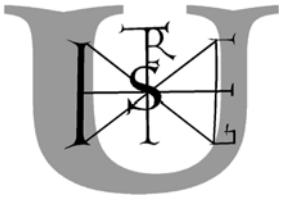

SZENT ISTVÁN UNIVERSITY

ECOLOGICAL EVALUATON OF INTERACTIONS OF RHIZOSPHERE BACTERIA AND HIGHER PLANTS

Thesis of Ph.D. dissertation

Bálint Oldal

Gödöllő

2006.

Doctoral School

Denomination: Ph.D. School of Environmental Science

Manager: Prof. Dr. Zoltán Menyhért
Professor, D.Sc.
Szent István University, Faculty of Agriculture and Environmental Science,
Institute of Environmenal Management

Program

Denomination: Agricultural and Environmental Microbiology, Soil Biotechnology

Manager: Prof. Dr. Mihály Kecskés
Professor, D.Sc.
World Council of Hungarian Professors

Discipline: Environmental Science, Environmental Microbiology

Supervisor: Prof. Dr. Mihály Kecskés
Professor, D.Sc.
World Council of Hungarian Professors

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Approval of School Manager Approval of Supervisor

AIMS

The aim of this work was on one hand to investigate and evaluate the plant growth regulating (PGR) and -promoting (PGPR) effects of culturable bacteria occurring in the rhizosphere (and rhizoplane) of hairy vetch (*Vicia hirsuta* Roth), white lupine (*Lupinus albus* L.) and rye (*Secale cereale* L.) plants from the long-term crop rotation field experiment which was established by Vilmos Westsik in 1929 for studying the amelioration of low-nutrient-supplied sandy soils by means of organic manures enriched with inorganic fertilizers in a low-input (organic management) system, in the Nyírség region in North-Eastern Hungary.

On the other hand, the cyanide and heavy metal pollution in the Tisza River of the year 2000 induced another investigation project (OLDAL et al. 2001, VARGHA et al. 2001, OLDAL et al. 2004), which provided a secondary base for strain selection from the water and floodplain of the river: What effect could have such another pollution event (already happened to various extent in the meantime) on the agricultural fields located in the soils developing on colluvial material of Tisza River (Anthropic Regosols), in the Nyírség region? The influence of the habitat on the tolerance, plant growth promoting beneficial effect and antagonistic ability against plant pathogenic microorganisms was eco-physiologically compared within the bacterial strains gained from the anthropogenic (crop rotation system) and natural (rather intact: shore, floodplain) ecosystems.

SCOPE

1.) Assessment of the occurrence of bacteria originated from rhizosphere, soil and water in cultivated and natural – floodplain – areas

- Isolation of bacteria from the rhizosphere of vetch, lupine and rye host plants of Westsik-type sand improving crop rotation long-term experiment and from water and floodplain soil of the Upper Tisza River in some characteristic sampling areas.
- Effect of different origins (treatments of the cop rotation experiment versus polluted and non-polluted areas) to the natural abundance: determination of interrelations between sampling sites and eco-physiological properties of bacterial strains.
- Formation of an own strain collection applicable for soil biotechnological purpose, by using strains dealing with known properties.

2.) Assessment of eco-physiological properties of the isolated bacteria by different methods

- Assessment of the plant growth promoting effects in axenic cultures using lupine, vetch and rye test plants; selection of effective strains.
- Screening and demonstration of hormone-like substances produced by the strains showing significant growth promotion.
- Assessment of the tolerance of selected bacterial strains against cyanide and several heavy metal compounds, as well as
- determination of their antagonistic capacity against some characteristically occurring plant pathogenic microscopic fungi.

3.) Selection of bacterial strains potentially applicable for plant inoculation

MATERIALS AND METHODS

Sampling sites

Characterization of sampling area of the Westsik-type crop rotation long-term experiment

Free-living bacteria were isolated from the rhizosphere and rhizoplane of rye (*Secale cereale* L.), hairy vetch (*Vicia villosa* ROTH) and white lupin (*Lupinus albus* L.) grown **(Table 1.)** in the plots of the Vilmos Westsik-type sandy soil improvement crop rotation long-term experiment.

Table 1. Treatments of Westsik's long-term experiment. Shading displays legumes.

Westsik-type code	Treatment, fertilizers	Crop rotation sequence
I.	Out of crop, weed ploughing, —	S, R, S
II.	Lupine green manure, P-K	L, R, S
III.	Lupine root manure, P-K	L, R, S
IV.	Sand improvement with straw, N-P-K	S, R, R
V.	Straw manure fermented with NH_4NO_3, N-P-K	R, S, R
VI.	Straw manure fermented with water, N-P-K	S, R, R
VII.	Non-fermented straw manure, —	R, S, R
VIII.	Lupine root- and green manure, P-K	S, R, L
IX.	Leguminous stringy forage crops, P-K	S, R, L
X.	Dual forage crops, —	S, V, R
XI.	Farmyard manure, P-K	V, S, R
XII.	Autumnal forage crop mix, P-K	R, S, R
XIII.	Second-sowed lupine green manure, N-P-K	S, R, R
XIV.	Second-sowed lupine green manure, N-P-K	S, R, R
XV.	Second-sowed lupine green manure, —	S, R, R

S: potato (*Solanum tuberosum* L.); **R**: rye; **L**: lupine; **V**: vetch with oats.

The soil of the experimental farm is comprised of a mixture of sand with little calcium content and carbonate-loess. The ratio of silty clay is 4-6%. The area was covered with unbroken forest during the soil formation phase; therefore the soils are weak humic (LAZÁNYI 1994). In the accumulation level, 2-3 cm thick iron trails 30-40 cm apart may be found, therefore the water and nutrient storage capability of the soil is better than that of shifting sand, because iron trails are able to store more water and nutrients due to their colloide content. The plots are located on a sandy hill oriented North-South close to the Érpatak Creek, which is supplied by the water from the pans among pins of sand.

Characterization of the soil geography and vegetation of the Upper Tisza River floodplain with regards to heavy metal pollution

The River Szamos, which carried the cyanide and heavy metal pollution to the River in 2000, flows into the River Tisza at Jánd above Vásárosnamény, where the river still has upper stream characteristics, but is relatively slow (its average water flow is 4-6 $km \cdot h^{-1}$). Heavy metals may have poisonous effect on living organisms primarily as a result of bioaccumulation processes. However, bacteria are a lot more resistant to cyanide than are eukaryotes. Several species even produce cyanide, which is used in biological protection against plant pathogenic microorganisms. Many bacteria, e.g. some *Pseudomonas* species are able to utilize cyanide as C and N sources (KUNZ et al. 1998).

Soil and water samples were obtained at the sampling sites recommended by the Environmental Inspectorate of the Upper Tisza Region (today: Environmental, Nature Conservancy and Water Policy Inspectorate of the Upper Tisza Region, Nyíregyháza), where – in order to assure representative water samples – the main stream was accessible by pedestrian bridge or ferry; these are: Tivadar (705.8 river km), Aranyosapáti (668.6 river km, the Szamos outlet, below and close to Vásárosnamény), Záhony (613.1 river km) and Balsa (544.1 river km). Soil type is Mollic Fluvisol everywhere (FAO 1998). The flora of the area is made more diverse by floodplain forest grassland, softwood groves, reedy shore zones and swamps, marshes and wetlands. The related flora and fauna show high diversity.

The agricultural landscape of the Upper Tisza Region and the Nyírség Area is made more colorful by semi-cultured meadows and grazing land. Plant cover on two sides of the rivers and creeks crossing the region have belted-zonal layout. Endangering factors in the area are chemicals originating from forest management and nearby agricultural areas.

Isolation, identification and characteristics of bacteria

Isolation from rhizosphere, soil and water

126 different bacterial cultures have been isolated with the TEPPER (1945) method from the rhizosphere and rhizoplane of vetch, lupine and rye. In addition, 48 *Pseudomonas* spp. strains were isolated from treatments I., IV., VI. and IX. (SIMON et al. 1973). Siderophore producers (26 strains) were selected out of the isolated bacteria, and their antagonistic abilities against plant pathogenic fungi were investigated.

Samples were collected from the water of the Upper River Tisza and the floodplain soil in the vicinity of four settlements, Tivadar, Aranyosapáti, Záhony and Balsa, with simple random sampling (HORVÁTH 1980) from a depth of 20 to 40 cm (level "A"). 314 separate colonies were isolated. Pure cultures were grouped phenotypically (Gram-staining, oxidase and catalase tests), while 43 characteristic strains were genotypically identified with the 16S rDNA method.

In order to obtain the widest possible range of culturable microorganisms, strains originating from rhizosphere, as well as soil and water were cultured and maintained on solid media suitable for various bacterial groups: Ashby's mannitol-phosphate Agar, King's B Agar, Nutrient and Endo Agar.

