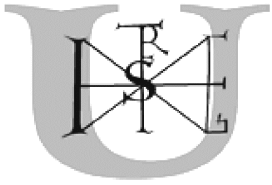

SZENT ISTVÁN UNIVERSITY

ECOPHYSIOLOGICAL SELECTION OF *PSEUDOMONAS* STRAINS, TOLERATING ENVIRONMENTAL STRESS FACTORS

Ph.D. thesis

Várady György

Gödöllő

2001.

Ph.D. School

Name: Soil, agrochemical, microbiological and agricultural basis of environmental management

Leader: Prof. Dr. Menyhért Zoltán
Professor, D.Sc.
Szent István University, Institute of Environment and Soil Management

Ph.D. subprogram

Name: Agricultural, environmental microbiology and soil biotechnology

Leader: Prof. Dr. Kecskés Mihály
Professor, D.Sc.
MTA Environmental Microbiology Institute Research Group

Discipline: Environmental Science

Consultant: Prof. Dr. Kecskés Mihály
Professor, D.Sc.
MTA Environmental Microbiology Institute Research Group

…….. ……
approval of school leader approval of consultant

PRELIMINARIES AND OBJECTS OF THE WORK

In consequence of intensive industrial and agricultural activity pollutants getting to the environment (pesticides, xenobiotics, dyes, detergents, etc.) damage the biosphere in increasing degree.

Decompose microorganisms fill the primary part the decomposing of the pollutants getting to the soils and waters. Microorganisms becoming resistant to environmental pollutant are able to survive permanently in the strongly contaminated soils. They occupy the ecological niche of less resistant microorganisms, which are not able tolerant the presence of pollutants. The biological diversity of the soils considerably decreases, so the microorganisms can less adapt to the other changes of the environment (disturbing the biological balance).

Nowadays the demand is increasing to prevent the harmful, often irreversible changes caused by chemical pollutants. The most important field of this is the protection of soils and natural waters. The extensively applied chemical compounds (mostly the slow degrading plant protecting compounds containing heavy metals) notify an increasing load to the microbial community of the soil.

The demand is increasing to replace the chemical protection methods applying in the agriculture by environmental friendly biological control methods. The single or combined applying of the adequately chosen microorganisms (mostly bacteria and microscopic fungi) in many instances can be cheap, but stable and successful.

The object of our oriented basic research in the soil-plant-microorganism system, considering the practical application of bacterium strains selected by us was the following:

Preliminary researches:

- Sampling from the basic soil types can be found in different region of Hungary;
- Isolation of fluorescent *Pseudomonas* strains from the soil samples and from the rhizosphere of plant roots grown in soil samples;
- Identification the species of the strains using classical and modern (BIOLOG) microbiological methods.

1. *In vitro* researches in Petri dishes:
   - Revealing the antagonistic effects of *Pseudomonas* strains against each other and against *Rhizobium* strains;
   - Antagonistic tests with *Pseudomonas* strains against some strains of frequent phytopathogen fungi *(Pythium, Phoma, Ascochyta, Fusarium*) and against the *Erwinia* (fire blight) strains;
   - Examination of antibiotic sensitivity of *Pseudomonas* strains by antibiotic discs method;
   - Determination the hidrogen-cianide (HCN), auxine production and pH tolerance of *Pseudomonas* strains;
   - Showing the influence of metal compounds on the growth and siderophore production of *Pseudomonas* stains by chromeasurol methods;
   - Determination of metal sensitivity of *Pseudomonas* stains measuring by photometer in liquid medium;
   - Seedling test showing the effects of *Pseudomonas* stains on the root elongation of pea.
2. Instrumental examinations:
   - Determination the quality and quantity of antibiotic-like secunder metabolites produced by *Pseudomonas* stains measured with HPLC in two different culture media.
3. Pot experiments:
   - Revealing the antagonistic effects of *Pseudomonas* stains and combinations of strains against phytopathogen fungi stains and their combinations.

MATERIALS AND METHODS

1. Isolation and identification of collected *Pseudomonas* strains, creation the strain collection

- Collecting and characterization of soil samples of basic soil types from the agricultural areas of Hungary;
- Isolation the **s**iderophore producing *Pseudomonas* strains:
  - from the suspension of collected soil samples on King's B culture medium by limiting dilution method (SIMON et al., 1973),
  - from the rhizosphere of pot plant roots grown in soil samples by the method of Tepper (TEPPER, 1945).
- Identification the strains:
  - by the classical methods: Gram staining, catalase, oxidase tests, morphological characterization, usage of carbon sources, fermentation ability, growing on different temperatures, nitrate reduction, presence of UV pigments (BERGEY'S MANUAL, (1984), JESSEN, (1965) and LÁNYI, (1980));
  - by BIOLOG test: on the basis of usage 95 different carbon sources, detection by the color changing of TTC, evaluation with the help of computer database;
- Storage of the isolated *Pseudomonas* strains in 50% glycerin in freezer.

