BENEFICIAL MICROORGANISMS IMPROVING YIELD QUALITY OF HORTICULTURAL CROPS AND SOIL FERTILITY

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2nd International Conference on Applied Microbiology and Beneficial Microbes
Osaka, Japan, 23-25 October 2017

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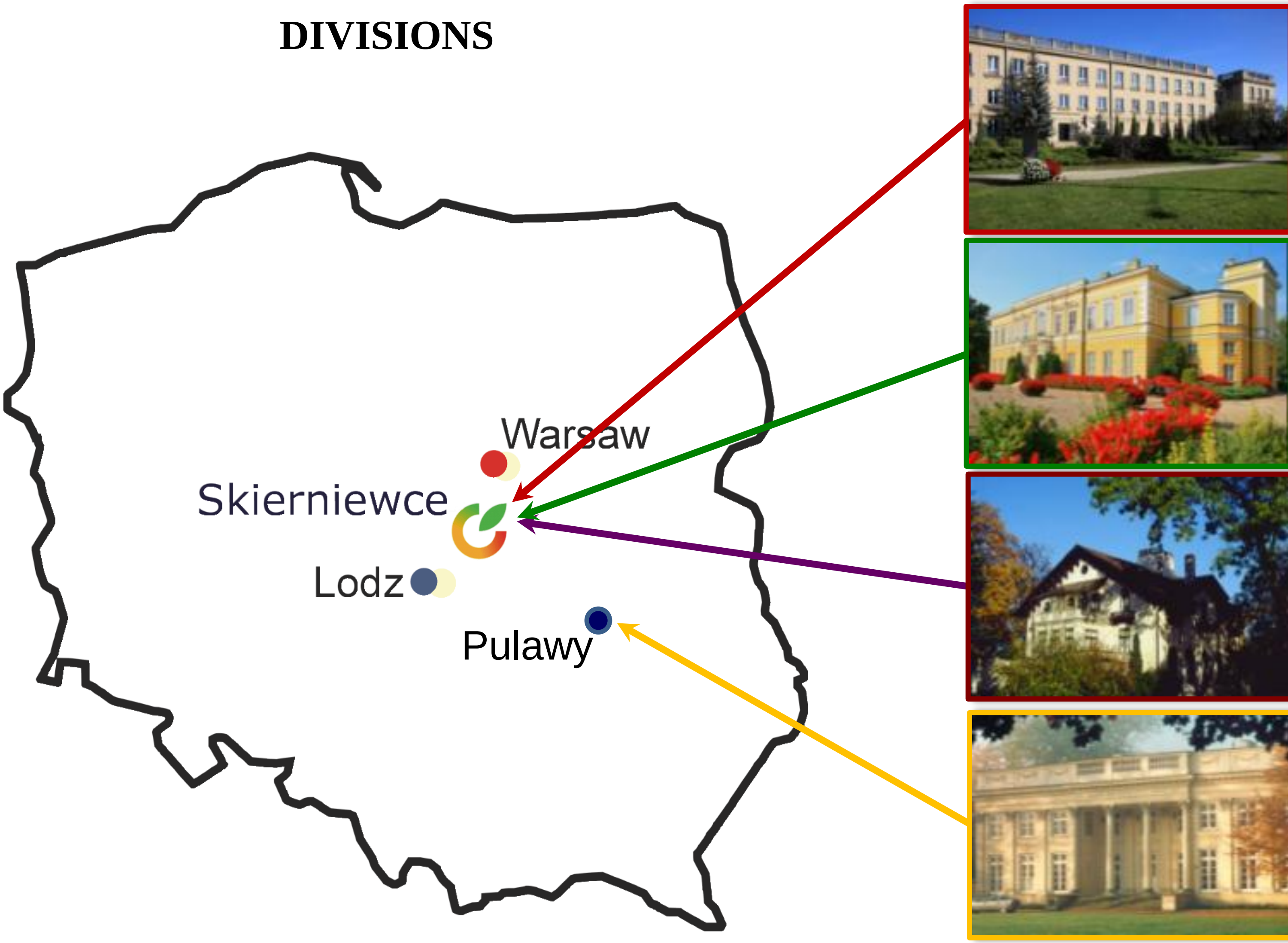
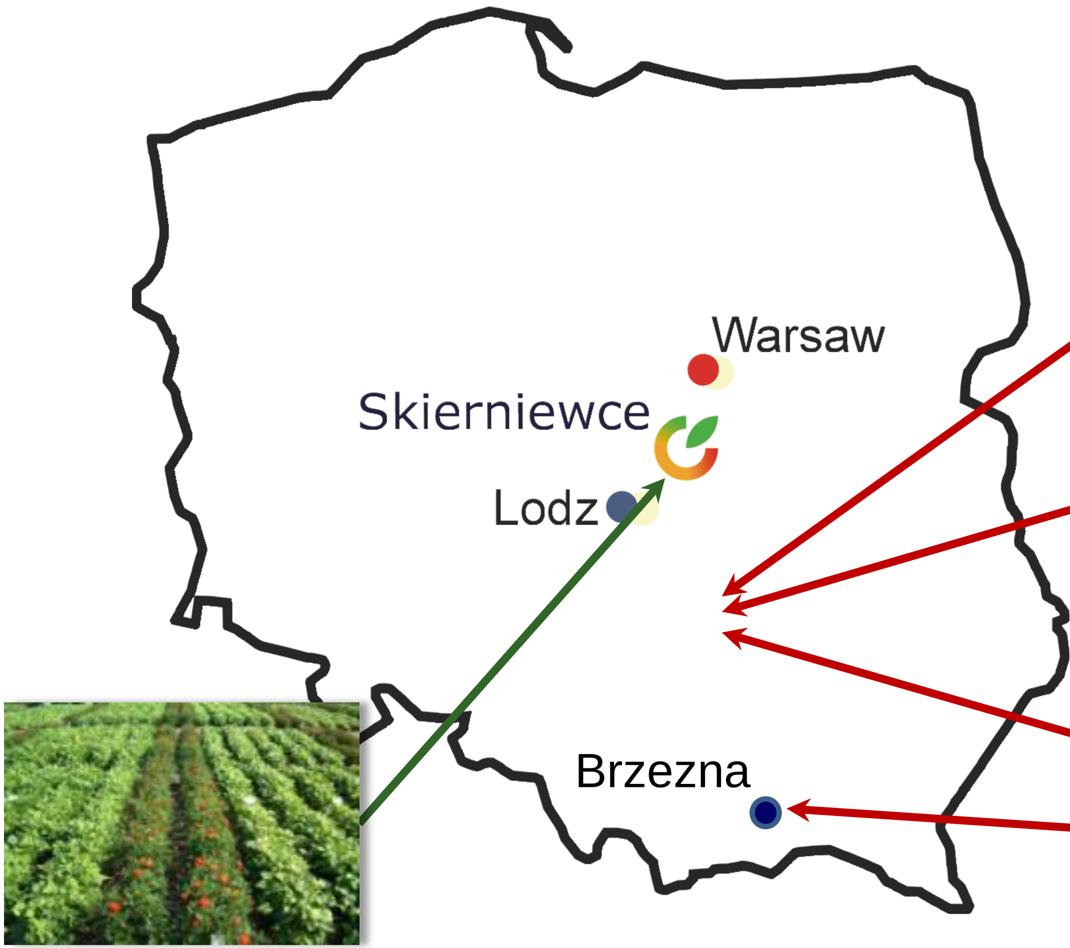
DIVISIONS



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.

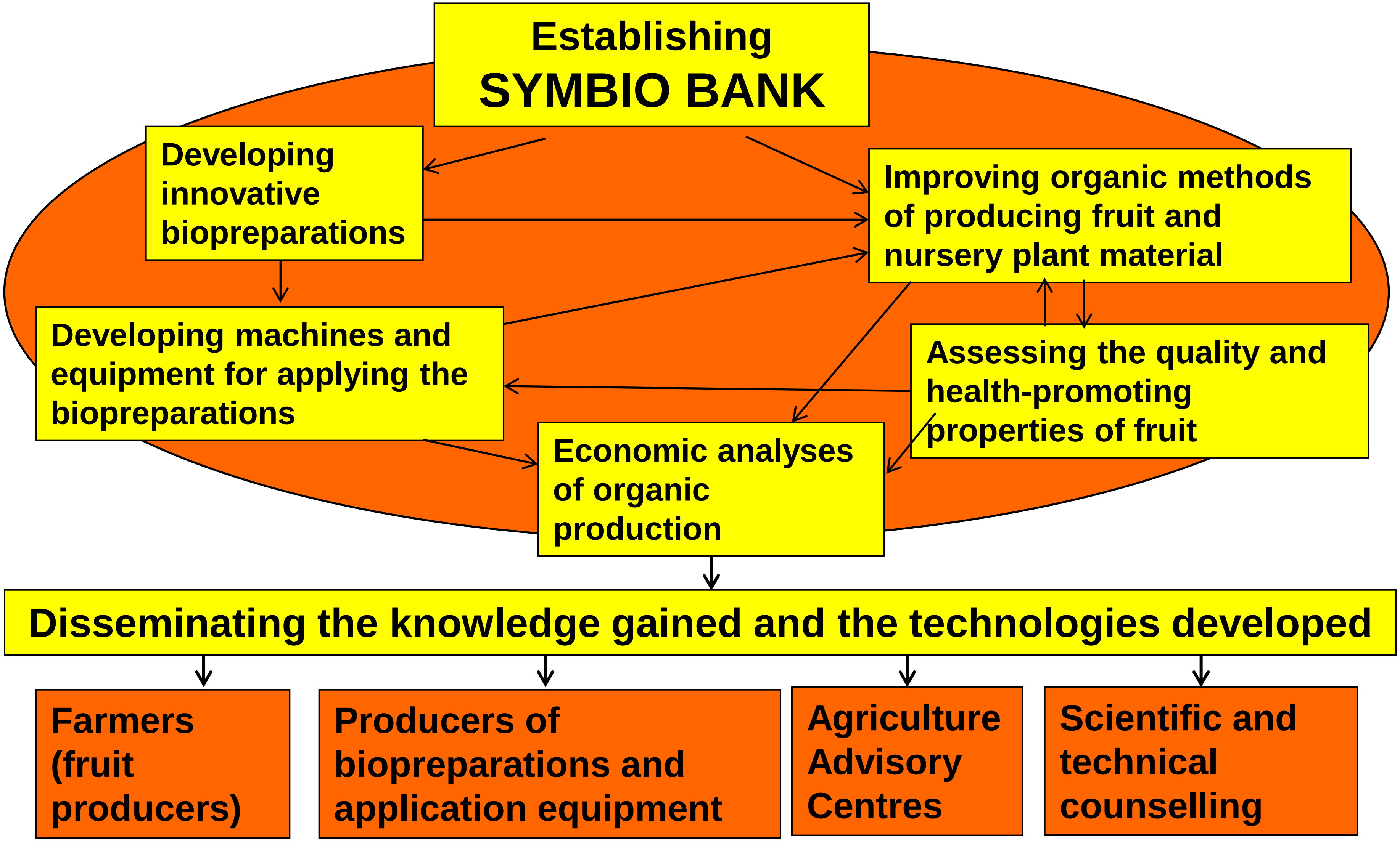
Ecological Vegetable Field Skierniewice

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.**

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,
contract No. UDA-POIG.01.03.01-10-109/08-00


Establishing
SYMBIO BANK
Developing innovative biopreparations
Improving organic methods of producing fruit and nursery plant material
Developing machines and equipment for applying the biopreparations
Assessing the quality and health-promoting properties of fruit
Economic analyses of organic production
Disseminating the knowledge gained and the technologies developed
Farmers (fruit producers)
Producers of biopreparations and application equipment
Agriculture Advisory Centres
Scientific and technical counselling

OBJECTIVES OF SYMBIO BANK

- **Isolation and characterization of symbiotic soil microorganisms naturally colonizing the roots of fruit plants or soil for horticultural practice**
- **Identification of beneficial species and strains of mycorrhizal fungi and rhizosphere bacteria using classical, biochemical and molecular techniques**
- **Determination of effectiveness – *in vivo* analyses to select the most beneficial species / strains of microorganisms for horticultural practice**
- **Preparation of native mycorrhizal and bacterial inocula**

THE COLLECTION IN SYMBIO BANK CONTAINS (NUMBERS)

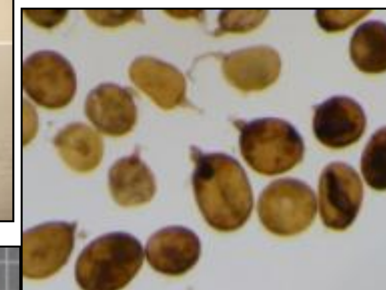
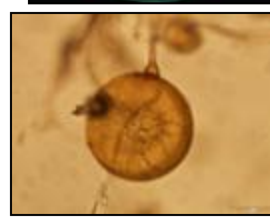
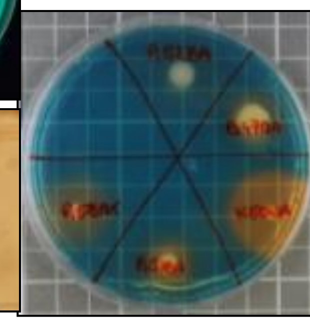
AMF spores isolated from the rhizosphere soil of the species:

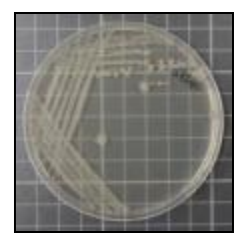
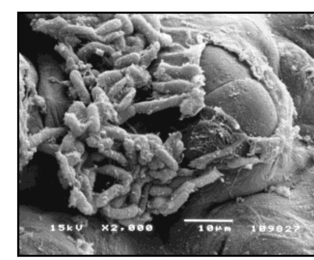
• strawberry	18.0 thousand
• apple	10.5 thousand
• sour cherry	1.5 thousand
• pear	14.0 thousand
• wild strawberry	9.0 thousand
Total	**53 thousand spores**

Isolates of bacteria:

• *Pseudomonas fluorescens*	300
• siderofore synthesis	500
• dissolving phosphorus compounds	200
• digesting cellulose	40
• producing spores	160
• fixing atmospheric nitrogen	100
• *Actinomycetes*	100
Total	**1400**



SYMBIO BANK COLLECTION OF SYMBIOTIC MICROORGANISMS

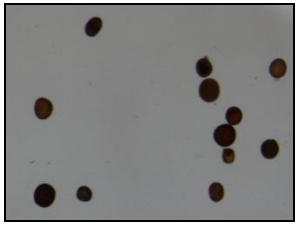
contains spores of AM fungi & PGPR bacteria isolated from the soil of organic orchards & plantations located in Skierniewice, Bieszczady, Białowieża areas (Poland).

SYMBIO BANK

Storage of spores of mycorrhizal fungi at low temperature

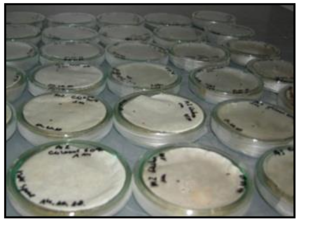
Storage of spores in cryoprotectant solutions (<u>sucrose, glycerol</u>, mannitol, trehaloze, glucose).
Storage of spores in calcium alginate envelopes based on cryoprotectant solutions.

Assessment of the condition and germination of spores after freezing



Spores

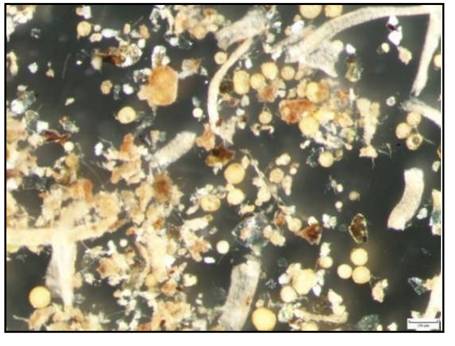
Freezing of spores



Assessment of germination

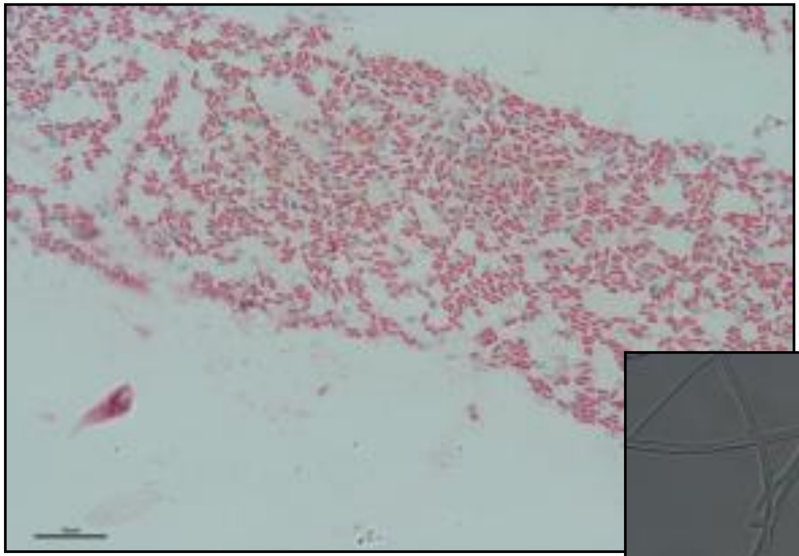
Germinating spore

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.

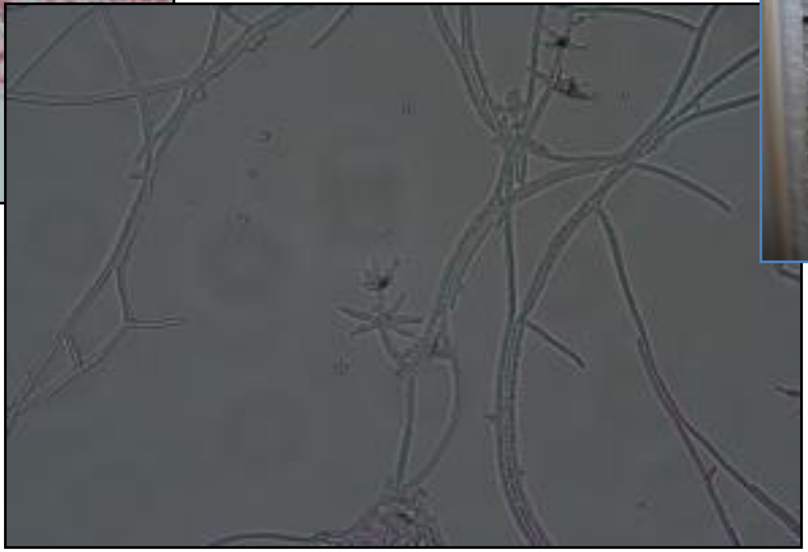


Spores of mycorrhizal fungi (AMF) during isolation

Development of innovative technologies for improving soil quality and increasing growth and yield of fruit plants using bioproducts and beneficial microorganisms collected in SYMBIO BANK



Pseudomonas fluorescens



Trichoderma sp.

SYMBIO BANK

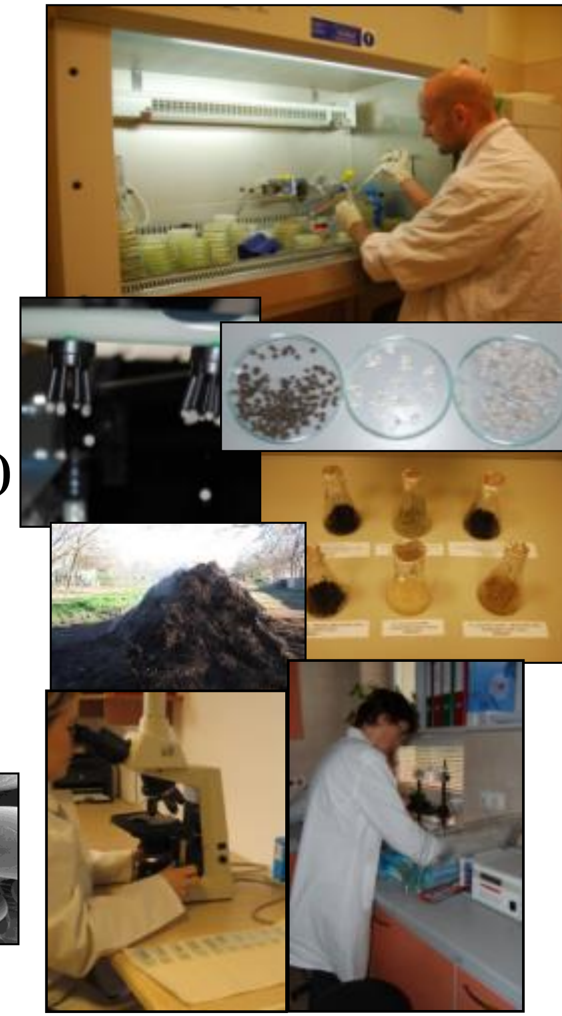
ACHIEVEMENTS OF THE PROJECT

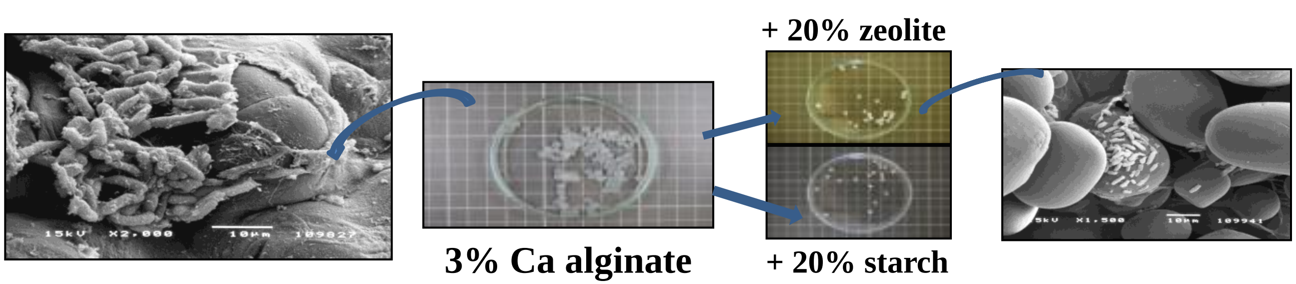
Developed compositions of bioproducts & technologies for their production:

- Microbial inocula (11 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



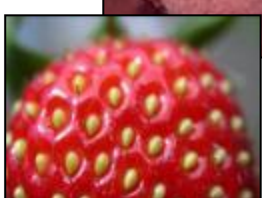


IMPROVED QUALITY OF APPLES, SOUR CHERRIES AND STRAWBERRIES FROM ORGANIC ORCHARDS & PLANTATIONS IN COMPARISON TO INTEGRATED PRODUCTION

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 ^oC

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

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

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

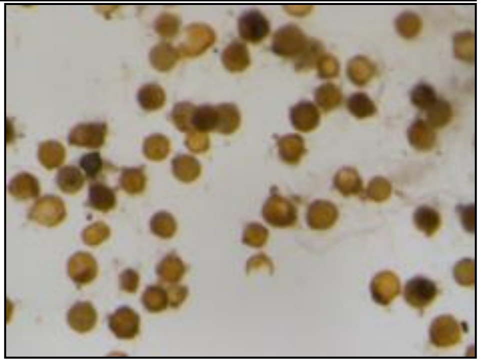
Application of liquid biopreparations to soil surface

TRAP CULTURES

Setting up trap cultures:

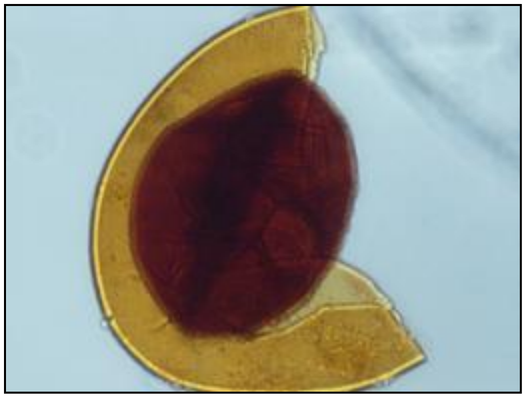
- **Collection of rhizosphere soil and preparation of trap cultures**
- **The layer thickness of spore walls was measured in freshly isolated spores & observed under a light microscope (Błaszkowski 2003).**
- **The observed AMF species were named according to Schüßler & Walker (2010) and Błaszkowski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



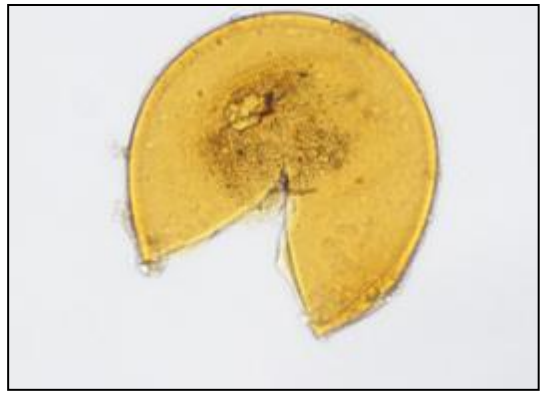


Mass multiplication of spores of am fungi in container cultures

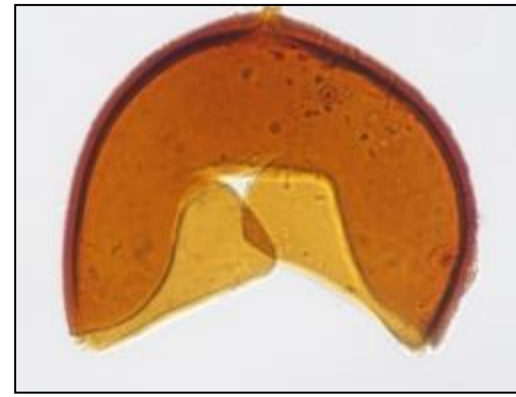
POMO MYCORRHIZA IMAGE GALLERY (BIESZCZADY)



Scutellospora dipurpurescens



Claroideoglomus claroideum



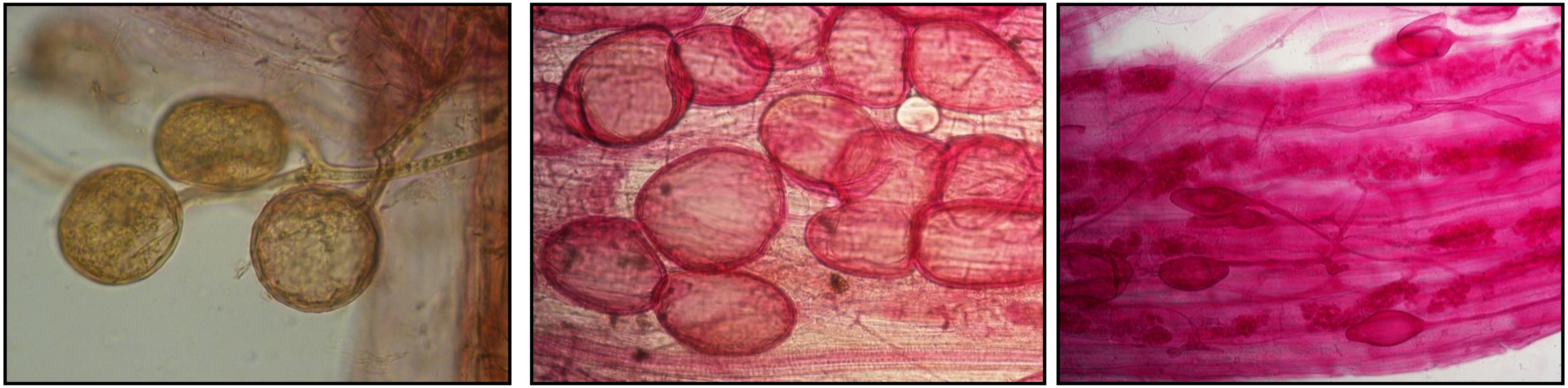
Septoglomus constrictum

Funneliformis mosseae

Glomus macrocarpum

Entrophospora infrequens

MYCORRHIZAL STRUCTURES IN THE ROOTS OF 'ELSANTA' STRAWBERRY PLANTS

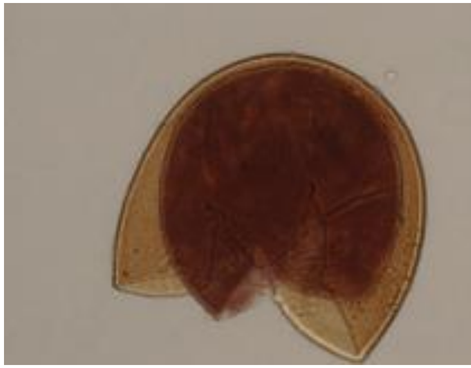


A B C

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)