Grouping and identification of isolated bacterial strains

Morphological characterization and grouping of bacteria on phenotypical basis

The 126 pure isolates obtained from the rhizosphere of vetch, lupin and rye of the Westsik-type sand improvement crop rotation long-term experiment were organized by colony morphology and Gram-staining in accordance with the instructions of the Bergey's Manual of Determinative Bacetriology (BUCHANAN and GIBBONS 1974), and in accordance with cell morphology determined with light microscope. Thereafter, bacterial strains were grouped by discontinuous native polyacrylamide gel electrophoresis (LAEMMLI 1970, MATTERELLI et. al. 1993 and 1998). Finally, the number of representative bacterial strains was decreased to fifty-six by regarding bacterial strains with identical pattern as identical strains.

Identification of bacterium strains with BIOLOG™ and API 20 NE™ methods

With the exception of the oxidase test (SZEGI 1979 and HORVÁTH 1980), to which each strain gave a positive reaction, the BIOLOG™ (BOCHNER 1989ab, FRANCO-BUFF et. al. 1998) identification method was applied instead of classical biochemical tests for the determination of bacteria isolated from the rhizosphere. Reactions were read visually after incubations of 4, 16 and 24 h at 30°C. The hierarchical clustering of the different bacterium strains was effected with an unweighted pair group method using arithmetic averages (UPGMA, SOKAL and MICHENER

1958, and ROHLF 1963) belonging to distance-optimizing combinatory methods (PODANI 1997), where the distance between two classes is the arithmetic average of the total Euclidean distances by pairs among classes.

The *Pseudomonas* spp. bacterial strains originating from treatments I., IV., VIII. and IX. of the Westsik-type crop rotation long-term experiment were characterized with API 20 NE™ (BioMérieux), which is suitable for the identification of Gram-negative, non-enteric bacteria isolated from environmental samples. The method of inoculation was identical with that of the BIOLOG™ test, but the cultures were suspended in physiological salt solution instead of a transport solution.

Identification of bacteria isolated from the Upper Tisza River by 16S rDNA base sequence

The representative bacterial strains from the water of the Upper Tisza River and the soil of its floodplain (12 and 17 strains, respectively), cultured on various media, were identified on a molecular basis: on the basis of 16S rDNA nucleotide sequences (RAINEY et al. 1996, ALTSCHUL et al. 1997, SAMBROOK et al. 1989). The resulting sequences were aligned with their closest known relatives with the help of the ARB software and on the basis of the GeneBank data base, and a distance matrix of the resulting consensus-sequences was created with a Kimura 2 parameter distance model (KIMURA 1980). This distance matrix was used to create the phylogenetic tree of the relationship of bacterial strains with the neighbour-joining method (SAITOU and NEI 1987).

Investigation of the effect of direct plant growth promoting effect of strains

Biotest with white mustard seedling

In the case of the white mustard seedling test, the effect of bacterial strains on plant growth was judged from the diameter of the first leaves of the seedling (1 pair; LETHAM 1968). According to the cited literature, this method is most suitable for screening for rough selection, because white mustard demands a high amount of nitrogen and nutrient, especially at seedling age. This biotest is based on the axenic interaction developing in a quasi-liquid culture of one kind of a bacterium and the seedling. 24 representative bacterial strains isolated from the plants of the Westsik-type crop rotation were included in this experiment.

Determination of nitrogen-fixing ability

Physiological test – In order to perform screening for the presence of nitrogen-fixing activity, the above-mentioned twenty-four representative bacterial strains were inoculated as submersed colony in N-free Ashby's mannitol-phosphate Agar in three replicates (HORVÁTH 1980). The development of the non-opaque zones (stains) around the colonies was observed during the seven-day incubation period (at 26 °C). The acids produced around the colonies performing intensive metabolism have a reaction with the $CaCO_3$ present in the culture medium, and the medium becomes clear. Since the culture medium was N-free in principle, it is possible that these strains have N-fixing activity.

Determination of nitrogen-fixing activity with the measurement of acetylene-reducing activity

The intensity of nitrogen-fixing may be measured precisely by gas chromatography, with the acetylene-reduction (ARA) test (DILWORTH 1966, KARDOS 1983) (this method is 10^3 – 10^4-times more precise than the ^{15}N-procedure). Acetylene inhibits the bonding of N_2-of the nitrogenase enzyme system, but nitrogenase reduces acetylene to ethylene, and the ethylene produced this way may be determined. The acetylene-reducing activity of the 24 representative bacterium strains from the Westsik-type crop rotation was determined with the GC-FID method described by HARDY and KNIGHT (1967) and modified by RÓŻYCKI et al. (1999). Nitrogenase activity of the strains was

expressed with the hourly amount of ethylene produced per culture (nM), calculated with the modified MÃRTENNSON equation (1993).

Assessment of the effect of some environmental factors on the representative bacterial strains

Determination of the cyanide- and heavy metal tolerance of bacterial strains originating from the Upper Tisza Region

The bacterial count of various sampling sites was determined with the method of ANGERER et al. 1998. Sensibility tests for cyanide and various heavy metals were executed with the following compounds and levels of concentration: KCN, $Zn(NO_3)_2$, $Cu(NO_3)_2$ and $Pb(NO_3)_2$: 1, 5, 10, 100, 200, 300 mg L^{-1}; $Pb(NO_3)_2$: in addition to the above, also 450 and 600 mg L^{-1}; equaling and exceeding the concentrations that occurred during the time of the pollution.

The effect of compounds on bacterial growth was determined after incubation in liquid Nutrient media at 28 °C for 24 hours (shaken culture, 90 round minutes^{-1}), with turbidimetry at a wavelength of 560 nm, against untreated control (containing no bacteria). According to MARTENSSON (1992), the effect of Cu^{2+} and Zn^{2+} ions strongly depends on the applied investigation method, thus e.g. the agar-containing liquid nutrient media may strongly adsorb them, thus equalizing and reducing their effect. With this conclusion in mind, and because of their better feasibility, the microfermentor method affected with liquid nutrient media was applied for further tolerance studies as well.

Determination of the heavy metal tolerance of selected bacterial strains from the Upper Tisza Region and Westsik's crop rotation site

On the basis of previous results, thirteen bacterial strains were selected for further examinations: *Pseudomonas* spp. W.1, *Pseudomonas corrugata* W.34, *Pasteurella* spp. W.30, *Pseudomonas* spp. W.37, *Pseudomonas gessardii* B.83, *Pseudomonas* spp. C.115, *Pseudomonas syringae* I.110, *Pseudomonas veronii* D.111, *P. veronii* III.119 and *P. veronii* VI.404, *Bacillus* spp. W.7, *Bacillus thüringiensis* C.69 and *B. thüringiensis* III.108.

Heavy metal tolerance of the selected strains was tested against $ZnCl_2$, $Zn(NO_3)_2$, $CuCl_2$, $Cu(NO_3)_2$, $CuSO_4$, $Pb(NO_3)_2$, $(NH_4)_6Mo_7O_{24}$, $Fe^{(III)}Cl_3$, $Fe^{(II)}SO_4$, $Fe^{(III)}(NO_3)_3$. Heavy metal compounds were established following the Ministry of Environment and Regional Development – current name: Ministry of Environment and Water information (http://www.kvvm.hu). The applied doses of concentration were: 50, 100, 200, 400, 800 and 1600 μM, in concentrations corresponding with the pollution of the River Tisza in 2000, and double of the maximum amount in that year to represent extreme loads (800 and 1600 μM). The effect of compounds on bacterial growth was determined with microfermentor method (incubation at 28 °C for 24 hours, turbidimetry at 560 nm). Bacterium representatives were grouped with hierarchical classification according to their level of tolerance.

The examination of the oligodynamic effect of heavy metals

A group of heavy metals (silver, copper, zinc, mercury, molybdenum) is toxic for microorganisms even at very high dilutions. Depending on the concentration level, these metals and their compounds may cause severe poisoning even to higher organisms, therefore the oligodynamic effect of these metals on selected bacterial strains capable of inoculating plants was examined individually.

The sensitivity of the strains to the above mentioned metal compounds was determined with the microfermentor method. These compounds were applied in the following concentration levels: 25, 50, 100, 200, 300, 400 μM (sulphates), and 25, 50, 100, 200, 300, 400, 500, 600, 800 μM (nitrates and chlorides). The maximum level of metal compound concentration tolerated by the strains was determined, and the compounds were classified accordingly.