2. *In vitro*, ecophysiological experiments with *Pseudomonas* strains (in Petri dishes)

- Antagonistic tests against *Pseudomonas* strains: on King's B culture medium, passing to small spots with one day bacterium suspension, one day after vaporizing the other strain, two days after evaluating the experiments by the measurement of the inhibition zone around the colony;
- Antagonistic tests against 6 *Erwinia amylovora* and 7 *Rhizobium* strains on King's B culture medium, and between *Rhizobium* strains on yeast-mannitol (YMA) culture medium by the previous method;
- Antagonistic tests against phytopathogen fungi strains on King's B and malt culture media, passing the strains or the mixtures of the strains to small spots with one day bacterium suspension and agar cubes covered with fungus mycelia. Two days later evaluating the experiments by the measurement of the inhibition zone around fungi, or the distortion of the fungus colonies;
- Antibiotic sensitivity tests with 22 types of RESISTEST antibiotic discs on King's B culture medium, spreading *Pseudomonas* strains with glass cane.

One day later evaluating the experiments by the measurement of the inhibition zone around antibiotic discs (CZIRÓK, 1999);

- Determination the hidrogen-cianide (HCN), auxine production, chitin and cellulose decomposition and pH tolerance of *Pseudomonas* strains;
- Determination the influence of 16 types metal compounds ($AgNO_3$, As_2O_3, $CdSO_4 \times {}^8/_3H_2O$, $CoSO_4 \times 7H_2O$, $CrK(SO_4)_2 \times 12H_2O$, $CuSO_4$, $HgCl_2$, $MnSO_4 \times nH_2O$, $Na_2MoO_4 \times 2H_2O$, $NiCl_2 \times 6H_2O$, $PbCO_3$, $SbCl_3$, $SnCl_2 \times 2H_2O$, Tl_2SO_4, V_2O_5, $ZnSO_4 \times 7H_2O$) in 8 concentrations (10, 20, 40, 80, 160, 320, 640 and 1280 µM) on the growth and siderophore production of *Pseudomonas* stains on modified chromeasurol King's B culture medium. Two days later evaluating the experiments by the measurement of the siderophore zone around colonies (SCHWYN and NEILANDS (1987));
- Determination the influence of culture medium on the siderophore production of *Pseudomonas* stains on modified chromeasurol King's B and malt culture media. Two days later evaluating the experiments the siderophore zone around colonies;
- Determination of metal sensitivity of 16 *Pseudomonas* stains in liquid medium against 3 metal compounds ($CoSO_4 \times 7H_2O$, $CrK(SO_4)_2 \times 12H_2O$, $AgNO_3$) in 4 concentrations (10, 40, 160 and 640 µM) on King's B liquid culture media. Evaluation after one day shaken incubation at 28 °C measuring the absorption by photometer at 550 nm (SPEKTROMOM photometer);
- Seedling test showing the effects of 24-hour liquid suspensions of *Pseudomonas* stains on the root elongation of the germinated pea seeds with 2-3 mm rootlet. Evaluation by the measurement of the roots after 5 day incubation at room temperature.

3. Determination of antibiotic-like secunder metabolites produced by *Pseudomonas* stains measured with HPLC

- Preparation of the samples: inoculation 20 ml liquid culture medium with 100 µl of 24-hour liquid suspensions of *Pseudomonas* stains in Erlenmeyer test-tube, shaken incubation for 2 days at 27 °C. Souring the suspension with HCl to pH 2, extraction with 2×20 ml ethylacetate in two pass with 5-5 minute shaking. Centrifuging the organic phase for 30 minutes at 250×g in Falcon tubes, storing the samples for a night at -20 °C. Vaporizing the organic phase with a rotating evaporator (Rotovapor, RE120) to a drop, dissolving it in 1 ml methanol, centrifuging it for 10 minutes at 500×g in Eppendorf tubes, pipetting the upper two-thirds to a HP tubes and sealing the samples;

– The process of the measuring: flowing the 10 µl prepared sample in methanol system at 10 MP pressure between 200 and 400 nm wavelength with 13 minutes retention time by high pressure liquid chromatographic method (HP-1090 HPLC) for determination the following metabolites: phloroglucinol, monoacetil-phloroglucinol, pyochelin, pyoluteorin and salicyl acid;
– Comparasion the quality and quantity of antibiotic-like secunder metabolites produced by *Pseudomonas* stains incubated on GCM and Karner's PDA (for high antibiotic production) culture media.