ARBUSCULAR MYCORRHIZAL FUNGI

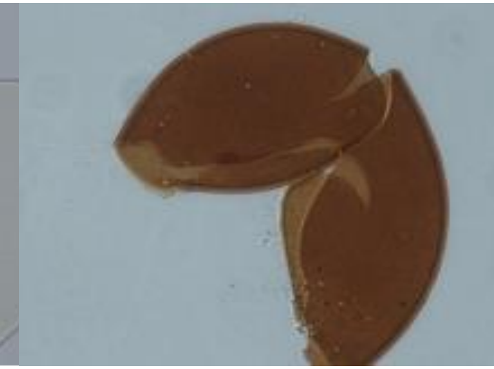
POMO MYCORRHIZA IMAGE GALLERY



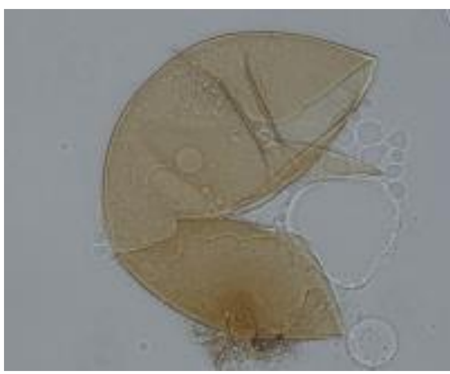
Scutellospora dipurpurescens

Claroideoglomus claroideum

Funneliformis mosseae



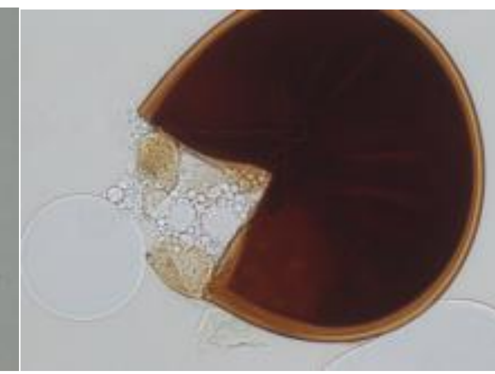
Septoglomus constrictum



Rhizophagus fasciculatus

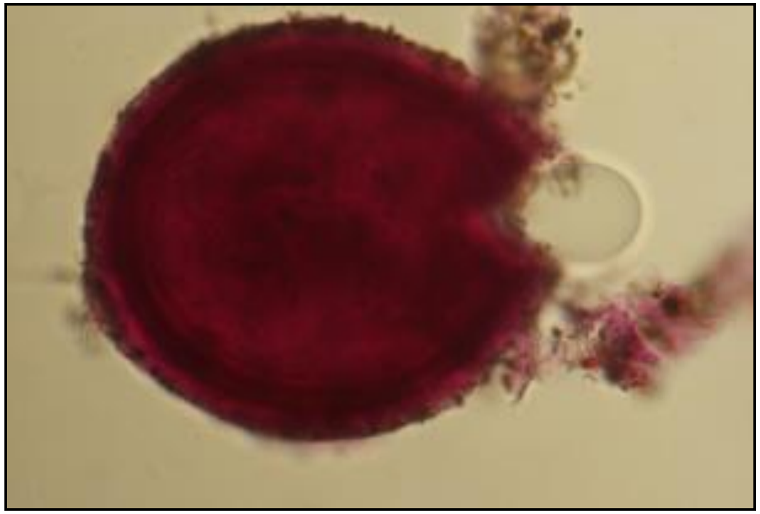
Septoglomus constrictum

Funneliformis mosseae

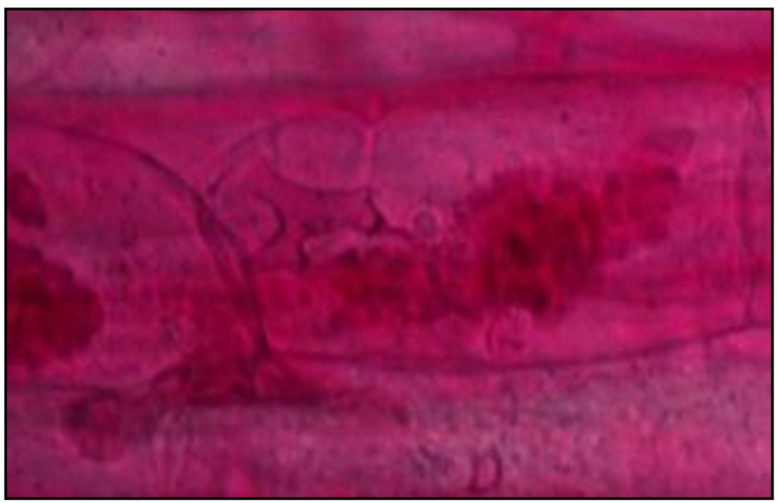


Scutellospora dipurpurescens

Mycorrhizal structures of *Claroideoglomus claroideum* in the roots of strawberry cv. ‘Elsanta’.

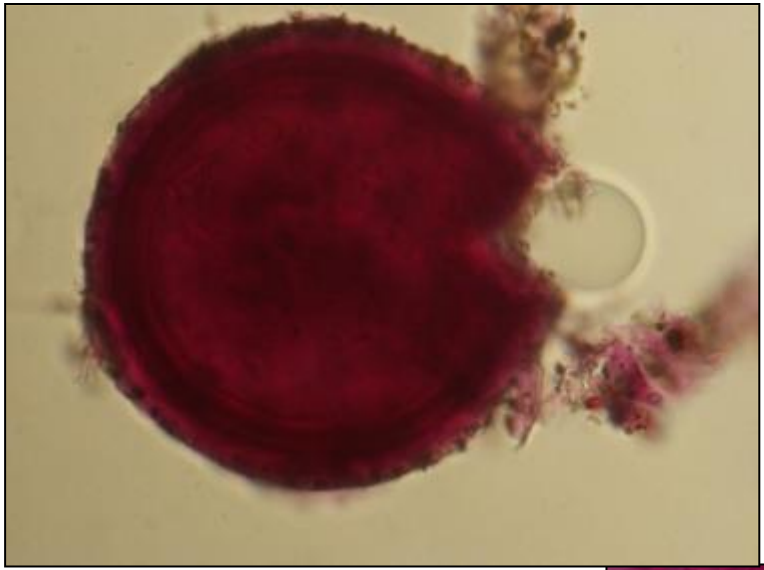


An AMF spore



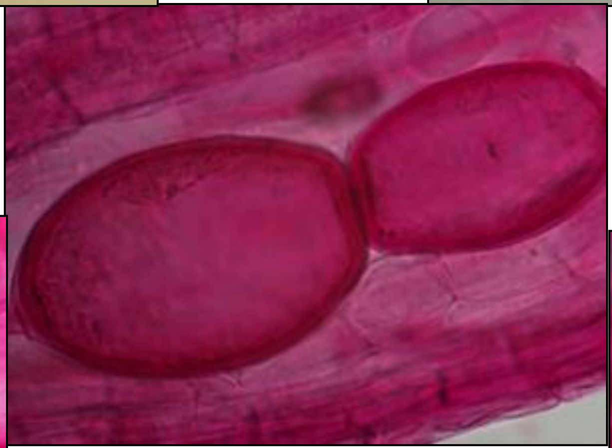
An AMF arbuscule

AMF arbuscules and mycelium

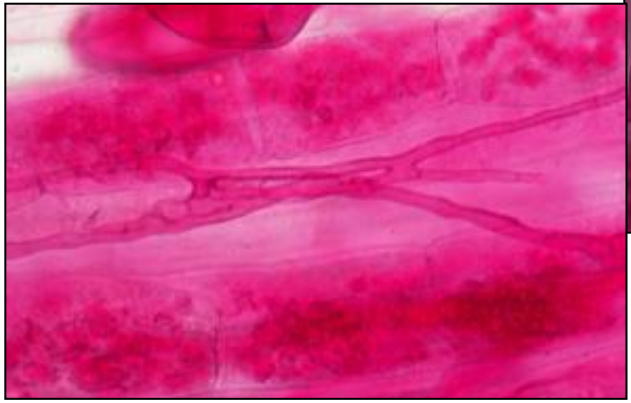


A spore in the roots of strawberry plants cv. ‘Elsanta’ (Dąbrowice, 2013)

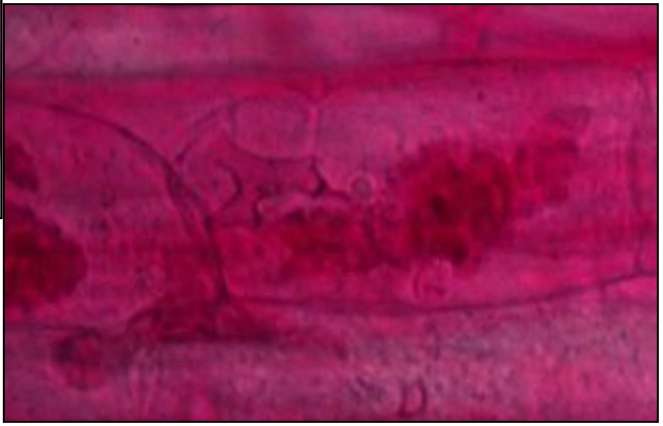
Spore in the roots of strawberry ‘Elsanta’(Skierniewice, 2012)



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)

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

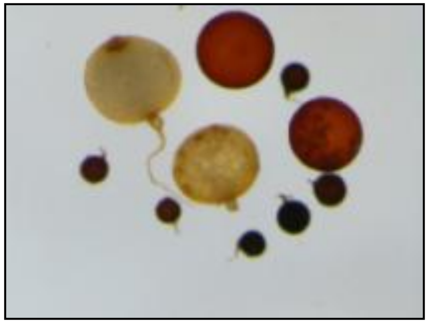
Treatment	Number of spores	
	Strawberry ‘Elsanta’	Strawberry ‘Honeoye’
Control	309	410
NPK	358	891
Manure	473	952
Micosat	481	926
Humus VP	367	900
Humus Active + Aktywit PM	476	2581
BF Quality	605	1355
BF Amin	534	1036
Tytanit	334	589
Vinassa	270	554
Total	4207	10194

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

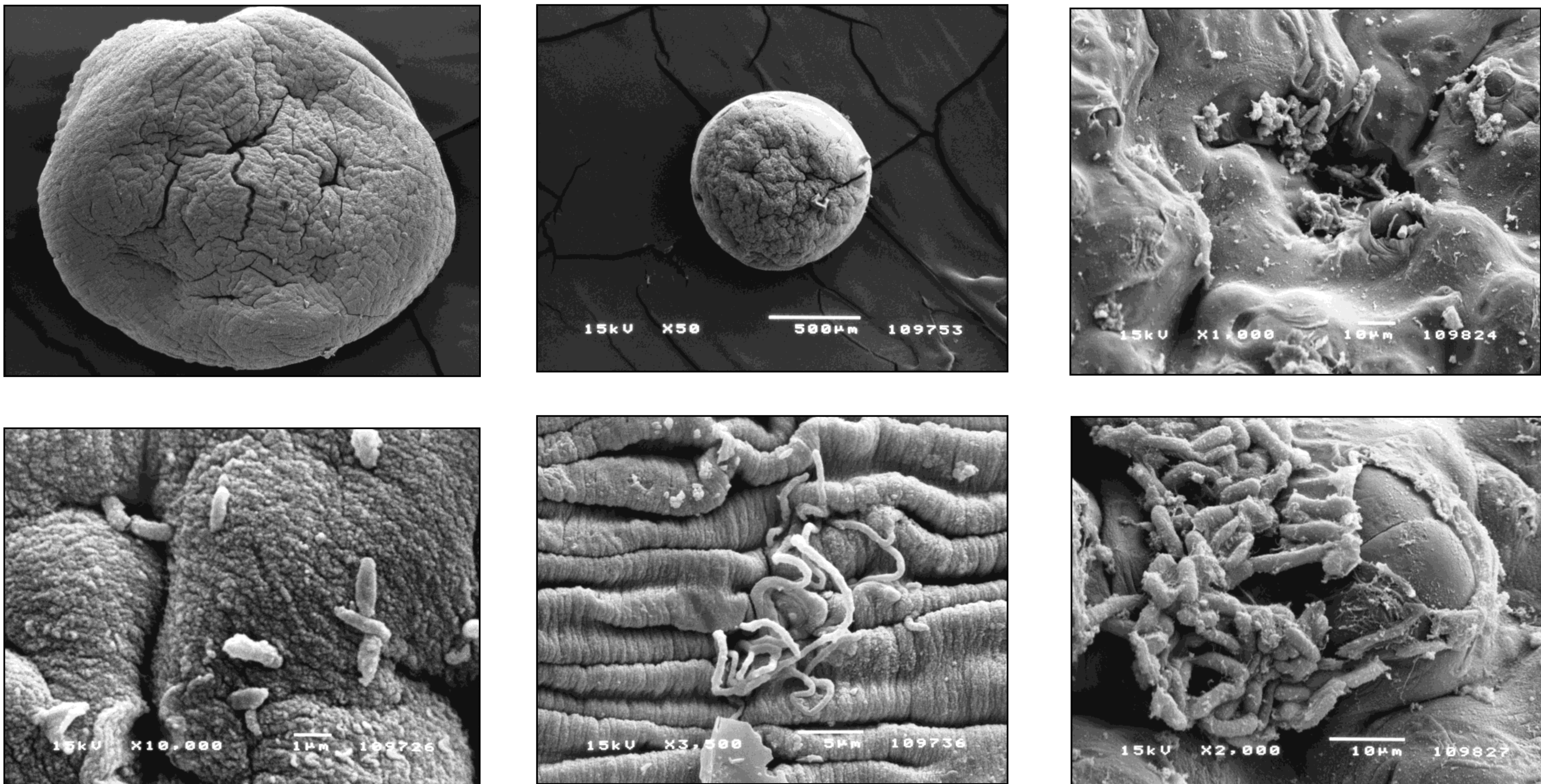
1. ***Septoglomus constrictum*** **(Trappe) Sieverd., G.A. Silva & Oehl**
2. ***Funneliformis mosseae*** **(T.H. Nicolson & Gerd.) C. Walker & A. Schüßler**
3. ***Funneliformis geosporus*** **(T.H. Nicolson & Gerd.) C. Walker & A. Schüßler**
4. ***Acaulospora capsicula*** **Błaszk.**
5. ***Acaulospora 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

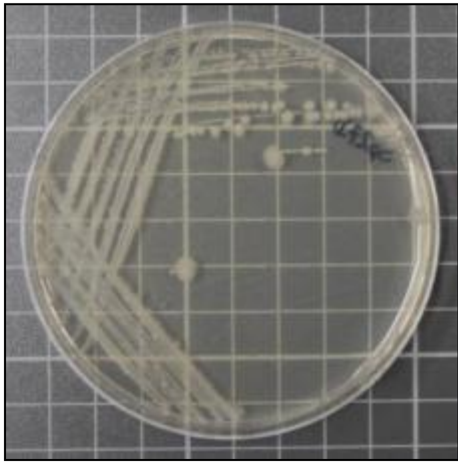
Rhizophagus fasciculatus



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

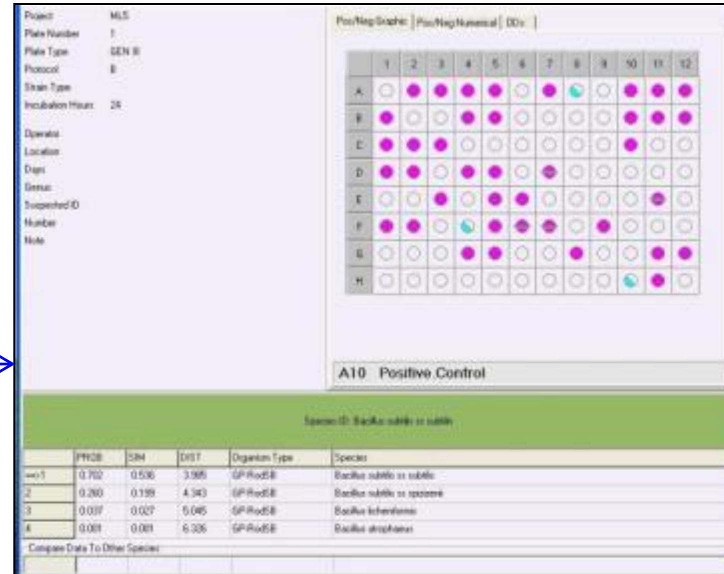


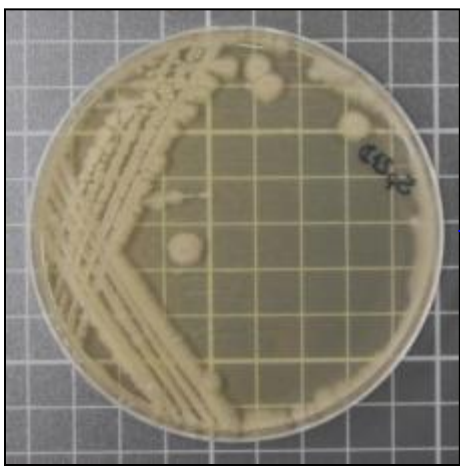
POMO PGPR IMAGE GALLERY



Growth of *Bacillus subtilis* Sp27D on nutrient agar medium

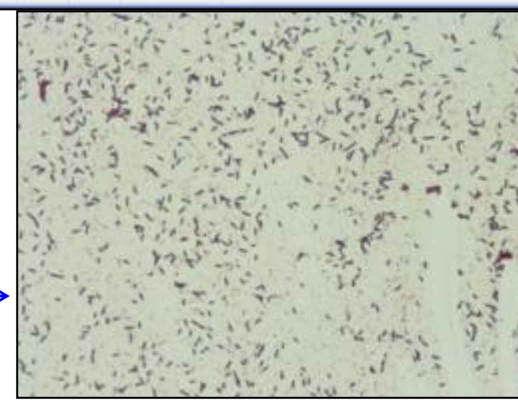
Biochemical properties of *Bacillus subtilis* Sp27D (BIOLOG)





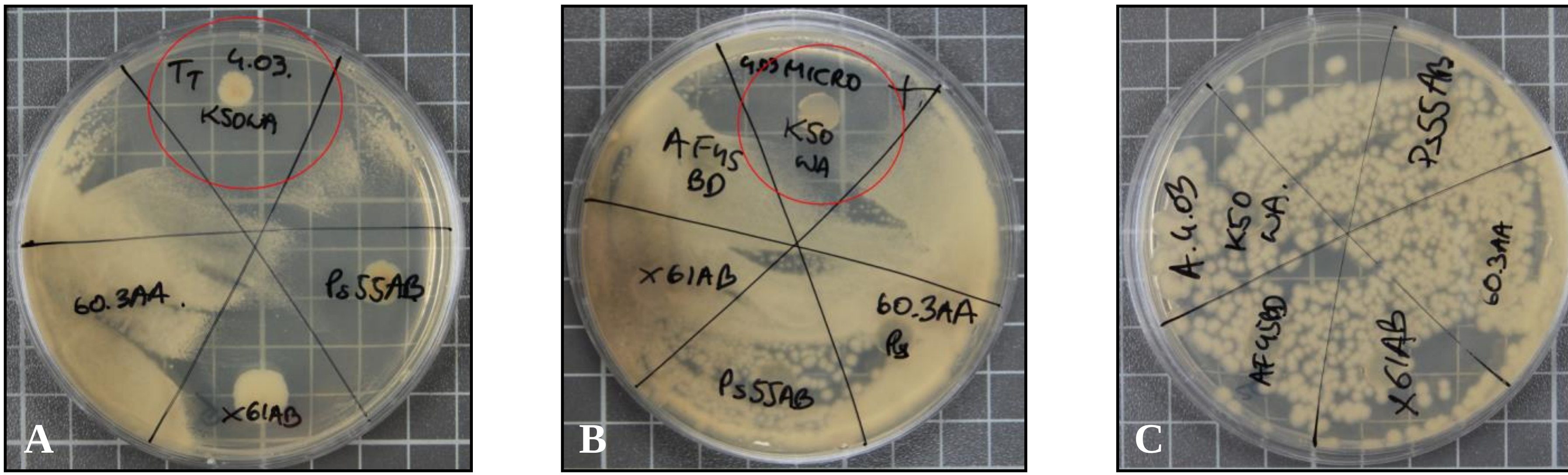
Growth of *Bacillus subtilis* Sp27D on TSA medium

Cells of *Bacillus subtilis* under microscope



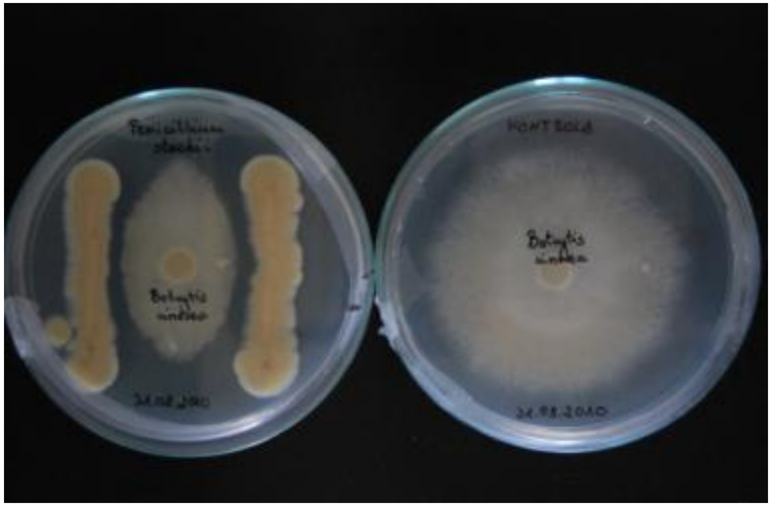
EVALUATION OF ANTAGONISTIC PROPERTIES OF BACTERIAL STRAINS COLLECTED IN SYMBIO BANK

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

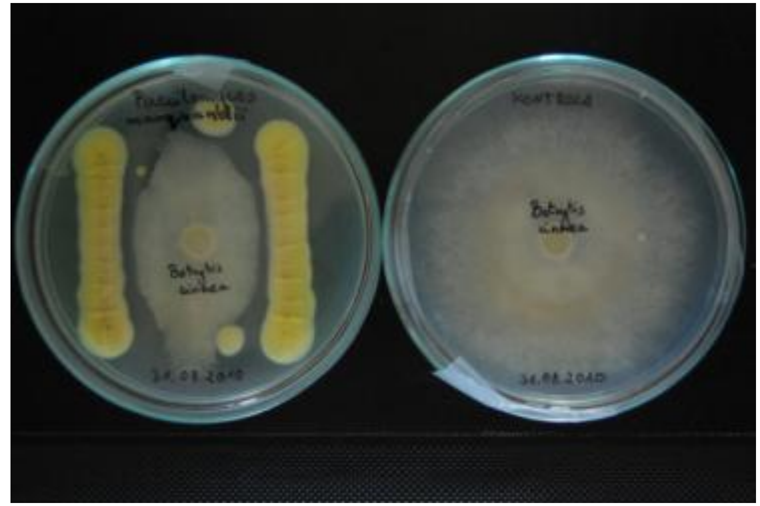


A – Test conducted in normal conditions – the highest production
B – Test conducted in an atmosphere with reduced oxygen content – medium production
C – Test conducted under anaerobic conditions –no production

BENEFICIAL MICROORGANISMS – PATHOGENIC FUNGI

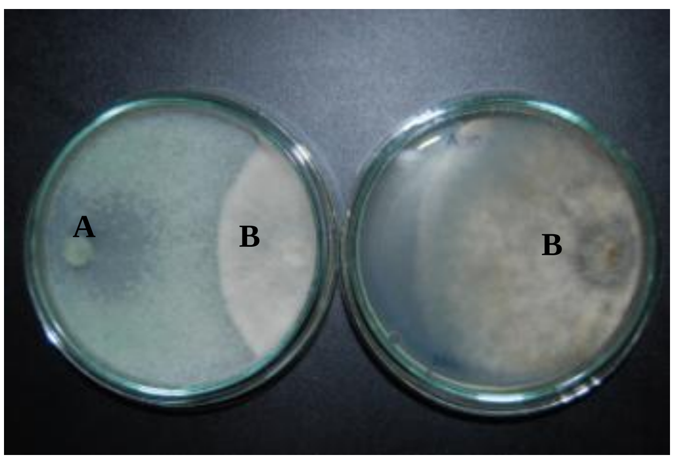


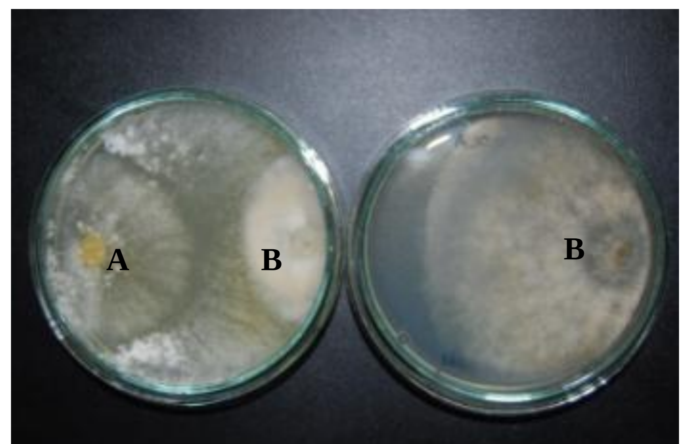
Inhibition of growth of *Botrytis cinerea* by the fungi *Penicillium steckii* and *Paecilomyces marquandii*



Control (*Botrytis cinerea*).

