BENEFICIAL MICROORGANISMS IN ORGANIC HORTICULTURE

RESEARCH INSTITUTE OF HORTICULTURE IN SKIERNIEWICE

Lidia Sas Paszt, Beata Sumorok, Anna Lisek,
Edyta Derkowska, Paweł Trzciński, Sławomir Głuszek, Eligio Malusá,
Michał Przybył, Mateusz Frąc, Krzysztof Weszczak

Research Institute of Horticulture
Department of Microbiology
e-mail: lidia.sas@inhort.pl

SME ORGANICS w Regionie Łódzkim 2018

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Research Institute of Horticulture

Lidia Sas Paszt
Lidia.sas@inhort.pl

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Skierniewice, Konstytucji 3 Maja 1/3, POLAND

CENTRAL EUROPE

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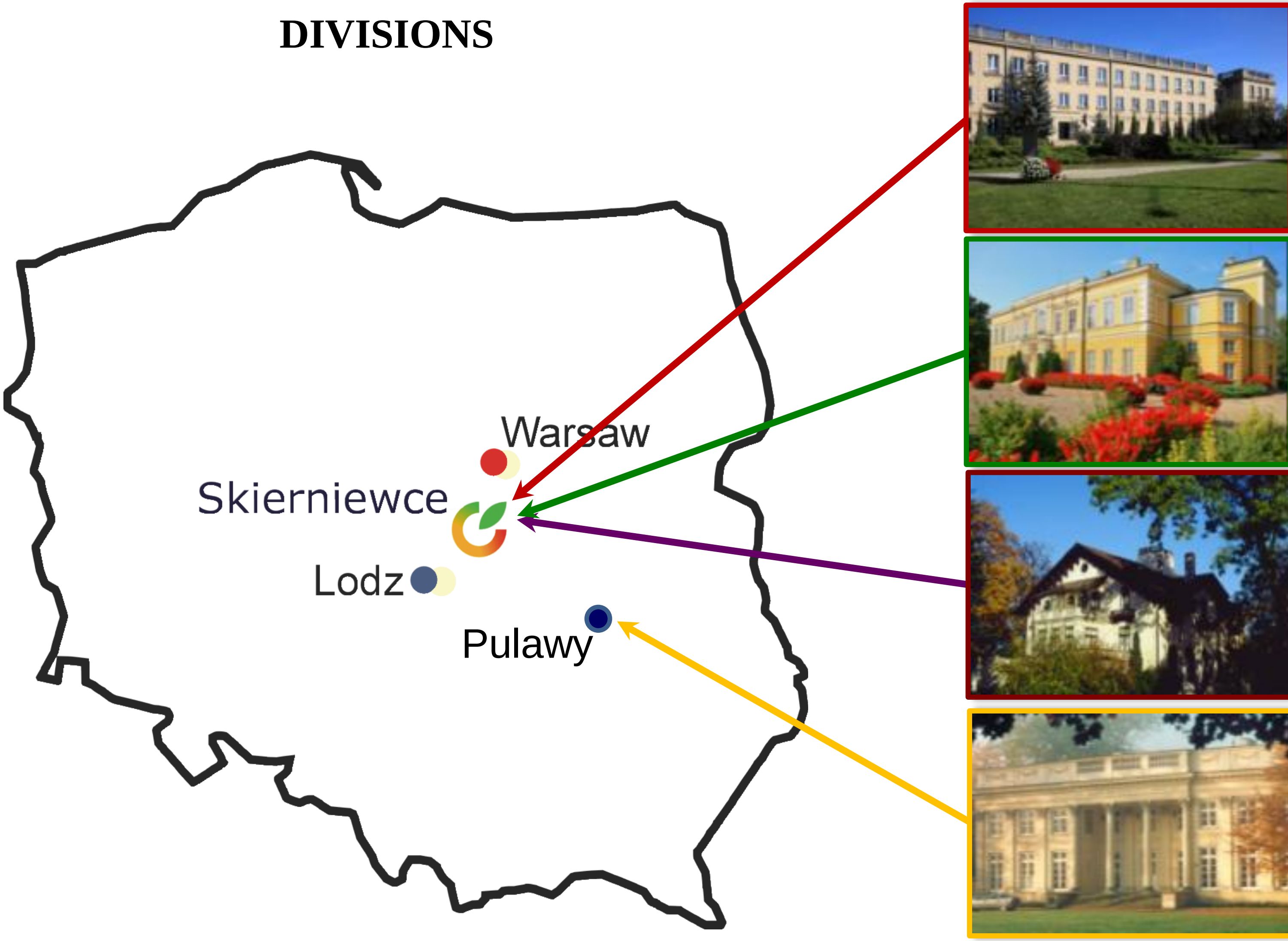
DIVISIONS



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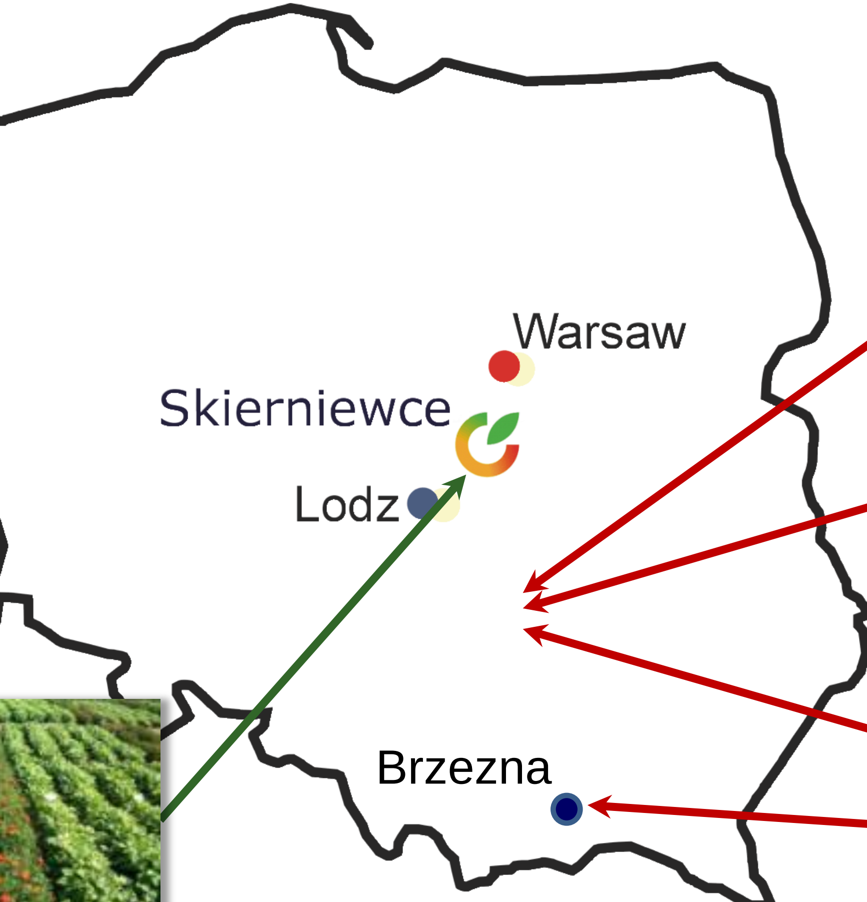
EXPERIMENTAL STATIONS



Experimental Station of Ornamental Plants Nowy Dwór Ltd.

Experimental Ecological Orchard, Nowy Dwór

Center For Elite Nursery Stock Prusy Ltd.

Fruit Experimental Station Brzezna Ltd.

Ecological Vegetable Field Skierniewice

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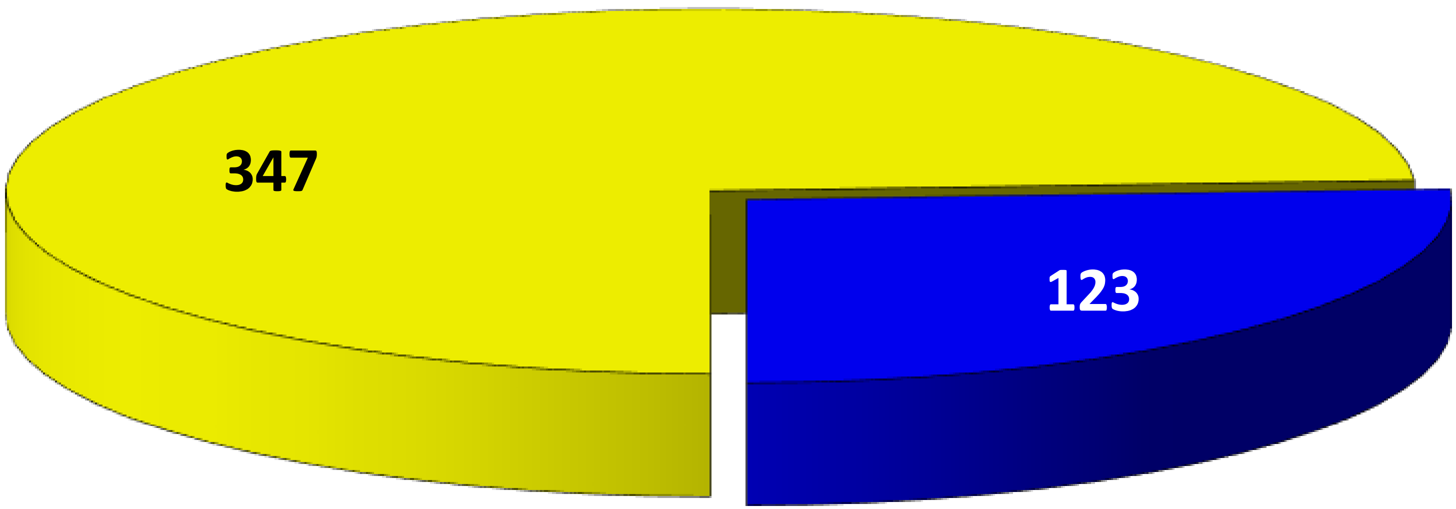
EMPLOYMENT

Total 470



Researchers and lab technicians

Administration, maintenance staff and field workers

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RHIZOSPHERE LABORATORY

- **The role of roots & the rhizosphere in the growth & yielding of fruit & vegetable crops.**
- **Development of sustainable methods of cultivation & fertilization of fruit plants for the production of high quality fruit & vegetables and to increase the natural fertility of the soil using PGPR rhizobacteria, AMF fungi and other components of the soil biosphere.**

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DEVELOPEMNT OF INNOVATIVE PRODUCTS & TECHNOLOGIES FOR THE ENVIRONMENTALLY-FRIENDLY CULTIVATION OF FRUIT PLANTS 2009-2015

The work has been supported by a grant from the EU Regional Development Fund through the Polish Innovation Economy Operational Programme,
<u>contract No. UDA-POIG.01.03.01-10-109/08-00</u>