4. Pot experiments with *Pseudomonas* stains

– Antagonistic tests with pea (Gloria de quimper) seeds coated with 35 *Pseudomonas* stains alone and pairs against mixtures of 2 *Pythium ultimum* or 6 *Phoma medicaginis* var. *pinodella* phytopathogen fungus strains;
  – Preparation the infested soil: incubation the cultures of *Pythium* stains for 3 days, the cultures of *Phoma* stains for a week in Erlenmeyer test-tubes at 24°C on sterile, moistened maize pellets. Mixing up the strains of fungi belonging to the same species, powdering the pellets into small crumb, creating the soil mixture (12-12 kg) infested with mycelia (1.5% infestation at the *Pythium*, 3% at the *Phoma*) and passing through a sieve. Putting in the pots (14×3 cm PVC tubes) with infested soil in the middle part, and not infested soil in the bottom and upper parts of pots;
  – Preparing *Pseudomonas* suspension: dispersing 250 µl one day incubated at 27 °C shaken bacterium suspension to King's B culture medium in Petri dishes. After 24 hours incubation gathering the bacterium layer in a 20-20 ml sterile solution of carboxymethile-cellulose (0,5% CMC);
  – Preparing the seeds treated with *Pseudomonas* strains: immersing the pre-germinated pea seeds with 2-3 mm rootlet (germinated in sterile watered agar-agar) to *Pseudomonas* suspension for a 10 minutes, and after this placing the treated and untreated (control) seeds to the infested soil layer;
  – Placing the pots of plants in the glasshouse for 16 hours with fluorescent light (150000 Lumen) and natural light at 20°C at night and 22°C in the daytime in watered soil, in random arrangement in 888 pots;
  – The evaluation of the experiment occurred by the calculation of the percent of survival plants, the determination of the level of infestation and the measuring the wet mass of plants.

RESULTS

1. Isolation and identification of *Pseudomonas* strains, creation the strain collection

– The analysis of soil samples collected from 22 places (30-50 kg/sample) was shown that the pH of the soils were neutral or faintly alkaline, in some samples the amount of certain metal compounds were high, other ones were rich in humus, but *Pseudomonas* strains could be found in all with 3 exceptions.
– The 22 intensive pigment-producing strains from about 300 fluorescent isolated colonies were identifyed by the classical methods. In consequences of comparing the results with the BIOLOG test, the following species were identified: 3 *P. corrugata* (PCO1-3), 8 *P. fulva* (PFU1-8), 6 *P. putida* (PPU1-6), 2 *P. tolaasii* (PTO 1-2) and one *P. synxantha* (PSY1). The first letters of their genus and species names and a number marked the strains. The authentic *P. fluorescens* and the identified (by us) strains with their spontaneous mutants (marking: strain name + $*,^{\times},^{+}$) were kept on at -20°C in cryovials in a 1:1 mixture of glycerin:culture medium in the strain collection of the research group (altogether 54 strains).

2. *In vitro*, ecophysiological experiments with *Pseudomonas* strains (in Petri dishes)

– The antagonistic characteristics of 35 different Pseudomonas strains were tested against each other in two repetitions with 1225 Petri dishes on King's B culture medium. It was established, that positive correlations were between the siderophore production and the antagonistic ability at the intensive fluorescent strains (e.g. PFU8, PFU3*, PFU2).
– The results of antagonistic tests against plant pathogenic fungi stains (*Alternaria*, *Botrytis*, *Fusarium*, *Rhizoctonia, Sclerotinia* és *Phoma*) were shown that the our *Pseudomonas* strains belonging to different species (PCO1, PFU2, PTO1) were appropriate efficiency in connection the measurement of the inhibition zone or the distortion of the fungus colonies. On the other hand, the two *P. fluorescens* reference strains (CHA0 and 2-93) proved to be stronger antagonistic influence especially against the representatives of *Fusarium* strains.
– *Pseudomonas* strains with the strongest antagonistic property were tested in pairs. The results were shown, that the effects between the strains were usually synergic, they enhanced each other (PPU3$^{\times}$–PAE1, CHA0$^{\times}$–PAE1, PTO1–PAE1), but in a few cases the strains decreased the effects of each other (PTO1–TMIA3, PTO1–PPU3$^{\times}$).
– By the application of the triple combination of strains from the best pairs (PAE1–PSP7–2-79, PAE1–PSP7–PTO1, PAE1–PSP7 PPU3$^{\times}$) the antago-

nistic effect become so strong, that they completely inhibited the multiplication of the *Ascochyta* fungus

