AZOSPIRILLUM STRAINS AS BIOFERTILIZERS AND BIOCONTROL AGENTS -A PRACTICAL REVIEW

Sergiu Fendrihan[1]
1Res Institute for Plant Protection-Ion Ionescu de la Brad nr 8, Bucharest , Romania
Florica Constantinescu[1],
1Res Institute for Plant Protection-Ion Ionescu de la Brad nr 8, Bucharest , Romania
Oana Sicuia[1]
1Res Institute for Plant Protection-Ion Ionescu de la Brad nr 8, Bucharest , Romania
Sorina Dinu[1],
1Res Institute for Plant Protection-Ion Ionescu de la Brad nr 8, Bucharest , Romania

ABSTRACT

Azospirillum biofertilizers have a history going back to the beginning of the XXth century. However, researches still have to find new and competitive strains and improve biofertilizers production and application techniques. They are one of te most used biofertilizers in organic farming. The present paper review and analyses the development of the new agriculture using such microorganism, and showing not only the biofertilization effect but some biocontrol properties of the presented strains. The products containing *Azospirillum* strains and their use begin to play an important role in modern agriculture and in special in some countries in course of development as an alternative to chemical expensive and some time not environmental friendly products. Countries from Asia, South America, use it in huge quantities. In developed area of the lobe, there are only some tiny initiative, mainly because the multinational companies, and because of the legislation in use.

Indexing terms/Keywords

Azospirillum, biofertilizer, biocontrol, nitrogen fixers microorganisms, inoculation in soil

Academic Discipline And Sub-Disciplines

Agriculture, eco agriculture; fertilization, plant protection

SUBJECT CLASSIFICATION

E.g., Mathematics Subject Classification; Library of Congress Classification

TYPE (METHOD/APPROACH)

Literature analysis, review of state of the art

1.INTRODUCTION

The need for nitrogen, phosphorus, .and other minerals are essential for plants growth. Legumes has, a symbiotic relation with nitrogen fixing bacteria from their roots. There are some diazotrophic non symbiotic strains are able use the atmospheric nitrogen which is use by the plants. In the modern agriculture and especially in organic farming this is the proper way to provide natural fertilizers for the cultivated plants. According to the International Food Policy Research Institute, 40% of the soils of farms, at international level, are degraded and this situation can be stopped by using the new technologies that are implementing biofertilizers, bioinoculants and biopesticides in the frame of the organic and integrated farming for a sustainable agriculture. According to Vessey (2003), the “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 plan”.

One of the first ideas that in the soil are present specific microbial communities was conceived by Hiltner (1904), which introduced the term of “rhizosphere” as the soil zone with high microbial activity. Indeed, in soils can be found *Actynomycetes* in the range of 10^4 -10^7 colony forming units (cfu) per gram of soil, bacteria in 10^8 cfu/g, algae in 10^3 -10^5 cells/g, protozoa 10^4, and fungi 10^5 spores or cells/g of soil, which are representing a huge microorganisms biomass. Inoculation with PGPB can lead to some minor changes in the local microbial community (Trabelsi and Mhadi, 2013).

There are many reports of *Azospirillum* isolation from both rhizosphere and roots, as endophyte in different non-leguminous plants (Franche et al., 2008). *Azospirillum* species were isolated from sugar cane roots and ryegrass (Gangwar and Kaur, 2009), from corn rhizosphere (Mehnaz et al., 2007), from wheat, barley and oats rhizosphere and inner tissues of roots (Venieraki et al., 2008).

1.1.Taxonomy

Azospirillum is a bacteria genus from α-Proteobacteria, Ord. Rhodospirillales, fam. Rhodospirillaceae, which contains free athmosphere nitrogen-fixing bacilli, Gram-negatives, aerobes, motiles, peritrichous, catalase and oxidase positives, containing Q-10 isoprenoid quinones as chemotaxonomic markers and $C_{18:1}\omega7C$ as predominant fatty acid according to Zhou et al. (2009). The Azospirillum bacteria are able to use various carbon sources, such as glucose,

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lactate, succinate, fructose, malate, pyruvate, and fumarate, reduces nitrate without requiring biotin. A table of species of this genus and some main characteristics, is presented in this paper (Table 1). The Azospirillum genus includes 19 species (table 1), six of them being identified in the last years, as novel species: Azospirillum agricola (Lin et al., 2016), A. fermentarium (Lin et al., 2013), A. formosense (Lin et al., 2012), A. humicireducens (Zhou et al., 2013), A. soli (Lin et al., 2015), and A. thiophilum (Lavrinenko et al., 2010). During time, diferents species of Azospirillum were reallocated taxonomically, Azospirillum fermenti becomes Niveispirillum fermenti, Azospirillum irakiense as Niveispirillum irakiense and Azospirillum amazonense (1983) as Nitrospirillum amazonense (Lin et al, 2014).

