Rhizobial Bioformulations: Past, Present and Future

4

Naveen Kumar Arora, Maya Verma, and Jitendra Mishra

Abstract

The nitrogen fixing bacterial group known as rhizobia are very important and are used as biological fertilizers for two main purposes; one is to fulfil the nutritional requirements of increasingly populated world and other to overcome the problems arising due to chemical fertilizers. Rhizobial bioformulations are in the market since more than a century and can be the solution for deficiency of nitrogen in our food and soils. Rhizobia maintain the soil fertility along with higher crop yields due to the capability of biological nitrogen fixation (BNF). Currently, various types of rhizobial biofertilizers are commercially available in the market all over the world for agricultural purposes. These can be solid carrier based formulations (organic and inorganic), liquid formulations (with and without additives), synthetic polymer based formulations or metabolite based formulations, but there still is a great room for improvement. However, over the years there have been subtle changes in the rhizobial inoculants in terms of production and application.

4.1 Introduction

Nitrogen (N) is one of the most important nutrients for plant growth. About 1–5 % of total plant dry matter consists of N. It is an essential constituent of proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites (Hawkesford et al. 2012). Due to its immense cellular need, N is required in large quantities. Although atmosphere contains 80% of dinitrogen (N_2) (Abd-Alla et al.

N.K. Arora (✉) • M. Verma • J. Mishra
Department of Environmental Microbiology, BBA University,
Lucknow 226025, Uttar Pradesh, India
e-mail: nkarora_net@rediffmail.com

S. Mehnaz (ed.), *Rhizotrophs: Plant Growth Promotion to Bioremediation*,
Microorganisms for Sustainability 2, DOI 10.1007/978-981-10-4862-3_4

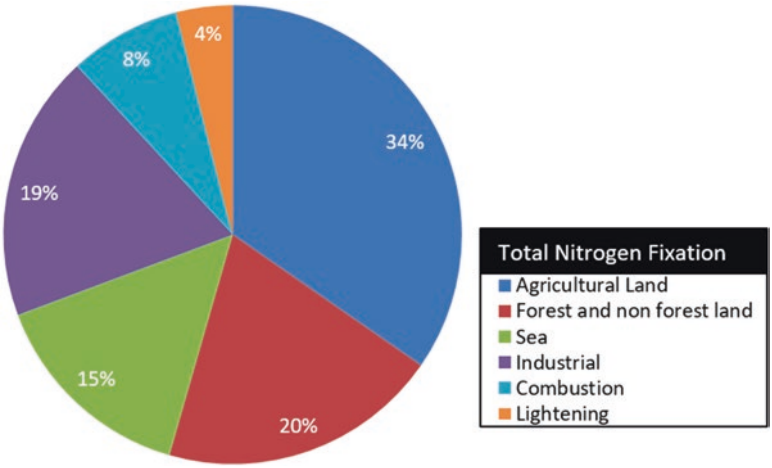

Fig. 4.1 Nitrogen fixation on earth (Modified from Bezdicek and Kennedy 1998)

2014a), it is relatively inert, and most organisms are unable to utilize it (Allito et al. 2015). Generally, the most reduced form of N that is ammonia (NH_3) or most oxidized form, nitrate (NO^{-}_3) are required to fulfil needs of plants. The fixed form of N is generated by conversion of N_2 to NH_3, a process also known as nitrogen fixation. Nitrogen fixation may occur by biological or non-biological means (Fig. 4.1). Non-biological fixation includes geochemical fixation by lightning (10% of the total N_2 fixation) (Bezdicek and Kennedy 1998) and industrial fixation by Haber-Bosch process (15% of total N_2 fixation) (Bezdicek and Kennedy 1998). However, BNF is carried out by some prokaryotes, including a small but diverse group of bacteria and archea, commonly referred as diazotrophs (Zehr et al. 2003; Kneip et al. 2007) (Fig. 4.2). They encode enzyme complex nitrogenase, that catalyses the conversion of N_2 gas to NH_3 (Santi et al. 2013). Amongst all of the fixed form of N_2, BNF is of immense importance. It is estimated that over half of the fixed N_2 is supplied biologically and has a profound agronomic, economic, and ecological impact (Smil 2001). Although BNF is a boon to agro-ecosystems, but N fertilizers are also used for meeting the total N requirement for agricultural production throughout the world. That is why a substantial increase has been noticed in demand for chemical N fertilizers as in comparsion to a century ago (Galloway et al. 2008). The increased use of chemical N fertilizers is problematic because it is susceptible to loss by leaching or denitrification (Luce et al. 2011) and regarded economically and environmentally undesirable. Moreover, manufacturing N fertilizers requires six times more energy than other fertilizers such as that of phosphorus or potassium (Da Silva et al. 1978).

There are various types of associations/interactions occurring between diazotrophs and their host plants (Santi et al. 2013). Amongst them most efficient are diazotrophic bacteria known as rhizobia, involved in the formation of root nodules in legumes (Santi et al. 2013). Currently, more than 98 species belonging to 14

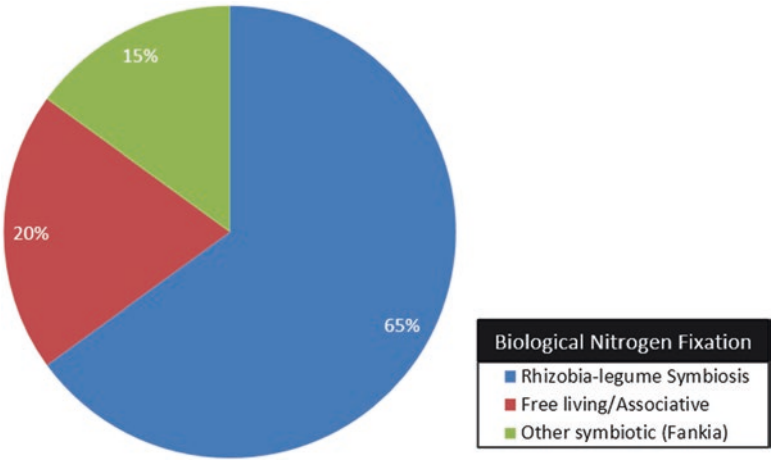

Fig. 4.2 Biological nitrogen fixation (Modified from Bouizgarne et al. 2015)

genera of α, β and ϒ proteobacteria have been described as rhizobia (Berrada and Fikri-Benbrahim 2014). Till date 12,000 nodulated legume species are known, and each has its own root nodulating partner(s) (Maróti and Kondorosi 2014). Legumes are second largest group of food and feed crops. These represent the third largest family of angiosperms and cover 12–15% of all available arable land which contributes more than 25% in the world's primary crop production (247 million tons of grain legumes annually; Ferguson et al. 2010). Rhizobia-legume symbiosis provides approximately 40 million tons of nitrogen into agricultural systems each year (Herridge et al. 2008) and plays a crucial role in increasing productivity and quality of crops specially protein content (Krapp et al. 2011). Besides this, these have been recognized as potential candidates to replace mineral N-fertilizers (Tairo and Ndakidemi 2013). It is estimated that the value of total nitrogen fixed by BNF process is equal to US $ 160–180 billion (Rajwar et al. 2013). Mostly the agricultural legumes have been studied for their symbiotic partner and others including wild and with little economic value are neglected (Ogasawara et al. 2003).

