



Department of Industrial and Environmental Microbiology

Information brochure

Department of Industrial and Environmental Microbiology is formed by four research team:

1. Supervisor :

dr hab. Mariusz Trytek, prof. UMCS

Mentors :

dr Anna Gromada
dr Mateusz Kutyla



2. Supervisor :

prof. dr hab. Adrian Wiater

2a. Supervisor : dr Kamila Wlizło

Mentors :

mgr Katarzyna Próchniak



3. Supervisor :

dr hab. Jolanta Jaroszuk-Ścisieł, prof. UMCS

Mentors :

dr Artur Nowak
mgr Anna Słomka



3a. Supervisor : dr Ewa Ozimek

4. Supervisor :

prof. dr hab. Monika Janczarek

Mentors :

Dr Paulina Adamczyk



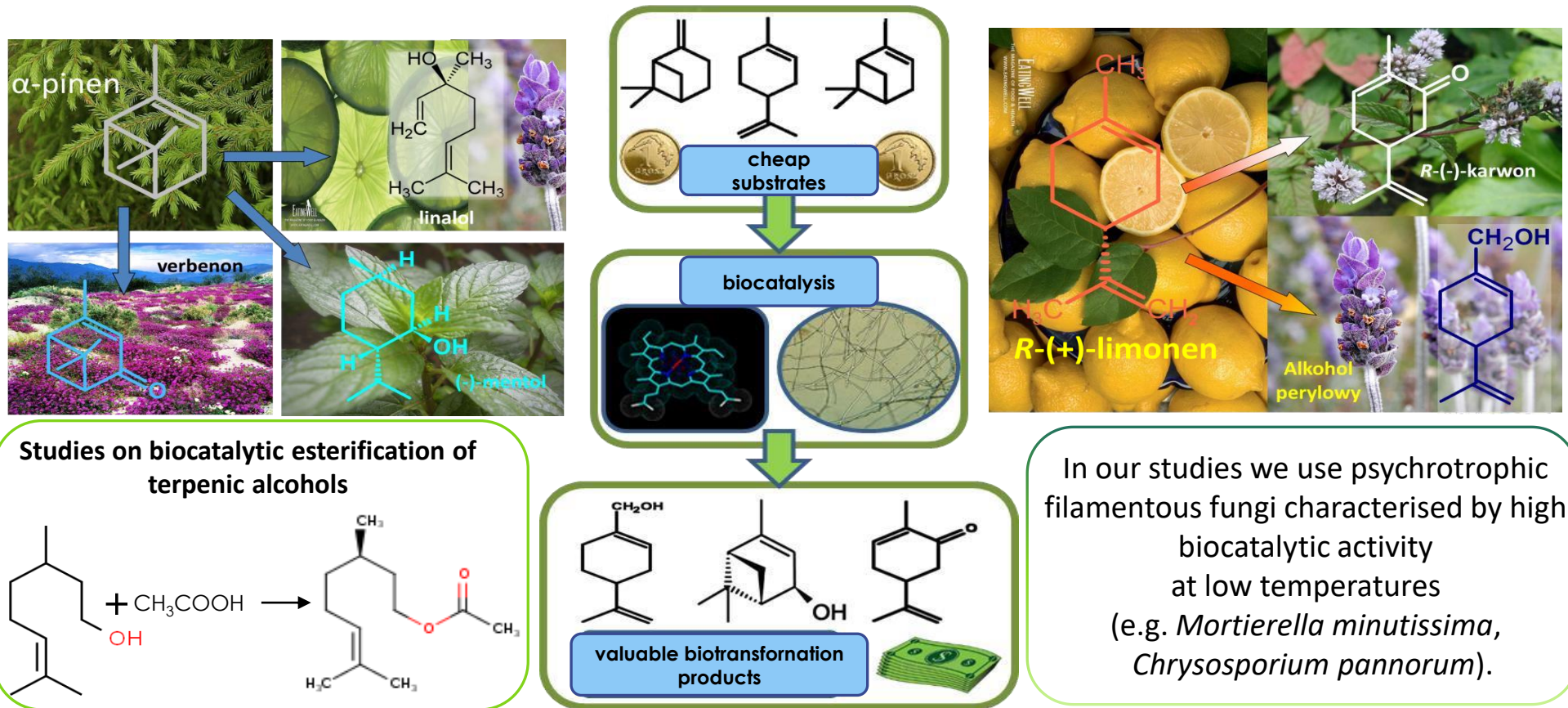
5. Supervisor :

dr hab. Małgorzata Majewska



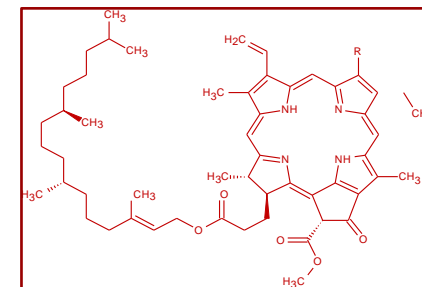
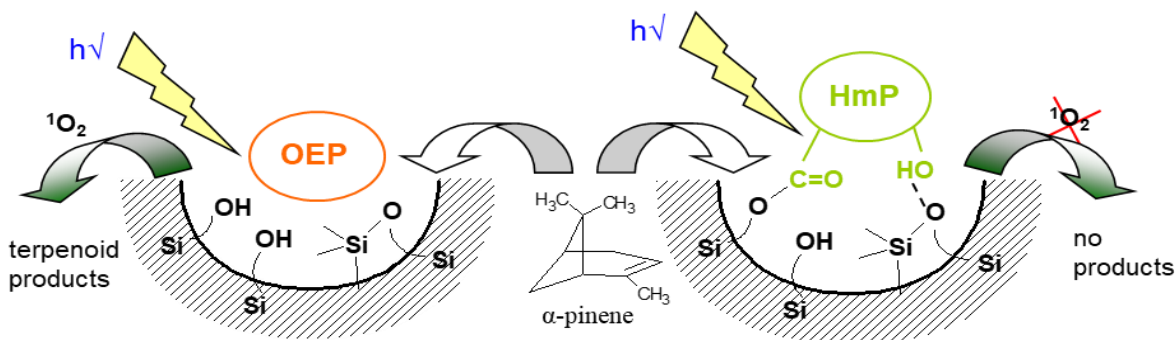
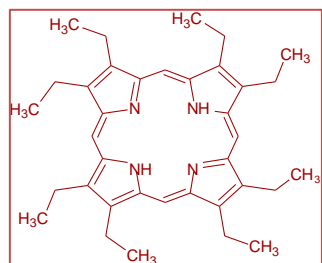
1. Biotransformation of terpenoids

The research aims to develop biotechnological ways of producing valuable bioactive products for use in the food, pharmaceutical and perfume industries. Experiments are performed on laboratory and bioreactor scale using cheap and natural precursors, e.g. limonene, pinene, citronellol, myrtenol. Determination of biological activity of the obtained compounds (anticancer, antifungal and antibacterial activity).



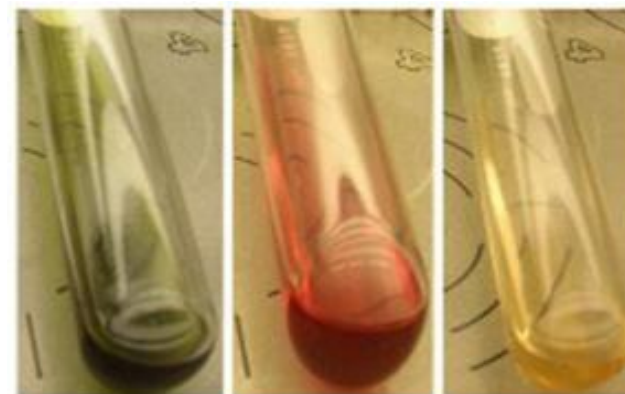
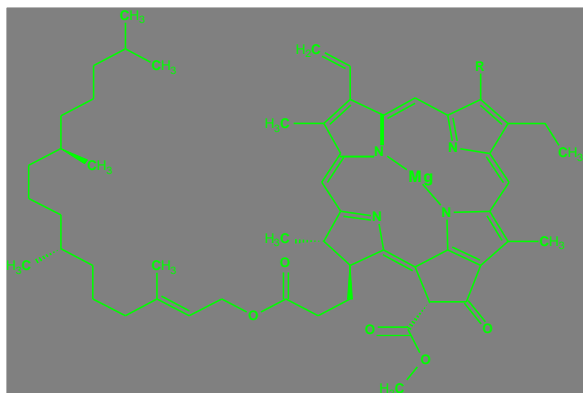
Methodological part of the research: preparation of biocatalyst, lyophilisation, extraction of biotransformation products with organic solvents, their concentration and analysis by gas chromatography-mass spectrometry (GC-MS).