Strain selection

Assessment of the regulating and promoting effect of bacteria on plant growth

Detection of the production of plant hormones in biotest

47 bacterial strains originating from the **1/** rhizosphere and rhizoplane (18 strains) of the rye, hairy vetch and white lupine plants grown at the Westsik-type crop rotation long-term experiment, the **2/** floodplain soil (17 strains) and **3/** surface water (12 strains) of the Upper Tisza River, taken at various sampling sites: Tivadar, Aranyosapáti, Záhony, Balsa were included in the assessment.

Plant growth regulating effect was detected with the modified semi-qualitative method of LETHAM (1968), with wheat as plant indicator against standard amounts (100 $mg \cdot l^{-1}$) of auxin and gibberellin (GA_3). The assessment was adjusted identically with the white mustard seedling test (LETHAM 1968), using nitrogen-free nutrient salt solution (HORVÁTH 1980) modified according to SARWAR and KREMER (1995). Following incubation at room temperature for seven days, the amount and mass of rootlings, and the length and mass of budlings were measured. The effect of bacterium strains was expressed as a percentage of the control.

Determination of siderophore production

The siderophore production of 26 *Pseudomonas* spp. strains was examined with cultivation on King's B solid medium containing chrome azurole, modified by SCHWYN and NEILANDS (1987) for testing *Pseudomonas* strains. The nature of the method is that, due to the very high affinity of siderophores to iron (III) ion, they can be demonstrated with colorimetry (orange coloring) regardless of their structure. The siderophore strains not older than 24 hours and cultured in liquid King's B culture medium were inoculated onto agar plates in groups of six, in spots of 5 µl, spread at equal distances. At the end of incubation (48 h at 28 °C), the diameter of the orange ring appearing around the colonies on the blue medium was measured. The activity of the bacterial strain inducing the largest ring was taken as 100%, and the activity of the other strains was expressed as a percentage of this strain.

Assessment of antagonism against plant pathogenic fungi

The antagonistic abilities of *Pseudomonas* spp. bacterium strains isolated from various treatments of the Westsik-type crop rotation long-term experiment (**Table 2.)** were tested against plant pathogenic microscopic fungi, such as *Rhizoctonia solani* (Kühn) DSM No.843 (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) = ATCC 13289 (American Type Culture Collection) and *Fusarium solani* F.00715 (Collection of Agricultural And Industrial Microorganisms, Szent István University, Budapest). On the basis of preliminary examinations, the bacteria belonged to the *P. aeruginosa* species and the *P. fluorescens-putida* group, the species-level identification of the latter was not possible with the API 20 NE™ method. Nevertheless, two strains, P65 and P80 are most probably the representatives of the *P. putida* species.

Table 2. Code and origin of the investigated PGPR *Pseudomonas* spp. strains

***Pseudomonas* spp.**	**Treatments of Westsik-type long-term experiment***			
	I. Out of crop	IV. Straw (26,1 t / ha / 3 yrs)	VIII. Root- and green manure	IX. Farmyard manure (26,1 t / ha / 3 yrs)
P. aeruginosa	A10; AX	A5/2; A35; A20	A6; A9; A34	16; 23/1; 28a; 30/2; 36
Fluorescens-putida type	F1; F2	F4; F8; F12	F15; F22; F38	F41; F44; F47; P65; P80

*Chemical fertilizers in treatments IV., VIII. and IX.: P 50 $kg \cdot ha^{-1}$ and K 16.2 $kg \cdot ha^{-1}$.

Axenic assessments were set up in solid malt and yeast extract media, with the use of two different techniques, spot inoculation and streaking, respectively. In the first case, two loops of culture of a bacterial strain were placed (following a 24-hour incubation) on opposite ends of a Petri-dish, and 1 agar disk, 5 mm in diameter, containing the culture of the plant pathogenic fungus was placed in the middle. In the second case, bacterial suspension was streaked on the surface of the media. The agar disk with a diameter of 5 mm, containing the culture of the plant pathogenic fungus was also placed in the middle of the dish. This method was repeated with the addition of iron ($Fe^{(III)}$: 0.01 g $FeCl_3 \times 6H_2O$ l^{-1}) to the nutrition medium. In this case, nutrition competition arising due to siderophore production is suppressed. Each combination of the plant pathogenic fungi and antagonistic bacteria was incubated in three parallel replicates at a temperature of 26 (±2) °C. The diameter of the fungal colonies was measured after eight days. The inhibiting effect of bacteria was expressed as a percentage of the control containing no bacteria [PI %] (Principal Inhibition).

Assessment of the *in vivo* interaction of bacterial strains and host plants

Rye, hairy vetch and white lupine plants originating from the Westsik-type long-term experiment were used for this assessment in addition to free-living bacteria isolated from their rhizosphere. Bacterial strains were grown in liquid Nutrient medium until reaching a cell count of 10^8 CFU ml^{-1} (24-36 h incubation at 26 °C). Surface-sterilized seeds were inoculated by dipping into the bacterial suspensions.

The plants were grown in 2.5 kg pots in light chambers, with long-day illuminations of 20 000 lx with 16-hour light (22-25 °C) and 8-hour dark (15 °C) periods. Growth medium was a 4:1 mixture of quartz sand (d = 0,6 mm) and pearlite. 8 pcs of rye, hairy vetch and white lupine seeds sterilized on the surface were planted in each pot, and the pots were irrigated twice a day for 40 days to reach 60% of the water capacity of sandy soil plough lands. Hoagland-solution and sterilized tap water were alternated during irrigation.

At the end of the 40-day period, the length of the primary root (legumes), and roots (rye) and that of the shoots (mm), and their mass (g) were measured. In the case of legumes, the number of offshoots and sprout shoots starting from the primary root was recorded, while in case of rye, the number of roots and shoots from the same seed was recorded. The parameters of the inoculated plants were expressed as the percentage of the untreated control.

Statistical methods applied during the assessments

Each assessment was repeated at least three times, and an average value was established. The difference between independent variables measured during biotests with white mustard seedling, biotests for the detection of plant hormones, the assessment of acetylene reducing activity, the cyanide and heavy metal tolerance of bacteria originating from various sampling sites, and the study of the interaction between selected PGPR bacterium strains and host plants was evaluated with analysis of variance (MANCZEL 1983, KEMÉNY and DEÁK 2000). As a result of variance estimation, null hypothesis was evaluated with F-test at a probability level of 95% (in the case of acetylene-reducing activity, 99%) and the statistically significant difference was determined.

In the case of heavy metal tolerance evaluation, bacterial strains were hierarchically classified using the group average method (SOKAL and MICHENER 1958; ROHLF 1963), and representative strains selected for further examination were chosen from the most resistant group, and from the next group in line (with slightly lower levels of tolerance).

In the assessment of antagonism against plant pathogenic fungi, bilateral probability interval was calculated from the inhibiting activity (PI%) of bacterial strains in the case of every inoculation method (KEMÉNY and DEÁK 2000) to a probability level of 95%, represented in a graph during the evaluation of results. The relationship between the origin, siderophore production and antagonistic effects of *Pseudomonas* spp. bacterial strains was determined with multiple variables ordination method (principal component analysis; PCA) with the use of the SYNTAX 2000 program (PODANI, 1997).

RESULTS

Description of bacterial strains derived from rhizosphere, soil and living water

Definition of the examined strains

A total of forty-seven strains have been examined, derived from **1/** rhizosphere and rhizoplane (18 strains: *Staphylococcus* spp. W.6, W.13, *S. cohnii* ssp. *cohnii* W.9, *Bacillus* spp. W.7, W.10, W.14, W21, *Bacillus megaterium* W.15, W.18, *Bacillus subtilis* W.32, *Corynebacterium* spp. W28, *Pseudomonas* spp. W.1, W.36, *Pseudomonas vesicularis* [currently *Brevundimonas vesicularis*, GARRITY et al. 2004] W20, *Pseudomonas corrugata* W.34, W.35, *Pasteurella* spp. W.30, W.31), of lupine, vetch and rye grown with Westsik's sand improving crop rotational long-term experiment; as well as from the **2/** floodplain (17 strains: *Pseudomonas* spp. I.401, II.109, II.407, III.103, III.106, III.108, V.104, VI.87, VI.404, VI.405, VII.101, VIII.97, VIII.102, VIII.120, *Pseudomonas syringae* I.110, *P. azotoformans* III.118, *P. jensenii* III.119) and **3/** water (12 strains: *Aeromonas media* A66, A.89, A.92, A.93, A.94, *Pseudomonas gessardii* B.83, *Bacillus thüringiensis* C.69, C.107, *Pseudomonas* spp. C.115, C.116, D.94, *Pseudomonas veronii* D.111) of Upper Tisza River. Thirty-six (36) representative strains (**Figures 1. and 2.**) were identified from the former source, and twenty-four (24) strains (**Figure 3.**) from the latter.

