THE BENEFICIAL MICROORGANISMS, SOIL BIOLOGY AND MODERN AGRICULTURE

Sergiu Fendrihan
Research- Development Institute for Plant Protection, Laboratory of Bacteriology
Bucharest, Romania

Research-Development Institute for Plant Protection
Bd. Ion Ionescu de la Brad nr.8, CP 013813,s 1,
Bucharest, ROMANIA
Tel.:004-021-2693231 (32,34)
Fax.: 004-021-2693239
E-mail: ecologos23@yahoo.com

Abstract. The problems imposed by the modern agriculture and sustainable development stimulated extended research in order to identify new technological and biological instruments to improve plant health and crop amounts and uses of less chemicals for fertilization and treatment to control pests and diseases. The use of the beneficial soil bacteria and fungi in order to improve agricultural production is of major importance for near future agriculture. This paper shortly review this problems.
Key words: beneficial microorganisms, arbuscular micorrhiza, phosphate solubilization bacteria

INTRODUCTION

The continuous and growing interest of the general public, farmers in new agricultural system and the production of so called bio-food poses challenging questions and problems to agronomic science in order to obtain eco-friendly products, free of pesticides and chemical fertilizations residues. According to Foster (1985), the agricultural soil is divided in rhizoplane, at the roots surface, rhizosphere, close to the roots, and the bulk soil. Some bacteria and fungi are able to penetrate in plants and live as endophytes, without having practically a relation of symbiosis or parasitism, their position being not entirely clarified.

It is evident that in the production process some elements used by plants are removed from soil and from agro ecosystem by crops, and there are a lack of some elements like N, K and P. The farmers must replace them with chemical inorganic or/and organic fertilizers containing the lacking elements. In the same time, the increase of the salt content of the soil is another challenge that must be resolved.

Soil biology system and microorganisms

The soil formation is a process of deterioration of the surface lithosphere due to their weathering and it is considered a real interface between lithosphere, atmosphere and biosphere, making a substrate for different ecosystems including the basis for agriculture. The soils of different categories provide shelter and nutrients not only for plants but for different other organisms and microorganisms entering in complex relations. The soils depending of it composition contains important communities and amount of microorganisms, fungi, bacteria, archaea, cyanobacteria, algae, protists and so on, some of them having an impact on plants and their biology and development.

One of the first ideas that in the rhizosphere there are specific microbial communities was conceived by Hiltner (1904) like a zone of high microbial activity. Indeed, in soils can be found *Actynomycetes* in the range of 10^6/gram of soil, bacteria -10^8/g soil, algae 10^4, protozoa 10^4, and fungi 10^5/go soil that are representing a huge microorganisms biomass. For example of beneficial soil non-symbiotic nitrogen fixer is *Azospirillum,* a bacteria genus from α-*Proteobacteria* and contains nitrogen fixing bacilli, Gram-negative, aerobes, motile, peritrichous, catalase and oxidase positives, containing Q10 isoprenoid quinones and as main fatty acids, C18:1ω7C and summed feature 3 (C16:1 ω7C and/or C16:1 ω6C) according to Zhou et al. (2009).

From the agricultural point of view, these components of soil microbiota are playing an important role in the plant cultivation systems. For a scheme see the fig. 1. They also interact with arbuscular (AM) fungi from soil (Vasquez et al., 2000) which have too a beneficial effect on plants development. Colonisation experiments with micorrhizae, showed to influence the composition of the microbiota from rhizosphere for example, *Glomus mosseae* increased the esterase activity of the microbial community. Some changes in the microbial communities occur by direct and indirect activity of mycorrhiza. Some of them are tolerant to an increased soil salinity, some of them resisting to over 300 nmol of salts (Usha et Kanimozhi, 2011). In fact, the mycorrhizae can enhance the phosphates dissolution in synergism with bacteria, enhancing the total phosphorus intake by plants from the soil (Roy-Bolduc, Hijri, 2010).

The complex system of soil microbial community interacting with others organisms in the soil and with the plants is presented in fig.1.

PGPB (plant growth promotion bacteria)

Nitrogen fixation. The process of nitrogen fixation from atmosphere is an essential process in the living world, because normally the plants and animals are not able to valorify it directly, but the bacterium does. Some diazotrophic strains are able to do it and to provide nitrogen in the appropriate form to the plants. In the modern agriculture and especially in organic farming this is a challenge to provide natural fertilizers for such cultures. According to the International Food Policy Research Institute, 40% of the soils of farms at the international level, are degraded and this situation can be stop by using the new technologies that are implementing biofertilizers and biopesticides in the frame of the new organic farming for a sustainable agriculture. According to Vessey (2003), a biofertilizer is a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. We can add that the biofertilizer bacteria, can release some growth stimulating factors like gibberellins and IAA, that's well known growth factors for plants. One of the most active are the strains of *Azospirillum* genus, wide spread in soils being isolated from rhizosphere of different wild and cultivated plants.

