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REVIEW ARTICLE

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Microbial fertilizers: A comprehensive review of current findings and future perspectives

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Abstract

Plant growth promoting microorganisms (PGPM) are an important group of microbial inoculants, which exist in rhizosphere and have the ability to inhabit the root of the plants and improve their development. Their positive influence is achieved through solubilization of phosphorus, nitrogen fixation, production of plant nutrients and phytohormones, protection from pathogens and recovery from stressful environmental conditions. This is the main reason for the increasing usage of many PGPMs which formulations are commonly known as microbial fertilizers. Microbial fertilizers represent an attractive replacement for chemical fertilizers that are polluting the environment. They are used to increase the crop yield in an eco-friendly way while relying on sustainable agriculture principles. The biggest problem nowadays is the very poor quality of such products, which results in the lack of confidence and makes commercialization much more difficult. In order to increase production and hence the commercialization of microbial fertilizers, desired quality and stability should be achieved. For this reason, many researches are done in this particular field. In order to develop an optimal product, it is important to know and understand the process, including the physiology of bacteria and plants, mass multiplication technological processes as well as the existing formulation and the specific effect on the desired plant. For this purpose, the aim of this review is to indicate the significance of microbial fertilizers and their beneficial effects on the plants, as well as to give a brief survey of the different aspects of production processes with a special emphasis on mass multiplication.

Additional keywords: biofertilizers; rhizosphere; inoculum; plant-growth-promoting-microorganisms; bioreactors; formulation.

Abbreviations used: AM (arbuscular mycorrhizas); CDM (chemically defined medium); CMC (carboxymethyl cellulose); DSM (Difco sporulation medium); EcM (ectomycorrhizae); JMM (Janson's modified medium); MTM (modified tryptone medium); PGPB (plant growth promoting bacteria) PGPM (plant growth promotion microorganisms); PVP (polyvinylpyrrolidone).

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Introduction

In recent years, there has been undoubtedly a significant increase in agricultural production due to increasing population growth and, therefore, the need for food (Hassen *et al.*, 2016). So far, the misuse of chemical fertilizers and pesticides has proven unsustainable and has contributed to the disturbance of ecological balance and pollution of the natural environment. Thus, the need to reduce their use is evident (Szilagyi-Zecchin *et al.*, 2016). There are many alternatives to the use of chemical fertilizers that can be adopted with the aim of reaching sustainable agriculture and environmental protection. Farmers can use crop rotations, integrated pest management or conservation tillage practices combined with applied management skills to increase the productivity and enhance the profitability (NRC, 1989). One of the possibilities with a great potential is to use microbial fertilizers. They have multiple roles: to fertilize the plant and to stimulate and control its growth in an ecologically sustainable way, which made them the main focus of research all over the world in the last years (Hassen *et al.*, 2016).

Plant Growth Promoting Microorganisms (PGPMs) can be found in the rhizosphere, a dense, narrow and nutrient rich zone of soil located nearby the plant's root. It is characterized with the presence of different root secretions and intense microbial activity (Zaidi *et al.*,

2015). Since the plant absorbs all the essential nutrients necessary for its growth and reproduction from the soil, plant growth may be affected by soil availability or by the presence of toxic metals. The interaction between microorganisms and roots significantly increases the uptake of essential compounds and prevents the accumulation of toxic compounds (Yadav *et al.*, 2015). The basic mechanisms that the PGPMs rely on to contribute to the increase of nutrient uptake are nitrogen fixation and phosphate solubilization. In addition, these microorganisms produce various plant hormones, siderophores, cyanides and lytic enzymes which have a phyto-stimulant effect on the plant and function as biopesticides and rhizomediators (Bjelić, 2014).

In order to take an advantage of the benefits PGPMs provide, it is necessary to formulate the appropriate inoculum, that is, microbial fertilizer (bio-fertilizer) which will contain living organisms capable of colonizing the rhizosphere and increasing plant growth. The development of a successful inoculant includes multiple steps (Shaikh & Sayyed, 2015; Vassilev *et al.*, 2015): *(i)* picking a suitable culture and isolation of effective microorganism; *(ii)* the characterization of the chosen microorganisms on an optimum medium with the appropriate growth conditions; *(iii)* microbial mass multiplication; *(iv)* choice of carrier; *(v)* formulation of the inoculum; and *(vi)* field studies; *(vii)* large scale studies and production at the industrial level; *(viii)* constructing a quality control and storage system. Each of these steps requires equal attention so that the process results in a microbial fertilizer of the desired quality. This paper offers an overview of the current research and existing technologies for the production of microbial fertilizers and the bio-fertilization of soil, with a special focus on each individual step within the process.

Nutrients necessary for plant growth

Plants require a great number of elements (macronutrients: N, P, K, Ca, Mg, S, C, O, H and micronutrients: Fe, B, Cl, Mn, Zn, Cu, Mo, Ni) and their compounds for growth and development. Those elements are used to create and maintain the cells and the necessary life processes such as growth, reproduction, respiration and photosynthesis. As a part of photosynthesis, plants create various forms of polysaccharides, lipids, proteins and other organic molecules (Kirkby, 2012). For normal functioning, the plant absorbs various chemical elements from the soil, which in combination with water and carbon-dioxide form compounds (White, 2012).

Numerous elements are widespread in the soil in forms that plants cannot assimilate. Because of that, the basic precondition for a plant to uptake certain element is its bioavailability (White, 2012). Water and nitrogen are considered to be the most important factors for development of a plant (Gonzalez-Dugo *et al.*, 2010). Plants are only able to absorb nitrogen in the form of nitrate and ammonia, while molecular form from the air remains unavailable to them. When the level of nitrogen in the plants is low vegetation is limited, leading to reduced productivity (Hassen *et al.*, 2016). Besides that, phosphorus usually originates from insoluble phosphate rock formations, and in spite of a large amount of phosphorus in the soil (400-1200 mg/kg), only a small part of it is available to the plant for plant metabolism processes. Phosphorus deficiency may cause slower growth of the plant and reduced leaf biomass (Cakmak, 2002; Avdalović *et al.*, 2015). Similarly, potassium regulates enzymatic reactions, salt stress resistance, stomata functions, photosynthesis and carbohydrate transport (Perrenoud, 1990). Lack of potassium in soil may cause plant functions disorder, resulting in poor crop quality (Lawton & Cook, 1954; Wang *et al.*, 2013). And furthermore, plant productivity is significantly influenced by the presence of other elements and plant hormones, thus, it is quite clear that every plant should be supplied with a sufficient amount of every nutrient. The long-term practice of enriching the soil through the use of chemical fertilizers has proven to be quite unfavorable for the environment and has led us to alternative solutions that will provide plants with the necessary compounds (Zaidi *et al.*, 2015). One of the more popular approaches is the use of microorganisms that promote plant growth, and their incorporation into microbial fertilizers which, when applied to seeds, plant itself or incorporated in the ground may provide all the nutrients plant need (Mrkovački *et al.*, 2012).

