International Journal of Microbiological Research 4 (3): 267-274, 2013
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2013.4.3.1110

Effect of Complex Microbial Inoculants on the Number and Diversity of Rhizospheric Microorganisms and the Yield of Soybean

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Abstract: In order to investigate the effects of *Ecovital* complex biological formulation on soybean, we carried out the field experiments on gray forest soil, Kiev region and on chernozem, Cherkassy region, under natural conditions. Our results showed that *Ecovital* application (co-inoculation with *Bradyrhizobium* and *Bacillus* strains) significantly improves soybean yield and soil fertility, as compare with inoculation by *B. japonicum* singly. Bacterization had lead to important changes in the structure of the indigenous microbial communities and shifts the ratio between nitrogen-fixing *Alphaproteobacteria* and *Firmicutes* in rhizosphere. Therefore, complex bacterial formulation was found to be prominent source for soybean crop production in order to minimize environmental problems linked to chemical fertilizers.

Key words: Microbial inoculants · *Bradyrhizobium* · *Bacillus* · Soybean · Microbial diversity

INTRODUCTION

The application of microbial inoculants plays an important role in agricultural systems and can substantially reduce the use of chemical fertilizers and pesticides [1]. Now there is an increasing number of inoculants being commercialized for various crops which can increase soil fertility and plant productivity due to activity of environmentally friendly microorganisms and particularly plant growth-promoting microorganisms (PGPMs). Significant increases in growth and yield of agronomical important crops in response to inoculation with PGPMs have been repeatedly reported [2,3,4]. The application of such microbes as bio-fertilizers may to minimize the use of expensive chemical fertilizers which can lead to soil salinity, heavy metal accumulation and water eutrophication, accumulation of nitrate, air pollution and greenhouse effect [5].

The mechanisms by which PGPMs promote plant growth are not fully understood, but it is believed that they enhance plant growth and yield either by direct or indirect mechanisms [6]. The direct growth promoting mechanisms are as follows: the ability to produce phytohormons like indol acetic acid, gibberellins, cytokinins and ethylene [7], non-symbiotic N_2 fixation [8], antagonism against phytopathogenic microorganisms by production of siderophores [9] and also solubilization of mineral phosphates and other nutrients [10]. The indirect mechanisms of plant growth promotion by PGPMs include the extracellular production of antibiotics, synthesis of antifungal metabolites, production of fungal cell wall lysine enzymes [11], depletion of iron from the rhizosphere, competition for sites on roots and induced systemic resistance [12].

Soil inoculants /biostimulants based on complex microbial formulas represent a next step in the agricultural industry. The effectiveness of microbial products based on single species is strongly tied to the conditions within the soil to which it is applied and reduced or eliminated in outside of optimal environment conditions.

A second key limiting is the nutrient needs of the microbial species. Many or most metabolic processes require multiple input factors. For example, in the case of nitrogen fixation, the primary inputs are atmospheric

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N_2 and the ATP molecules, a source of cellular energy for an energy intensive process. Therefore, if a single strain microbial formula is used and the conditions are not correct for the ATP generation, microbial products often fail to perform the task for which they are intended.

Complex microbial products include the essential microbial ecosystem needed to perform designed product capabilities and to provide commercially important processes in a wide variety of soil conditions. Nitrogen-fixing (NFB) and phosphorus-solubilizing bacteria (PSB) are important for crop plants as they increase N and P uptake and play a crucial role as PGPMs in the bio-fertilization. Phosphorus bio-fertilizers increase the availability of accumulated P, efficiency of biological N_2-fixation and the availability of some trace elements, due to generation of plant growth promoting substances [13]. Co-inoculation studies with PGPMs and rhizobia have shown increased plant nodulation and nitrogen fixation [3; 4]. A variety of microorganisms, including *Bacillus* and *Pseudomonas* species, are commonly found in the rhizosphere of leguminous and non-leguminous crops [14]. By virtue of their rapid colonization of the rhizosphere and stimulation of plant growth, there is currently considerable interest in exploiting. A greater number of nodules and dry weight were registered in soybean and alfalfa under co-inoculation with rhizobia and phosphate solubilizing *Pseudomonas* strains [15]. Dual inoculation of soybean seeds with *B. japonicum* and PSB significantly increased plant height, nodule fresh weight per plant, number of pods per plant, number of seeds per pod and per plant, seed yield, total N and P compared to the other treatments [16].