1.2. Genetics

The *Azospirillum* genome size varies from 4.8 Mbp to 9.7 Mpb, depending on the species (Caballero-Mellado et al., 1999; Martin-Didonet et al., 2000). The complete genome of an *Azospirillum* strain, the first to be sequenced, was reported to have 7.6Mbp (Wisniewski-Dyé et al., 2012). The *Azospirillum* sp. B510 complete genome consist in a single circular chromosome of 3 311 395 bp and 6 plasmids: pAB510a, pAB510b, pAB510c, pAB510d, pAB510e and pAB510f (Kaneko et al., 2010). Research in these aspects are in real progress, and other strains o different *Azospirillum* species have their complete genome sequenced, like *A. lipoferum* 4B, *A. brasilense* Sp245, CBG497 and Az39, and soon to be completely sequenced, such as *A. amazonense* Y2 (Cecagno et al., 2015). For the moment, no other complete genome sequence is available for *Azospirillum*, but different information on the genome are available in the scientific articles, on GenBank or other internet data bases.

1.3.Ecology

The plant microbe interaction and relations between microorganisms in soil are considerably important for the agroecosystem. Their communication is mediated by signaling molecules. The agroecosystem microbiome influence plants behavior and contribute to ecologically shaping of the ecosystem (Rout and Southworht, 2013). The strains of *Azospirillum* are wide spread in soils however their distribution is less studied as it could have be. Still, it was observed that is not very frequent in sandy soils (Pereira e Silva et al., 2013). *Azospirillum* was isolated from rhizosphere of different wild and cultivated plants. There was also reported in forest soil (Zhou et al., 2009). 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., 1993). A salt tolerant *A. lipoferum* strain was isolated from a soda lake (Lonar), located in Buldhana, India (Hingole and Pathak, 2013). Therefore, *Azospirillum* seems to be a versatile bacterial genus that can fit to different soil and humid environments, especially in plants rhizosphere. The amount of differents salts in soil (Na Mg, Ca) in high amounts reduce Azospirillum number and increase the time required for a culture to develop (Suhail and Mahdi 2011). In some stress conditions produced by high concentration of Zinc, these bacteria become immobile and make a kind of cyst (Gowri and Srivastava 1996). The periods of dryness reduce the concentration of of IAA, GBA, trans zeatin ribozide production, but good production of abscisic acid by the bacterial strains from the cultivations exposed to drought stress (Ilyas, 2012). The negative impact of drought conditions seemed to be diminished when *A.brasiliense* is applied as agro-inoculants to wheat culture. Wheat seedlings, inoculated with *Azospirillum* strains, benefit of an improved water status during salt and osmotic stresses, due to wider xylem vessels that are induced by the bacteria, which also might enhanced coleoptile water conductance (Pereyra et al., 2012). Similar aspects were also noticed in tomato plants, when *Azospirillum brasilense* inoculation increased xylem vessel area and stem hydraulic conductivity, and thus mitigated water stress imposed by a vascular disease (Romero et al., 2014). Pesticides, like monocrotophos, chlorpyrifos and others can be tolerated at low concentrations, but higher concentrations determine antagonistic effects for *Azospirillum* strains and decrease the ammonification process (Srinivasulu et al., 2012). The activity of stimulation of plant growth, is explained by Bashan et Bashan (2010) by a multifactorial process (multiple mechanism, which is presented like a hypothesis). This can be the truth, taking into account the multiple parameters necessary for plants growth and development. Its include phytohormones production, nitrogen fixation, environmental stress reduction, possible influence against phytopathogens. The strains of *Azospirillum* showed their dispersal not only in rhizosphere of mother-plants, but in stolons and new daughter-plants of strawberry(Guerrero-Molina et al, 2011), assuring their colonization.

