Introduction and Objectives

Microbial biomass is a small but labile component of soil organic matter and plays an important role in cycling and other nutrient elements. Because of its importance in the function of different ecosystems, synthesis/dynamics of microbial biomass and its role in plant nutrition under different ecosystem conditions has assumed greater significance.

Microbial populations are the key components of the soil-plant systems where they are immersed in a framework of the interaction affecting plant developments. Research on plant growth-promoting rhizobacteria (PGPR) has been increasing at an ever increasing rate since the term was first used by KLOEPPER and Co-workers in the late 1970s (KLOEPPER & SCHROTH 1978, SUSLOW et al. 1979). For example, the term PGPR appears in 11 citations from the 1980s in the USDA's Agricola Electronic Database (AED) of National Agricultural Library, Washington, DC; during the first half of the 1990s the term appears in 34 citations, and during the second half of the decade, 72 citations. Likewise, the term biofertilizer appears to have come into common use in the scientific literature in the late 1970s. The AED indicates approximately 60 citations per decade, which use the term biofertilizer during the 1980s and 1990s. This survey demonstrates the trend of an increasing rate of research on PGPR, but is not an absolute measure of the activity in the area. Many papers investigating bacterial-facilitated promotions of PG, but which did not use the terms PGPR or biofertilizer, are not reflected in the survey.

Microbial inoculant technology involves the selection and multiplication of plant-beneficial rhizomicrobes, such as those used for improved plant nutrition (biofertilizers), and for improved biological control of pests, weeds, and diseases (biocontrol agents). The concept of microbial inoculation of plants goes back almost one hundred years and has been commercialised over the last decade. Many rhizomicrobes may enhance the nutrient uptake of plants. Those microorganisms that have a direct beneficial effect on the plant may have considerable potential as biofertilizers. Three groups of plant-beneficial rhizomicrobes can be distinguished: N_2-fixing, mycorrhiza and PGPR.

Plant growth-promoting rhizobacteria: The impact of rhizobacteria (those members of the total rhizosphere bacteria that are able to colonize roots) on plant growth may be neutral, deleterious or beneficial. The term PGPR was given to beneficial rhizobacteria in 1978. Most PGPRs are fluorescent *Pseudomonas* or strains of *Bacillus* sp. PGPR can be inoculated on to some crops and can subsequently improve growth. The beneficial effects of PGPR fall into two categories: 1) PG and 2) plant disease suppression (PDS). PG is evidenced by increases in seedling emergence, vigour, seedling weight, root system development, and yield. The different mechanisms of PGPR, which enhance growth, are not yet well understood. Some PGPR strains are supposed to increase plant growth by positively interacting with various plant-symbiotic rhizomicrobes, such as *Rhizobium*, *Bradyrhizobium*, *Frankia* and mycorrhizal fungi. Several *Pseudomonas* and *Bacillus* spp. are capable of enhancing modulation. There are also PGPRs, which produce plant growth hormones in culture, but there is no actual proof of such activity under normal field conditions. Some PGPR strains do not promote growth *per se*, but the inoculated bacteria are controlling minor root pathogens which are not producing enough obvious symptoms to be noticed, but which hinder the complete expression of the plant's potential.

Biocontrol via PGPR is usually directed toward minor root pathogens. These PGPRs are supposed to suppress plant disease by producing siderophores (chelating compounds with a special affinity for iron) which reduce the availability of Fe for deleterious microbes, or by producing toxic compounds like antibiotics and HCN which are active against deleterious rhizobacteria. A disadvantage of microbial inoculants is their sensitivity to environmental changes in the field. The use of PGPR fluorescent *Pseudomonas* provides one of most promising strategies to reduce plant diseases caused by soilborne phytopathogens, employing a safe and an environment-friendly technology. The beneficial activities of PGPRs have been established in many crops including the strategic plants, vegetables, fruit trees etc. A large proportion of phosphorus in soil is present in insoluble form and therefore not available for plant nutrition. The ability to convert insoluble P to an accessible form like orthophosphate is an important trait from a PGPR for increasing plant yields. Reports on non-symbiotic N_2-fixation in many crops have stimulated appreciation for the importance of rhizobacteria in plant production and plant protection. Apart from P solubilization and biological N_2-fixation,

improvement of other plant nutrient uptake and phytohormone production like IAA are some examples of mechanisms that directly influence plant growth.
Since the 1980s PGPRs and other microorganisms have been investigated as possible replacements for chemicals used to control a broad range of plant diseases. Isolates from genus *Pseudomonas* have been tested due to their widespread distribution in soil, ability to colonize the rhizospheres of host plants, and ability to produce a range of compounds antagonistic to a number of serious plant pathogens. The endophytic bacteria of *Pseudomonas* are known to act as biocontrol agents either directly, by inhibiting plant pathogens or indirectly, by inducing plant systemic resistance to pathogens.
Root colonizing rhizobacteria that exert beneficial effects on plant development via direct or indirect mechanisms have been defined as PGPR. Root colonization and induction of an Fe stress regulated promoter for siderophore production by fluorescent *Pseudomonas* strains is an important study nowadays especially in the rhizosphere of plants grown in salinized environment. The introduced PGPR must colonize plant root system and persist in the rhizosphere during the growing season for plant promotion to be manifested.
Fe is an essential for energy metabolism in microorganisms. Although abundant in most soils, the concentration of Fe^{3+} in solution is usually extremely low, except under anaerobiosis conditions or low pH. Microorganisms, therefore, have to compete for Fe^{3+}.
Under Fe-limiting conditions, many microorganisms have been shown to produce low-molecular-weight Fe-chelating agents called siderophores, which facilitate transport of Fe^{3+} into the cells. Since Fe availability is extremely low in aerobic, neutral- to high-pH environments, one important factor in microbial competition is the production and utilization of siderophores. The capacity to utilize a wide variety of Fe-chelates may increase the competitiveness of a given strain. This could include not only microbial siderophores but also plant derived chelators such as phytosiderophores. On the other hand, some microbial siderophores can be taken up and utilized as Fe sources by plants. Plants may be simultaneously exposed to unfavourable root-zone salinity condition especially in the arid and saline areas.
The fungal and bacterial pathogens play a major role in the development of diseases on many important field and horticulture crops, often resulting in poor plant yields, as well as the salinity of the soil. Considering the cost of chemical pesticides and hazard involved, biocontrol of plant diseases is now increasingly capturing the imagination of plant microbiologists and soil microbiologists as well.
In the present study the efficacy of isolated fluorescent *Pseudomonas* strains from different soil types were evaluated for mobilizing the essential nutrient uptake in field growing common bean and maize and thereby enhancing the total plant production. The study was stated by examined the culture conditions that favour for growth of fluorescent *Pseudomonas* strains and siderophore production under Fe limitation and salt stress as well as their ability to antagonize a range of plant pathogens under Fe limitation and salt stress.

MATERIALS AND METHODS

- **Soil samples**

1. Four Hungarian uncultivated soil samples were collected from brown forest sandy soil of Experimental Station of Szent István University (Gödöllő), Szarvas, Hortobágy and Nyíregyháza. Two more Libyan soil samples (Tripoli and Asaba) were used for isolation of different fluorescent pseudomonads. Brown forest soil was used in greenhouse experiments.
2. Artificial soil sample (BLUH-FIX) is defined as the moist natural fine and homogenous structure of the planting soil ensures the optimal growing conditions for the young plants at the starting period. The compositions of the planting soil is especially favourable for developing healthy roots as it contains reduced amount of peat and humus in an optimal dose according to the plant needs. That is the reason to study the root colonization compatibility of the selected strains with this soil under greenhouse conditions.

- **Test plants**

The test plants were selected according to their economical importance:
1. Field growing common bean (*Phaseolus vulgaris* L.)
2. Maize (*Zea mays* L.)