- 19 *Pseudomonas* strains with antagonistic features were tested against 6 *Erwinia amylovora* strains on King' B culture medium. Beside the strong antagonistic characteristic of reference strains (TMIA3, 2-79, CHA0) some of our strains (PFU8, PAE1) showed outstanding antagonistic features. The lack of inhibition zone at the examination of *Erwinia* strains against *Pseudomonas* strains only the surface modification of the *Erwinia* colony showed the scale of antagonism.
- The antagonistic characteristics of 35 different Pseudomonas strains were tested against 7 *Rhizobium* strains. The greater part of the *Pseudomonas* strains were inhibitory effect on the growth of beneficial N_2-binding bacteria, but could be found some such strains (PCO1, PTO1) which could be compatible for seed inoculation together with *Rhizobium,* and, in addition to they had good antagonistic property against phytopathogens.
- 7 *Rhizobium* strains were tested against each other, but apart from one case, inhibiting effects were not detected.
- Examinations were carried out to determine the antibiotic sensitivity of 54 *Pseudomonas* strains against 22 type antibiotics. Some straind (PFU4*, PCO3, PSY1) proved increased sensitivity against many antibiotics, especially in case of the kanamycin, tobramycin and paromycin, but the effects of the penicillin, nystatin and ampicillin were negligible.
- From the 33 tested *Pseudomonas* strains 10 possessed the ability of the hydrogene-cianide production, auxine production however could be shown only at the CHA0 reference strains. Between pH 6.0-7.5 every *Pseudomonas* strains were able to multiply, around pH 5 however some strains already were not able to multiply. The strains wich could tolerate acid condition above all, were able grow at pH 4.4, but all of them – except the PSP5 strain – were not capable to produce siderophores.
- Examinations were carried out to detect the effect of 16 metal compounds in 8 concentrations to the siderophore production of 54 *Pseudomonas* strains. The toxicity order of the applied metal compounds from the most toxic to the mildest effect was the following: Hg>Co>Sb>Ag>Tl>As>V>Cd>Ni>Cu>Mo>Pb>Mn>Zn>Sn>Cr. The higher concentrations of the first 8 metal compounds showed strongly toxic effect. These compounds inhibited not only the siderophore production, but the growing of the *Pseudomonas* strains also. The nickel and the following metals caused significant growth inhibition neither in the highest applied concentration. At some strains could be observed the

increasing of the siderophore production at the growing concentrations of metal compounds (e.g. the PFU8 strain at the influence of antimony, copper, lead, manganese, and zinc and tin ions).

– We revealed at the comparison of siderophore production of 54 *Pseudomonas* strains on proteose-pepton containing culture medium, that the strains possessed outstanding siderophore production (CHA0$^{\times}$, PFU4, PCO3) produced order of magnitude more iron chelate, than the strains with the slightest fluorescence intensity. In case of malt culture medium the siderophore production was order of magnitude lower at all strains, than on the King's B medium.
– In the metal sensitivity tests of 16 *Pseudomonas* stains in liquid medium against 3 metal compounds were established that the silver ions were the most toxic. The growth of all strains except PFU3 and PTO1 was inhibited at the 160 µM silver concentration. The cobalt ions were less toxic, and the least the chrome ions, where slight growth stimulation was detected at the lower concentrations (up to 40 µM).
– At the pea seedling tests we found out, that from the 19 *Pseudomonas* strains the PFU2^{+} markedly stimulated the root growth of tested plants. We detected strong root growth inhibition in many instances at the strains possessed exceptional antagonistic property.

3. Determination of antibiotic-like secunder metabolites produced by *Pseudomonas* stains measured with HPLC

– The most common antibiotic-like compound was the 2,4- diacetyl-phloroglucinol (PHL) from 35 examined *Pseudomonas* strains. It was formed in somewhat higher quantity on CGM culture medium, than on Karner's PDA. Except for some strains (PFU5, PFU6*, PPU3, PPU6, PSP1) it was occurred at the most *Pseudomonas* strains. The CHA0 reference strain showed PHL production in a large quantity on both culture media. From our strains only the PFU8 and PTO1 strains were able to produce in bigger quantity on CGM culture medium. However in case of the culture medium with potato extract (Karner's PDA), the PCO2 strain produced this benzene derivative responsible for antagonism in an enormous quantity and the PCO1 strain in medium quantity.
– The pyoluteorin (PLT) was occurred relatively rare, and the produced amount of it was lower, than the PHL. Only the two reference strains (CHA0, 2-79) produced on PDA culture medium, but on GCM culture medium the PFL1, the PFU7 and the PPU5 strains produced in a large quantity also. Except 8 slightly PLT producer strains the others were not able to synthetize it.