1.4. Biofertilization

1.4.1. General plant growth stimulation

The concept of Effective Microorganisms was developed by prof. Higa from Japan and consists in the isolation and inoculation of beneficial microorganisms alone or in combinations in order to enhance productivity in agriculture (Higa and Parr, 1994). The same authors developed the idea of microorganisms control in soils.A literature survey conducted by Veresoglou et Menexes (2010), shown the beneficial effects of inoculants with the strains *A brasilense* and *A lipoferum* on the wheat production, with an increase yield of seeds of 8,9%, the *A lipoferum* having a greater effect than *A brasilense*. The main plants growth stimulation activity is the athmospheric nitrogen fixation provinding one of the main nutrients for plants. This strain *Azospirillum* became a real model of plant growth stimulating bacteria (Cassan et al 2014). There are many scientific reports on wheat growth stimulation by *Azospirillum* inoculation. For example a study showed that not only the phytohormones interfere with plant stimulations, but the lipopolisaccharides of membrane has the role of adhesion and in the same time stimulates in the roots cells an active cells division in meristemes and stimulate plants response to bacterial cells (Evseeva et al., 2011). A survey of literature of about 27 years, showed, after a careful data statistical meta-analyses, a production of seed of 9 % more dry mass and 18 % more than non-inoculated cultures (Veresoglu et al, 2012). Important information is that *Azospirillum* strains are able to solubilize phosphate, giving to plants the possibility to use it as an important nutrient (Tahir et al., 2013). The authors find in the rhizosphere of wheat, strains of *Azospirillum, Bacillus*, and *Enterobacter*, some of them having phosphate solubility abilities, and increasing the grain yield. Other aspect is the phosphates solubilisation effect of the *Azospirillum* strains, together with some *Bacillus* and *Enterobacter* strains in the wheat culture (Tahir et al, 2013). The absorption of N, P K elements, with *Azospirillum sp.*, manure and mycorrhiza application in combination, is significant improved (Ardakani et al, 2011), even in condition of low water availability. The combination between *Glomus* and *Azospirillum*, helps the cultivated sugar cane, improving nitrogen availability and absorbtion in plants (Bellone et al, 2012), increasing plants biomass. The combination of *Azospirillum lipoferum*, *Pseudomonas putida* and *Azotobacter chroococcum*, improved growth of sunflower (*Helianthus anuus*) with some manure, considered the best combination in order to not use so much chemical fertilizers (Mehran et al, 2011). A combination of *Azospirillum* and *Bradyrhizobium*, improved the growth of soya plants (Marks et al, 2013) due to their metabolites.

14.2. Nitrogen fixation

The nitrogen fixing bacteria are acting generally like a consortium that can be retrieve like symbiotic strains or non symbiotic associate with leguminous and non leguminous plants (Franke et Lindstrom 2008). The non-symbiontic forms consortiums in the rhizosphere invoving bacteria and even cyanobacteria. Nitrogen fixation ability of some of soil microbial community are very appreciated, being the main benefit offered by *Azospirillum* strains. Thats why bioprospecting was developped in this field of activity. Some new methods in computing bioinformatics and screening by metagenomics of those community are now used (Wang et al, 2013) like both which allow to determinate in a large number of sample the *nif* genes, and correlate with ecological environmental conditions. In facts, this that is the main characteristic and use of such strains. The main fertilization now a day is still the use of chemical fertilizers which can pollute the soils and contaminate the production. The cultures named bio and eco, are supposed to not use any chemicals in its agrotechnical scheme