The knowledge of the survival of inoculated bacterial strains in the field and their effects on the indigenous microbial communities has been of great interest because of large-scale practical use of selected natural or genetically modified microorganisms. Soil inoculation or seed bacterization may lead to changes in the structure of the indigenous microbial communities, which is important with regard to the safety of introduction of microbes into the environment. On the other side, to exert their beneficial effect on plants, successful colonization and persistence in the plant rhizosphere are required for PGPMs. Unfortunately, the interaction between PGPMs and plants can be unstable. The good results obtained *in vitro* usually are not reproduced under field conditions. Root colonization, which is a complex process, is under the influence of various parameters such as bacterial traits, root exudates, biotic and abiotic factors [17]. Some of the factors influencing the survival and activity of bacteria in the rhizosphere are physical (texture, temperature and humidity), while others are chemical, such as pH, nutrient availability and organic matter content.

After being introduced into the soil, the inoculants may affect the indigenous microbial population and, conversely, the indigenous microorganisms can affect the inoculants. Some groups of microorganisms may be stimulated, some may be inhibited or there may be no effect of the introduced microorganisms on the structure of the indigenous population. The effect of bacterial inoculation depends on the conditions in soil, plant species, adaptation ability of the introduced microorganisms, etc. [7].

The present investigation aimed to evaluate the efficiency of new complex microbial formulation *Ecovital* in order to use it as a bio-fertilization for improvement soil properties and nutrient availability and yield of soybean, as far as determine metabolic activity and diversity of bacterial communities in rhizosphere of inoculated soybean plants.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions: Complex bacterial formulation *Ecovital* is co-culture of living cells of *Bradyrhizobium japonicum* UCM B-6018 and *Bacillus megaterium* UCM B-5724 [18]. For seed inoculation in field experiments *B.japonicum* was cultured at 28°C using yeast mannitol medium (YM) and *B.megaterium* – on media for phosphorus solubilizing bacteria. Liquid cultures were grown in flasks with rotary agitation (200 rpm) and culture densities were estimated by optical density by A_{600}. Bacterial suspensions (10^9 colony formed unit (CFU) ml^{-1}) were mixed in ratio 2:1. Highly efficient *B.japonicum* UCM B-6035, a component of commercial *Nitragin* formulation, was applied for mono-inoculation.

Site Description, Sampling and Measurements: Field experiments were conducted at the Experimental stations on gray forest soil, Kiev region, for *Alisa* and *Gorlitsa* soybean biovars and on chernozem, Cherkassy region, for *Romantica* biovar. The soybean seeds were treated with microbial suspension (sterile water in control variant) about 1 hour before sowing (final - 10^7 CFU). The plants were grown without mineral or organic fertilizers and pesticides.

Soil and plants were sampled at mid-flowering and mature phases and after harvesting. Mean number, weight and area of green leaf per plant and CFU count of soil cultivable bacteria were evaluated as described earlier [19]. Total DNA was isolated directly from soil samples with Power Soil Kit (MoBio, USA) and was used for amplification and further cloning analysis of bacterial *nifH* genes as described in [20].

Statistical Analysis: The data were analyzed statistically by analysis of variance and differences among the significant treatments were determined by least significant difference (LSD) test. All data presented were the mean of three different plots, i.e., $n = 3$.

RESULTS AND DISCUSSION

Phenotypic Characteristics of Bacterial Components of *Ecovital* Formulation: *B.japonicum* UCM B-6018 actively forms root nodules with soybean of different biovars and is not sensitive to antibacterial extracts of soybean seed coats. The strain is pectinolytic, resistant to acid pH, low soil water content and heavy metals pollution.

Dissolved mineral phosphates *B.megaterium* UCM B-5724 is Gram-positive spore-forming rod with active complex of phosphohydrolases. The strain can produce different plant growth promoting substances, such as phytohormons, organic acids, antagonistic compounds against phytopathogenic bacteria *Pseudomonas syringae, Pseudomonas fluorescens, Erwinia coratovora, Clavibacter michiganensis subsp. michiganensis* and phytopathogenic fungi *Fusarium tricinåtum, Fusarium lactis, Alternaria alternatà, Cladosporium sphaerospermum, Cochliobolus spicifer*.