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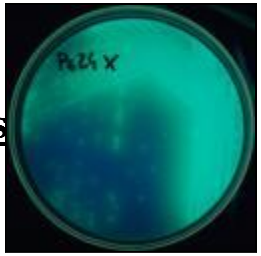
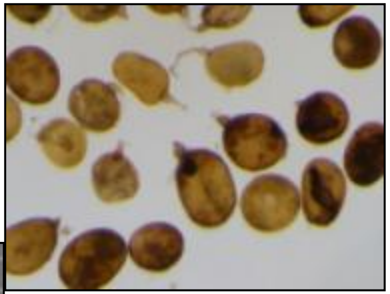
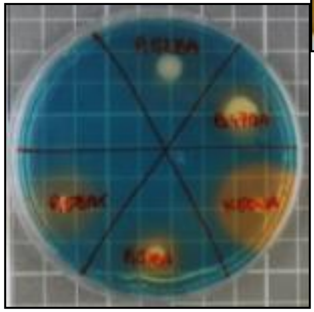
THE COLLECTION IN SYMBIO BANK CONTAINS (NUMBERS)

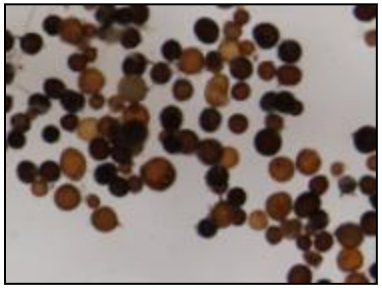
AMF spores isolated from the rhizosphere soil of the species:

• strawberry	18.0 thousand
• apple	10.5 thousand
• sour cherry	1.5 thousand
• pear	14.0 thousand
• wild strawberry	9.0 thousand
Total	**53 thousand spores**



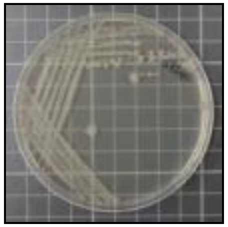


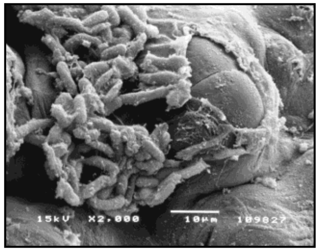




Isolates of bacteria:

• *Pseudomonas fluorescens*	300
• siderofore synthesis	500
• dissolving phosphorus compounds	200
• digesting cellulose	40
• producing spores	110
• fixing atmospheric nitrogen	100
• *Actinomycetes*	100
Total	**1350**





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SYMBIO BANK COLLECTION OF SYMBIOTIC MICROORGANISMS contains spores of AM fungi & PGPR bacteria isolated from the soil of organic orchards & plantations located in Skierniewice, Bieszczady, Białowieża areas (Poland).

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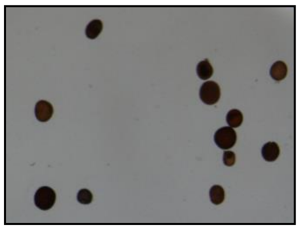
SYMBIO BANK

Storage of spores of mycorrhizal fungi at low temperature

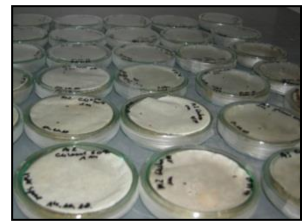
Storage of spores in cryoprotectant solutions (<u>sucrose, glycerol</u>, mannitol, trehaloze, glucose).
Storage of spores in calcium alginate envelopes based on cryoprotectant solutions.

Assessment of the condition and germination of spores after freezing



Spores

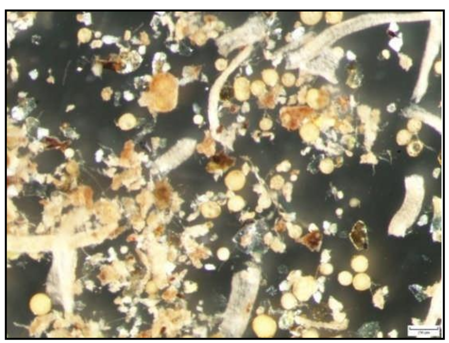
Freezing of spores



Assessment of germination

Germinating spore

After 12 months in storage at -80°C, spores stored in cryoprotectant solutions survived freezing better & retained greater ability to germinate than spores stored in envelopes of calcium alginate + cryoprotectants.



Spores of mycorrhizal fungi (AMF) during isolation

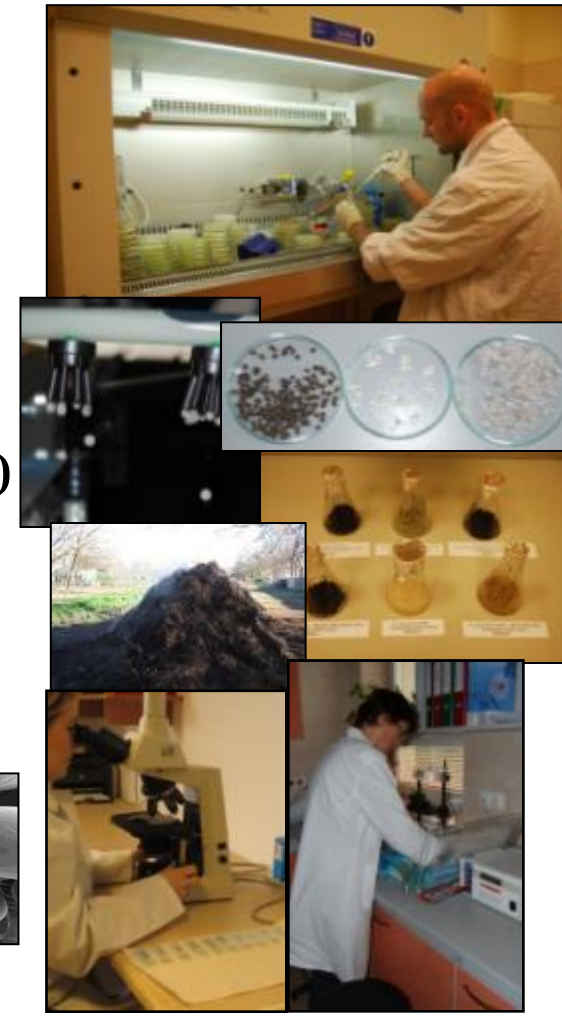
ACHIEVEMENTS OF THE PROJECT

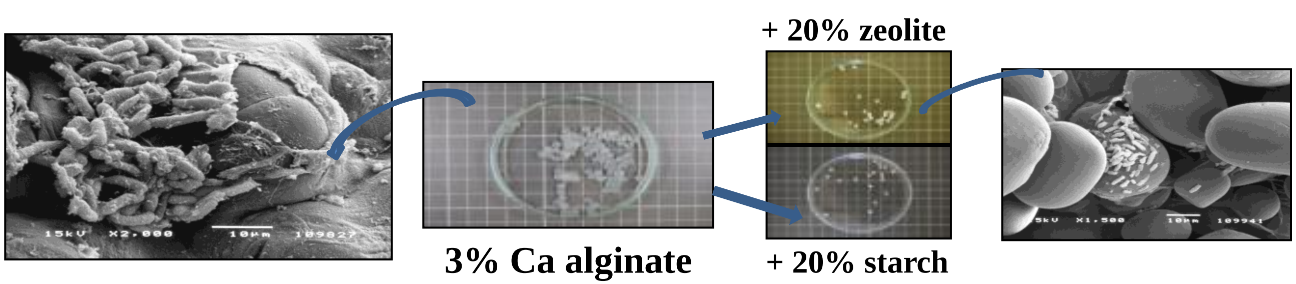
Developed compositions of bioproducts & technologies for their production:

- Microbial inocula (3 patents)
- 4 microbilogically enriched bio-fertilizers
- 12 lignite-based composts (nominated the best 4)
- 3 microbiologically enriched liquid biostimulators
- plant protection products for organic production

• Tested 10 carrier media (calcium alginate, carrageenan, perlite)

• Applied for 3 patents for machines for applying bioproducts



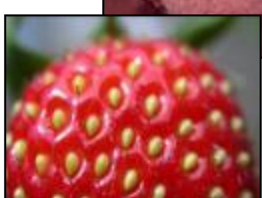


IMPROVED QUALITY OF APPLES, SOUR CHERRIES AND STRAWBERRIES FROM ORGANIC ORCHARDS & PLANTATIONS IN COMPARISON TO INTEGRATED PRODUCTION

Quality features:

- **Internal quality**
- **Sensory evaluation**
- **Safety assessment of fruit consumption**

Preparation of pulp from apples, sour cherries and strawberries for clinical studies



Initial grinding

Grinding in CO_2

Individual 500 g portions prepared for testing and storage at -25 ^{o}C

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POSITIVE EFFECT OF DIET ENRICHED WITH ORGANICALLY GROWN FRUIT ON CONSUMERS' HEALTH

- **Clinical studies in humans and animals – guinea pig**

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INNOVATIVE MACHINES DEVELOPED FOR APPLYING BIOPRODUCTS

Test stand for assessing viability of microorganisms

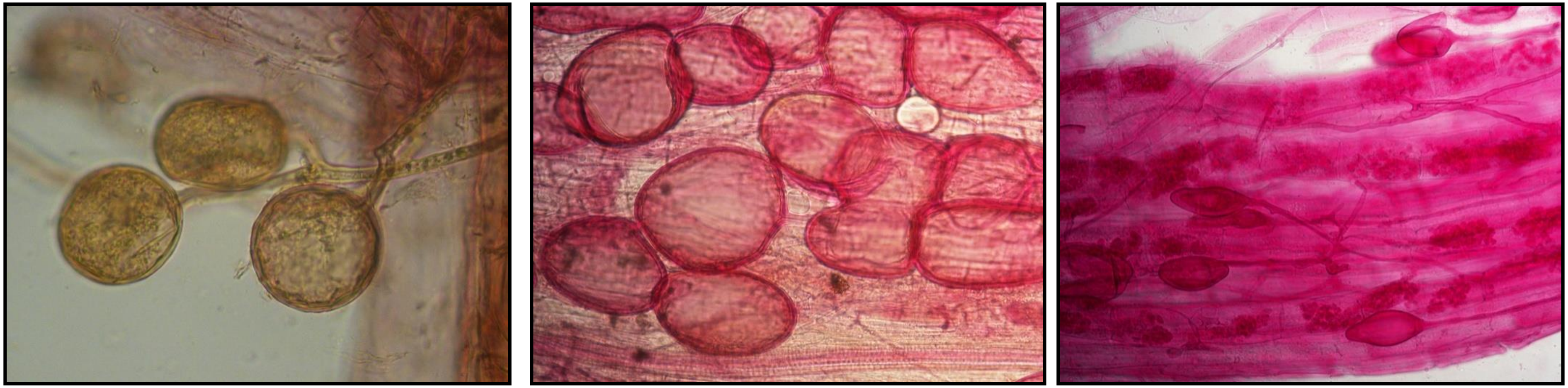
Feeder for applying mycorrhizal preparations under soil surface close to the root system