Out of the Gram-positive strains derived from Westsik's crop rotational long-term experiment, from the rhizosphere of legumes and from the soil, members of three orders and suborders of two phyla (*Firmicutes* and *Actinobacteria*) have been isolated. Concerning Gram-negatives, bacteria from a total of eight orders belonging to four classes of two phyla have been isolated. Bacteria belonging to similar taxonomic categories have recently been described (ÇAKMAKÇI et al. 2005), their sources being areas rich in organic substances (under intensive cultivation) and areas with organic or minimal cultivation.

Bacterial species isolated and maintained from along the Upper Tisza stretch between Tivadar and Balsa can predominantly be classified under the phyla *Alphaproteobacteria* and *Gammaproteobacteria* (GARRITY et al. 2004). Samples derived from the stretch around Tivadar – which can be regarded as a control, owing to its being free of contamination – were mainly *Pseudomonas* bacteria, while those strains that were obtained from stretches at Aranyosapáti, Balsa and Záhony were scattered around the phylogenetic tree (**Figure 3.**). Similar results were produced by RÓŻYCKI et al. (1999) and ELO et al. (2000), who isolated and selected free-living bacterial strains that were diazotrophic and producers of indoleacetic acid and siderophores.

The effects of bacterial strains on the growth of white mustard seedling in biotests

Strains that were isolated from the rhizosphere of legumes of Westsik's sand improving crop rotational long-term experiment had different effects on the development of the examined seedlings. The size of the leaves reached that of the untreated control in 13 cases and exceeded that in 6 cases, from a total of 24 strains involved.

According to FRENYÓ (1958 and 1985) nitrate can be traced in many plants when they are starved, even if they obtain no nitrate whatsoever from the environment. In his experiment he grew seedlings from tiny seeds, including white mustard, so that their reserves would be used up quicker, as the nitrate produced in plant cells correlates with the depletion of metabolic energy and organic materials. The atmosphere as an oxidative environment might help the reversal of nitrate reduction if the reduction potential is diminishing. The process is likely to be facilitated by dehydrogenating enzymes which can possibly be produced by bacteria.

As the plant's reserves are finished, the balance might shift from nitrate reduction towards oxidation, which could curb growth. Therefore it cannot be ruled out that the presence of bacteria enhanced the exudate production of young roots (while the produced exudates could be consumed) and thereby helped bringing about a starved state.

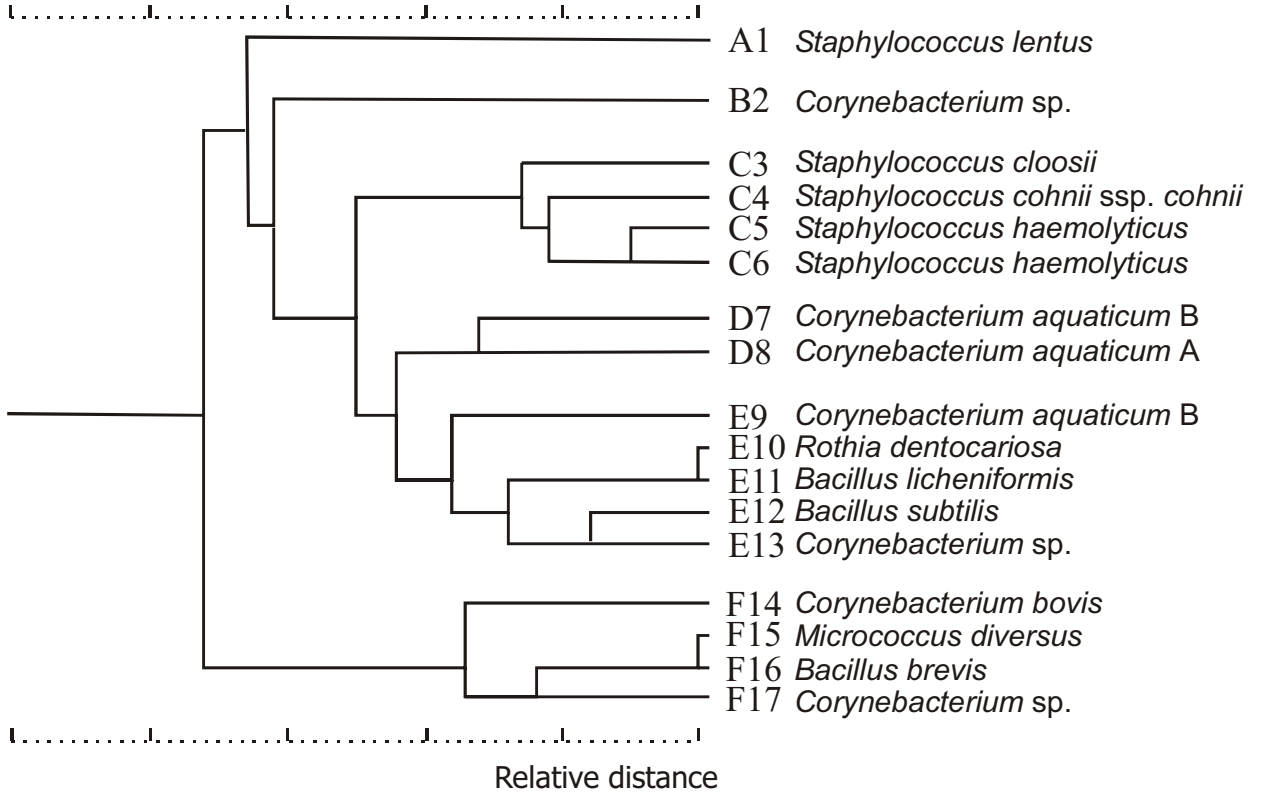

Figure 1. Grouping and identification of Gram-positive bacteria isolated from the rhizosphere of vetch, lupine and rye of Westsik's crop rotation by BIOLOG™ system. Dendrogram is drawn using UPGMA method. Distance of two classes is expressed with the arythmetic average of the total Euclidean distances by pairs among classes.

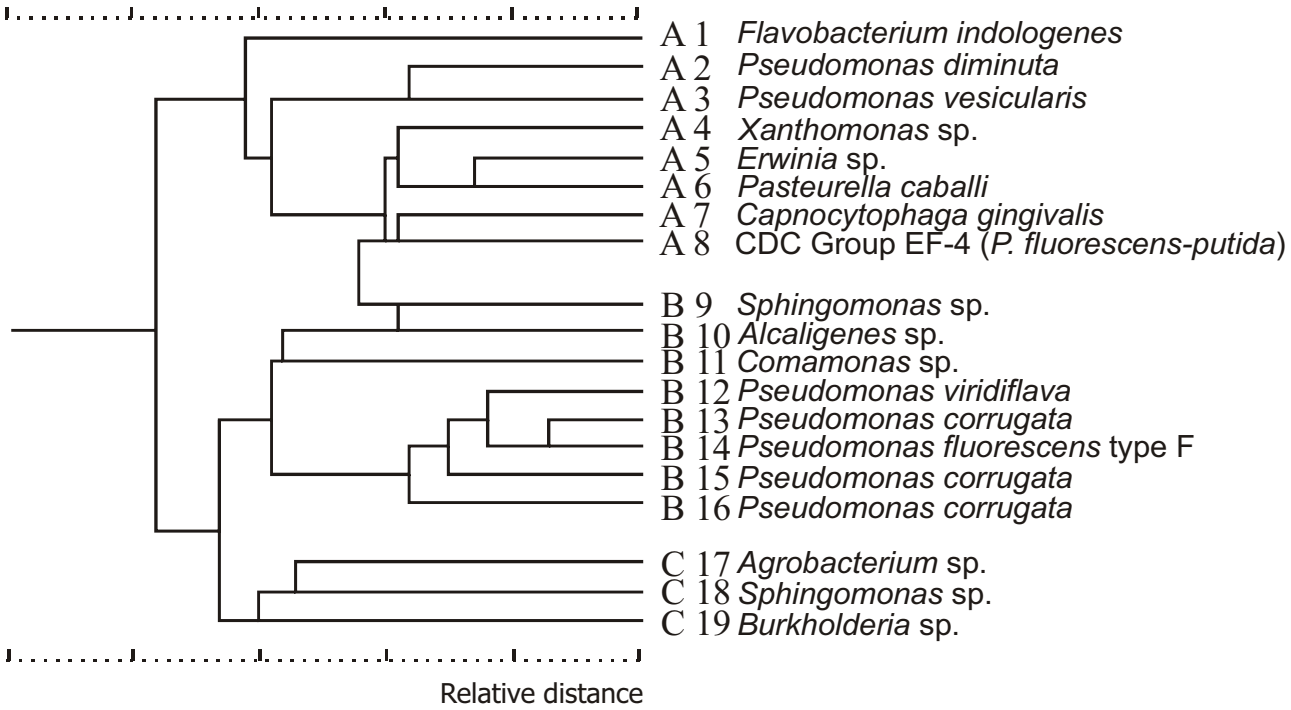

Figure 2. Grouping and identification of Gram-negative bacteria isolated from the rhizosphere of vetch, lupine and rye of Westsik's crop rotation by BIOLOG™ system. Dendrogram is drawn using UPGMA method. Distance of two classes is expressed with the arythmetic average of the total Euclidean distances by pairs among classes.