– The monoacetyl-phloroglucinol (MPHL) is one of a precursor of PHL, so PHL and MPHL usually occurred together. The best producer strains were the CHA0 and the PFL1 strains on GCM culture medium, but the PTO1, the PTO2 and the PCO3 strains also synthetized it in a significant quantity. On PDA culture medium the CHA0 and the PCO2 strains produced a lot of it, and the PFU7, PFL1, PPU5 and PSP1 strains produced it in a medium amount. In case of 13 strains it was not detectable on neither culture media
– The pyochelin was the most infrequent occurring metabolite. The CHA0 strain synthetized it in a significant quantity on both culture media. The PPU5 and the PSP1 strains produced it in a lot of amount on GCM culture medium, and other 3 strains (PTO2, PTO2*, 2-79) produced it in a limited concentration. In a small amount of pyochelin could be detected at the PCO1 and the PCO2 strains on Karner's PDA culture medium. In case of other strains it was not detectable.
– The greatest difference was appeared at the salicylic acid production between the effect of two culture media. Apart from the good salicylic acid producer CHA0, other *Pseudomonas* strains could produced it only a limited concentration on GCM culture medium. At 14 strains the presence of it could not be observed. On PDA culture medium the amount of produced salicylic acid was much higher, and could be shown at all strains. Especially the strains originated from Érd (PCO2 and PCO2*) stood out wit there extraordinary level salicylic acid production.

4. Pot experiments with *Pseudomonas* stains

– On the basis of the results of 35 *Pseudomonas* strains tested against *Pythium* could be shown, that even the reference strains (CHA0, 2-79) with *in vitro* outstanding antagonistic property were not able to give a suitable protection against the rapid fungal infection. On the basis of the surviving plants numbers the efficiency of the most advantageous strains did not reach the 50 %. In case of some strains (PFU2, PPU5 and PSP2) could be detected worse results than the controls without bacteria. The numbers of surviving plants at *Phoma* treatments were not showed significant differences. On the basis of average masses of plants the best effect could be obtained with application of PTO1, PSP1 and PPU4 strains, and the worst effect could be found in case of TMIA3, CHA0 and PFU7 strains.
– The PAE1 strain in four combinations (PAE1-PTO1, PAE1-PPU3$^{\times}$, PAE1-TMIA3, and PAE1-PSP7) produced outstanding results. The *Pythium* performed more thorough destruction of plants in these cases than the *Phoma.*

NEW SCIENTIFIC ACHIEVEMENTS

1. With the exception of 3 most extreme soil samples with high sodium content (Nádasladány: marsh soil; Apaj: solonchak; Kunszentmiklós: solonetz) fluorescent *Pseudomonas* strains could be found in all samples.
2. With the application of BIOLOG test and classical identifying methods could be proofed, that between the strains isolated by us and belonging to the *P. fluorescens-putida* species-group essential differences existed in relationship. One part of identified strains belonged to the plant growth promoting rhizobacteria (PGPR) (e.g. *P. fluorescens*, *P. putida, P. fulva*), the others could be facultative pathogens (e.g. *P. corrugata, P. tolaasii, P. aeruginosa*). In the knowledge of proper places of species could not be found correlation between the occurrence frequency of isolated strains (species) in a given soil type and the certain area or soils of Hungary.
3. At the experiments with *Pseudomonas* against phytopathogen fungi the own isolated strains (PCO1, PFU2 and PTO1) provided similar good results, than the reference CHA0 and the 2-79 strains. The level of antagonism was strongly influenced by the applied culture medium and methods, and the phytopathogen fungi. The tests with *Pseudomonas* strains in pairs was shown, that using together the strains with moderate antagonistic property (PAE1, PPU3$^{\times}$, PTO1) the joint effect sometimes was stronger, because the effects between the strains were usually synergic, and the strains enhanced the antagonistic influence of each other. The outstanding effects of these compatible pairs of strains could be enhanced between certain circumstances applying a third strain (PSP7). These facts especially confirm the importance of strain selection.
4. According to the results of examinations against *Erwinia amylovora* the *Pseudomonas* strains producing relatively big inhibition ring (PFU8, 2-79, CHA0, TMIA3, PAE1) could mostly restricted the pathogens of fire blight.
5. In connection with the change of siderophore production of *Pseudomonas* strains for the influence of metal compounds it have to emphasize, that the higher concentrations of applied metals (Hg, Co, Ag, Cd) compounds influenced definite inhibitory effect not only on the siderophore production, but on the growth of strains also. On the other hand the zinc and the tin had a stimulating effect on the siderophore production of strains even the highest tested concentration.
6. The pea seedling tests revealed, that some of the *Pseudomonas* stains with a prominent antagonistic ability (PFU2$^{\times}$, PFU8) were able to affect positively the growth of the roots of pea seedlings, the others however caused the inhibition of root growth.

7. Between the results of *in vitro* antagonistic test and pot experiments could not revealed unambiguous positive correlation considering the degree of antagonism. At the application of *Pseudomonas* strain pairs the adequate partners were able to increase the advantageous effect of each other.
8. Between the antibiotic production and the volume of antagonistic property not every time could be found correlation. The level of antagonistic property mainly depended on the siderophore production of strains. The strains with a weak antagonistic property could produce only salicylic acid, or outside of this a little amount of other antibiotics.