Plant growth promoting microorganisms (PGPMs)

As previously stated, PGPMs naturally inhabit the rhizosphere, favorably affect the plant, improving its productivity and resistance to pathogens (Mrkovački *et al.*, 2012). Some of the PGPMs, which in general can be divided into bacteria and fungi, include the following strains: A*zospirillum, Azotobacter, Rhizobium, Pseudomonas, Enterobacter, Bacillus, Paenibacillus, Klebsiella, Flavobacterium, Gluconobacter, Penicillium, Trichoderma* and *Streptomyces* (Nadeem *et al.*, 2015). Microorganisms such as *Rhizobium, Klebsiella, Clostridium, Bacillus megaterium, Penicillium* sp*., Trichoderma viride* improve growth and crop yield, while *Pseudomonas aureofaciens, Trichoderma, Streptomyces* sp. may act as biocontrol agents against pests and plant disease (Abhilash *et al.*, 2016).

Based on their interaction with the plant, plant growth promoting bacteria (PGPB) can be divided into symbiotic or free-living bacteria (Zaidi *et al.*, 2015). They can further be divided into extracellular and intracellular. Extracellular PGPBs inhabit the space of the rhizosphere, the root surface or the intracellular space of the root cells, while the intracellular PGPBs inhabit the root cells, penetrate the cell wall and integrate with the plant, forming new organ on the plant tissue – nodule, that provides optimal conditions for the bacteria (Kiprovski, 2012; Owen *et al.*, 2015). On the other hand, plant growth promoting fungi include arbuscular mycorrhizas (AM), ectomycorrhizae (EcM) and root fungi such as *Penicillium*, *Trichoderma* and *Aspergillus*. They produce organic acids and enzymes that inhibit pathogens or dissolve insoluble compounds (Owen *et al.*, 2015).

Knowing that every plant has a defense system against pathogens, it is interesting to discuss the way plants actually detect and distinguish beneficial microbes from a pathogenic kind. It is believed that every plant has a receptor with microbe-associated molecular patterns which are the key elements in a plant-microbe communication (Finkel *et al.*, 2017). In that process, different signaling mechanisms are involved (chemoattraction, initiation of the nodulation process, release of volatile compounds etc.) and a variety of chemical compounds (organic acids, sugar, flavonoids, volatiles) are released. Presence of a certain compound is actually signal for starting off root colonization or nodule forming process. After colonizing the plants root, bacteria start to show their beneficial effects (Lugtenberg, 2015). Beneficial effects are various and depend on a particular plant and microorganism applied (Table 1).

There is a wealth of literature covering different possibilities of inoculation of wide range of plants, even in horticulture and fungiculture (Rainey, 1991; Qin *et al.*, 2016). Rhizobia is used by great number of commercialized fertilizers designed for legume crops, although some studies show other choices might be successful as well (Marks *et al.*, 2015). For example, *Pseudomonas aeruginosa* was used to promote growth of faba beans and common beans at the same time preventing root rotting caused by *Fusarium culmorum* (Haddoudi *et al.*, 2017). Cereals inoculation have been particularly studied as well. Numerous researches show that inoculation of maize or wheat provides excellent results. Different species have been tested out (*Burkholderia capacia, Bacillus subtilis Azotobacter* sp.*, Azospirillum* sp.*, Pseudomonas* sp.) in a form of a single or mixed inoculum showing significant growth promotion in terms of plant's dry weight, root length and yield (Boddey *et al.*, 1986; Bevivino *et al.*, 1998; Gholami *et al.*, 2009; Sachin, 2009; Yazdani *et al.*, 2009; Rojas-Tapias *et al.*, 2012; Kavamura *et al.*, 2013; Mumtaz *et al.*, 2017). Accomplished results may differ based on the potting medium used. Earlier study (Bevivino *et al.*, 1998) shows that sand-peat/manure mixture gave better results in terms of fresh plant weight, when compared to the soil which is original bacterial inhabitant. This indicates the importance of choosing a suitable carrier and delivery system, which will be discussed later in the review.

PGPM mechanisms of action

PGPMs affect plants by increasing crop yield and plant resistance to stressful environmental conditions and pathogens (Garcia-Fraile *et al.*, 2015). These bacteria can directly affect the plant by producing substances that can regulate growth and improve the yield. Besides, they can increase water uptake, nutrient uptake and essential elements uptake, all of them having a beneficial effect on the plant (Zhao *et al.*, 2010; Rojas-Tapias *et al.*, 2012; Grobelak *et al.*, 2015; Owen *et al.*, 2015). Indirect mechanisms include the inhibition of pathogens through the production of antibiotics and enzymes. Among that, PGPMs increase the availability of micronutrients (uptake of Fe, Zn, Se) through the processes of solubilization chelating and oxidation/reduction reactions in the soil (Abhilash *et al.*, 2016). An overview of the mechanisms of action of certain PGPMs is shown in Table 2.

Direct PGPM mechanisms of action

The main mechanisms PGPMs use to contribute to the increase of nutrients in the soil are nitrogen fixation and phosphate solubilization, along with solubilization of other minerals. After photosynthesis, nitrogen fixation is the most important biological process in nature, enabling the circulation of nitrogen in the biosphere (Wani *et al.*, 2016). Symbiotic bacteria from the group *Rhizobium* and *Frankia,* and non-symbiotic bacteria such as *Azospirlillum* sp., *Azotobacter* sp. and *Acetobacter* sp. have the ability to assimilate N_2 from the atmosphere and convert it into NH_3^- as part of a mechanism well-known as nitrogen fixation (Szilagyi-Zecchin *et al.*, 2016). Nitrogen fixation is controlled through the amount of oxygen and the availability of nitrogen and is carried out with the help of the nitrogenase, enzyme produced by bacteria (Vijayabharathi *et al.*, 2016). The transformation of nitrogen takes place through ammonification, nitrification, nitrogen fixation and denitrification (Bjelić, 2014).

The conversion of insoluble forms of phosphorus into forms that are more available to the plant in the rhizosphere is achieved by means of bacteria called phosphate-solubilizers (Szilagyi-Zecchin *et al.*, 2016).

Table 1. Effects of inoculation of plants using different microorganisms.