The rhizosphere constitutes a special habitat for different bacteria, fungi and other microorganisms which enter in complex relationship between them and between this community and plants. Some of them enter in the superficial layers of roots tissue but do not have pathogenic effect on plants. This genus looks like to be ubiquist and can fits to different soil and humid environments, but in special in the rhizosphere of different plants many isolation being reported in the literature as shown above. An isolation of a new strain from forest soil was reported too (Zhou et al., 2009).

Effect of the PGPR bacteria was demonstrated on corn culture parameters as for example stems and seed germination. In the same time, the bacteria promote the plant yield in seeds and six bacterial strains including *P.putida* strain R-168, *P.fluorescens* strain R-93, *P.fluorescens* DSM 50090, *P.putida* DSM291, *A.lipoferum* DSM 1691, *A.brasilense* DSM 1690 were used (Gholami et al., 2009). Okon (1985) showed that the use of *Azospirillum* strains is beneficial for plants by their effect on enhancing root growth, increasing the rate of minerals and water intake. The studies obtained a big number of isolated microorganisms from the medicinal plants rhizosphere as *Ocimum sanctum* L., *Coleus forskholii Briq*, *Catharanthus roseus* (L.) G. Don. and *Aloe vera* which have in their rhizosphere beneficial bacteria genera as *Azospirillum*, *Azotobacter* and *Pseudomonas* (Kathikeyn et al., 2008).

It is now obvious that a main role in the PGP have the auxins secreted by them for example strains which are able to stimulate plants growth and release ethylene inhibitors (Glick et al., 2005) The strains of *Azospirillum* are wide spread in soils being isolated from rhizosphere of different wild and cultivated plants. Some of them have effects as biocontrol agents, for example, the siderophores of *Azospirillum brasilense* showed an antifungal effect against the antracnose, a fungal disease produced by *Colletotrichum acutatum* in strawberries culture (Tortora et al., 2011).

Some strains were isolated from contaminated soils with tars (Lin et al., 2009) or with oil (Young et al., 2008) or even from different extreme environments like sulphide springs (Lavrimenko et al., 2010) or acidic environments (Magalhaes et al., 1983). After Maneswari (2011), PGPR can affect plant growth by different mechanisms providing a very wide class of actions like enhacing nutrient solubilisation, nutrient fixation, repression of soil borne pathogens-releasing siderophores, antibiotics, etc; improve the tolerance to stress produced by abiotic factors like drought, metals, salinity; producing phyto hormones and enhancing plant growth; releasing of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, the enzyme hydrolyze the ACC, a precursor of the growth inhibitory ethylene in plants., the result being the stimulation of root formation (Glick, 2005) one of them being the well known *Pseudomonas fluorescens*. Other effect is the enhancing of production to cereals like wheat and maize, to legumes and fruits for example the strawberries culture the production is greater using bio fertilization with PGPB *Pseudomonas* and *Bacillus* strains, in some experimental variants the number of fruits plant growth and nutrient yield is semnificative higher than in normal culture non inoculated culture (Etsiken et al., 2009). A 20 years studies with PGPB, like strains of *A. brasilense* and *A. lipoferum* showed to different species of cultivated plants an increasing of production yield of about 5-30% (Okon et Labandera-Gonzales, 1994). The studies with this strains are not new, many years of experience showing a real enhancement of the agricultural production of different cultivated plants and many aspect of the relation at the molecular level with the plants,adherence to the plant, releasing of stimulating substances and others were described (Bashan et al., 2004).

Phosphate solubilisation. Phosphate occurs in soil like precipitated form-orthophosphates adsorbed by oxides of Al, Fe and Ca (Awasti et al., 2011) and the bacteria phosphate solubilizers reduce the soil pH by producing acids and secrete exoenzyme acid phosphatase which interfere with organic phosphate, making available for plants the insoluble phosphate forms as soluble form.

