formulation of microbiologically-enriched bioproducts for use in fruit-growing practice. The most effective strains and species of microorganisms will be registered in Poland as bacterial and mycorrhizal inocula to be used in fruit production and in phytoremediation of heavy metal pollution. The establishment of the bank of spores of mycorrhizal fungi will contribute to the understanding and maintenance of the biodiversity of these symbionts, the knowledge of their biology and ecology, as well as to the development of ecological technologies of fertilization of fruit plants in Poland and the protection of the natural environment and human health.*

Keywords: bacteria, microscopic fungi, AMF, isolates

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Introduction

Plant-soil microorganisms can modulate the uptake of mineral nutrients through feedback processes that reflect plant responses to environmental conditions. The intimate interrelation between the root and symbiotic arbuscular mycorrhizal fungi and the resulting enhancement in plant uptake of N and P, is further expanded by the interactions between the fungus and bacteria present in both the rhizosphere and mycorrhizosphere. Numerous species of plant growth promoting bacteria form biofilm when colonizing roots, which can affect bio-geochemical processes and can result in increased availability of poorly available mineral nutrients. The complexity of the relationship between plant and rhizosphere microorganisms is further complicated by the effects on both rhizobacteria and mycorrhizas of protozoan grazers, which are also directly affecting the root system. The soil biotic component include complex communities with different trophic levels that function in very heterogeneous environments. These communities are formed by several kinds of microorganisms that can live symbiotically or in association with roots. Four major groups of microorganisms are considered as beneficial to plants: arbuscular mycorrhizal fungi (AMF) (Jeffries *et al.* 2003), plant growth promoting rhizobacteria (PGPR) (Vessey 2003), nitrogen-fixing rhizobia, which are usually not considered as PGPR, and microbial biocontrol agents, which are composed of several kinds of microorganisms (viruses, bacteria, yeasts and fungi).

An important part the project called EkoTechProduct, which is carried out at the Research Institute of Horticulture in Skierniewice (Poland), is to establish and maintain a Bank of Symbiotic Microorganisms, called the SYMBIO BANK. At present, the collected material of isolated spores of mycorrhizal fungi and PGPR bacteria comes from organic orchards and plantations located around Skierniewice (Poland), and organic orchards and plantations in the Bieszczady and Białowieża areas (Poland).

Material and Methods

To distinguish more than 80 isolates of *Pseudomonas* bacteria and bacteria dissolving phosphorus compounds, acquired from the soil in the root zone of apple and sour cherry trees, the technique of rep-PCR was employed, based on an analysis of DNA polymorphism. The tests allowed the selection of bacterial isolates that were different or belonged to the same strain. For the detection of arbuscular mycorrhizal fungi (AMF) in the roots of strawberry plants, the nested PCR technique was used, based on the amplification of fragments of the large subunit ribosomal gene (LSU rDNA) using specific primers. The analyses were performed on the DNA extracted from the roots of strawberry cultivars ‘Honeoye’, ‘Elsanta’ and ‘Elkat’ that had been treated with bio-preparations and fertilized with NPK. The tests helped to determine the presence or absence of mycorrhizal fungi of the genera *Glomus, Acaulospora* and *Scutellospora* in the roots. The results were used to determine the effect of the bio-preparations on the presence of mycorrhizal fungi in the roots of strawberry plants.

Over 600 strains of *Pseudomonas fluorescens* and other beneficial bacteria and fungi (*Rahnella aquatilis*, *Bacillus* sp., *Trichoderma* sp.) have been collected. *Pseudomonas fluorescens* strains were isolated from selective S1 medium (Gould *et al.* 1984). Other beneficial bacteria were isolated from differentiating CAS agar medium (siderophores production) or Pikovska medium (dissolving phosphate compounds) (Husen *et al.* 2003).

Ten bacterial strains with most potent beneficial abilities were identified and characterized by the BIOLOG system (Holmes *et al.* 1994; Pires & Seldin 1995) and used in the further screening studies under greenhouse conditions. Screening studies under greenhouse conditions indicate that three strains (Ps49A - *Pseudomonas fluorescens*, Pi3A and Pi5A -

Rahnella aquatilis) enhance growth of strawberry plants. However further and more complex studies must be conduct.

Identification of spores:

- Trap cultures were set up with narrowleaf plantain in 0.5 L pots filled with a mixture of rhizosphere soil and autoclaved sand, at a ratio of 1:1 v/v (Błaszkowski 2003). Pots were placed in SunBags (Sigma).
- After six months, 200g samples of the pot substrate were taken from the trap culture combinations and spores were isolated by wet sieving and centrifuging in a sucrose gradient (Brundrett *et al.* 1996).
- The isolated spores were divided into morphotypes according to size, shape, and colour of spores.
- The layer thickness of spore walls and germination walls was measured in freshly isolated spores which were crushed in PVLG or PVLG+Melzer's reagent (1:1, v/v) and observed under a light microscope equipped with a micrometer eyepiece (Błaszkowski 2003).
- The observed AMF species were named according to Schüßler *et al.* (2001) and Błaszkowski (2003).

All the results were statistically evaluated with analysis of variance. Comparisons of means were at $p \leq 0.05$ with the Duncan test.