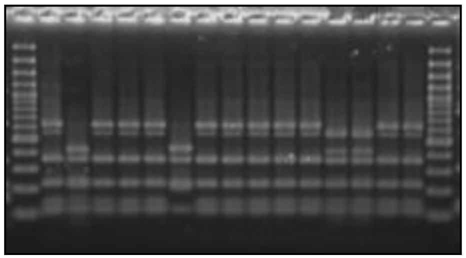
ANTAGONISTIC ACTIVITY OF FUNGI



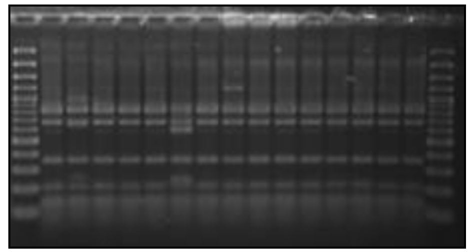


Antagonism of fungi of the genus *Trichoderma sp.* towards *Botrytis cinerea*,
A – fungus of the genus *Trichoderma sp.*, B - *Botrytis cinerea* causing grey mould

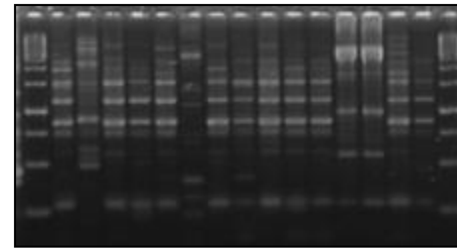
IDENTIFICATION AND ASSESSMENT OF GENETIC SIMILARITY OF RHIZOSPHERE BACTERIAL ISOLATES OF *PSEUDOMONAS* SPP. USING MOLECULAR TECHNIQUES



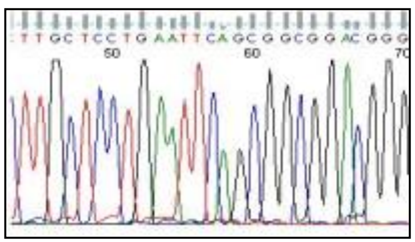
RFLP analysis of 16S rRNA gene



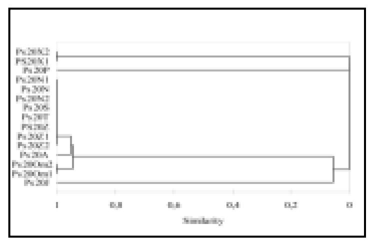
RFLP analysis of 16S-ISR-23S region



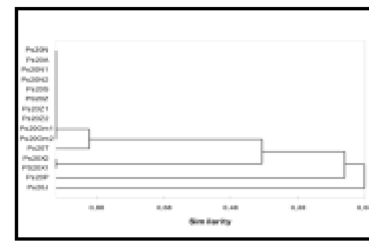
Analysis of repetitive sequences in the genome (rep-PCR)



Sequence analysis of the 16S rRNA gene



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



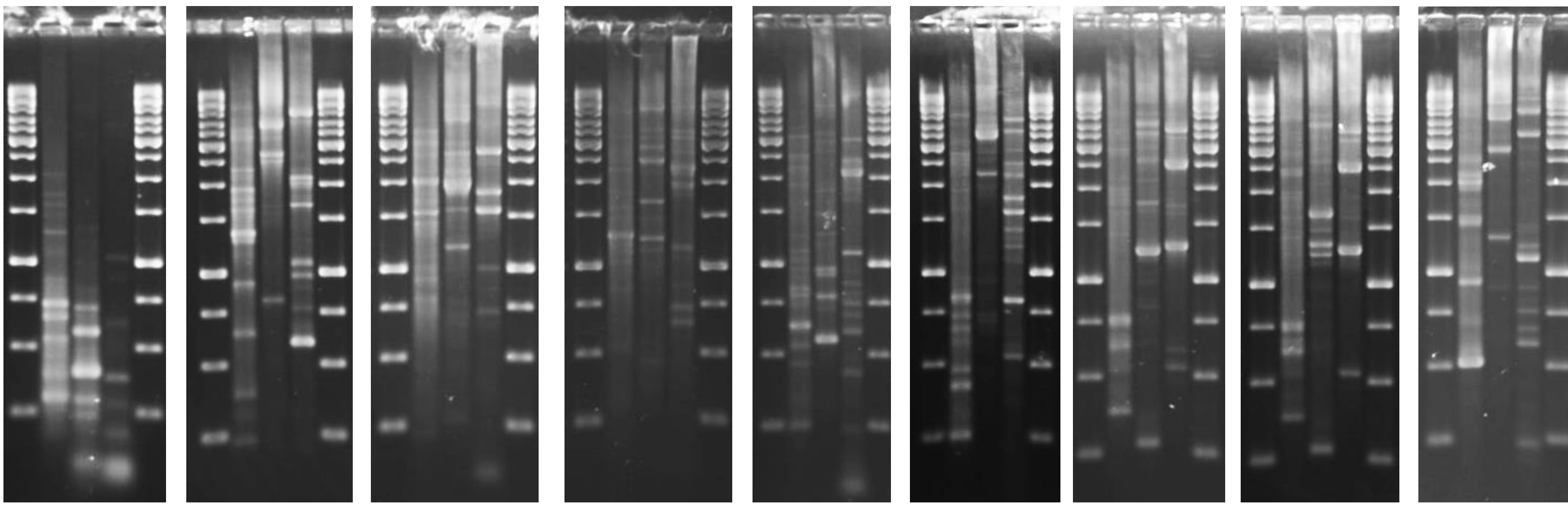
Genetic similarity of soil bacterial isolates, obtained on the basis of RFLP analysis

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.

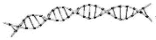
DNA PROFILES OF SELECTED BENEFICIAL BACTERIAL STRAINS

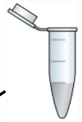


K50WA AF65AL X29L Pi22B Ps49A Ps1/2 Pi3A X31E Sp27D

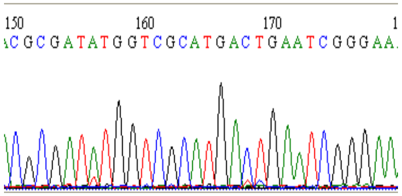
IDENTIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGI USING MOLECULAR METHODS

Extraction of DNA from plant roots or fungal spores

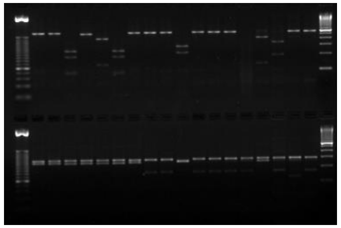




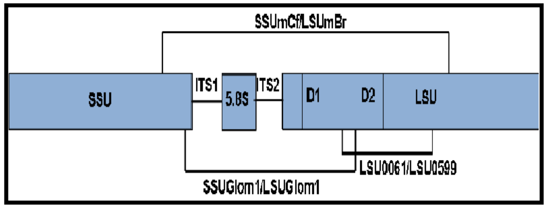
Nested PCR



Sequence analysis



RFLP



rDNA gene and primers allowing amplification of its regions

SELECTION AND IDENTIFICATION OF SOIL BACTERIAL STRAINS FOR POTENTIAL USE IN SOIL BIOREMEDIATION

Size of products obtained in reactions with primers specific to lipopeptide groups.

Lipopeptides	Primers*	Size of the PCR products obtained (bp)
Surfactins	As1-F/Ts2-R	480
Mycosubtilins	Am1-F/Tm1-R	420, 520
Plipastatins	Ap1-F/Tp1-R	960
Fengycins	Af2-F/Tf1-R	Lack of PCR products

*Primers sequences according to Tapi et al. 2010. Appl. Microbiol. Biotechnol. 85: 1521-1531

Identification of soil bacterial isolates showing the ability to produce surfactins.

Bacterial isolates producing surfactins	Identifiication
Sp41CA, Sp41DA, Sp7D, Sp10C, Sp4Ę, Sp6A”, Sp3B, Sp3/8, Sp4G, Sp5E, Sp17XA, Sp27E	*Bacillus* spp.
AF72AB2, Sp27D	*Bacillus subtilis*
Sp2M, Sp2O, Sp17DA, Sp17E, AF61AA, Sp7N, Sp1F, Sp3F	*Paenibacillus* spp.

Analysis of the 16S rRNA gene and the *tuf* gene for 88 bacterial isolates indicated 99-100% similarity of DNA sequences to the genera *Bacillus*, *Paenibacillus* and *Brevibacterium*.

The tests helped to select 33 lipopeptide-producing bacterial strains. The strains belong to the genus *Bacillus*, including the species *Bacillus subtilis*, and to the genus *Paenibacillus*.

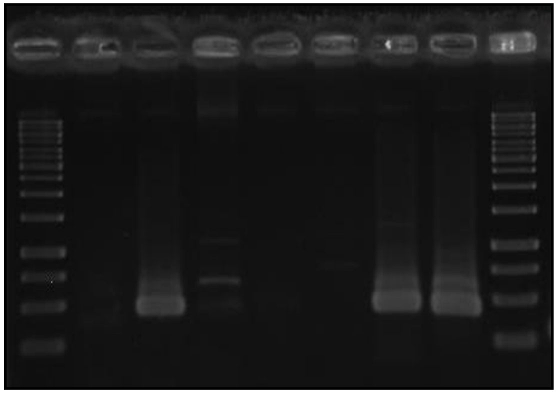
The highest number of the selected soil bacterial strains (25%) showed the ability to produce surfactins.

Identification of soil bacterial isolates showing the ability to produce plipastatins.

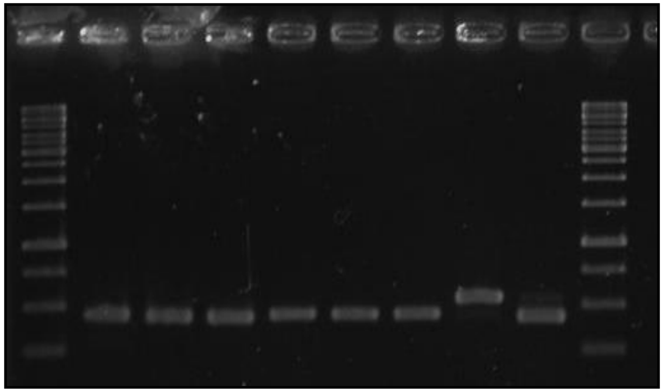
Bacterial isolates producing plipastatins	Identifiication
Sp27E	*Bacillus* sp.
AF75AB2, Sp27D	*Bacillus subtilis*

Identification of soil bacterial isolates showing the ability to produce mycosubtilins.

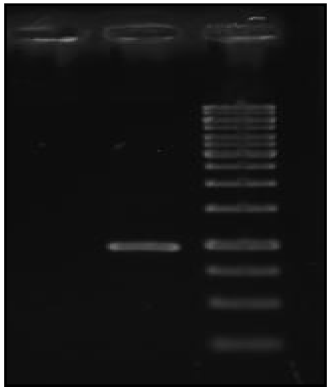
Bacterial isolates producing mycosubtilins	Identification
Sp5E, Sp6B, Sp6D, Sp6E, Sp6G, Sp6H, Sp6J	*Bacillus* spp.
AF61AA	*Paenibacillus* sp.



Selection of bacterial strains with the ability to synthesize surfactins.



Selection of bacterial strains with the ability to synthesize mycosubtilins.



Selection of bacterial strains with the ability to synthesize plipastatins.

The ability to produce mycosubtilins was found in 9.1% of the strains, while the ability to produce plipastatins was observed in 3.4% of the strains. None of the strains tested showed the ability to produce fengycins.

It was found that 29 strains showed the ability to produce one type of lipopeptides. Strains Sp27D and AF75AB2 of *Bacillus subtilis* and Sp27E of *Bacillus* sp. showed the ability to produce two types of lipopeptides – surfactins and plipastatins, while strain AF61AA of *Paenibacillus* sp. showed the ability to produce surfactins and mycosubtilins.

The selected bacterial strains will find application in the development of microbiologically enriched bioproducts intended for bioremediation of soils contaminated with aromatic hydrocarbons.

EXPERIMENTAL COMBINATIONS

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

VISUALIZATION OF pH CHANGES IN RHIZOSPHERE AND ACQUISITION OF ROOT EXUDATES

BIO-PHYSICO-CHEMICAL ANALYSES

Samples of:

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

APPLICATION METHODS

The aim of the research was to design and conduct field and greenhouse experiments and to determine the most effective methods of application of microbiological inocula and bioproducts in organic cultivation of fruit plants.

Greenhouse experiment

Field experiment

Application of biopreparations

RESEARCH METHODOLOGY

Greenhouse experiment with strawberry plants in cylinders (IO Greenhouse, 2015)

- Greenhouse experiments in growth cylinders with strawberry cvs 'Elkat' and 'Honeoye', apple cv. 'Topaz', and sour cherry cv. 'Sabina'

- Bioproducts used:
- Humus UP, Humus Active + Aktywit PM, BF Quality, Vinassa,

- Control combinations:
- non-fertilized plants (zero control)
- plants fertilized with NPK (NPK control)
- plants fertilized with manure

- Solid bioproducts were mixed with soil, and liquid biostimulants were applied to the leaves, 3 times, at 2-3 week intervals.

Applications of biopreparations Humus Active + Aktywit PM, BF Quality and Vinassa increased growth and yield of strawberry plants.

GREENHOUSE EXPERIMENTS

‘Topaz’ apple trees
BF Quality

‘Topaz’ apple tree
NPK Control

‘Topaz’ apple tree
BF Quality

‘Topaz’ apple trees
0 Control

‘Sabina’ sour cherry tree
NPK Control

‘Sabina’ sour cherry trees

‘Sabina’ sour cherry tree
Humus Active+Aktywit PM

‘Topaz’ apple tree
NPK Control

‘Topaz’ apple tree
BF Quality

Experiment in growth cylinders with
‘Topaz’ apple trees

‘Sabina’ sour cherry tree
NPK Control

‘Sabina’ sour cherry tree
Humus Activ+Aktywit PM

Experiment in growth cylinders with
‘Sabina’ sour cherry trees

Applications of bioproducts Humus Active + Aktywit PM, BF Quality and Vinassa increased vegetative growth of roots and shoots of apple trees cv. ‘Topaz’ and sour cherry cv. ‘Sabina’.

EXPERIMENT IN STONEWARE POTS

Applications of bioproducts increased population size, survival rate and effectiveness of beneficial microorganisms in organic cultivation of strawberry plants cv. ‘Elsanta’ (Experimental Orchard, Dąbrowice, 2015).

Ingrowth-core method enabled to investigate the effects of various bioproducts on root growth in two strawberry cvs 'Elsanta' and 'Elkat' in open field

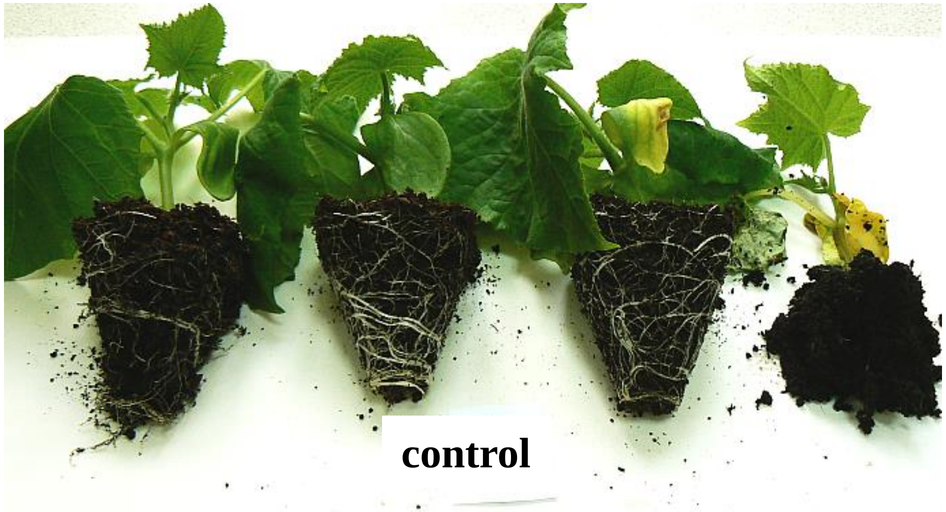
- The strongest stimulation of the development of the root system was observed after application of preparations: Micosat F, Humus UP, Humus Active + Aktywit PM.
- Plants fertilized with NPK had the most poorly developed root system.

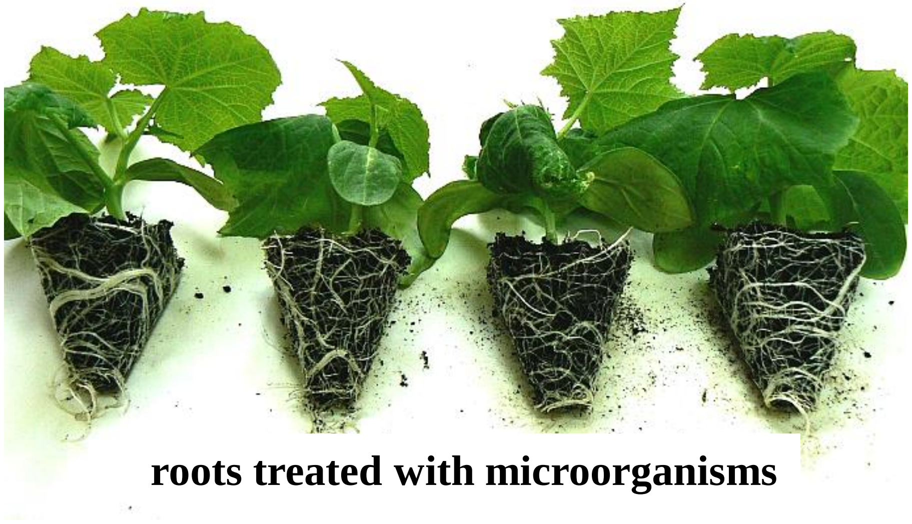
Ingrowth cores in field experiment with strawberry plants
(Experimental Orchard, Dąbrowice, 2015)

Microbiological bioproducts showed high effectiveness in stimulating vegetative growth and yielding of strawberry, apple and sour cherry plants (Experiment in stoneware pots, Dąbrowice, 2015r.).

BENEFICIAL MICROORGANISMS IN THE CULTIVATION OF PLANTS

- Improve seed germination
- Stimulate development of the root system
- Improve vegetative growth and yield – atmospheric nitrogen fixation
- Make nutrients availabe to plants
- Increase resistance of plants to pathogens and pests and abiotic stresses

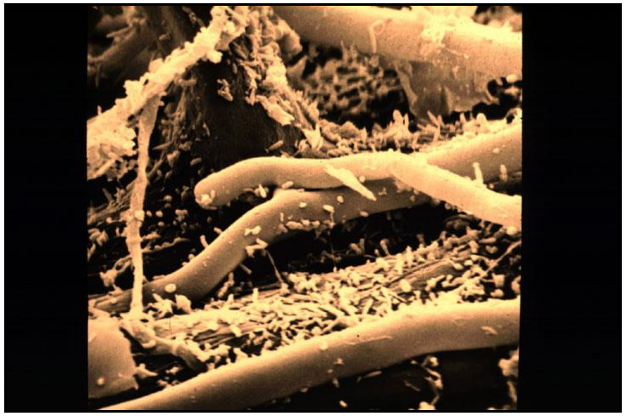




del Carmen Montero-Calasanz et al. 2013; Kasim et al. 2013; Egamberdieva & Lugtenberg 2014; Mia et al. 2014

MICROORGANISMS STIMULATE GROWTH AND YIELD OF VARIOUS CROP SPECIES

- **Potato – *Pseudomonas* spp. (5-33% increase in yield)**
- **Carrot and oat – *Bacillus subtilis* (48% increase in yield)**
- **Sugar beet - *Pseudomonas* spp. (4-8 t/ha increase in yield)**
- **Wheat – *Pseudomonas fluorescens* Strain 2-79 (17% increase in yield)**
- **Apple – *Bacillus subtilis* Strain EBW-4 & BACT-1 (65-179% increase in TCSA)**



Sobiczewski P. 2002; Ghyselinck et al. 2013; Smirnova et al. 2013; Papageni et al. 2014

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

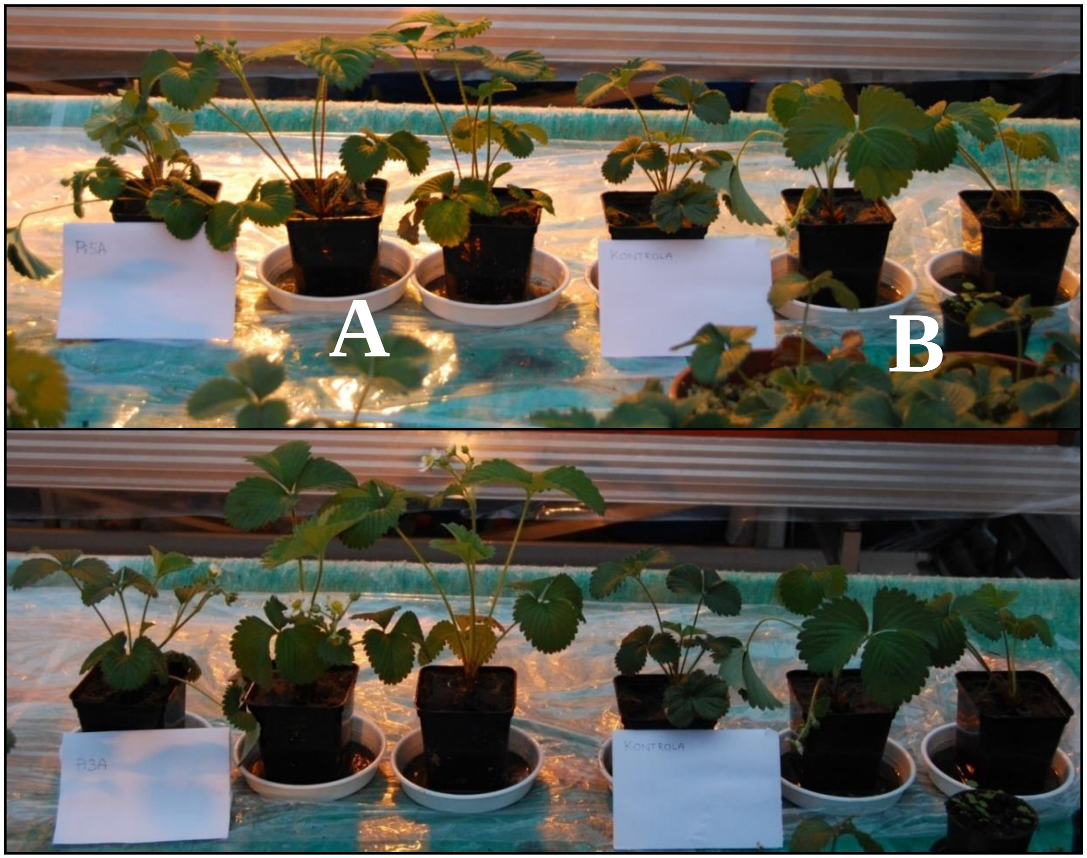
A – non-inoculated plants (control)

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)

Beneficial effect of *Rahnella aquatilis* on growth of 'Elsanta' strawberry plants in greenhouse



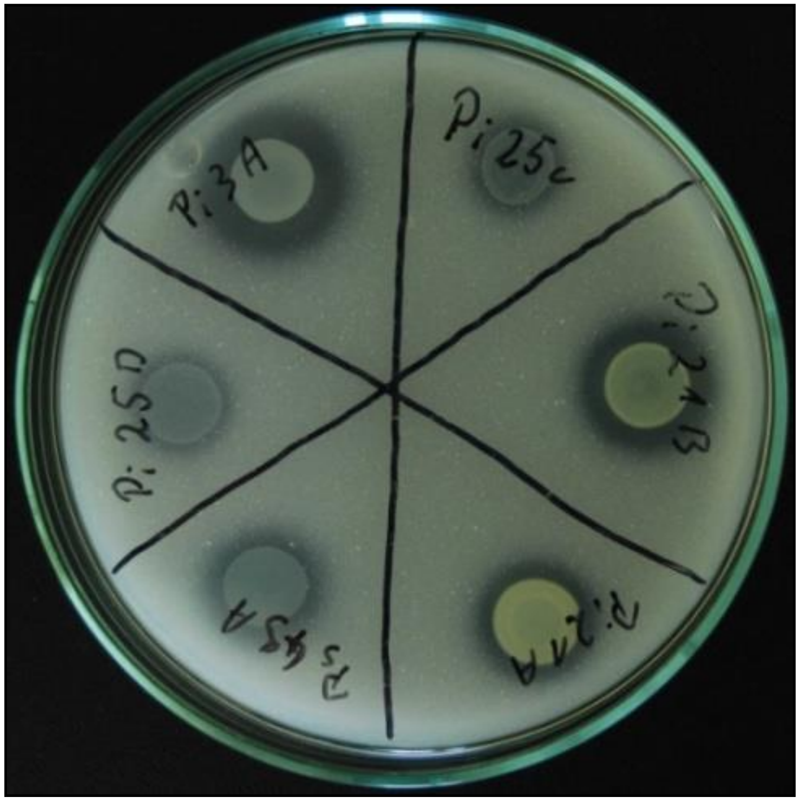
Strawberry plants inoculated with strains of the bacterium *Rahnella aquatilis*

A - *Rahnella aquatilis*
B - control

Trzciński et al. 2012

IO Rhizosphere Laboratory

Chitinolytic activity of bacteria

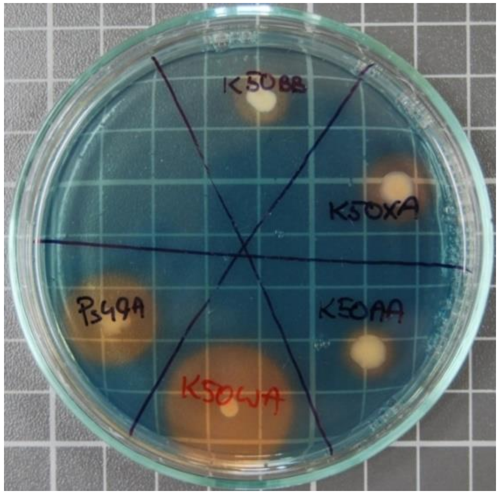


Phosphate solubilizing activity of bacteria (colorless 'halo' around colonies)

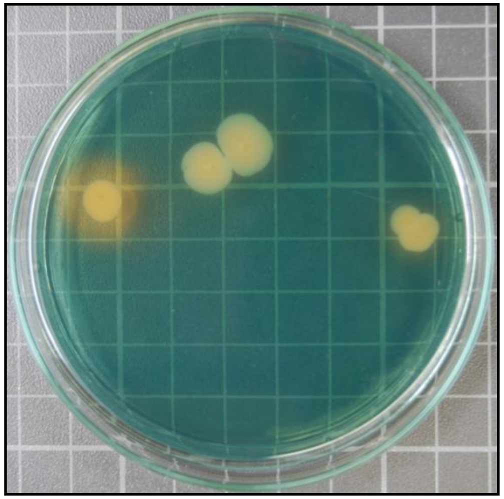
Ability of bacterial isolates to produce chitinase (colloidal chitin medium)

Trzciński et al. 2012, unpublished data

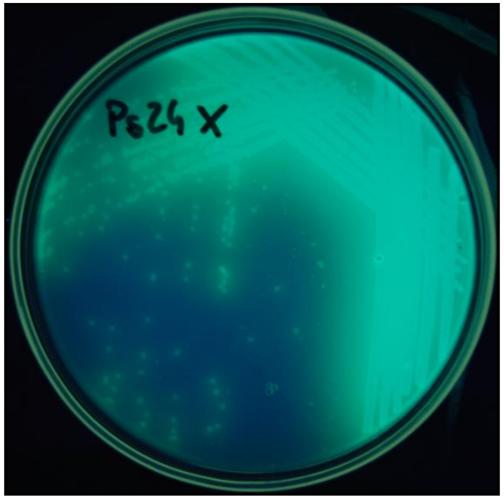
Production of siderophores by beneficial bacteria inhibiting growth and development of pathogenic microorganisms



A



B



C

A. & B. CAS agar medium – orange 'halo' around bacteria indicates production of siderophores.

C. *Pseudomonas fluorescens* bacteria on S1 medium seen under UV light.
The yellow-green pigment produced by these bacteria is a siderophore.