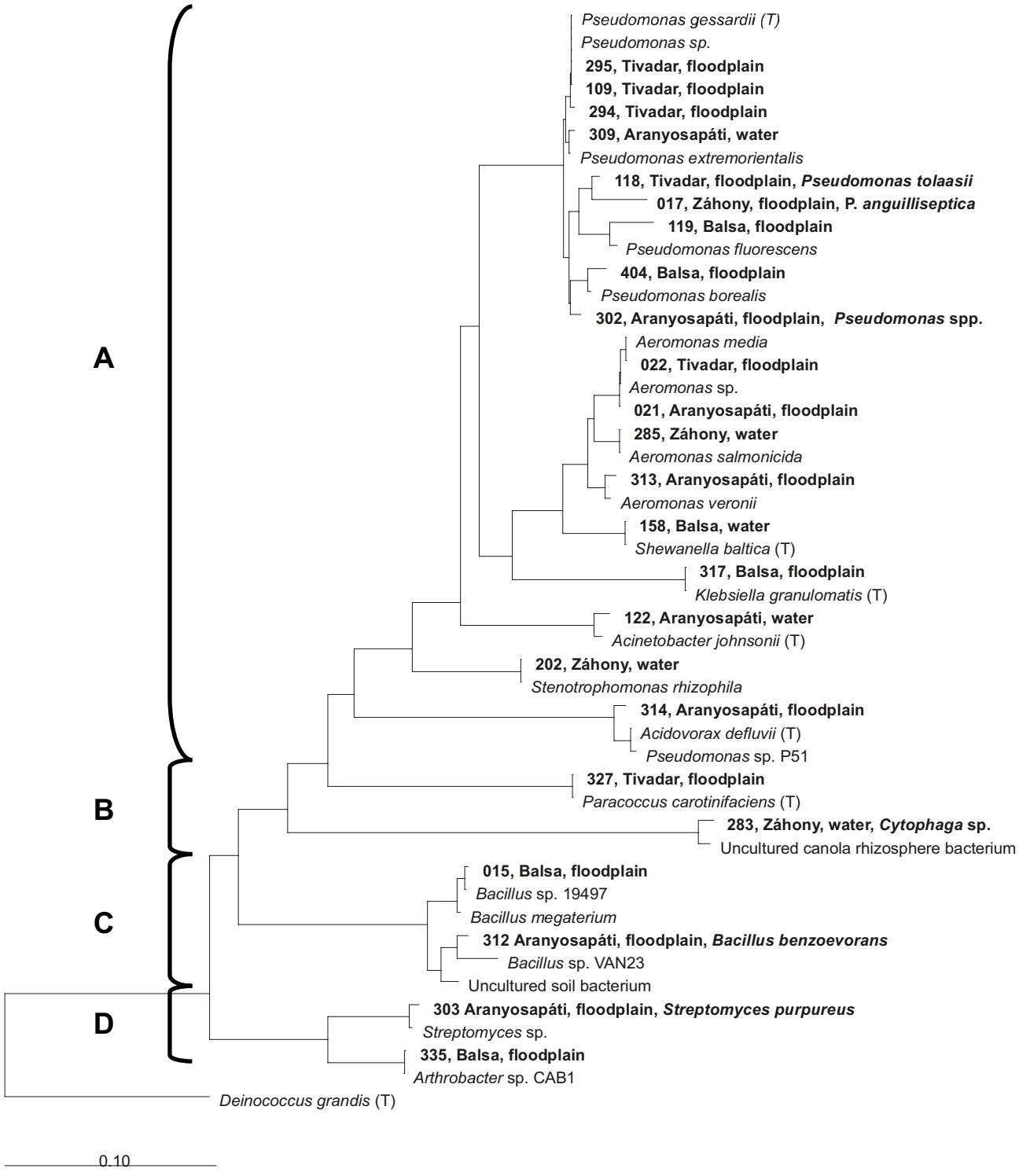

Figure 3. Phylogenetic tree of twenty-four representative bacterial strains isolated from different compartments of the Upper Tisza River. Drawing is calculated by neighbour-joining method; dash displays 0.1 substitute per nucleotide. A: Phylum BXII *Proteobacteria*, Class III *Gammaproteobacteria*. B: Class I *Alphaproteobacteria*. C: Phylum BXIII *Firmicutes*. D: Phylum BXIV *Actinobacteria*. T: type strain.

Nitrogen-fixing ability of free-living bacterial strains isolated from the rhizosphere

Diazotrophic activity has successfully been demonstrated in one group of bacterial strains originating from Westsik's sand improving crop rotation. Through physiological testing, no significant difference was observed between strains based on the diameter of clean zones formed around the colonies. However, the application of ARA-testing provided measurable results. Active strains predominantly belonged to the genus *Pseudomonas*, two *Bacillus* species and one strain of *Flavobacterium indologenes.* The majority of bacteria living in the rhizosphere and having a nitrogenase enzymatic system are likely to fix only an amount of N_2 that is essential for their existence. Members of certain species of bacteria (e.g. *Bacillus* spp. W.33 or *Pseudomonas corrugata* W.39) showed significantly greater activity, while some strains might be particularly active.

The diazotrophic activity of members of these genera has recently been demonstrated by several authors. N_2-fixing strains, isolated from the rhizosphere of free living, wild and cultivated plants, were selected based on their production of indoleacetic acid and gibberellins and on their antagonistic ability against plant pathogenic fungi (*Fusarium*, *Rhizoctonia* and *Verticillium* spp.). These strains have successfully been applied as a stimulant of the growth of host plants in an axenic culture (RÓŻYCKI et al. 1999, ELO et al. 2000, PAL et al 2001, EGAMBERDIYEVA and HÖFLICH 2004 and ÇAKMAKÇI et al. 2005).

Effects of environmental factors on representative bacterial strains

Cyanide and heavy metal tolerance of strains derived from the Upper Tisza area

Generally no connection has been found between the tolerance levels of strains belonging to different taxonomic categories. There was no significant difference in their cyanide, Zn and Cu tolerance levels. However, in the case of lead the cumulative tolerance of strains (MTC, maximum tolerated concentration) and the place of their isolation (origin) showed correlation (**Figure 4.**).

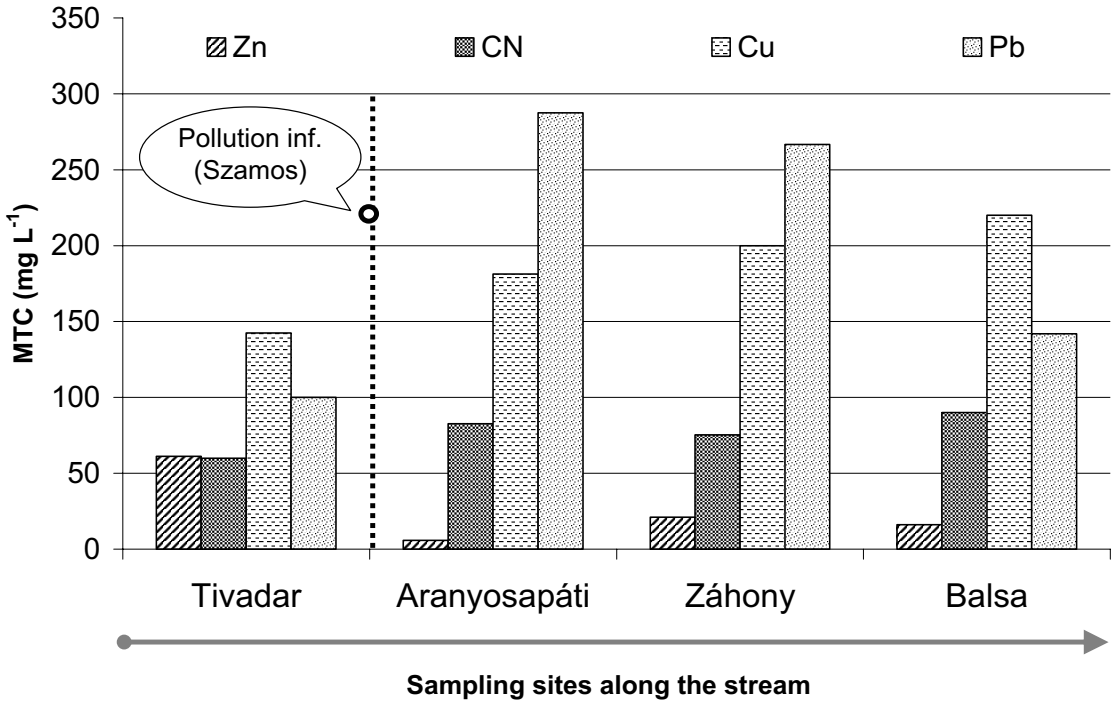

Figure 4. Cyanide and heavy metal tolerance of twenty-five bacterial strains originated from different compartments of Upper Tisza River ($SD_{P95\%}$ = 163.2). Tivadar serves as an unpolluted control.