Injecting a preparation below soil surface near plant roots

Application to the soil. Granular fertilizer spreader for strawberry

Application of liquid biopreparations to soil surface

MYCORRHIZAL STRUCTURES IN THE ROOTS OF ‘ELSANTA’ STRAWBERRY PLANTS



A B C

A – Mycorrhizal mycelium and spore in the roots - Micosat (mag. 10 x 40),
B – Vesicles in the roots – Biochar + rhizosphere bacteria (mag. 10 x 40),
C – Root fragment with mycelium, vesicles and arbuscules inside it - Humus UP (mag. 10 x 10)

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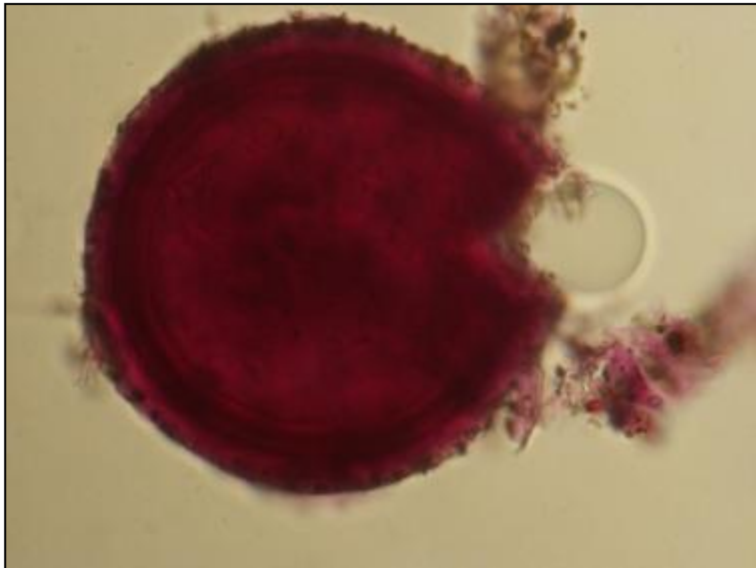
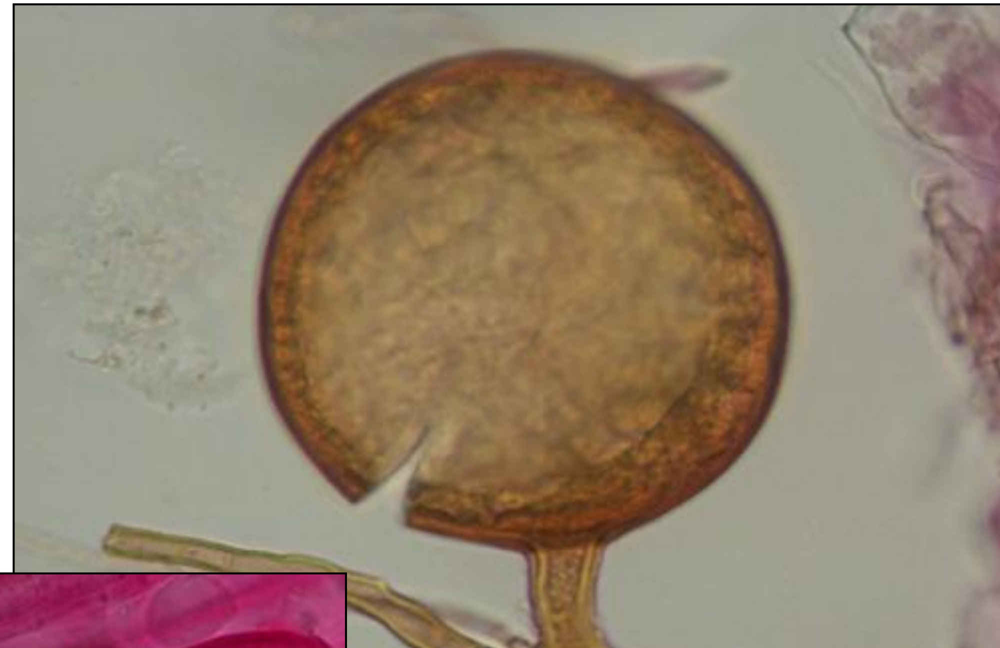


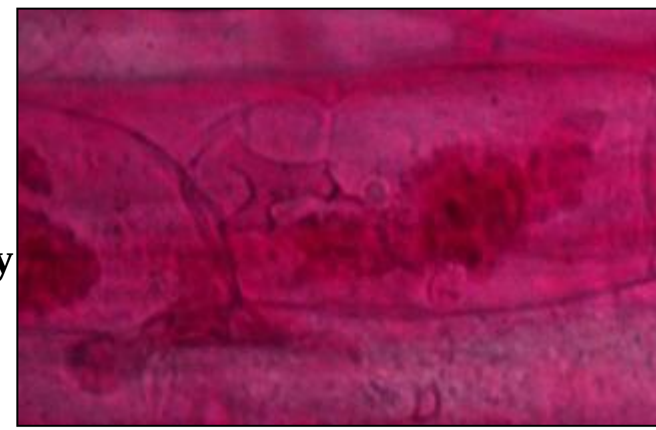
A spore in the roots of strawberry plants cv. 'Elsanta' (Dąbrowice, 2013)



Spore in the roots of strawberry 'Elsanta'(Skierniewice, 2012)

Vesicles in the roots of strawberry 'Elkat' (Skierniewice, 2014)

Arbuscule in the roots of strawberry 'Elsanta' (Skierniewice, 2014)



Arbuscule in the roots of strawberry cv. 'Elkat' (Dąbrowice, 2013)

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APPLICATION OF MICROBIAL BIOPRODUCTS INCREASED FORMATION OF SPORES IN THE RHIZOSPHERE OF STRAWBERRY PLANTS

Treatment	Number of spores	
	Strawberry 'Elsanta'	Strawberry 'Honeoye'
Control	309	410
NPK	358	891
Manure	473	952
Micosat	481	926
Humus VP	367	900
Humus Active + Aktywit PM	476	<u>2581</u>
BF Quality	<u>605</u>	1355
BF Amin	534	1036
Tytanit	334	589
Vinassa	270	554
Total	4207	<u>10194</u>

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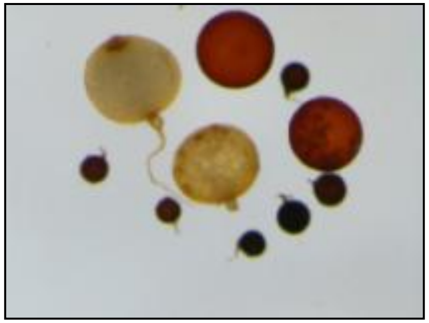
AMF SPECIES OF THE GENERA *GLOMUS, ACAULOSPORA, FUNNELIFORMIS, SCUTELLOSPORA, GIGASPORA, RIZOPHAGUS* FOUND IN THE RHIZOSPHERE OF FRUIT PLANTS (BIESZCZADY)

1. ***Septoglomus constrictum*** **(Trappe) Sieverd., G.A. Silva & Oehl**
2. ***Funneliformis mosseae*** **(T.H. Nicolson & Gerd.) C. Walker & A. Schüßler**
3. ***Funneliformis geosporus*** **(T.H. Nicolson & Gerd.) C. Walker & A. Schüßler**
4. ***Acaulospora capsicula*** **Błaszk.**
5. ***Acaulospora paulinae*** **Błaszk.**
6. ***Claroideoglomus claroideum*** **(N. C. Schenck & G. S. Sm.) C. Walker & A. Schüßler**
7. ***Glomus macrocarpum*** **Tul. & C. Tul.**
8. ***Funneliformis caledonius*** **(T.H. Nicolson & Gerd.) C. Walker & A. Schüßler**
9. ***Entrophospora infrequens*** **(I.R. Hall) R.N. Ames & R.W. Schneid.**
10. ***Acaulospora scrobiculata*** **Trappe**
11. ***Rhizophagus fasciculatus*** **(Thaxt.) C. Walker & A. Schüßler**
12. ***Gigaspora margarita*** **W.N. Becker & I.R. Hall**
13. ***Acaulospora cavernata*** **Błaszk.**
14. ***Glomus pansihalos*** **S.M. Berch & Koske**
15. ***Scutellospora dipurpurescens*** **J.B. Morton & Koske**
16. ***Acaulospora*** **1 sp.**
17. ***Glomus*** **1 sp.**
18. ***Gigaspora*** **1 sp.**