♦ **Natural mineral salty water**

The "Ferenc József" natural pharmacological alkaline water which contains different cationic and anionic ions in 700 ml. This water type was used to determine the sensitivity of the fluorescent pseudomonads isolates to natural combinations of different chemical forms as their present in nature.

METHODS

♦ **Isolation of fluorescent pseudomonads**

Different PGPRs fluorescent *Pseudomonas* were isolated from uncultivated soil, rhizosphere or rhizoplane of common bean and maize plants cultivated in different soil types. To isolate soil, rhizosphere and rhizoplane inhabitant of fluorescent pseudomonads, seeds of common bean and maize plants were grown and maintained in greenhouse for 2 weeks. After 15 days, suspension of soil, rhizosphere or rhizoplane was prepared. A serial dilution technique was followed to obtain 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} of each soil sample. One ml of each suspension was plated on 12 ml of the following agar media: Casamino Acids, Cetrimide, King'-B (KING et al 1954), GSP, MG (MARSCHNER et al 1997), Pseudosel, *Pseudomonas* F, *Pseudomonas* P, Synthetic (SCHER & BAKER, 1982) and Trypticase Soya. The plates were incubated for 24 - 48 h at 28°C. The separated single yellowish green water-soluble pigment producing rhizobacterial colonies were isolated and grown on King's-B (KB) and MG media. The isolated colonies were compared with the growth of the standard strains of *P. fluorescens* (B77 and W91). The ability of isolates to produce fluorescent siderophore was tested by plating the isolated bacterial colonies on KB agar and incubating for 2 days at 28°C. Plates were then inspected under 336-nm UV light, and fluorescence was compared visually to those of *P. fluorescens* (B77 and W91) as positive control. The selected colonies were purified using dilution technique and streak plate method. Purified colonies were used for further identification through the classic and modern methods. The pure colonies were stored in respective medium and maintained at 4°C.

♦ **Identification of the isolates**

The further identification methods were carried out by non-fermentable BBL crystal Programme, and GN plates of BIOLOG system uses a 96-well carbon utilization assay. For 16S rDNA, design of *Pseudomonas*-specific amplicon and probe. An alignment of 16S rRNA genes was analyzed to identify conserved regions suitable for developing a fluorescent real-time Polymerase Chain Reaction (PCR) assay to detect 16S rRNA gene sequences specific to the genus *Pseudomonas*. Using the Sequence Express software (ABI), specific forward Pf (21-mer [5'-GGGTGGTGGAATTTCCTG-TGT-3']) reverse Pr (20-mer [5'-GAAGCGGTGACCACAAGGAA-3']) primers were designed to amplify a 65-bp amplicon from *Pseudomonas* 16S rRNA genes, and an MGB-TaqMan assay for *Pseudomonas*, using a primer-probe (Pp) set that identified a *Pseudomonas*-specific implication within the 16S rRNA gene (19-mer [5'-GTGAAATGCGTAGATATAG-3']) to allow quantification of this amplicon. The identification was done according to JOHNSON et al. (1999), JOHNSON & NIELSEN (1999), AAGOT et al. (2001), STACH et al. (2001) and TARNAWSKI et al. (2003).

♦ **Growth of fluorescent Pseudomonas strains at different time intervals**

Detection of the rhizobacterial growth on different growth media was investigated. The growth was checked after 24 and 48 h of incubation at 28°C. Similarly, the bacterial growth was detected in KB broth at 28°C for different time intervals (15, 19, 21, 25, 37, 43, 47, 63, 87 and 110 h) of incubation.

♦ **Determination of siderophores**

According to SCHWYN & NEILANDS (1987): In 100 ml Erlenmeyer flask containing 25 ml of KB broth medium. The inoculum used was consisted of 1 ml of 10^6 CFU/ml cell suspension. The cultures were incubated under shaking at 28°C for 2 days. The bacterial growth was estimated by measuring the absorbance at 600 nm. The bacterial suspensions were removed by centrifugation at 10000 rpm for 15 min. After pH was adjusted to 7, a 800 µl of a regularly renewed $FeCl_3$ solution (1 M) was added, and the medium stirred for 20 min. Siderophore was estimated by measuring the absorbance at 380 nm. Similarly, the siderophore production of fluorescent *Pseudomonas* isolates was detected at different time intervals (15, 19, 21, 25, 37, 43, 47, 63, 87 and 110 h) of incubation at 28°C. Siderophore was detected using different volume ratios (2:1 and 1:1) of supernatant of bacterial free culture and CAS reagent. The effect of frost on the siderophore production was carried out by growing the rhizobacterial isolates for 15 h at 28°C and then stored in the freeze (- 4°C) for 3 and other set for 4 months. The detection of the siderophore in the frozen cultures was carried out as mentioned above.

According to ALEXANDER & ZUBERER (1991), LOPER & HENKELS (1999) and SHARMA & JOHRI (2003a): Siderophore production was carried out using the different media and was detected after employing the universal CAS assay. Plates were spotted with overnight grown cultures. By the formation of orange or yellow halos surrounding the bacterial colonies on CAS – HDTMA agar after 2 days of incubation at 28°C. The cultures were compared to the control.

♦ **Antagonistic effect on phytopathogenic fungi**

The antagonistic activity of native fluorescent pseudomonads isolated from different habitats *in vitro* against *Alternaria tenuis*, *Botryodiplodia theobromae, Fusarium dianthy, F. graminearium, F. oxysporum, F. solani*, *Phoma medicaginis, Pythium ultimum, Rhizoctonia solani* and *Trichoderma* sp. was investigated on PDA and KB agar media, as described by DILEEP et al. (1992) and modified by us. A loopful of heavy suspension ($1x10^6$ CFU ml^{-1}) of fluorescent rhizobacterial isolates was streaked on the four inner sides of Petri plates to form rectangular shape (90x120 mm). Six mm in diameter of actively mycelial disc of one of test fungi was cut from freshly growing culture, and was placed at the centre of rectangular formed by the rhizobacterial streak lines of 15 or 30 mm away from the edges of the plate and the fungal growth zone was noted after 7 days at 28±2°C. To verify whether the inhibition was due to the presence of siderophores, the above test was repeated using media amended with 80, 160, and 240 µM of $FeCl_3$ or NH_4VO_3. Measurements of inhibition zones between the edges of bacterial growth and the edge of growing fungal colony were taken after 5 to 7 days of incubation.

♦ **Assays for lytic enzyme activity**

The **chitinolytic** activity was assessed using a solid minimal medium containing (gl^{-1}) 0.8 K_2HPO_4, 0.2 KH_2PO_4, 0.5 $(NH_4)_2SO_4$, 0.2 $MgSO_4.7H_2O$, 2 Casamino acids, 15 purified agar and an equal volume of a 1% (w/v) of colloidal chitin suspension as well as containing (mgl^{-1}) 10 $CaCl_2$. $2H_2O$, 10 $FeCl_3.6H_2O$, 1 $ZnSO_4.7H_2O$. The **proteolytic** activity was studied on plates containing (gl^{-1}) 100 skim milk, 1.5 yeast extract, and 15 agar. Using the procedure described by DUNNE et al. (1997), the fluorescent *Pseudomonas* isolates were streaked directly on the plates or spotted onto the centre of the plates. The plates were incubated overnight at 28°C. Both chitin- and skim milk- containing plates are opaque and enzyme activity was identified by the development of a clear zone around the colonies**.**

♦ **Phosphate solubilization ability**

The PO43- solubilizing assay was done on the surface of the agar plate containing a medium described by Pikovskaya (1948) and according to the procedure of Goldstein (1986). The colonies forming clarification zones considered as PO_4^{3-} solubilizers. The diameter of the clearing zone was measured.