Strains obtained from the stretch above Aranyosapáti – where the contamination empties into the water – belong to far more species and genera than other samples. These isolates collectively have proved to be more tolerant than their counterparts from unpolluted stretches. Besides selection exerted by cyanide and heavy metal compounds, the above findings may imply that along polluted stretches bacterial communities capable of adapting to environmental changes may have appeared.

Heavy metal tolerance of bacterial strains obtained from the Upper Tisza area and Westsik's crop rotation

Simultaneous testing has also been carried out on the tolerance of strains, isolated at different time periods and obtained from two different sampling sites, against ten heavy metal compounds. The most important observations are as follows:

- There was no significant difference between the heavy metal sensitivity spectra of strains originating from Westsik's sand improving crop rotation, the water of the Upper-Tisza and from floodplain soil respectively. This refers to their specific own resistance, therefore they are likely adopt to the conditions over a longer period, which well exceeds the duration of laboratory testing.
- The species composition of strongly versus weakly resistant groups did reveal differences: more tolerant strains belonged to the phylum BXII *Proteobacteria* of the domain of *Bacteria* and were predominantly from the Class III of *Gammaproteobacteria.* They overwhelmingly belong to the genus *Pseudomonas.* On the other hand, more sensitive groups were mostly Gram-positive rods and cocci, which are members of the phylum BXIII *Firmicutes,* their majority being of the *Bacillus* species. The reason of this divide presumably lies in the different composition, structure and permeability of Gram-negative (*Pseudomonas* spp.) and Gram-positive (*Bacillus* spp.) cell walls.
- The reproduction of strains was generally inhibited to a greater extent by sulphate forms of heavy metals. The nitrate form compensated the toxic effects of any given metal, which were manifested in the stimulation of reproduction in small concentrations.
- Based on MTC values of the examined strains, the toxicity order of metal compounds are as follows:

  $(NH_4)_6Mo_7O_{24}$, > $CuSO_4$, > $ZnCl_2$, > $Cu(NO_3)_2$, > $Zn(NO_3)_2$, > $CuCl_2$, $Pb(NO_3)_2$, > $Fe^{(II)}SO_4$, > $Fe^{(III)}Cl_3$, > $Fe^{(III)}(NO_3)_3$.

Selection of strains beneficial to soil-microbe-plant system

Characteristics of bacterial strains that regulate and stimulate plant growth

Production of plant hormones

It has been found that bacterial strains (pre-selected based on their heavy metal and cyanide tolerance, derived from lupine, vetch and rye grown with Westsik's sand improving crop rotational long-term experiment and from the water or the soil of the floodplains of the Upper-Tisza River and predominantly belonging to the genera *Bacillus* and *Pseudomonas*) stimulated the growth of wheat seedlings (*Triticum aestivum* L.) in comparison to auxins and gibberellins, regarding the growth of both shoots and the root.

Compared with indoleactic acid (auxin), most strains stimulated the growth of the root mass along with the length and mass of the shoot. In comparison with gibberellic acid (GA_3) only the length and the mass of the shoot showed significant increase. The above may be explained by the different allocation of the two hormones in the plant as auxin enhances the lengthwise growth of roots and the development of hairy roots, while gibberellins stimulate proliferation, leaf formation and cell elongation in the shoot and they can accelerate germination (LOPER and SCHROTH 1986,

ARSHAD and FRANKENBERGER 1995). The thirteen most efficient strains significantly enhanced the growth of both the root and the shoot in the following descending order:

Pseudomonas gessardii B.83 > *Pseudomonas* spp. W.35 (CDC group EF-4) > *Bacillus* spp. W.7 > *Pasteurella* spp. W.30 > *Pseudomonas* spp. C.115 > *Pseudomonas syringae* I.110 > *Pseudomonas* spp. W.1 > *Pseudomonas veronii* D.111 *P. veronii* > III.119 > *P. veronii* VI.404 > *Bacillus thüringiensis* C.69 > *B. thüringiensis* III.108 > *Pseudomonas* spp. W.36.

Antagonism against plant pathogenic fungi

The inhibitory effect of some *Pseudomonas* spp. bacteria (originating from the rhizosphere and fostering plant fitness) against two microscopic and plant pathogenic fungi present in the soil was examined with two different laboratory techniques. The preliminary biotest showed that the bacterial strains produced siderophore-like substances.

The streaking method produced an almost 100% inhibition rate while spot inoculation yielded a mere 50%. This supports the significance of direct contact between the antagonistic bacteria and the target agent in the formation of biological interaction. Both methods can be applied to determine antagonistic traits, which is the simple preliminary screening of strains that can be used for biological control purposes. It can reasonably be assumed that the siderophore production of the analyzed *Pseudomonas* samples is involved in the formation of the antagonism, since all presented strains produce siderophore-like secondary metabolic products which empower them with an advantage in the feeding competition against other microorganisms in their environment (ELLIOTT et al. 1984, COOK 1993).

In the case of the fungus *Fusarium solani* F.00715 the antagonistic effect of bacteria is likely to have been manifested due to the siderophore production, since in a Fe-enriched medium the inhibition on the growth of the fungus was greatly reduced. However, inhibition against the strain *Rhizoctonia solani* ATCC 13289 was primarily caused by other mechanisms (e.g. the production of antibiotics). In this case iron may serve as a mediator of the production of numerous secondary bacterial metabolic products.

The relative siderophore production of strains F08, P65 and F80 was significantly greater than that of other strains, although it was only strain F80 that exerted strong inhibitory effects on both fungi. Bacterial strains from plots IV. (4) and VIII. (8) of Westsik's crop rotation (F04, F12, F22, F38, F41 and F47) displayed uniform behaviour against the *Fusarium solani* fungus both in normal and Fe-enriched culture media. Their inhibitory effects were reduced to about half of when siderophores were present (with the exception of strains F22 and F41) yet the reduction was still minimal compared to inhibition in a normal medium and that of other strains. It can therefore be concluded that in these cases antagonism is not caused solely by siderophore compounds, but by the production of other secondary metabolic products as well (**Figure 5.**).

The inhibitory effect of *Pseudomonas* bacteria on plant pathogenic fungi through antibiotics production has been examined in greater detail by VÁRADY (2001). The results of this work confirm the widespread presence of antibiotics production in different microenvironments, but the findings concerning the fungus *Fusarium solani* reinforces the role of other mechanisms (notably siderophore production) in determining the applicability of certain strains in plant inoculation and biological protection. Várady verified the siderophore production of *Pseudomonas* strains derived from different sources and their consequent stimulating effect on the growth of various test plants, but he never examined the direct antagonist effects of siderophores. This latter antagonism has directly been proved in this work, albeit only in the case of one pathogenic fungus.

The inhibitory effect of *Pseudomonas aeruginosa* bacterial strains on plant pathogenic fungi is a field that has seen only limited research, mainly due to the species being an opportunistic human pathogen as well. Still they are ubiquitous members of soil ecosystems; therefore they are in constant interaction with other microbes in the soil. In this experiment *P. aeruginosa* strains showed a significantly greater inhibitory effect compared to non pathogenic *Pseudomonas* spp. strains. Pot

experiments (BUYSENS et al. 1996, BIRÓ et al. 1998) have confirmed that *fluorescens-putida* group can also be successfully used against plant pathogens present in the soil (e.g. protection against soil sickness – replant disease – of apple). Following the experiment of the author, *Pseudomonas aeruginosa* strains have been eliminated from further research as their potential dermoallergenic effect on humans could not be ruled out.