Glomus rubiforme



Rhizophagus fasciculatus

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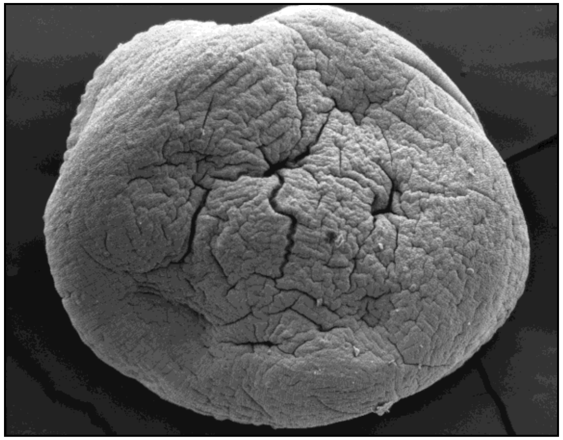
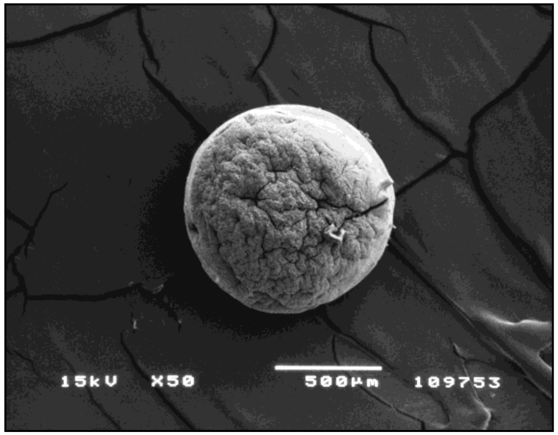
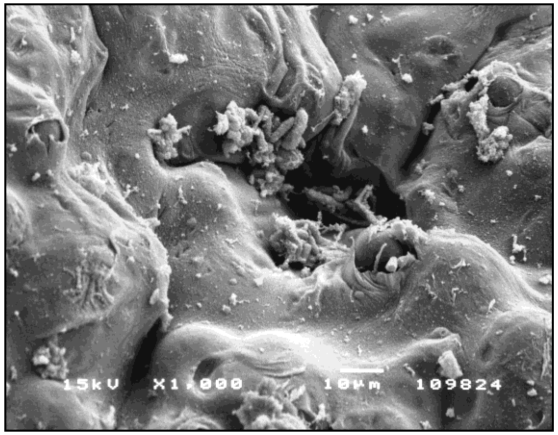
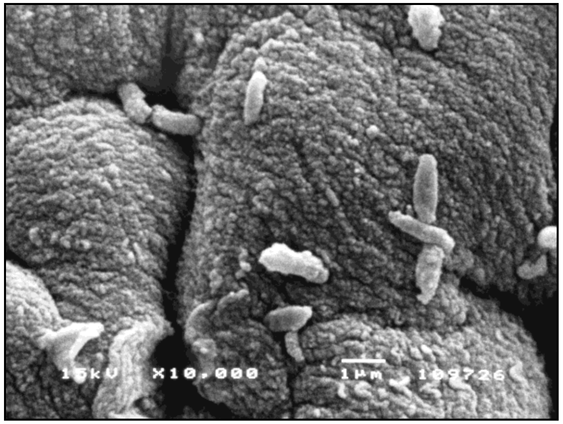
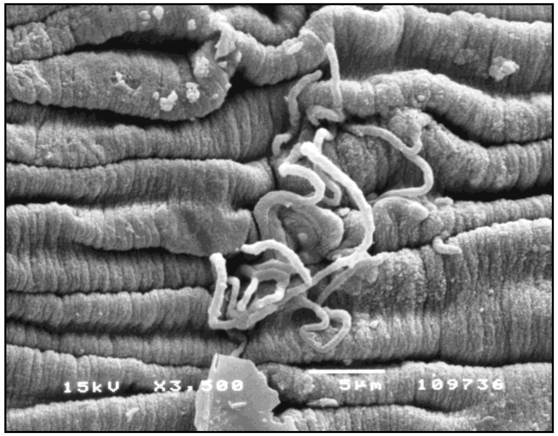
SCANNING ELECTRON MICROSCOPY IMAGES OF DIFFERENT BENEFICIAL MICROORGANISMS (*Pseudomonas*, *Actinomycetes*) EMBEDDED IN ALGINATE BEADS

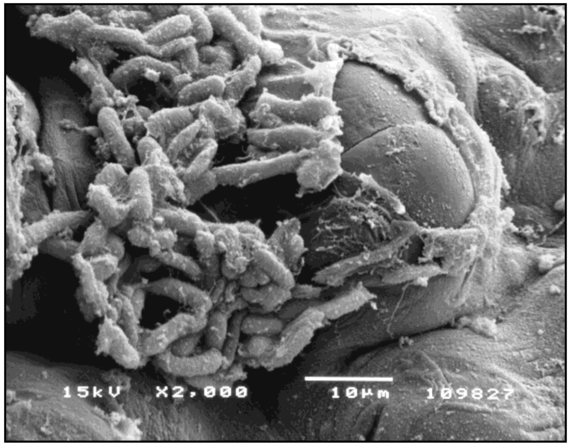












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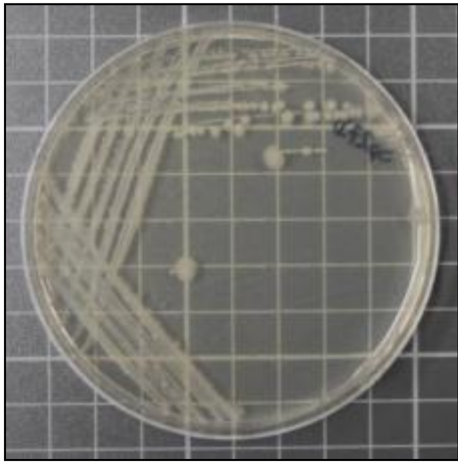
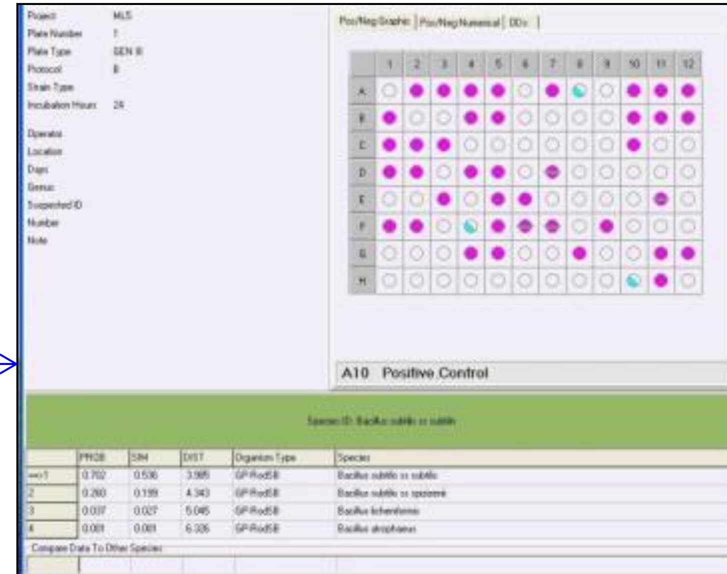
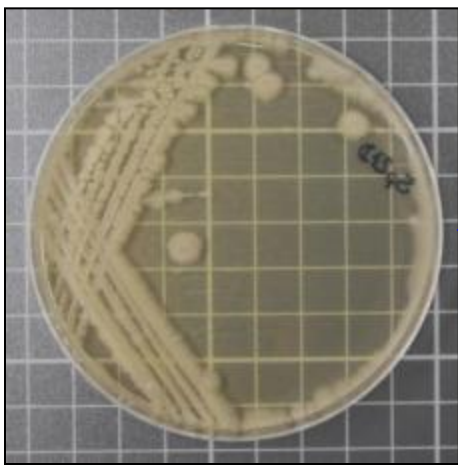
POMO PGPR IMAGE GALLERY



Growth of *Bacillus subtilis* Sp27D on nutrient agar medium

Biochemical properties of *Bacillus subtilis* Sp27D (BIOLOG)





Growth of *Bacillus subtilis* Sp27D on TSA medium

Cells of *Bacillus subtilis* under microscope

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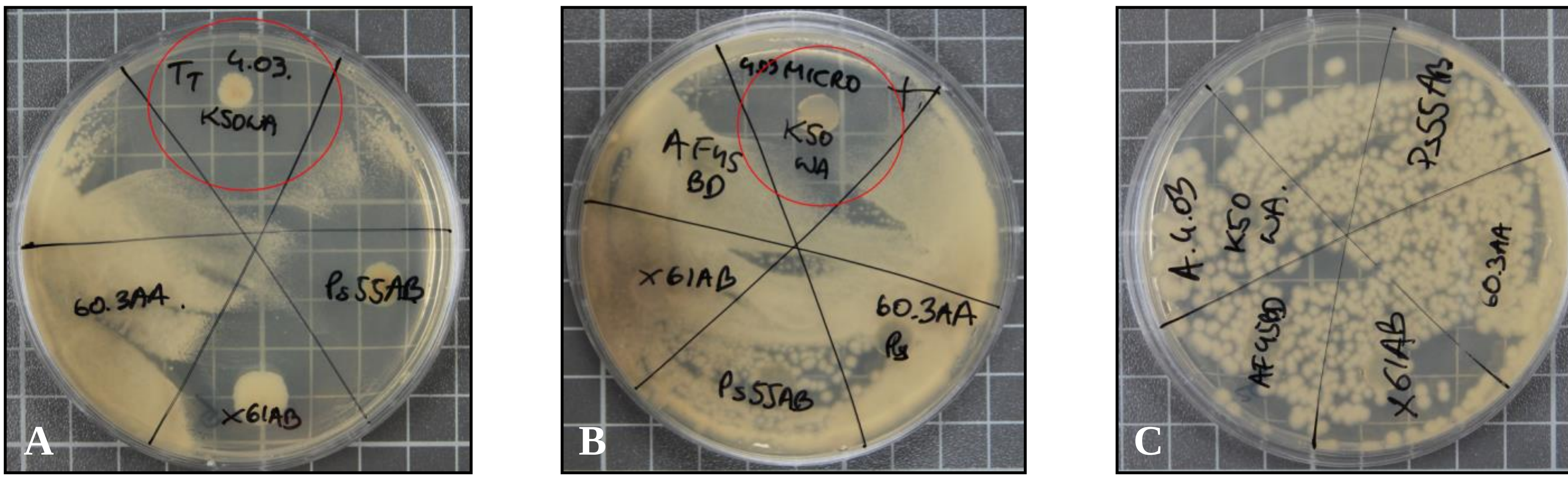
EVALUATION OF ANTAGONISTIC PROPERTIES OF BACTERIAL STRAINS COLLECTED IN SYMBIO BANK

Production of metabolites toxic to *Verticillium dahliae* by rhizosphere bacteria at different oxygen levels



A – Test conducted in normal conditions – the highest production
B – Test conducted in an atmosphere with reduced oxygen content – medium production
C – Test conducted under anaerobic conditions –no production

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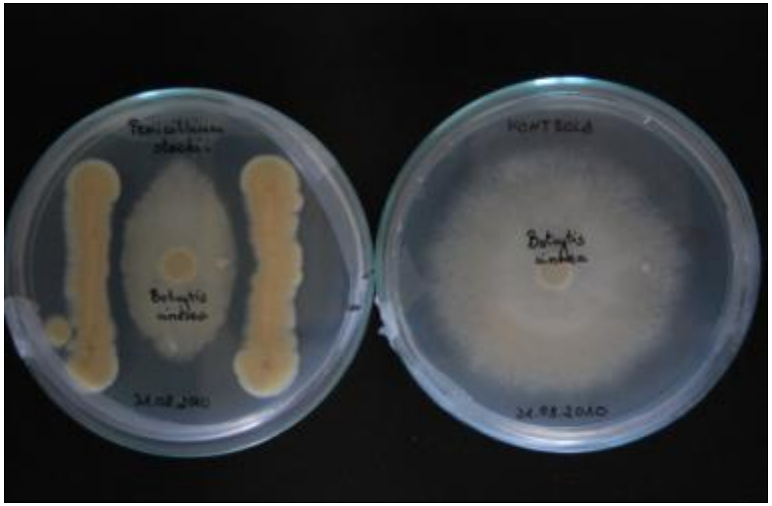
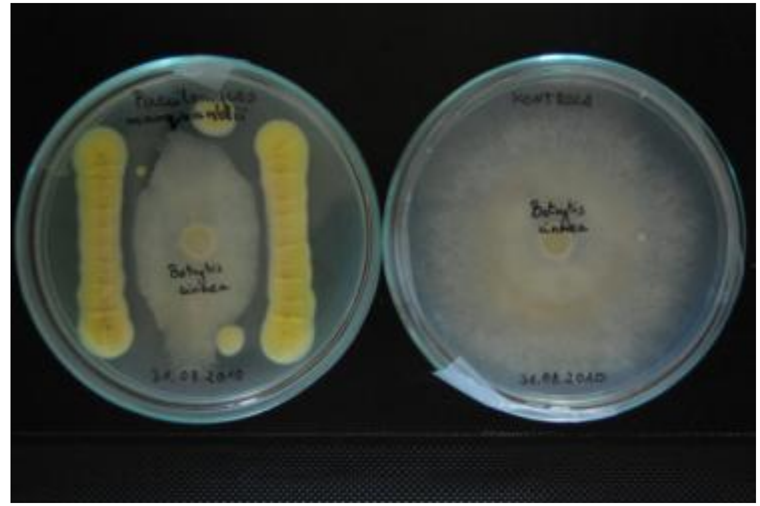
BENEFICIAL MICROORGANISMS – PATHOGENIC FUNGI