♦ **Tolerance to fungicides**

The tolerance to Copperoxychloride 50 WP, Dithane M-45, Mikal 75% WP, and Orthocide 50 WP was measured at 0, 0.1, 1.0, 10 and 100 mgl^{-1} in KB broth inoculated with 100 µl of cell suspension of a 24 h fresh culture and incubated at 28°C for 48 h in shaking incubator (150 rpm). The tolerance was estimated by measuring the rhizobacterial growth at 600 nm.

♦ **Tolerance to heavy metal**

The tolerance of the bacterial isolates to Al_2Cl_3, $(NH_4)_6Mo_7O_{24}$, NH_4VO_3, $CdCl_2.2.5H_2O$, $Co(NO_3)_2$, $CuCl_2$, $FeCl_2.6H_2O$, $La_2Cl_3.7H_2O$, $PbCl_2$, $MnCl_2.4H_2O$, $NiCl_2$, and $ZnCl_2.6H_2O$ was measured at the concentrations of 0, 40, 80, 160 and 320 µM and were determined in KB broth medium according to ROANE (1999) and ROSSBACH et al. (2000). The inoculation, incubation and the estimation of the tolerance were similarly as in fungicides.

♦ **Minimal inhibitory concentrations of some bacterial growth inhibitors**

The KB broth was modified by inclusion of heavy metals at different concentrations and was inoculated with 100 µl of cell suspensions from pre-culture of 24 h. The tested concentrations of heavy metals were 250, 500, 1000, 2000, 4000, 8000, and 16000 µM. The assay was carried out in microplates. The results were obtained after 24 h incubation at 28°C.

♦ **Effect of alkalinity and salinity**

The alkalinity and salinity tolerance of the rhizobacterial isolates to $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, K_2CO_3, KCl, K_2HPO_4, K_2SO_4, Na_2CO_3, NaCl, Na_2HPO_4, and Na_2SO_4 at 0, 10, 20, 40 and 80 mM while in pharmaceutical natural mineral water the concentrations were 0, 75, 150, 300, 450 and 600 mgl^{-1}. Similar method was used as in the tolerance of heavy metals.

♦ **Siderophore production under the salts stress and heavy metals**

The detection of siderophore production by fluorescent *Pseudomonas* isolates under the stress of heavy metals and salts used in the above experiments was carried out. The cell-free supernatants were mixed with CAS reagent and the optical density at 380 nm was recorded after short incubation for 10 min at room temperature in the dark.

♦ **Seed bacterization root colonization and at different treatments of NaCl, $FeCl_3$**

The experiment of ***seed bacterization*** was carried out in two steps:

A. Using sterilized artificial soil (BLUH-FIX)
B. Using natural sterilized brown forest of Gödöllő soil.

When the artificial soil was used, the effect of 45 experimental factors on the common bean and maize plant growth parameters (fresh and dry weights of the shoot and roots) were studied. Depending on the results obtained, the second experiment was continued with the natural brown forest soil using ten experimental factors instead of 45. These ten factors showed the best results in the first experiment using the artificial soil. Subsequently, the preparation of rhizobacterial suspension of GH-041, HH-0410, AL-042, and TL-041 strains to obtain 10^6 $CFUml^{-1}$ was adjusted by UV visible spectrophotometer at 600 nm.

Seed bacterization was carried out by a method of DILEEP et al. (1992) and modified by DILEEP et al. (2001). Common bean and maize seeds were surface sterilized. Four strains of fluorescent *Pseudomonas* (GH-041, HH-0410, Al-042 and TL-041) were grown in KB medium, that were harvested and suspended in 20 ml of 1% sterile carboxymethylcellulose (CMC) suspension. The experiment was carried out in brown forest soil.

- A five grams of surface sterilized seeds were steeped in one of four fluorescent *Pseudomonas* (GH-041, HH-0410, Al-042 and TL-041) suspension for 60 min. The bacterized seeds were examined for CFU on KB medium and adjusted to give 10^6 CFU/seed for individual treatment.
- The control and bacterized seeds were sown into plastic pots containing sterile soil. The seeds were grown under greenhouse conditions with a 14 h day time temperature of 22–25°C, and a 10 h night time with a temperature of 15–17°C. The illumination was supplemented with four 400-W metal halide lamps providing a natural light intensity of 1000 µmol photons $m^{-2}S^{-1}$, and 80% relative humidity. The growth in terms of shoot and root fresh and dry weight was recorded after 50 days of germination. During the growth and development, plants were watered with distilled water as needed.
- The populations of strains were estimated in the soil bulk and in the rhizosphere of common bean and maize plant under/ or without CMC treatment.

Root colonization was carried out using 15 fluorescent *Pseudomonas* isolates.

- In 250 g sterilized acid-wash quartz in pots, the experiment was conducted to estimate the root colonization of common bean and maize plants and bacterial population after seed bacterization and seed germination.
- The sterilized system was watered by sterile distilled water as well as with the nutrient solution that was supplied by soil extract medium (0.02 gl^{-1} K_2HPO_4, 0.02 gl^{-1} $MgSO_4.7H_2O$, 100 ml soil extract and 900 ml distilled water. The soil moisture was always kept at 60%.
- The roots of the seedlings were suspended in 50 ml sterile water and vigorous shaking in horizontal shaker at 25°C for 30 min, the solution was serially diluted, planted on KB agar medium, and incubated at 28°C for 48 h. All colonies formed units of tested rhizobacteria were re-isolated and considered to be rhizoplane colonizers.

♦ **Enzymatic potential activities in plant rhizosphere**

The potential activities of the following enzymes in the rhizospheres of common bean and maize plants grown in a 2 kg pots containing Gödöllő brown forest soil were measured under the stress at different concentrations of 0.25% NaCl, 0.25% $FeCl_3$, and the inoculation of rhizosphere by 4 different fluorescent *Pseudomonas* (GH-041, HH-0410, Al-042 and TL-041) strains or in a combination with 0.25% NaCl and 0.25% $FeCl_3$.

Dehydrogenase was assessed according to GARCÍA et al. (1993) and it is expressed as µg of iodonitrotetrazolium formazan g^{-1} dry soil.

Catalase was determined using the method of TABATABAI & BERMNER (1970), and it is expressed as µmol O_2 min^{-1} g^{-1} dry soil.

Urease was detected according to the method of NANNIPIERI et al. (1980), and it is expressed as μmol of NH^+_4-N released g^{-1} dry soil h^{-1}.

Protease was measured in the same way as for urease (NANNIPIERI et al. 1980). Protease is expressed as μmol of NH^+_4-N released g^{-1} dry soil h^{-1}.

Phosphatase was measured according to TABATABAI & BERMNER (1969), and it is expressed as μmol of P-nitrophenol g^{-1} dry soil and incubation time (h).

β-glucosidase was measured according to MASCIANDARO et al. (1994), and it is expressed as μmol of P-nitrophenol g^{-1} dry soil and incubation time (h).

♦ **Nutrition status in the soil and plant**

The total organic C content of soil and plant was measured by the loss on ignition at 560°C, by the Walkley-Black method (NELSON & SOMMERS 1982). Total N in soil was estimated by diacid digestion and Kjeldahl distillation (BREMMER & MULVANEY 1982). The total P and K were analyzed by triacid digestion and estimation by standard procedures of JACKSON (1967).

♦ **Statistical analysis**

All tests were carried out at least in triplicates. Group differences across metric dependent variables based on set of categorical (non-metric) variables were assessed by multiple analyses of variance (MANOVA). Differences in means were evaluated by F-probe according to Sváb (1981). Excel 5.0 statistical functions were used for calculations and graphic presentation of data. Standard deviation (SD) and Least Significant Difference at 5% level ($LSD_{0.05}$) were calculated as well.

RESULTS

Seedling inoculation with PGPR and exerting a suppression of plant roots pathogens and improving the soil fertility might be a promising tool in future eco-agricultural biotechnology.