2. Photo-oxidation of terpenes in biomimetic systems



The aim of the research is to develop an efficient method for terpenes oxidation, using biomimetic catalysts - natural porphyrin derivatives (e.g. chlorophylls and pheophytins), synthetic porphyrins, and modified porphyrins of natural origin, e.g. protoporphyrin IX. The research will also include immobilisation of the most active biocatalysts in silica matrices obtained by sol-gel method. Analytical techniques such as UV/Vis spectroscopy, visible light and infrared spectrofluorimetry as well as gas chromatography and mass spectrometry will be used.

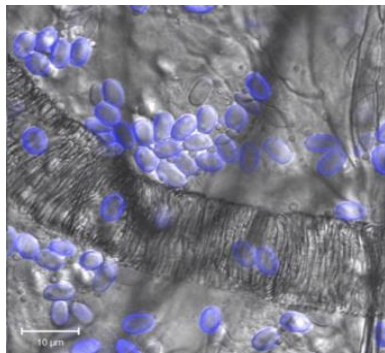
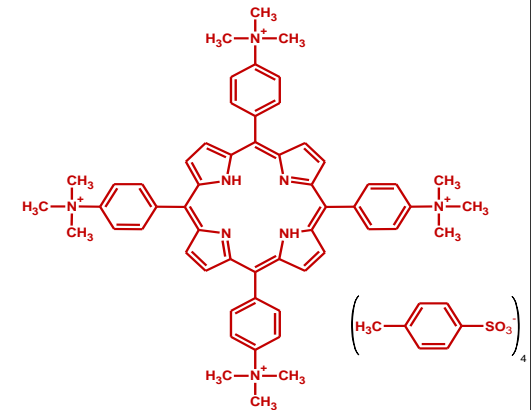
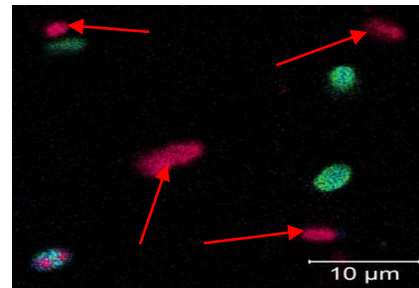
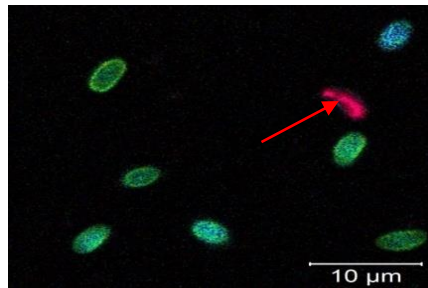
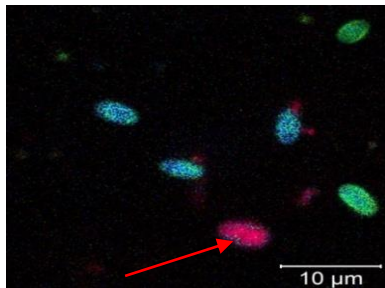
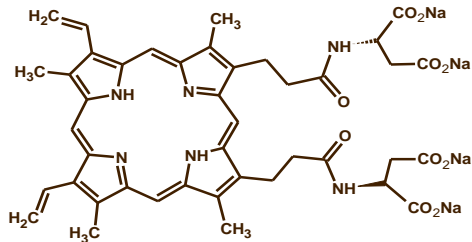
The research is conducted in collaboration with the Department of Inorganic Chemistry, the Department of Organic Chemistry of Maria Curie-Skłodowska University and the Institute of Organic Chemistry of the Polish Academy of Sciences.



3. Effect of porphyrinoid compounds on the activity of intracellular parasites of *Nosema* spp.



Research on the inactivation of *Nosema* spores causing the contagious honey bee disease nosema - which contributes to the extinction of bee colonies.



- ❖ Research is conducted using:
 - ✓ Light microscopy,
 - ✓ Scanning fluorescence microscopy,
 - ✓ Scanning electron microscopy (SEM)

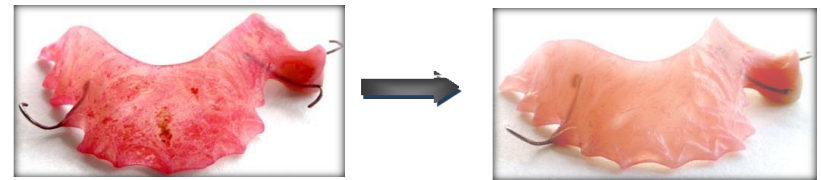
Research are conducted in collaboration with the University of Life Sciences in Lublin, Institute of Organic Chemistry of the Polish Academy of Sciences, Institute of Immunology and Experimental Therapy, PAS in Wrocław, Department of Molecular Biology, Department of Immunobiology, Department of Cell Biology of the Faculty of Biology, Department of Biology and Biotechnology, Maria Curie-Skłodowska University.

1. Plaque-degrading enzymes



Mutanases are the only group of enzymes that have the ability to degrade mutans, the polymers that make up plaque, which is the cause of tooth decay. The bacteria that cause tooth decay grow in the form of a biofilm (plaque) which, in addition to them, includes a matrix made up of bacterial polymers. The structure of these polymers is crucial to the biofilm's ability to cause caries, as it affects its physical and biochemical properties.

These polymers increase the adhesion of microorganisms to teeth and to each other, i.e. they bind the biofilm together; they serve as a reserve energy source for bacteria; they protect microorganisms from hostile environmental influences and determine what enters and leaves the biofilm. Of the numerous polymers that make up dental plaque, the following play a decisive role in causing tooth Decay water-insoluble, fibrous, and therefore difficult to remove α -1,3-glucan, called mutan.



The elimination of this plaque component through its breakdown by mutanase can be used to prevent and combat tooth decay. Microbial mutanase can be used as an active substance in oral care products such as mouthwashes, toothpastes, dental gels, chewing gums, etc.



Natural anti-caries

As a result of cooperation with, among others, the Department of Pharmacognosy of the Medical University of Białystok, the Department is working on phytochemical research on selected plant species and the assessment of their anti-caries activity.

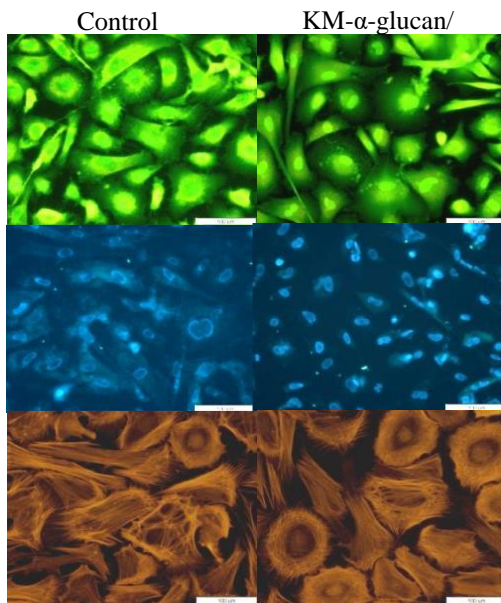
So far, it has been found that various extracts from selected *Potentilla* species can be a source of bioactive substances, mainly polyphenolic compounds such as tannins, phenolic acids and flavonoids with antitumour effects. The investigated preparations may prevent caries, as they show inhibitory effect on streptococcal glucosyltransferase activity, limit bacterial biofilm formation in vitro and have diverse antibacterial activity, thanks to these features they may be a component of pharmacological agents used in the prevention and treatment of caries. Further research will focus on bioactive substances also isolated from other plants and other antimicrobial substances.