1.4.3. Phytohormones production

Azospirillum can synthesize a broad spectrum of plant hormones and can also interfere in different plant metabolic pathways involved with phytohormone synthesis. The main phytohormones mediated by *Azospirillum* are the indole 3-acetic acid (IAA) (Malhotra and Srivastal 2008) as auxin hormone, gibberellic acid (GA3) as gibberellin hormone, zeatin (Z) as cytokinin hormone (Perrig et al, 2007), all three with a stimulating role in plant growth and development. This bacterium is also interfering in ethylene (ETH) and abscisic acid (ABA) synthesis (Cohn et al 2009), these two acting as aging hormone and dormancy hormone, respectively. The phytohormones production is not directly induced by the microorganisms, and plants are not thoroughly dependent on microbial phytohormone production. There is also a complex interaction between soil microorganisms prior to the hormone synthesis, for example *Pseudomonas fluorescens* releases a secondary metabolite 2,4-diacetylphloroglucinol (DAPG) which acts as signal molecules for the *Azospirillum,* inducing the expression of plant-beneficial genes related with the synthesis of plant growth promoting substances (Combes-Meynet et al., 2011). The content of micronutrients in soil, like Zn, Mo, Fe, can influence the production of IAA, gibberellins and exo-polysaccharides by the *Azospirillum* strains (Ganapathy et Salvagi, 2006). Plant-bacteria interactions also influence the phytohormones synthesis. When strawberries plants were treated with *A. brasilense* REC3, RLC1, PEC5 strains, fruit production was enhanced. The process was initiated by the plant roots whose exudates promote the positive chemotactics in *Azospirillum* and than bacteria release the phytohormone-like substances which contribute to plant growth promotion and development (Pedraza et al., 2010; Meddipour et al., 2012; Drogue et al., 2013). *A. brasilense* inoculum was also used in plant tissue cultures for micropropagation, where it enhanced *in vitro* rooting of shoots, with 21 days before indole-3-butyric acid (IBA) pulse treatment (Larraburu et Llorente, 2015). Other experiments shown the importance of nitric oxide produced by the strains of *Azospirillum* in the presence of IAA, on branching and formation of the architectures of the roots systems of tomato (Molina –Favero et al., 2008).

1.4.4. Lectins production

The epiphytic and endophytic *A. brasilense* strains are lectin producers. These compounds are able to influence the activity of acidic (pH 3.5), neutral (pH 6.8), and alkaline (pH 7.8) proteinases. They alter the ratio between different protease activities in germinating seeds (Alenkina et Nikitina, 2015). A lectin binding activity was discovered to outer membrane of *Azospirillum brasilense* and is recognized by EPS produced by the aggregated cells (Mora et al., 2008) and can play a role in aggregation of cells.

1.4.5. Phosphates solubilization

Another important information, is that *Azospirillum* strains are able to solubilise phosphate, giving to plants the possibility to use it as an important nutrient (Tahir et al., 2013). The authors found in the rhizosphere of wheat strain of *Azospirillum, Bacillus* and *Enterobacter*, some of them having phosphate solubilization abilities, and increasing the grain yield. Other aspect, is the phosphates solubilization effect of the *Azospirillum* strains, together with some *Bacillus* and *Enterobacter* strains in the wheat culture (Tahir et al, 2013). The absorbtion of N, P K elements, with *Azospirillum sp.*, manure and mycorrhiza application in combination, is significantly improved (Ardakani et al, 2011), even in condition of low water availability.

1.5.Polymers production

Some *Azospirillum* strain are able to produce different polymers like PHB (Kamenev et al., 2012), like glycol-polymers (Kanevska et al, 2010) indirectly biostimulation by ensuring the adherence of bacteria and to its protection.

1.6. Biocontrol with *Azospirillum* strains

The strains from *Azospirillum* genera are not classical agents for bio-control, but some of them can help by their secretion to control some pathogens. For example, the siderophores of *Azospirillum brasilense* showed an antifungal effect against the anthracnose, a fungal disease produced by *Colletotrichum acutatum* in strawberries culture (Tortora et al. 2011) inducing a systemic resistance in plants to phytopathogens. The *Azospirillum* strains inoculated in maize can induce resistance of the plants to attack of *Diabrotica* (Santos et al, 2014), due to the induction of production of sesquiterpenes by plants tissues, which is not agreed by *Diabrotica* larvae. The general systemic resistance to abiotic factors stress is induced too (Yang et al, 2009). Some of the bioproducts use *Azospirillum brasilense* together with *Pseudomonas fluorescens* in flocs (Joe et Sivakumaar, 2010) being effective not only like bio- fertilizer, but like biocontrol agent against *Pyricularia oryzae* too. Another example is the antagonistic effect of *Azospirillum brasilense* s245 against *Rhizoctonia solani* (Vettori et al. 2010).