Microorganism	Plant	Result	Reference
Pseudomonas fluorescens and *Azospirillum brasilense*	Paddy rice	Increased biomass production, harvest index, and grain yield. Reduced number of chaffy grains	García de Salamone *et al*. (2012)
Pseudomonas fluorescens	Peanuts	Reduced incidence of leaves dots caused by pathogens	Meena *et al*. (2002)
Pseudomonas sp.	Carnation	Prevention of wilt triggered by *Fusarium* sp.	van Peer *et al*. (1991)
Pseudomonas putida	Melon	Prevention of melon wither	Bora *et al*. (2004)
Pseudomonas chlororaphis TSAU13	Cucumber and tomato	Protection of plants from pathogens and control of the production of phytohormones	Egamberdieva & Adesemoye (2016)
Pseudomonas aeruginosa	Faba beans and common beans	Enhances shoot and dry weight (25% and 110%) and root dry weight (29% and 67%) in faba beans and common beans respectively.	Haddoudi *et al*. (2017)
Bacillus sp.	Maize	Increasing leaf area and dry biomass weight	Kavamura *et al*. (2013)
Bacillus amyloliquefaciens	Potato	Reduction of diseases caused by *Ralstonia solanacearum*	Wei *et al*. (2011)
Bacillus aryabhattai	Grapevine	Significantly increased the growth of *Vitis vinifera* L. Cabernet Saugvinon	Liu *et al*. (2016)
Burkholderia cepacia	Maize	Growth promotion in terms of plant fresh weight	Bevivino *et al*. (1998)
Azospirillum sp.	Wheat	Promoted nitrogen accumulation	Boddey *et al*. (1986)
Azospirullum spp. enriched with metabolites of *Rhizobium tropici*	Maize	Statistically significant increase in grain yield in relation to non-inoculated control in 5 out of 6 experiments	Marks *et al*. (2015)
Azospirillum brasilense	Cucumber	Increasing resistance to Fe-limiting growth conditions, increase in Fe content of leaves and biomass	Pii *et al*. (2015)
Azotobacter chroococcum	Maize	Phosphorus solubilization, production of auxin, catalase activity. Protecting plants from the inhibitory effects of NaCl.	Rojas-Tapias *et al*. (2012)
Azotobacter chroococcum	Maize	Increased root length and plant's dry weight	Sachin (2009)
Rhizobium sp.	Legumes	Yield increased 15-30%	Dobbelaere *et al*. (2001)
1. Rhizobium leguminosarum bv. phaseoli *2. Azotobacter chroococcum* *3. Rhizobium leguminosarum bv. phaseoli + Azotobacter chroococcum + Streptomyces* sp.	Green beans	Individual culture *Rhizobium* spp. and *Azotobacer* spp. have contributed to an increase in the number of pods per plant and grain weight, while a mixed inoculum did not give significant results	Jarak *et al*. (2010)
1. Pseudomonas sp. *PGERs17* *2. Rhizobium leguminosarum*	Lentil	Increased resistance to thermal stress. Increased root length and production of metabolites	Mishra *et al*. (2011)
Bacillus cereus, Staphylococcus sp., *Pseudomonas fluorescens*	*Cerasus sachalinensis*	Improved root viability, root carbohydrate concentration and seedling growth	Qin *et al*. (2016)

Table 1. Continued

Microorganism	Plant	Result	Reference
Bacillus flexus, Bacillus megaterium, Sinorhizobium meliloti	Maize	Improved phosphorus nutrition and growth to the same level accomplished before with addition of soluble phosphorus-fertilizer at 40 w/v	Ibarra-Galeana *et al*. (2017)
Bacillus arybhattai and *Bacillus subtilis*	Maize	Improved shoot length, root length, plant fresh and dry biomass	Mumtaz *et al*. (2017)
Azotobacter chroococcum, Azotobacter vinelandii, Azospirillum lipoferum and *Bacillus subtilis*	Sugar beet	Increase in root yield and white sugar yield	Govedarica *et al*. (2002)
Azotobacter chroococcum, Azospirillum lipoferum, Bacillus megaterium	Maize	Increase of yield. The highest yield was obtained with the application *A. chroococcum* and *A. lipoferum*	Hajnal-Jafari *et al*. (2012)
Azotobacter chroococcum	Sugar beet	Increase in sugar beet yield by 7% and crystal sugar yield by 6%.	Mrkovački *et al*. (2007)
Pseudomonas fluorescenes Pseudomonas putida Azospirillum lipoferum Azospirillum brasilense	Maize	Increased height and dry weight of plants, leaf area, weight of 100 grains and number of kernels per piston	Gholami *et al*. (2009)
Bacillus lentus Azotobacter chroococcum Azospirillum brasilense Pseudomonas putida	Maize	Increased grain yield, weight of the piston, the number of grains per piston	Yazdani *et al*. (2009)

Some of the PGPMs, including *Pseudomonas* sp., *Bacillus* sp., *Burkholderia* sp., *Rhizobium* sp. and *Flavobacterium* sp., have the ability to solubilize some insoluble phosphate compounds. The usage of these bacteria as a part of bioinoculants may enhance the assimilation of phosphate and offers numerous advantages to the direct stimulation of plant growth (Hassen *et al.*, 2016). In some cases, bacteria from the group of *Bacillus, Pseudomonas, Serratia* and *Streptomyces* can take part in the solubilization and mineralization at the same time (Milošević & Govedarica, 2001; Gopalakrishnan *et al.*, 2014). Ibarra-Galeana *et al.* (2017) proved that *Sinorhizobium meliloti, Bacillus flexus* and *Bacillus megaterium* have the possibility to solubilize tricalcium phosphate and hydroxyapatite. During that process, some extracellular enzymes (various phosphatases) and important compounds (pH lowering organic acids, siderophores and hydroxyl ions) are released in order to dissolve the minerals. While substrate is being degraded, phosphorus is delivered into the soil (Sharma *et al.*, 2013; Souza *et al.*, 2015; Basu *et al.*, 2017).

Knowing the importance of other macro- and micronutrients, it is important to research strains that will enhance their absorption from the soil. It has been recently reported (Mumtaz *et al.*, 2017) that inoculation with Zn-solubilizing bacteria can help to enhance Zn nutrition by plants, therefore improving the growth of plant. In this particular study *Bacillus aryabhattai* and *B. subtilis* were used to inoculate maize, which resulted in better growth of the plant. On the other hand, *Azosprillium brasilense* is proved to be Fe-solubilizing bacteria increasing the Fe and biomass content in cucumber plants (Pii *et al.*, 2015), which is attributed to production of siderophores and will be discussed in next section of this review.

PGPMs are also able to make phytohormones which stimulate plant growth, thus the mechanism of their activity is known as biostimulation. Some of the most important phytohormones are auxins, cytokinins, gibberellins and abscisic acid (Gopalakrishnan *et al.*, 2014).