2. Studies on the biological activity of fungal (1→3)- α -D-glucans

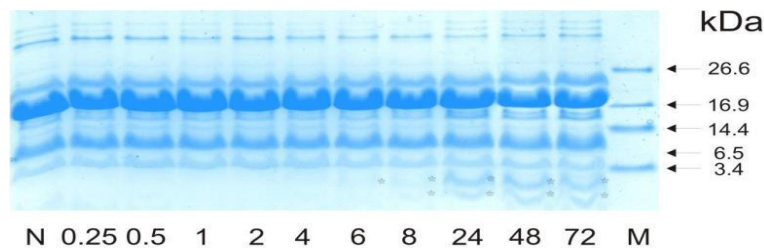
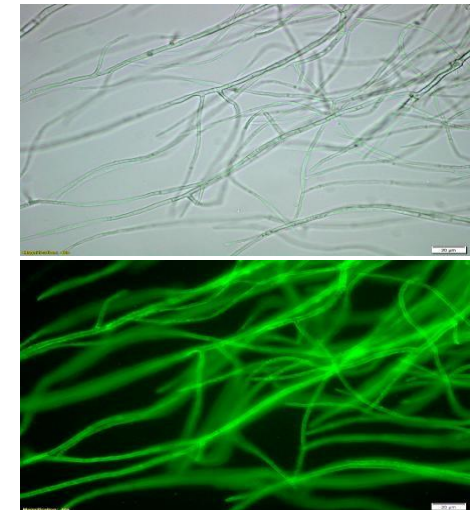
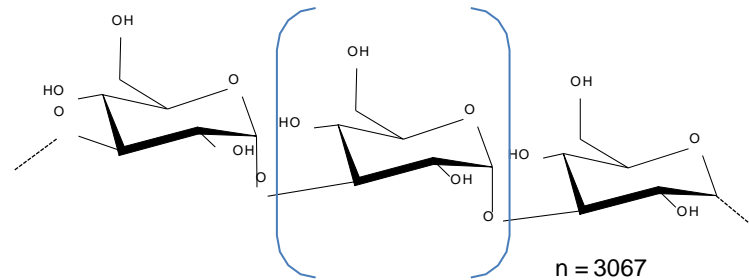


The aim of this study is to evaluate the biological potential of (1→3)- α -D-glucans. These polymers are isolated from vegetative mycelium and fruiting bodies of macrofungi.

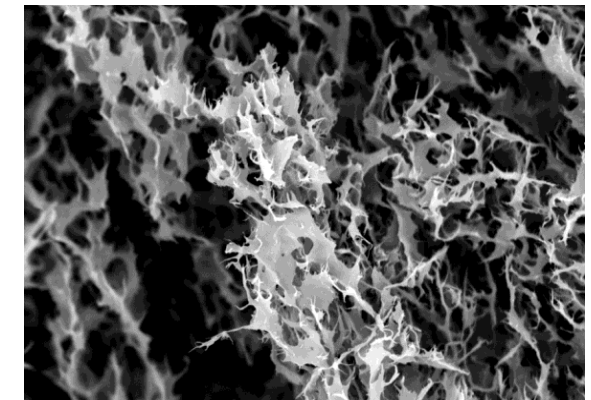
(1→3)- α -D-glucans are used, among others, as a carrier of hydrolytic enzymes, heavy metal ion sorbents, stimulators of plant and animal immunity induction, a source of oligosaccharides with prebiotic potential or structural components of biodegradable coatings.



Human intestinal epithelial myofibroblasts (CCD-18Co)

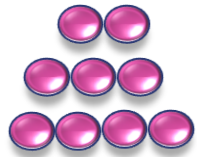


Time after immunization [h]



3. Nigerooligosaccharides - new compounds with prebiotic properties

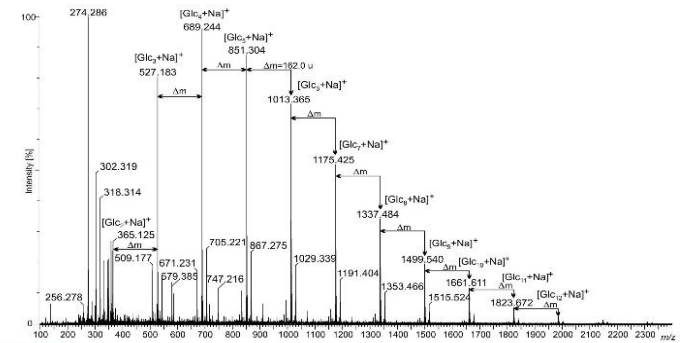
Prebiotics



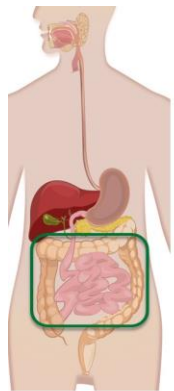
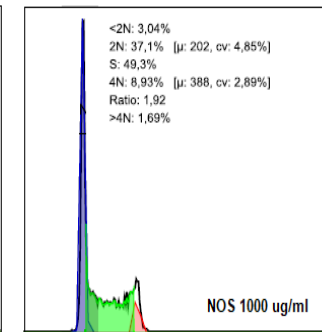
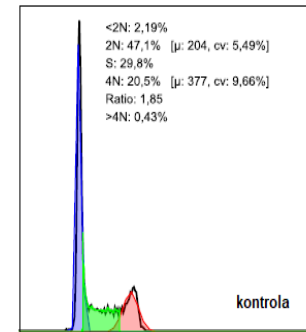
selective fermentation
e.g. by bifidobacteria



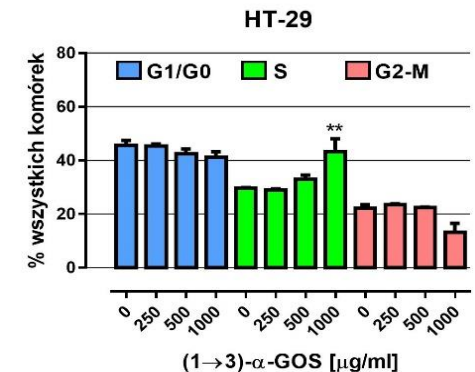
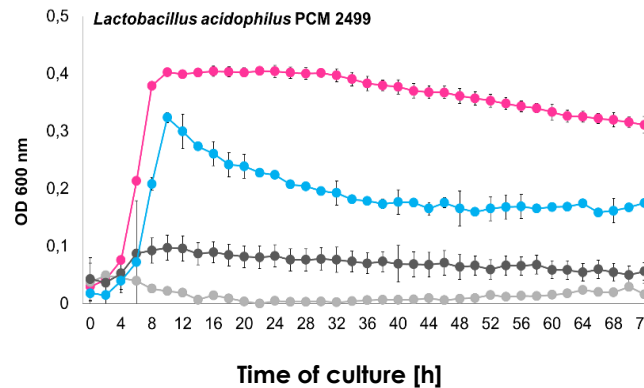
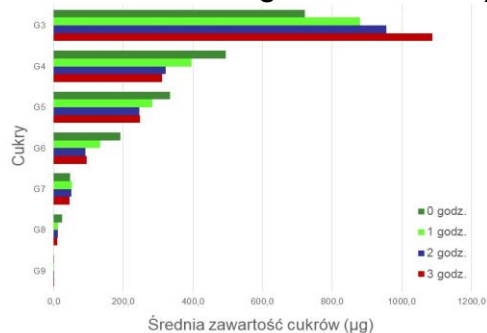
- lactic acid
- acetate acid
- propionic acid
- butyric acid



Prebiotics are selectively fermented food ingredients that enable specific changes in the composition and/or activity of the microflora of the gastrointestinal tract, thus having a beneficial effect on the health of the host. Prebiotics include, among others, oligosaccharides, i.e. carbohydrates with low molecular weight. Such compounds include the poorly known nigerooligosaccharides. These compounds are obtained by controlled hydrolysis of fungal (1→3)-α-D-glucans, isolated from fruiting bodies of macrofungi. Currently, in-depth studies with nigerooligosaccharides are being conducted in our Department to verify the 5 basic requirements for prebiotics as defined by Wang [Food Research International, 2009, 42, 8-12].