Selected Physicochemical Properties of Soils: Soil analysis of the experimental field has shown that the gray forest soil was moderately acidic (pH=5.4) and sandy loamy in texture. The cation exchange capacity (CEC) of the soil was found to be 2.4 meq/100 g which is low. This low CEC may be attributed to the hag percentage (47%) of sand which is low in CEC and also the acidic nature of the soil. The available nitrogen and phosphorous contents of the soil before sowing were found to be 40 and 110 mg kg^{-1}, whereas exchangeable potassium content was 88 mg kg^{-1}.

Low-humic podzolized chernozemic soil was moderately acid (pH=5.6-5.8) and heavy loamy in texture. The CEC of the soil was 2.8- 3.2 meq/100 g and humus content in top soil was 3.3%. The available nitrogen and phosphorous were found to be 100-110 and 110-120 mg kg^{-1} and exchangeable potassium content was 80-90 mg kg^{-1}.

Uninoculated variants (K) did not show any differences in soil properties as compare with soil before sowing. On the other hand, inoculation of soybean seeds resulted in changes in NPK content. At harvesting time of soybean a highly significant ($P<0.01$) increase in available P contents as compared to the controls (Table 1) was evaluated in all inoculation experiments with *Gorlitsa* biovar. Available N has been increased significantly ($P<0.01$) under application of bio-fertlizer, whether only bradyrhizobial inoculant (BJ) or binary bacterial complex *Ecovital* (ECV) were applied for *Gorlitsa* and *Alisa* biovars. The content of free amino acids increased in experiment with *Ecovital* inoculation of *Gotrlitsa* soybean.

Characteristics of Photosynthesis Activity of Soybean Plants: Characteristics of soya green leaves were evaluated for *Romantica* biovar at mid-flowering and

Table 1: Effect of bacterial inoculation on fertility characteristics of gray forest soil.

Experimental ID	Soybean biovar	Easily hydrolyzed nitrogen by Cornfield, mg kg $soil^{-1}$	Labile phosphorus (P_2O_5) by Kirsanov, mg kg $soil^{-1}$	Exchangeable potassium (K_2O) by Kirsanov, mg kg $soil^{-1}$	Free amino acids, mg kg^{-1}
K	*Gorlitsa*	40	112	90	3.0
BJ		46	128	102	2.3
ECV		46	170	104	3.2
K	*Alisa*	39	102	NA*	NA
BJ		44	125	NA	NA
ECV		44	160	NA	NA

*Not available

Abbreviations for this table and all figures: K- without inoculation, BJ- inoculated with *B. japonicum* UCM B-6035, ECV – inoculated with *Ecovital*.

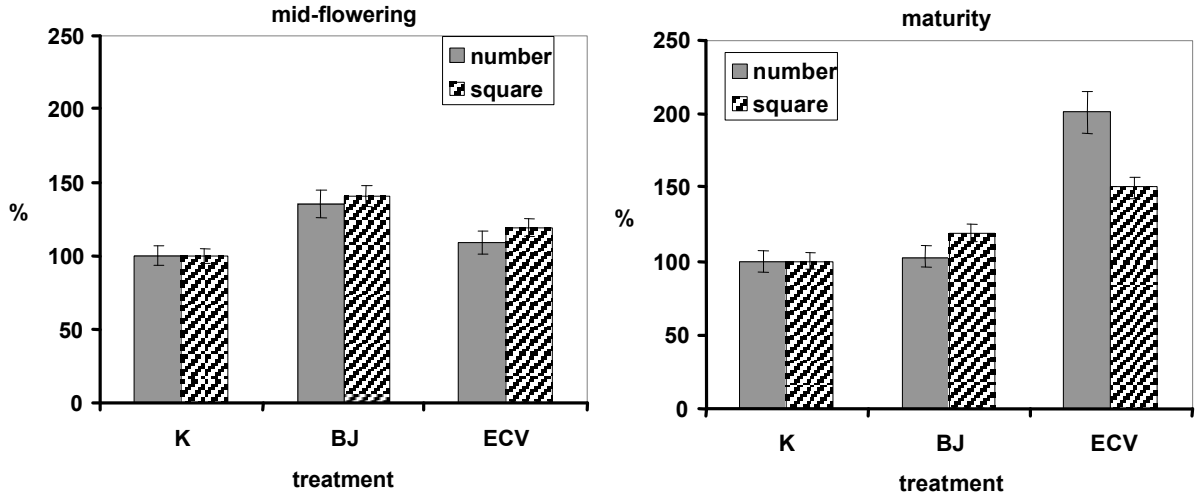

Fig. 1: Effect of bacterial fertilization on structural characteristics of soybean green leaves (number and square for one plant) at mid-flowering and mature phases in field experiments with *Romantica* biovar in comparison with non-inoculated seeds, %.