Results

As part of the SYMBIO BANK, trap cultures were set up and used to isolate and identify spores of the following species of arbuscular mycorrhizal fungi: *Ambispora fennica, A. gerdemannii, Gigaspora margarita, Glomus aggregatum, G. caledonium, G. claroideum, G. constrictum, G. drummondii, G. fasciculatum G. macrocarpum, G. microaggregatum, G. mosseae, G. pallidum, G. rubiforme, Scutellospora dipurpurescens.*

The collection in the SYMBIO BANK contains (approximate numbers):

Spores isolated from the soil of the following plant species:

- strawberry 16.0 thousand
- apple 6.5 thousand
- sour cherry 1.1 thousand
- pear 8.1 thousand

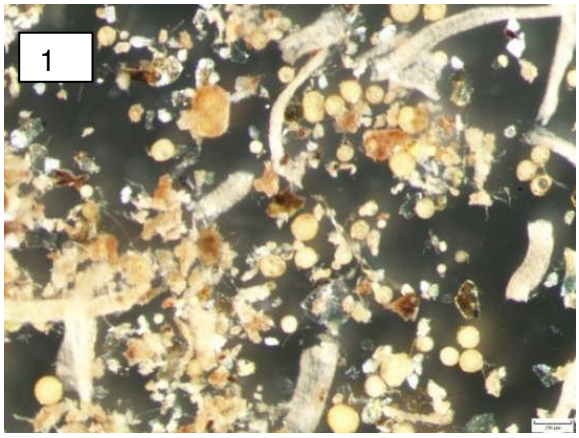

Photo. 1 and 2. Spores of mycorrhizal fungi (AMF) during isolation

Isolates of bacteria:

- *Pseudomonas fluorescens* 170
- dissolving phosphorus compounds 40
- digesting cellulose 40
- producing spores 110
- fixing atmospheric nitrogen 10
- actinomycetes 40

Isolates of microscopic fungi, a total of 50, including

- *Trichoderma* sp. 30

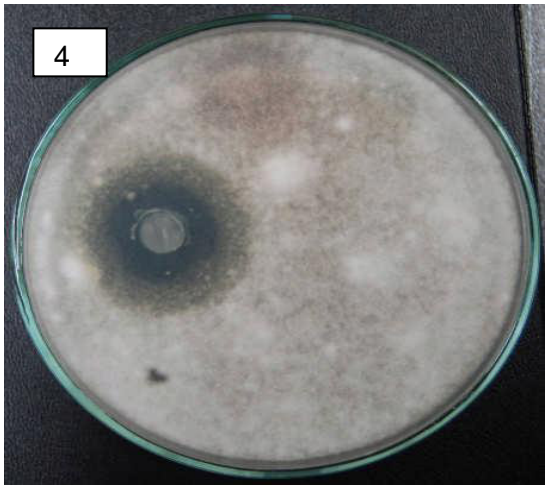

Photo. 3. Inhibition of the growth of *Botrytis cinerea* fungus by antagonistic fungi

Photo. 4. Isolates of rhizosphere bacteria, potential antagonists of soil pathogens (zone of growth inhibition caused by the bacteria)

Isolation and identification of the species and strains of AM fungi and PGPR bacteria is continued. After they have been collected, the microorganisms are identified, characterized, catalogued and stored. The strains of beneficial bacteria are stored in a cryoprotectant (glycerol) at -80°C. The AM fungi are stored in trap cultures in a greenhouse and in the form of spores immersed in various cryoprotectants and frozen at -80°C.

Discussion

A website of the SYMBIO BANK will be launched very soon and will contain a list of strains and species of beneficial soil microorganisms held in the collection. Their descriptions will serve as a source of key information for the identification of the species of AM fungi and PGPR bacteria in Poland. This will contribute to the knowledge of the biodiversity of these symbionts and will help in the formulation of microbiologically-enriched bioproducts for use in fruit-growing practice.

Within the EkoTechProduct project the performance of all the rhizosphere components influenced by microbial inoculation practices will be also studied, e.g. modification of the rhizospheric environment and microbial development by the plant root system. For example, roots may release chemical signals that can be recognized by the microbes, which in turn respond either by modifying morphological features (Buee *et al*. 2000) or by producing feedback signals that set the plant to allow root colonization (Bais *et al*. 2006;

Hirsch *et al*. 2003; Vierheilig & Piché 2002). Furthermore, several compounds exudated by roots can mimic quorum sensing signals that affect the bacterial communities (Bauer & Mathesius 2004). On the other hand, plants respond to microbial root colonization by increasing the release of exudates and by modifying their composition (Phillips *et al*. 2004; Kamilova *et al*. 2006; Steinkellner *et al*. 2007).

All the plant-soil-microbial interactions will be studied after application of soil microbial inocula and their beneficial influence on plant growth, development and yielding.

The most effective strains and species of microorganisms will be patented and registered in Poland as bacterial and mycorrhizal inocula. They will be used in horticultural production and in phytoremediation of degraded soils. The establishment of the bank of spores of mycorrhizal fungi and rhizospheric PGPR bacteria will contribute to the understanding and maintenance of the biodiversity of these symbionts, the knowledge of their biology and ecology, as well as to the development of ecological technologies of fertilization of fruit plants in Poland and the protection of the natural environment and human health.

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