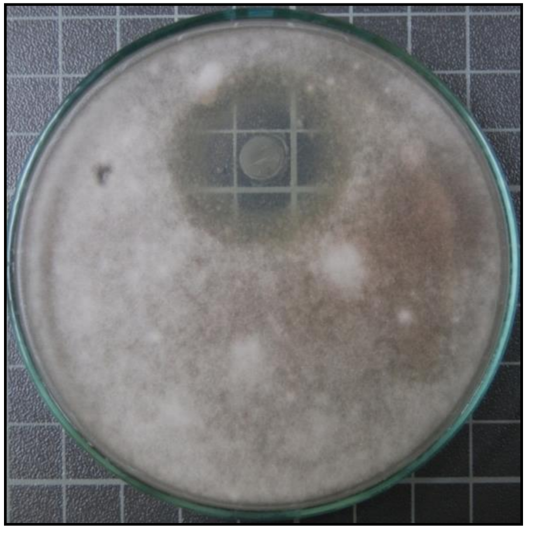
***Pseudomonas* spp. are among the largest producers of siderophores in the soil, synthesizing eg. pseudobactin, pyochelin, pyoverdine, chinolobactin, and salicylic acid.**

Trzciński et al. 2011, unpublished data

Beneficial bacteria inhibit growth of pathogenic fungi

- ➢ *Botrytis cinerea*
- ➢ *Fusarium oxysporum*
- ➢ *Verticillum dahliae*

which cause losses in arable and horticultural crops



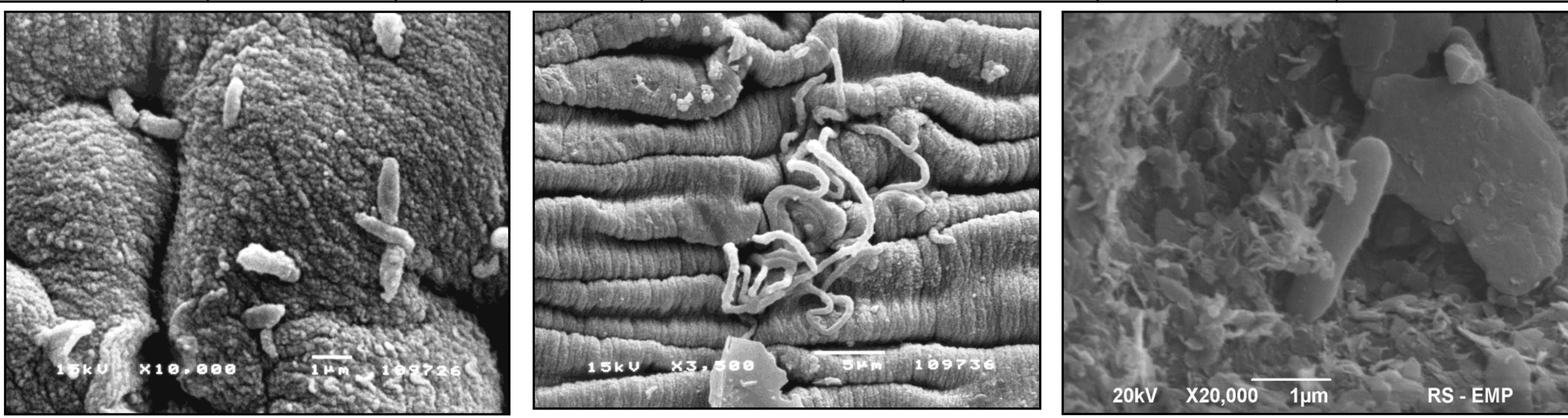
Effect of biofertilizers and beneficial microorganisms on vegetative growth and yield of apple trees cv. ‘Topaz’ (Experiment in stoneware pots, Skierniewice, 2015)

Treatment	Fruit yield kg/tree	Number of shoots per tree	Mean shoot length [cm]
Control NPK	0.639 b	4.27 a	33.29 a
Control NPK + PGPR bacteria	0.559 ab	3.66 a	36.80 a
Manure	0.577 ab	4.22 a	32.66 a
Manure + PGPR bacteria	0.541 ab	3.19 a	36.86 a
Mycorrhizal substrate	0.722 b	2.54 a	55.09 a
Mycorrhizal substrate + PGPR bacteria	1.299 d	3.75 a	35.86 a
Humus UP	1.120 cd	3.29 a	46.07 a
Humus UP + PGPR bacteria	1.796 e	3.77 a	35.92 a
Humus Active + Aktywit PM	0.827 bc	3.29 a	38.21 a
Humus Active + Aktywit PM + PGPR bacteria	1.366 de	3.09 a	42.69 a
BF Quality	0.526 ab	3.19 a	52.49 a
BF Quality + PGPR bacteria	0.786 b	2.45 a	48.30 a
BF Amin	0.542 ab	3.27 a	41.03 a
BF Amin + PGPR bacteria	0.773 b	2.92 a	45.90 a
Yeast	0.388 a	2.69 a	54.94 a
Yeast + PGPR bacteria	1.222 cd	3.23 a	44.89 a
Vinassa	0.479 ab	2.59 a	50.42 a
Vinassa + PGPR bacteria	1.281 cd	4.22 a	31.71 a
Florovit Natura	0.629 b	2.41 a	54.98 a
Florovit Natura + PGPR bacteria	1.132 cd	2.05 a	60.68 a
Florovit Eko	0.746 b	4.12 a	37.24 a
Florovit Eko + PGPR bacteria	0.947 c	2.68 a	45.55 a

Application of microbiologically enriched bioproducts increased average length of shoots and yield of ‘Topaz’ apple trees.

Effect of bioproducts on the number of groups of microorganisms in rhizosphere of strawberry cv. 'Elsanta' (Experimental Orchard, Experiment in stoneware pots, Dąbrowice, 2015)

Treatment	Total number of bacteria [cfu × 10^5 g^{-1}]	Total number of spore-forming bacteria [cfu × 10^4 g^{-1}]	Total number of *Pseudomonas* fluorescent bacteria [cfu × 10^3 g^{-1}]	Total number of diazotrophs [cfu × 10^5 g^{-1}]	Total number of actinomycetes [cfu × 10^4 g^{-1}]	Total number of microscopic fungi [cfu × 10^3 g^{-1}]
Control (no fertilization)	65.6 de	28.6 ab	21.7 b	48.2 g	38.4 c-e	58.3 ab
NPK fertilization	55.5 bc	29.6 ab	99.9 e	31.1 ef	52.9 fg	121.8 de
Mycorrhizal substrate	28.2 a	44 b-e	0.5 a	14.9 a-c	20.5 a	53.9 a
PGPR A	29.5 a	53.2 d-f	1.1 a	15.2 a-c	21.8 ab	71.6 a-c
PGPR B	44.4 ab	65 fg	0.6 a	3.7 a	58.3 gh	53.4 a
PGPRC (A+B)	34.4 a	51.8 c-f	7.2 a	29.1 d-f	19.7 a	89.2 c
Mycorrhizal substrate + PGPR C	70.9 cd	60.9 e-g	1.2 a	35.8 ef	69 h	128.1 e
Compost + PGPR C	78 de	71.4 g	1 a	40.9 fg	83 i	79.2 a-c
Vinassa + PGPR C	132 f	107.8 h	3.7 a	86.9 h	60.4 gh	97.3 cd
Compost	56.2 bc	33.8 a-c	36 c	26.8 c-e	45.9 ef	98.1 cd
Vinassa	40 ab	28.6 ab	47.6 d	11.8 ab	33.8 b-d	84.5 bc
Humus UP	92.2 e	65.3 fg	0.7 a	18.8 b-d	66.8 h	84.9 bc
Humus UP + PGPR C	41 ab	24.2 a	0.6 a	13.2 ab	42.8 d-f	76.5 a-c
Rhizocell	40.7 ab	41.5 a-d	0.7 a	10.2 ab	28.6 a-c	59.8 ab

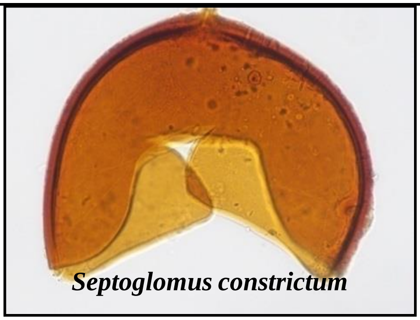


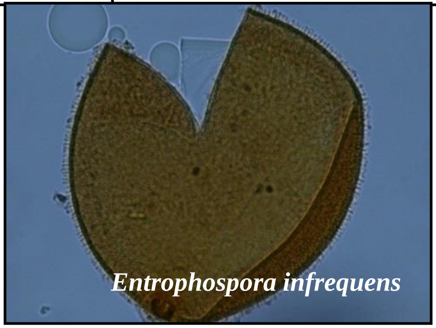
Beneficial microorganisms *Pseudomonas*, *Actinomycetes* in rhizosphere of strawberry cv. 'Elsanta'

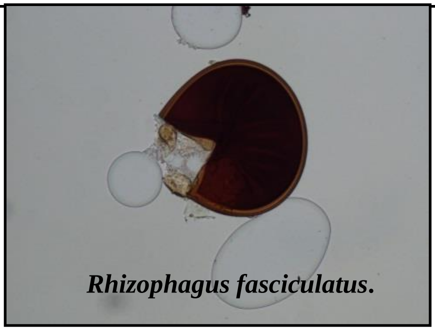
Application of Vinassa together with PGPR contributed to significant increase in total number of bacteria, spore-forming bacteria and diazotrophs.

Effect of bioproducts on the number of spores and species of AM fungi in rhizosphere of strawberry cv. 'Elsanta' (Experiment in stoneware pots, Dąbrowice 2015)

Treatment	Number of spores in 100 g soil	AMF species
Control	14 ab	*Claroideoglomus claroideum*
NPK	8 a	*Claroideoglomus claroideum*
Compost	23 b	*Claroideoglomus claroideum*
PGPR A	26 b	*Claroideoglomus claroideum, Funneliformis mosseae*
PGPR B	33 cd	*Claroideoglomus claroideum, Funneliformis mosseae*
PGPR C	28 bc	*Claroideoglomus claroideum, Funneliformis mosseae*
Vinassa	31 cd	*Claroideoglomus claroideum, Funneliformis mosseae*
Rhizocell	23 b	*Claroideoglomus claroideum, Funneliformis mosseae*
Bacterial consortium	29 bc	*Claroideoglomus claroideum, Funneliformis mosseae*
Mycorrhizal substrate	35 d	*Claroideoglomus claroideum, Funneliformis mosseae*
Mycorrhizal substrate + PGPR C	36 d	*Claroideoglomus claroideum, Funneliformis mosseae*
Florovit Natura + NPK	26 b	*Claroideoglomus claroideum, Funneliformis mosseae*
Vinassa + PGPR C	29 bc	*Claroideoglomus claroideum, Funneliformis mosseae*
Compost + PGPR C	35 d	*Claroideoglomus claroideum, Funneliformis mosseae*


Septoglomus constrictum


Entrophospora infrequens

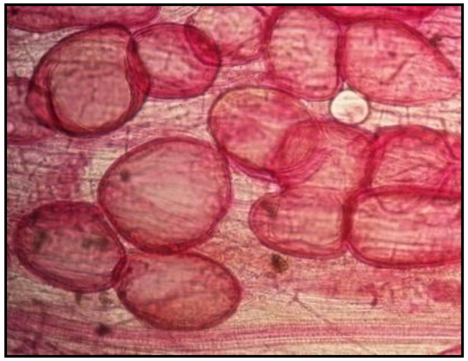

Rhizophagus fasciculatus.

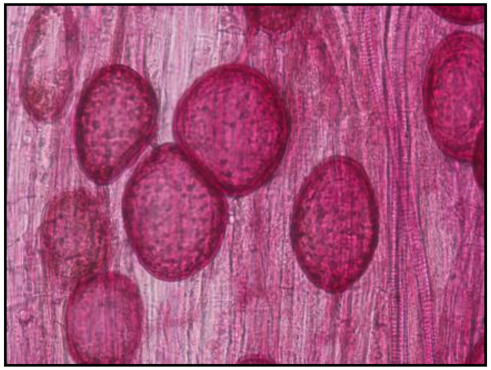

Scutellospora dipurpurescens

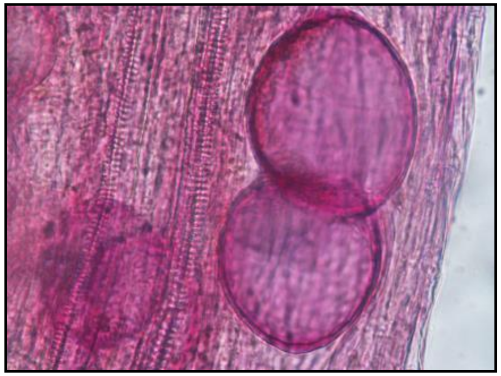
Effect of microbial consortia and biopreparations on mycorrhizal frequency (F%) and mycorrhizal intensity (M%) in the roots of strawbery cv. 'Elsanta' (Field experiment, Dąbrowice, 2015)

Treatment	F%	M%
Control – no fertilization	15.56 ab	0.16 ab
NPK fertilization	8.89 a	0.09 a
Compost	23.33 b	0.23 b
PGPR A	25.56 b	0.26 b
PGPR B	34.44 cd	0.34 bc
PGPR C	28.89 bc	0.29 b
Vinassa	30.0 c	0.30 bc
Rhizocell	23.33 b	0.23 b
Bacterial consortium	28.89 bc	0.29 b
Mycorrhizal substrate	34.44 cd	0.34 bc
Mycorrhizal substrate + PGPR C	36.67 d	0.37 cd
Florovit Natura + NPK	25.56 b	0.26 b
Vinassa + PGPR C	28.89 bc	0.29 b
Compost + PGPR C	34.44 cd	0.34 bc

Spores in roots of strawberry 'Elsanta' after application of mycorrhizal substrate





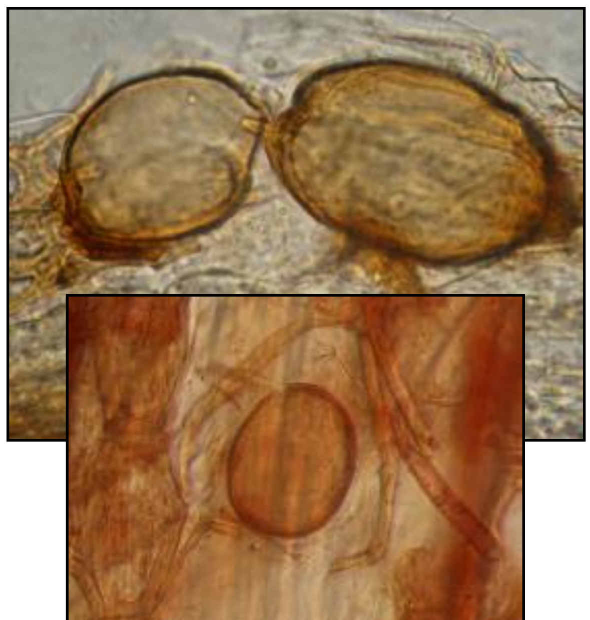


Vesicles in the roots of strawberry 'Elsanta' after application of mycorrhizal substrate

Positive effect of microbial consortia and bioproducts on mycorrhizal association in roots of strawberry, apple and sour cherry

- **Application of microbial consortia PGPR A, B and C and mycorrhizal substrate had a positive effect on the presence of mycorrhizal fungi in the roots of strawberry plants, compared to control plants.**
- **Compared to control, combined application of Humus Active and Aktywit PM and Micosat contributed to the greatest extent to increasing mycorrhizal frequency in roots of apple trees.**
- **Biopreparations Micosat and Vanessa in field cultivation of sour cherry cv. 'Sabina' and 'Debreceni Bötermö' increased mycorrhizal frequency in the roots of plants.**

Spores in roots of strawberry cv. 'Elsanta' – mycorrhizal substrate



Vesicles and mycorrhizal mycelium in roots of apple cv. 'Topaz'

Mycelium in roots of sour cherry cv. 'Sabina' – Humus Active + Aktywit PM

APPLE TREE ROOTS AFTER APPLICATION OF BIOCHAR

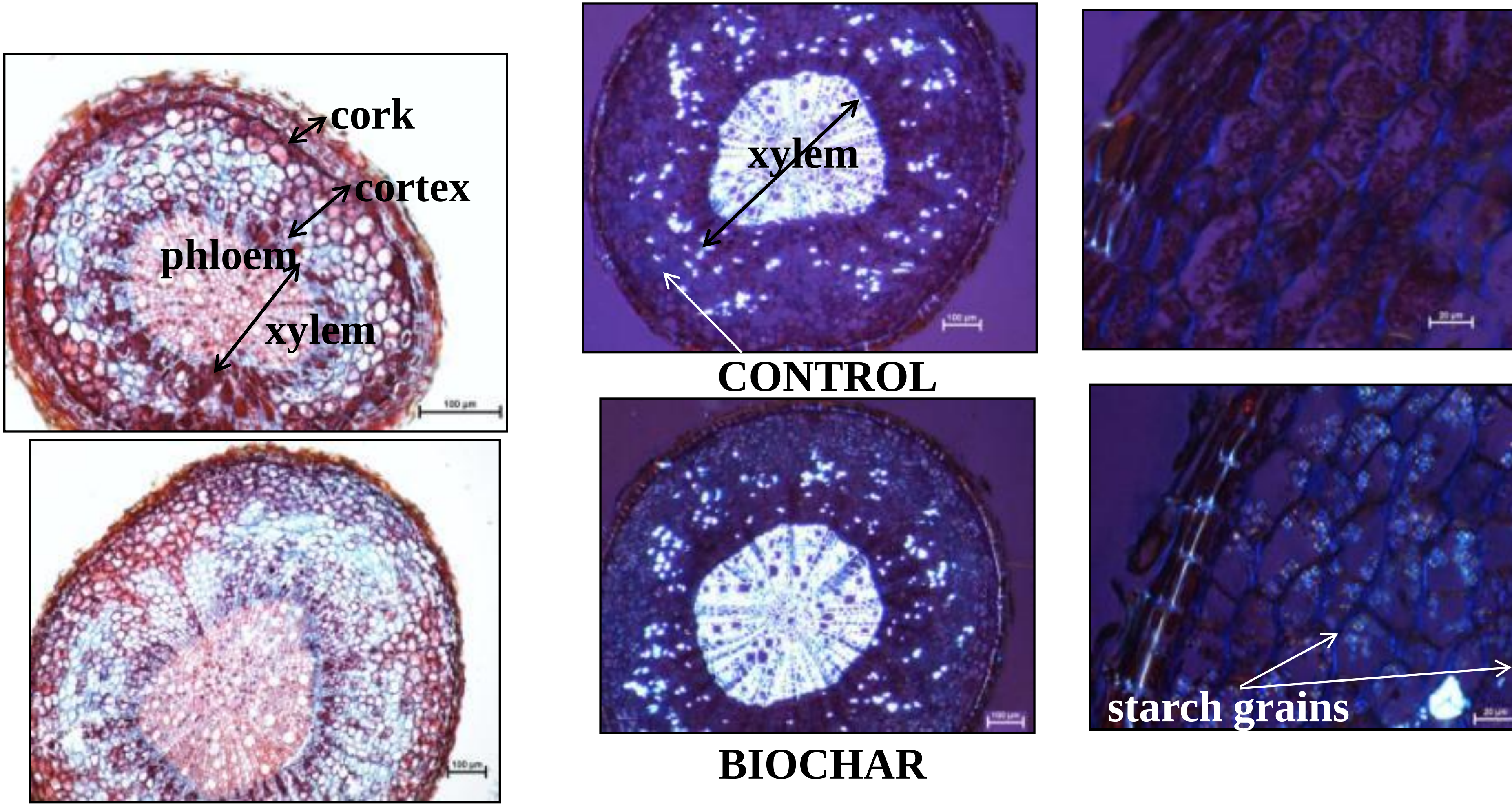
CONTROL

BIOCHAR + MICROORGANISMS

BIOCHAR

Application of biochar and biochar with microorganisms contributed to a multiple increase in length and number of roots compared to control roots.

EFFECT OF BIOCHAR ON CELL STRUCTURE IN APPLE ROOTS



Apple root sections under normal and polarized light

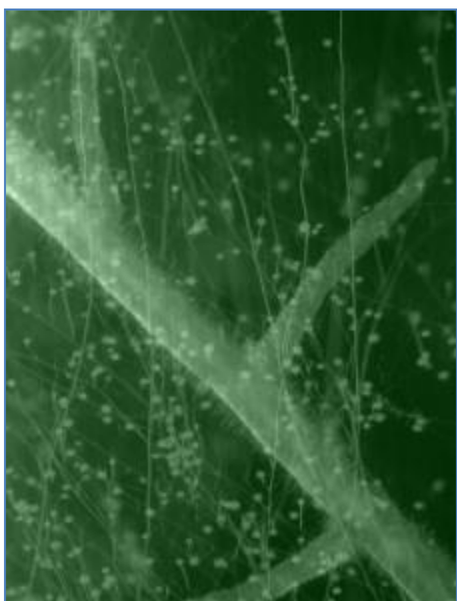
Application of biochar contributed to increased formation of starch in cells of apple tree roots.

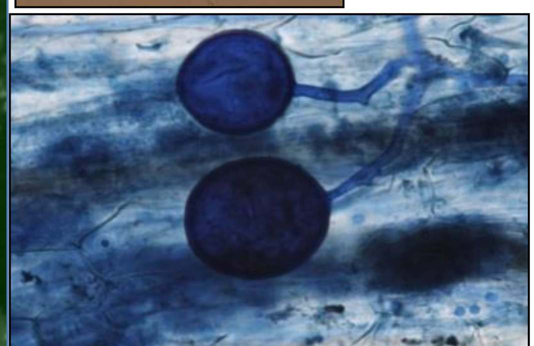
Beneficial bacterial strains and their practical applications:

<u>Bactericides</u>	**Beneficial action**
Pseudomonas fluorescens **A506**	**Prevents fire blight**
Agrobacterium radiobacter **K84**	**Rhizosphere bacterium, prevents crown gall**
Agrobacterium radiobacter **K1026**	**Rhizosphere bacterium, interspecies competition**
Bacillus subtilis	**Rhizosphere bacterium, interspecies competition**
Bacillus circulans	**Rhizosphere bacterium, interspecies competition**
Bacillus amyloliquefaciens	**Rhizosphere bacterium, interspecies competition**
<u>Fungicides</u>	
Streptomyces lydicus **WYEC 108**	**Soil bacteria used to control pathogenic fungi eg.** ***Fusarium, Rhizoctonia, Pythium, Phytophthora, Phytomatotricum, Aphanomyces***
Pseudomonas syringae **ESC-10**	**Controls grey mould on fruits**
Pseudomonas chlororaphis	**Rhizosphere bacterium, interspecies competition**

Benefits of mycorrhizae

- better uptake of nutrients and water
- production of growth regulators and other substances that stimulate plant growth
- protection of the root system against soil-borne diseases
- improvement in growth and yielding of crop plants
- improvement in soil fertility and structure – degree of aggregation and mechanical resistance of soil



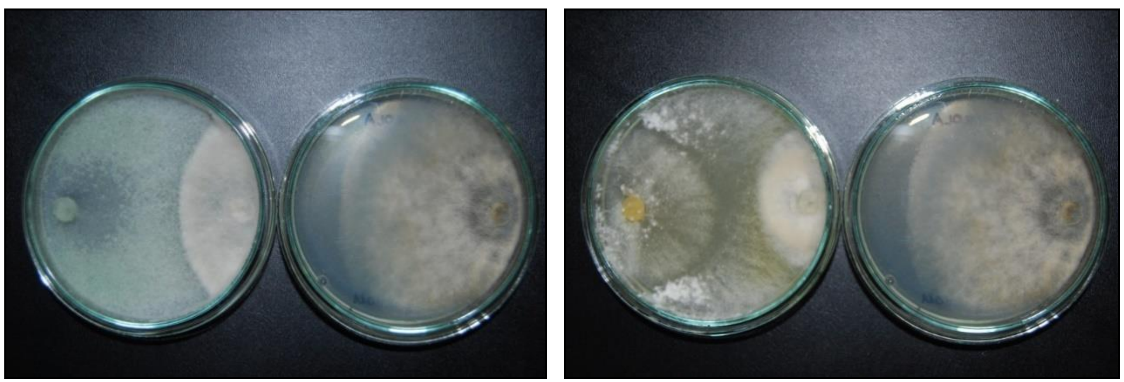


http://shachar-hill.plantbiology.msu.edu/wp-content/uploads/2009/06/plantphyscover11.jpg

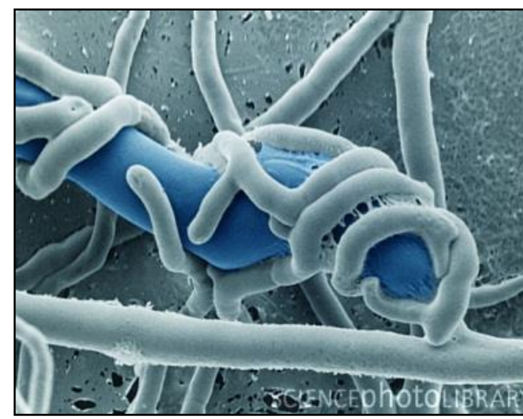
Siddiky et al. 2012; Leung et al. 2013; Yang et al. 2014; Leifheit et al. 2015; Meier et al. 2015

Beneficial action of free-living fungi
Plant Growth-Promoting Fungi

- **Stimulate growth and yielding of crop plants**
- **Solubilize phosphates – species of the genera *Aspergillus* & *Penicillium***
- **Control development of pathogenic fungi, occupy ecological niches, compete for food**
- **Restrict hyphal growth of pathogenic fungi – *Rhizoctonia*, *Botrytis*, *Colleotrichum***



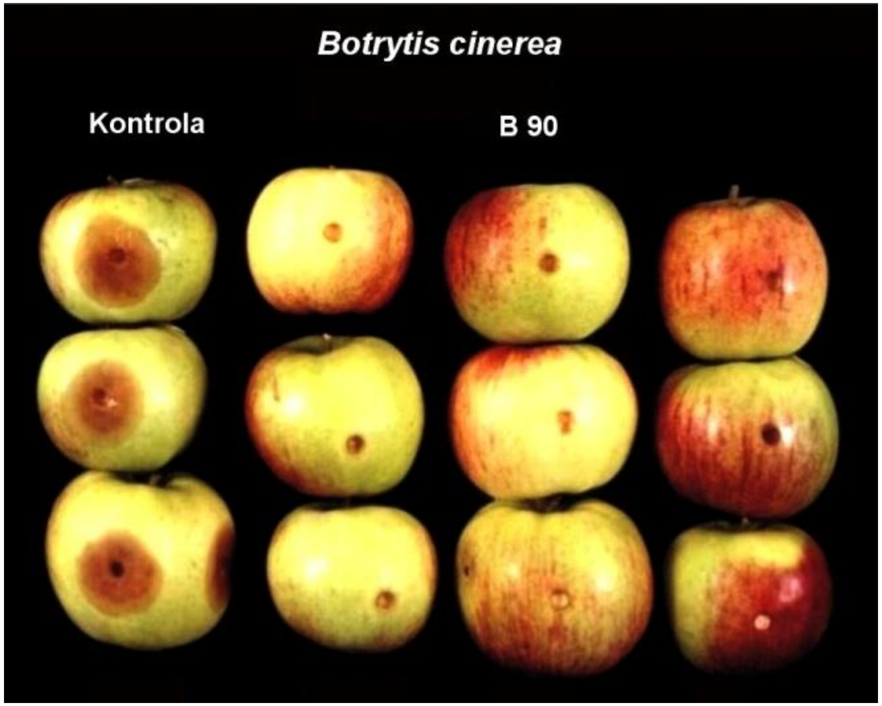
Antagonism of *Trichoderma* fungi towards *Botrytis cinerea* after 6 days of incubation at 26°C on PDA medium. A - *Trichoderma* fungus; B - *Botrytis cinerea* (grey mould)