CONCLUSIONS AND SUGGESTIONS

1. The siderophore production performing importance for production of antagonistic effect is considerably depended on the available Fe^{3+} ion content of soil, what is significantly influenced by the pH of the soils. Therefore the selection of strains suitable for given plant-soil system has special importance.
2. In the interaction of *Pseudomonas* strains between each other the production of siderophores has a great importance in the producing of intragenetic antagonistic effect.
3. At the preliminary selection of *Pseudomonas* strains testing of pH toleration did not prove essential point of view, because below the pH 5, where the growth of certain *Pseudomonas* strains already inhibited, the solubility of $Fe(OH)_3$ becomes satisfactory, so the taking up the iron by bacteria already is not impeded, for this reason siderophores is not produced. However the pH of the most important Hungarian arable soils is in the range of 6.0-7.5.
4. During the seed inoculation together with *Rhizobium* strains only application of such *Pseudomonas* strains is suitable, which have good antagonistic property against phytopathogens, but did not affect adversely the growth of root nodule forming bacteria.
5. The quality and quantity of antibiotic-like secunder metabolites in our strains – similarly to the siderophores –was strongly affected by the applied culture medium. This confirm the fact, that the different conditions of soils can essentially influence the availability and effectiveness of siderophore producing *Pseudomonas* strains using them for plant growth promotion and against phytopathogens.
6. At the application of *Pseudomonas* strains has to take into consideration also the importance and possibility of the strain selection using them for preventive or causative plant protection.

LIST OF SCIENTIFIC WORKS RELATED TO THE SUBJECT OF THE DISSERTATION

REVISED, REFERRED PUBLICATIONS:

1. NAÁR ZOLTÁN, **VÁRADY GYÖRGY**, KISS ZITA, KUCSMA NÓRA, KECSKÉS MIHÁLY (1995): Különböző fajokhoz tartozó *Trichoderma* törzsek kadmium érzékenysége, Agrokémia és Talajtan, 44., 3-4. 457-462.
2. BAYOUMI HAMUDA, KISS ZITA, KUCSMA NÓRA, **VÁRADY GYÖRGY**, BALÁZSY S., KECSKÉS M. (1995): Különböző talajtényezők hatása a *Rhizobium leguminosarum* törzsek szaporodására és túlélésére, Agrokémia és Talajtan 44., 3-4. 463-472.
3. BAYOUMI, BALÁZSY SÁNDOR, **VÁRADY GYÖRGY**, KISS ZITA, KUCSMA NÓRA, KECSKÉS MIHÁLY (1995): Lóbab *Rhizobium* törzsek talaj-fungicid érzékenysége, Agrokémia és Talajtan, 44., 3-4. 473-480.
4. BAYOUMI HAMUDA, KUCSMA NÓRA, **VÁRADY GYÖRGY**, KISS ZITA, BALÁZSY S., KECSKÉS M. (1995): Néhány alumínium vegyület hatása különböző Rhizobium fajokhoz tartozó törzsek szaporodására, Agrokémia és Talajtan, 44., 3-4. 481-490.
5. BAYOUMI HAMUDA, KISS ZITA, **VÁRADY GYÖRGY**, BALÁZSY SÁNDOR, KUCSMA NÓRA, KECSKÉS M. (1996): The Influence of Nitrapyrin and sodium azide on the growth and respiration of some *Rhizobium* strains, Microbiological Research 151. 247-251.
6. BAYOUMI HAMUDA H.E.A.F., KUCSMA N., **VÁRADY GY.**, KISS Z., KECSKÉS M. (1996): Nehézfémek és kombinációik hatása különböző *Rhizobium leguminosarum* törzsek szaporodására. Agrokémia és Talajtan, 45., No. 1-2., 153-168.
7. BAYOUMI, H. E. A. F., KISS Z., **VÁRADY**, **GY.**, BALÁZSY S., KUCSMA, N., KECSKÉS M. (1996): A N-serve és a nátrium-azid hatása endoszimbionta rhizobiumok szaporodására és légzésére. Agrokémia és Talajtan, 45., No. 1-2., 169-175.
8. BIRÓ B., MAGYAR K., **VÁRADY GY.**, KECSKÉS M. (1998): Specific replant disease reduced by PGPR rhizobacteria on apple seedlings. Proc. 4th Intern. Symp. Replant Probl. Acta Horticult. 477**:** 75-81.
9. **VÁRADY GY.**, KUCSMA N., BIRÓ B., BAYOUMI, H.E.A.F., KECSKÉS M. (2002): Környezetkárosító fémvegyületek hatása a *Pseudomonas*ok szaporodására és sziderofor termelésére. Agrokémia és Talajtan, In press.
10. **VÁRADY GY.**, KUCSMA N., BIRÓ B., BAYOUMI, H.E.A.F., KECSKÉS M. (2002): The influence of antibiotic and siderophore production on the antagonistic effects of *Pseudomonas* strains against fungal pathogens. Int. Microbiol., In press.