In the era of intensive agriculture, rising costs of N fertilizers and their adverse effects on environment have posed a threat to agroecosystems. Using rhizobial inoculants in the form of biofertilizers in place of N fertilzers has been considered as cheap and sustainable alternative (Arora et al. 2001; Kennedy et al. 2004; Mia et al. 2007). Use of biofertilizers is becoming more popular at the global level. The market trends also indicate that this could further increase in near future. The global biofertilizers market was estimated at US $ 535.8 million in 2014 and projected to reach up to US $ 1.88 billion by 2020 (Markets and Markets 2015). Amongst all types of biofertilizers, nitrogen fixers contribute maximum (75%) in agriculture (Grand View Research 2015) (Fig. 4.3).

Rhizobial biofertilizers have been applied to crops since more than a century. However, in last few years, research on field application of rhizobia has addressed

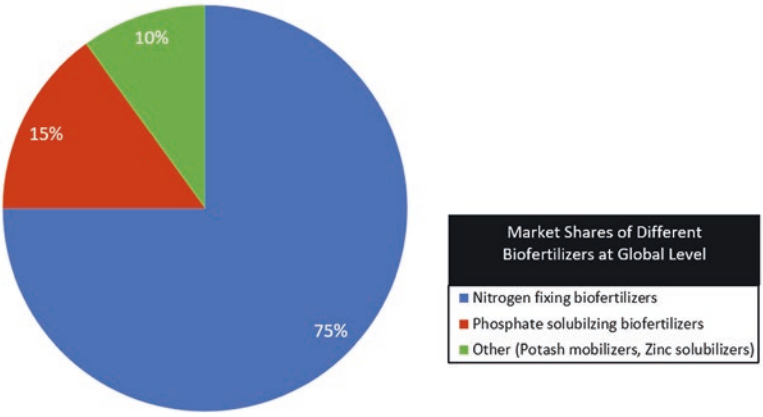

Fig. 4.3 Market share of different types of biofertilizers at global level (Grand View Research 2015)

the task of identifying the essential conditions required for its survival, usability and quality in the formulation products for enhancing crop production. Generally, the term bioformulation entails application of microorganism(s) as partial or complete substitute for chemical fertilizers/pesticides (Arora et al. 2010) but in a broad sense it is essential to define role of an active ingredient, a carrier material and an additive in preparation of bioformulation (Mishra and Arora 2016). Various types of bioformulations having different rhizobial species as active ingredients are being used, and indeed, have a profound effect on crop N requirement. However, newer techqinues for identification of different carriers, additives and delivery systems have provided robustness to convential bioformulations and show potential to subside the current need of mineral N fertilizers. In the present review, the journey of development and advances in rhizobial inoculants since their inception in the market, are discussed.

4.2 History

Legumes have been used as a food source since ancient times and also known as soil improvers. However, real role of rhizobia was identified much later. German botanist Leonhard Fuchsius, published the first drawings of nodulated legumes in 1542 (Fuchsius 1542). Malpighi (1679) also observed nodules on the bean roots (*Phaseolus vulgaris* and *Vicia faba*). BNF by legumes was first time proposed by Boussingault (1838) when he was doing crop rotation experiments with legumes and found increased N content causes superior nutritive quality in legumes and benefits to the soil. Lachmann (1858) during the microscopic study of nodules found that the nodules contain vibrio like particles. Further, these particles were also described as bacteria like and Woronin (1866) validated that root nodules in legumes were formed by a specific group of bacteria. Another milestone in the field of rhizobia-legume symbiosis was the discovery of the ability of root nodules to fix

gaseous N which was demonstrated by German scientists Hellriegel and Wilfarth, in 1886 and two years later, they published their observations (Hellriegel and Wilfarth 1888). In the same year Dutch microbiologist Beijerinck, first time isolated a bacterium from root nodules and named it as *Bacillus radicicola* (Beijerinck 1888) and later Frank renamed it *Rhizobium leguminosarum* (Frank 1889).

The first commercial N biofertilizer of rhizobia, 'Nitragin' was patented by Nobbe and Hiltner (1896). Famous agricultural chemist Guthrie stated about rhizobial inoculants "one of the most valuable contributions ever made by science to practical agriculture" (Guthrie 1896). In the nineteenth century, Löhis and Hansen (1921) classified the rhizobia into two groups according to their growth patterns as slow growers and fast growers. Baldwin and Fred (1929) proposed the cross inoculation between some leguminous host plants and rhizobia. This cross inoculation concept was suggested for taxonomic characterization of rhizobia, based on cross inoculation groups (Eckhardt et al. 1931; Fred et al. 1932).

Before the discovery of rhizobia, the inoculation of seed or soil was done in the crop by "soil transfer method", in which soil from legume grown field to field or field to seed were applied before planting (Fred et al. 1932). However, artificial inoculation techniques by using pure cultures on agar slants and broths also began (Nobbe and Hiltner 1896; Fred et al. 1932). Inoculant production techniques started to change from those of the early 1900s, and use of solid carrier based formulations were started for legume inoculants, which were developed to enhance the shelf life and field efficacy of inoculants. In this context, soil and peat formulations were used (Fred et al. 1932). Peat was the most important carrier for long term storage of inoculants because of some beneficial properties, e.g., high water holding capacity, chemical and physical evenness, non-toxic and environment friendly nature (Ferreira and Castro 2005). Although peat was being used as the most common type of carrier for rhizobia based inoculants (Bezdicek et al. 1978; Khavazi et al. 2007; Albareda et al. 2008) but some constraints were also reported, such as it may contain inhibitory factors for the microbes (Brockwell 1985) and lack of availability in many parts of the world. Hence, the interest in using other carrier materials was also on. The carrier materials such as lignite (Kandasamy and Prasad 1971), filter mud (Philpotts 1976), coal-bentonite mixture (Deschodt and Strijdom 1976), cellulose (Pugashetti et al. 1971), coal (Crawford and Berryhill 1983), bagasse, wheat straw, compost of coir dust and soil, charcoal, manure, compost, powdered coconut shells, ground teak leaves also started to find their way in rhizobial inoculants (Tilak and Subba Rao 1978). Solid carrier based inoculants were also developed as granular inoculants for direct application in soil (Brockwell et al. 1980), and key benefits of this technique were easy storage, handling, and application (Smith 1992).