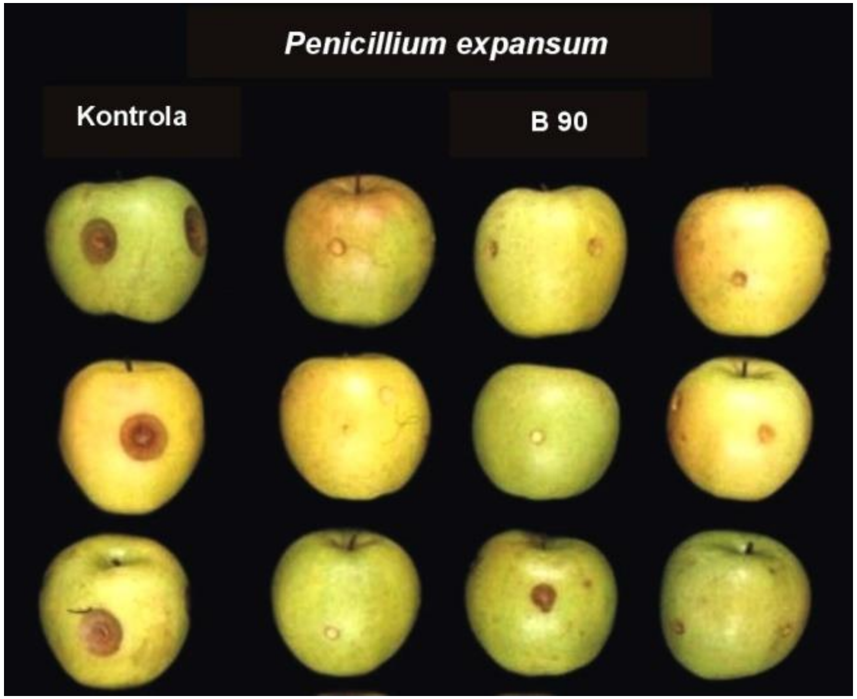


http://www.sciencephoto.com/media/156724/enlarge

Hermosa et al. 2013; Khan & Lee 2013; Zhang et al. 2013; Gong et al. 2014; Martínez-Medina et al. 2014

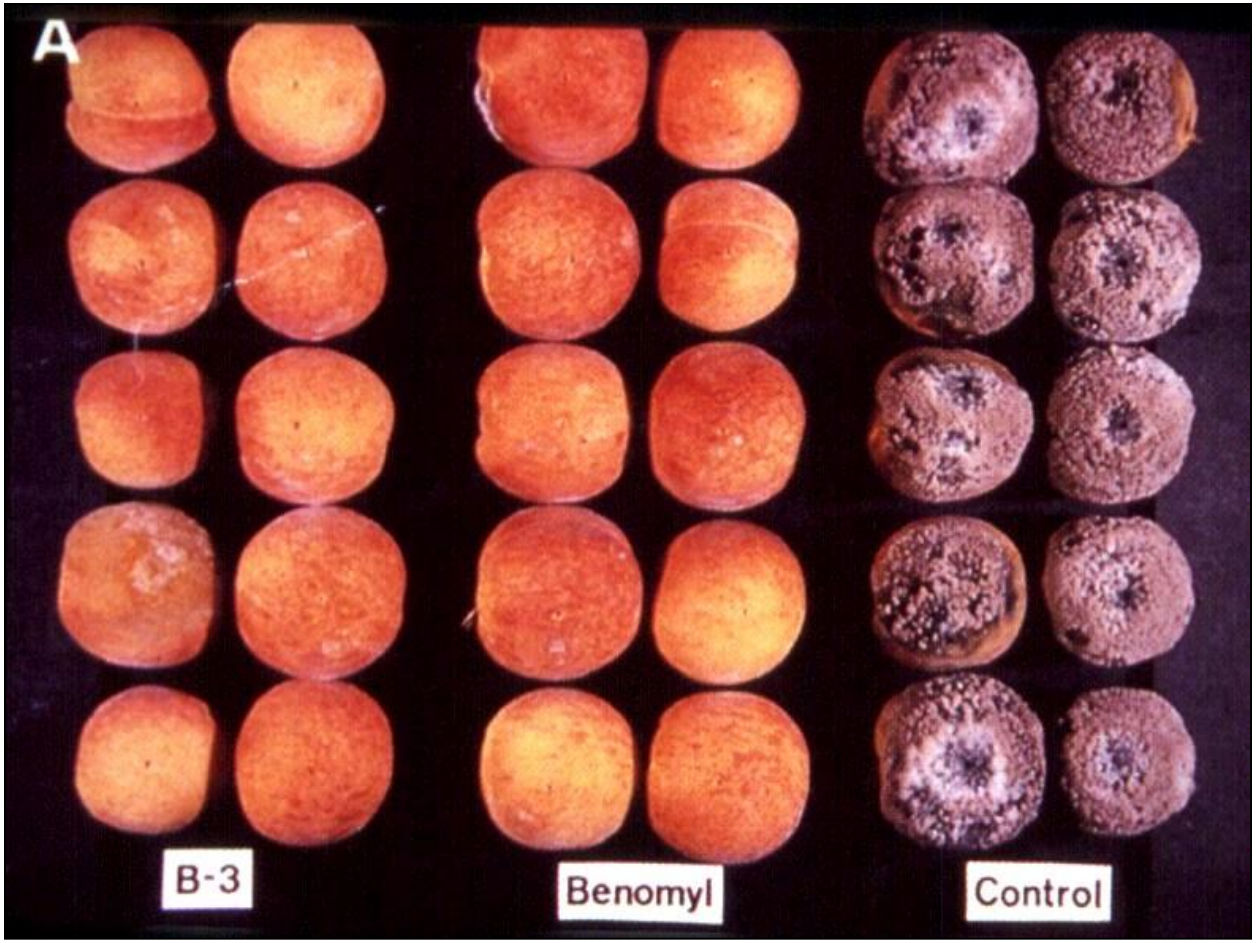
Effective protection of apples against grey mould (*Botrytis cinerea*) and blue mould rot (*Penicillium expansum*) by *Pantoea agglomerans* bacteria





Phot. H. Bryk & D. Rasz-Zając; Sobiczewski et al. 2007; Poppe et al. 2003

Bacillus subtilis protects peaches against brown rot



Pusey 1989; Arrebola et al. 2010; Yánez-Mendizábal et al. 2013

Beneficial microorganisms as components of bioproducts

- **Biofertilizers**
- **Plant growth and yield promoters**
- **Plant resistance stimulants**
- **Biopesticides**
- **Microbiological preparations for preserving fruit and vegetables**
- **Microbial inocula for improving soil quality**

BIOFERTILIZERS

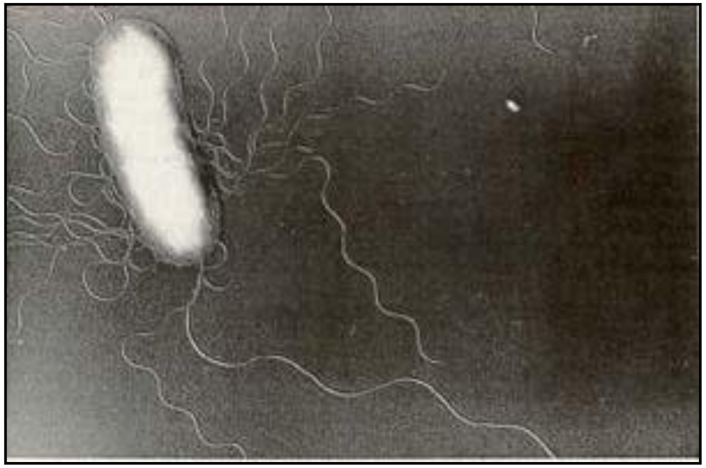
are organic products containing live microorganisms, macro and micronutrients, amino acids, oligopeptides, sugars, vitamins, plant hormones and other biologically active substances.

Biofertilizers applied to seeds, plant surfaces or soil colonize the rhizosphere and roots, increasing nutrient uptake.

Microorganisms with biofertilizing action include, eg.:

- **nitrogen assimilating, symbiotic (*Rhizobia* in *Fabaceae*, *Frankia* in *Alnus*) and free-living (eg.: *Azospirillum*, *Acetobacter diazotrophicus*) bacteria**
- **bacteria and fungi solubilizing forms of phosphorus unavailable to plants**

Rhizobia on roots of leguminous plants



***Azospirillum brasilense* ATCC 29145**

http://web.mst.edu/~microbio/BIO221_1999/A_brasilense.html

Lim et al. 2012; Qiu et al. 2012; Mehnaz 2015

MICROBIOLOGICAL BIOPESTICIDES

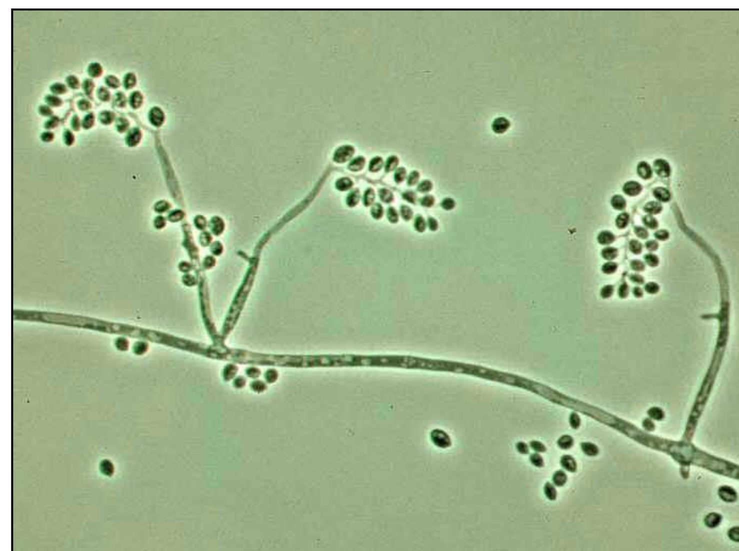
Microbiological biopesticides contain live microorganisms, mainly bacteria and fungi, which are the active ingredients.

They are used as: fungicides, bactericides, herbicides, nematicides, insecticides, etc. These preparations have different mechanisms of action: hyperparasitism, competition for ecological niches and resources, release of biocidal substances into the environment.

http://www.forestryimages.org/browse/detail.cfm?imgnum=1223007

Beauveria bassiana **– fungus parasitizing on insects, used as bioinsecticide**

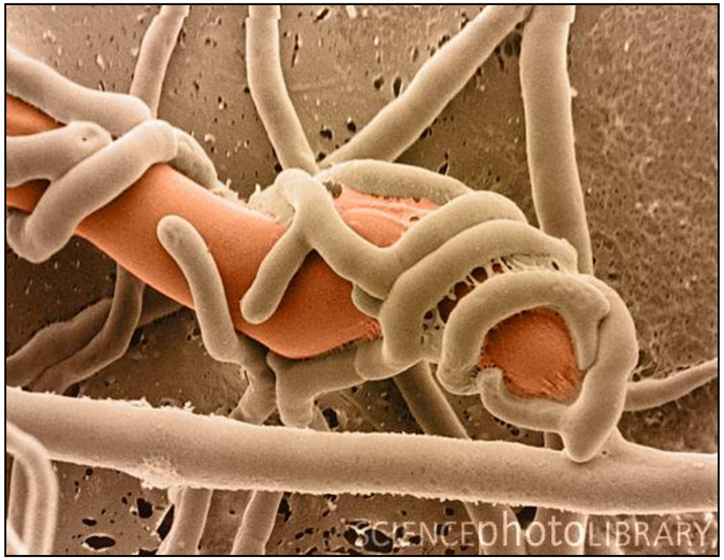
http://www.senasa.gob.pe/0/modulos/JER/JER_Interna.aspx?ARE=0&PFL=2&JER=42



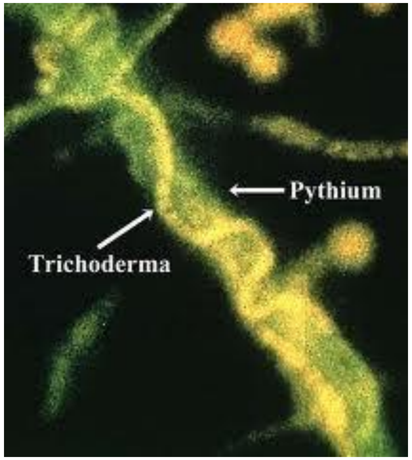
http://ecaaser3.ecaa.ntu.edu.tw/weifang/Hort/screens/thrips.htm

Fang et al. 2012; Harper 2013; Lamareddine et al. 2013; Wang & Leger 2014

MICROBIOLOGICAL BIOPESTICIDES

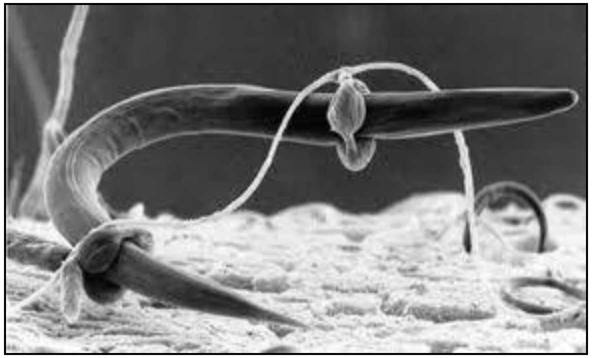


Trichoderma mycoparasite attacking hyphae of *Rhizoctonia*



Trichoderma mycoparasite attacking hyphae of *Pythium*

Preparations containing *Trichoderma* fungi



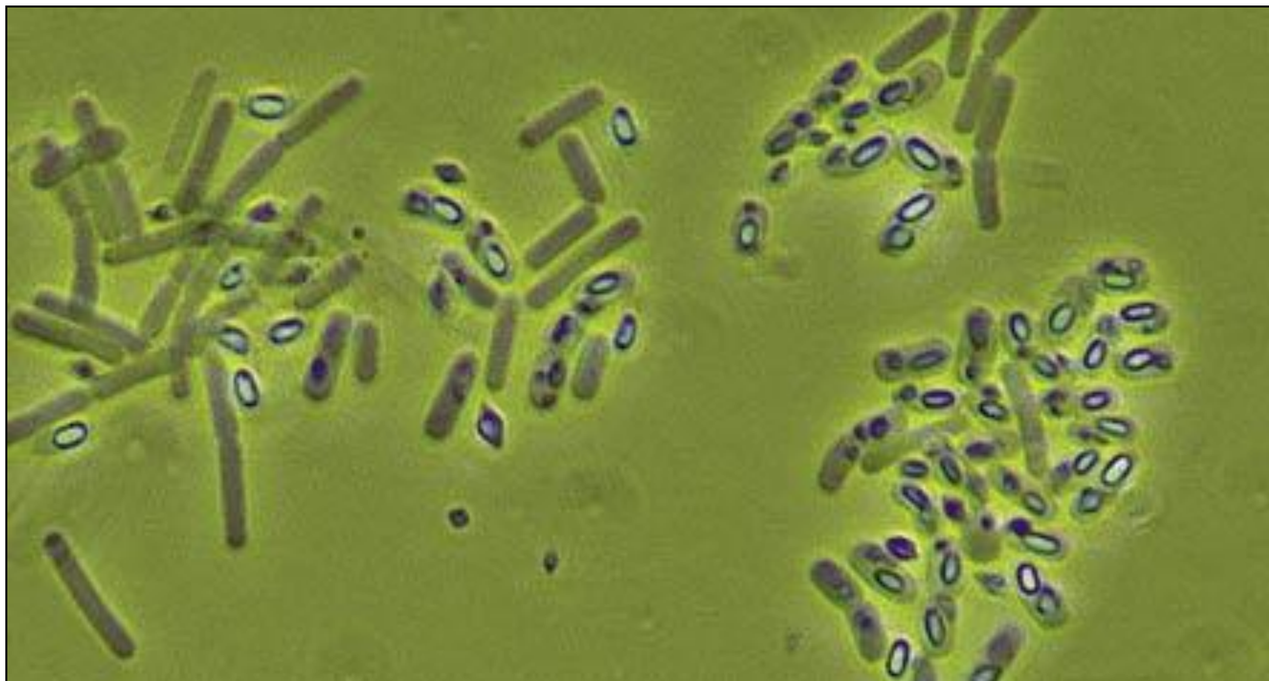
Arthrobotrys predatory fungus specializing in 'fishing' for nematodes

Ji et al. 2014; Woo et al. 2014; XueHui et al. 2014; Soytong 2015

MICROBIOLOGICAL BIOPESTICIDES

- **Biochemical pesticides containing active metabolites of microbial origin (eg. preparations containing toxin of bacterium *Bacillus thuringensis*)**

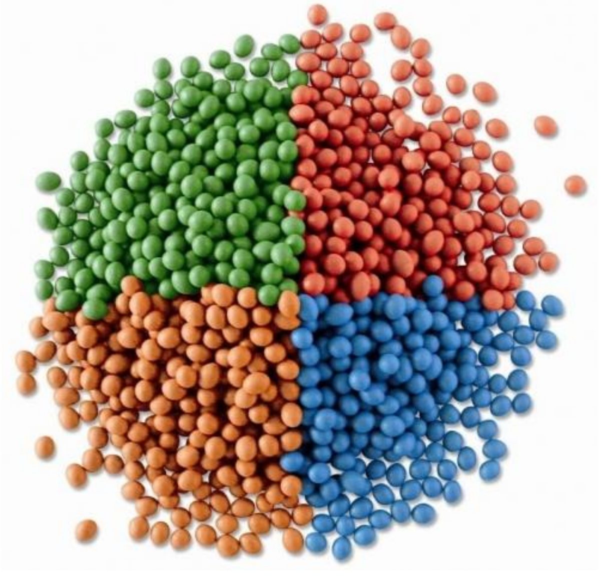
Preparation containing *Bacillus thuringensis*

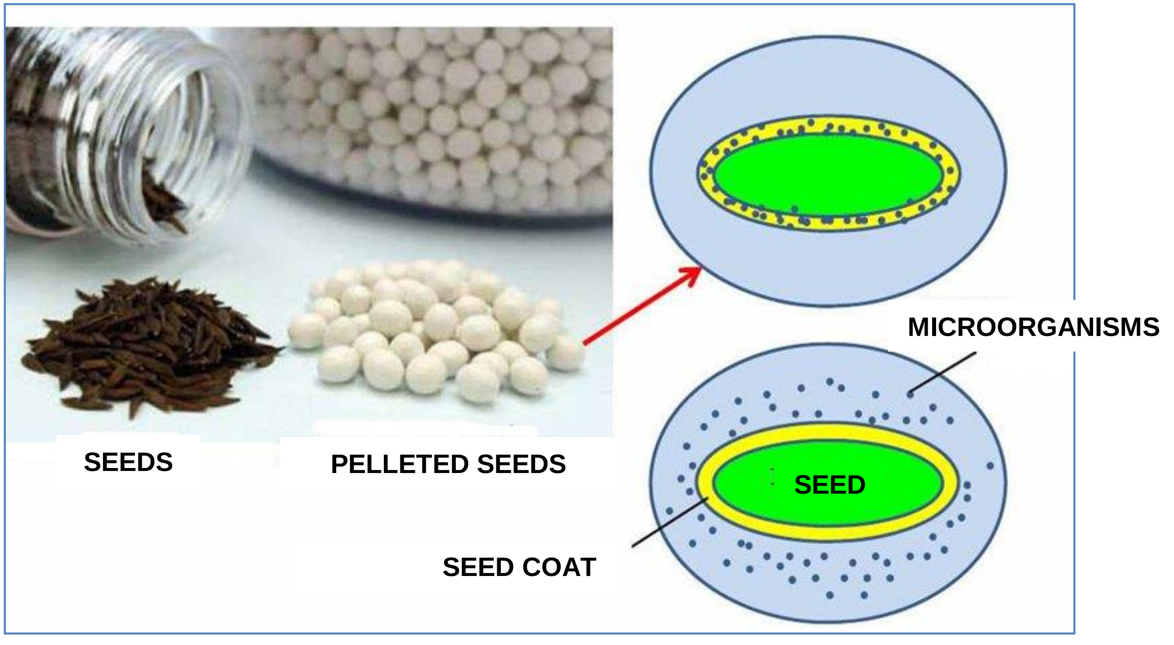


***Bacillus thuringensis* cells**

FUNCTIONAL FORMS OF MICROBIOLOGICAL PREPARATIONS

- **powders, granules, pellets**
- **liquid products (aqueous, oily, colloidal suspensions)**
- **capsules (alginate, gelatin)**
- **pelleted seeds**
- **organic substrates with AMF spores and PGPR**





Mohammadi & Sohrabi 2014; Wang et al. 2015

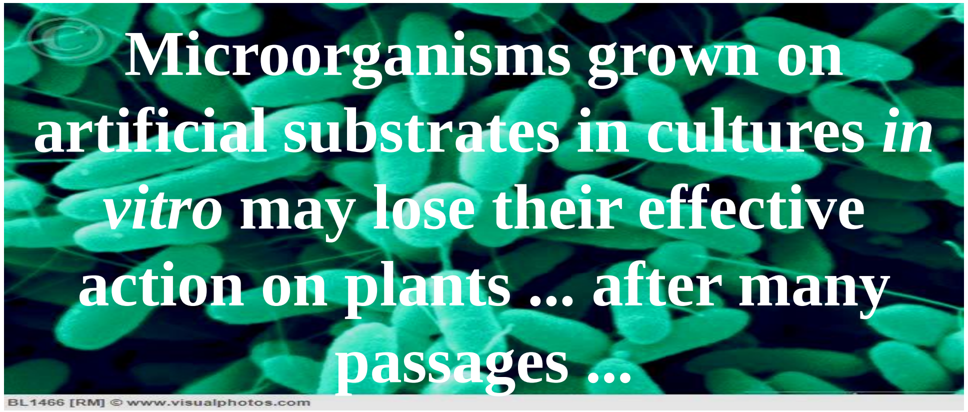
PROBLEMS IN DEVELOPMENT AND PRACTICAL APPLICATION OF MICROBIAL INOCULA

Will the microorganisms intended for use in practice be effective?

product formulation

product stability

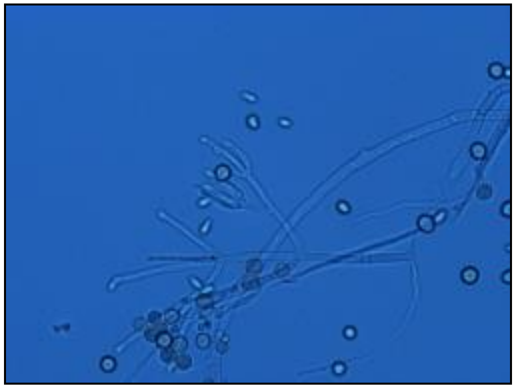
application methods



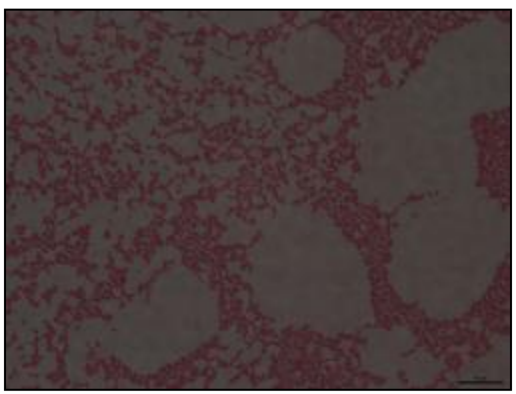
proving a positive effect on plants

Sethi et al. 2014, Owamach et al. 2014

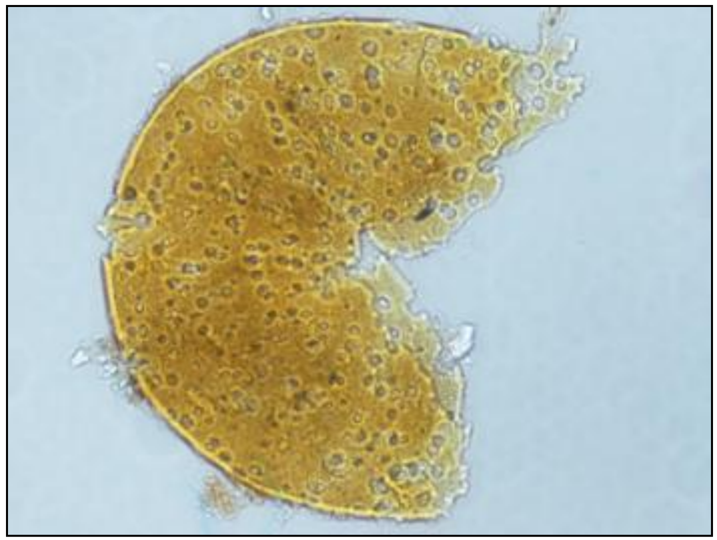
CONSORTIUM STIMULATING PLANT GROWTH AND YIELD



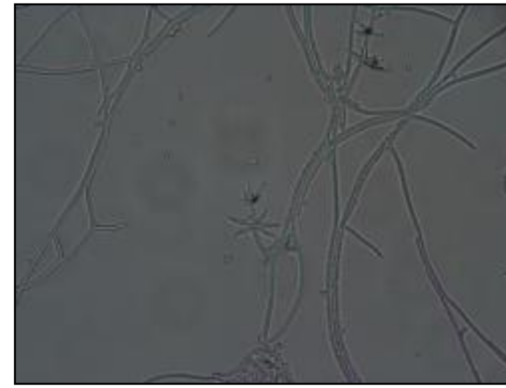
Paecilomyces lilacinus **– limiting populations of nematodes.**



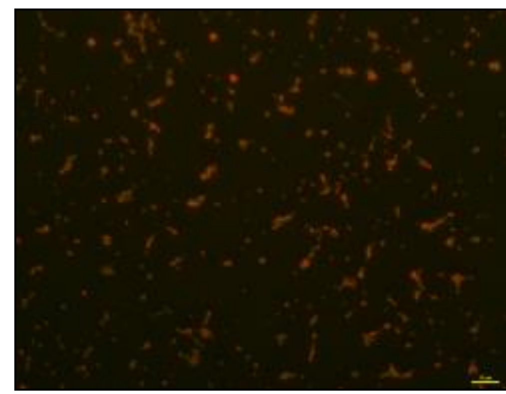
Pseudomonas fluorescens **– stimulating the vegetative growth of plants (stained by Gram's method).**



Funneliformis mosseae

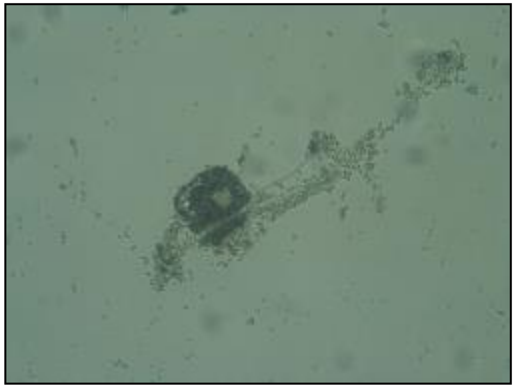


Trichoderma **sp. – limiting populations of pathogenic fungi.**

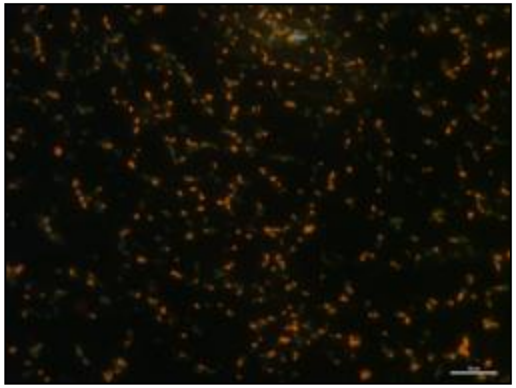


Rahnella aquatillis **– stimulating the vegetative growth of plants (stained with acridine orange).**