Auxins are plant hormones with a cardinal role to modulate the development of a plant. As much as 80% of the PGPMs can synthesize the indole acetic acid (IAA), which has an important role in the stimulation of cellular division and differentiation (Mrkovački *et al.*, 2012). IAA induces the occurrence of lateral roots among dicotyledons and adventive roots among monocotyledons, improves secondary thickening of the walls and an increase in xylem cells, which results in better minerals and water uptake (Hassen *et al.*, 2016; Vijayabharathi *et al.*, 2016). *Azospirillum* sp., fluorescent *Pseudomonas* sp., and several other PGPMs secrete IAA (Hassen *et al.*, 2016; Tabatabaei *et al.*, 2016).

Gibberellins take part in cellular elongation and division, as well as the internodium elongation. The mechanisms

Table 2. Short review of different mechanisms conducted by plant growth promoting microorganisms

Microorganisms	Mechanism of action	Reference
Pseudomonas sp	ACC-deaminase activity, solubilization of phosphorus, nitrogen fixation. Synthesis of: indole acetic acid, ACC deaminase, siderophore, HCN, exopolysaccharides, ammonium, alginic acid	Lockwood & Schippers, 1984; Manwar *et al*., 2004; Grobelak *et al*., 2015; Tabatabaei *et al*., 2016
Bacillus sp.	Zinc solubilization, phosphorus solubilization. Synthesis of: exopolysaccharides, indole acetic acid, siderophore, ACC deaminase, HCN, cellulases, organic acids, different enzymes.	Grobelak *et al*., 2015; Liu *et al*., 2016; Vidhyalakshmi *et al*., 2016
Rhizobium sp.	CoQ10 production, phosphate solubilization, exopolysaccharide production.	Wu *et al*., 2003; Duta *et al*., 2004; Alikhani *et al*., 2006; Huo *et al*., 2012
Azotobacter sp.	Amelioration of saline stress, increased resistance to NaCl. Synthesis of: Indole acetic acid, siderophore, protease, and alginates	Sachin, 2009; Díaz-Barrera *et al*., 2011; Rojas-Tapias *et al*., 2012; Wani *et al*., 2016
Azospirillum sp.	Antifungal activity Production of: siderophore, indole-3-acetic acid	Ona *et al*., 2005; Tortora *et al*., 2011

which improve plant growth through gibberellins are as yet unknown (Vijayabharathi *et al.*, 2016). Some authors believe that gibberellins increase the density of the absorbent hairs on the root that soak up water and nutrients, which contributes to the formation of greater sized fruits, an increased number of buds, prevents the dormant stage of the bulb and stimulates parthenocarpy. The lack of gibberellins is responsible for the occurrence of dwarf plants (Gopalakrishnan *et al.*, 2014).

Cytokinins stimulate cellular division in some plants and in some cases the development of the root and absorbent hairs on the root (Gopalakrishnan *et al.*, 2014). In addition, they take part in the growth of plant callus and help the differentiation of the shoots (Vijayabharathi *et al.*, 2016). Ninety percent of rhizosphere microorganisms have the ability to produce and release cytokinins, while approximately 30 compounds from the group of cytokinins that promote growth have a microbial origin. Existing data indicates that *Rhizobium* sp. produces cytokinins (*Gopalakrishnan et al.*, 2014).

Abscisic acid regulates the physiological processes in plant (Vijayabharathi *et al.*, 2016). In part, it is synthesized in the chloroplasts, while its entire biosynthesis primarily takes place in the leaves, initiated by the stressful environmental conditions such as a lack of water and low temperatures (Gopalakrishnan *et al.*, 2014). It helps the germination of the seed, the closing of stomata and tolerance to environmental stress (Vijayabharathi *et al.*, 2016).

Indirect PGPM mechanisms of action

Various pathogenic bacteria, fungi and nematodes may infect the plant and thus reduce crop yield to a great extent. As previously indicated, PGPMs significantly influence the induction of plant resistance to pathogens by synthesizing various antibiotics, siderophores, cyanides or lytic enzymes (Bjelić, 2014).

One of the main mechanisms for the control of pathogens is the ability to synthesize one or more antibiotics. Many PGPMs with the ability to synthesize antibiotics also produce cyanide, which in most cases has a synergistic effect when combined with antibiotics (Glick, 2015). Furthermore, with the aim to prevail over the restricted supply of iron in the soil, some PGPMs are able to produce siderophores. Siderophores are low molecular mass organic compounds with strong chelating affinity towards ions of iron (Fe^{+3}). In presence of oxygen, most of the iron particles are only partly soluble and thus are not completely available to the living organisms (Gopalakrishnan *et al.*, 2014). Bacterial siderophores have a positive effect on the growth of plants, functioning as a source of iron that is readily usable to the plant (Hassen *et al.*, 2016). Certain studies have indicated that *Pseudomonas,* which produces siderophores, influences antifungal activity towards different pathogenic fungi (Manwar *et al.*, 2004), while *Bacillus cereus* has a potential in biocontrol of rice fungi (Etesami & Alikhani, 2017). *Pseudomonas* strains have been studied way back in 1984 when it was proved that they inhibit growth of

six fungi by virtue of siderophore production (Lockwood & Schippers, 1984). *Pseudomonas putida* efficiently controlled tomato foot and root tor caused by *Fusarim oxysporum* in laboratory experiments as well as at industrial level (Validov *et al.*, 2009).

In addition, PGPMs have a positive effect on the characteristics of the soil itself and their consortiums are successfully used in the processes of bioremediation. This is how nutrient poor and polluted soil becomes arable and available to agricultural production, since a transformation occurs in the hydrocarbons and other pollutants into less detrimental forms (Beškoski *et al.*, 2012). Microorganisms that effectively break down hydrocarbons and oil-based pollutants include *Nocardia* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Flavobacterium* sp., *Micrococcus* sp., *Arthrobacter* sp., *Corynebacterium* sp., *Mycobacterium* sp., *Bacillus* sp., etc. (Milic *et al.*, 2009; Beškoski *et al.*, 2011). AM fungi are also studied in the phytoremediation processes, indicating their role in improving soil conditions and enhancing plant tolerance to heavy metals (Barea *et al.*, 2005). Some studies have shown that commonly known PGPM (*Bacillus* sp., *Pseudomonas* sp., *Agrobacterium* sp., etc) not only improve the plant growth, but also reduce uptake of heavy metals by plants (Ahemad, 2015; Ullah *et al.*, 2015). For example, *Microbacterium* sp. successfully prevented chromium toxic effect on pea by simply reducing its bioavailability in soil (Soni *et al.*, 2014). On the other hand, *Pseudomonas putida* is capable of simultaneously degrading naphthalene in soil, protecting the seed and the plant from possible lethal effect (Lugtenberg & Kamilova, 2009).