1.7. Bioremediation

The strains *Azospirillum* and *Pseudomonas stuzeri* were used in bioremediation of oils and hydrocarbs polluted soils and the authors describe a possible system (Galazka et Galazka, 2015). The same, some scientists proved that the complex of *Azospirillum* and microalga *Chlorella vulgaris* co-immobilized in alginate beds, can contribute to better removal form wastewaters the ammonium and the soluble phosphates ions than only the algal cells alone (de Bashan et al, 2002). Another bioremediation is the use of different plants to fix and to remediate mining wastes. In this environments, *Azospirillum* use to enhance growth of quail bush which are essential for shaping the landscape and the environment, including influenced the microbiota from soil. The plants resist better to saline conditions in soil. For example, experiments with seeds of *Lactuca sativa* L., treated with *Azospirillum* suspensions showed an increased resistance a better germination rate and better growth, even exposed to 80 Mol/mc NaCl (Barasi et al, 2006). The use of a mixture of nitrogen fixers, fungi and other microorganisms improve the recultivation with *Medicago* of heavy contaminated soils with Zn and Pb (Ogar et al. 2015). The relative density of the *Azospirillum* strains in agricultural and heavy metals contaminated soils were similar, but the strains from contaminated soils were tolerant strains to heavy metal content and in the same time to salt content (Moreira et al., 2008).

2. APPLICATION OF *AZOSPIRILLUM* CONTAINING PRODUCTS

2.1. Conditioning of the *Azospirillum* strain products

The conditioning of the bacterial strains for commercial production, have several question.

1.One is the problem of compatibility between the strains used in the same formulation as for example Azotobacter and Azospirillum and of course with the AM fungi;

2.Another problem is the compatibility with the conditioning support;

3.Their viability under different storage conditions;

4. Application technology that's fits with their plant stimulation activity;

5. Very important thing is the set up and establishment of some quality standard for the commercial products.

From the point of view of the development, some undeveloped country or in course of development are in the first line of the research and production of such bio-fertilizers due to their lower income and weak industrial development, they focus on the cheaper alternatives to fertilizers and pesticides. Bangladesh scientists consider them a good solution for fertilisation of their cultures (Hossain et Jahan, 2015). We have practically no sound information about the quality of those products. In the top is India which looks like to develop local SME that are producers of biofertilizers in cheap production facilities and with local distribution. In the same time patents of biofertilizers and their application were registered to different world wide patent offices. In this very moment there are a lots of biofertilizers of different origin for sale in shop or internet. Some of the bioproducts are not only pure Azospirillum but they are co-aggregates with other strains like Pseudomonas (Joe and Sivakumaar, 2010), Azotobacter, Azorhizobium or strains from the same genus (Joe and Sivakumaar, 2010). The co-aggregates of Azospirillum and Azotobacter showed the most long term survival of bacterial cells in vermiculite. Two culture media for mass production were proposed by Bashan and coauthors (2011). Singh and co authors (2011) proposed a medium from glutamate production process wastewaters. Azospirillum brasilense and Pantotea dispersa immobilized in clay and olive wastes were used for inoculation in field of Cistus albidus in arid soil, for bioremediation and enhancement of plant growth (Schoebitz et al, 2014). An older paper, propose the immobilization of the cells in poly sodium acrilate (Wang et al, 2004). The Azospirillum strains were used in differents formulations alone or together with different substances.Another proposal is to use as carrier is the composted sawdust with a Bradyrhizobium/Rhizobium/Azospirillum mixture that enhance production of soybean for example, with 34-62% (Kostov et Lynch, 1998). Liquid bioinoculants formulations with trehalose (10mM) were proposed for a long term survival of Azospirillum (Kumaresan et Reeta, 2011).

2.2. Use of *Azospirillum* strains in agriculture

There are many reasons to use the microbial products in agriculture for bioferitlization and control of pathogens and pests:to have a sustainable, conservative agriculture (Carvajal-Muñoz et Carmona-Garcia, 2012), to develop the plants cultures without using chemicals or use less chemical products, to mobilize from soils minerals nutrients, to stimulate plants growth in order to have an increased crops.