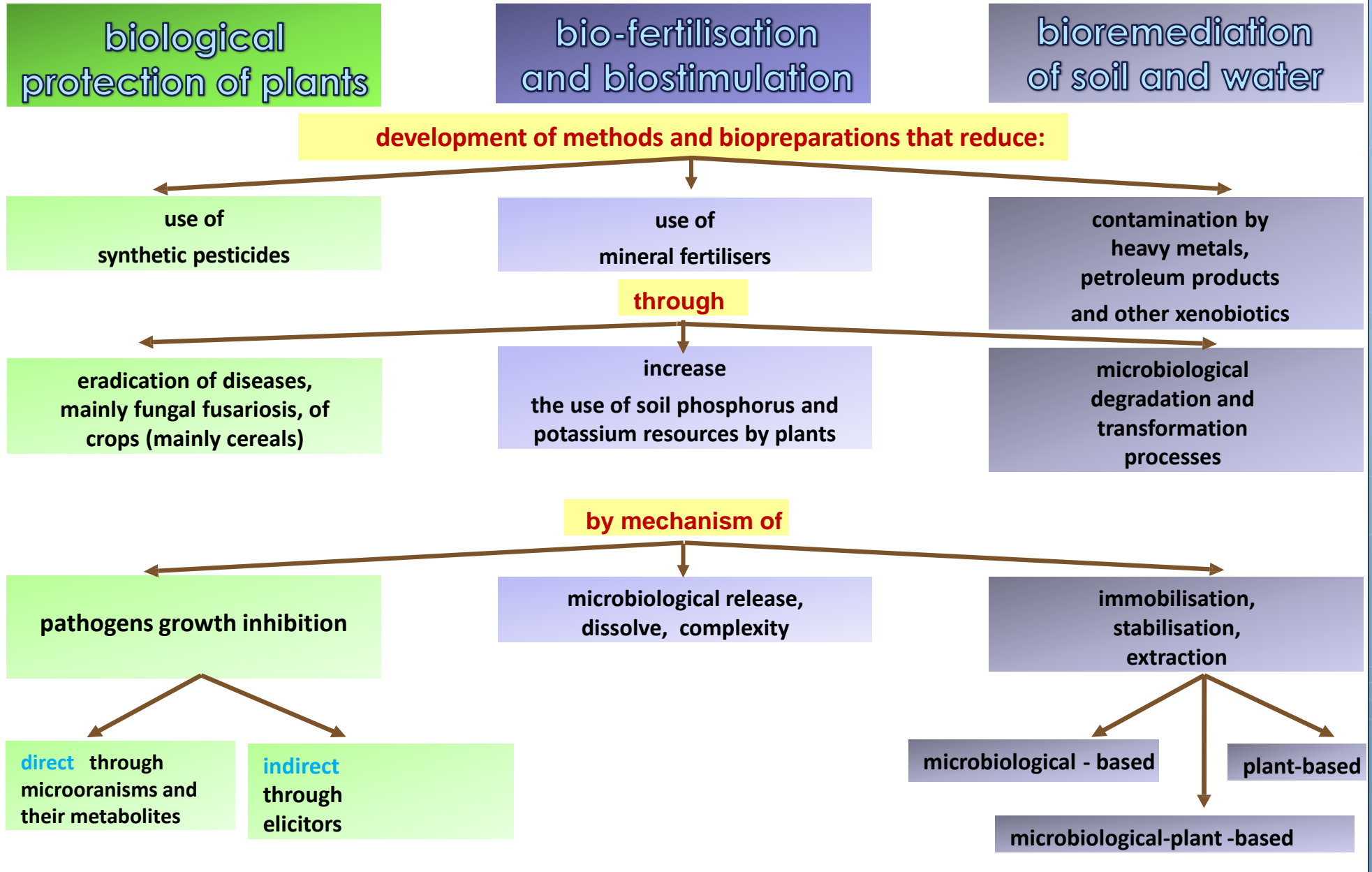
Digestion of (1→3)-α-d-glucoligosaccharides under conditions simulating intestinal activity



Examples of titles of diploma theses in the field of industrial microbiology

| Supervisor | Thesis topics |
|---------------------------------------|--|
| | Master thesis |
| dr hab. Mariusz Trytek, prof. UMCS | <ol style="list-style-type: none"> 1. Biotransformation of β-pinene using the psychrotrophic fungus <i>Chrysosporium pannorum</i> 2. Biotransformation of α-pinene using selected porphyrins as catalysts in heterogeneous systems with addition of cationic surfactant CTAB 3. Optimisation of lipase biosynthesis conditions using the fungus <i>Chrysosporium pannorum</i> A-1 4. Screening of porphyrin and terpenoid compounds for growth inhibition of <i>S. cerevisiae</i> mutants with deletion of MAP1 and MAP2 genes 5. Optimization of myrtenol biotransformation conditions in a photocatalytic system |
| dr hab. Adrian Wiater, prof. UMCS | <ol style="list-style-type: none"> 1. Structural characterization of (1\rightarrow3)-α-D-glucans from <i>Penicillium lanosum</i> and <i>P. notatum</i> mycelium and evaluation of their ability to biosorb heavy metals. 2. Selection of optimal conditions for purification of a fungal mutanase by affinity chromatography on α-(1\rightarrow3)-glucan 3. Characterisation and function of (1\rightarrow3)-α-D-glucanase from <i>Trichoderma viride</i> 4. The effect of extracts of selected species of <i>Potentilla</i> on the growth of streptococci, their synthesis of mutans and the formation of carious films with their participation 5. Evaluation of the anticancer activity of polysaccharides obtained from aqueous extracts of fruiting bodies of the birch whitethorn <i>Fomitopsis betulina</i> |
| | Bachelor thesis |
| dr hab. Mariusz Trytek, prof. UMCS | <ol style="list-style-type: none"> 1. Use of polysaccharides isolated from fruiting body fungi in cancer control 2. Tejxobactin as a representative of a new group of antibiotics |
| dr hab. Adrian Wiater, prof. UMCS | <ol style="list-style-type: none"> 1. Directions for improvement of brewer's yeast |
| dr Kamila Wliżło | <ol style="list-style-type: none"> 1. Personalised food 2. Application of polysaccharides in breast cancer therapy 3. Effects of Bisphenol A on the human body 4. Bioremediation of wastewater from micropollutants by fungal organisms 5. Elimination of environmental micropollutants by enzymes isolated from macrofungi |

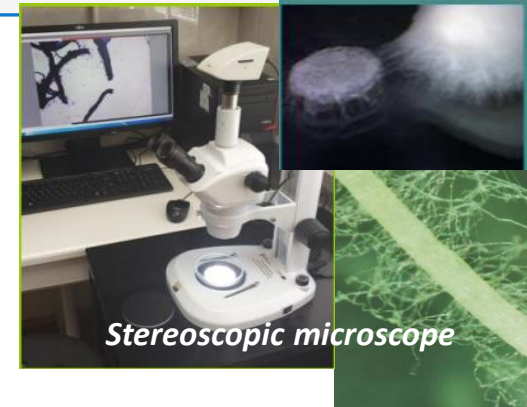
Research topics in environmental microbiology



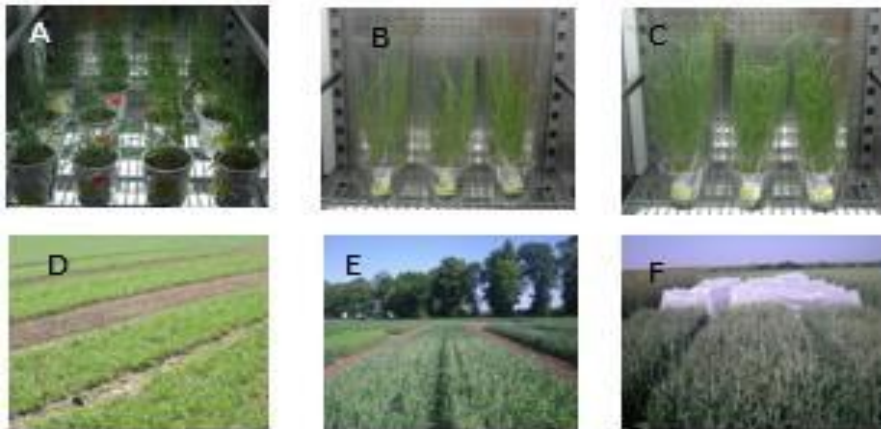
WITHIN THE FRAMEWORK OF ENVIRONMENTAL RESEARCH, WE OFFER:

Gain practical skills in:

- ✓ isolation, purification and cultivation of fungi and bacteria, biochemical analysis, spectrophotometric methods
- ✓ determination of enzyme activity in vitro and in vivo (soil, plants)
- ✓ purification and characterisation of enzymes;
- ✓ determination of microbial and plant secondary metabolites, e.g. phytohormones; chelating compounds
- ✓ determination of ecophysiological profiles of micro-organisms
- ✓ determination of cytological changes associated with pathogenesis and induction of resistance;
- ✓ differentiation of heavy metal occurrence forms;
- ✓ determining the bioavailability of heavy metals;
- ✓ determination of concentrations of heavy metals and xenobiotics (petroleum derivatives) in the environment;
- ✓ formulation of microbial vaccines and bio-fertilisers on suitable media;
- ✓ preparation and characterisation of fungal, bacterial and plant cell wall and extracellular polymers



testing of plant protection and fertiliser biopreparations under phytotron, greenhouse and field conditions

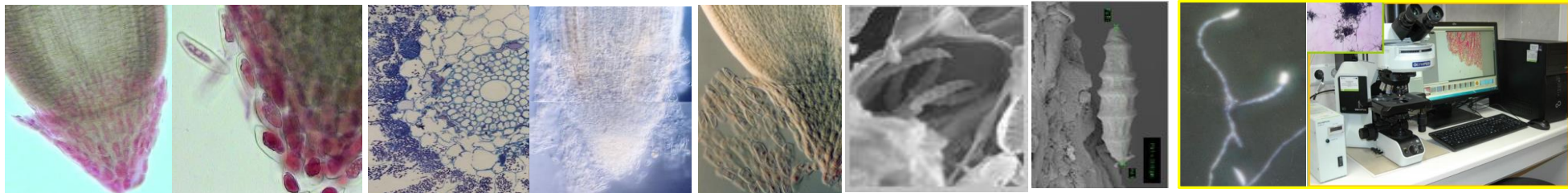


(1) **Phytotronic:** inoculated rye and wheat seedlings at early tillering stage (A), rye at late tillering stage (B), wheat at late tillering stage (C),