It is reported that liquid based formulations for rhizobia were developed as alternatives and were effective (Van Schreven et al. 1953; Singleton et al. 2002). Smith et al. (1981) reported that nodule numbers increased when applied with liquid rhizobial inoculants. The freeze-dried inoculants based on lyophilization techniques were first time identified as commercially beneficial in 1958 (Brockwell 1982). Bonish (1979) applied diluted soil samples to inoculate clover seedlings growing in lab conditions. At that time gel based microbial inoculants were also developed as

alternatives to powdered carrier-based inoculants by entrapping rhizobia in polymer gels such as polyacrylamide-entrapped *Rhizobium* (PER) (Dommergues et al. 1979), alginate-entrapped *Rhizobium* (AER), xanthan-entrapped *Rhizobium* (XER); which gave satisfactory results in wet conditions (Jung et al. 1982). Kremer and Peterson (1982) reported that freeze-dried rhizobia suspended in dried oil were resistant against high soil temperatures. Brockwell et al. (1988) found whole soil inoculation technique (WSIT) suitable for clover production. In this method, rhizobial populations of soil were used to inoculate plants for assessing the N_2 fixing capability of that soil. Thies et al. (1991) developed simple functions to predict the need for inoculation based on numbers of rhizobia in the soil and soil nitrate levels.

Use of flavonoids (genistein/daidzein) for enhancing soybean yield was patented by Smith and Zhang (1999) which was commercialized as SoyaSignal™ (Leibovitch et al. 2001). Ballard and Charman (2000) used the Brockwell technique (WSIT) to evaluate the symbiotic N_2-fixing potential of soil samples. Other formulation types also appeared in the market, such as the vermiculite-based Gold Coat TM *Rhizobium* inoculant (Paau et al. 1991), liquid seed-applied soybean inoculant, Cell-Tech® (Smith 1995), liquid in-furrow inoculant LIFT (Smith 1995) and air-dried clay powder for alfalfa, Nitragin® Gold (Smith 1995). It can be said that rhizobial species have been successfully marketed globally and inoculants are produced and used in many countries in all continents of the world (Nelson 2004).

Hussain et al. (1995) introduced precursor based inoculum technology for growth enhancement of lentil crop in which indole acetic acid (IAA) precursor (tryptophan) was added with inoculum. The commercialisation of a genetically engineered strain of *Sinorhizobium meliloti* was approved in 1997 (EPA 1997). After few years, modifications in liquid inoculants were proposed; Singleton et al. (2002) developed additives and cell protectants based liquid formulations for improved growth performance. The additives promote cell survival in storage and after application to seed or soil (Singleton et al. 2002). Commonly used additives for rhizobial inoculants were polyvinyl pyrrolidone (PVP), carboxymethyl cellulose (CMC), gum arabic, sodium alginate and glycerol. PVP is a synthetic vinyl polymer that improves survival of rhizobia by protecting it from desiccation and also from harmful seed coat exudates (Singleton et al. 2002). The CMC has important rheological property and increases the gel viscosity of carriers to make it more suitable for viability of rhizobial cells (Rohr 2007). Gum arabic is a complex carbohydrate extracted from Acacia and commonly used as adhesive to protect the rhizobia against desiccation (Wani et al. 2007). Sodium alginate is non-toxic compound and used to enhance the survival of inoculant because it has limited heat transfer property and high water activity (Jung et al. 1982). Glycerol is used as additive because it protects rhizobial cells from desiccation by slowing the drying rate (Manikandan et al. 2010).

Besides above mentioned carriers for rhizobial inoculants, waste water sludge was also used as a carrier and it was firstly reported by Ben Rebah et al. (2002a). There are various other types of formulations with different carriers developed for rhizobial inoculants and some of them have been patented, e.g., the patent no. 521.850 (Belgian) for *Rhizobium* which uses diatomaceous earth and colloidal silica; the British patent no. 1.777.077 for the use of bentonite for *Rhizobium*. For

more advancement in formulation techniques, genetic modification of rhizobia is also being done to increase the efficiency of N_2 fixation such as genes that regulate the Hup system (recycles the hydrogen released during nitrogen fixation) have been identified and transferred (Brito et al. 2002).

From the beginning of twentieth century, extensive research has been carried out for development of state of the art rhizobial bioformulations and advents of newer techniques have provided inputs in this direction. There are various modifications related to rhizobial inoculants that have been done since their inception and the major events in the history are compiled in Fig. 4.4. The rhizobial inoculants are being used from long time in diverse types of formulations and detail of some formulations are given in Table 4.1.

4.3 Present Scenario

The application of rhizobial bioformulations is one of the cheapest and eco-friendly approaches for improving production of leguminous plants and fixation of atmospheric nitrogen (Thakare and Rasal 2000). It has been estimated that 2000 tons of rhizobial inoculants of worth US$ 50 million are produced worldwide every year (Ben Rebah et al. 2007) and this quantity is sufficient to inoculate 20 million hectares of legumes (Herridge et al. 2002). Currently, there are various types of rhizobial bioformulations available in the market for agricultural purposes and all are categorized mainly into two major groups - solid formulations and liquid formulations (Burges and Jones 1998). Peat is still most common carrier material in rhizobial inoculant production (Kaljeet et al. 2011) and other carrier materials such as coal, bagasse, coir dust, perlite are also used (Albareda et al. 2008). In a recent study, Ruíz-Valdiviezo et al. (2015) worked on the granular peat based and perlite based bioformulations. The additive based solid inoculants are also used such as sawdust based formulations amended with CMC (Aeron et al. 2012).

Liquid formulations are used for legume inoculation as more suitable technique for mechanical sowing in large areas (Fernandes-Júnior et al. 2009). Liquid formulations typically contain aqueous, oil or polymer based products and these formulations may have the desired strain and its nutrients, which are more tolerant to adverse conditions (Brahmaprakash and Sahu 2012). One of the methods for liquid formulations is water-in-oil emulsions (Vandergheynst et al. 2007), which is beneficial for desiccation sensitive organisms as it slows down water evaporation. Currently, additive based liquid formulations are in greater use and demand (Rivera et al. 2014; Ruíz-Valdiviezo et al. 2015).