Soil analysis

Studies on soil composition from Hungary and Libya reported many significant differences in pH, sticky point, total salts, humus, low and high cations and anions exchange capacity, and the content of K, P and Fe.

Identification of fluorescent pseudomonads isolates

According to the production of clear fluorescent yellow-green pigments that give a fluorescent colour under UV light are functioning as siderophores. The 107 isolates was reduced to 36 with more purified and differentiated morphologically and microscopically. On the basis on the classical and modern methods of identification, studies to distinguish between the isolates based on the biochemical reactions were carried out using BBL plates and BIOLOG GN plates. The 36 isolates were reduced to 14 different strains depending on their biochemical reaction and antagonistic activities. The 14 strains further tested for their growth rates at different time intervals and their siderophore production after storage in freeze phase, PO_4^{3-} solubilization, lytic enzyme activity, growth and siderophore production on the surfaces of different media, fungicide-, salt-, heavy metal tolerance and their population size and colonizing the roots of the test plants in quartz soil culture. Depending on the results obtained, the most tolerant and characteristic four strains were further identified according DNA hybridization and based on 16S rRNA method. The results of PCR and 16S rRNA method of identification identified these four fluorescent *Pseudomonas* strains as: GH-041 (*P. fulva),* HH-0410 (*Pseudomonas* sp. (strain NN016909)), AL-042 (*P. borealis)* and TL-041 (*P. putida)* and were used in the study of plant–microbe interaction under the stress of different environmental factors.

Growth of fluorescent Pseudomonas strains at different time intervals

The lowest growth of the tested strains was found on the nutrient agar plates. It was found that the growth rate of the strains was directly proportional with the production of siderophore. In KB broth medium, it was found that maximum growth was obtained when the rhizobacteria grown for 47 and 63 h, while by increasing the incubation time the growth rate was decreased. Also, the log phase of most strains was obtained during the first 15 h of incubation. This indication was observed for the two Gödöllő strains (GH-041 and GH-043) and the Hortobágy strains (HH-041, HH-042, HH-045, HH-49, HH-0410 and HH-0416) except the growth of the HH-0410 strain that had a maximum growth range between 43 and 47 h, and its growth after 63 h was in the range of the growth of other strains. For

Libyan strains, the growth rates of the strains isolated from Tripoli (TL-041, TL-046, TL-0410 and TL-0412) were similar to those of the Hungarian origin. The growth of strain AL-042 was at maximum when incubated for 87 h, while the growth of the other strain AL-043 was similar to those of the Hungarian strains. It can be seen that the log phase of the two strains was different, and the cell number of AL-043 was lower than of AL-042.

Determination of siderophores production

On agar plates: The growth of fluorescent *Pseudomonas* showed maximum siderophore production in SSM, SM, KB medium followed by TSM and was low in NA. This was irrespective of time of incubation from 12 to 48 h. The complex media of KB, TSM and NA are rich media having the ingredients like peptone, beef extract, which have trace of Fe concentrations. This suggested that synthetic media are better for siderophore production in comparison to complex medium to avoid the unwanted Fe concentration.

In liquid: It was found that when the mixing ratio was two part of supernatant and one part of CAS reagent, results demonstrated that the GH-043 strain produced more siderophore than GH-041 after 47 h of incubation and after 63 h, the strain GH-041 produced more siderophore than the strain GH-043. The results indicated that the other six Hungarian strains isolated from Hortobágy had maximum siderophore production when they were incubated for 47 h. The strain HH-0410 gave the maximum amount of siderophore, while the lowest siderophore production was detected by HH-049. The levels of siderophore produced by the Libyan strains were different from the Hungarian strains. The results indicated that the amounts of siderophore produced by the Libyan strains were decreased when the strains incubated for longer time. When the mixing ratio was in equal parts, the results showed that the highest amount of siderophore produced by the two Gödöllő strains (GH-041 and GH-043) was obtained when they incubated for 63 h. Meanwhile, the strains isolated from Hortobágy soil were divided into two groups, first one gave the highest amount of siderophore after 47 h (HH-041, HH-049 and HH-042), and the second group (HH-0410, HH-046 and HH-045) produced the highest amount after 63 h incubation. The highest siderophore production from both groups was obtained by HH-0410 and HH-041. For Libyan strains, AL-042 was produced the highest amount of siderophore after incubation for 63 h, while a maximum amount of siderophore was produced by AL-043 strain when incubated for 47 h. The results demonstrated that all Tripoli strains produced siderophore after 63 h incubation and the strain TL-046 produced the highest amount. Comparatively, higher amounts of siderophore production by the strains stored in freeze for four months than those strains stored in freeze for three months.

Antagonistic effect on phytopathogenic fungi

Fifteen strains of the promising fluorescent *Pseudomonas* were used for testing their antagonistic potential activity against some phytopathogenic fungi (*Alternaria tenuis*, *Botryodiplodia theobromae, Fusarium dianthy, F. graminearium, F. oxysporum, F. solani*, *Phoma medicaginis, Pythium ultimum, Rhizoctonia solani*, and *Trichoderma* sp.). Among the isolates, HH-041 showed maximum inhibition of the mycelium growth of the tested fungi. Significant differences were observed among the 15 fluorescent *Pseudomonas* toward their antagonistic effects on the growth of phytopathogenic fungi. The obtained results revealed that the fluorescent *Pseudomonas* strains GH-041, HH-041, HH-042, HH-0410, PF-ELTE, AL-042, AL-043, TL-041, TL-046 highly inhibited the mycelial growth of *A. tenuis*, while the strains GH-041, HH-042, HH-0410, TL-041, TL-0410 significantly inhibited the growth of *B. theobromae*. The mycelial growth of *F. dianthy* was highly inhibited by strains GH-041, GH-043, HH-041, HH-045, HH-0410, HH-0416, PF-ELTE, AL-042, AL-043, TL-041, TL-0410. The growth colony diameter of *F. graminearium* was significantly reduced when grown in the presence of GH-041, GH-043, HH-041, HH-042, HH-045, HH-049, HH-0410, HH-0416, AL-042, AL-043, TL-041, TL-046, TL-0410, TL-0412. But all the fluorescent *Pseudomonas* strains GH-041, GH-043, HH-041, HH-045, HH-0410, HH-0416, PF-ELTE, AL-042, AL-043, TL-041, TL-0410 had highly antagonistic effect on the growth of *F. oxysporum*. *F. solani* colony diameter was inhibited by the effect of the growth and the metabolic material produced by all strains except HH-045. The colony forming unit of *Phoma medicaginis* was inhibited by the effect of the growth of GH-041, HH-041, HH-042, HH-0410, AL-042, AL-043, TL-041, TL-046. Also, the present results showed that the strains GH-041, GH-043, HH-041, HH-042, HH-045, HH-0410, HH-0416, AL-042, AL-043, TL-041, TL-0410, TL-0412 were highly reduced the mycelial growth of *Pythium ultimum*. However, the

growth of *Rhizoctonia solani* was inhibited by the antagonistic effect of GH-041, GH-043, HH-042, HH-045, HH-049, HH-0410, AL-042, TL-041, TL-046, TL-0412. The results revealed that GH-041, GH-043, HH-041, HH-042, HH-049, HH-0410, HH-0416, PF-ELTE, AL-042, AL-043, TL-041, TL-046, TL-0410, TL-0412 inhibited the growth of *Trichoderma* sp. The results demonstrated that 240 µM $FeCl_3$ or NH_4VO_3 was able to decrease the antagonistic potential activities of the strains. It was found that 240 µM of NH_4VO_3 was more active to remove the antagonistic effect of the strains on the fungal phytopathogens than 240 µM $FeCl_3$.