SHORT PUBLICATIONS:

11. KUCSMA, N., BAYOUMI HAMUDA, H.E.A.F., **VÁRADY**, **GY**. BIRÓ, B., KECSKÉS, M. (1994): Effect of metal-combination on some strains of various *Rhizobium* species. Abst. Ann. Meet. Hung. Microbiol. Soc. 1994. Aug. 23-25., Szolnok, Hungary, pp. 111.
12. BAYOUMI HAMUDA, H.E.A.F., **VÁRADY**, **GY.**, KISS, Z., BALÁZSY, S., KUCSMA, N., KECSKÉS, M. (1995): Ecological factors influencing the saprophytic competence of endosymbiont *Rhizobium leguminosarum* strains introduced to brown forest soil. Acta Microbiol. Immunol. Hung. 43, 274.
13. BAYOUMI HAMUDA, H.E.A.F., KISS, Z., **VÁRADY**, **GY.**, BALÁZSY, S., KUCSMA, N., KECSKÉS, M. (1995): Nitrification and respiratory process inhibitors influencing

growth and respiration of some *Rhizobium* species. Acta Microbiol. Immunol. Hung. 43, 273-274.

14. KUCSMA NÓRA, H.E.A.F. BAYOUMI, **VÁRADY GYÖRGY**, BÍRÓ BORBÁLA, KECSKÉS MIHÁLY (1995): Effect of metal combinations on some strains of various *Rhizobium* species, Acta Microbiol. Immunol. Hung. 42, 117-118.
15. BIRÓ, B., KUCSMA, N., KISS, Z., **VÁRADY**, **GY**., KÖVES-PÉCHY, K., VÖRÖS, I., KECSKÉS**,** M. (1996): Interaction between rhizosphere microorganisms and red clover at metal stress. Acta Microbiol. Immunol. Hung. 44**:** 89-90.
16. KECSKÉS, M., BIRÓ, B., BAYOUMI, H.E.A.F., KISS, Z., KUCSMA **VÁRADY**, **GY**., NAÁR, Z. (1997): Heavy metal ion toxicity on various soil and rhizosphere microorganisms. Acta Alimentaria. 26, 313-315.
17. HEGEDŰS, A., FARKAS, S., **VÁRADY**, **GY**., KECSKÉS**,** M. (1999): The role of growth of *Trichoderma* spp. inoculates in the growth of Gerbera plants. Acta Microbiol. Immunol. Hung. 46: 122.

PROCEEDINGS

18. KECSKÉS, M. BIRÓ, B., BAYOUMI, H.E.A.F., NAÁR, Z. , **VÁRADY**, **GY**., KISS, Z., KUCSMA, N. (1996): Microbial component of combined preparations used for plant. Proc. Internat. Multidispl. Conf., North Univ. Baia Mare, Baia Mare, Romania, 21-23 May, 1996. pp. 4-14.
19. KECSKÉS**,** M., BAYOUMI, H.E.A.F., NAÁR, Z., **VÁRADY**, **GY**. (1997): Metal ion sensitivity and tolerance of soil microorganisms. Proc. Internat. Multidispl. Conf., North Univ. Baia Mare, Baia Mare, Romania, 28-29 November, 1997. Sci. Bull. Vol. XI. North Univ. Baia Mare pp. 73-82.
20. **VÁRADY**, **GY**., KECSKÉS**,** M. (1997): Antagonstic effect of *Pseudonomas* strains in connection of their antibiotic production. Proc. Internat. Regional Sem. Environment Protection: Modern studies in Ecology and Microbiology. Uzhgorod, Ukraine. May 13-16, 1997 Vol. 2. pp. 167-172.
21. HEGEDŰS, A., FARKAS, S., **VÁRADY**, **GY**., KECSKÉS**,** M. (1997): Interactions of *Pseudomonas* and *Fusarium* strains on root of seedlings of wheat cultivars. Proc. Internat. Regional Sem. Environment Protection: Modern studies in Ecology and Microbiology. Uzhgorod, Ukraine. May 13-16, 1997 Vol. 2**.** pp. 173-176.

LECTURES AND POSTERS

1. KUCSMA NÓRA, H.E.A.F. BAYOUMI, **VÁRADY GYÖRGY**, BÍRÓ BORBÁLA, KECSKÉS MIHÁLY: Effect of metal combinations on some strains of various *Rhizobium* species, A Magyar Mikrobiológiai Társaság Nagygyűlése, Szolnok, 1994. aug. 23-25.
2. BAYOUMI HAMUDA, WELDON KIMBERLY, KUCSMA NÓRA, KISS ZITA, **VÁRADY GYÖRGY**, KECSKÉS MIHÁLY: Effect of various soil states on the multiplication of *Rhizobium leguminosarum* strains, A talaj környezetvédelmének problémái, III. Országos Konferencia, Nyíregyháza, 1994. szeptember 28-29.
3. BAYOUMI HAMUDA, WELDON KIMBERLY, KUCSMA NÓRA, **VÁRADY GYÖRGY**, KISS ZITA, KECSKÉS MIHÁLY: Combined effect of different PGPR and NH_4NO_3 PAE1els on symbiotic characters of *Vicia faba*. Az MTA Szabolcs-Szatmár-Bereg megyei Tudományos Testülete III. Tudományos Ülése, Nyíregyháza, 1994. október 1.
4. BAYOUMI HAMUDA, WELDON KIMBERLY, KUCSMA NÓRA, **VÁRADY GYÖRGY**, KECSKÉS MIHÁLY: Growth and survival of *Rhizobium leguminosarum* strains af-