Mass multiplication of microorganisms

Mass multiplication of microorganisms is achieved through the application of a batch, semi-continuous (fed-batch) or continuous cultivation in various growth media (submerge or solid-state). Continuous fermentation is considered an experimental procedure and is rarely used on an industrial scale (Glick, 2015). Batch fermentations seem to be most commonly used, as in that case, it is easy to set up and control the bioreactor. In the case of the application of a semi-continuous fermentation process, it is possible to perform: fed-batch fermentation with pulse feeding, exponential fed-batch fermentation and linear fed-batch fermentation (Öztürk *et al.*, 2016). The addition of nutrients during the semi-continuous processes extends the exponential and stationary phase, thus resulting in higher biomass concentration. Many experiments have proven that semi-continuous fermentation can lead to better results, giving higher yields of desired products (Ona *et al.*, 2005; Reis *et al.*, 2005; Monteiro *et al.*, 2014). Still, this process is not used widely because it requires much more attention, nonstop control and monitoring, which can lead to higher production costs (Glick, 2015). In order to achieve desired growth, it is necessary to provide required conditions of fermentation, that is, parameters such as medium composition, temperature, pH values, mixing speed and concentration of oxygen (Garcia-Ochoa & Gomez, 2009).

Choosing a culture

The choice of culture depends primarily on the objective of the formulation of fertilizers, that is, the effects that should be achieved, as well as the plants on which they will be applied. In addition, it is necessary to consider the complexity of the procedure for replicating the desired culture and to consider whether the final product will consist of a pure or mixed culture (Herrmann & Lesueur, 2013).

Research has shown that the greatest benefit for the plant is achieved by using mixed inoculants (Yazdani *et al.*, 2009; García de Salamone *et al.*, 2012; Hajnal-Jafari *et al.*, 2012; Mrkovacki *et al.*, 2012). The idea to study mixed inoculants came from a fact that the natural habitat of microorganisms consists of various species living at the same place. This, in turn, is connected to their close relationship with the plant and other microorganisms included in the natural processes of defense and struggle for location and/or nutrients (Owen *et al.*, 2015). The advantage of using a mix of cultures when compared to individual strains is also demonstrated in the processes of cleaning up the environment using microorganisms through a process of bioremediation. Namely, both in the laboratory and at the industrial level, microbial units have proven to have a significantly greater effect and longer life-span in contaminated soil (Beskoski *et al.*, 2011; Gojgic-Cvijovic *et al.*, 2012). Moreover, mixed inoculants also participate in some of the key processes such as the humidification of anthropogenically contaminated terrains, which is how microorganisms practically prepare the soil for cormophyte growth (Miletić *et al.*, 2014).

Medium composition

The medium for microbial growth and multiplication needs to be cheap, easily available and should contain the necessary nutrients. In most cases, fermentation is initiated on a liquid surface, while solid-state fermentation is usually used for producing fungi (Shaikh & Sayyed, 2015). The medium used for fermentation should be sterile. High temperatures

during the process of sterilization could affect some of the thermo-degradable compounds, which could change the content of the medium and the outcome of the fermentation (Doran, 2013). To prevent this from happening continuous monitoring is required during the whole process in order to ensure the desired quality (Glick, 2015). Some of the most commonly used substrates are glucose and sucrose (as a source of carbon) and ammonium sulfate (as a source of nitrogen). Besides that, most of the bacteria require the presence of some trace elements and vitamins. For example, in the case of *B. subtilis* thiamine is required to start the sporulation, while addition of calcium enhances the sporulation process (Monteiro *et al.*, 2014). When it comes to cell viability, addition of different compounds (*e.g.*: polyvinylpyrrolidone – PVP or carboxymethyl cellulose – CMC) have also proven very efficient. Namely, it has been found that in many cases lysis of cells and reduction of the number of cells in the fermentation broth occurs, which is regulated by the addition of the mentioned substances (Leo Daniel *et al.*, 2013). That is why many researchers prefer to use a chemically defined medium (Table 3) which allows them to adjust the ratio of specific substances and achieve the best result with the desired microorganism (Zhang & Greasham, 1999). Besides that, in recent years different agriculture waste materials have been applied into systems based on solid-state fermentations. It has been suggested that this method provides best quality spores at a low price and it is expected that people will soon take advantage of its potential (Vassilev *et al.*, 2015).

Oxygen addition

Irrespective of the type of fermentation, oxygen is added to the bioreactor (usually in the form of sterile air), anti-foaming agents, as well as acids and bases for the regulation of pH values (Glick, 2015). Oxygen concentration should be on a specific level during the whole fermentation process and it is necessary to permanently provide a sufficient amount of oxygen for the growth of aerobic bacteria. Still, importation of oxygen into the bioreactor can create bubbles which can affect the cellular growth and lower the volumetric mass transfer coefficient (Garcia-Ochoa & Gomez, 2009). Since gas content is highly affected by agitation and type of gas distributor, this problem can be solved using an appropriate stirring speed (Kiełbus-Rąpała & Karcz, 2011). An effective sporulation and higher biomass concentration are achieved when there is an excess of oxygen. In environment limited in oxygen concentration, supplied with essential nutrients, *B. subtilis* has the ability to produce bioactive compounds which are highly active against phytopathogenic bacteria. In anaerobic conditions, this microorganism does not show growth, except in the case of the addition of pyruvate, when it comes to growth through fermentation and anaerobic respiration (de Carvalho *et al.*, 2010).

Mixing

Mixing is very important in every fermentation process for a variety of aspects of fermentation. Effective mixing will fasten the process, enhance mass transfer and reduce the hydraulic retention time (Kiełbus-Rąpała & Karcz, 2011). Mixing is also necessary to prevent thermal stratification, maintain the desired pH values, to increase contact between the substrate and microbial culture, prevent foaming and to provide uniform distribution of the substrate and microorganisms (Liu, 2012). Besides that, good mixing can prevent the accumulation of toxic metabolites in the areas of the bioreactor which are characterized by weaker mixing (Glick, 2015). Successful mixing is easily accomplished in bioreactors of a smaller size, but it represents the main problem when the extent of the fermentation rises. Large-scale operations require additional researches, since the results can vary depending on the volume of bioreactor (AL-Mashhadani *et al.*, 2015). Adequate mixing is also providing the effective control of the oxygen concentration in the reactor (de Carvalho *et al.*, 2010). An overview of the research related to cultivation of various PGPBs is given in Table 4.