Azospirillum lipoferum together with *Bacterium megaterium* was used to enhance the mobility of the N and P and solubilisation of calcium phosphate in wheat culture soils (EL-Komy, 2005). Different strains of *Pseudomonas* and *Bacillus* (*B. megaterium, B. circulans, B. subtilis, B. polymyxa, B. sircalmous*), *Rhizobium* and *Enterobacter* are good solubilizers of phosphates from soils (Khan et al., 2009). Other important genera containing solubilisers of mineral P are *Achromobacter, Aereobacter, Agrobacterium, Bacillus, Burkholderia, Erwinia, Flavobacterium Microccocus, Rhizobium* and *Pseudomonas*.

Many of these strains also exhibit other properties, as production of siderophores, phytohormones and enzymes. The ability of the rhizobacterial isolates to solubilize insoluble phosphate was primarily described by the so called solubilization index (Muleta et al., 2012).

Arbuscular mycorrhiza. The soil fungi which are stimulating plant growth is the so called arbuscular mycorrhiza as *Glomus intraradices* and *Gigaspora margarita* wich are able to stimulate the growth of medicinal plants and its useful content in active substances. As example we have the proved effects of the fungi for *Echinacea purpurea*, the plant biomass are 3 time more developed than the normal untreated plants and the concentration if useful active products are (caffeic acids, pigments terpenoids and other) are growing considerable (Gualandi, 2010).

Inoculation with *Azospirillum*, do not affect anyhow the fungi but inoculation of the maize with *Pseudomonas* and *Tricoderma*, well-know biocontrol agents, does. Colonisation with micorrhizae, showed to influence the composition of the microbiota from rhizosphere-*Glomus mosseae* increased the esterase activity of the microbial community. Some changes in the microbial communities occur by direct and indirect activity of mycorrhiza. Roy-Bolduc et Hijri (2010) showed that the mycorrhiza can improve the nutrient intake of zinc and potassium, enhance water uptake and increase plant resistance to drought, and improve soil structure.

Use of mixed biofertilizants. For some ornamental plants like *Delonix regia*, from tropical area, the treatment of seedlings with bioinoculant containing *Azospirillum* strain alone or in different combination with other strains and with arbuscular mycorrhyza (AM) fungi are beneficial and enhance the growth, the biomass, the nitrogen yield, protein and chlorophyll content (Meenaksidundaram et al., 2011).The same result was obtained for the growth promotion and stimulation of *Capsicum chinense* in Mexico using *A. brasilense* and other PGPB and AM (Constantino et al., 2008). Following those studies, the effect of co-inoculation with PGPB and AM fungi together produces a lower colonization with fungi of the roots than in the case of single AMF application and the effect of plant growth promoting was better when AMF and PGPB was applied separately.

Application of a mixture of bacteria (*Azotobacter chroococcum, Azospirillum lipoferum* and *Bacillus megatherium)* enhances the biomass and essential oil content of *Foeniculum vulgare* in comparation with the chemical fertilized cultures (Mahfouz et Sharah- Eldin, 2007) and some strains of *Burkholderia gladioli* 10216, *Burkholderia gladioli* 10217, *Enterobacter aerogenes* 10208 and *Serratia marcescens* 10238 produced an effect of biomass and glucosides, stevioside (ST) rebaudioside-A at *Stevia rebaudiana* (Mamta et al. 2010). In the same time, the PGPB bacteria, can act like antagonists of plant seed borne fungi pathogens, for example in the case of tomatoes (Mogle et Mane, 2009).

Integrating beneficial microorganisms in modern agricultural and plant protection systems

The new integrated systems of cultivated plants are important for both modern and so called bioagriculture. Regarding in the past, practically the archaic and classical agriculture, was completely bio (but the notion is something very new) until the middle of 19th century, when the first chemicals for fertilizing and pest control were applied. The concept of bioproducts and agriculture is a term that's differing from the old classical agricultural concept because some modern mass production methods are involved in the new concept. There are new methods of growing plants and producing agricultural products lacking residues of chemicals, both fertilizers and pest control products. The tendency is to obtain mass production of healthier food integrating the new and older methods of cultivation, for a high quality and in the same time amount of production in order to cover the necessities of human kind. The new system proposes the reduction of chemicals inputs which are required for the compensation of the important biomass and chemicals output from the system (removed by crop). In this matter the use of biological fertilizers, antagonists and insects can play a main role to set up a bio- or a partially bio-agrosystem. In the laboratory of RDIPP, were performed experiments with granulated formulations of antagonists of pathogenic fungi like *Pythium debarianum* and *Fusarium oxysporum* f *radici lycopersici* with good results (fig. 2).

The specialists in agriculture and in agricultural research must focus on the methods and the means of cultivated plant development in order to obtain a good quality production. In the same time not only food agriculture production is in focus but the production of medicinal, technical, industrial ornamental plants, legumes and fruits that must be organically grown and must benefit from the biofertilizers applications.