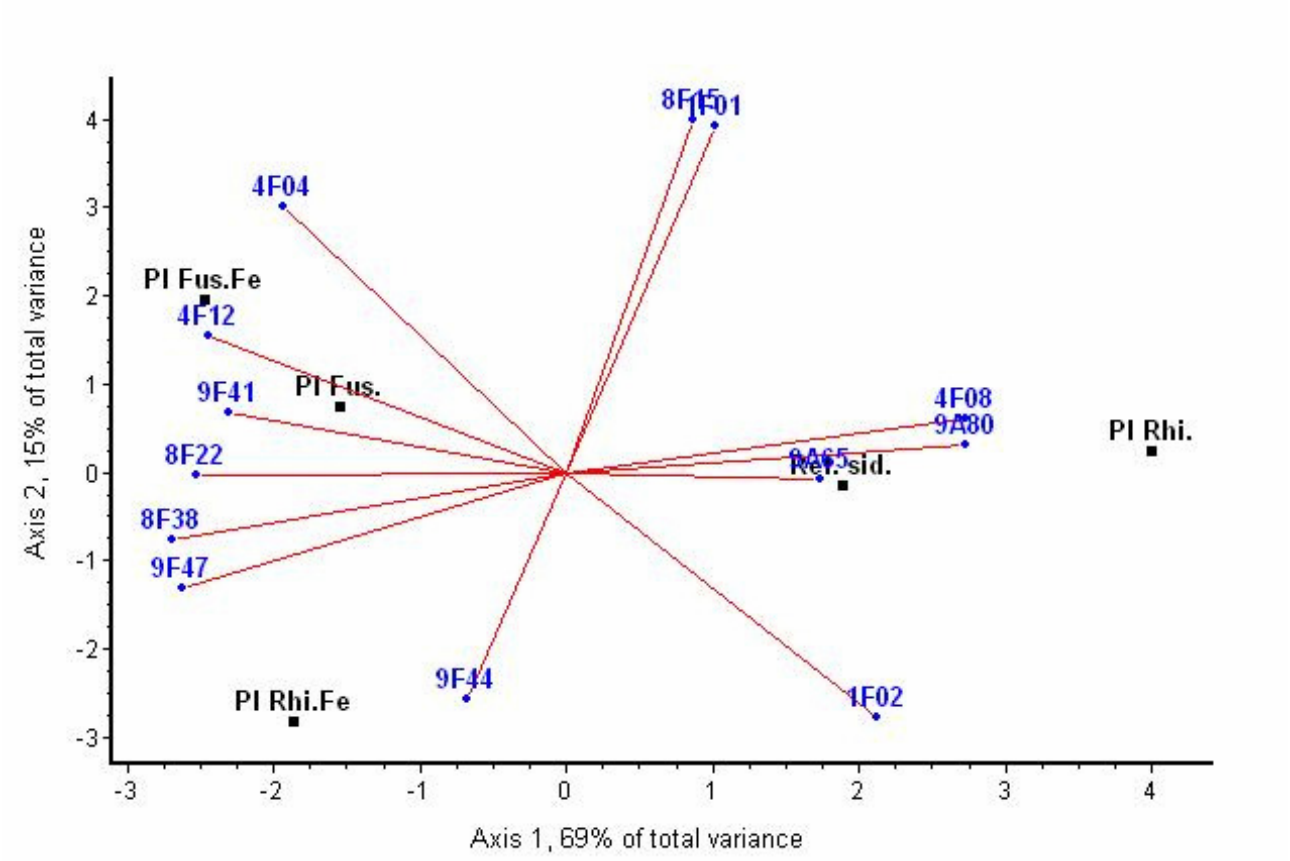

Figure 5. Euclidean biplot: relationship between the origin, siderophore production and antagonistic effect on *Fusarium solani* and *Rhizoctonia solani* plant pathogenic fungi of the *Pseudomonas* spp. bacterial strains (F: *P. fluorescens-putida* group, P: *P. putida*). PI: inhibition rate (control %), Fe: Fe-enriched medium, Fus.: *Fusarium*, Rhi.: *Rhizoctonia*, Rel.sid: siderophore production rate (control %). Westsik's plot treatments as origins of the bacteria are referred with Arabic numbers corresponding to the Roman numbers of **Tables 1. and 2.**

Growth stimulatory effects of selected bacterial strains on host plants in axenic cultures

The tested lupin, vetch and rye plants (which are also host plants of bacterial strains living in the rhizosphere and isolated from Westsik's sand improving crop rotational long-term experiment) reacted in different ways to their seeds being inoculated with bacteria. In the case of lupine practically all bacterial strains caused significant growth in the length and mass of a given part of the plant, located either above or under the ground, when compared to the untreated control. The bacterial activity was most evident in the increase of the root's length and mass, which hints at the presence of metabolic products similar to the hormone auxin. The selected strains could colonize the hairy roots of the lupine following inoculation.

The vetch reacted either by growth of the shoot or in several cases with the growth of the mass of the root. The former implies the production of secondary metabolic products acting like gibberellin (GA_3). However, it can be assumed that the products of the bacteria were not allocated in the shoot alone, as gibberellins can exert many of their physiological effects in cooperation with auxins, by increasing their endogenous concentration (EGAMBERDIYEVA and HÖFLICH 2004, PARK et al. 2005).

Rye is a monocotyledon, which responded with significant root mass growth to the presence of bacteria in some cases. This reaction shows the growth induced by auxin but it was presumably the intensity of the plant's nutrient intake that increased greatly, thereby leading to a boost of wet mass. Taking into account the effects of bacterial strains of all strains, the following seven strains have been selected as potential components of inocula (all preserved within the author's own culture collection as lyophilisates): *Bacillus subtilis* W.32, *Bacillus thüringiensis* C.69, *Pseudomonas* spp. W.1, *Pseudomonas corrugata* W.34, *Pseudomonas gessardii* B.83, *Pseudomonas veronii* D.111 and *P. veronii* W.10.

New scientific accomplishments

1. 56 representative bacterial strains have been produced from Westsik's crop rotation, using the discontinuous native PAGE method. 36 of these have been defined using the BIOLOG™ method. From plots exhibiting sand improvement by means of no-cropping, root- and green fertilization and farmyard manure, a further 26 *Pseudomonas* spp. strains have been isolated and then defined using the API 20 NE™ test. From the sampling sites of the Upper Tisza, 24 representative bacterial strains have been identified using the 16S rDNS molecular technique and their phylogenetic location has been illustrated.
2. The nitrogen fixing activities and the direct plant-growth stimulating effects of 24 bacterial strains derived from Westsik's crop rotation have been compared with seedling tests. There was a correlation between the results of ARA and physiological tests, but the seedling test has proved to be inappropriate for a quick and reliable analysis of growth-promoting effects.
3. From the 47 bacterial strains selected from Westsik's crop rotation and the floodplain area of the Upper Tisza, the following 13 strains have significantly stimulated the growth of both roots and shoots in the semi-qualitative seedling test (presented in descending order):

   Pseudomonas gessardii B.83 > *Pseudomonas* spp. W.35 (CDC group EF-4) > *Bacillus* spp. W.7 > *Pasteurella* spp. W.30 > *Pseudomonas* spp. C.115 > *Pseudomonas syringae* I.110 > *Pseudomonas* spp. W.1 > *Pseudomonas veronii* D.111 *P. veronii* > III.119 > *P. veronii* VI.404 > *Bacillus thüringiensis* C.69 > *B. thüringiensis* III.108 > *Pseudomonas* spp. W.36.
4. The antagonistic capability of *Pseudomonas* spp. strains derived from different plots of Westsik's crop rotation can be realized by the iron competition caused by their siderophore production and by the production of antibiotic secondary metabolites. The siderophore production of some strains in itself showed antagonistic effects against the fungus *Fusarium solani.*
5. The tolerance of cyanide and 10 heavy metal compounds in the selected bacterial strains (from cultivated and natural sampling sites and from the Upper-Tisza area) has proved to be specific to strains and does not correspond to the origin (cultivated or natural – wild – areas) of any given strain. However, the tolerance and taxonomic diversity of samples obtained from uncontaminated stretches of the Tisza has always been lower.
6. The strains with the highest level of resistance to aforementioned environmental factors and with indirect plant growth promoting effect after seed inoculation into test plants (lupin, vetch and rye) have been selected and have been preserved within the author's own culture collection as lyophilisates:

   Bacillus subtilis W.32, *Bacillus thüringiensis* C.69, *Pseudomonas* spp. W.1, *Pseudomonas corrugata* W.34, *Pseudomonas gessardii* B.83, *Pseudomonas veronii* D.111 and *P. veronii* W.10.