Polymer gel based formulation techniques and synthetic polymer based techniques are also in focus (Fernandes-Júnior et al. 2009). Synthetic formulations based on a mixture of polymers have been continuously investigated (Bashan 1998; John et al. 2011). Alvarez et al. (2010) used silica gel as efficient formulation technique for rhizobial inoculants. Denardin and Freire (2000) reported that blends of natural or synthetic polymers are able to maintain viability of rhizobial cells for over 6 months. For agricultural and environmental uses, these polymers include alginate,

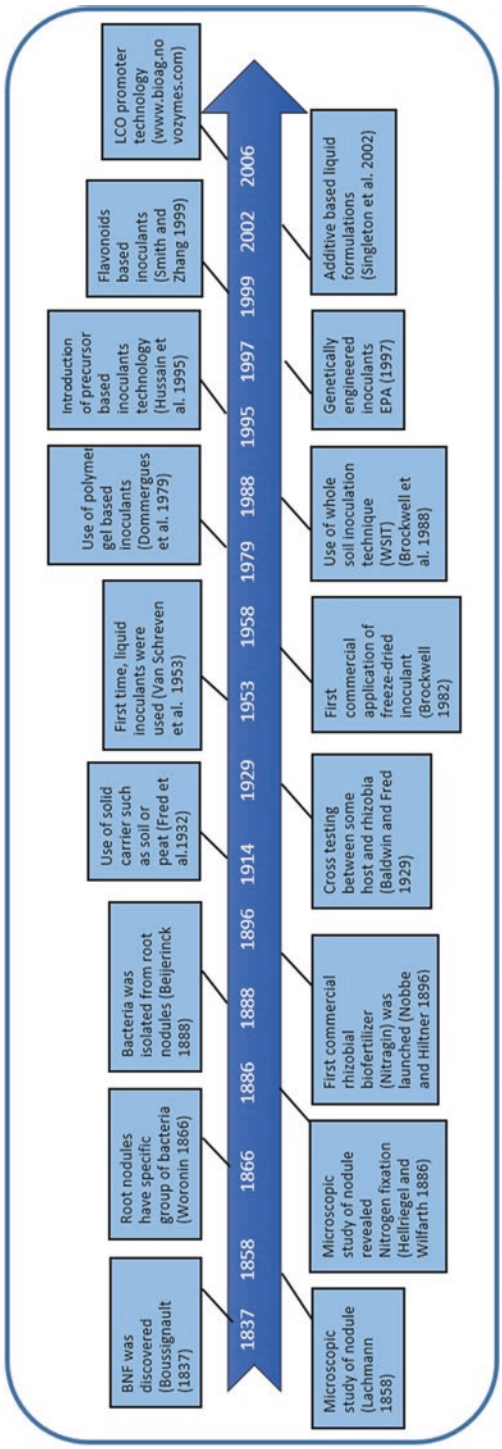

Fig. 4.4 Timeline of major discoveries in the development of rhizobial bioformulations

Table 4.1 Overview of some of the rhizobial bioformulations around the globe

Formulation type	Additives	Microorganism	Plant species	References
Liquid (culture media or water)	Glycerol, PVP, trehalose, FeEDTA	*Bradyrhizobium japonicum*	Soybean	Singleton et al. (2002)
	PVP; FeEDTA	Several rhizobia	Soybean	Albareda et al. (2008)
		B. japonicum		
	Unknown (commercial)	*B. japonicum*	Soybean	Maurice et al. (2001)
	Gum Arabic	*Bradyrhizobium* sp.; *Rhizobium* sp.	*Acacia mangium*, Greengram, *Leucaena leucocephala*	Diouf et al. (2003), Gamal-Eldin and Elbanna (2011), and Wani et al. (2007)
Organic inoculants (peat)	None or with undisclosed additives	*B. japonicum*;	Chickpea, faba beans, maize, pea, soybean	Clayton et al. (2004), Hamaoui et al. (2001),Hungria et al. (2010), Hynes et al. (2001), Khalid et al. (2004), and Revellin et al. (2000)
		Rhizobium sp.;		
	Applications as:	*Rhizobium leguminosarum*		
	Seed coating and pellets	*bv. viceae*		
	Vermiculite	Rhizobia	*Calliandra calothyrsus*	Kokalis-Burelle et al. (2003) and Odee et al. (2002)
	Pyrophyllite (hydrous aluminum silicate)	*Trichoderma virens* and *Burkholderia cepacia*	Bell pepper	Meyer et al. (2001)
	Arabic gum	Several *Rhizobium* and *Bradyrhizobium*	Bean, *Lupinus*, *Hedysarum*	Albareda et al. (2009) and Temprano et al. (2002)
Coir dust/coco peat vermicompost/ earthworm compost	Lignite	*Bacillus megaterium* and *R. leguminosarum*	Soybean	Raja Sekar and Karmegam (2010)
Sawdust	Composted by inoculation with *Cephalosporium* sp. and *Azospirillum Brasilense*	*B. japonicum, B. Arachis, R. meliloti, R. lotus*	Groundnuts, lucerne and a grass mixture of bird's foot trefoil and ryegrass, soybean	Kostov and Lynch (1998)

(continued)

Table 4.1 (continued)

Formulation type	Additives	Microorganism	Plant species	References
Sawdust	None	*R. leguminosarum* and *Pseudomonas fluorescens*	*Trifolium repense*	Arora et al. (2008)
Fibers from brewer's spent barley grain grape bagasse, cork compost	Gum Arabic, CMC	Several rhizobia; *B. japonicum*	Soybean	Albareda et al. (2008)
Wastewater sludge	Acid, alkaline and oxidative pre-treatments	*S. meliloti, R. leguminosarum* bv *viciae*, *B. japonicum* and *B. elkanii*	Not tested	Ben Rebah et al. (2002a, b)
Clay soil	Elemental sulfur	*Rhizobium* sp. and *Thiobacillus* sp.	Groundnut	Anandham et al. (2007)
Loess soil	None	Phosphate solubilizing bacteria (PSB) and *Rhizobium* sp.	None	Li et al. (2011)
Clay minerals, perlite	Gum Arabic, CMC	Several rhizobia; *B. japonicum;* *B. megaterium*	Soybean	Albareda et al. (2008)
Local soils	None	Rhizobia	*Calliandra calothyrsus*, rice	Hashem (2001) and Odee et al. (2002)
Perlite	Gum Arabic	*Rhizobium* and *Bradyrhizobium*	Bean, *Lupinus*, *Hedysarum*, Soybean	Temprano et al. (2002)
Alginate	None	*Rhizobium* spp.	*Leucaena* *Leucocephala*	Forestier et al. (2001)
CMC/corn starch	MgO	Rhizobia *Azospirillum amazonense*, *B. tropica*	Cowpea	Fernandes-Júnior et al. (2009)

Modified from Bashan et al. (2014)

agar, λ and κ carrageenan, pectin, chitosan, bean gum, and proprietary polymers (Bashan et al. 2014). Granular vermicompost, produced from essential oil bearing crop, scented geranium (*Pelargonium graveolens*), is used as efficient carrier for rhizobia (Ben Rebah et al. 2007). Some natural compounds such as cow urine and calliterpenone added to rhizobial bioformulations increased the numbers of rhizobial cells by tenfold when supplemented at 12.5–25.0 µl/ml (Kalra et al. 2010).