Anyway, independent of the national research programs and projects, the private companies developed products based on PGPB, packed in different forms and are available on the markets in special in Asia, where there are a lots of companies producing and selling such biofertilizers. Some of the bioproducts are not only pure *Azospirillum* but they are co-aggregates with other strains like *Pseudomonas*, *Azotobacter, Azorhizobium* or strains from the same genus

(Joe and Sivakummaar, 2010). The co-aggregates of *Azospirillum* and *Azotobacter* showed the most long term survival of bacterial cells in vermiculite.

Some of the bioproducts uses *Azospirillum brasilense* together with *Pseudomonas fluorescens* in flocs (Joe et Sivakumaar, 2010) being effective not only like bio- fertilizer, but acting like biocontrol agent against *Pyricularia oryzae* too. Co-inoculation of tomatoes with *Azospirillum brasilense* Sp 245, and *Bacillus subtilis* strain 101, have no synergistic effects on plant biomass in comparation with single strain application and are registered even some roots alterations (Felici et al., 2008). A new tendency is to use in organic eco-farming system a combination of biopreparates with bacteria and fungi and with biocompost and vermicompost (Densilin et al., 2010). For example, interesting studies regarding the composition and yield in essential oils for some medicinal plants like *Rosmarinus officinalis* was performed showing a favourable essential oils content after application of biofertilizers (Abdelaziz et al., 2009).

Problems of application of microbial biopreparates in organic (bio) production agriculture and not only

1. the potential users (farmers) are not very well informed about the benefits of biofertilizer use in organic farming;
2. some scientists and users are reluctant to this idea. The scientists think about the unequal and less reproducible results of the biofertilization methods, and the need for improvement of the quality, reproducibility of the result and finding new performance strains and more experiments with them;
3. the quality control and problems related with standardisation. The quality of the final products is a real challenge and in the same time is very hard but is necessary to establish a kind of standardization of the products;
4. some safety problems are necessary to clarify in order to contribute to safety of the workers in agriculture, avoiding potential harmful, pathogenic strains;
5. maintenance of the final conditioned products, the shelf life of the products must be enhanced in order to have good results in the fields even after a long time from the production;
6. the legal aspects of accreditation of the production, of complying with laws and regulations are necessary for further developments;
7. gaps between research and technological transfer; it took a very long time for examination of the patent and releasing patents and transfers it to some interested companies.

Development of the market of biopreparates containing beneficial microorganisms

According to the studies of Danish Technological Institute presented by Anne-Belinda Bjerre (2012) the needs for fertilizers in the world and in the EU are increasing for fertilizers, and in specially NPK. Providing the elements, can be done by

-inorganic fertilizers potash, phosphate rock, urea, ammonium and so on
-organic fertilizers compost, peat moss, manure, wood ash, guano
-biofertilizers containing selected bacterial strain
-single strains
-multiple strains

The author showed that the necessity of fertilizers is increasing following increasing of agricultural products for food and green energy production.

In the same time, organic agriculture rules and good practice requires the interdiction of inorganic fertilizers and other chemicals for plant protection. She evaluates the necessities for the bio-fertilizers in some EU and Denmark. For example, the turnover of biofertilizer consumptions can be only for Germany to the 49,2 mil euro for conventional farming and about 65 mil euro for organic farming as potential markets level of sales and there is still not market there for this kind of products.

The situation is different in Asia being a dynamic market and sales for biofertilizers where the Indian companies are selling many biofertilizers products (Examples: Green Aegies Agro Pvt. Ltd., Pune, Shree Biocare India (*Rhizobium, Azotobacter, Azospirillum* and Phosphate solubilizing microorganisms-PSM), Biomate India Pvt Ltd, New Delhi (www.indianmart.com). Currently, there are about 114 producers of organic inputs and bio-fertilizers with an installed capacity of 18,500 tones. According to estimates of the National Bio-fertilizer Development Center (NBDC) and the Bio-Tech Consortium of India Ltd (BCIL) about 344,800-507,032 tones of bio-fertilizers are required for Indian agriculture. In reality, however, their use is limited (citation from Dewasthale et Bondre 2008). The commercial production and trade is unbalaced the most are produced by little companies in especially in Asia and in India there are a lot of companies like this. Important companies in Occident like Novozym, Bioworks they are producing and selling in Europe and America, other were absorbed by bigger companies like Monsanto (Constantinescu and Sicuia, 2013). The total amount of business in this area was, for the beginning of the 21 century, of about 10-20 millions dollars/year that's means still a low level comparing with chemical fertilizers companies.