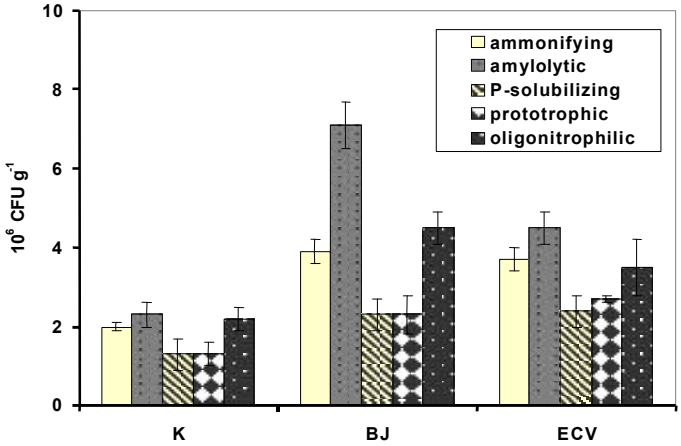

Fig. 2: Effect of bacterial fertilization on number of microorganisms in rhizosphere of *Gorlitsa* biovar at mid-flowering stage

maturity stages (Fig.1). Inoculation of seeds by *Bradyrhizobium* alone (BJ) had no significant ($P<0.01$) influence on leaf number and average area in comparison with non-inoculated seeds (K). Co-inoculation with *Bacillus* (ECV) produced the highest number of leaves at maturity stage. These findings were confirmed in experiments with *Gorlitsa* biovar. At mid-flowering stage the leaf number and area for *Ecovital* treatment increased up to 40%.

Microbial fertilizers enhanced the chlorophyll content in green leaves of *Romantica* biovar. This enhancement was insignificant among control and mono-inoculation, but for *Ecovital* application showed significantly more chlorophyll (36%) than control. A similar result was reported for soybean by Zarei *et al*. [21].

Number and Diversity of Rhizosphere Microorganisms: The use of bacterial fertilization had a positive effect on the number of all the investigated rhizosphere microorganisms (Fig.2). We have focused on soil cultivable bacteria (CFUs) of different physiological groups, which ecological characteristics are reasonably well defined. Rhizosphere soil of *Gorlitsa* biovar was sampled for this investigation at the mid-flowering stage of soybean.

Number of aerobic ammonifying bacteria (MPA-growing) is a good indicator for the soil status evaluation and for all changes that can occur under effects of different factors. The number of these bacteria were from 2.0 to 3.9 × 10^6 CFU g^{-1} dry soil and fell well within the theoretical and practical limits reported in

the literature [22]. The lowest abundance of heterotrophs was determined in the variant without inoculation, while the highest abundance in the variants with inoculation (Fig.2). All bio-fertilizers strongly affected the increase of the number of heterotrophic microorganisms, which certainly affects the increase of their biomass that may be a prerequisite for the raise of the soil productive capacities. The number of heterotrophs is only one aspect of the population, other parameters such as structure and diversity may be also affected by the crop rotations as other works on the effect of soil management on bacteria conclude [23].

The number of amylolytic microorganisms was 2-3 times higher in all variants with inoculation. Microorganisms secreting amylases are widely distributed in bulk and rhizospheric soil and hydrolyze starches, one of the most important naturally occurring glucose plant polymers. Plant starches are typically degrades by soil clostridia. Many of these bacteria produce ammonia, which is oxidized to nitrate by nitrifying microorganisms and contribute to nitrogen content of soil and available nitrogen for crops.

The effect of bio-fertilization on oligonitrophilic and prototrophic bacteria was less clear, although the number of microorganisms increased compare with uninoculated variant ($p< 0.01$). Oligonitrophilic bacteria are important group, including in part free living nitrogen-fixing bacteria that reduce atmospheric nitrogen and convert it into organic forms.