In case of rhizobial inoculants, mono-inoculation, co-inoculation or multistrain inoculation are also being used (Arora et al. 2014; Malusá and Vassilev 2014; Verma et al. 2014). The application of rhizobia in combination with other plant growth promoting rhizobacteria (PGPR) are used as suitable alternatives to promote plant growth both under normal and stress conditions, for example, plant growth promoting bacteria (PGPB) and rhizobia enhance nodulation, nitrogen fixation symbiotically (Rodrigues et al. 2013; Arora et al. 2014; Chibeba et al. 2015) and increase the grain yield by involving diverse mechanisms (Hungria et al. 2015). It is reported that Rubiya (2006) developed the "Multigeneric diazotrophic co-flocs" (*Azospirillum, Azotobacter* and *Rhizobium*) and reported good improvement in rice yield. The biofilm based inoculants containing a fungal-rhizobia consortium were also applied significantly for increased N_2 fixation in legumes (Jayasinghearachchi and Seneviratne 2004). The soil-made inoculants are also used by mixing clay soil inoculant with powdered elemental sulphur and inoculation of sulphur oxidizing bacteria (*Thiobacillus* sp.) with rhizobia synergistically promoted the yield and oil content of groundnut in sulphur-deficient soils (Anandham et al. 2007). Khare and Arora (2011) reported that on applying pyocyanin-producing pseudomonads together with rhizobia, an enhancement in nodulation ability is observed, which causes better growth and productivity of groundnut even in the presence of fungal phytopathogens. The consortium based liquid bioformulation technique is now being used as an important way for sustainable agriculture (Pindi and Satyanarayana 2013). Some of the recent trends include arbuscular mycorrhizal fungi (AMF) and rhizobia co-inoculation, which enhance the growth and yield of crops due to higher nutrient uptake (Abd-Alla et al. 2014b). AMF causes the enhancement in nitrogen uptake process by rhizobia and their association with legumes (Tajini et al. 2011). Meng et al. (2015) stated that AMF and rhizobia simplify the nitrogen uptake process and transfer in soybean/maize inter-cropping system. This AMF and rhizobia co-inoculation has great potential for stressed soils. Zhu et al. (2016) reported that AMF and nitrogen fixing bacteria enhance alfalfa yield under saline conditions.

There is also an emerging formulation technique in which the addition of microbial/plant associated secondary metabolites to bioformulations increases agricultural productivity by improving the inoculants efficiency (Morel et al. 2015). In the current market, metabolite based formulations for rhizobial inoculants are highly focused and the additions of flavonoids and phytohormones are being used for rhizobial inoculants. Plant inoculation with rhizobial cells, previously induced with flavonoids during growth, significantly alleviates the effects of adverse conditions (Nápoles et al. 2009; Muñoz et al. 2014). The addition of flavonoids to inoculated crops enhances the nitrogen fixation (Dashti et al. 2000), improves the rhizobial competitiveness and nodulation (Pan and Smith 2000). Although flavonoids are expensive, these act at very low concentrations and produced industrially for sustainable agriculture (Mishra and Arora 2016; Morel et al. 2016).

The phytohormones produced by microbes or plants show positive effects on plant growth (Hedin and McCarty 1994; Dazzo et al. 2000; Chandra et al. 2007; Tank and Saraf 2010; Kudoyarova et al. 2015). The inoculations of plants with phytohormones producing rhizobia have positive effects on plant development and seed

priming with phytohormones (IAA, gibberellins, abscisic acid and ethylene) increase the germination rate and finally crop yields (Roberto et al. 2012; Bianco et al. 2014; Kudoyarova et al. 2014; Qiu et al. 2014). Addition of phytohormones to bioformulations increases plant development and yield in comparison to bioformulations alone (Morel et al. 2016). Although various types of rhizobial bioformulations are commercially available in the current market having diverse types of additives, adjuvants and metabolites, it needs more exploration for future use.

4.4 Limitations: With the Current System

The demand of bioinoculants, mainly rhizobial inoculants, is high and these are produced commercially at global level (Brockwell and Bottomley 1995). Although rhizobial bioformulations have very significant value in agriculture, these also have some limitations. It is obvious that on application of inoculants in field, the introduced microbes face a very hostile environment and sometimes their population decreases, which leads to failure (Bashan and Levanony 1988; Arora et al. 2001). In some cases, applied rhizobial inoculants are unable to increase sufficient crop yield because of competitions faced by indigenous rhizosphere microflora of plants (Merwe et al. 1974; Olufajo and Adu 1993; Mazid and Khan 2014). Generally, predatory organisms, protozoans and bacteriophages are already present in the soil (Somasegaran and Hoben 1994). The environmental conditions also affect the inoculant efficacy and adverse abiotic stresses (hot, dry and saline conditions) can cause rapid decrease in rhizobial populations (Mazid et al. 2011; Deshwal et al. 2013). Other factors such as bacterial survival on the seed are mainly affected by three factors: desiccation, the toxic nature of seed coat exudates and high temperatures (Deaker et al. 2004). Bacterial survival on seed directly affects the total legume/crop yield (Brockwell and Bottomley 1995). Sterilization techniques of inoculant carrier (Strijdom and van Rensburg 1981) and compatibility towards crop also affect the applicability of inoculants (Lupwayi et al. 2006). Shelf life of inoculants is a very major factor for their efficacy which mainly depends on several factors (production technology, carrier and packaging material used, transport activity) to sustain the quality of inoculants (Arora et al. 2010). Often import and proper storage of inoculants are also problematic because in absence of proper care, viability of inoculants decreases with loss in their beneficial properties (Kaljeet et al. 2011). The storage of bioformulations needs special facilities and skills, which most producers, shopkeepers, and farmers do not possess (Arora et al. 2010). The use of genetically improved rhizobia as inoculants has some legislative constraints because it requires permission from environmental protection agencies to release into the environment and the second problem is less understanding of microbial ecology (Geetha and Joshi 2013).