Assays for lytic enzyme activity

Under *in vitro* conditions, colonies produced clearing zones on plates containing chitin or casein, as an indicator of extracellular chitinase or protease activities, respectively. For chitinase, it was found that HH-0410 strain gave the widest clear zone (16.7 mm) followed by the Libyan strain TL-041 (16.4 mm), AL-042 (14.2 mm), GH-041 (13.7 mm) and HH-0416 (13.1 mm), while the smallest zones were given by the strains of TL-0410 (8.8 mm), TL-0412 (8.9 mm), and HH-049 (8.9 mm). Generally the Hungarian strains were more chitinase produced than Libyan strains. In the study of protease detection, it was found that TL-041 was the highly secreted protease with 17.3 mm clear zone diameter followed by GH-041 with 16.4 mm, AL-042 with 15.4 mm and PF-ELTE (15.3 mm).

Phosphate solubilization ability

The results showed that fluorescent *Pseudomonas* strains produced 24.5±0.4 to 34.7±0.2 mm of clearing zones indicated the P solubilization. HH-049 strain of Hortobágy soil showed the lowest ability to solubilize P while the maximum clearing zone was observed with AL-042 strain isolated from Asaba. The results indicated that throughout the Hungarian strains, GH-041 produced the maximum clear zone (33.5) around the inoculum. The following strains produced clear zones of P solubilize over 30 mm: AL-042 (34.7), GH-041 (33.5), TL-041 (32.7), HH-042 (30.1), HH-045 (31.3) and HH-0410 (31.9).

Tolerance to fungicides

It was found that the Hungarian strains proved the more tolerant than the Libyan strains. The results showed that Orthocide was more toxic comparing with Dithane M-45 and the tolerance of the strains depended on the strain itself regardless to its origin. The most tolerant strain was GH-041, HH-0410, AL-042 and TL-041. Mikal had more toxic effect on the growth rates of the strains than Dithane and less than Orthocide and Copperoxychloride. The results illustrated that HH-0410, HH-416, AL-042, GH-041 and TL-041 were the most tolerant strains, while the most sensitive strain was TL-0410.

Tolerance to heavy metal

The results indicated that all tested concentrations of $AlCl_3$ significantly reduced the growth rates of the Hungarian and Libyan strains, except 40 µM which slightly increased the growth rates of HH-0410, AL-042, TL-041 and TL-0412. These strains were the most tolerant, while the TL-046 strain was the most sensitive. The most tolerant strains to $CdCl_2$ were GH-041, HH-0410, TL-041 and TL-0410. All strains were sensitive to 40 µM, while the growth rates of the strains GH-041, HH-0410, TL-041 and TL-0410 were improved by this concentration. The most sensitive strain was HH-049. It was found that $CdCl_2$ was less toxic to the strains than $AlCl_3$. The growth rates of the strains were less than the controls at all $Co(NO_3)_2$ concentrations, while at 40µM, the growth of HH-041, AL-042 and TL-041 was slightly increased. The most sensitive strain was TL-046. Results illustrated the strains HH-0410, AL-042 and TL-041 were the most tolerant, while minimal growth rate of TL-046 showed its high sensitivity to the metal. The 40 µM concentration of $FeCl_2$ improved the growth rates of the strains GH-041, GH-043, HH-0410, AL-042 and TL-041. The most sensitive strain was HH-045.

It was found that $LaCl_3$ was less toxic than $AlCl_3$. The 40 µM concentration of $LaCl_3$ slightly improved the growth of GH-041, AL-042 and TL-0412. The most sensitive strain was TL-046. The concentrations of $MnCl_2$ were improved the growth of the strains more than those of $FeCl_2$. The results demonstrated that 40 µM improved the growth rates of all Hungarian and Libyan strains, and 80 µM slightly increased the growth rates of AL-042 and TL-041. The most sensitive strains were HH-049, and TL-0410. It was found that 40 µM $(NH_4)_6Mo_7O_{24}$ significantly increased the growth rates of HH-0410, AL-042 and TL-041 and slightly of the strains AL-043 and TL-046. The most sensitive strain was TL-0412. There was no differentiation among the Hungarian strains, except the strain HH-0410 that had a slight improvement in the growth rate. It was found that the growth rates of all strains at 320 µM $NiCl_2$ were more than those at the above mentioned metal compounds. The concentration 40 µM

$NiCl_2$ increased the growth rates of the HH-0410, AL-042, TL-041, TL-046 and TL-0410, while it decreased the growth of the other strains. The strains HH-0410, AL-042, TL-041 proved to be the most tolerant strains. The results indicated that $PbCl_2$ is one of the most toxic compounds, where the all concentration reduced the growth of the investigated strains except the concentration 40 µM slightly increased the growth of HH-0410 and TL-041. The most sensitive strain was HH-041. $ZnCl_2$ was less toxic than $PbCl_2$. Comparatively; the growth rates of the strains were improved by 40 µM in some (GH-041, HH-0410, AL-042 and TL-041) while the growth of other strains at this concentration was near to the control. It was found that the growth of the Hungarian strains was more improved than the growth of Libyan strains. The most tolerant strains were GH-041, HH-0410, AL-042 and TL-041. In case of NH_4VO_3, the growth of Libyan strains was more than of the Hungarian strains. From the above, it was found that the most toxic heavy metal on the growth of the strains was $PbCl_2$.

Minimal inhibitory concentration of bacterial growth inhibitors

The results of MICs of the 12 heavy metals shown that strain HH-0410 was found to be the most resistant strain to all investigated heavy metals followed by the strain TL-041, GH-041, AL-042, TL-046, which showed their resistance lay between 16000 and 8000 µM for at least four metals, and 8000 – 4000 µM for at least four other metals. The resistance decreasing order of the remaining strains toward the tested heavy metals was HH-041, GH-043, TL-0410, HH-042, HH-045, HH-049, AL-043, TL-0412 which were resistant to at least 4000 µM for at least three metals. While, the most sensitive strain was HH-0416, which illustrated its sensitivity to three metals at 500 µM and to other three metals at 1000 µM.

Effect of alkalinity and salinity

The results of natural mineral water indicated that the concentrations up to 300 mgl^{-1} increased the growth rates of all Hungarian strains (except GH-043 and HH-041). While, the concentration of 450 mgl^{-1} decreased the growth rate of the strains HH-042, HH-045, HH-0410 and HH-0416, but increased the growth rate of HH-049. At 600 mgl^{-1} concentration, the growth rates of all Hungarian strains were significantly decreased. The most sensitive strain was the HH-041, while the most tolerant strain was HH-049. However, the effect of natural mineral water on the Libyan strains was less than its effect on the Hungarian strains, and this may be due to the origin of the strains. It was found that the concentration 450 mgl^{-1} increased significantly the growth rates of all strains more than the controls. But, the concentration of 600 mgl^{-1} significantly reduced the growth of all the strains.

When the strains were grown in salinized broth medium with various concentrations of pure chemicals, it was found that $CaCl_2$ significantly improved the growth rates of all Hungarian and Libyan strains. It was found that the growth rate of PF-ELTE of *P. fluorescens* strain improved more than the other Hungarian strains at 10 and 20 mM while the strain of HH-042 was gradually decreased. It was illustrated that the growth rates of Hungarian strains in the growth medium containing various concentrations of K_2CO_3. The results of this study showed that the growth rates of the Hungarian strains (except HH-042) were significantly improved up to 40 mM. The growth rates of the strains at 20 mM were generally similar (except GH-041). Meanwhile, the concentration 80 mM reduced the growth of all strains. Results showed that the Libyan strains were more tolerant to K_2CO_3, and the strains were able to grow even at the highest concentration (80 mM) as in case of TL-0412. The results indicated that the strains isolated from soil of Tripoli were more tolerant to the salt than those isolated from soil of Asaba.