What could be of much help to develop the market will be the new Common agricultural policy containing recommendations on soil preservation and good agricultural conditions (Bjerre, 2010), the requirements and encouraging the organic agriculture according to Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91 (Bjerre, 2010) and of course the cooperation between farmers, scientists, officials, and policy makers in agriculture, financial help for the farmers for biofertilizer use, start up and government help project for IMM producers. In the same time we must note that it is a tendency to take care for biodiversity of soils (European Commission-DG ENV Soil biodiversity: functions, threats and tools for policy makers) in the EU.

An important topic is the improvement of shelf life of the products. Some thinks that the liquid form is better for applications than solid granules. The improvement of the shelf life and survival of *Azospirillum brasilense* was tested using different protectant substances like trehalose, glycerol polyvinyl pirrolidone, gum arabica and so on (Kumaresan and Reeta, 2011), the trehalose and glycerol being the best protectant, the survival after 11 month of storage being the best, 108 cell/ml .The others like polyvinyl alcool, PEG supported the same amount of bacteria in less time extent.

CONCLUSIONS

The organic agriculture products are a growing market, and the organic farming is strictly regalemented and requires the use of biofertilizers, biopesticides and other non synthetic chemicals, and a very strict content of chemical residues at the laboratory analysis in order to pass the accreditation of the organic farm and production. That's why in the last decades, there are an increase demand for biopesticides and biofertilizers. Unfortunately, due to the legislation and formalities, the validation and market release of the bioproducts are very expensive and time consuming in the EU countries. The Asian market is very developed and dynamic, but in the same time there are doubt of efficiency and long time maintenance of the biofertilizers containing beneficial microorganisms.

The main biofertilizers are bacteria and fungi from the plant photosphere, rhizoplane and even endophytes, having complex interaction in the microbiota communities and with plants and surrounding soil microbiota. There are still many unknown and unclear problems regarding those relations due to the fact that are a multifactorial problem.

The release of new products containing microorganisms, require the establishment of some standards of strain selection, production and maintenance, increasing of the shelf life.

Scientifically speaking, there are many problem to solve, the biochemical and biological relation with plants and with other bacterial or fungal strains, the real effect of enhancement of agricultural production and useful substances and nutrient plant content, some aspects of resistance to stress factors, and the criteria of products standards. There are many aspects of biofertilizers biology, fate in the environment and their application methods improvement to be clarified by new scientific research projects.

ACKNOWLEDGMENTS

Many thanks to colleagues Florica Constantinescu, Oana Sicuia, Dinu Sorina, Dinu Luminita for providing materials and for supporting the researches and to Ana-Maria Andrei for advice and support.