(2) **field:** wheat at the tillering stage grown from grain treated with *F. culmorum* strains (D), rye inoculated by seed treatment (E) and at the milk maturity stage (F)

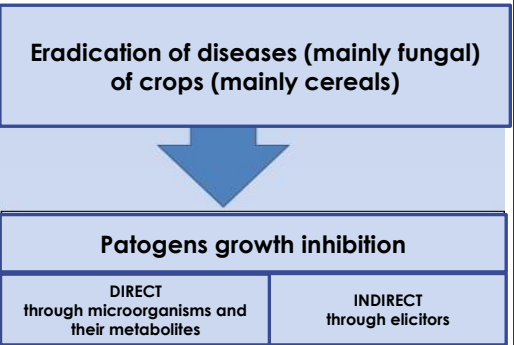
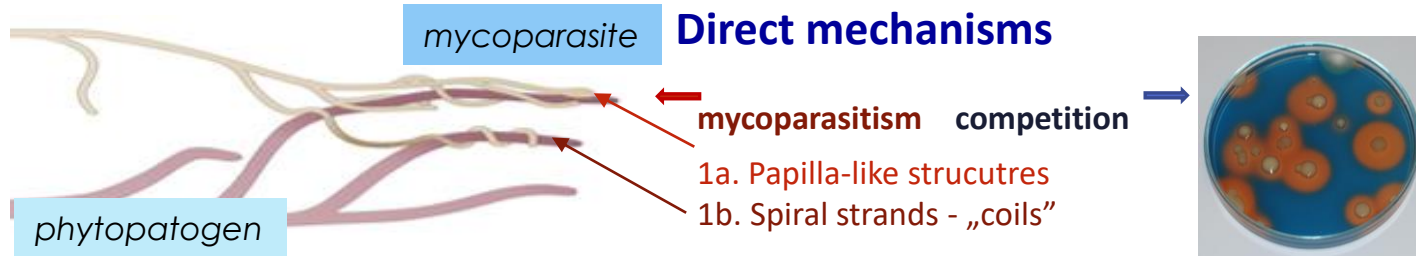


cytological preparation and documentation of microscopic images

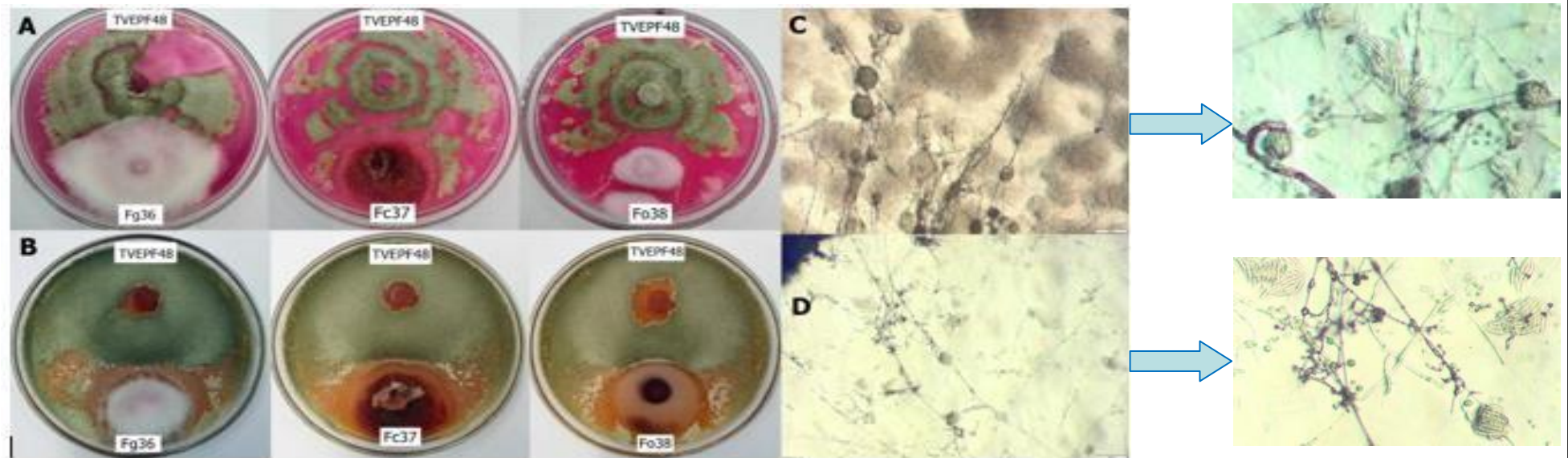


Fluorescence microscope

1. Biological protection by direct and indirect methods (induction of plant resistance by elicitors) and plant growth stimulation

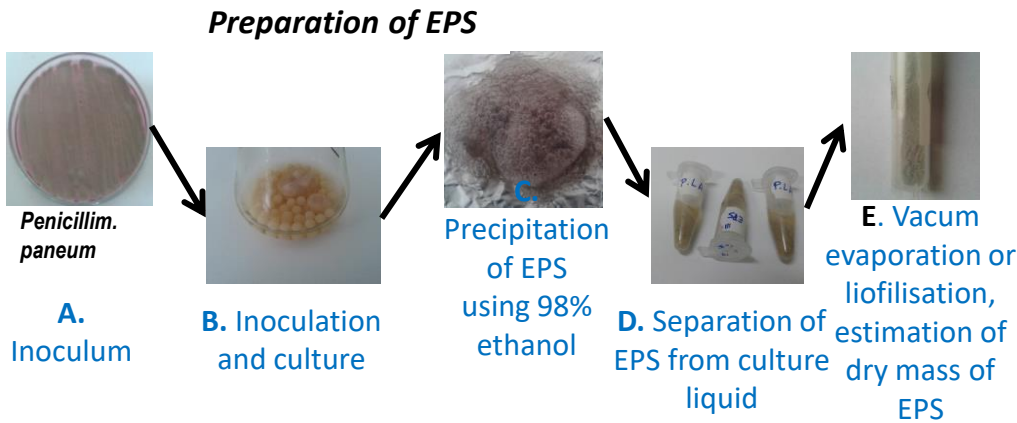


DEVELOPMENT AND TESTING OF BIOPREPARATIONS FOR PLANT PROTECTION AND GROWTH STIMULATION

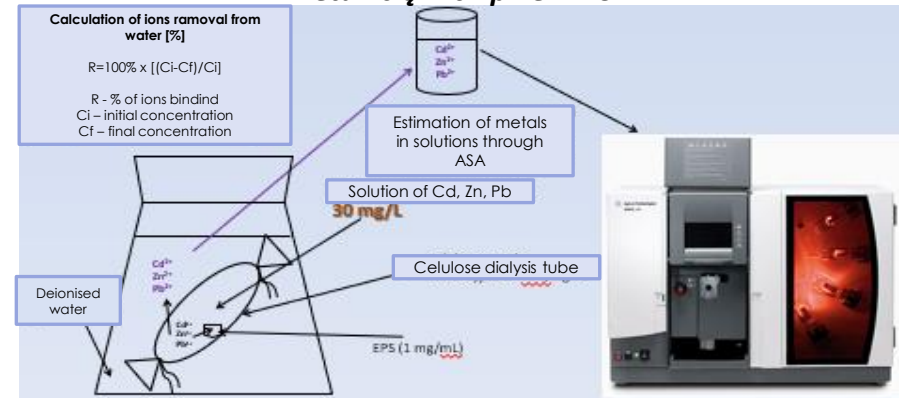


Biotic effect between *Trichoderma velutinum* strain TVEPF48 and pathogenic *Fusarium* strains on Martin's medium (A); on PDA medium (B); growth inhibition of pathogenic *Fusarium* strains: *F. graminearum* (Fg36), *F. culmorum* (DEMFc37) and *F. oxysporum* (DEMFc38) on both media with the effect of colony encirclement and inhibition zone formation; microscopic image (LM) of the effect of strain TVEPF48 on pathogenic strain *F. culmorum* Fc37 - *T. velutinum* strain TVEPF48 (dark and thin filaments) interacts with filaments and phialides of macroconidia of *F. culmorum* Fc37 surrounding them and causing cell wall lysis (C, D).