Carriers in the development of formulations

The commercial application of microbial fertilizers depends on the development of formulations with sustainable carriers which can enable the longer viability of the applied microorganism. “Primitive” inoculants, bacterial cultures without supplemental carriers, are rarely used commercially and they can only be seen during starting experiments, since they are easy to use (Bashan *et al.*, 2014). Considering the fact that the formulations represent the final form of the product which will be sold on the market, their function is to stabilize the microorganism, help its release onto the desired plant, protect the microorganism and increase its functionality (Shaikh & Sayyed, 2015). The choice of carrier depends on desired viability of microorganism as well as on the type of application (liquid, powder or seed coating) (Nakkeeran *et al.*, 2005). Bacteria can also be subjected to lyophilization and stored without carriers, albeit with the application of cryoprotectants such as mannitol or microcrystalline cellulose. The addition of a carbon source or cellular protectant can

Table 3. Different media used for mass multiplication of plant growth promoting microorganisms

Medium[1]	Content	Microorganism	Reference
DSM	Nutrient broth, KCl, $MgSO_4$, $Ca(NO_3)_2$, $MnCl_2$, $FeSO_4$	*Bacillus subtilis*	Monteiro *et al*., 2014
CDM1	$C_6H_8O_7$, KH_2PO_4, K_2HPO_4, NaH_2PO_4, $(NH_4)_2SO_4$, $MgCl_2 \cdot 6H_2O$, $(NH_4)2HPO_4$, $FeCl_3$, thiamine-HCl i $Ca(NO_3)_2$, glucose	*Bacillus subtilis*	Monteiro *et al*., 2014
CDM2	Glucose, peptone, yeast extract, meat extract, NH_4NO_3, KH_2PO_4, $MgSO_4$, NaCl, $(NH_4)_2Mo_4$, $Ca_3(NO_3)_2$, $MgSO_4 \cdot 7H_2O$, $(NH_4)_2SO_4$, PVP-40, $FeCl_3$, EDTA, CaO, EDTA, salt solution, NH_4HPO_4	*Bacillus subtilis*	Ilić, 2016
CDM3	Glucose, yeast extract, NH_4NO_3, KH_2PO_4, $MgSO_4$, NaCl, $Ca_3(NO_3)_2$, $(NH_4)_2SO_4$, PVP-40, $FeCl_3$, salt solution, NH_4HPO_4, glycerol	*Bacillus megaterium*	Ilić, 2016
CDM4	Glucose, KH_2PO_4, $(NH_4)_2SO_4$, yeast extract, $CaCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 7H_2O$, trace elements	*Bacillus licheniformis*	Reis *et al*., 2005
CDM5	$MgSO_4 \cdot 7H_2O$, $Na_2HPO_4 \cdot 12H_2O$, KH_2PO_4, trace elements	*Pseudomonas putida*	Davis *et al*., 2015
CDM6	$(NH_4)_2HPO_4$, K_2SO_4, $MgSO_4 \cdot 7H_2O$, NaCl, trace elements solution, $FeSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot 5H_2O$, $CaCl_2 \cdot 2H_2O$, $Na_2B_4O_7 \cdot 10H_2O$, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	*Pseudomonas putida*	Ramadan *et al*., 2013
CDM7	Glucose, sucrose, peptone, yeast extract, corn steep, KH_2PO_4, Na_2HPO_4 $MgSO_4 \cdot 7H_2O$, steep liquid	*Rhizobium radiobacter*	Wu *et al*., 2003
CDM8	Glucose, L-glutamate, yeast extract, KH_2PO_4, $(NH_4)3PO_4$, $MgSO_4$, NaCl, $CaCl_2$, $MnSO_4$, $ZnSO_4$, $FeSO_4$	*Bacillus subtilis*	Tavares *et al*., 2013
NFB	Malonic acid, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, NaCl, $CaCl_2$, $FeSO_4$, Na_2MoO_4, $MnSO_4$, KOH, NH_4Cl, H_3BO_4	*Azospirillum brasilense*	Trujillo-Roldán *et al*., 2013
Modified NFB blue	Malic acid, K_2HPO_4, $MgSO_4$, NaCl, $CaCl_2$, Na_2MoO_4, $MnSO_4$, FeEDTA, glycerol, bromothymol blue, KOH, biotin	*Azosprillum brasilense*	Leo Daniel *et al*., 2013
MTM	Tryptone, yeast extract, *D*-glucose, NaCl, $MgSO_4 \cdot 7H_2O$, K_2HPO_4 $CaCl_2$, K_2SO_4, Na2SO4, $NaHCO_3$, Na_2CO_3, Fe(III), EDTA	*Azospirillum brasilense, Azospirillum lipoferum*	Bashan *et al*., 2011
JMM	Sucrose, K_2HPO_4, $MgSO_4$, NaCl, $FeSO_4$, Na_2MoO_4, glycerol	*Azotobacter chrococcum*	Leo Daniel *et al*., 2013
Modified Pikovaskaya broth	Yeast extract, dextrose, $Ca_3(PO_4)_2$, KCl, $MgSO_4$, $MnSO_4$, $FeSO_4$, $(NH_4)_2SO_4$, glycerol	*Bacillus megaterium*	Leo Daniel *et al*., 2013
L-malate minimum	L-malate, tryptophan and tetracycline	*Azospirillum brasilense*	Ona *et al*., 2005
CDM9	Malic acid, NaOH, $MgSO_4 \cdot 7H_2O$ $CaCl_2$, NaCl, NH_4Cl, yeast extract, $FeCl_3$, $NaMoO_4 \cdot 2H_2O$, $MnSO_4$, H_3BO_3, $Cu(NO_3)_2 \cdot 3H_2O$, $ZnSO_4 \cdot 7H_2O$, K_2HPO_4, KH_2PO_4	*Azospirillum brasilense, Azospirillum lipoferum*	Bashan *et al*., 2011

[1]DSM: Difco sporulation medium. CDM: Chemically defined medium. NFB: Nitrogen free bromothymol. MTM: Modified tryptone medium. JMM: Janson's Modified medium.

increase the life span and effectiveness of the fertilizer. Glucose, sucrose, maltose, trehalose, molasses and glycerol are some of the cellular protectors that are often used (Garcia-Fraile *et al.*, 2015). An ideal carrier should be resistant to the external environment, biodegradable, economic and easily available. It should extend the lifespan of the formulation and have a good buffer capacity (Leo Daniel *et al.*, 2013; Garcia-Fraile *et al.*, 2015).