All inoculant producers claim that their products promote crop productivity but actually most of the products are available in the market without robust scientific data to favour their efficacy (Herrmann et al. 2015). In this regard, Brockwell et al. (1995) stated that most of the inoculants (90%) have no practical impact on the yield of target crop. Similarly, Olsen et al. (1996) reported that commercially available

rhizobial inoculants lack proper population of rhizobial cells. Recently, Herrmann et al. (2015) analysed various inoculants and reported that more than 50% of the inoculants have high levels of contamination. It is also reported that contaminants have detrimental effects on the quality of rhizobial inoculants (Sparrow and Ham 1983; Rodriguez-Navarro et al. 1991) and 25% contaminants of the commercial inoculants can be opportunistic human pathogens (Olsen et al. 1996; Gomez et al. 1997).

The quality of inoculants is judged by their efficacy on application in field and if their quality and field performance is poor, then the product become unsuccessful in the market. Hence a lot of inoculants produced globally are of poor quality and the reason behind this issue is the lack of efficient quality control programmes (Somasegaran 1991; Brockwell and Bottomley 1995; Catroux et al. 2001). This drawback can lead to a negative impact on the future of the inoculant industry. In this context, various researchers suggested the need of an appropriate regulatory quality control program at international level for successful production and use of inoculants by end users (farmers) (Olsen et al. 1994; Herrmann et al. 2015; Arora et al. 2016). Thus, there is a requirement of strict regulations for rhizobial bioformulations to overcome above mentioned problems related with its worldwide productions and applications. All these factors have to be taken into accord to develop future rhizobial inoculants.

4.5 Future Prospects

The future of rhizobial formulations is directed to overcome or improve the lacunae associated with the present systems. As there are various types of rhizobial formulations available in the market, but each product has some limitations especially regarding their use, efficacy, survival or market availability. Hence, it is challenging task to develop a state of art formulation technology that fulfils all the required traits and make it available to farmers with global acceptancy. In this context, novel scientific approaches and information has also led to some good results and promise. For example, genotypic diversity of rhizobia can be assessed accurately by polymerase chain reaction (PCR) fingerprinting techniques such as enterobacterial repetitive intergenic consensus PCR (ERIC- PCR; Pongslip 2012), repetitive element palindromic PCR (rep-PCR; Menna et al. 2009) and enterobacterial repetitive sequences (BOX- PCR; Granada et al. 2014). These techniques help in the characterization of rhizobial strain which controls quality of inoculants (Pongslip 2012). Similarly, two primers random amplification polymorphic DNA (TP-RAPD) and amplified fragment length polymorphism (AFLP) are highly discriminating fingerprinting techniques and differentiate at species or below species level (Gzyl et al. 2005). However, emphases should also be given on techniques for increasing population density and survival of rhizobial strains in inoculants. Damasceno et al. (2013) devised electrospinning technique of rhizobia immobilization in nanofibers. They showed that encapsulation of rhizobial cell with polyvinyl alcohol (PVA) nanofibers enhances protection from dehydration and also minimized the effects of

toxic chemicals. Nanoparticles made of inorganic or organic materials may enhance the quality of carrier-based microbial inoculants (Malusa et al. 2012). Sivasakthivelan and Saranraj (2013) also stated that survival of cells is mandatory for better commercialization of rhizobial inoculants in the global market. Suman et al. (2016) used the hydrogel based inoculants and experimentally proved that this method has low-cost and long shelf-life and also increased plant development in drought-prone environments. There has been a conflict in choosing a carrier for rhizobia inoculant development, as the carrier choice differs greatly in different inoculants. It is clear that same basic carriers are still in use. Various workers addressed that to increase the inoculant quality and efficiency, and to reduce costs and environmental impacts, alternative carrier materials have to be explored (Ben Rebah et al. 2007; Albareda et al. 2008). Polymeric inoculants (Bashan et al. 2014) and alginate beads (Sivakumar et al. 2014) have already been tested and need more exploration for their future use in inoculants.

Recently, Arora and Mishra (2016) provided their view on using metabolites and additives in bioformulations. Metabolites such as EPS can be used as carriers and also can play role in protection of cells and help in the nodulation process. The rhizobia nodulation genes *nod* ABC are involved in the synthesis of lipo-chitin oligosaccharides (LCOs) metabolites which are synthesized in the response of root exudates or flavonoids (Abdel-Lateif et al. 2012). LCOs increase plant growth development on applying alone or in combination with rhizobia and have given better results in field conditions (Miransari and Smith 2009; Marks et al. 2013). LCOs also act as growth regulators in a wide variety of plants, including non-legumes (Zhang and Smith 2002; Prithiviraj et al. 2003). Novozyme, first started to manufacture bioformulations containing LCOs and this technology is known as LCO promoter technology. These days, single formulations containing LCOs (Ratchet® and Torque®) or formulations with bacteria (OptimizeII®, Signum® and DynaStartMax®) are being marketed (Bioag.novozymes.com 2015).

Precursor in inoculant technology is also emerging as promising technique for efficient rhizobial bioformulations development (Naveed et al. 2015). Qureshi et al. (2012) found that the co-inoculation of *Rhizobium* and *Bacillus* sp. in the presence of a phytohormone precursor L-tryptophan (L-TRP) improved the pod and straw yield. Similarly, Qureshi et al. (2013) showed that interaction of L-TRP and rhizobial species increased the fresh fodder and dry matter yield in comparison to their separate application. Micronutrients have important role in nitrogen fixation and in this regard, Arora et al. (2009) recommended that the supplementation of Mo and Fe (up to certain concentrations) in soils along with the rhizobial formulations enhance the symbiotic nitrogen fixation process. Mmbaga et al. (2014) reported that the rhizobial inoculants supplemented with exogenous nutrients (phosphorus and potassium) improved photosynthesis, nutrient uptake, nodulation, growth and yield of the crop. According to Marks et al. (2013) and Morel et al. (2015), the bioformulations based on a mixture of various compounds, e.g., phytohormones, LCOs, flavonoids may be very useful for higher plant growth, and further research is required in this field.