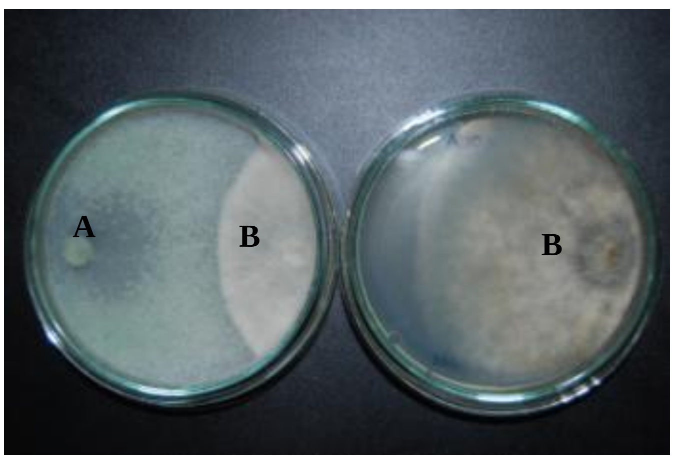


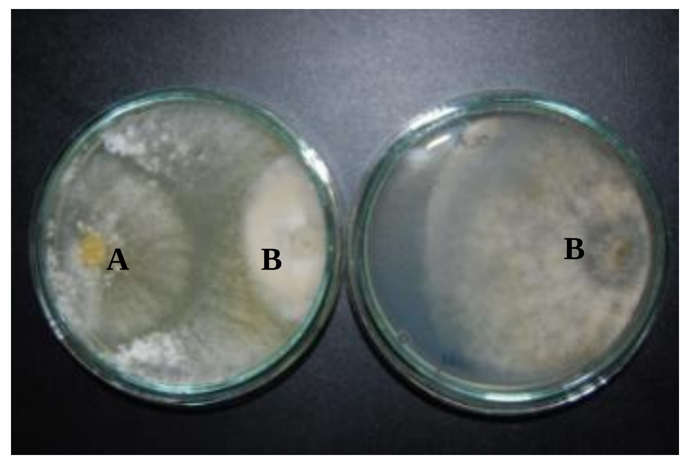
Inhibition of growth of *Botrytis cinerea* by the fungi *Penicillium steckii* and *Paecilomyces marquandii*



Control (*Botrytis cinerea*).

ANTAGONISTIC ACTIVITY OF FUNGI





Antagonism of fungi of the genus *Trichoderma sp.* towards *Botrytis cinerea*,
A – fungus of the genus *Trichoderma sp.*, B - *Botrytis cinerea* causing grey mould

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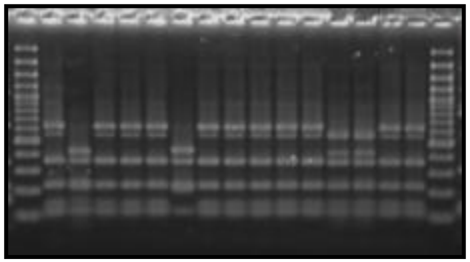
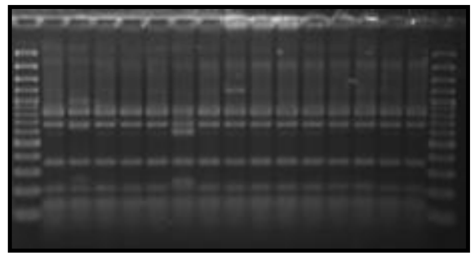
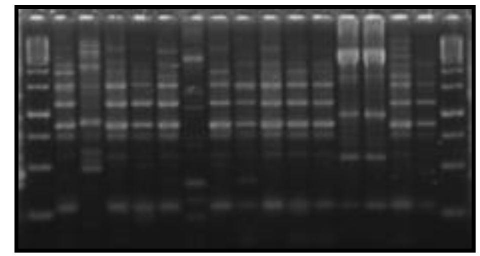
IDENTIFICATION AND ASSESSMENT OF GENETIC SIMILARITY OF RHIZOSPHERE BACTERIAL ISOLATES OF *PSEUDOMONAS* SPP. USING MOLECULAR TECHNIQUES



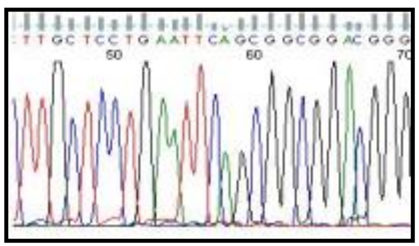
RFLP analysis of 16S rRNA gene



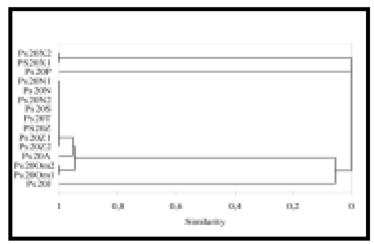
RFLP analysis of 16S-ISR-23S region



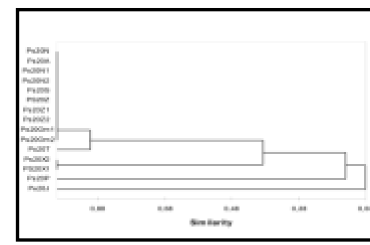
Analysis of repetitive sequences in the genome (rep-PCR)



Sequence analysis of the 16S rRNA gene



Genetic similarity of soil bacterial isolates, obtained on the basis of rep-PCR analysis.



Genetic similarity of soil bacterial isolates, obtained on the basis of RFLP analysis

Bacterial strains were identified in the rhizosphere of sour cherry as belonging to *Pseudomonas fluorescens*, *Pseudomonas putida* or *Pseudomonas* spp.

The greatest differentiation of isolates within the clusters was obtained after using the rep-PCR technique with REP and ERIC primers.

The identified, the most valuable bacterial strains of *Pseudomonas* with beneficial effects on plants will be used in commercial fruit growing.

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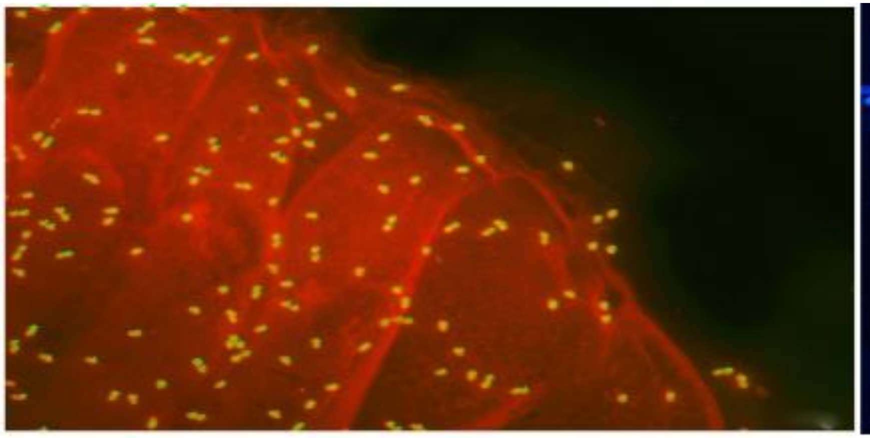
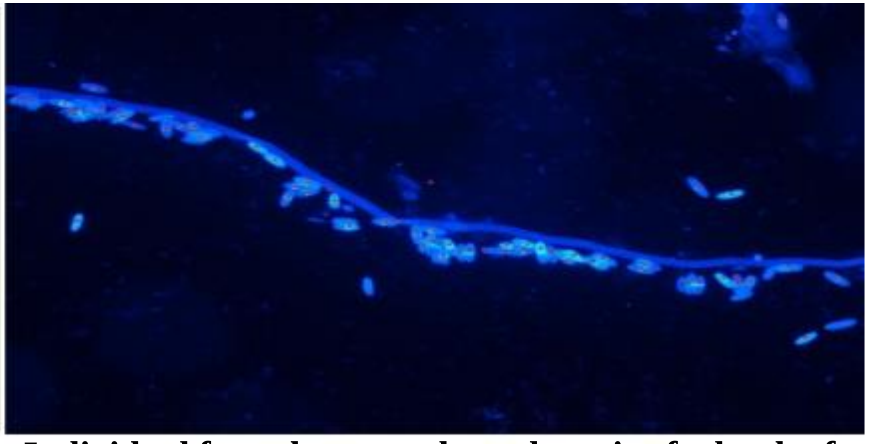
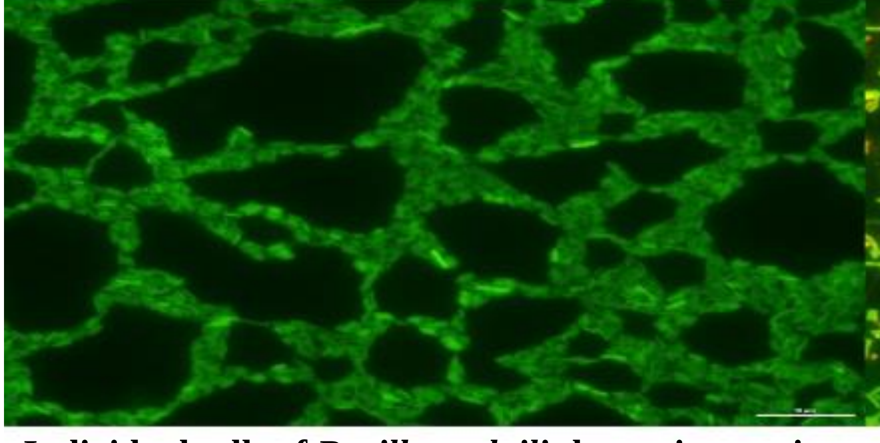
MICROBIAL COMPONENTS OF BIOPRODUCTS



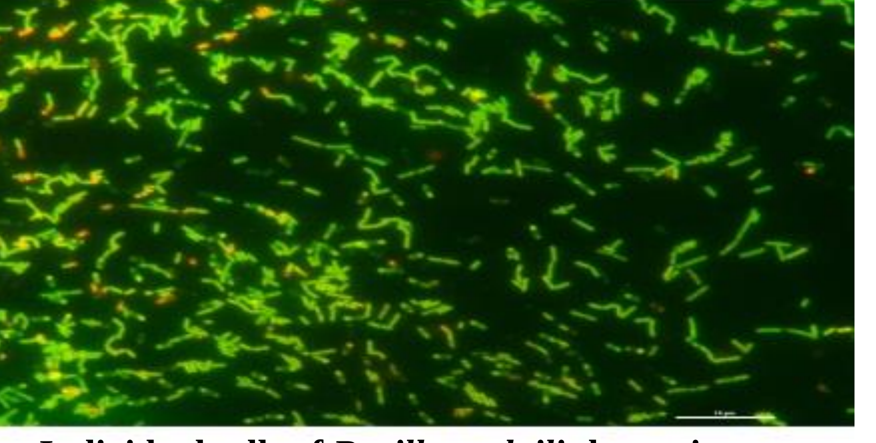
Individual cells of *Azotobacter* sp. bacteria - from rhizosphere of carrot (stained with acridine orange)



Individual fungal spores along the axis of a hypha from rhizosphere of parsley (stained with calcofluor white).