REFERENCES

1. Abdelaziz M., Pokluda R., Abdelawahab M. (2007) Influence of compost, microorganisms and NPK fertilizers upon growth, chemical composition and essential oil production of *Rosmarinus officinalis Not. Bot. Hort. Agrobot. Cluj*, 35, (1) Print ISSN 0255-965X; Electronic ISSN 1842-4309
2. Awasti H, Tewari R., Nagyyar H. (2011) Synergy between Plants and P-Solubilizing Microbes in soils: Effects on Growth and Physiology of Crops *International Research Journal of Microbiology* (IRJM) (IS SN: 2141-5463) Vol. 2(12) pp. 484-503
3. Bashan Y, Holgun G, De-Bashan LE. (2004). *Azospirillum*-plant relationships,physiological, molecular, agricultural, and environmental advances (1997–2003). *Can J Microbiol* 50, 521–577
4. Bjerre A-B, (2012) Theme 1: European Biofertilizer Policy and Potential Market, Danish Technological Institute, presentation March 2012, Putraya, Malaysia
5. Constantino M., Gomez-Alvarez R., Alvarez-Solis J. D., Geissen V., Huerta E., Barba E. (2008) Effect of Inoculation with Rhizobacteria and Arbuscular Mycorrhizal Fungi on Growth and Yield of *Capsicum chinense* Jacquin *J. of Agric. Rural Develop. Trop. Sbtrop*. 109 (2):169–180.
6. Constantonescu F, Sicuia O. (2013)) Combaterea biologica a bolilor plantelor cultivate
7. Dimpka C.O. Zeng J., McLean J.E., Britt D.W., Zhan J., Adersson AJ. (2012) Production of Indole-3-Acetic Acid via the Indole-3-Acetamide Pathway in the Plant-Beneficial Bacterium *Pseudomonas chlororaphis* O6 Is Inhibited by ZnO Nanoparticles but Enhanced by CuO Nanoparticles Appl Environ Microbiol.; 78(5): 1404–1410.
8. Desilin DM, Srinivasans, Manju P, Sudha S (2011) Effect of Individual and Combined Application of Biofertilizers, Inorganic Fertilizer and Vermicompost on the Biochemical Constituents of Chilli (Ns - 1701). *J Biofertil Biopest.* 2:106.
9. Esitkem A, Yildiz HE., Erclisi S. , Fiden Donmez M., Turan M, Gunes A (2010) Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry *Scientia Horticulturae* 124 62–66.
10. El-Komy H.M.A.: (2005) Coimmobilization of *A. lipoferum* and *B. megaterium* for Plant Nutrition *Food Technol. Biotechnol.* 43(1) 19–27
11. Foster R.C. (1986) The Ultrastructure of the Rhizoplane and Rhizosphere *Ann. Rev. Phytopathol* 24: 211-234
12. Felici C, Vettori L, Toffani A., Nuti M. (2008) Development of strain-speci¢ genomic marker fo rmonitoring a Bacillus subtilis biocontrol strain in the rhizosphere of tomato *FEMS Microbiol Ecol* 65 289–298.
13. Gholami A., Shahsavani S., Nezarat S. (2009) The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *Intl J. Biol. Life Sci*., 5:35-40.
14. Gualandi R.J., Gwinn K.D., Owley B. H., Auge R.M. (2009) The role of fungal endophytes in the production of natural products in *Echinacea purpurea Phytopathology* 100:S44.
15. Hiltner L. (1904) Ueber neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie und unter besonderer Berucksichtigung der Grundungung und Brache. *Arb. Deut. Landw. Gesell*, 98:59-78.
16. Joe M. M., Sivakumaar P. K. (2009) Long term survivability of *Azospirillum* co-aggregates: Bioinoculation effect on the growth and yield of sunflower *Agricultura* 6: 71-77 (2009)
17. Khan G., Jilani L, Aktar M. S.,. Naqvi S. M. S, Rasheed M. (2009) Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production Agriculture University Rawalpindi, Pakistan *J. agric. biol. sci*. 1 (1):48-58