The discovery of novel stress tolerating rhizobial species is also thought to be imperative in developing bioformulations that will survive in stress conditions (high temperature, drought, salinity) (Arora et al. 2012; Laranjo et al. 2014; Rao 2014). Currently, various types of rhizobial species have been discovered but only few of them are used as inoculants (Table 4.2). Stress tolerant bacteria have lots of scope for bioformulations production and also have important role in reclamation of waste lands (Arora et al. 2000, 2006). In a study, Karuppasamy et al. (2011) showed that

Table 4.2 Classification of rhizobia and their inoculants used in global market

Genus	Species	Inoculant crop	References
Rhizobium	*leguminosarum*	*Lactuca sativa and Daucus carota, Pea*	Flores-Félix et al. (2013) and Clayton et al. (2004)
	galegae	*Galega orientalis*	Vassileva and Ignatov (2002)
	tropici	*Zea mays*	Marks et al. (2015)
	endophyticum	*P. vulgaris*	López-López et al. (2010)
	phaseoli	*Vigna radiate*	Zahir et al. (2010)
	fabae	*V. faba*	Tian et al. (2008)
	etli	*P. vulgaris*	Soares et al. (2006)
	undicola	*Neptunia natans*	de Lajudie et al. (1998)
	gallicum	*P. vulgaris*	Sassi-Aydi et al. (2012)
	giardinii	*P. vulgaris*	Amarger et al. (1997)
	hainanensis	NA	NA
	huautlense	*Sesbania herbacea*	Wang and Martínez-Romero (2000)
	mongolense	*Medicago ruthenica*	Van Berkum et al. (1998)
	yanglingense	NA	NA
	larrymoorei	NA	NA
	indigoferae	NA	NA
	sullae	*Hedysarum coronarium*	Fitouri et al. (2012)

(continued)

Table 4.2 (continued)

Genus	Species	Inoculant crop	References
	loessense	NA	NA
	cellulosilyticum	*P. vulgaris*	Diez-Mendez et al. (2015)
	miluonense	*Lespedeza chinensis*	Gu et al. (2007)
	multihospitium	NA	NA
	oryzae	*Glycine max*	Waswa (2013)
	pisi	NA	NA
	mesosinicum	NA	NA
	alamii	*Helianthus annuus*	Alami et al. (2000)
	alkalisoli	NA	NA
	tibeticum	*Trigonella foenumgraecum*	Abd-Alla et al. (2014c)
	tubonense	NA	NA
	halophytocola	NA	NA
	radiobacter	*Graminaceous crops*	Humphry et al. (2007)
	rhizogenes	NA	NA
	rubi	NA	NA
	vitis	NA	NA
	nepotum	NA	NA
Ensifer	*meliloti*	*Medicago truncatula, Mucuna pruriens*	Olah et al. (2005) and Aeron et al. (2012)
	fredii	*G. max*	Albareda et al. (2008)
	sahelense	NA	NA
	terangae	NA	NA
	medicae	NA	NA
	arboris	NA	NA
	kostiense	NA	NA
	xingianens	NA	NA
	adhaerens	NA	NA
	kummerowiae	NA	NA
	americanum	*P. vulgaris*	Mnasri et al. (2012)
	mexicanus	*P. vulgaris*	Lloret et al. (2007)
	numidicus	NA	NA
Shinella	*kummerowiae*	NA	NA

(continued)

Table 4.2 (continued)

Genus	Species	Inoculant crop	References
Mesorhizobium	*loti*	*Lotus corniculatus*	Karaś et al. (2015)
	huakuii	NA	NA
	cicero	*Cicer arietinum*	Rokhzadi et al. (2008)
	tianshanense	NA	NA
	mediterraneum	*Hordeum vulgare, Cicer arietinum*	Peix et al. (2001) and Dudeja et al. (2011)
	plurifarium	NA	NA
	amorphae	NA	NA
	chacoense	NA	NA
	septentrionale	NA	NA
	temperatum	*G. max*	Waswa (2013)
	thiogangeticum	NA	NA
	albiziae	*Albizia kalkora, G. max*	Wang et al. (2007) and Waswa (2013)
	caraganae	NA	NA
	gobiense	NA	NA
	tarimense	NA	NA
	australicum	NA	NA
	opportunistum	NA	NA
	metallidurans	NA	NA
	alhagi	NA	NA
	camelthorni	NA	NA
	abyssinicae	NA	NA
	muleiense	NA	NA
	hawassense	NA	NA
	qingshengii	NA	NA
	robiniae	NA	NA
	shonense	NA	NA
	shangrilense	NA	NA
	silamurunense	NA	NA
	tamadayense	NA	NA
Phyllobacterium	*trifolii*	*Fragaria ananassa*	Flores-Felix et al. (2015)
Methylobacterm	*nodulans*	*Crotalaria perrottetii*	Jourand et al. (2004)

(continued)

Table 4.2 (continued)

Genus	Species	Inoculant crop	References
Microvirga	*lupine*	NA	NA
	lotononidis	*Leobordea sp.*	Ardley et al. (2013)
	zambiensis	NA	NA
Ochrobactrum sp.	*cytisi*	*Cucumis sativus*	Xu et al. (2015)
	lupine	*Lupinus albus*	Trujillo et al. (2005)
Azorhizobium	*caulinodans*	*Leucaena leucocephala*	Waelkens et al. (1995)
	dobereinereae	NA	NA
	oxalatiphilum	NA	NA
Devosia	*neptuniae*	*Neptunia natans*	Rivas et al. (2003)
Bradyrhizobium	*japonicum*	*G. max*	Zerpa et al. (2013)
	elkanii	*Vigna unguiculata*	Soares et al. (2006)
	iaoningensese	NA	NA
	yuanmingense	*G. max*	Soe and Yamakawa (2013)
	betae	NA	NA
	canariense	NA	NA
	iriomotense	NA	NA
	jicamae	NA	NA
	lablabi	NA	NA
	huanghuaihaiense	NA	NA
	cytisi	NA	NA
	daqingense	NA	NA
	denitrificans	NA	NA
	oligotrophicum	NA	NA
	pachyrhizi	NA	NA
Burkholderia	*caribensis*	*Amaranthus cruentus* and *A. hypochondriacus*	Parra-Cota et al. (2014)
	cepacia	*P. vulgaris*	Peix et al. (2001)
	tuberum	*Macroptilium atropurpureum*	Annette et al. (2013)
	phymatum	*P. vulgaris*	Talbi et al. (2013)

(continued)

Table 4.2 (continued)

Genus	Species	Inoculant crop	References
	nodosa	NA	NA
	sabiae	NA	NA
	mimosarum	NA	NA
	rhizoxinica	NA	NA
	diazotrophica	NA	NA
	endofungorum	NA	NA
	heleia	NA	NA
	symbiotica	*Mimosa cordistipula*	Sheu et al. (2012)
	ambifaria	*Zea mays, A. cruentus and A. hypochondriacus*	Ciccillo et al. (2002) and Parra-Cota et al. (2014)
	vietnamiensis	*Oryza sativa*	Choudhury and Kennedy (2004)
Cupriavidus	*taiwanensis*	*Rhynchosia ferulifolia*	Garu et al. (2009)
Pseudomonas	NA	NA	Zhao et al. (2013)

Modified from Berrada and Fikri-Benbrahim (2014)
NA Not Available

the growth of tree legumes *Samanea saman* could be improved by application of stress tolerant rhizobia. Ahmad et al. (2013) also stated that halo-tolerant, auxin producing *Rhizobium* strains improve osmotic stress tolerance in mung bean. Recently, benefits of using exopolysaccharides (EPS) in bioformulation is documented (Tewari and Arora 2014; Thenmozhi and Dinakar 2014). EPS protects inoculated rhizobial cells from stress factors such as salinity, dessication and pH (Ophir and Gutnick 1994; Qurashi and Sabri 2012). Use of EPS as efficient alternative carriers for inoculant production is also reported (Rodrigues et al. 2015). Maheshwari et al. (2012) suggested that the combined application of microbial inoculants and fertilizer worked as better choice for farmers to reduce the risk and expenses of chemical fertilizers.