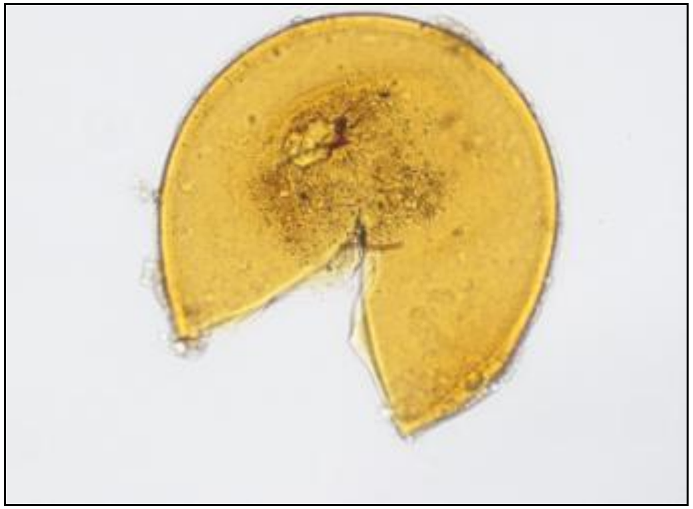
CONSORTIUM MOBILIZING PHOSPHORUS COMPOUNDS IN SOIL



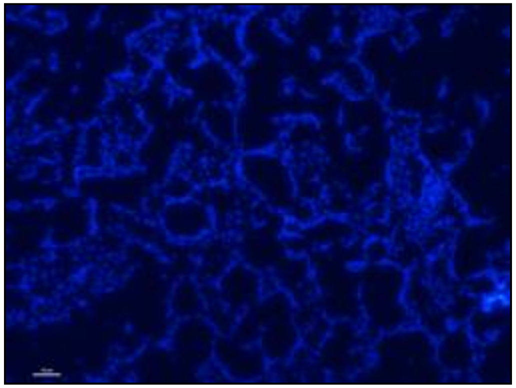
Penicillium sp. – dissolving phosphorus compounds



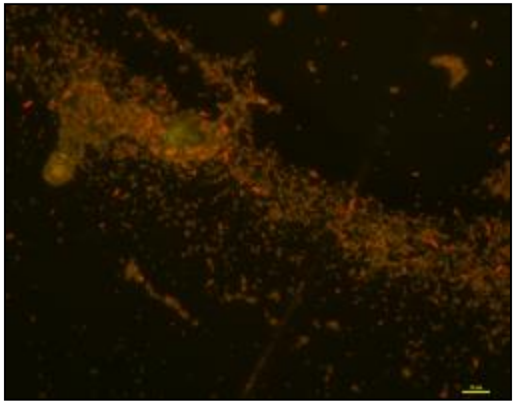
Pseudomonas fluorescens – dissolving phosphorus compounds (stained with acridine orange)



Claroideoglomus claroideum



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



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

CONSORTIUM AGAINST SOIL PATHOGENS

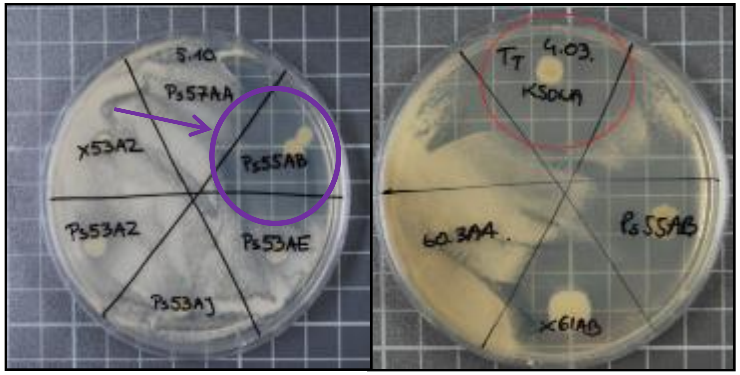
Description and composition of consortium against *Verticillium dahlie, Fusarium oxysporum* and *Botrytis cinerea*: *Serratia plymuthica*, strain x61AF, x61AB, *Pseudomonas* sp, strain K50WA, *Lysobacter* sp, strain 60.3AA, bacteria producing metabolites toxit to fungi and chitinolytic enzymes, actinomycetes N45PO, N45BD, AF45DO, *Bacillus* and other gram+ rods Sp27d, AF74AA, *Paenibacillus* sp, strain AF74AA, strain Sp17DA, AFG1AA, density min. 10^9 cfu/ml and strains of *Trichoderma* fungi WT11AC, Tr43, Tr52 with population size approx. 10^6 cfu/ml.

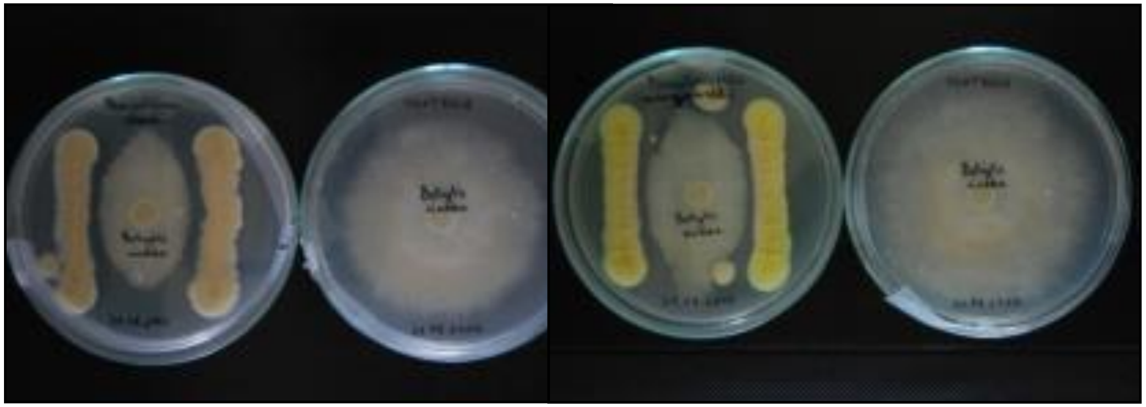
Mode of action: The bacteria produce metabolites toxic to fungi of the genera *Verticillium* and *Fusarium* limiting their growth. *Trichoderma* fungi parasitize on *B. Cinerea*, among others, reducing their occurrence.

Directions for use in crops: Consortia of beneficial microorganisms are applied to the soil (against *Fusarium* and *Verticillium*) or to the leaves (against *Botrytis cinerea*) in the form of 2-10% aqueous solution at a dose of 200-1000 litres per hectare, 2-3 times during the growing season, at 2-3-week intervals, preferably in combination with organic fertilization.

Benefits: Reduction in the occurrence of soil pathogens and stimulation of the development of beneficial microorganisms. Increased resistance to abiotic stresses, particularly drought; improved yield in the cultivation of crops without irrigation.

Antagonistic bacterial strain inhibiting growth of *Verticillium dahliae*





Antagonistic fungi inhibiting growth of *B. cinerea*

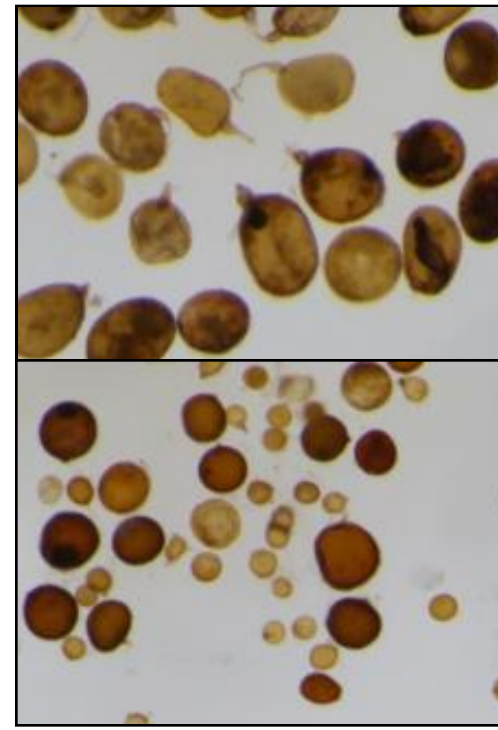
BACTERIAL-MYCORRHIZAL CONSORTIA

PGPR bacteria:

Pi22B	***Pseudomonas fluorescens***
Ps49A	***Pseudomonas fluorescens***
Ps1/2	***Pseudomonas fluorescens***
Pi3A	***Rahnella aquatilis***
x31E	***Rahnella aquatilis***
x31N	***Rahnella aquatilis***
Sp27D	***Bacillus subtilis***

Mycorrhizal fungi:

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



BACTERIAL-MYCORRHIZAL CONSORTIUM for stimulating root growth and rhizosphere processes

Description and composition: species of mycorrhizal fungi – *Claroideoglomus claroideum*, *Gigaspora margarita*, *Septoglomus constrictum*, *Funneliformis mosseae*, *Scutellospora dipurpurescens*, *Glomus macrocarpum*, *Funneliformis caledonius*, *Rhizophagus fasciculatus*, and strains of rhizosphere bacteria and fungi synthesizing auxins and other hormones: *Rahnella aquatilis* Pi3A, x31E, x31N, *Pantoea* sp./*Erwinia* sp. Pi21B, *Pseudomonas* sp. Ps54GF, filamentous fungi *Trichoderma* sp. Tr43, Tr52.

Directions for use: soil or foliar application, 2-10% aqueous solution (200-1000 litres/ha), 2-3 times during the growing season at 2-3 week intervals, preferably in combination with organic fertilization.

Benefits: Increased resistance of plants to water stress, improved rooting, increased absorptive surface area of roots and availability of micronutrients to plants, stimulation of plant growth and resistance to pathogens.

Blossoming sour cherry trees cv. 'Sabina' – Humus Active+Aktywit PM

Root system of apple tree cv. 'Topaz' treated with Humus UP.

Root system of apple tree cv. 'Ariwa' treated with Humus Active + Aktywit PM.

PGPR CONSORTIUM FOR INCREASING AVAILABILITY OF MINERAL IONS

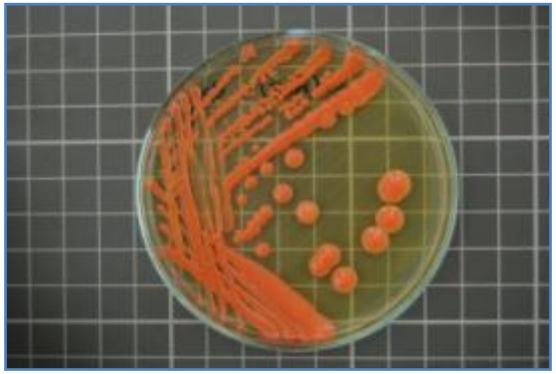
Description and composition: rhizosphere bacteria and mycorrhizal fungi:

- Bacteria *Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptomyces* spp. (10^6 cfu·g^{-1}), microorganisms producing enzymes that degrade cellulose and other compounds: actinomycetes 7G2, TACT11, TACT10, TACT8A, TACT7A, fungi *Trichoderma* WT8, gram- rods 60.3AA *Lysobacter* sp.
- Spores and hyphae of 5 species of AM fungi of the genus *Glomus* and fragments of roots colonized by them.

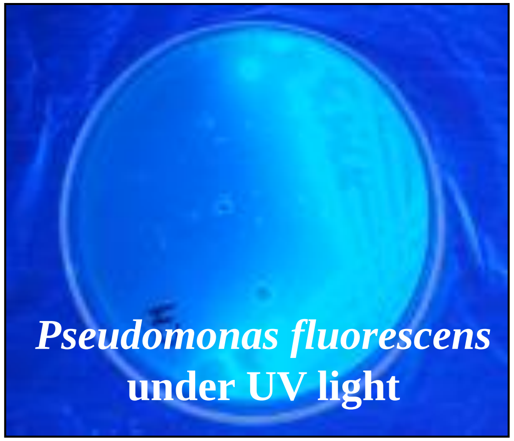
Directions for use: as wettable powder. Addition of 25% solution of molasses or Vinassa or 1% powder milk to aqueous suspension for a few hours prior to treating seeds increases the effectiveness of inoculation. The bacterial-mycorrhizal substrate can be used together with an organic fertilizer, 2-3 times during the season at 2-week intervals at a dose of 50-100 kg/ha; also used for seed dressing.

Benefits of using PGPR: synergistic effects with mycorrhizal fungi, stimulation of mineral uptake by plants, increased development and working range of roots, beneficial effect, especially in non-irrigated crops.

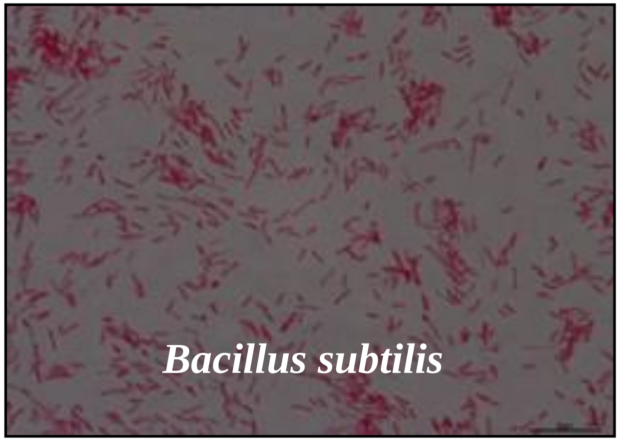
Glomus macrocarpum



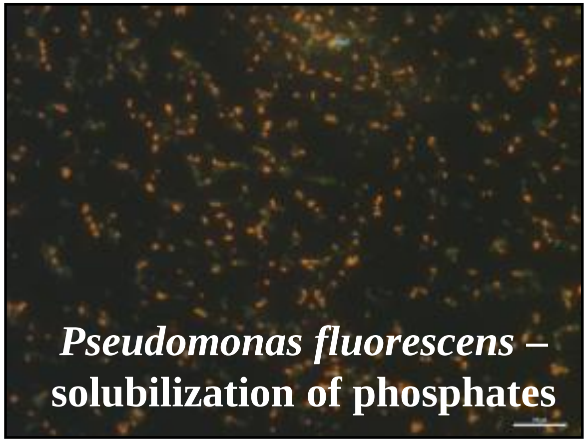
Strain of *Rhodotorulla* sp. (PDA medium)



***Pseudomonas fluorescens* under UV light**



Bacillus subtilis



***Pseudomonas fluorescens* – solubilization of phosphates**

BIOPRODUCTS STIMULATE GROWTH OF ROOTSTOCKS AND SOUR CHERRY AND APPLE TREES IN NURSERIES

BIOPRODUCTS STIMULATE FRUITING OF APPLE TREES

‘Topaz’

‘Ariwa’

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

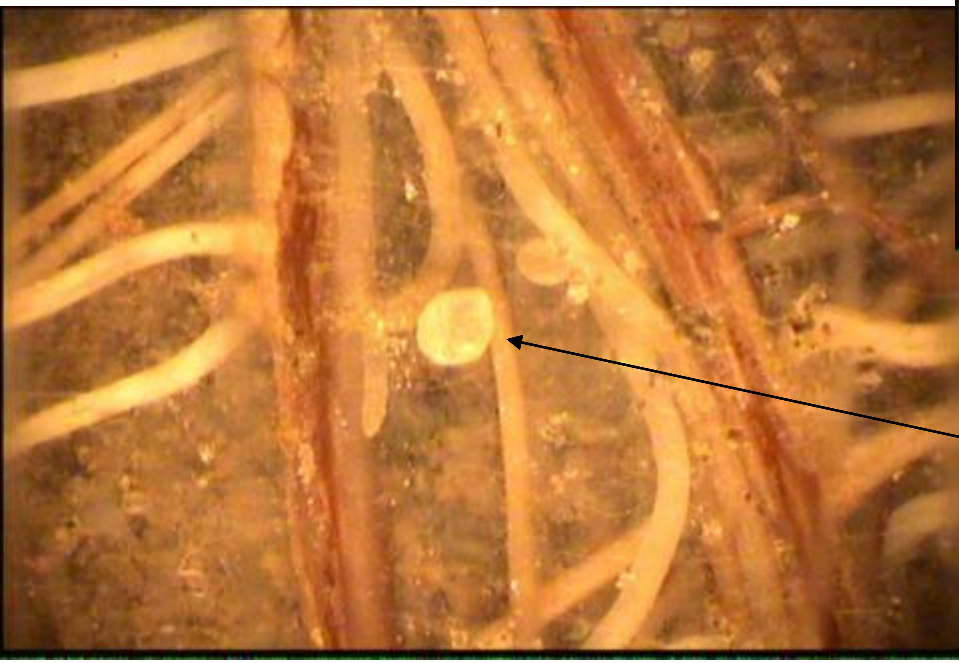
- significantly reduced bud graft mortality in winter, stimulated root development & branching of maiden trees, improving their quality.

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.

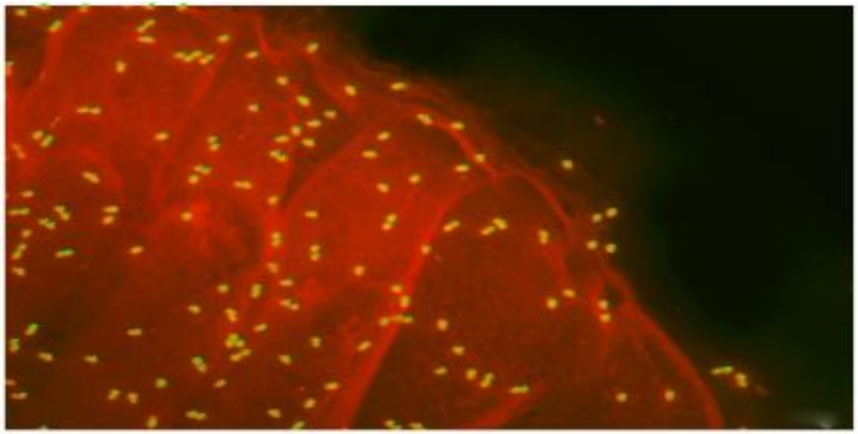
POSITIVE EFFECT OF ORGANIC MULCHES ON GROWTH AND SURVIVAL RATE OF APPLE ROOTS

FINE ROOTS
A THICKER SKELETAL ROOT
IN THE BACKGROUND

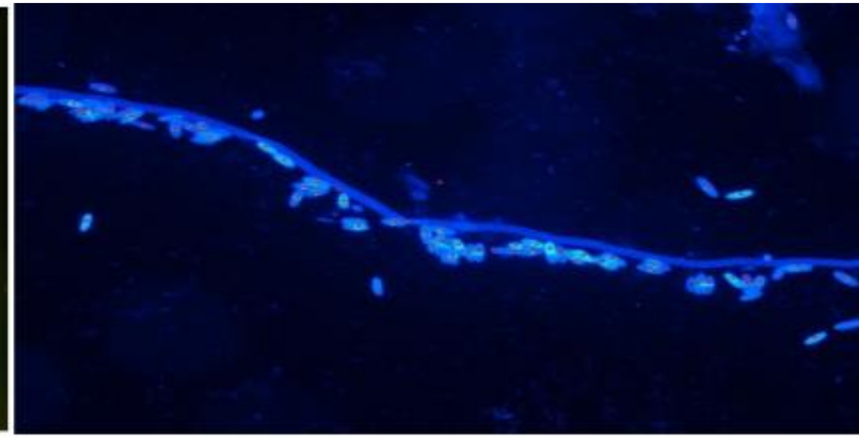


SPORE
OF AN ARBUSCULAR
MYCORRHIZAL FUNGUS

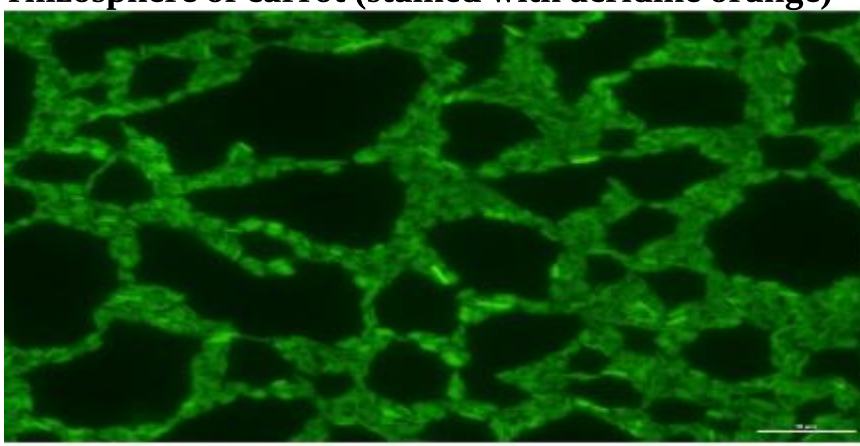
MICROBIAL COMPONENTS OF BIOPRODUCTS



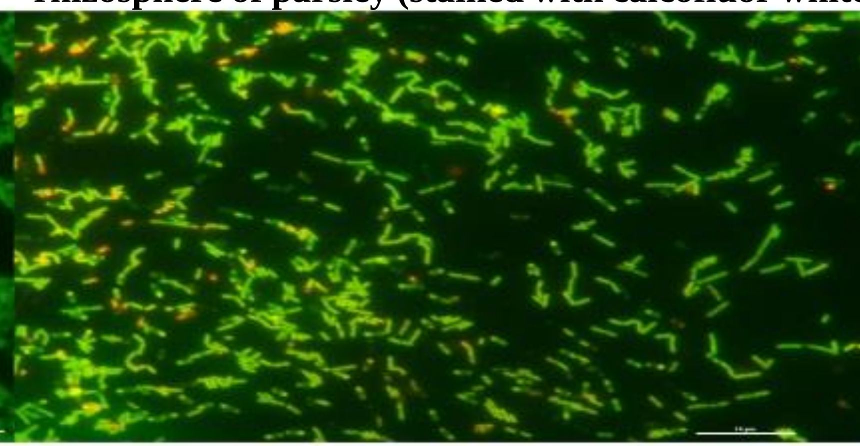
Individual cells of *Azotobacter* sp. bacteria - from rhizosphere of carrot (stained with acridine orange)



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

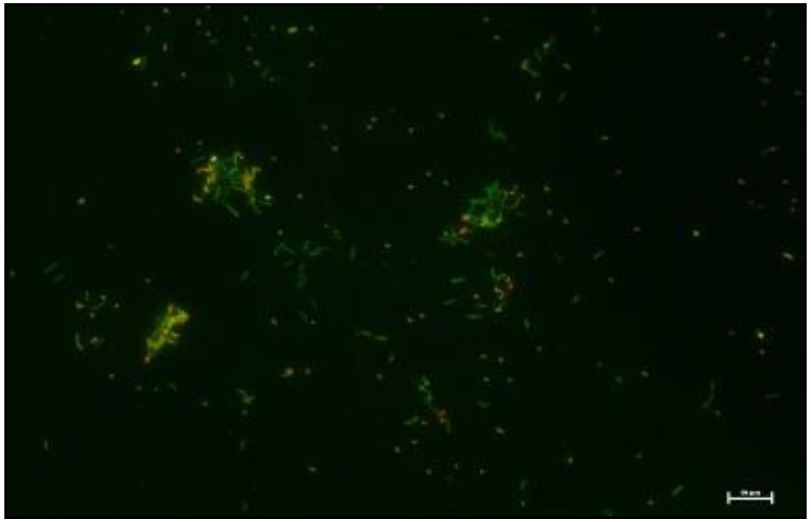


Individual cells of *Bacillus subtilis* bacteria growing on potato-glucose medium (stained with acridine orange).

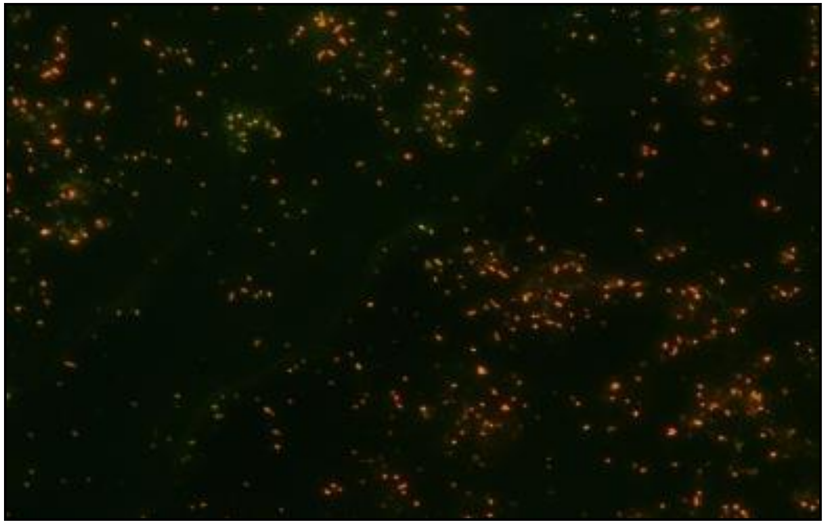


Individual cells of *Bacillus subtilis* bacteria growing on potato-glucose medium (stained with acridine orange).

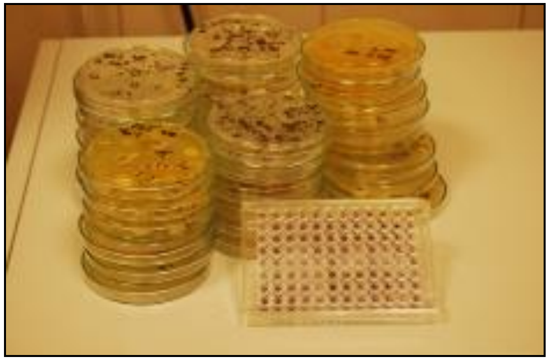
MICROBIAL COMPONENTS OF BIOPRODUCTS



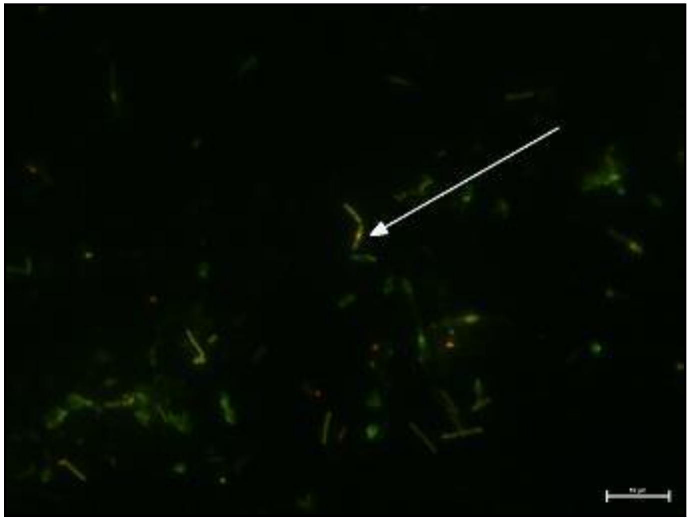
Rhizosphere bacteria in the root zone of carrot (stained with acridine orange)



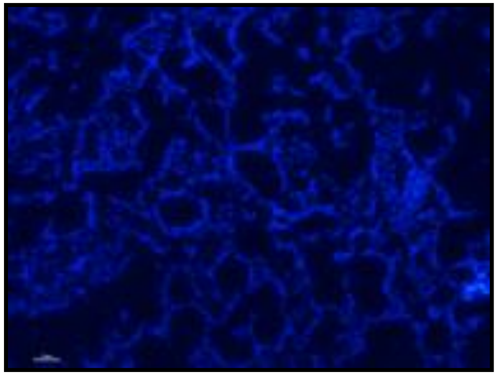
Rhizosphere bacteria from the root zone of parsley (stained with acridine orange)



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



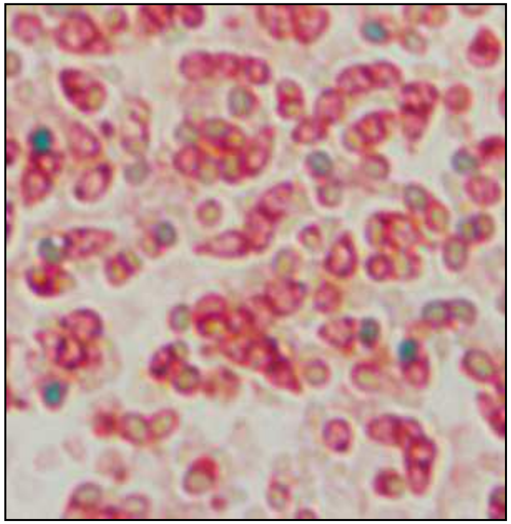
A *Bacillus* bacterial cell with a spore visible



***Pantoea* sp.**

IMAGES OF THE STUDIED GROUPS OF MICROORGANISMS

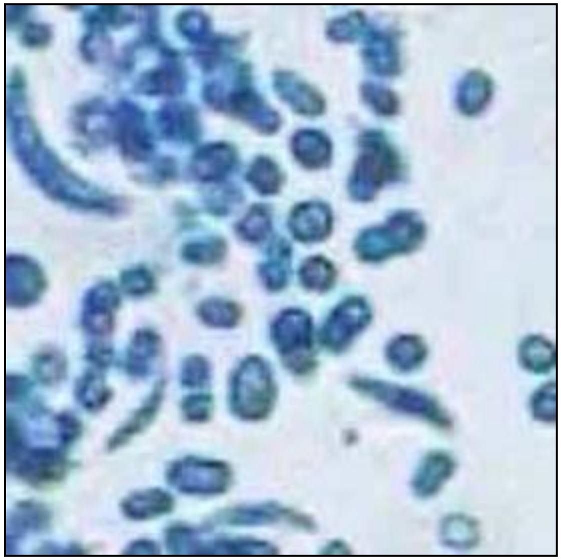
Bacillus megaterium



Megaterium endospors

Rhizobium meliloti

Serratia



Desulfovibrio

Development of innovative bioproducts and technologies for improving the quality of soils in organic cultivation of vegetable crops – 2016

Funded by the Ministry of Agriculture and Rural Development

Research area 1. Organic cultivation of vegetables, including herbs.