2. Research on preparation of exopolymers and their use in bioremediation and biocontrol of plants

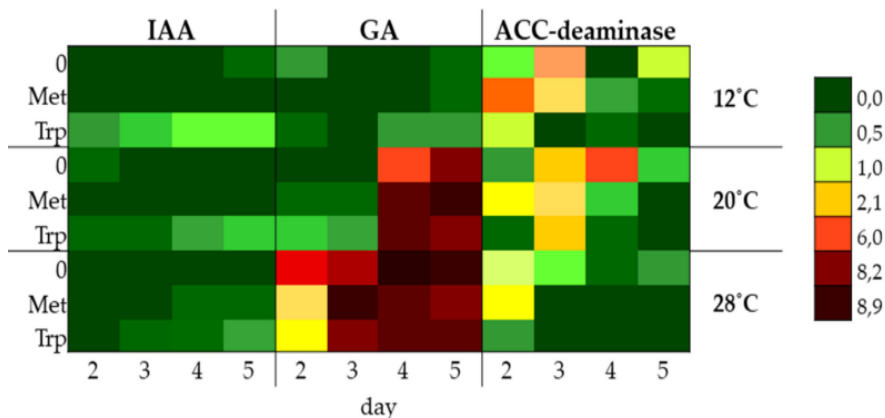


Schemat oznaczania poziomu chelatowania jonów metali ciężkich przez EPS



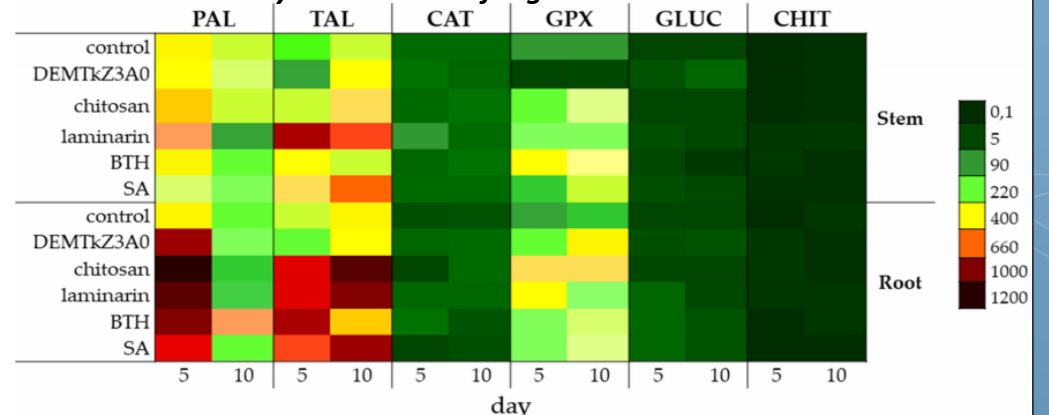
3. Research on microbial regulation of phytohormones and induction of plant immunity

Comparison of phytohormone production and ACC deaminase activity under different bacterial and fungal culture conditions



Concentration of IAA, GA, activity of ACC-deaminase

Comparison of resistance marker levels in roots and stems of plants induced by bacterial and fungal inoculation and elicitors



PAL—phenylalanine lyase, TAL—tyrosine lyase, CAT—catalase, GPX—guaiacol peroxidase, GLUC—glucanase, CHIT—chitinase

BIOFERTILISATION AND BIOSTIMULATION

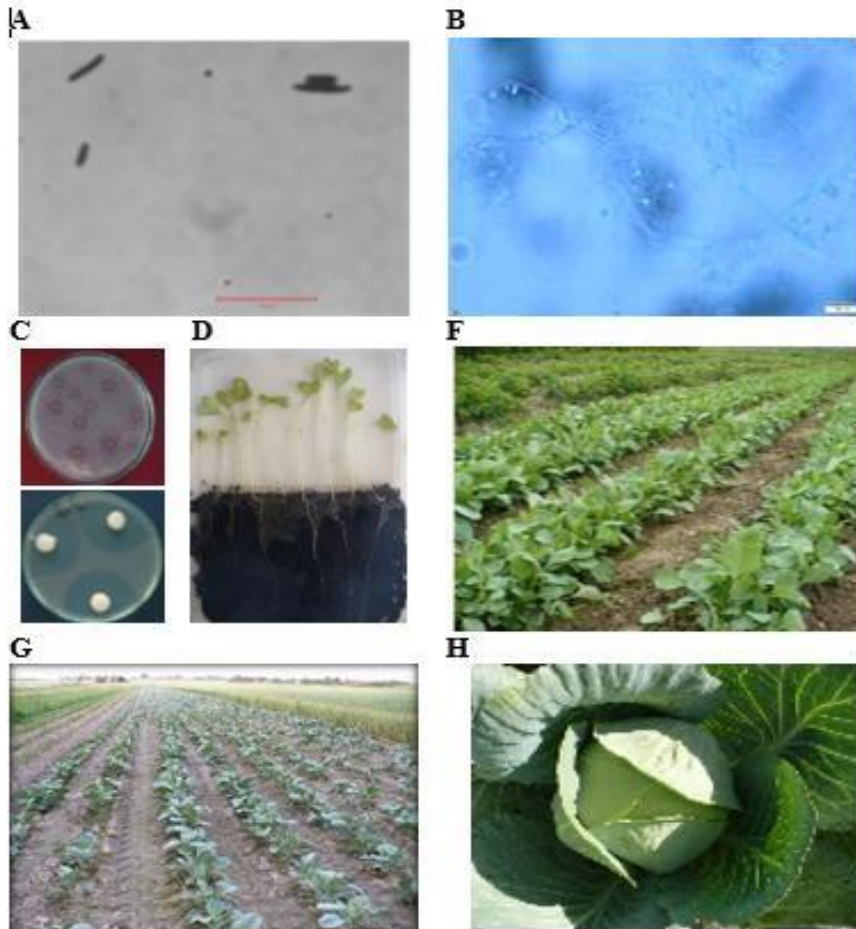
1. Development of biopreparations and methods to reduce environmental contamination by mineral fertilisers

2. Increasing plant use of soil
PHOSPHOR and **POTASSIUM**



3. MICROBIOLOGICAL
✓ release
✓ dissolve
✓ complexity

Use of selected PSM (Phosphate Solubilizing Microorganisms) fungal strains in biofertilization

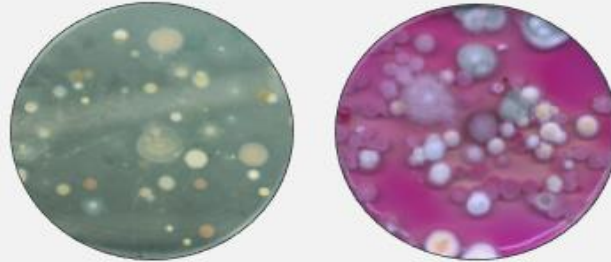


Pseudomonas luteola BN0834, PSM strain isolated from soil near Skierniewice - photo taken with Axiovert200M microscope equipped with LSM 5 PASCAL head, in Nomarsky contrast (A); *Mortierella alpina* 2 PSM strain, selected from Spitzbergen soils; photo taken with Olympus BX53 light microscope (B); Selection of bacterial and fungal PSM strains (C); Testing of selected PSM strains in a phytotron experiment in Fitobox (D); Application of selected PSM strains in the production of seedlings of white cabbage (*Brassica oleracea* L. convar capitata - medium late variety) to increase the fraction of bioavailable P - field experiment (E); White cabbage obtained in field cultivation from seedlings obtained from PSM grafted seeds (G, H)

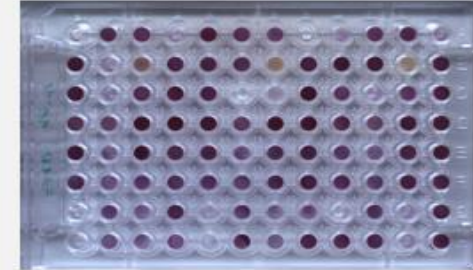
1. Assessment of microbial biodiversity of heavy metal contaminated environments (establishment of liquid and plate cultures of microorganisms)



Collection of material from a heap subjected to reclamation by afforestation



Diversity of bacterial and fungal colonies isolated from material sample

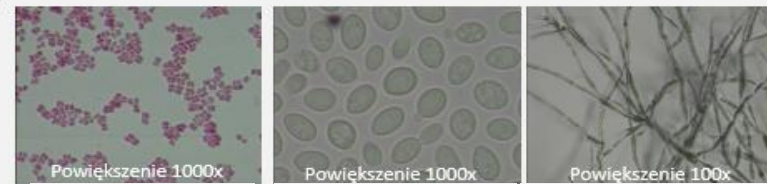


Metabolic diversity of microorganisms estimated using Biolog® EcoPlate

2. Metabolism, interactions and use of microorganisms in soil bioremediation (macroscopic and microscopic observations, biochemical analyses)