In general, carriers can be divided into organic and inorganic, based on the nature of their composition. Based on their state, formulations can be liquid or solid (Nakkeeran *et al.*, 2005). Microorganisms can be formulated in the form of concentrated dry or wet dust, granules and briquettes which are easy to store, transport and apply. Dry powder and dry granules are the best choices for spore forming microorganisms,

Table 4. Cultivation of plant growth promoting bacteria

Type of microorganism/ Cultivation type	Medium[1]/ Substrate	Optimum experimental conditions				Results/Conclusions	Reference
		Temperature (°C)	Shaking speed (rpm)	Incubation time (h)	Aeration rate/ O_2 concentration		
Bacillus subtilis/ Shaken flasks	DSM, CDM1/ glucose, vitamins, ammonium-sulphate	37	150	48	-	-Vitamins affect the sporulation significantly. -There is no sporulation without thiamine. -By adding glucose up to 20 $g \cdot L^{-1}$ sporulation increases.	Monteiro *et al.* (2014)
Bacillus subtilis/ Batch	DSM/ CDM1	37	100-1200	48	2 $L \cdot min^{-1}$ 30%	Maximum vegetative cells concentration ($1.3 \cdot 10^{10}$ $cells \cdot mL \cdot L^{-1}$) has been achieved at the end of the exponential phase. -Cell lysis has been noticed. 48% of cells formed spores with final concentration: $6.3 \cdot 10^{9}$ $spores \cdot L^{-1}$	Monteiro *et al*. (2014)
Bacillus subtilis/ Fed-batch (3 phases)	DSM/ CDM1/ glucose, ammonium-sulphate	37	100-1200	48	2 $L \cdot min^{-1}$/ 30%	-An excess of glucose compared to ammonium prevents the lysis of cells and allows the cells to sporulate. -Best result ($3.6 \cdot 10^{10}$ $spores \cdot mL \cdot L^{-1}$) is achieved with a glucose concentration of 20 $mg \cdot L^{-1}$, ammonium concentration under 900 $mg \cdot L^{-1}$ and with the addition of Ca.	Monteiro *et al.* (2014)
Bacillus subtilis/ Shaken flasks and Petri dishes	DSM or F medium	37	200	24 and 72	-	The best result was achieved on F medium after 72 h (more than 10^{9} $spores \cdot mL^{-1}$ after purification)	Tavares *et al.* (2013)
Bacillus subtilis/ Batch	CDM2	30	200-300	72	-	Obtained cell concentration was $5 \cdot 10^{8}$ $CFU \cdot mL^{-1}$	Ilić (2016)
Bacillus subtilis/ Fed-batch (2 phases)	CDM2/ glucose, Ca	30	200-300	72	-	Obtained cell concentration was $5 \cdot 10^{8}$ $CFU \cdot mL^{-1}$	Ilić (2016)
Bacillus megaterium/ Batch	CDM3	30	200-300	72	-	Obtain cell concentration was $1.55 \cdot 10^{9}$ $CFU \cdot mL^{-1}$	Ilić (2016)
Bacillus megaterium/ Fed-batch (2 phases)	CDM3	30	200-300	72	-	Obtain cell concentration was $7 \cdot 10^{9}$ $CFU \cdot mL^{-1}$	Ilić (2016)
Bacillus megaterium var, phospaticum/ Shaken flasks	Modified Piko-vaskaya broth	28	200	96	-	-Addition of PVP 2% has influenced better cell maintenance. -Achieved cell concentration: $3.7 \cdot 10^{10}$ $CFU \cdot mL^{-1}$	Leo Daniel *et al.* (2013)
Bacillus licheniformis/ Fed-batch (4 phases)	CDM4	45	1500	-	-	Starving period has increased the number of cells with de-polarized membrane, which indicates that the stress was caused by lack of glucose.	Reis *et al*. (2005)

Table 4. Continued

Type of microorganism/ Cultivation type	Medium[1]/ Substrate	Optimum experimental conditions				Results/Conclusions	Reference
		Temperature (°C)	Shaking speed (rpm)	Incubation time (h)	Aeration rate/ O_2 concentration		
Azospirillum brasilense/ Shaken flasks	NFB	30	100-250	-	-	The best growth has been noticed at shaking speed of 220 rpm and volumetric mass transfer coefficient, K_{La}=31 h^{-1}	Trujillo-Roldán *et al.* (2013)
Azospirillum brasilense/ Batch	NFB	30	205	-	5 L·min^{-1}	Final biomass concentration was not affected by the change of K_{La} (83 h^{-1}), although growth rate was reduced by 35%.	Trujillo-Roldán *et al.* (2013)
Azospirillum brasilense/ Pilot-scale submerged system (capacity:1400 L)	NFB	30	52	-	500 L·min^{-1}	Achieved cell concentration: 3.5-7.5·10^8 CFU·mL^{-1}	Trujillo-Roldán *et al.* (2013)
Azospirillum brasilense/ Batch	L-malate minimum	30	50-500	-	3%	Aerobic growth inhibits the synthesis of indole-3-acetic acid. Tryptophan is necessary for cell multiplication and production of indole acetic acid	Ona *et al.* (2005)
Azospirillum brasilense/ Fed-batch	L-malate minimum	30	50-500	-	3%	During semi-continuous production, there were no significant quantities of indole acetic acid.	Ona *et al*. (2005)
Azospirillum brasilense/ Shaken flasks	Modifed NFB blue	28	200	96	-	3.88· 10^{10} CFU·mL^{-1} and 3.56·10^8 CFU/mL were achieved using PVP 2% and CMC 0.1 %, respectively.	Leo Daniel *et al.* (2013)
Azospirillum brasilense and *Azospirillum lipoferum*/ Shaken flasks	Nutrient agar, OAB medium, MTM/ glycerol, Na-gluconate	36	120	24	-	Highest cell number (10^{11} cells·mL^{-1}, 10^9 CFU·mL^{-1}) was obtained on a medium containing natirum gluconate and glycerol, which significantly improve bacterial growth.	Bashan *et al.* (2011)
Azotobacter chrococcum/ Shaken flasks	Modifed Jenson's medium	28	200	96	-	4.1·10^8 CFU·mL^{-1} and 4.64·10^{10} CFU·mL^{-1} have been achieved using 0.1% CMC and 2% PVP, respectively.	Leo Daniel *et al.* (2013)

Table 4. Continued

Type of microorganism/ Cultivation type	Medium[1]/ Substrate	Optimum experimental conditions				Results/Conclusions	Reference
		Temperature (°C)	Shaking speed (rpm)	Incubation time (h)	Aeration rate/ O_2 concentration		
Pseudomonas putida/ Batch	Glucose and NH_4Cl	30	324	-	-	-Specific growth rate, μ=0.67 h^{-1}. -Yield coefficient on biomass on substrate, =0.45 $g \cdot g^{-1}$ -Yield coefficient on biomass on oxygen, =1.2 $g \cdot g^{-1}$ -Cell dry weight, CDW= 3.5 $g \cdot L^{-1}$ (after 7 h).	Davis *et al.* (2015)
Pseudomonas putida/ Fed-batch, exponential feeding, 3 procedures	CDM5/ glucose, $(NH_4)_2SO_4$	-	500	-	7 $L \cdot min^{-1}$	-Procedure 1: CDW= 53 $g \cdot L^{-1}$ (for 22 h) μ=22 h^{-1} ; -Procedure 2: CDW=102 $g \cdot L^{-1}$, biomass productivity: 3.1 $g \cdot L^{-1} \cdot h^{-1}$ (for 33 h) -Procedure 3: Maximum specific growth rate, μ_{max} =0.67 h^{-1} (after 9 h), CDW= 50 $g \cdot L^{-1}$. Highest biomass productivity: 2.4 $g \cdot L^{-1} \cdot h^{-1}$ for 18 h (43 $g \cdot L^{-1}$).	Davis *et al.* (2015)
Pseudomonas putida/ Batch	CDM6/ glucose and $(NH_4)_2H$-PO_4	32	1000	40	-	The most productive medium is the one containing the highest amount of glucose and salt. Achieved growth was 114 g dry cells $\cdot L^{-1}$	Ramadhan *et al.* (2013)
Rhizobium radiobacter/ Shaken flasks	Glucose, peptone, yeast extract, NaCl	30	200	24	-	High concentration of dissolved oxygen favor the cell growth	Wu *et al.* (2003)
Rhizobium radiobacter/ Fed-batch	CDM7	30	400-1500		0.5-2.5 v^{-1} v^{-1} min^{-1}	50% better results were achieved in fed-batch conditions (CoQ10 concentration: 51.1 $mg \cdot L^{-1}$ and *CDW*= 23.9 $g \cdot L^{-1}$).	Wu *et al.* (2003)