There was a high positive effects on the growth of the Hungarian and Libyan strains when grown in broth medium salinized by K_2HPO_4. The results showed that among the Hungarian strains PF-ELTE was the most tolerant strain, while the growth rate of the strain HH-041 was the lowest comparatively with the others. Also, the Libyan strains demonstrated high tolerance to the salinized medium.

The results showed that the PF-ELTE, GH-041 and HH-0410 were the most tolerant strains, and HH-042 was the most sensitive strain to K_2SO_4. By increasing the concentration of the salt, the growth rate of the strain decreased. Similarly, strains isolated from soil of Tripoli were more tolerant to the K_2SO_4-containing broth medium than those strains isolated from Asaba, in which 40 mM significantly reduced the growth of the Asaba strains more than those strains of Tripoli. The strain TL-041 was the most tolerant Libyan strain, while the most sensitive strain was AL-043.

The concentration 20 mM of KCl significantly improved the relative growth rates of Hungarian strains, while 40 mM increased the growth rates of the HH-042, HH-0410 and PF-ELTE strains, and at the same time decreased the growth of the other strains. The most tolerant strain was HH-0410.

Comparatively, the Libyan strains were more tolerant to the KCl concentrations than the Hungarian strains. The Hungarian strains were more tolerant to the concentrations of $MgSO_4$ than the Libyan strains. The Libyan strains were more tolerant to the concentrations of Na_2CO_3 than the Hungarian strains. The results indicated that the Hungarian strains were more tolerant to the concentrations of Na_2HPO_4 than the Libyan strains. The growth of GH-041 decreased by increasing the concentration of NaCl in the broth medium, while the 20 mM increased the growth rate of the strains isolated from soil of Hortobágy. The most sensitive strain to the investigated salt was the PF-ELTE. The HH-0410 was the most tolerant strain to NaCl among the Hungarian strains. But among the Libyan strains, AL-042 was the most tolerant to NaCl followed by AL-043 and TL-041. The most sensitive strain was TL-046. In this study, it was found that 80 mM Na_2SO_4 significantly improved the growth rates of the Hungarian strains except HH-0416 and PF-ELTE. Among the Hungarian strains, HH-041 was the most tolerant strain and HH-0416 was the most sensitive strain to Na_2SO_4. The growth rates of the Libyan strains were differentially increased among themselves, and the Libyan strains were more tolerant to Na_2SO_4 than the Hungarian strains. On the basis of the salt effects, it was found the most tolerant strains were, in order: GH-041, HH-0410, AL-042, and TL-041.

Siderophore production under salt stress and heavy metal

Effect of salt stress

It was found that 20 mM $CaCl_2$ increased the amount of siderophore produced by the Hungarian strains except HH-041. While by increasing the concentration, the production of siderophore was decreased. The highest amount of siderophore at 20 mM concentration was produced by strain PF-ELTE. Meanwhile, The Libyan strains were able to produce siderophore at 20 mM and 40 mM as in the cases of TL-041, TL-046 and TL-0410. The production of siderophore by the Hungarian strains was proved at 10 mM of K_2CO_3, but it was found that GH-041 ad HH-0410 produced siderophore in the broth medium containing 40 mM of K_2CO_3. All the Libyan strains produced siderophore at 20 mM of K_2CO_3 and decreased by increasing the concentration. It was found that the strains were able to produce the siderophore even at 40 mM K_2HPO_4except HH-041. The strain PF-ELTE proved to be the less siderophore producer, and by increasing the concentration of the salt, the amount of siderophore decreased. The strains GH-041, HH-042 and HH-0410 were the most siderophore producing strains. The Libyan strains were less siderophore producing strains than the Hungarian strains at high concentrations, Most of them produced the siderophore only at 10 mM. Some strains like TL-041, TL-046 and TL-0410 had the ability to produced siderophore at 20 mM. The amount of siderophore at high concentration was low. The strains GH-041, HH-042, HH-0410 and PF-ELTE were able to produce siderophore at 20 mM of K_2SO_4. While the other strains were able to produce siderophore at 10 mM. The Libyan strains AL-042 and AL-043 and TL-041 can produce siderophore at 40 mM of K_2SO_4, and the other strains TL-0410 and TL-0412 were able to produced siderophore at 20 mM. The lowest amount of siderophore was produced by TL-046. Therefore, the Libyan strains had the ability to produce higher amounts of siderophore than the Hungarian strains.

All the Hungarian strains were produced siderophore even at 40 mM of KCl, but at 80 mM the amount of siderophore was reduced. The maximum amount was detected with HH-041. Results illustrated the siderophore production by Libyan strains in the growth medium containing various concentrations of KCl. Strains of Asaba were more siderophore producing strains than the Tripoli strains, except TL-041, which produced the highest amount of siderophore. The results indicated that this salt reduced the amount of siderophore production by the strain TL-0412 even at the lowest concentration. The strain GH-041 was the strain which was able to produce the highest amount of siderophore, and even at 40 mM $MgSO_4$, while the other isolated strains produced siderophore at 20 mM except PF-ELTE. Still strains of Asaba produced higher amounts of siderophore than the Tripoli strains. But the Tripoli strain TL-041 produced siderophore at 40 mM. The salt Na_2CO_3 potentially reduced the production of siderophore of the Hungarian strains but less than the Libyan strains. The strains GH-041 and HH-0410 were produced siderophore at 20 mM, while the all strains produced it at 10 mM. Also, the AL-042 and TL-041 strains produced the siderophore at 20 mM while the other strains produced it at 10 mM. The AL-042 was the highest siderophore production among the Libyan strains. All the Hungarian and Libyan strains produced siderophore at 10 mM, while 20 mM Na_2HPO_4 reduced the amount of siderophore less than the controls, except HH-0410 which produced siderophore even at 40 mM. 20 mM of the salt improved the siderophore production by AL-042, TL-041 and TL-046. NaCl highly reduced the production of siderophore by the strains. All the strains produced siderophore at 10 mM,

and GH-041, HH-041, HH-0410, HH-0416, AL-042, AL-043, TL-041 and TL-0412 were highly produced siderophore at 20 mM, while AL-042 was produced siderophore at 40 mM. All strains produced siderophore at 20 mM Na_2SO_4 except TL-046, TL-0410 and TL-0412, which produced it at 10 mM only. HH-0410 was the only strain, which produced siderophore at 40 mM. Generally, it was found that NaCl was the most effective salt, which reduced the production of siderophore.

Effect of heavy metals

It was shown that the siderophore production by all strains were less than in controls. Increasing the concentrations of $AlCl_3$ decreased the production amounts of siderophore. $CdCl_2$ was less inhibitory for producing siderophore than $AlCl_3$. The most siderophore producer strain was HH-0410 while the lowest amount of siderophore was produced by TL-046. $Co(NO_3)_2$ was able to improve the siderophore production by Hungarian strains more than the Libyan strains. It was found that the strains isolated from soil of Hortobágy were the most siderophore producers like in case of $CdCl_2$ and $AlCl_3$. Also, the strains of Gödöllő were more producing siderophore than the Tripoli strains. The most siderophore producer was HH-0410 and the lowest was TL-041. $CuCl_2$ was able to differentiate between the production of siderophore among the strains and within the strains too. HH-0410 was the most siderophore producer. Among the Libyan strains TL-041 and AL-042 were the most siderophore producers. $FeCl_2$ was the highest siderophore production inhibitor, and the Hungarian strains were more siderophore producers than the Libyan strains. $LaCl_3$ was less toxic than $AlCl_3$ regarding to the production of siderophore. The results obtained show that the Hungarian strains were able to produce siderophore more than the Libyan strains. In the growth medium containing various concentrations of $MnCl_2$ or $(NH_4)_6Mo_7O_{24}$, or $NiCl_2$, there was no difference among the Hortobágy strains in the production of siderophore except that HH-0410, which was the highest producer. Also, similar results obtained with $PbCl_2$. The results of the study showed that NH_4VO_3 was less inhibitor than $FeCl_2$. There was a similarity between the strains of Gödöllő, Hortobágy and Asaba in the production of siderophore. The most producer was HH-0410 and the lowest amount was produced by TL-046. The Hungarian strains proved to be higher siderophore than the Libyan strains in $ZnCl_2$ treated medium. The highest producer was HH-0410, and the lowest producer was TL-0410.