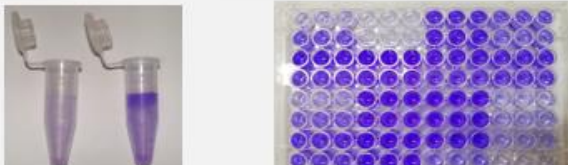
Cultures showing interactions between bacteria and fungi



bacteria

yeast

filamentous fungi



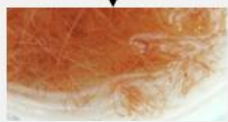
Biofilm stained with crystal violet



Biochemical API test to determine the ability of microorganisms to assimilate, ferment or degrade specific chemical compounds

3. Assessment of bioavailability of metals in contaminated soils and their bioaccumulation in plant tissues

- macroscopic and microscopic observations
- simple and sequential extractions of heavy metals from soil
- mineralisation and determination of Cd concentration (spectrophotometric or ASA method)



roots of plant from control soil



roots of plant from contaminated soil



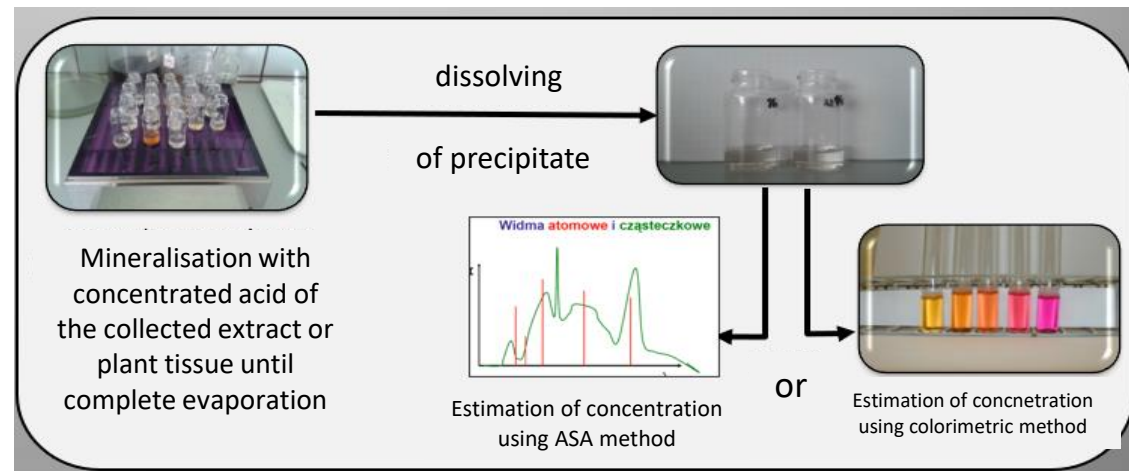
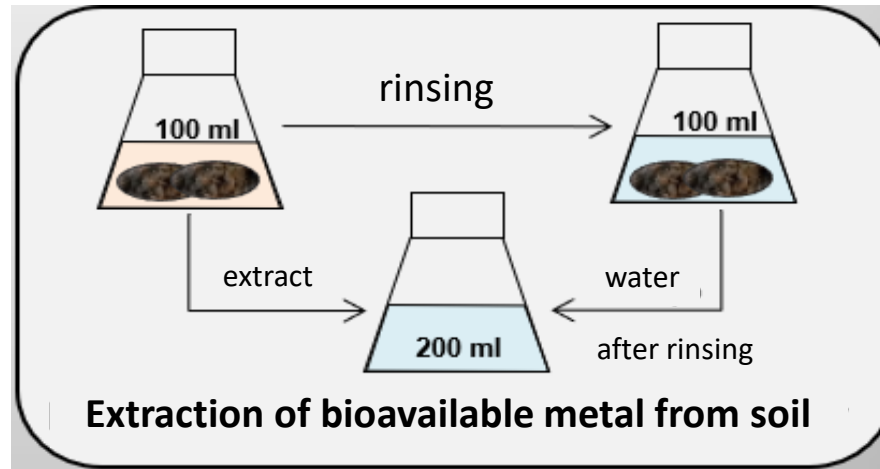
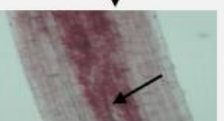
Normal growth of side root



Deformation of side root



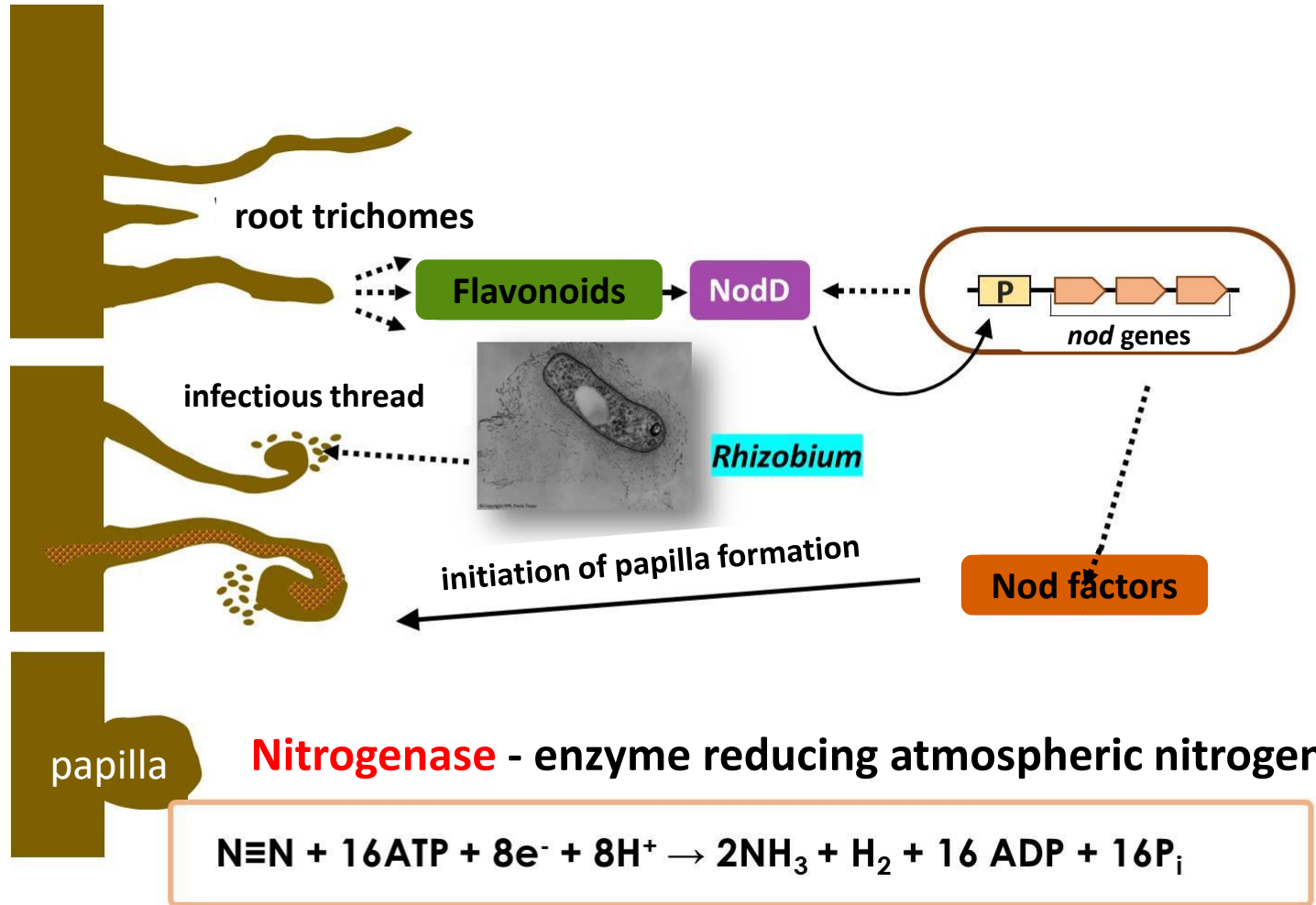
Accumulation of Cd on conductive beam in the root after ditizone staining