18. Karthikeyan B, C. Jaleel A., Lakshmanan G.M.A. Deiveekasundaram M. (2008) Studies on rhizosphere microbial diversity of some commercially important medicinal plants *Colloids and Surfaces B: Biointerfaces* 62 (1) 15 :143–145.
19. Kumaresan G., Reeta D. (2011) Survival of Azospirillum brasilense in liquid formulation amended with different chemical additives. J Phytol. 3(10):48-51.
20. Lavrimenko K., Chemousova E., Gridneva E., Dubinina G., Akimov V., Kuever J., Lysenko A., Grabiovich M.: *Azospirillum thiophilum* sp. nov., a diazotrophic bacterium isolated from a sulfide spring. *Int*. *J*. *Syst*. *Evol*. *Microbiol*., 2010, 60, 2832-2837.
21. Lin S.Y., Young C.C., Hupfer H., Siering C., Arun A.B., Chen W.M., Lai W.A., Shen F.T., Rekha P.D., Yassin A.F.:(2009) *Azospirillum picis* sp. nov., isolated from discarded tar. *Int*. *J*. *Syst*. *Evol*. *Microbiol*., 2009, **59**, 761-765.
22. Magalhaes F.M., Baldani J.I., Souto S.M., Kuykendall J.R., Dobereiner J.: (1983) A new acid-tolerant *Azospirillum* species. *An*. *Acad*. *Brasil Ciênc*., 1983, **55**, 417-430
23. Meenakshisundaram M., Santhaguru K., Rajenderan K. (2011) Effects of Bioinoculants on Quality Seedlings Production of *Delonix Regia* in Tropical Nursery Conditions *Asian J. Biochem. Pharm. Res.* 1 (1) :98-107.
24. Mahfouz, S.A., Sharaf-Eldin M.A., (2007) Effect of mineral vs. biofertilizer on growth, yield and essential oil content of fennel (*Foeniculum vulgare* Mill.). Int. Agrophysics, 21: 361-366.
25. Maneswari DK. (2011) Bacteria in Agrobiology Crop ecosystems Springer Verlag Berlin Heildelberg 2011. (Aeron a, Kumar S.,Pandey P.)
26. Mamta R.P.,Pathania,V.,Gulat ,A.,Singh B. Bhanwra R.K., Tewari, R.(2010) Stimulatory effect of phosphate-solubilizing bacteria plant growth, stevio-side and rebaudioside- contents of *Stevia rebaudiana* Berton *Appl.Soil Ecol*. 46, 222–229.
27. Mogle U.P, Maner R Y (2010) Antagonistic effect of Bio-fertilizers against Seed Born Mycoflora of Tomato (*Lycopersicun esculentum*) *Res. J. Agric. Sci.* 1(3): 255-258
28. Okon, J. (1985). *Azospirillum* as a potential inoculant for agriculture. *Trends Biotechnol*. 3: 223-228
29. Roy Bolduc A, Hijri M (2011) The Use of Mycorrhizae to Enhance Phosphorus Uptake: A Way Out the Phosphorus Crisis. *J Biofertil Biopest*. 2:104.
30. Okon Y., Labandera-Gonzales C.A. (1994) Agronomic applications of *Azospirillum*: An evaluation of 20 years worldwide field inoculation Soil Biol. Biochemi. 26 (12):1591–1601.
31. Tortora ML, Diaz-Ricci JC, Pedraza RO (2011) *Azospirillum brasilense* siderophores with antifungal activity against Colletotrichum acutatum. *Arch Microbiol.* 193(4):275-286
32. Usha DK, Kanimozhi K (2011) Isolation and characterization of saline tolerant *Azospirillum* strains from paddy field of Thanjavur district *Adv. Appl. Sci. Res*., 2 (3): 239-245
33. Vasquez, E AL. (2000) Interaction between arbusclar mycorrhizal fungi and plants *Appl. Soil Ecol*. 15, 261-272.
34. Vessey, J.K. (2003) Plant growth promoting rhizobacteria as bio-fertilizers. *Plant Soil* 255, 571-586
35. Weller DM,.(2007) *Pseudomonas* biocontrol agents of soilborne pathogens: Looking back over 30 years. *Phytopathology* 97:250-256.
36. Zhou Y, Wei W, Wang X, Xu L, Lai R (2009) *Azospirillum palatum* sp. nov., isolated from Forest soil in Zhejiang province, China. J Gen Appl Microbiol 55:1–7
37. Directive 2001/36/EC relating to plant protection products based on micro-organisms;
38. Regulation 852/2004 on food hygiene
39. Regulation no. 834/2007 on organic production, Directive 91/414 concerning plant protection products.
40. Directive SEC (2006) 914 on the sustainable use of pesticides.
41. European Commission - DG ENV Soil biodiversity: functions, threats and tools for policy makers.