Special focus was be on phosphate solubilizing bacteria (PSB), which are a diverse group of unrelated bacteria able to readily solubilize insoluble forms of P. This bacterial group is vital to the P cycle in soil and some of them may be used in order to enhance the availability of P in soil. Although there are numerous studies regarding the ecology of this group of soil bacteria [24], information about the effect of agricultural practices on abundance and diversity of PSB is scarce. Populations of PSB ranged from 1.3×10^6 to 2.4×10^6 CFU g^{-1} dry soil. Populations in control site were significantly lower than those in inoculated with *B. japonicum* and *Ecovital*. Application of *Ecovital* formulation was the most effective and increase of PSB was up to 85%. The activity of PBS resulted in a highly significant ($P<0.01$) increase in available P contents in soils with bacterial fertilization (Table 1).

By driving crucial soil processes, such as decomposition of organic materials and nutrient cycling, soil bacteria are key players in ecosystem functioning. The structure of the microbial community in soil, the distribution of microbial biomass and enzyme activity may be affected by several factors, such as farming systems, crop rotation, soil type, soil pH, etc. This is why it is also important to take into consideration microbiological indicators when evaluating soil quality and effectiveness of plant-microbial interactions. For soybean the effective symbiosis with rhizobia is the key limiting factor for plants growth, mainly due to nitrogen fixation. To our knowledge, no previous study has addressed to evaluate the diversity of NFB community as indicators of soil quality and crop productivity.

The main hypothesis tested in this study was that the relative abundance of selected taxa of soil NFB could be used as indicator of the impact of agronomic management at a regional scale. Based on molecular analysis of *nifH*, evolutionary conservative marker gene of nitrogen fixation, we have found that the combined use of the abundance of two bacterial phyla could potentially fulfill this task.

Composition of soil diazotrophs was found to be distinct in different variants of field experiments (Fig.3). Phylogenetic analysis of translated NifH sequences shown that the representatives of phylum *Proteobacteria,* clustered with *Alphaproteobacteria* and demonstrated similarity to members of the genera *Leptospirillum*, *Derxia* and *Azohydromonas,* were predominated in experiments without bacterial fertilization. Mono-inoculation and application of *Ecovital* formulations have changed the community structure of diazotrophic community. Predominating nitrogen-fixing bacteria have formed a compact cluster together with the obligate (*Clostridium*) and facultative (*Paenibacillus*) anaerobic bacteria (Fig. 3). So, we concluded that the balance between *Alpaproteobacteria and Firmicutes* in nitrogen fixing community of soybean rhizosphere may be the indicator for effectiveness of bacterial fertilization. *Clostridium* and *Paenibacillus* are active nitrogen-fixing and PGPMs bacteria and increase of their number in rhizosphere may be of great value for crop growth.

Yield and Yield-Related Parameters: The inoculated soybean produced maximum seed yield under *Ecovital* inoculation of *Gorlitsa* (2600 kg ha^{-1}), minimum seed yield (1380 kg ha^{-1}) was obtained in the un-inoculated plots of *Romantica* (Fig.4). This data are in accordance with findings [15; 16] that the highest grain yield was achieved by the co-inoculation of rhizobia and phosphate solubilizing bacteria. Soybean is a protein plant and contains significant amounts of all the essential amino

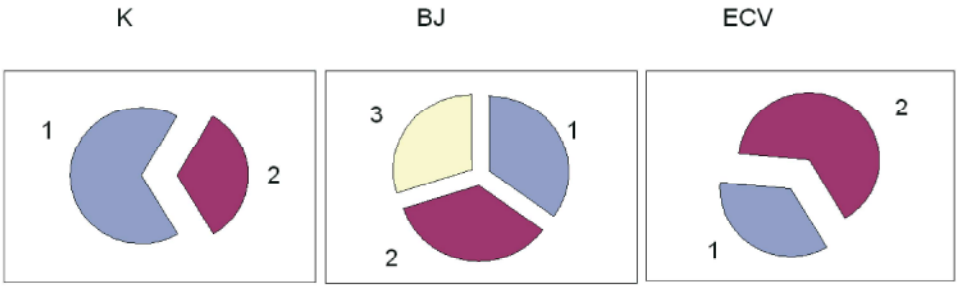

Fig. 3: Effect of bacterial fertilization on *nifH* gene diversity in rhizosphere of *Gorlitsa* biovar at mid-flowering stage. Abbreviations: 1 – *Alphaproteobacteria,* 2- *Clostridium*, 3- *Paenibacillus*