Examples of titles of diploma theses in the field of environmental microbiology

| Supervisor | Thesis topics |
|---|--|
| | Master thesis |
| dr hab. Jolanta Jaroszuk-Ścisiel, prof. UMCS | <ol style="list-style-type: none"> 1. Effect (biotic effect) of Gram negative bacterial strains of <i>Pseudomonas</i> (<i>P. luteola</i>, <i>P. fluorescens</i>) and <i>Chryseobacterium</i> sp. on the growth of three phytopathogenic strains of <i>Fusarium</i> spp. 2. Effects (biotic effect) of <i>Bacillus</i> sp. on the growth of phytopathogenic strains of <i>Fusarium</i> spp. (<i>F. culmorum</i>, <i>F. graminearum</i>, <i>F. oxysporum</i>) 3. Exopolymers and growth rate of <i>Fusarium</i> spp. (<i>F. oxysporum</i>, <i>F. avenaceum</i>, <i>F. graminearum</i>) on heavy metal (Cd, Pb, Zn) media 4. Exopolymers and growth rate of fungi <i>Trichoderma</i> spp. (<i>T. harzianum</i>, <i>T. koningiopsis</i>, <i>T. reesei</i>) on heavy metal (Cd, Pb, Zn) media |
| dr hab. Małgorzata Majewska | <ol style="list-style-type: none"> 1. Sorption of cadmium by selected soil components in the presence of humic acids 2. Antifungal activity of selected bacterial strains isolated from Spitsbergen soils 3. Antagonism of bacteria isolated from Spitsbergen soils towards fungi of the genus <i>Fusarium</i> 4. Effects of rapeseed and uranyl ions on soil microbial activity and diversity 5. Metal-contaminated soils as a source of microorganisms with bioremediation potential - screening of plant growth promoting microorganisms |
| dr Ewa Ozimek | <ol style="list-style-type: none"> 1. Metabolic activity of soils inoculated with <i>Mortierella</i> spp. strains studied (in vitro) at different temperatures |
| | Bachelor thesis |
| dr hab. Jolanta Jaroszuk-Ścisiel, prof. UMCS | <ol style="list-style-type: none"> 1. Shaping the microbiome and the physical and chemical properties of the soil environment by biocontrol and bionavigation biopreparations 2. Usable microbial polymers of different composition and microbial degradation of natural and synthetic polymers 3. Antimicrobial substances - directions of the search for new compounds and mechanisms |
| dr hab. Małgorzata Majewska | <ol style="list-style-type: none"> 1. Cometabolism phenomenon in bioremediation of soils contaminated with organic xenobiotics 2. The role of the plant microbiome in the phytoremediation of contaminated soils 3. The role of siderophores in the bioremediation of heavy metal contaminated soils |
| dr Ewa Ozimek | <ol style="list-style-type: none"> 1. Microbiological pollution of urban air 2. Plastic pollution of aquatic environments 3. Role and origin of indolyl-3-acetic acid in nature 4. Bacteria in the human small intestine |

Study of symbiotic interactions Rhizobium - faba bean plant



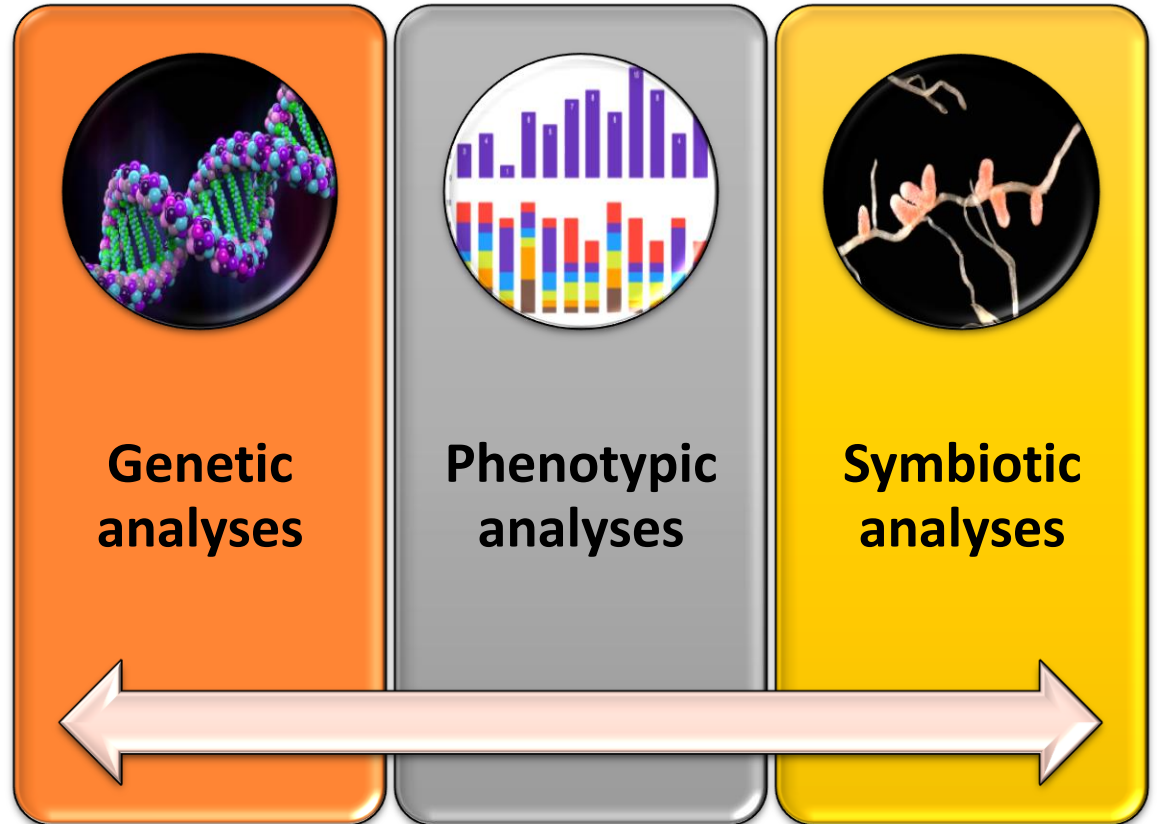
The total global N₂ fixation by the symbiotic BNF process is about **200 million tonnes per year**



Meadow clover
(*Trifolium pratense*)

• Research topics:

1. Genetic and facultative characterisation of meadow clover microsymbionts from two climate zones: subpolar and temperate,
2. Characterisation of the exopolysaccharide (EPS) biosynthetic pathway of *Rhizobium leguminosarum*: functional analysis of proteins involved in EPS synthesis and regulation of this proces,
3. Investigating the effects of heavy metals on the symbiosis of *R. leguminosarum* bv. *trifolii* with clover and the role of EPS in the adaptation of these bacteria to different stress conditions, including low temperature and heavy metal stress.



The research will be partly carried out within the project " Investigation of genetic and functional diversity of grass clover (*Trifolium pratense*) microsymbionts from two climate zones: subpolar and temperate in order to identify strains with potential use in agriculture" (no. 2018/31/B/NZ9/00663) funded by the National Science Centre

Examples of titles of diploma theses in the field of genetics

| Supervisor | Thesis topics |
|--------------------------------|---|
| | Master thesis |
| prof. dr hab. Monika Janczarek | <ol style="list-style-type: none"> 1. Determination of surface properties of <i>Rhizobium leguminosarum</i> bv. <i>trifolii</i> 24.2 wild-type strain and its derivatives differing in the level of EPS synthesis 2. Phenotypic characterisation of <i>Rhizobium leguminosarum</i> strains isolated from root papillae of red clover 3. PCR analysis of genes involved in quorum sensing and polysaccharide synthesis of <i>Rhizobium leguminosarum</i> bv. <i>trifolii</i> 4. Effect of environmental factors on the expression of genes involved in polysaccharide synthesis of <i>Rhizobium leguminosarum</i> bv. <i>trifolii</i> 5. Transcriptional analysis of the <i>gmsA</i> gene involved in glucomannan synthesis in <i>Rhizobium leguminosarum</i> bv. <i>trifolii</i> 6. Characterisation of the <i>pssA</i> gene involved in the synthesis of the exopolysaccharide of <i>Rhizobium leguminosarum</i> bv. <i>trifolii</i> |
| | Bachelor thesis |
| prof. dr hab. Monika Janczarek | <ol style="list-style-type: none"> 1. Mitochondrial DNA and its application in forensic science and medicine 2. Signals of <i>Fabaceae</i> plants involved in symbiosis with <i>Rhizobiaceae</i> 3. Molecular signals, surface structures and extracellular proteins of rhizobia involved in symbiosis with <i>fabaceae</i> 4. Bacterial biofilms and their role in chronic diseases 5. Characteristics of bacteria of the genus <i>Pantoea</i> and their biotechnological potential 6. Quorum-sensing phenomenon in Gram-negative bacteria |