Seed bacterization and root colonization

Population dynamics

The present study showed the population size of 15 tested rhizobacteria fluorescent bacteria in the rhizoplanes of maize and common bean at 21st and 35th days after germination. It was significantly increased to 7.22 when the common bean seedlings were bacterized by HH-0410. Also, it was increased to 7.21 when common bean seedlings were bacterized by AL-042 after 21st day. The results indicated that the population size of the bacteria in the rhizoplane of maize after 21st day was increased significantly to 7.272 by HH-0410 or to 7.241 by GH-041. After 35th day of plantation, the population size of induced bacteria to the rhizoplane of bean was at maximum when inoculated by HH-0410 (7.258) or by TL-041 (7.243) or by AL-042 (7.241). Similarly, the highest population size was found by HH-0410 (7.328) or by TL-041 (7.295), GH-041 (7.292) or by AL-042 (7.241). This population was maintained till the end of the assayed period without considerable changes. The results of using CMC with seed bacterization showed that the population size of the four test strains in soil bulk, and rhizospheres of common bean and maize was improved more than the population size of the rhizobacteria bacterized the plant seeds directly without applying CMC. The HH-0410 was the most abundant strain in all cases, while the AL-042 strain had the lowest population size in all conditions.

Total dry matter production

In the study carried out using the artificial soil "BLUH-FIX", the highest dry matter accumulation was found when the plant bacterized by one of used bacterial (GH-041, AL-042, TL-041, and HH-0410) strains as well as in combination with low concentration of $FeCl_3$ (0.25%). Under all conditions, the plants inoculated by HH-0410 strain gave the highest fresh and dry weight of shoot and root of maize as well as the bean roots. The lowest plant weights (fresh or dry) was obtained when the plant was grown in soil treated with $FeCl_3$ (0.5%) and NaCl (0.5%). According to potential effect of bacteria on the weights of the maize plants, it was found that the decreasing order of bacterial effect was HH-0410 > AL-042 > TL-041 > GH-041 alone or in combined with low concentration of NaCl, while it was found that this order changed to the following when the bacterial strains inoculated to the soil-plant system treated with high concentration of NaCl, $FeCl_3$, or at low or high NaCl combined with low or high $FeCl_3$. The order was HH-0410 > AL-042 > GH-041 > TL-041. According to potential effect of

bacteria on the weights of common beans, it was found that the decreasing order of bacterial effect alone or in combined with 0.25% NaCl or with 0.25% or 0.5% $FeCl_3$ or with 0.25% NaCl combined with 0.25% $FeCl_3$ was HH-0410 > TL-041 > AL-042 > GH-041.
The second pot experiment which conducted with only 10 treatments using the "Gödöllő brown forest soil" as natural agroecosystem. The results indicated the higher dry matter accumulated occur in plants bacterized by fluorescent *Pseudomonas* (GH-041, HH-0410, AL-042 and TL-041) strains compared to the control. The higher common bean dry matter accumulation was found according to the following decreasing order of the bacterial growth promoting activity HH-0410 > TL-041 > GH-041 > AL-042. While in maize plants, it was HH-0410 > AL-042 > TL-041 > GH-041. Meanwhile, similar results were obtained when the plants were bacterized and grown in soil system treated with low NaCl combined with low $FeCl_3$. The understanding of the mechanisms of action of these strains will help in the development of a biotechnological product.

Enzymatic potential activities in rhizosphere

It was found that when the soil bulk or the rhizospheres of the common bean and maize plants treated with 0.25% NaCl + 0.25% $FeCl_3$ and inoculated with one of the four fluorescent *Pseudomonas* strains, the potential activity of urease was at maximum. The potential activity of the enzyme in the rhizosphere of maize was higher than its activity in the common bean rhizosphere. The highest potential activity of the enzyme was determined when the strain HH-0410 was bacterized the seeds. Similar results were observed in case of measuring the potential activity of protease. While, the highest enzyme potential activity was recorded at HH-0410. The potential activity of phosphatase was more than 1.5 x of the activity in control. The results showed the similarity of the activity as in case of urease and protease. The decreasing order of potential activity of the phosphatase in the ecosystem of plant bacterized by HH-0410, TL-041, AL-042, and GH-041. The potential activity of β-glucosidase in the treated conditions was nearly 2 x compared with the control. Also, the highest potential activity of the enzyme was illustrated at TL-041. The potential activity of dehydrogenase was significantly positive effected by the treatments, where it was highly in the pots carried the HH-0410 except in the case of maize rhizosphere, the maximum potential activity of the enzyme was found to be: HH-0410, TL-041, GH-041 and AL-042. It was higher in maize rhizosphere than in common bean and soil bulk. The determined value of the potential activity of catalase was nearly 2 x more than the control and significantly high when the pots contained the inoculum of HH-0410.

Estimation on total C and available N, P and K in soil and plant uptake

The accumulations of P and K nutritional status were higher in the rhizospheres of maize and common bean plants than in the soil bulk. Because of native *Rhizobium phaseoli* habitat the Gödöllő brown forest soil, the amount of N accumulation in the rhizosphere of common bean was higher than the detected amount in maize rhizosphere followed by soil bulk. The highest amounts of C, N, P, and K were observed in the soil bulk (16.37, 11.93, 89.23 and 308.33 mg kg^{-1}, respectively) and in rhizospheres of common bean (54.27, 53.73, 101.53 and 306.67 mg kg^{-1}, respectively) and maize (47.00, 31.80, 108.10 and 319.33 mg kg^{-1}, respectively) plants when inoculated with HH-0410. The uptake of C, N, P and K in bacterized plants was higher compared to the control. All the strains significantly increased the total N content in common bean and maize plants, the highest uptake value being by HH-0410. Highest P and K accumulation was observed in common bean and maize plants bacterized with HH-0410. The highest C accumulation in common bean or maize plants was observed in the plants bacterized with TL-041 (30.1, 40.333 mg kg^{-1}, respectively). The total C and N, P and K accumulation in the plants showed significant change over control plant and gave an evidence of the PGPR characters of the tested bacterial strains.

THE NEW SCIENTIFIC RESULTS

The results of the present study with common bean and maize confirmed the efficiency of the selected PGPR fluorescent *Pseudomonas* strains in plants growth promoting test. However, the growth promoting capacity varied with the rhizobacterial isolates.

- Four plant growth promoting fluorescent *Pseudomonas* rhizobacterial strains were evaluated for their efficiency of growth promotion. GP was carried out with field growing common bean and maize. A concentration of $1x10^6$ CFU ml^{-1} was identified as optimum for seed bacterization

since at this concentration maximum germination was registered. Overall, results of the experiments identified the fluorescent *Pseudomonas* rhizobacterial strain HH-0410 of the Hungarian collection followed by the Libyan strain AL-042 to enhance maximum growth compared to control.

- Assessment of common bean and maize plant growth by PGPR strains under greenhouse conditions identified enhancement in emergence rate, germination rate, fresh and dry weights of the plants raised from bacterized seeds by the fluorescent *Pseudomonas* rhizobacterial strains.
- The bacterization of common bean and maize seeds by the four fluorescent *Pseudomonas* rhizobacterial strains resulted in an easy mobilization of essential nutrients (total C, N, P and K) in the plant rhizosphere and an enhanced uptake, which reflected in increased plant biomass. These PGPR can be explored for the effective management of the plant growth and health.
- The data revealed the potential of isolates for collateral plant growth promotion, biocontrol and bioremediation. The selected strains may serve as an important bioresource for development of effective bioinoculants.
- Fluorescent pseudomonads are known to exert extensive antagonistic effect as biocontrol action against soil- and root-borne phytopathogens through releasing of antimicrobials and siderophores.