Individual cells of *Bacillus subtilis* bacteria growing on potato-glucose medium (stained with acridine orange).



Individual cells of *Bacillus subtilis* bacteria growing on potato-glucose medium (stained with acridine orange).

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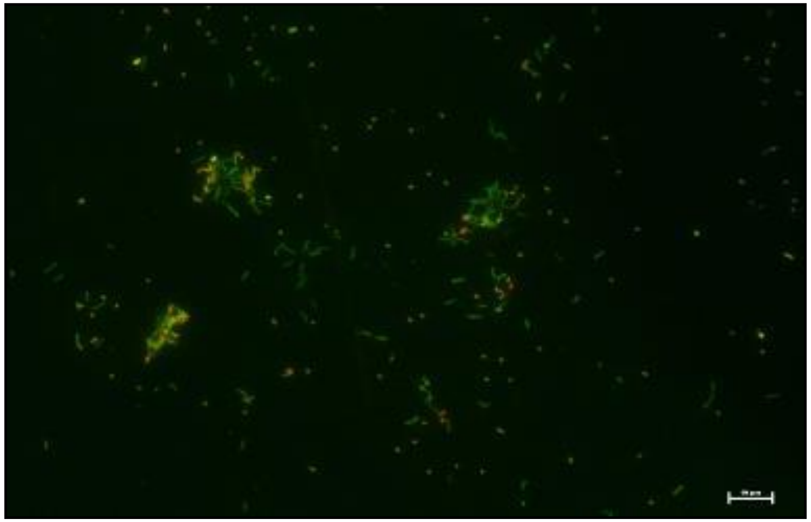
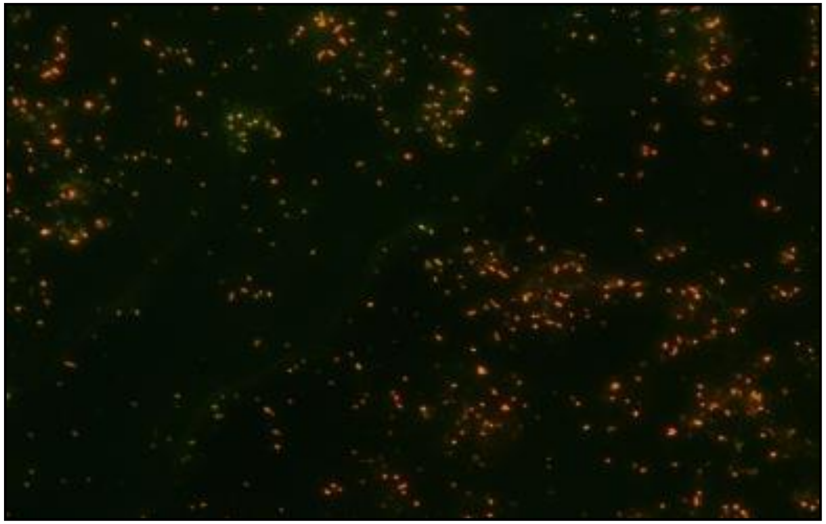
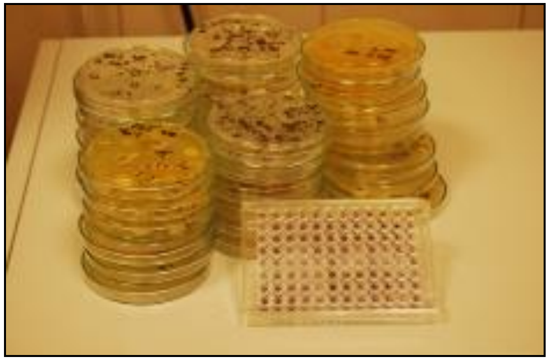
MICROBIAL COMPONENTS OF BIOPRODUCTS



Rhizosphere bacteria in the root zone of carrot (stained with acridine orange)



Rhizosphere bacteria from the root zone of parsley (stained with acridine orange)



Identification and selection of strains of beneficial microorganisms (Rhizosphere Laboratory, 2015)

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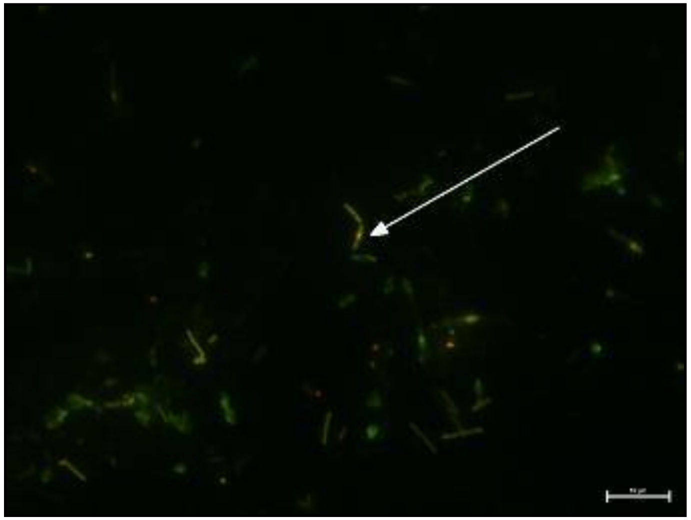
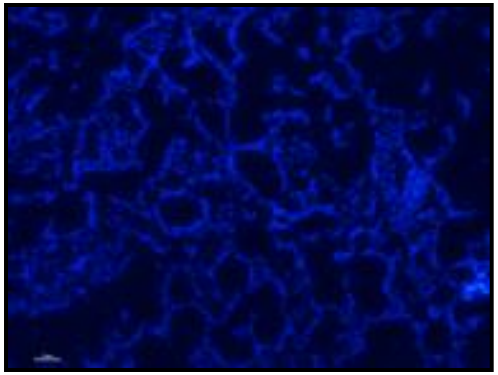


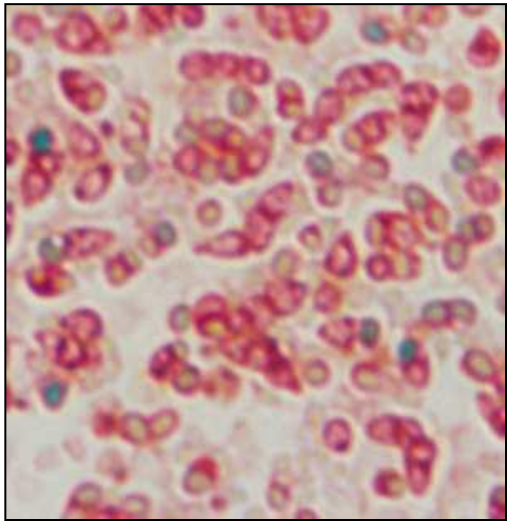
A *Bacillus* bacterial cell with a spore visible



Pantoea sp.

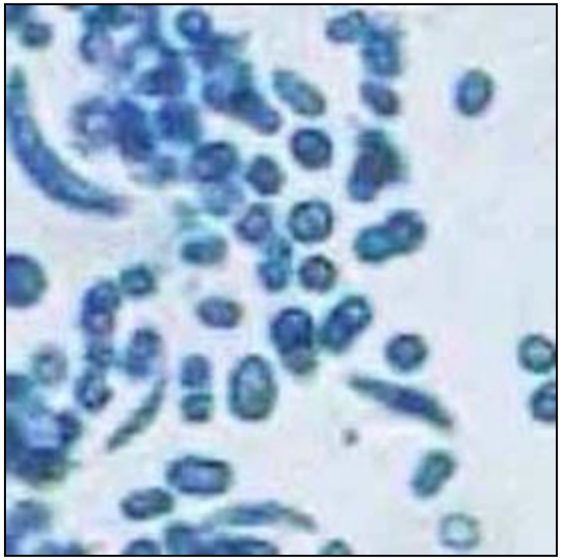
IMAGES OF THE STUDIED GROUPS OF MICROORGANISMS


Bacillus megaterium


Megaterium endospors


Rhizobium meliloti


Serratia


Desulfovibrio

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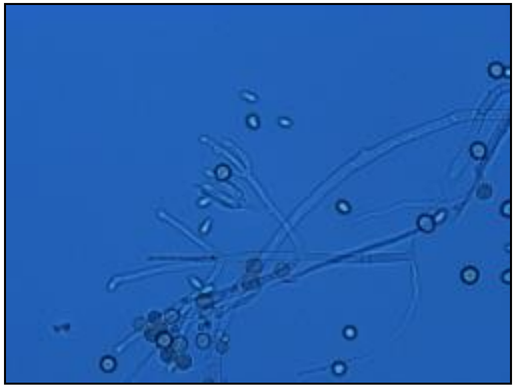
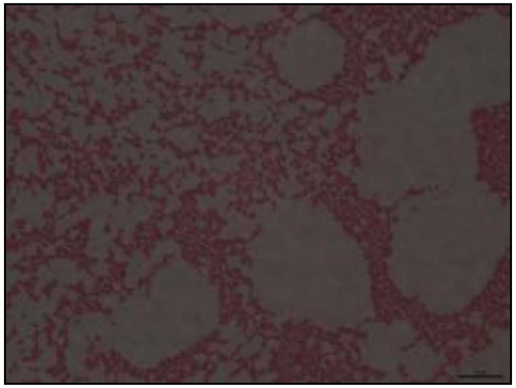
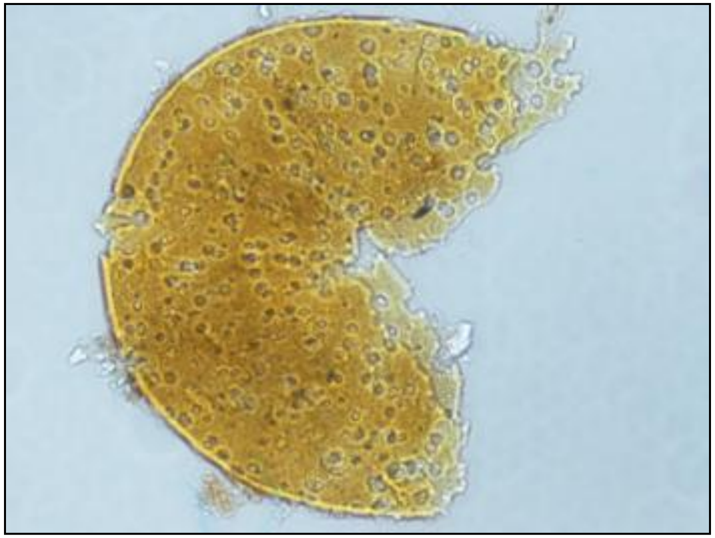
CONSORTIUM STIMULATING PLANT GROWTH AND YIELD