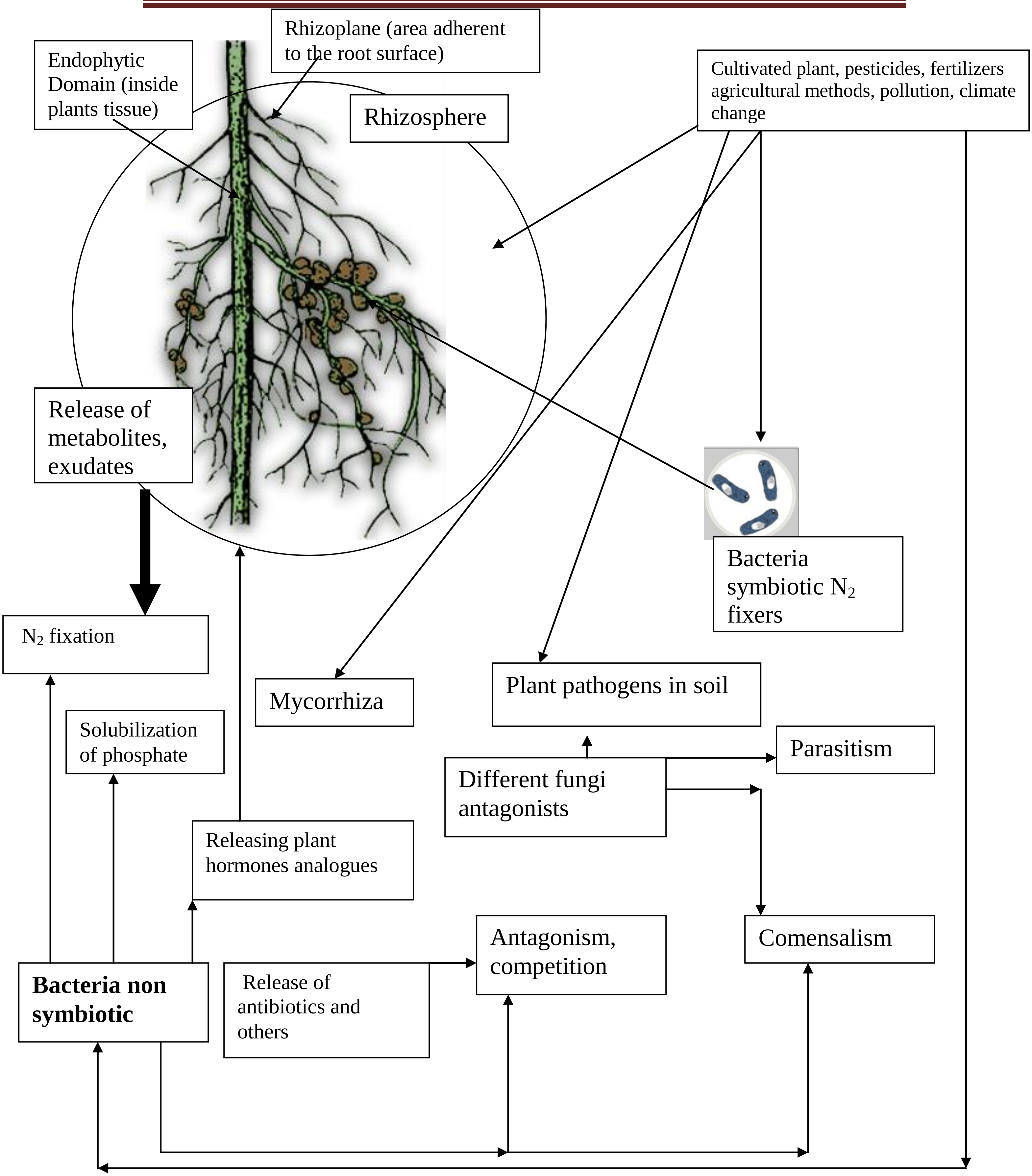

Fig. 1 Scheme of relation inside microbiota and between microbiota and plants

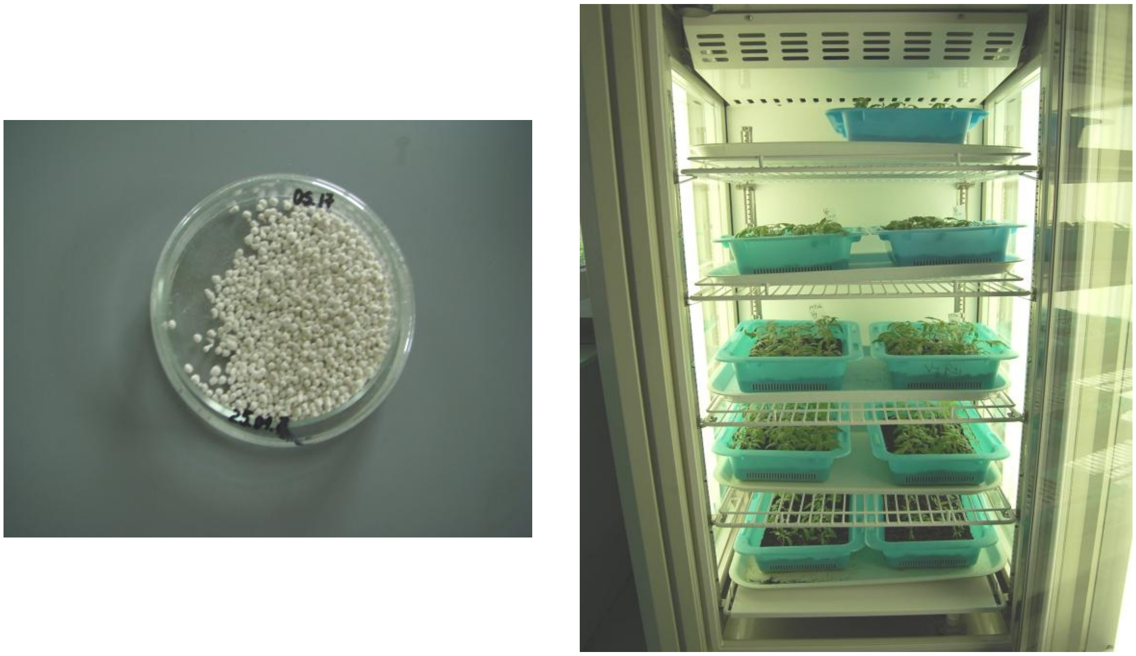

Fig. 2 Test in laboratory are done with granulated biofertilizers containing bacteria in the range of 10^8 (left photo, S. Fendrihan); an experiment with tomatoes in laboratory controlled conditions (right photo, Sorina Dinu).