fected by different sodium salt concentration, IX. Mikrobiológiai Tudományos Ülés, Nyíregyháza, 1994. okt. 7-8.

5. NAÁR ZOLTÁN, **VÁRADY GYÖRGY**, KISS ZITA, KECSKÉS MIHÁLY, Antagonista trichoderma, pszeudomonasz és foszfátoldó mikroorganizmusok előfordulása a Westsik homoki tartamkísérlet kezeléseiben, Országos Homokkutatási Konferencia, Nyíregyháza, 1995. június 9-10.
6. BAYOUMI HAMUDA, **VÁRADY GYÖRGY**, KISS ZITA, BALÁZSY SÁNDOR, KUCSMA NÓRA, KECSKÉS MIHÁLY: Nitrification and Respiratory Process Inhibitors Influencing Growth and Respiration of some *Rhizobium* Species. A Magyar Mikrobiológiai Társaság Nagygyűlése, Budapest, 1995. augusztus 23-25.
7. BAYOUMI HAMUDA, KISS ZITA, **VÁRADY GYÖRGY**, BALÁZSY SÁNDOR, KUCSMA NÓRA, KECSKÉS MIHÁLY: Ecological Factors Influencing the Saprophytic Competence of Endosymbiont *Rhizobium leguminosarum* Introduced to Brown Forest Soil, A Magyar Mikrobiológiai Társaság Nagygyűlése, Budapest, 1995. aug. 23-25.
8. **VÁRADY GYÖRGY**: Rhizospheric fluorescens pseudomonads antagonists of some phytopathogen fungi, International symposium on current topics in microbiology, Uzghorod, 7-8. February 1996.
9. BÍRÓ BORBÁLA, KUCSMA NÓRA, **VÁRADY GYÖRGY**, KECSKÉS MIHÁLY: Heavy metal sensitivity of some Rhizobium - leguminous plant system, International symposium on current topics in microbiology, Uzghorod, 7-8. February 1996.
10. FARKAS SÁNDOR, HEGEDŰS ANTAL, **VÁRADY GYÖRGY**, KECSKÉS MIHÁLY: Néhány xenobiotikum hatása *Escherichia coli*, *Rhizobium meliloti* és *R. leguminosarum* törzsek szaporodására. A Magyar Mikrobiológiai Társaság Nagygyűlése, Nyíregyháza, 1996. augusztus 21-23.
11. KUCSMA, N., BAYOUMI HAMUDA, H.E.A.F., KECSKÉS, M., **VÁRADY, GY.**: Abiotikus és biotikus talajfaktorok hatása a *Rhizobium leguminosarum* bv. *viciae* törzsek szaporodása laboratóriumi feltételek között. Az MTA Sz-Sz-B m. Tud. Testülete, az MMT Mezőgazd. és Élelmiszer Mikrobiol. Szekciója. XII. Mikrobiológiai Tudományos Ülése. 1997. október 10-11, Nyíregyháza.

REFERENCES

BERGEY'S MANUAL of Systematic Bacteriology (1984): Vol. 1. Williams and Wickins, Baltimore, London.

CZIRÓK, É. (főszerk.) (1999): Klinikai és járványügyi bakteriológia, Budapest, Melania Ltd.., 592-603.

JESSEN, O. (1965): *Pseudomonas aeruginosa* and other green fluorescent pseudomonads. A taxonomic study. Munksgaard, Copenhagen. pp. 243.

LÁNYI, B. (szerk.), (1980): Járványügyi és Klinikai bakteriológia. Módszertani útmutató. OKI, Budapest, p. 588.

SCHWYN, B. AND NEILANDS, J. B. (1987): Universal Chemical Assay for the Detection and Determination of Siderophores. Anal. Biochem. 160, 47-56.

SIMON, A., ROVIRA, A. D. AND SAUDS, D. C. (1973): An improved selective medium for the isolation of fluorescent pseudomonas. J. Appl. Bacter. 36, 141-145.

TEPPER, E. C. (1945): Metodika ucseta tipicsnüh bakterij rizoszferü szelstkohozjajsztvennüh rasztenij. TSZHA dokladü, vüp. 2, 131.