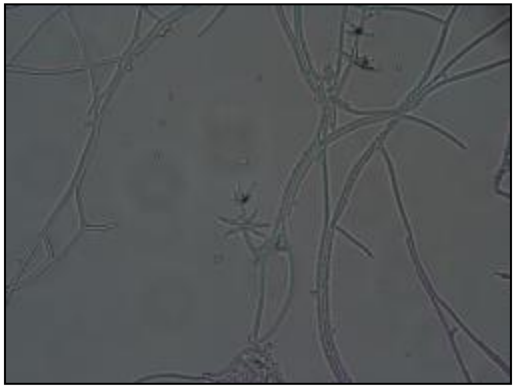
***Paecilomyces lilacinus* – limiting populations of nematodes.**



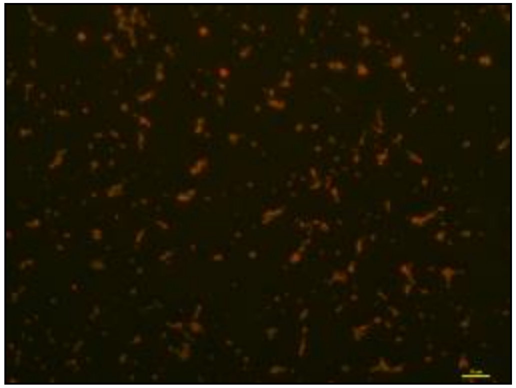
***Pseudomonas fluorescens* – stimulating the vegetative growth of plants (stained by Gram's method).**



Funneliformis mosseae



***Trichoderma* sp. – limiting populations of pathogenic fungi.**



***Rahnella aquatillis* – stimulating the vegetative growth of plants (stained with acridine orange).**

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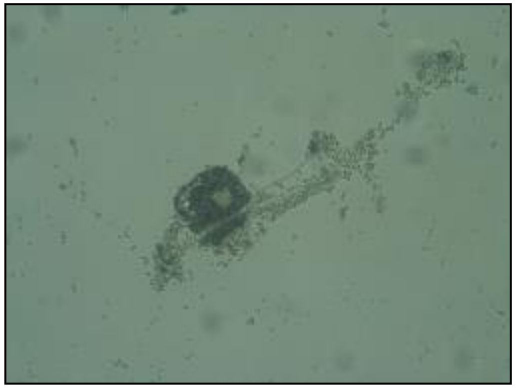
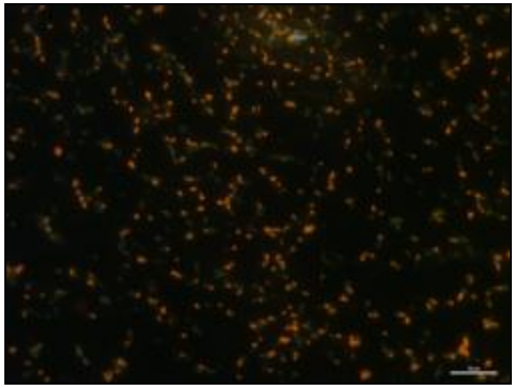
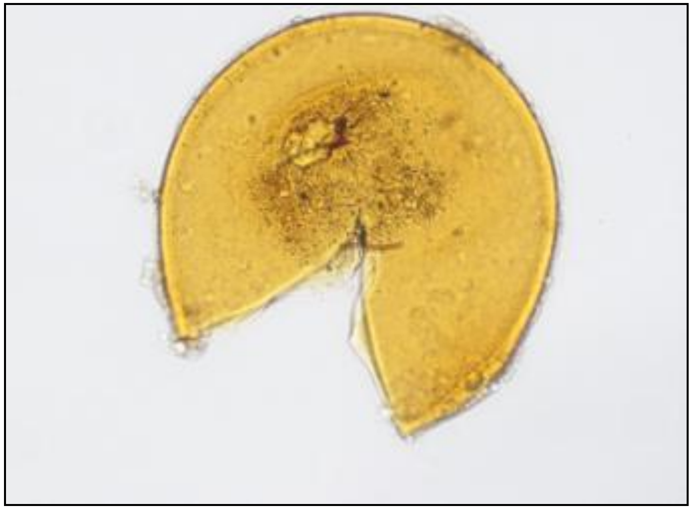
CONSORTIUM MOBILIZING PHOSPHORUS COMPOUNDS IN SOIL



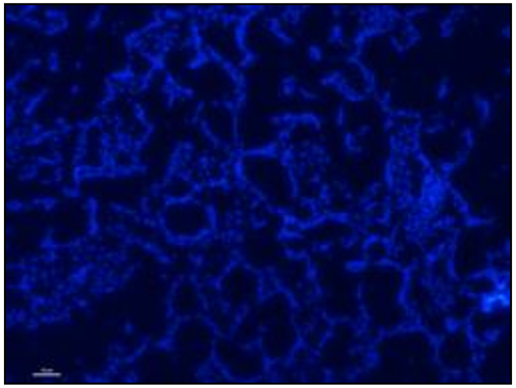
Penicillium **sp. – dissolving phosphorus compounds**



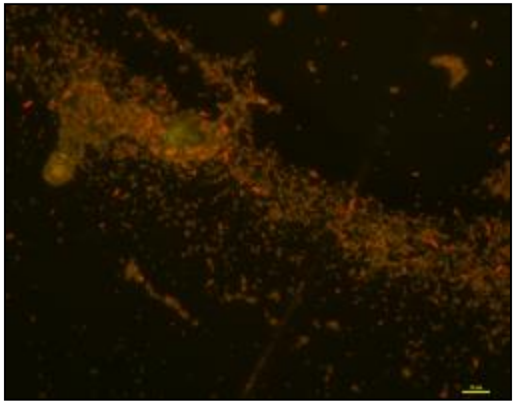
Pseudomonas fluorescens **– dissolving phosphorus compounds (stained with acridine orange)**



Claroideoglomus claroideum



Pantoea **sp. – dissolving phosphorus compounds (stained with DAPI)**



Rahnella aquatillis **– dissolving phosphorus compounds (stained with acridine orange)**

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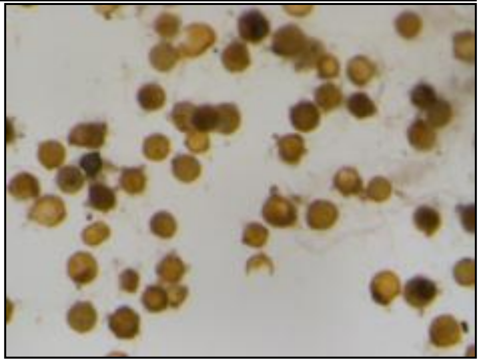
TRAP CULTURES



<u>Setting up trap cultures</u>:

- **Collection of rhizosphere soil and preparation of trap cultures**
- **The layer thickness of spore walls was measured in freshly isolated spores & observed under a light microscope (Błaszkowski 2003).**
- **The observed AMF species were named according to Schüßler & Walker (2010) and Błaszkowski (2003).**

Mass multiplication of spores of mycorrhizal fungi in container cultures

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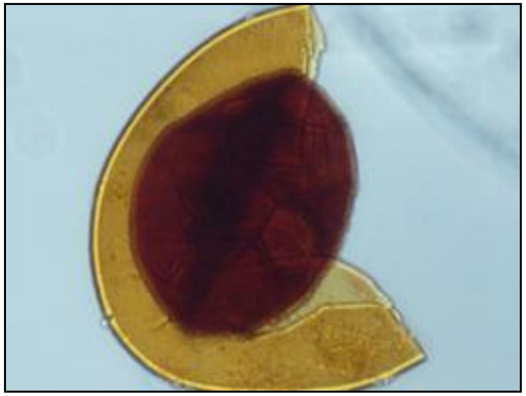
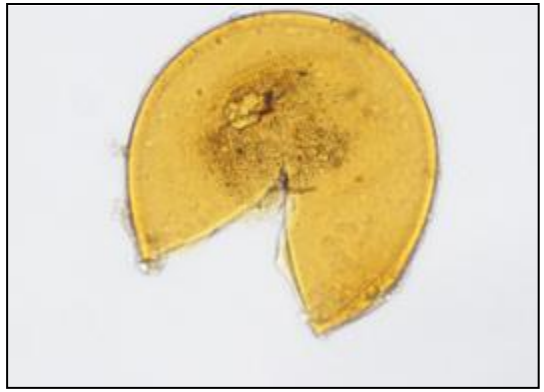
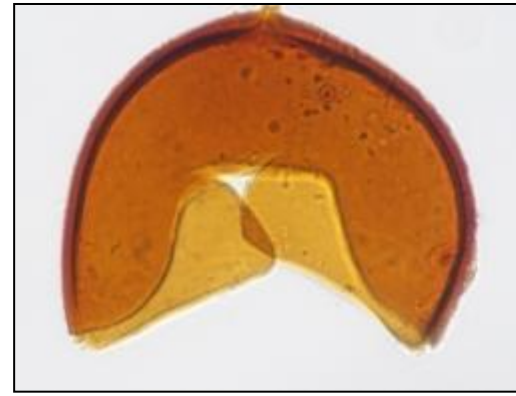
POMO MYCORRHIZA IMAGE GALLERY (BIESZCZADY)



Scutellospora dipurpurescens



Claroideoglomus claroideum



Septoglomus constrictum

Funneliformis mosseae

Glomus macrocarpum

Entrophospora infrequens

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POSITIVE EFFECT OF RHIZOBACTERIA ON POST-VITRO ADAPTATION & GROWTH OF 'ELKAT' STRAWBERRY PLANTS

A – non-inoculated plants (control)

B – plants inoculated with *Pseudomonas fluorescens* (strain Ps1/2)