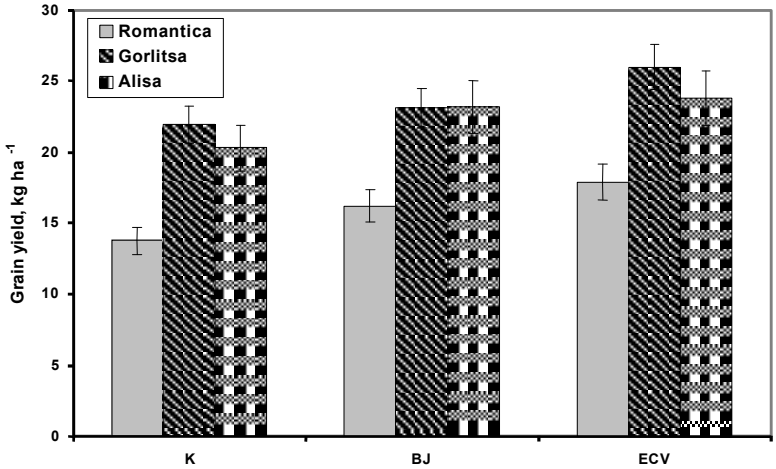

Fig. 4: Effect of bacterial fertilization on grain yield of different soybean biovars.

acids for humans. Several strategies were developed in order to improve the production of soybean proteins. Most of them, including application of chemical or organic fertilizers and pesticides, developing of transgenic plants have a negative impact on the environment. Present study was focused on the improving the soybean protein production using rhizobacteria as bio-fertilizers. *Gorlitsa* plants inoculated with bio-fertilizers produced beans that contained more soluble protein. This difference was small but statistically significant ($P< 0.01$) and the increase of protein production can be translated into 60 kg of soybean protein ha^{-1} in variants under rhizobia inoculation and 180 kg under binary inoculation. Such improvement in total protein production is comparable to effect achieved changes in the genome of transgenic soybean plants [25] or by usage of important amounts of chemical or organic fertilizers.

CONCLUSIONS

Bacterial inoculants are the potential tools for sustainable agriculture for the future. For this reason, there is an urgent need for research to clear definition of what bacterial cultures are useful and necessary for different environmental conditions and plants, so that optimal bacterial strains can either be selected and/or improved. Combinations of beneficial bacterial strains that interact show a promising trend in the field of inoculation technology. It is inferred from this investigation that co-inoculation of N-fixing and P-solubilizing microorganisms (*Ecovital* formulation) is very effective in increasing grain yield of soybean. We concluded that available soil P content was improved following inoculation with P-solubilizing bacteria and *Bacillus* can influence on the efficacy of *Bradyrhizobium* in increasing soil N level.

In conclusion, the results of the present study showed that complex bio-fertilizers can be used for certain proposes with influence on availability of NPK, number and diversity of soil microbial communities, plant morphological changes, grain yield and protein content. It is important to promote the appropriate use of complex bio-fertilizers through national fertilizer programs. Efforts should be made, wherever possible, to introduce and popularize these innovative eco-friendly technologies.

ACKNOWLEDGEMENTS

The reported study was supported by RFBR, research project No. 13-04-90442 Ukr_a and NASU, research project No F53.4/028.