FUTURE TRENDS

- PGPR offer an environmentally sustainable approach to increase crop production and health. The application of molecular tools is enhancing our ability to understand and manage the rhizosphere and will lead to new products with improved effectiveness.

These can be done throughout:

- Basic research on rhizosphere biology.
- Molecular and biochemical aspects of plant microbial interaction.
- Development of microbial consortia with a broad spectrum of biological activity for growth promotion and disease suppression.
- Product development, quality evaluation and commercialisation among the farming community.

SUMMARY

The use of PGPRs in agriculture is not merely a possibility for the future; there are already a variety of products on the market. In the present study the efficacy of isolated fluorescent *Pseudomonas* strains from different soil types were evaluated for mobilizing the essential nutrient uptake in maize and common bean and thereby enhancing the total plant production. The study was stated by examining the culture conditions that favour for growth of fluorescent *Pseudomonas* strains and siderophore production under Fe limitation and salt stress as well as their ability to antagonize a range of plant pathogens under Fe and V. In the present study, our results indicated that:

Four fluorescent rhizobacterial strains were selected depending on their predominance from a collection of 107 isolates, and the *in vitro* assays (growth and siderophore production on different media, antagonism, heavy metal and fungicide, alkalinity and salinity tolerance, production of siderophore under the stress of different concentrations of heavy metals and salts, lytic enzymes, phosphate solubilization), originally isolated from different soil types and plant rhizospheres for studying their effect on the root colonization, nutritional status and the plant growth in the presence of Fe under the stress of salt. These rhizobacteria were grown in a complete cultural medium. Investigations were made to determine their effects on the following biometrics parameters: fresh and dry weights of plant shoots and roots, plant growth index, total C, N, P and K uptake and the population stability in the rhizosphere of common bean and maize plants. The enzyme potential was activated in the plant rhizospheres. The positive influence of these fluorescent rhizobacterial strains on the plant growth is probably due to these beneficial properties.

Among various media tested, standard succinate promoted maximum siderophore production. There were low concentrations of siderophore production in complex agar media like King's-B, trypticase soya and nutrient. Supplement with $FeCl_3$ resulted in decreased siderophore production. Native

fluorescent pseudomonads isolated from the soil, and rhizospheres of common bean and maize were screened *in vitro* for their antagonistic activity against the 10 phytopathogenic fungi. The 14 fluorescent strains were showed their potential to react with all phytopathogenic fungi. These strains produced siderophores, but addition of Fe or V to the medium decreased the inhibition of phytopathogenic fungi. Lytic enzymes such as chitinase and protease were detected in 42.7% of the total bacterial isolates. Sterile CMC was successful as a carrier for the four (GH-041, HH-0410, TL-041 and AL-042) fluorescent *Pseudomonas* strains. The most antagonistic strains were selected for artificial inoculation of common bean and maize seedlings. Two types of soils were used in pots under greenhouse conditions. The treatments were seeds and roots of common bean and maize seedlings soaked in the bacterial suspension of selected, antagonistic PGPR strains before planting, the same procedure was supplemented by adding and mixing the suspension into the soil, and repeated irrigation treatments with the antagonists.

Pseudomonas strain HH-0410 inhibited the growth of different phytopathogens *in vitro*. Larger inhibition zones were obtained on King's B and nutrient agar media compared to potato dextrose agar. The addition of 240 μM of $FeCl_3$ or NH_4VO_3 to the medium decreased the inhibition of fungal growth, suggesting the involvement of siderophores and other antifungal secondary metabolites. Strains of fluorescent *Pseudomonas* were found effective in promoting growth of maize and common bean plants and evaluated for their nutrient mobilization capacity in the rhizospheres of test plants. P solubilization potency of the strains was proved *in vitro*. In plant studies, the strains significantly increased the final plant accumulation dry matter production in the treated plants compared to control. The role of fluorescent pseudomonads mediated nutrient flux in the soil pot in plant growth promotion was confirmed with the higher uptake of nutrients by the bacterized plants after 50 days of treatment and plantation. Significant uptake of N and P was noticed in (regarding to N accumulation: HH-0410, and for highest P accumulation was observed in plants bacterized with HH-0410) treated plants as it is related to the dry matter yield. The uptake of K although was higher in bacterized plants, compared with the control. The highest amount of K in common bean plants was observed in those bacterized by HH-0410. While in case of maize bacterized with HH-0410 showed significantly at higher levels. The bacterial population associated with the roots maintained a minimum population of logarithmic numbers even up to 50 days after treatment. The study revealed the enhanced nutrient mobilization in the rhizospheres of maize and common bean with PGPR treatment, which resulted in enhanced plant production. Generally, it was found that the highest amounts of nutritional elements C, N, P, and K were observed in the soil bulk (16.37, 11.93, 89.23 and 308.33 mg kg^{-1}, respectively) and rhizospheres of common bean (54.27, 53.73, 101.53 and 306.67 mg kg^{-1}, respectively) and maize (47.00, 31.80, 108.10 and 319.33 mg kg^{-1}, respectively) plants when inoculated with HH-0410. Regarding to plant treated with bacterial strains, the highest C accumulation in common bean or maize plants was observed in the plants bacterized with TL-041 (30.1, 40.333 mg kg^{-1}, respectively), but when bacterized with HH-0410 strain and grown in soil treated with low NaCl and low $FeCl_3$, the amount of fixed C was 37.2, 41.6 mg kg^{-1}, respectively. The total C and NPK accumulation in the plants showed significant positive changes over the control plant and gave an evidence of the PGPR characters of the tested bacterial strains.

The results of the present study with common bean and maize confirmed the efficiency of the selected PGPR fluorescent *Pseudomonas* isolated strains in promoting growth test plants. However, the growth promoting capacity varied with the rhizobacterial isolates.

- Four plant growth promoting fluorescent *Pseudomonas* rhizobacterial strains were evaluated for their efficiency of growth promotion. GP was carried out with field growing common bean and maize. A concentration of $1x10^6$ CFU ml^{-1} was identified as optimum for seed bacterization since at this concentration maximum germination was registered. Overall, results of the experiments identified the fluorescent *Pseudomonas* rhizobacterial strain HH-0410 of the Hungarian collection followed by the Libyan strain AL-042 to enhance maximum growth compared to control.
- Assessment of common bean and maize plant growth by PGPR strains under greenhouse conditions identified enhancement in emergence rate, germination rate, fresh and dry weights of the plants raised from bacterized seeds by the fluorescent *Pseudomonas* rhizobacterial strains.
- The bacterization of common bean and maize seeds by the four fluorescent *Pseudomonas* rhizobacterial strains resulted in an easy mobilization of essential nutrients (total C, N, P and K) in

the plant rhizosphere and an enhanced uptake, which reflected in increased plant biomass. These PGPR can be explored for the effective management of the plant growth and health.

- The data revealed the potential of isolates for collateral plant growth promotion, biocontrol and bioremediation. The selected strains may serve as an important bioresource for development of effective bioinoculants.
- Fluorescent pseudomonads are known to exert extensive antagonistic effect as biocontrol action against soil- and root-borne phytopathogens through releasing of antimicrobials and siderophores.

From these studies, it is investigated that, the PGPR, rhizobiofertilizer, antagonize fungi, and plant growth regulators alone or in compatible combinations as chelator or fungal toxin or nutrients, are promising. These may be exploited in formulation and development of suitable rhizobacterial pesticide for disease management as well as PGP by an economically sustainable, eco-friendly technology.

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