There are many scientific reports on wheat growth stimulation by *Azospirillum* inoculation. For example a study showed that not only the phytohormones interfere with plant stimulations, but the lipo-poly-saccharides (LPS) of membrane has the role of adhesion and in the same time stimulates in the roots cells an active cells division in meristemes and stimulate plants response to bacterial cells (Evseeva et al., 2011). A survey of literature of about 27 years, showed, after a careful data statistical meta-analyses, a production seed growth of 9 % dry mass, and total growth with 18 % more than non inoculated cultures (Veresoglu et al, 2012). *Azospirillum brasilense* mutant for ammonium binding site of the glutamine synthetase, which means the bacteria release ammonium in the environment. This results in special effect on growth of wheat parameters (Van Dommelen et al. 2008).

The production of bioflocs is improved by addition in the culture media of potassium nitrate and fructose (Joe et al, 2010) The same strains, were used for inoculation of wheat, having as results the increased aerial biomass by 12%, root biomass by 40% and grain yield by 16% (Naiman et al, 2009).

The co-inoculation of tomatoes with *Azospirillum brasilense* Sp 245, and *Bacilus subtilis* strain 101, have no synergistic effects on plant biomass in comparison with single strain application, even some roots alterations occurs (Felici et al, 2009). Some strains of *Azospirillum* were identified in soil rhizosphere of *Coffea arabica* in some organic coffee farms from Brasil, and were supposed to be use in the future as inoculants (Caldwell et al., 2015).

Inoculation in soya bean plants with *Azospirillum* strains have as effect the growth of the roots and nodulation (Molla et al. 2013), in special co-inoculation with *Bradirhizobium*. The inoculation with *Azospirillum* sp. Strain B510, in dose of 1×10^8 cfu/ ml, of the rice seedling produces a growth promotion effect in culture (Isawa et al 2010) with a clear difference and yield comparing with non-inoculated seedlings. For some ornamental plants like *Delonix regia*, form tropical area, the treatment of seedlings with bioinoculant containing *Azospirillum* strain alone or in different combination with other strains and with mycorrhyza (AM) fungi are beneficial and enhance the growth, the biomass, the nitrogen yield, protein and chlorophyll content (Meenakshisundaram et al, 2011). The same strain was used to inoculate maize seed. Following inoculations, biomass of shoots and roots grows, and in the same time the production and amount of secondary metabolites like benzoxazine was modified, and the effect of stimulation depending of the strain of bacteria and of the cultivar of maize used (Walker et al., 2011). Inoculation with *Azospirillum* culture of 200g /ha, in the condition of lack of chemical fertilization, growth of the production is enhanced by 15,4% in 2001 of the crop (do Carmo Lana et al, 2012).

Azospirillum application improved the growth of wheat (Arzaneh et al, 2011) and prepared together with a microbial metabolite (*Rhizobium tropici* strain CIAT 899 lipo-chitooligosaccharides) showed an increase in grain yield and shoot dry yield and N yield in maize (Marks et al, 2015). Different formulations of *Azospirillum* products, were reviewed by Bashan et de Bashan, 2015).

As a market analysis shown that the microbial inoculants (Bullion et Winder, 2016), including *Azospirillum*, total sale of 280mil USD to 5 billions USD. Leaders of the market are Novozymed and BASF in North and South America, USA and Brazil being the major markets. Another important region is the Asia Pacific area with a clear tremendous activity in India and China with little companies producing and selling its microbial fertilizer and biocontrol products. Other market analysis reports (2016) prnewswire.com/news-release/biofertilizers-market-by-type-microorganisms,mode of application, crop type, form, and region global forecast to 2022) showed that the market will grow with 14, 8% until 2022. This report shows that the Asia-Pacific region dominated the market in 2015, and will continue in 2022. The strongest companies are Novozymes A/S (Denmark) National Fertilizers LTD (India) Madras Fertilizer LTD (India), Gujarat State Fertilizers & Chemicals Ltd. (India), Rhizobacter SA (Argentina) T Stanes & Comp. Ltd (India), Camson Bio Technologies Ltd. (India) Lallemand Inc. (Canada), Nutramax Laboratories Inc. (USA).An important thing, is the legislation which permit the selling of such products on the market. The problem of legislation has many aspects as establishing definitions (Malusá et Vassilev, 2014), procedures for production, standards for this kind of products. According to EU Commision regulation 889/2008 on organic production, the microorganism are only for plant protection (biocontrol), and some products on mycorrhizal fungi were accepted and registered after the same legislation as biological control substances. The authors showed that India has a very complete legislation of production and use of the biofertilizers (containing microorganisms). There are concerns in the field of sustainable agriculture, conservation of biodiversity and soil management, and biotechnology projects in the frame of research programme Horizon 2020.