[1]DSM: Difco sporulation medium. CDM: Chemically defined medium. NFB: Nitrogen free bromothymol. MTM: Modified tryptone medium. JMM: Janson's Modified medium.

while wet dust and granules contain metabolically active microorganisms (Ramadan *et al.*, 2013; Mishra & Arora, 2016). On the other hand, liquid formulations contain different compounds apart from microorganisms. Those compounds protect the cell and support prolonged cellular life and resistance to environmental conditions (Herrmann & Lesueur, 2013). Liquid formulations are typically water-based, oil-based on polymer products. Polysaccharides such as resin, CMC and polyvinyl alcohol derivatives are often used to change the characteristics of the fluid in liquid formulations (Shaikh & Sayyed, 2015).

When it comes to organic carriers, peat and compost are used the most. When peat and microorganisms are mixed, microorganisms maintain their metabolic activity and in some cases continue to multiply during storage (Bashan *et al.*, 2014; Figueiredo *et al.*, 2016). Similar shortcomings can be cited for plant waste material which is generally not used for commercial purposes. On the other hand, sawdust

and other waste material from the wood-woodworking industry have found wide application in other forms of biotechnological processes which include the use of microorganisms, such as bioremediation, where they function as carriers, but also as an alternative source of carbon and energy (Ramadan *et al.*, 2013). Furthermore, compost can be considered as a possible carrier, especially for the production processes that include specific cells. For example, the addition of diazotrophs or phosphate-solubilizing bacteria in the compost increases the amount and availability of the nitrogen and phosphorus in the final product (Malusá *et al.*, 2012).

Polymers are new materials among carriers for microbial fertilizers which encapsulate or "immobilize" microorganisms and release them gradually in the process of degradation (Herrmann & Lesueur, 2013). Even though they offer numerous advantages, the viability of the inoculant is still an issue. This is why the addition of nutrients into inoculant is being studied and tested to enable the prolonged life span of the microorganism (Díaz-Barrera *et al.*, 2011). Among them, hetero copolymers excite great interest. They are the result of grafting natural products such as microbial produced levan and polystyrene, which combine the best features of both types of material, and where the final product has better features than the initial components with an increased biodegradability (Kekez *et al.*, 2016).

Current state and perspectives

Even though many researches confirm the beneficial effect of PGPMs on plants and there is an abundance of tested inoculums with various combinations of microorganisms (some of them commercially available), the results are not always consistent and in some cases unexpected (Shaikh & Sayyed, 2015; Abhilash *et al.*, 2016; Keswani *et al.*, 2016). Study taken in Belgium during the 1999-2000 season, tested out inoculation of winter wheat with *Azospirillum brasilense* and *Azospirillum iraknse*. Although early studies during growth season proved positive effect in terms of plant and root dry weight and number of shoots, the ultimate result was not that encouraging. At the end of the season, due to bad weathering conditions, final yield of winter wheat didn't reach its full potential. Similar results have been witnessed in Mexico, implying the main barrier to commercial application of PGPMs (Dobbelaere *et al.*, 2001).

Experience has proven that microbial fertilizers are not accepted to a great extent by agricultural producers because it is not easy to replicate their effect on the field. It is obvious that if the inoculant is not made or applied in the appropriate manner, the useful features of the fertilizer will not be manifested (Mishra & Arora, 2016). Reduced number of viable cells and bad colonization of the root have been noted in the case of many biofertilizers available on sale. A contamination of the inoculant was also noted in many of commercially available products, which leads to a lack of trust in microbial fertilizers and influences their commercial potential (Vassilev *et al.*, 2015). Handling these products requires attention wherefore improper use represents the main reason that such a large number of microorganisms give good results in laboratory conditions, but not in the field, and pretty much explains why the commercialization of these products is not easy (Bashan *et al.*, 2014).

There is a proposal to determine the natural microbiome of each plant and to design microbial fertilizer especially for that specific plant, based on its needs and environment (Chebotar *et al.*, 2015). A study about inoculation of wheat with *Azospirillum* sp. clearly indicates that strains isolated from wheat roots performed better in the experiment when compared to strains isolated from other places (Boddey *et al.*, 1986). Certain authors have given a suggestion for developing fertilizers that will be enriched with different metabolites along with microorganisms, according to plants requirements (Marks *et al.*, 2015). Also, it is considered that the biofertilizers will have a greater application in the future if we are able to develop inert material that can increase product stability, lengthen its life span and its effectiveness in the field itself (Abhilash *et al.*, 2016). One of the ways in which these problems could be overcome is the development of the so-called "tailor-made" products, which will be structured according to the needs of the buyers and can lead to a greater price of the final product (Shaikh & Sayyed, 2015). In addition, it is interesting to note that most or researches are mainly focused on the characterization of microorganisms that are suitable for plants as well as on the further application in the soil-plant system in controlled conditions. Processes of mass multiplication and formulating procedures have been studied to a much smaller extent, even though they have the most significant importance for the effectiveness of the product (Vassilev *et al.*, 2015).

Conclusion

Microbial fertilizers have been in a focus of researches for quite a long time. They are considered to be ecologically acceptable alternative to chemical fertilizers and agrochemicals, which are overused and harmful to the environment. Although this idea is not brand new and has been subject of plenty of scientific papers for years now, many questions still remain unanswered and there is a lot of place for improvement. The production of microbial fertilizers does not depend

solely on the detailed knowledge of the physiology of plants and microorganisms, but also on the large number of technological challenges such as the fermentation process, type of formulations, the population of microorganisms and their system of release. Thus, the development of a stable bioformulation is possible through combining knowledge from microbial and technical aspects. Additional research is necessary in order to enhance the production process and, what's most important, to improve the products reliability and practical usage. One thing is certain: since they are ecologically acceptable, biofertilizers will have a very significant function in modern agriculture.

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