REFERENCES

1. Figueiredo, M.V.B., L. Seldin, F.F. de Araujo and R.M.R. de Lima, 2010. Plant Growth Promoting Rhizobacteria: Fundamentals and Applications. Plant Growth and Health Promoting Bacteria. Berlin: Heidelberg, pp: 22-36.
2. Gray, E.J. and D.L. Smith, 2005. Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. Soil Biology Biochemistry, 37: 395-412.
3. Figueiredo, M.V.B., H.A. Burity, C.R. Martinez and C.P. Chanway, 2007. Plant growth-promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (*Phaseolus vulgaris* L.). World Journal of Microbiology and Biotechnology, 24: 1187-1198.
4. Egamlierdieva, D., G. Berg, K. Lindstrom and L.A. Rasanen, 2010. Co-inoculation of *Pseudomonas spp.* with *Rhizobium* improves growth and symbiotic performance of fodder galega (*Galega orientalis* Lam.). European Journal of Soil Biology, 3-4: 269-272.
5. Serpil, S., 2012. An agricultural pollutant: chemical fertilizer. International Journal of Environmental Science and Development, 3(1): 77-80.
6. Glick, B.R., 1995. The enchancements of plant-growth by free-living bacteria. Canadian Journal of Microbiology, 41: 109-117.
7. Egamberdiyeva, D., 2007. The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Applied soil ecology, 36: 184-189.
8. Salantur, A., A. Ozturk and S. Akten, 2006. Growth and yield response of spring wheat (*Triticum aestivum* L.) to inoculation with rhizobacteria. Plant Soil Environment, 52: 111-118.
9. Scher, F.M. and R. Baker, 1982. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to *Fusarium* with pathogens. Phytopathology, 72: 1567-1573.
10. Cattelan, A.J., P.G. Hartel and J.J. Fuhrmann, 1999. Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Science Society American Journal, 63: 1670-1680.
11. Bharathi, R., R. Vivekananthan, S. Harish, A. Ramanathan and R. Samiyappan, 2004. Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. Crop Protection, 23: 835-843.
12. Glick, B.R., B. Todorovic, J. Czarny, Z. Cheng, J. Duan and B. McConkey, 2007. Promotion of plant growth by bacterial ACC deaminase. Critical Reviews in Plant Sciences, 26(5-6): 227-242.
13. Kucey, R.M.N., H.H. Janzen and M.E. Leggett, 1989. Microbially mediated increases in plant-available phosphorus. Advances in Agronomy, 42: 199-228.
14. Li, D.M. and M. Alexander, 1988. Co-inoculation with antibiotic producing bacteria to increase colonization and nodulation by rhizobia. Plant and Soil, 108: 211-219.
15. Rosas, S.R., G. Avanzini, E. Carlier, C. Pasluosta, N. Pastor and M. Rovera, 2009. Root colonization and growth promotion of wheat and maize by *Pseudomonas aurantiaca* SR1. Soil Biology Biochemistry, 41: 1802-1806.
16. Argaw, A., 2012. Evaluation of co-inoculation of *Bradyrhizobium japonicum* and phosphate solubilizing *Pseudomonas spp*. effect on soybean (*Glycine max* L. (Merr.)) in assossa area. Journal of Agricultural Science and Technology, 14: 213-224.
17. Shiri-Janagard, M., Y. Raei, K. Gasemi-Golezani and N. Aliasgarzad, 2012. Influence of *Bradyrhizobium japonicum* and phosphate solubilizing bacteria on soybean yield at different levels of nitrogen and phosphorus. International Journal of Agronomy and Plant Production, 3(11): 544-549.
18. Tytova, L.V., N.O. Leonova, I.S. Brovko and G.A. Iutynska, 2013. Complex microbial formulation Ecovital for leguminous crops seeds inoculation. Patent UA 101388 C2 (Ukraine), IPC (2013.01) C05F 11/00, C12P 39/00. Pub.25.03.2013, Bull. #6.
19. Tytova, L.V., N.O. Leonova and A.F. Antipchuk, 2010. Nitrogen fixing microorganisms in microbial-plant systems. Bioregulyatsiya mikrobno-rastitel'nykh system (Bioregulation of Microbial–Plant Systems). Kiev: Nichlava, pp: 99-195.
20. Kizilova, A.K., L.V. Titova, I.K. Kravchenko and G.A. Iutinskaya, 2012. Evaluation of the diversity of nitrogen-fixing bacteria in soybean rhizosphere by *nifH* gene analysis. Microbiology, 81(5): 621-625.

21. Zarei, I., E.M. Khah, G. Mohammadi and S. Petropoulos, 2011. Assessment of growth and yield components following the application of different biological fertilizers on soybean (*Glycine max L.*) cultivation. Australian Journal of Crop Science, 5(13): 1776-1782.
22. Kennedy, A.C., 1999. Bacterial diversity in agroecosystems. Agriculture, Ecosystems, Environment, 74: 65-76.
23. Ceja-Navarro, J.A., F.N. Rivera-Orduña, L. Patiño-Zúñiga, A. Vila-Sanjurjo, J. Crossa, B. Govaerts and L. Dendooven, 2010. Phylogenetic and multivariate analyses to determine the effects of different tillage and residue management practices on soil bacterial communities. Applied and Environmental Microbiology, 76: 3685-3691.
24. Khan, M., A. Zaidi and P. Wani, 2006. Role of phosphate-solubilizing microorganisms in sustainable agriculture – A review. Agronomy for Sustainable Development, 26: 29-43.
25. Li, H.Y., Y.M. Zhu, Q. Chen, R.L. Conner, X.D. Ding, J. Li *et al*., 2004. Production of transgenic soybean plants with two anti-fungal protein genes via *Agrobacterium* and particle bombardment. Biologia Plantarum, 48: 367-374.