3.CONCLUSIONS

The non symbiontic nitrogen fixer from Azospirillum genera were used in some countries and are in use under differents formulation with success for improving plant nutrition and their growth, which means we can do it in Europe and Romania too in order to have new ecological products for our sustainable agriculture.

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Author' biography with Photo

Sergiu Fendrihan is Senior scientist and associated professor of microbiology, coworker in many projects in country and abroad, of agricultural microbiology, astrobiology, environmental microbiology, founder of the first Bioresource Center from Romania. He wrote over 12 chapter of books ,10 books and many scientifc articles, about 50 presentations at scientifical conferences. He are working for many years in the Institute of Plant Protection from Bucharest.

Florica Constantinescu is the Director of the Research Institute for Plant Protection and leader of Bacteriology laboratory, with many articles and presentation, and patents in this field of activity, with stages of work abroad in prestigious institutions.

Oana Sicuia is a young very skilled researcher at the RDIPP working in the field of organic horticulture using plant beneficial microorganisms.

Sorina Dinu is scientific researcher worked on research topics regarding biological control and plant nutrition, collaborator in the frame of COST - 830 position (Development of bacterial inoculants with beneficial effects on crop legumes, cereals and technical plant), NUCLEU (Plant protection microbiological means, sustainable alternative to chemical products) programs.

Table 1. Table with the species of genus Azospirillum

Nr	Species	GenBank	Properties	Isolated from	References
1	*Azospirillum agricola* Lin et al.,2016	The type strain is CC-HIH038^T (=BCRC 80909^T = JCM 30827^T).	was ubiquinone Q-10 fatty acids found in strain CC-HIH038^T $C_{16:0}$, iso-$C_{18:0}$, $C_{16:0}$ 3-OH, $C_{14:0}$ 3-OH/iso-$C_{16:1}$ and $C_{18:1}\omega7c/C_{18:1}\omega6c$.	Cultivated soil	Lin et al. ,2016
2	*Azospirillum brasilense* corrig. Tarrand et al. 1979	DSM 1690T	Use glycerol as a carbon source optimal temperature 37ºC, pH 6-8, nitrate red+ urease+	From roots of tropical grasses	Tarrand et al 1979
3.	*Azospirillum canadense* Mehnaz et al. 2007	DS2T, DS2T DS2T (=NCCB 100108T=LMG 23617T).	free-living diazotrophic strain Q-10 quinone system; major fatty acid 18:1ω7c) pH-5-7, T 25-30ºC, nitrate reduction+	Corn rhizosphere	Mehnaz et al 2007
4	*Azospirillum doebereinerae* Eckert et al. 2001	GSF71T as the type strain (l DSM 13131T; reference strain Ma4lDSM 13400) GSF71T as the type strain (lDSM 13131T; reference strain Ma4lDSM AJ238567 and AJ238568, respectively	Curved or S-shaped rods, 1.0–1.5 µm in width and 20–30 µm in length, G-, motile polar flagellum. They grow at 30ºC and pH- 6-7. They didn't growth at 37ºC, nitrate reduction-, urease +	bacteria was isolated from the roots of the C4-gramineous plant Miscanthus.	Eckert et al 2001
5	*Azospirillum formosense*, sp. nov Lin et al. 2012.	CC-Nfb-7T (5BCRC 80273T5JCM 17639T5DSM 24137T).	G-, spiral or rod-shaped, non-spore-forming diazotrophic bacterium Oxidase+, catalase+,pH-5-9, temp. 30ºC	Agricultural soil	Lin et al, 2012
6.	*Azospirillum halopraeferens* Reinhold et al. 1987	Strain Au 4 (= LMG 7108) isDSM 3675.	G-, 0.7- 1.4 µm 2.4 -5 µm are gram negative, vibrioid to S shaped; motile flat circular colonies can tolerate 0.25 % NaCl and 41ºC, pH-6-8, intracellular granules of poly-P-hydroxybutyrate	with the roots of Leptochloa fusca (L.) Kunth	Reinhold et al 1987
7.	*Azospirillum humicireducens* sp. nov Zhou et al 2013	The type strain is SgZ-5T (5CCTCC AB 2012021T5KACC 16605T).	G-, facultative anaerobic, motile, spiral, straight-to-slightly curved rod-shaped and nitrogen-fixing strain	Microbial fuel cell	Zhou et al 2013
8	*Azospirillum largomobile* corrig. (Skerman et al. 1983) Ben Dekhil et al 1997	DSM 9441T (former Conglomeromonas largomobilis)	Polymorphic cells, 1-10 side flagella,and a polar flagella use acetylglucosamine, D glucose, glycerol, D mannitol, D Ribose, D Sorbitol, grow at 28ºC optimum, nitrate reductase-	Fresh lake water	Ben Dekhil et al. 1997
9	*Azospirillum lipoferum* (Beijerinck 1925).	DSM 1691T	Polymorphic cells, use as carbon sources N acetylglucosamine, D glucose, glycerol, D mannitol, D Ribose, D Sorbitol, growth at 37ºC, pH 5,7-6,8. require biotin for growth	Root of tropical grasses	Tarrand et al. 1979