Research area 2. Research on innovative solutions for commercial organic cultivation of vegetables and herbs.

Cucumber plants treated with biopreparations that stimulate plant growth and yielding (IO Experimental Field, 2016).

Carrot plants treated with biopreparations that stimulate plant growth and yielding (IO Experimental Field, 2016).

The aim: development of innovative technologies for improving the quality of soils, with the use of bioproducts and beneficial soil microorganisms.

- **Improvement of the growth and yielding of vegetable crops in organic cultivation, using bioproducts**

- **Determination of Soil Quality Indicators in organically grown vegetable crops after application of bioproducts**

- **Development of fertilization recommendations**

METHODOLOGY

Carrot plants 'Nipomo' & cucumber '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*).

The controls were:

- plants not treated with bioproducts (control)

- manure

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

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

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

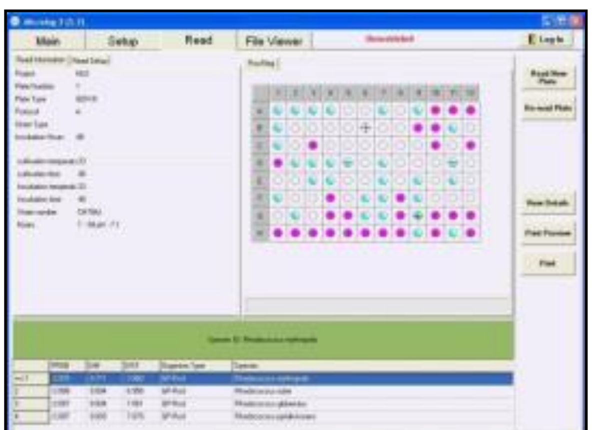
- **Development of bacterial-mycorrhizal inoculum and microbiologically enriched bioproducts for field experiments.**
- **Determination of mycorrhizal frequency of arbuscular mycorrhizal fungi (AMF) in the roots of vegetable plants from field experiments.**
- **Influence of microbiologically enriched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

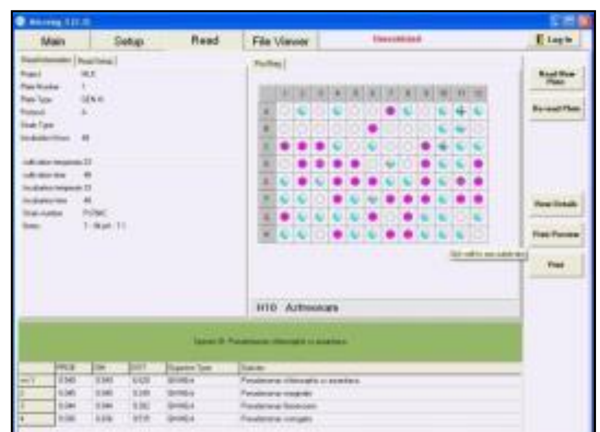
Biological protection of carrot and cucumber plantings against diseases and pests (IO Experimental Field, 2016).

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

RESTORATION OF SOILS WITH LOW ORGANIC MATTER CONTENT IN ORGANIC CULTIVATION OF CUCUMBER AND CARROT, USING MICROBIOLOGICAL BIOPRODUCTS

- **Determination of soil biological properties: abundance of major groups of microorganisms in soil and rizosphere.**
- **Development of methods and substrates for effective multiplication of selected microorganisms.**
- **Identification of microorganisms in soil before and after the application of microbiologically enriched biopreparations.**
- **Monitoring of microbiological stability of newly developed bioproducts and bacterial-fungal inocula.**
- **DNA extraction and DGGE analysis (denaturating gradient gel electrophoresis) of the 16S rRNA bacterial gene.**





Biochemical identification of bacteria and microscopic fungi

Total number of bacteria, actinomycetes, spore-forming bacteria and fungi in the soil from plots where carrot plants were grown

Treatment	Bacteria * × 10^6	Actinomycetes × 10^6	Spore-forming bacteria × 10^5	Microscopic fungi* × 10^4	Yeast × 10^4
	Population size in cfu / g of soil dry weight				
Control (no fertilization)	38.6 a	13.1 a	74.3 a	16.6 b	0.94 c
Bacterial-mycorrhizal inoculum	23.4 a	12.3 a	54.2 a	12.1 c	4.64 b
Microbiologically enriched Bioilsa	36.2 a	10.7 a	88.9 a	24.7 b	11.9 b
Microbiologically enriched compost	30.5 a	12.3 a	51.5 a	37.9 ab	26.0 ab
Microbiologically enriched humic acids	34.5 a	16.7 a	70.8 a	44.2 a	34.3 ab
Microbiologically enriched biochar	12.3 a	4.2 a	28.2 a	25.4 b	20.0 b
Manure	28.6 a	5.1 a	53.9 a	26.3 ab	86.5 a

*Total number of bacteria; *all fungi (filamentous and yeast); ** single yeast colonies in Petri dishes

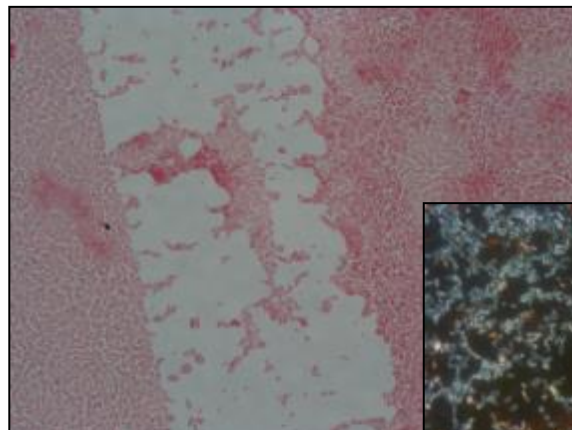
- **Microbiologically enriched Bioilsa, compost, humic acids, biochar showed a tendency to increase the population of fungi in the soil.**

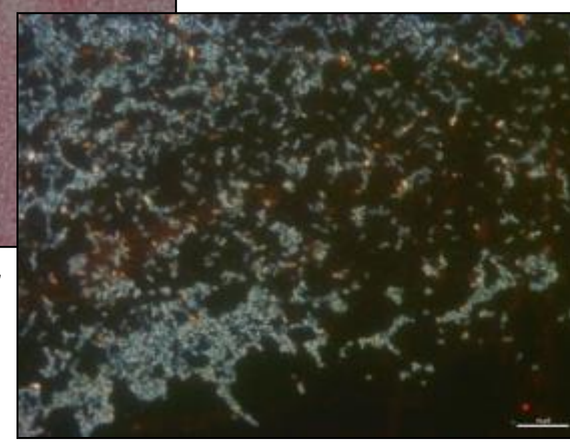
- **All the tested biopreparations increased the yeast population in the soil.**

Number of bacteria of the genus *Pseudomonas* in the soil of the plots where carrots were grown

Treatment	*Pseudomonas* × 10^3	Fluorescent *Pseud.* × 10^3
	Population size in cfu / g of soil dry weight	
Control (no fertilization)	52.8 a	3.5 a
Bacterial-mycorrhizal inoculum	83.0 a	5.7 a
Microbiologically enriched Bioilsa	171.0 b	23.7 b
Microbiologically enriched compost	107.2 ab	12.5 ab
Microbiologically enriched humic acids	95.6 a	3.1 a
Microbiologically enriched biochar	78.5 a	5.3 a
Manure	246.6 c	23.7 c

The biopreparations used showed a tendency to favourably increase the abundance of *Pseudomonas* bacteria in the soil.





Pseudomonas fluorescens

Clean yeast culture

Mean dehydrogenase activity in soil samples from the plots where carrot and cucumber plants grew

Treatment	Mean dehydrogenase activity [µmol TPF g^{-1} d.w. $24h^{-1}$		
	16.06.2016	17.08.2016	10.10.2016
Carrot			
Control (no fertilization)	0.22	0.23	0.18
Bacterial-mycorrhizal inoculum	0.18	0.22	0.17
Microbiologically enriched Bioilsa	0.23	0.20	0.19
Microbiologically enriched compost	0.16	0.31	0.20
Microbiologically enriched humic acids	0.17	0.19	0.20
Microbiologically enriched biochar	0.26	0.24	0.13
Manure	0.15	0.24	0.24
Cucumber			
Control (no fertilization)	0.21	0.28	0.31
Bacterial-mycorrhizal inoculum	0.24	0.41	0.32
Microbiologically enriched Bioilsa	0.28	0.50	0.39
Microbiologically enriched compost	0.33	0.54	0.44
Microbiologically enriched humic acids	0.33	0.51	0.34
Microbiologically enriched biochar	0.33	0.62	0.37
Manure	0.31	0.62	0.40

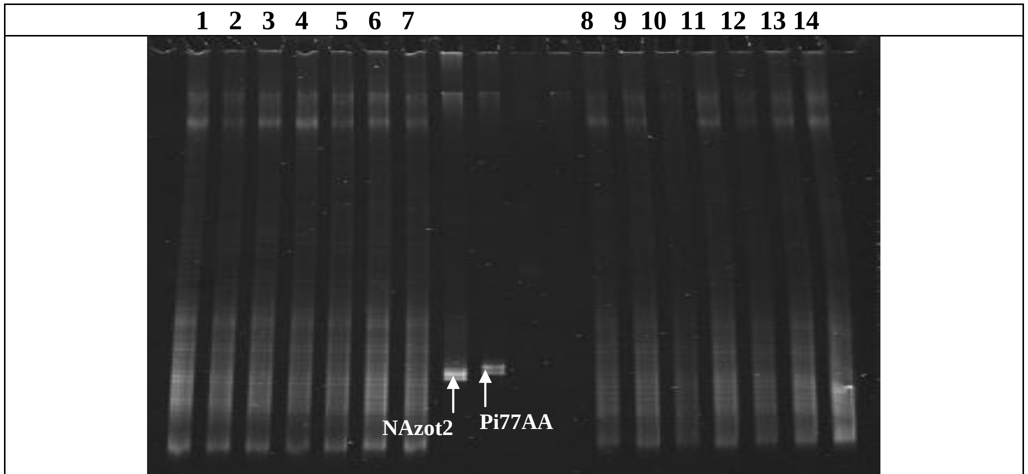
- **The applied biopreparations increased activity of the dehydrogenase enzyme in August in the soil collected from the plots on which ‘Adam’ cucumber plants were grown.**
- **This correlation was not observed in the samples of the soil in which carrot plants grew.**

Effect of carrier and temperature on the abundance and survival of bacteria after 60 days

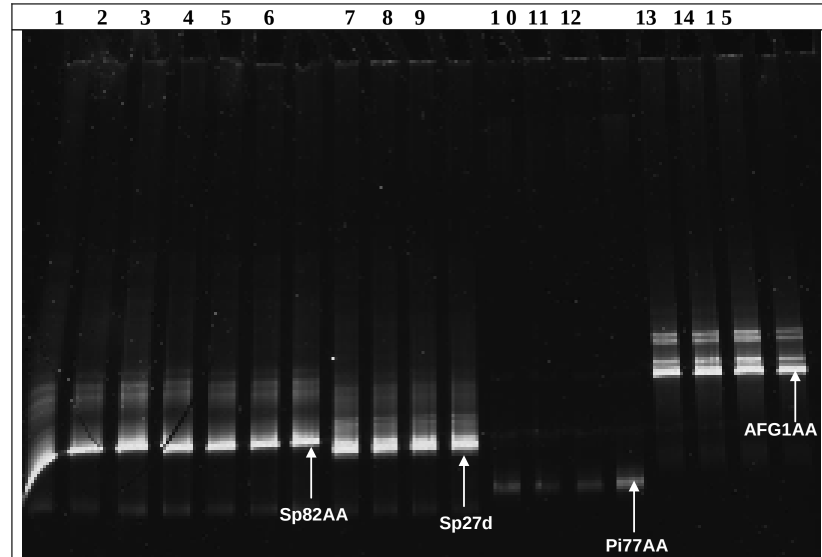
Sample	Number of bacteria on Day 1 × 10^5 cfu · g^{-1}	Number of bacteria on Day 60 × 10^5 cfu · g^{-1}	Survival rate [%]
Ps1/2 lyophilized (room temperature)	**1.27 ± 0.15**	**1.18 ± 0.34**	**93%**
Ps1/2 lyophilized + charcoal (room temperature)	**142.5 ± 3.54**	**144.81 ± 12.1**	**100%**
Ps1/2 lyophilized + charcoal (4°C)	**142.5 ± 3.54**	**114.28 ± 19.68**	**80%**
Sp82AA lyophilized (room temperature)	**3437.071 ± 68.1**	**3295.37 ± 56.9**	**96%**
Sp82AA lyophilized + charcoal (room temperature)	**556.22 ± 36.11**	**510.59 ± 67.36**	**92%**
Sp82AA lyophilized + charcoal (4°C)	**556.22 ± 36.11**	**395.05 ± 12.28**	**71%**
Sp27d lyophilized (room temperature)	**744.35 ± 58.1**	**695.2 ± 14.91**	**93%**
Pi77AA lyophilized (room temperature)	**1.8 ± 0.08**	**1.59 ± 0.08**	**88%**
AFG1AA lyophilized (room temperature)	**89.5 ± 7.78**	**74.68 ± 3.14**	**84%**

- **The results showed high survival rate of bacterial strains in the soil and in the bioproducts after two-month storage at various temperatures.**

Monitoring of microorganisms in the soil before and after application of microbiologically enriched biopreparations



Rys. 2. Profile DGGE uzyskane w wyniku amplifikacji genu 16S rRNA ze starterami GC357-F /907-R. 1-7: DNA z prób gleby pobranej z okolic korzeni marchwi (7 kombinacji), 8-14: DNA z prób gleby pobranej z okolic korzeni ogórka (7 kombinacji). Strzałkami oznaczono produkty amplifikacji kontrolnych szczepów bakterii.



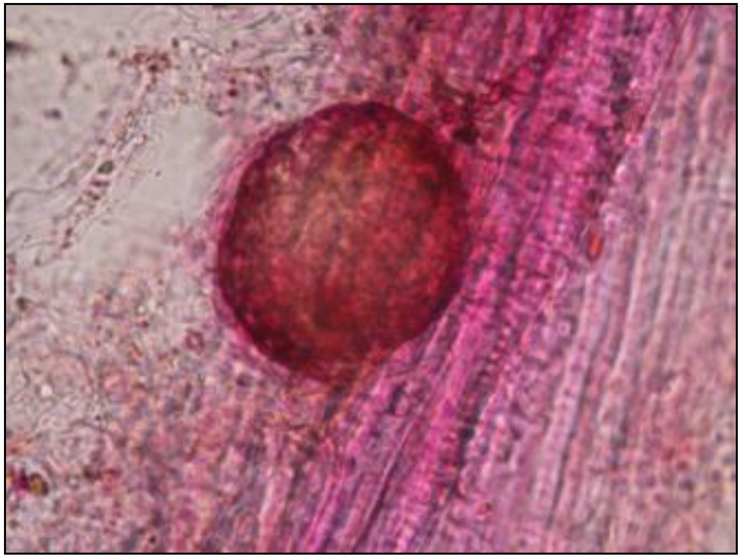
Rys. 4. Profile DGGE uzyskane w wyniku amplifikacji genu 16S rRNA ze starterami GC357-F /907-R. 1 – 15 oznaczenia bioproduktów. Strzałkami oznaczono produkty amplifikacji kontrolnych szczepów bakterii.

The results showed that NAzot2 strain of *Klebsiella* sp. bacteria exhibited viability in the soil for over two months after the application of the bioproducts.

Effect of the newly developed bioproducts on mycorrhizal frequency (F%) and mycorrhizal intensity (M%, m%) in the roots of 'Nipomo' carrot plants (IO Experimental Field, 2016)

Treatment	F%	M%	m%
Control	11.11 a	0.01 a	0.33 a
Bacterial-mycorrhizal inoculum	22.22 ab	0.2 ab	6.6 ab
Microbiologically enriched Bioilsa	21.11 ab	0.1 ab	3.3 ab
Microbiologically enriched compost + 10% Biochar	21.11 ab	0.1 ab	3.3 ab
Microbiologically enriched humic acids (5%)	21.11 ab	0.1 ab	3.3 ab
Microbiologically enriched biochar	21.11 ab	0.1 ab	3.3 ab
Manure	21.11 ab	0.1 ab	3.3 ab

After the application of bacterial-mycorrhizal inoculum, the roots of carrot plants were more frequently colonized by arbuscular mycorrhizal fungi than the roots of control plants.

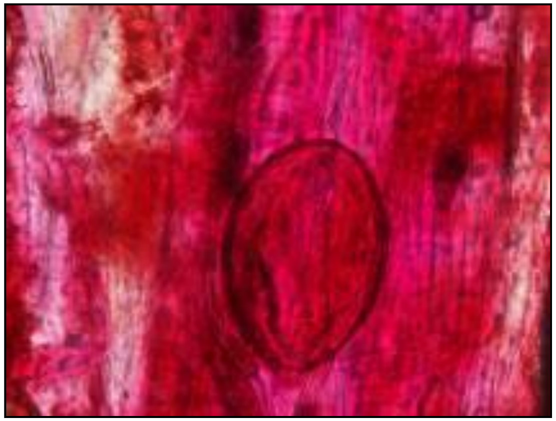


Spore in the roots of 'Nipomo' carrot after application of bacterial-mycorrhizal inoculum

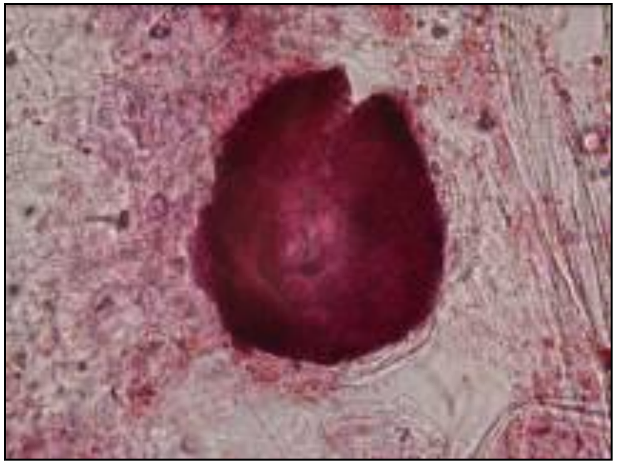
Effect of th enewly developed bioproducts on mycorrhizal frequency (F%) and mycorrhizal intensity (M%, m%) in the roots of 'Adam' cucumber plants (IO Experimental Field, 2016)

Treatment	F%	M%	m%
Control	18.89 a	0.09 a	1.0 a
Bacterial-mycorrhizal inoculum	27.78 c	0.18 b	1.0 a
Microbiologically enriched Bioilsa	24.44 b	0.14 ab	1.0 a
Microbiologically enriched compost + 10% Biochar	23.33 ab	0.13 a	1.0 a
Microbiologically enriched humic acids (5%)	27.78 c	0.18 b	1.0 a
Microbiologically enriched biochar	22.22 ab	0.12 a	1.0 a
Manure	24.44 b	0.14 ab	1.0 a

Compared to the control, the application of bacterial-mycorrhizal inoculum and microbiologically enriched humic acids had the greatest influence on the increase in mycorrhizal frequency in the roots of 'Adam' cucumber plants.



Vesicle in the roots of 'Nipomo' carrot after application of bacterial-mycorrhizal inoculum



Spore in the roots of 'Adam' cucumber after application of bacterial-mycorrhizal inoculum

Mycelium in the roots of 'Adam' cucumber after application of bacterial-mycorrhizal inoculum

Effect of microbiologically enriched products on the intensity of photosynthesis and maximum efficiency of photosystem II of 'Adam' cucumber plants (IO Experimental Field, August 2016)

Treatment	Intensity of photosynthesis (µmol CO_2 m^{-2} s^{-1})	Maximum efficiency of photosystem II (Fv/Fm)
Control	10.51 b	0.70 a
Bacterial-mycorrhizal inoculum	14.45 f	0.71 a
Microbiologically enriched Bioilsa	13.24 e	0.72 ab
Microbiologically enriched compost + 10% Biochar	12.07 c	0.84 c
Microbiologically enriched humic acids	16.47 g	0.80 bc
Microbiologically enriched biochar	12.14 d	0.83 c
Manure	10.27 a	0.82 bc

- **The highest intensity of photosynthesis for cucumber was obtained after the application of microbiologically enriched humic acids, microbiologically enriched compost in combination with biochar, and after applying microbiologically enriched biochar.**

Effect of microbiologically enriched bioproducts on the growth characteristics of above-ground parts of 'Nipomo' carrot plants (IO Experimental Field, 2016)

Treatment	Fresh weight of above-ground parts [g]	Dry weight of above-ground parts [g]	Surface area of above-ground parts [cm^2]
Control	20.15 a	6.16 a-c	697.61 b
Bacterial-mycorrhizal inoculum	16.73 a	5.68 a-c	573.36 b
Microbiologically enriched Bioilsa	26.86 a	7.85 bc	749.87 bc
Microbiologically enriched compost + 10% Biochar	20.45 a	4.91 ab	630.53 b
Microbiologically enriched humic acids	27.18 a	8.31 c	934.43 c
Microbiologically enriched biochar	23.20 a	7.53 bc	726.39 b
Manure	15.60 a	3.46 a	384.97 a

'Nipomo' carrot plants after application of microbiologically enriched compost

'Nipomo' carrot plants after application of microbially enriched humic acids

The use of microbiologically enriched humic acids increased the growth characteristics of the above-ground parts of carrot plants.

Effect of microbiologically enriched bioproducts on root growth characteristics of 'Nipomo' carrot plants (IO Experimental Field, 2016)

Treatment	Root fresh weight [g]	Root dry weight [g]	Root length [cm]	Root surface area [cm^2]	Root diameter [mm]	Root volume [cm^3]	Number of root tips
Control	155.43 a	46.20 a	18.87 a	171.60 a	33.32 a	113.56 a	419 a
Bacterial-mycorrhizal inoculum	179.86 a	52.51 a	18.81 a	184.32 a	36.80 ab	122.84 ab	352 a
Microbiologically enriched Bioilsa	227.36 a	86.58 a	20.96 a	212.57 a	42.09 b	135.60 b	409 a
Microbiologically enriched compost + 10% Biochar	199.33 a	57.91 a	20.50 a	199.99 a	38.41 ab	131.58 ab	316 a
Microbiologically enriched humic acids	219.16 a	81.30 a	18.09 a	196.28 a	43.17 b	142.15 b	359 a
Microbiologically enriched biochar	193.43 a	53.63 a	18.42 a	188.92 a	39.31 ab	128.07 ab	383 a
Manure	196.20 a	43.55 a	18.02 a	185.17 a	41.79 b	134.89 b	406 a

The highest values for root growth parameters of carrot plants were obtained after the application of microbiologically enriched biofertilizer Bioilsa.

'Nipomo' carrot plants treated with microbiologically enriched biochar

'Nipomo' carrot plants after application of microbiologically enriched biofertilizer Bioilsa

Effect of microbiologically enriched bioproducts on marketable yield and fresh weight of 'Nipomo' carrot plants (IO Experimental Field, 2016)

Treatment	Marketable yield [no./plot]	Marketable yield [kg/plot]	Non-marketable yield [no./plot]	Non-marketable yield [kg/plot]
Control	37.0 a	8.1 a	22.5 ab	5.6 bc
Bacterial-mycorrhizal inoculum	87.0 c	13.7 b	22.8 ab	4.3 a-c
Microbiologically enriched Bioilsa	45.3 ab	9.5 ab	30.3 b	6.2 c
Microbiologically enriched compost + 10% Biochar	76.0 bc	12.8 ab	11.5 a	2.6 a
Microbiologically enriched humic acids	51.0 ab	11.0 ab	22.8 ab	5.0 a-c
Microbiologically enriched biochar	73.3 bc	13.3 b	18.0 ab	3.6 ab
Manure	64.3 a-c	11.5 ab	20.8 ab	4.3 a-c

'Nipomo' carrot control plants

'Nipomo' carrot plants treated with bacterial-mycorrhizal inoculum.

- The use of bacterial-mycorrhizal inoculum resulted in a significant increase in marketable yield of carrot.
- Application of microbiologically enriched Bioilsa increased non-marketable yield.