CONCLUSIONS AND RECOMMENDATIONS

Based on the findings, the prospects of using bacterial strains selected through laboratory methods in biological plant protection are promising. During the experiments the following conclusions and recommendations have been made:

a) During the categorization of strains, differences have been found – using the discontinuous native PAGE method – between isolated bacteria that were obtained from Westsik's crop rotation and were morphologically similar. These could be utilized during the selection of representative strains. As a further step, most isolated bacterial strains were identifiable – at least on a genus level – using the BIOLOG™ kit. The predominant sample among Gram-negative bacteria was *Pseudomonas*, while most Gram-positive species were either *Corynebacterium* or *Staphylococcus.* It has been admitted by the manufacturer that the BIOLOG™ test is more suited for identifying metabolic types than it is for identifying species. It has however been successfully applied in categorizing other specific micro cosmoses (e.g. intestinal microbiota, medical appliances, etc.). Incorporating the results of more recent research, BIOLOG EnviroPlate™ was released in 2000, having a wider spectrum and database. It has successfully been used with environmental samples. Unfortunately it might never be applicable for identifying microorganisms that are diverse but appear only in small numbers, therefore are viable but non-culturable.

   The efficiency of the API 20 NE™ test – used for identifying *Pseudomonas* spp. bacterial strains only – fell short of expectations. Out of 48 strains, only 26 resulted in approximate species identification, out of which only 13 *P. aeruginosa* definitions were correct. A further 13 strains that were possibly related to *P. fluorescens* and *P. putida* species were collectively categorized as „*P. fluorescens-putida*".

b) As opposed to the above points, isolated samples from the water and the floodplain soil of the Upper-Tisza were almost always identifiable using the 16S rDNS molecular method. In this case the number of identified taxons was also much higher, despite the fact that the examination was carried out using not direct environmental samples but with cultured isolates. Here the crucial point is planning the appropriate primer, because according to the findings of NIKOLAUSZ et al. (2004, 2005), the widely used 27f primer – a section approximately 500–1500 bp long – cannot multiply the relevant DNA region of all bacteria.

c) It has been shown through two methods (physiological and ARA-testing) that a group of free-living bacterial strains – isolated from the rhizosphere of the Westsik crop rotation's vetch, lupine and rye – were capable of fixing atmospheric nitrogen. The results of culturing on Ashby's solid medium and those of the ARA-test correlated well, which makes it possible to use the medium for the selective isolation of aerobic diazotrophic bacteria, not only anaerobic ones. To obtain appropriate results, it is imperative to assure the cleanliness of all chemicals used and to make sure no ammonia gets into the medium during incubation. However, the results of the white mustard (*Sinapis alba* L.) seedling test only correlate with the results of the previous tests in the case of the most active bacterial strains. Therefore in the latter case other factors, such as the bacterial uptake of root exudates, might also influence the development of seedlings. Many of the examined strains actually inhibited plant development. This process might have triggered the endogenous nitrate formation of seedlings, which did not allow the beneficial effects of strains with lesser diazotrophic activities be felt.

d) It has been found that bacterial strains (pre-selected based on their heavy metal and cyanide tolerance, derived from lupine, vetch and rye grown with Westsik's sand improving crop rotational long-term experiment and from the water or the soil of the floodplains of the Upper-Tisza River and predominantly belonging to the genera *Bacillus* and *Pseudomonas*)

stimulated the growth of wheat seedlings (*Triticum aestivum* L.) in comparison to auxins and gibberellins, regarding the growth of both shoots and the root.

e) The 26 *Pseudomonas* spp. bacterial strain isolated from different plots of Westsik's crop rotation produce siderophores. 13 of these, belonging to the *fluorescens-putida* group have been tested for their antagonistic effects on *Fusarium solani* and *Rhizoctonia solani* plant pathogenic fungi using two different biological tests (streaking and spot inoculation). In the case of spot inoculation less inhibition was observed, but the bacteria were still able to block the development of pathogens during the entire incubation period. The difference between the two techniques was statistically reinforced, since these methods share the immanent feature that antagonism is more efficient with streak inoculation due to the pathogens and the biological control agent being in direct contact. The antagonistic capability of the above strains can be realized by the iron competition caused by their siderophore production and by the production of antibiotic secondary metabolic products. The former strategy was particularly efficient against *Fusarium solani*, while the latter worked better against *Rhizoctonia solani* microscopic fungi. In the case of *Fusarium* direct antagonism caused by siderophores has also been proved.

f) In connection with the cyanide and heavy metal contamination of the Tisza River in 2000, representative bacterial strains were isolated from the water and the floodplain soil of the Upper Tisza. These strains were examined together with ones from Westsik's crop rotation and their tolerance of cyanide and some heavy metals was determined. Lead compounds exerted only limited inhibition on the reproduction of strains compared to compounds of copper and zinc. Therefore it can be assumed that the bacteria adapted to the contamination of the sampling sites and they tolerated higher lead levels. Yet the extent of disturbance the cyanide and heavy metal contamination caused along the Tisza underlines the fact that significant damage was done to the ecosystem (e.g. widespread devastation of fish populations). This way bacterial strains selected from "hotspots" (places triggering alternative metabolic pathways, such as highly contaminated stretches of rivers) can play an important role as bioremedies.

g) The oligodynamic effect of heavy metals was tested with bacterial strains that were selected from the above mentioned sources and based on their regulating and stimulating effects on plant growth together with their antagonistic capabilities. The reactions of individual strains to heavy metals were different, yet there was no significant difference between the heavy metal sensitivity of samples taken from Westsik's sand improving crop rotation or from the Upper Tisza's floodplain area. This leads to the conclusion that their resistance might be specific, probably determined by the differences in the structure of their cell walls. Sulphate forms of heavy metals generally inhibited the reproduction of strains to a greater extent, while nitrate forms compensated the toxic effects of a given metal, thereby at small concentrations stimulated bacteria reproduction. In the case of Fe-compounds the microelement-effect might also be involved, as supported by the high level of tolerance against compounds of iron.

h) The recolonization ability and the plant growth promoting effect of the selected bacterial strains were tested in axenic cultures with pot experiments. The plants (hairy vetch, white lupine and rye) differed in their reactions to seed inoculation with bacteria. Dicotyledons such as vetch and lupine generally displayed an increase in root length and mass, which can be attributed to the presence of metabolic products with the effects of the auxin hormone. As for the vetch, some strains greatly increased the mass of the shoot. The latter phenomenon means the possible presence of secondary metabolic products acting like gibberellin (e.g. GA_3). Rye, which is a monocotyledon, responded with significant root mass growth to the presence of bacteria in some cases. The most efficient strains stimulated the growth of both the root and the shoot of the host plant. This can be connected with the observation that gibberellins may exert many of their physiological effects in cooperation

with auxins, by increasing their endogenous concentration (EGAMBERDIYEVA and HÖFLICH 2004, PARK et al 2005).

* * *

Summing up the aforementioned points it has been concluded that by applying appropriate selection methods more resistant and more efficient strains have been obtained, despite their generally weak tolerance of environmental factors and their highly differing plant-growth stimulating effects. Surveying biotic and abiotic factors, along with the quest for less sensitive bacterial strains suitable for producing inocula is important both in an ecological and in economical sense.

Results from laboratories and climate chambers cannot be extrapolated to field conditions without further examinations (e.g. co-inoculation, compatibility with other rhizosphere bacteria) but the experiences of the author and other quoted researchers draw attention to the preliminary screening of potential inoculant strains with laboratory techniques. Bacterial strains that stimulate and promote (through their antagonistic abilities against two plant pathogenic fungi) the growth of test plants and that resist some contaminating agents are truly promising in securing strong seedling emergence of crops grown on sandy soil, notably lupine, vetch and rye.

Possibilities for further research

The root recolonization ability of bacterial strains selected with laboratory methods and stimulating plant growth and applicable for plant inoculation has been concluded based on their growth-promoting effect in pot experiments. Recolonization can be demonstrated by in situ UV or immunofluorescent microscopy, e.g. applying the LiveDead BackLight kit or Auroprobe LM – Amersham – dyeing (DALTON et al 2004). An appropriate and probably more cost-effective alternative is the Fluorescent In Situ Hybridization method (LOY et al. 2003).

Only indirect proof is herewith available for the existence of metabolic products that are responsible for the plant growth-stimulating effects of bacterial strains. A precise qualitative (e.g. plant hormones, vitamins, antibiotics, etc.) and quantitative definition of these products is possible using HLPC or GC-MS techniques. It would be useful to carry these out on the author's own strain collection.

The possible growth-stimulating effects of different bacterial metabolic products on other host plants and the way these plants might utilize these also merits further research, e.g. finding those factors that determine whether any given excretum is translocated in the root- or shoot system of a plant (FRENYÓ 1985).

To achieve all the above, it would indeed be useful to carry out research concentrating on specific subfields and carried out on host plants present under special ecological conditions (e.g. leguminous forage plants, crops or species that can be grown on shifting sand) and on the bacteria isolated from the rhizosphere of such plants, possible including specific groups (e.g. *Pseudomonas*, *Bacillus*, *Micrococcus*, *Flavobacterium*, etc.)

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