10	*Azospirillum melinis Peng et al. 2006*	*TMCY 0552T (=CCBAU 5106001T=LMG 23364T=CGMCC 1.5340T) as the type strain.*	*Bacteria G- straight or slightly curved, non-motile forming colonies on NFB medium 3 mm circular, convex and translucent. Facultatively anaerobic chemo-organotrophic, ph 4-8, temp. 20- 33ºC, nitrate reduction+*	*endophytic diazotrophs of Melinis minutiflora*	*Peng et al. 2006*
11	*Azospirillum oryzae Xie and Yokota 2005.*	*COC8T (=IAM 15130T=CCTCC AB204051T).*	*Cells are spiral or vibrioid, motile via a single polar flagellum. PHB granules are pre-sent in the cells.pH 6-7, temp. 30C, nitrate reduction +, ureaze+*	*roots of the rice plant Oryza sativa*	*Xie and Yokota 2005.*
12	*Azospirillum palatum Zhou et al 2009*	*WW 10T (=LMG 24444T=KCTC 13200T=CCTCC AB 207189T)*	*rods G- strictly aerobic, motile, peritrichous flagella, colonies yellow flat circular. Grow at 30-37ºC and pH 6.0-8.0.*	*Forest soil*	*Zhou et al 2009*
13	*Azospirillum picis Lin et al. 2009*	*The type strain is IMMIB TAR-3T (5CCUG 55431T 5DSM 19922T*	*G- aerobic, motile rods, Cat +, Ox+ pink-coloured colonies,pH 6-9, temp.37ºC, nitrate reduction+ urease+*	*isolated from discarded road tar Taichung City Taiwan*	*Lin et al. 2009*
14	*Azospirillum rugosum Young et al. 2008*	*GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMMIB AFH-6T is AM419042. IMMIB AFH-6T (5CCUG 53966T5DSM 19657T).*	*G-, light-orange rods motile, tolerates 2.0 % (w/v) NaCl, temp 22-37, nitrate+, urease+*	*from an oil-contaminated soil sample*	*Young et al. 2008*
15	*Azospirillum soli Lin et al. 2015*	*The type strain is CC-LY788T (=BCRC 80569T=JCM 18820T).*	*aerobic, G-, rod/spiral-shaped, pH 6-8,nitrate +*	*Agricultural soil Taiwan*	*Lin et al. 2015*
16	*Azospirillum thiophilum Lavrinenko et al. 2010*	*Strain BV-ST*	*mixotrophic growth under microaerobic conditions with simultaneous utilization of organic substrates and thiosulfate,polymorphic cells,tolerates 3% NaCl, pH 6,5-8,5, temp 37ºC, urease – nitrate +*	*sulfur bacterial mat collected from a sulfide spring of the Stavropol Krai, North Caucasus, Russia.*	*Lavrinenko et al. 2010*
17	*Azospirillum zeae Mehnaz et al. 2007*	*N7T (5NCCB 100147T5LMG 23989T) as the type strain.*	*G- rods, 0.9–1.5 µm, motile single polar flagellum. It forms pink colonies tolerate 0.5–1.0 % NaCl. pH-5-7, temp. 30ºC, nitrate +, urease+ do not grow with NaCl 3%.*	*Roots of corn*	*Mehnaz et al. 2007*

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