Effect of microbiologically enriched bioproducts on marketable and non-marketable crop weight of 'Adam' cucumbers (IO Experimental Field, 2016)

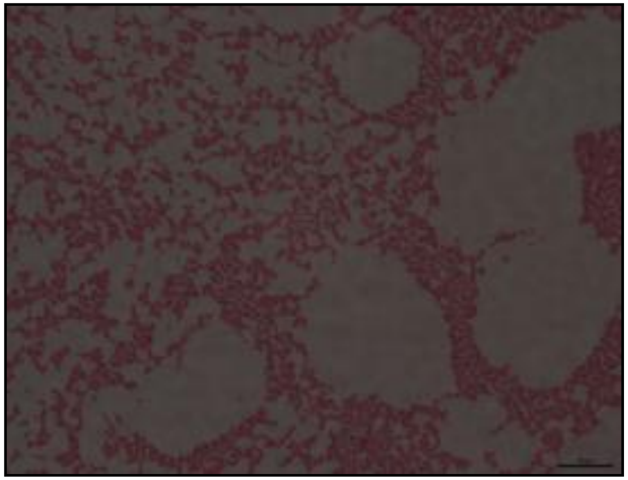
Treatment	Marketable crop weight [kg]	Non-marketable crop weight [kg]	Total crop weight [kg]
Control	7.0 ab	4.9 d	13.0 b
Bacterial-mycorrhizal inoculum	8.4 bc	4.3 cd	12.7 b
Microbiologically enriched Bioilsa	8.5 bc	3.5 a-c	12.0 ab
Microbiologically enriched compost + 10% Biochar	6.9 ab	2.5 ab	9.4 ab
Microbiologically enriched humic acids	7.6 ab	3.6 bc	11.2 ab
Microbiologically enriched biochar	5.3 a	2.5 ab	7.8 a
Manure	5.9 a	2.2 a	8.1 a

Cucumber fruit after the use of bacterial-mycorrhizal inoculum (left) and microbiologically enriched Bioilsa (right); IO Experimental Field, 2016

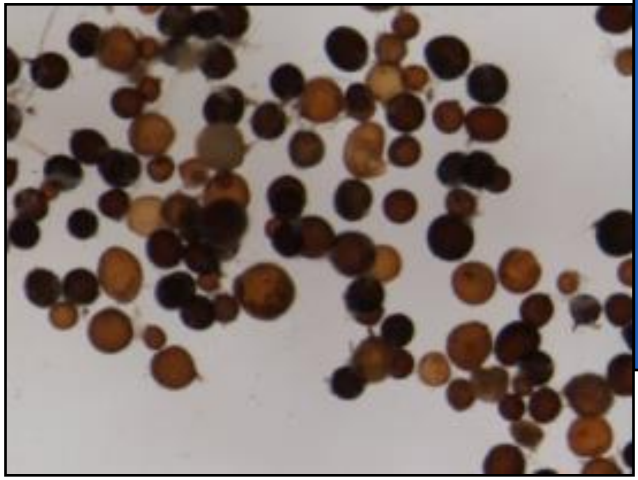
The use of bacterial-mycorrhizal inoculum and microbiologically enriched Bioilsa resulted in an increase in the marketable crop weight of 'Adam' cucumbers.

DETERMINATION OF SOIL QUALITY INDICATORS IN ORGANICALLY GROWN VEGETABLE CROPS BEFORE AND AFTER APPLICATION OF MICROBIOLOGICALLY ENRICHED BIOPRODUCTS

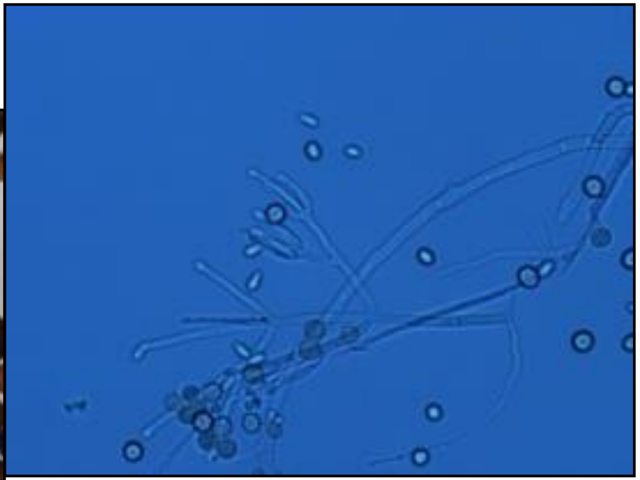
- **Assessment of biochemical properties of soils in organic vegetable crops.**
- **Determination of chemical properties of soils and plant material (pH, macroelements, microelements).**
- **<u>Assessment of the abundance of microorganisms before and after the use of bioproducts</u>.**



Pseudomonas fluorescens



Spores of mycorrhizal fungi

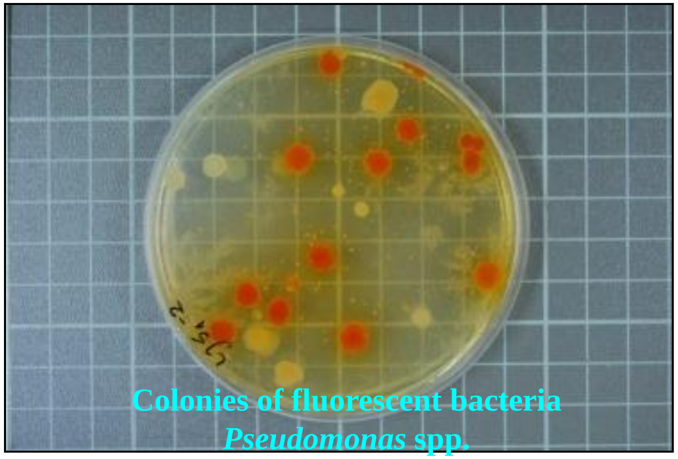


Paecilomyces lilacinus

Abundance of *Pseudomonas* bacteria from plots before and after seed sowing

Treatment	Before seed sowing		After seed sowing	
	Pseudomonas × 10^4	Fluorescent *Pseud.* × 10^4	*Pseudomonas* × 10^4	Fluorescent *Pseud.* × 10^4
	Population size in cfu / g of soil dry weight			
Control (no fertilization)	22.6 d	5.1 b	4.4 b	1.1 a
Bacterial-mycorrhizal inoculum	22.1 d	3.5 c	9.7 b	4.7 a
Microbiologically enriched Bioilsa	138.7 a	84.4 a	11.1 b	2.8 ab
Microbiologically enriched compost	21.6 d	2.1 d	5.7 b	1.6 a
Microbiologically enriched humic acids	78.3 b	3.2 c	5.2 b	1.3 a
Microbiologically enriched biochar	106.2 ab	51.4 a	9.5 b	2.4 ab
Manure	42.5 c	6.5 b	32.7 a	3.9 ab

- **Application of microbiologically enriched Bioilsa, biochar and manure had a significant influence on the increase in the growth of *Pseudomonas* bacteria.**



Colonies of fluorescent bacteria *Pseudomonas* spp.

Total number of bacteria, actinomycetes and spore-forming bacteria in the soil before and after sowing cucumber seeds

Treatment	Before seed sowing			After seed sowing		
	Bacteria * $\times 10^6$	Actinomycetes $\times 10^6$	Spore-forming bacteria $\times 10^5$	Bacteria * $\times 10^6$	Actinomycetes $\times 10^6$	Spore-forming bacteria $\times 10^5$
	Population size in cfu / g of soil dry weight					
Control (no fertilization)	31.9 b	4.7 c	36.2 b	35.1 a	8.0 a	47.1 a
Bacterial-mycorrhizal inoculum	72.0 a	13.7 a	73.4 a	23.5 a	13.7 ab	57.6 a
Microbiologically enriched Bioilsa	32.8 b	4.0 c	41.3 b	28.4 a	11.4 a	56.8 a
Microbiologically enriched compost	50.7 ab	9.0 ab	43.4 b	24.7 a	11.6 ab	61.1 ab
Microbiologically enriched humic acids	47.5 ab	5.7 b	57.5 a	24.7 a	11.2 a	55.4 a
Microbiologically enriched biochar	76.8 a	10.0 ab	70.0 a	23.7 a	10.0 a	56.7 a
Manure	41.7 b	6.1 b	43.8 b	17.1 a	7.0 a	53.1 a

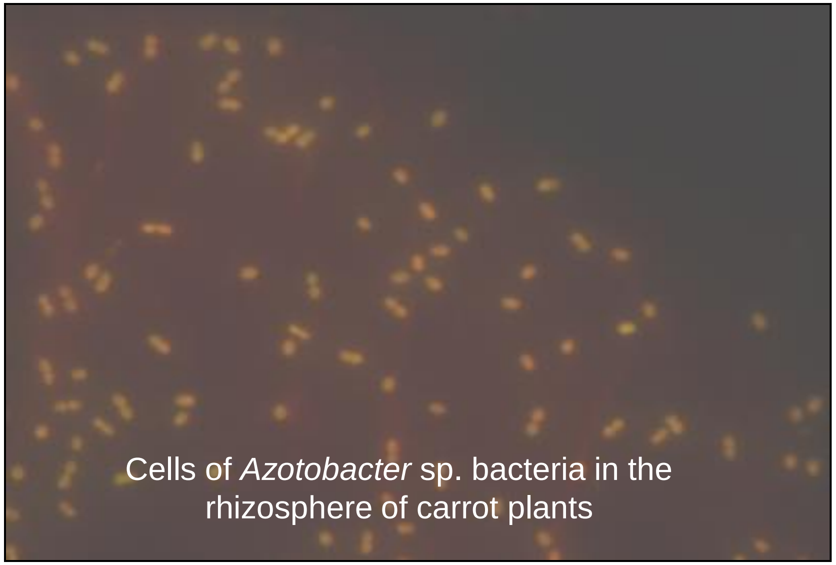
* total number of bacteria

Applications of bacterial mycorrhizal inoculum, microbiologically enriched compost and humic acids contributed to the increase in the number of actinomycetes and spore-forming bacteria.

Abundance of free-living nitrogen-fixing *Azotobacter* bacteria in the soil before and after sowing cucumber and carrot seeds

Treatment	*Azotobacter** before seed sowing		*Azotobacter** after seed sowing	
	Cucumber	Carrot	Cucumber	Carrot
Control (no fertilization)	7.7	8.7	8.7	7.5
Bacterial-mycorrhizal inoculum	8.7	7.3	7.4	6.4
Microbiologically enriched Bioilsa	12.6	10.8	8.4	9.1
Microbiologically enriched compost	12.3	11.7	4.3	10.1
Microbiologically enriched humic acids	9.7	12.5	4.2	7.4
Microbiologically enriched biochar	9.3	9.2	5.7	6.2
Manure	11.1	11.3	10.5	10.6

* Number of *Azotobacter* colonies growing around 20 microsamples of soil placed in 1 Petri dish. Average for 10 dishes.

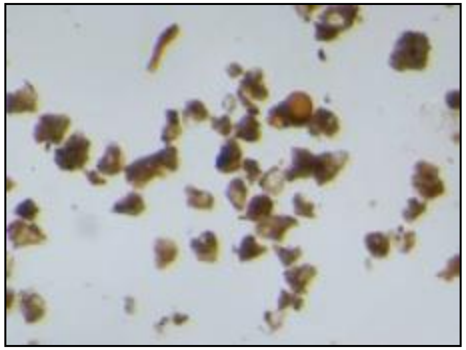


Cells of *Azotobacter* sp. bacteria in the rhizosphere of carrot plants

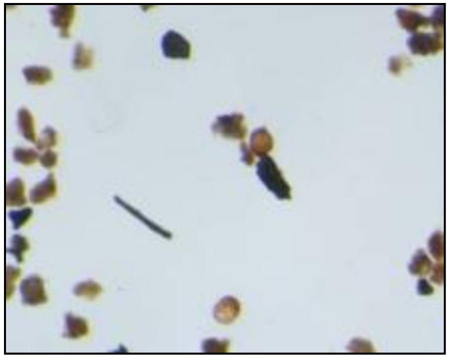
Application of microbiologically enriched Bioilsa, compost and manure increased the abundance of *Azotobacter* bacteria in the rhizosphere of carrot plants.

Number of AMF spores in the rhizosphere of 'Nipomo' carrot plants (IO Experimental Field, 2016)

Treatment	Number of spores in 100 g of soil
Control	50 ab
Bacterial-mycorrhizal inoculum	81 cd
Microbiologically enriched Bioilsa	90 de
Microbiologically enriched compost + Biochar	75 c
Microbiologically enriched humic acids	62 b
Microbiologically enriched biochar	100 e
Manure	85 cd



AMF spore in the rhizosphere of 'Nipomo' carrot roots after application of bacterial-mycorrhizal inoculum.

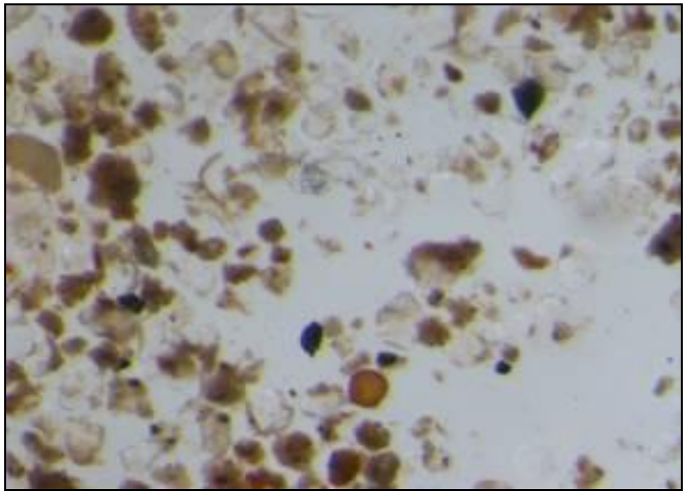


AMF spore in the rhizosphere of 'Nipomo' carrot roots treated with microbiologically enriched biochar.

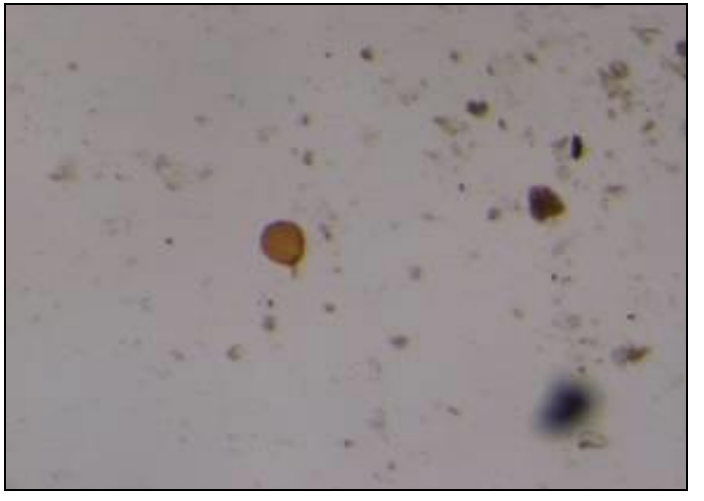
Application of microbiologically enriched biochar had the greatest effect on the formation of the highest number of spores of arbuscular mycorrhizal fungi in the rhizosphere of carrot plants.

Number of AMF spores in the rhizosphere of 'Adam' cucumber plants (IO Experimental Field, 2016)

Treatment	Number of spores in 100 g of soil
Control	20 a
Bacterial-mycorrhizal inoculum	92 cd
Microbiologically enriched Bioilsa	85 c
Microbiologically enriched compost + Biochar	63 b
Microbiologically enriched humic acids	93 cd
Microbiologically enriched biochar	140 e
Manure	100 d



AMF spore in the rhizosphere of 'Adam' cucumber roots after application of bacterial-mycorrhizal inoculum.



AMF spore in the rhizosphere of 'Adam' cucumber roots treated with microbiologically enriched biochar.

Application of microbiologically enriched biochar resulted in the formation of the largest number of spores in the rizosphere of cucumber plants.

DEVELOPMENT OF FERTILIZATION RECOMMENDATIONS

- **Optimisation of fertilization doses of bioproducts in relation to the nutritional requirements of the species and varieties of organically grown crop plants, the dates and frequency of their application.**
- **A technology of bioproduct application was developed to improve the fertility of soils in organic vegetable crops.**

Appearance of cucumber fruit towards the end of vegetation, after application of bacterial-mycorrhizal inoculum (IO Experimental Field, 2016)

Recommendations for the newly developed bioproducts:

BACTERIAL-MYCORRHIZAL INOCULUM

Bacterial strain of *Pseudomonas fluorescens* that is part of the bacterial-mycorrhizal inoculum

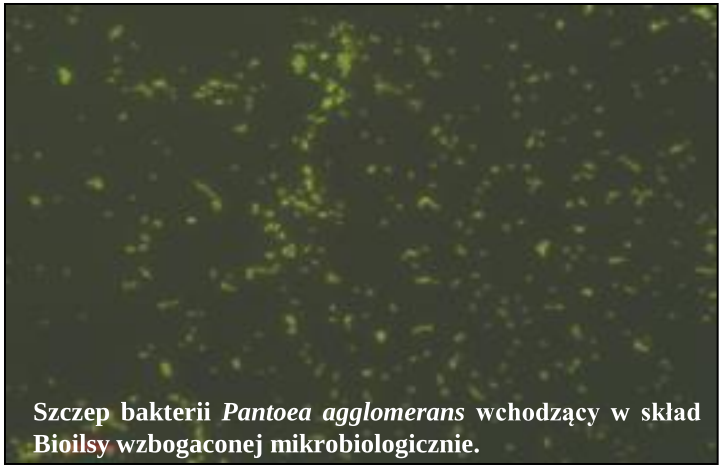
Composition: **NAzot2 strain of bacteria *Klebsiella oxytoca*, IAA – indolylacetic acid, Pi77AA (*Pantoea agglomerans*), Ps1/2 (*Pseudomonas fluorescens*), N52AD (*Pantoea / Erwinia* sp), and species of mycorrhizal fungi *Claroideoglomus claroideum*, *Gigaspora margarita*, *Funneliformis mosseae*, *Scutellospora dipurpurescens*, *Rhizophagus fasciculatus.***

Mode of action: **bacteria *Klebsiella oxytoca, Pantoea agglomerans, Pseudomonas fluorescens* oraz *Pantoea / Erwinia* sp. stimulate plant growth, dissolve phosphorous compounds, fix atmospheric nitrogen in soil, make iron available to plants, produce siderophores. Mycorrhizal fungi increase plant resistance to water stress, increase absorptive surface of roots and promote root growth, increase the availability of phosphorus, nitrogen, potassium, iron, manganese and other microelements to plants and stimulate their growth and yielding.**

Directions for use: **The preparation can be applied in furrows, along the rows of plants. It is recommended to use the biopreparation once during the growing season, preferably during planting / sowing. The use of the preparation should be combined with fertilization with manure or other organic fertilizer.**

Benefits: **reduces the doses of traditional fertilizers, both mineral and organic, stimulates the development of the root system.**

MICROBIOLOGICALLY ENRICHED BIOFERTILIZER BIOILSA



Szczep bakterii *Pantoea agglomerans* wchodzący w skład Bioilsy wzbogaconej mikrobiologicznie.

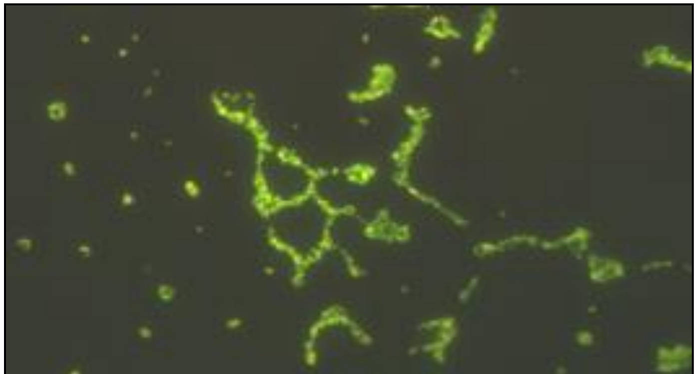
<u>Composition:</u> product contains organic nitrogen (N – 12.5%, including organic nitrogen soluble in water 5%), organic carbon of biological origin (C – 42%, including extracted organic carbon – 95%) and NAzot2 strain of bacteria *Klebsiella oxytoca* (synthesis of IAA – indolylacetic acid), Pi77AA - *Pantoea agglomerans*, Ps1/2 - *Pseudomonas fluorescens*.

<u>Mode of action:</u> bacteria *Klebsiella oxytoca*, *Pantoea agglomerans* and *Pseudomonas fluorescens* stimulate plant growth, make phosphorus compounds available to plants, make iron available to plants by altering its valency, and fix atmospheric nitrogen in the soil.

<u>Directions for use:</u> product in the form of granules applied to the soil in the amount of 700 kg per hectare, once only, prior to seed sowing. In addition, the biopreparation can be used for dressing vegetable seeds with an aqueous suspension.

<u>Benefits:</u> The use of microbiologically enriched Bioilsa increases the growth and yielding of plants, and the uptake and amounts of minerals in the roots and above-ground parts of plants, i.e. nitrogen, potassium, phosphorus, magnesium, iron.

MICROBIOLOGICALLY ENRICHED COMPOST



Strain of bacteria *Klebsiella oxytoca*, which is part of microbiologically enriched compost

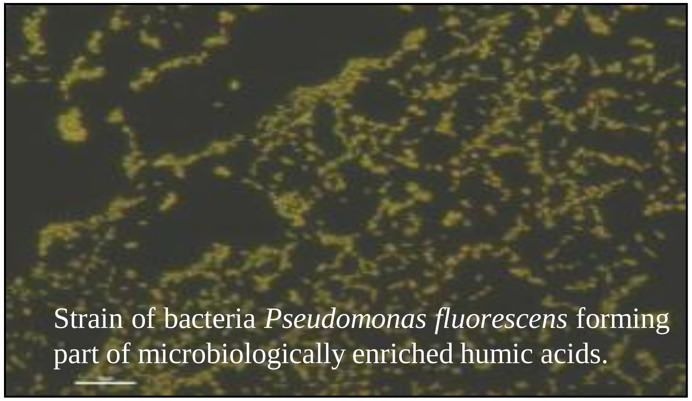
<u>Composition:</u> brown coal dust, NAzot2 strain of bacteria *Klebsiella oxytoca* (synthesis of IAA – indolylacetic acid), Pi77AA – *Pantoea agglomerans*, Ps1/2 – *Pseudomonas fluorescens* and food industry waste such as Vinassa and whey. The newly developed compost contains a suitable C : N ratio (from 30 : 1 to 40 : 1).

<u>Mode of action:</u> stimulates the uptake of elements such as potassium, phosphorus, nitrogen, copper, manganese, iron, sodium, and their subsequent metabolism, particularly in terms of stimulation of plant growth and yielding.

<u>Directions for use:</u> Compost is applied to the soil. It should be used in the amount of 40 t / ha together with soil fertilization with a nitrogen fertilizer or another biofertilizer. It is also recommended to use compost in combination with foliar fertilization with an organic fertilizer (e.g. Vinassa or sea algae extract).

<u>Benefits:</u> The use of compost stimulates the growth and yielding of plants, and improves the physicochemical and microbiological properties of the soil, which helps to reduce mineral fertilization of plants. The use of compost increases the organic matter content in the soil.

MICROBIOLOGICALLY ENRICHED HUMIC ACIDS



Strain of bacteria *Pseudomonas fluorescens* forming part of microbiologically enriched humic acids.

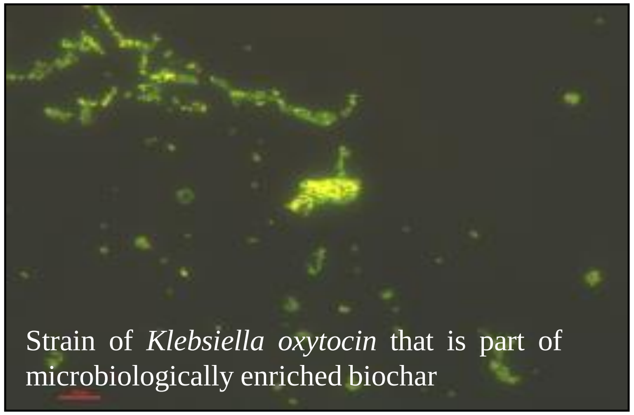
<u>Composition:</u> humic acids from brown coal in liquid form and NAzot2 strain of bacteria *Klebsiella oxytoca* (synthesis of IAA – indolylacetic acid), Pi77AA – *Pantoea agglomerans*, Ps1/2 – *Pseudomonas fluorescens*.

<u>Mode of action:</u> The effectiveness of humic acids enriched with beneficial microorganisms is due to the high levels of humic compounds (humic and fulvic acids), thanks to which the minerals in the soil are more readily available to plants and improve the microbiological activity of the soil.

<u>Directions for use:</u> Microbiologically enriched humic acids are applied to the soil at a conc. of 5%, and their aqueous solution should be prepared immediately prior to application. The product can be applied 2-3 times from the beginning of vegetative growth, at 2-3 week intervals. The dose depends on the type of the crop plant and ranges from 50 to 100 litres per hectare during the growing season.

<u>Benefits:</u> Improvement in the uptake of minerals from soil by plants, increased amounts of humus compounds in the soil improve its bio-physico-chemical properties, including water capacity, which is particularly important in non-irrigated crops.

MICROBIOLOGICALLY ENRICHED BIOCHAR



Strain of *Klebsiella oxytocin* that is part of microbiologically enriched biochar

<u>Composition:</u> organic and loamy parts from brown coal and biochar; molasses and additions of components causing extraction of humic acids, and NAzot2 strain of bacteria *Klebsiella oxytoca* (synthesis of IAA – indolylacetic acid), Pi77AA – *Pantoea agglomerans*, Ps1/2 – *Pseudomonas fluorescens*.

<u>Mode of action:</u> improves soil properties, bioremediation of contaminated soils, increases water capacity and pH of soils, prevents leaching of nutrients and binds organic and inorganic contaminants, reduces nitrogen losses during composting. It is also used to reduce groundwater and surface water pollution by retaining biogenic nutrients in the soil and for neutralization of landfill waste.

<u>Directions for use:</u> Microbiologically enriched biochar is applied to the soil in the form of granules, 3-6 mm in dia. The dose depends on the type of crop plant and soil fertility, and ranges from 10 to 50 t / ha. It should be used in conjunction with soil fertilization with a nitrogen fertilizer or with foliar fertilization with an organic fertilizer (e.g. sea algae extract).

<u>Benefits:</u> increases plant growth and yielding, and improves soil properties. Biochar used as bedding retains moisture, reduces erosion, and enriches the soil with organic matter and humus, which is simultaneously beneficial for the development of soil microflora.

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*.**

- **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.**

RECOMMENDATIONS FOR PRACTICE

- **Greenhouse and field experiments demonstrated high effectiveness of biostimulants and natural microbiologically enriched fertilizers on increasing growth and yield of fruit and vegetable plants and quality of arable soils.**

- **Application of bioproducts contributed to increasing the levels of minerals and organic matter in the soil and the population size, biodiversity and survival of rhizosphere bacteria and mycorrhizal fungi in rhizosphere soil of horticultural crops.**

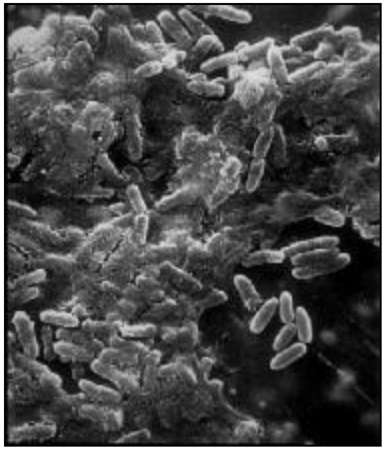
- **Biopreparations containing beneficial soil microorganisms can be used for fertilizing plants and reducing the occurrence of soil pathogens.**

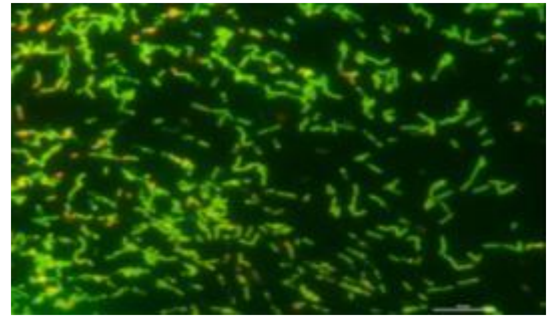
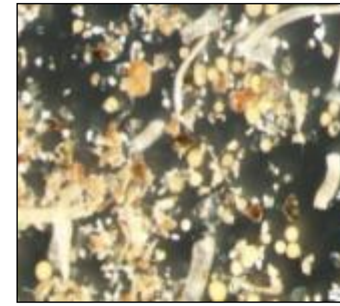
- **The use of bioproducts, i.e. mycorrhizal substrate, Humus UP, Humus Active + Aktywit PM, BF Quality and Vinassa, and organic fertilizers Florovit Natura and Florovit Eko, is an effective and economically viable technology of fertilizing of horticultural plants.**

Blossoming apple trees cv. 'Topaz' in field experiment

Compost

Vinassa



THANK YOU