A – non-inoculated plants (control)

B – plants inoculated with a consortium of bacteria – prof. Kloeppert (Consortium 2)

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INNOWACYJNA GOSPODARKA
NARODOWA STRATEGIA SPÓJNOŚCI

UNIA EUROPEJSKA
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ROZWOJU REGIONALNEGO

VISUALIZATION OF pH CHANGES IN RHIZOSPHERE AND ACQUISITION OF ROOT EXUDATES

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EXPERIMENTAL COMBINATIONS

- **Control**
- **NPK**
- **Manure**
- **Micosat + ½ manure**
- **Humus UP**
- **Humus Active + Aktywit PM**
- **BF Quality + ½ manure**
- **BF Amin + ½ manure**
- **Manure + Tytanit**
- **Manure + Vinassa**

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BIO-PHYSICO-CHEMICAL ANALYSES

Samples of:

- rhizosphere soil
- leaves
- roots
- root exudates
- bioproducts

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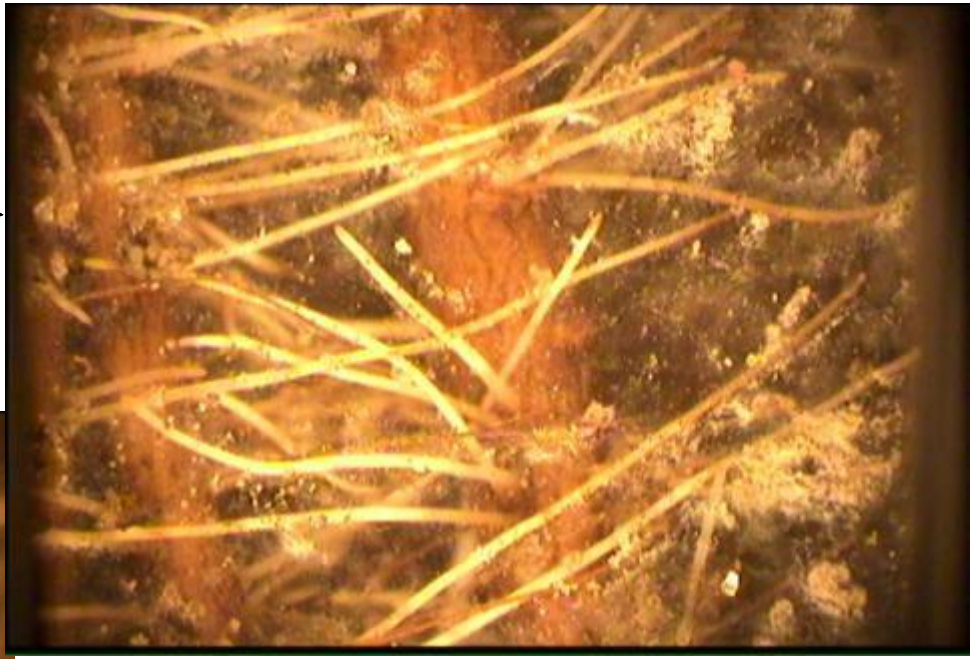
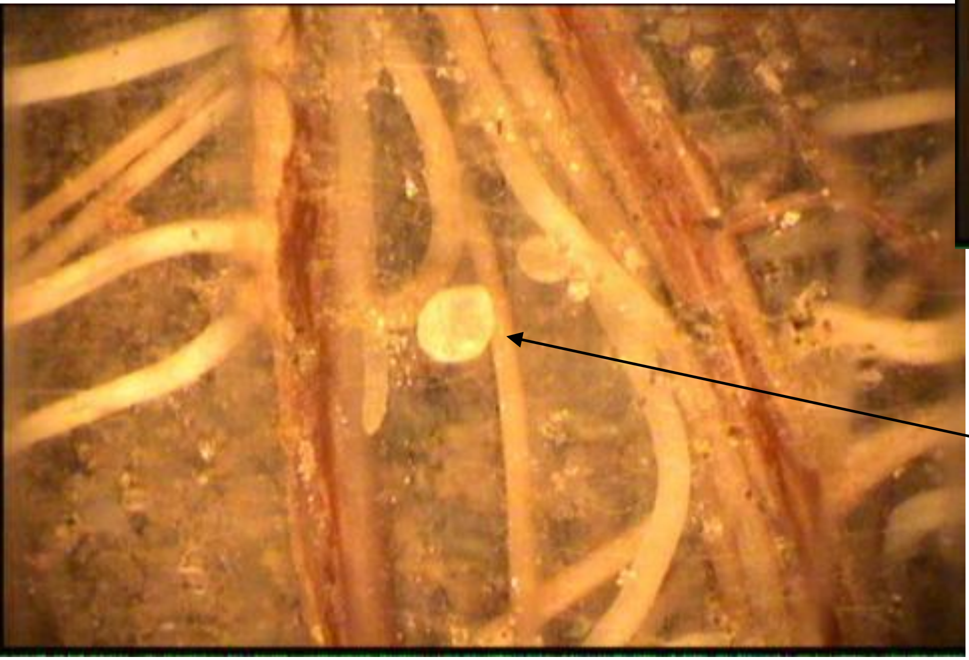
POSITIVE EFFECT OF ORGANIC MULCHES ON GROWTH AND SURVIVAL RATE OF APPLE ROOTS

FINE ROOTS
A THICKER SKELETAL ROOT
IN THE BACKGROUND





SPORE
OF AN ARBUSCULAR
MYCORRHIZAL FUNGUS

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POSITIVE EFFECT OF INNOVATIVE ORGANIC FERTILIZERS ON THE GROWTH OF APPLE AND SOUR CHERRY TREES IN NURSERY

Vinassa, BioFeed Amin, BioFeed Quality, Humus UP, Humus Active

- significantly reduced bud graft mortality in winter, stimulated root development & branching of maiden trees, improving their quality.

Micosat, BioFeed Quality, BioFeed Amin and Vinassa

- used in the nursery had a positive after-effect on the growth and fruiting of apple and sour cherry trees in orchards.

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Carrot plants cv. 'Nipomo' and cucumber plants cv. 'Adam' were treated with the bioproducts:

- Bacterial-mycorrhizal inoculum

(*Klebsiella oxytoca*, *Pantoea agglomerans*, *Pseudomonas fluorescens*, *Pantoea/Erwinia* sp, *Claroideoglomus claroideum*, *Gigaspora margarita*, *Funneliformis mosseae*, *Scutellospora dipurpurescens*, *Rhizophagus fasciculatus*).

- Microbiologically enriched Bioilsa

(organic nitrogen, organic carbon, *Klebsiella oxytoca*, *Pantoea agglomerans*, *Pseudomonas fluorescens*).

- Microbiologically enriched compost

(brown coal dust, *Klebsiella oxytoca*, *Pantoea agglomerans*, *Pseudomonas fluorescens*, Vinassa and whey).

- Microbiologically enriched humic acids

(humic acids from brown coal in liquid form, *Klebsiella oxytoca*, *Pantoea agglomerans*, *Pseudomonas fluorescens*).

- Microbiologically enriched biochar

(organic and loamy parts from brown coal, molasses, *Klebsiella oxytoca*, *Pantoea agglomerans*, *Pseudomonas fluorescens*).

Carrot and cucumber plants treated with biopreparations stimulating growth and yielding of plants (IO Experimental Field, 2016).

The controls were:

- plants not treated with bioproducts (control)

- manure

The experiments were conducted in the Certified Ecological Field of the Research Institute of Horticulture in Skierniewice.

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IMPROVING THE GROWTH AND YIELDING OF ORGANICALLY GROWN VEGETABLE PLANTS USING BIOPRODUTS ENRICHED WITH BENEFICIAL MICROORGANISMS

- **Development of bacterial-mycorrhizal inoculum and microbiologically enriched bioproducts for field experiments.**
- **Determination of mycorrhizal frequency of arbuscular mycorrhizal fungi (AMF) in the roots of vegetable plants from field experiments.**
- **Influence of microbiologically enriched bioproducts on photosynthesis intensity and maximum efficiency of photosystem II of 'Adam' cucumber plants.**
- **Assessment of the vegetative growth and yield of plants.**

Biological protection of carrot and cucumber plantings against diseases and pests (IO Experimental Field, 2016)

Carrot and cucumber plants treated with biopreparations (IO Experimental Field, 2016)

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CONCLUSIONS

- **Beneficial microorganisms from SYMBIO BANK stimulate vegetative growth and yielding of strawberry, apple, sour cherry, cucumber, tomato and other species of horticultural plants.**
- **The bacterial strains have a protective effect against *Botrytis cinerea*, *Fusarium oxysporum* and *Verticillum dahliae*.**
- **Application of beneficial microorganisms increases the size of population of beneficial microorganisms in the rhizosphere of horticultural crops.**
- **The most effective strains and species of microorganisms are components of newly developed biostimulants, composts and bacterial-mycorrhizal inocula.**
- **Synthetic NPK fertilizers were found to have a negative effect on biodiversity and activity of beneficial soil microorganisms.**
- **Consortia of beneficial microorganisms, biofertilizers and microbiologically enriched composts are an effective and economically viable alternative to standard NPK fertilization.**
- **Innovative microbiologically enriched bioproducts are being implemented into agricultural practice for improving crop growth and yield and soil fertility.**


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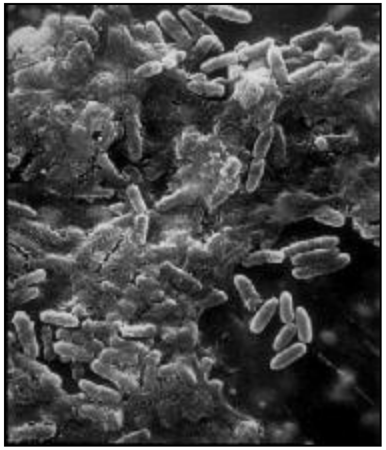

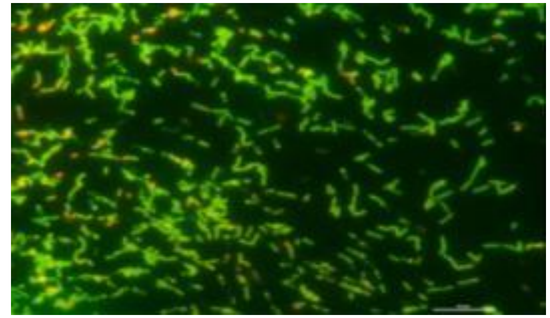

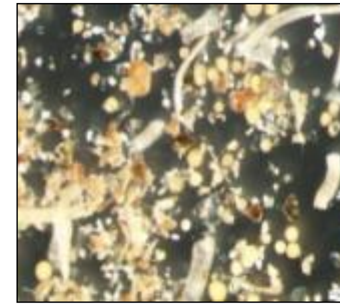

THANK YOU