Use of omics-based approaches (genomics and proteomics) can also be very useful in enhancing our understanding of rhizobia-legume symbiosis (Ramalingam et al. 2015). Omics based techniques including genomics, proteomics and metabolomics can go a long way in designing state of the art bioformulation for a particular soil and crop. Discovery of very similar signalling pathway in cereals as used by legumes to fix nitrogen has opened the door of non-legume fixation (de Bruijn 2016) and according to research by Rogers and Oldroyd (2014), in near future cereal crops capable of fixing nitrogen will also be available by the application of synthetic biological approaches.

4.6 Conclusion

Nitrogen is one of the key and limiting nutrients for agricultural ecosystems. The most important process to bring the atmospheric nitrogen in use for living creatures is BNF. Amongst microbes possessing the capacity to carry out BNF, rhizobia are the most important. Rhizobial inoculants are being used since long time around the globe, but still there is room for improvement so as to enhance the efficacy, productivity and credibility amongst the farmers. Liquid formulations are now picking up the pace in comparison to solid carrier based ones. Different types of additives and metabolites are also now being incorporated into the formulations to enhance their shelf life, performance and efficacy. Gel based encapsulated inoculants are also being used and with emergence of biotechnology, genetically improved inoculants and co-inoculants of rhizobia with other PGPRs/AMFs are also being worked upon in present time. Use of biotechnological tools and improvement in regulations can go a long way in designing a rhizobial bioformulation which will be more reliable and effective. To design a tailor made state of the art rhizobial formulation, it is very important to further our knowledge on plant-microbe interactions by using the latest tools and techniques. Also it would be better if the government and non-government organizations help in spreading the knowledge of usefulness of such bioformulations amongst the end-users. This is particularly required in developing nations. In future, we must see an even greater role and share of rhizobial inoculants in the market for sustainable supply of nitrogen to the future generations.

References

Abd-Alla MH, Issa AA, Ohyama T (2014a) Impact of harsh environmental conditions on nodule formation and dinitrogen fixation of legumes. In: Ohyama T (ed) Advances in biology and ecology of nitrogen fixation. InTech Open, Rijeka, p 282

Abd-Alla MH, El-Enany AE, Nafady NA et al (2014b) Synergistic interaction of *Rhizobium leguminosarum* bv. *viciae* and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. Microbiol Res 169:49–58

Abd-Alla MH, Bashandy SR, Bagy MK et al (2014c) *Rhizobium tibeticum* activated with a mixture of flavonoids alleviates nickel toxicity in symbiosis with fenugreek (*Trigonella foenumgraecum* L.) Ecotoxicology 23(5):946–959

Abdel-Lateif K, Bogusz D, Hocher V (2012) The role of flavonoids in the establishment of plant roots endosymbioses with arbuscular mycorrhiza fungi, rhizobia and Frankia bacteria. Plant Signal Behav 7(6):636–641

Aeron A, Khare E, Arora NK et al (2012) Practical use of CMC-amended rhizobial inoculant for *Mucuna pruriens* cultivation to enhance the growth and protection against *Macrophomina phaseolina*. J Gen Appl Microbiol 58(2):121–127

Ahmad M, Zahir ZA, Nazli F et al (2013) Effectiveness of halo-tolerant, auxin producing *Pseudomonas* and *Rhizobium* strains to improve osmotic stress tolerance in mung bean (*Vigna radiata* L.) Braz J Microbiol 44(4):1341–1348

Alami Y, Achouak W, Marol C et al (2000) Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium* sp. strain isolated from sunflower roots. Appl Environ Microbiol 66:3393–3398

Albareda M, Rodríguez-Navarro DN, Camacho M et al (2008) Alternatives to peat as a carrier for rhizobia inoculants: solid and liquid formulations. Soil Biol Biochem 40:2771–2779

Albareda M, Rodriguez-Navarro DN, Temprano FJ (2009) Use of *Sinorhizobium* (*Ensifer*) *fredii* for soybean inoculants in south Spain. Eur J Agron 30:205–211

Allito BB, Ewusi-Mensah N, Alemneh AA (2015) Rhizobia strain and host-legume interaction effects on nitrogen fixation and yield of grain legume: a review. Mol Soil Biol 6(2):1–6

Alvarez GS, Pieckenstain FL, Desimone MF et al (2010) Evaluation of sol-gel silica matrices as inoculant carriers for *Mesorhizobium* spp. cells. In: Mendez-Vilas A (ed) Current research, technology and education topics in applied microbiology and microbial biotechnology. Formatex, pp 160–167

Amarger N, Macheret V, Laguerre G (1997) *Rhizobium gallicum* sp. nov., and *Rhizobium giardinii* sp. nov., from *Phaseolus vulgaris* nodules. Int J Syst Evol Microbiol 47(4):996–1006

Anandham R, Sridar R, Nalayini P et al (2007) Potential for plant growth promotion in groundnut (*Arachis hypogaea* L.) cv. ALR-2 by coinoculation of sulfur-oxidizing bacteria and *Rhizobium*. Microbiol Res 162:139–153

Annette A, Andrew L, Michelle L et al (2013) Nodulation and effective nitrogen fixation of *Macroptilium atropurpureum* (siratro) by *Burkholderia tuberum*, a nodulating and plant growth promoting beta-proteobacterium, are influenced by environmental factors. Plant Soil 369(1/2):543

Ardley JK, Reeve WG, O'Hara GW et al (2013) Nodule morphology, symbiotic specificity and association with unusual rhizobia are distinguishing features of the genus *Listia* within the southern African crotalarioid clade *Lotononis s.l.* Ann Bot 112(1):1–15

Arora NK, Mishra J (2016) Prospecting the roles of metabolites and additives in future bioformulations for sustainable agriculture. Appl Soil Ecol. doi.org/10.1016/j.apsoil.2016.05.020

Arora NK, Kumar V, Maheshwari DK (2000) Isolation of both fast and slow growing rhizobia effectively nodulating a medicinal legume, *Mucuna pruriens*. Symbiosis 29:121–137

Arora NK, Kumar V, Maheshwari DK (2001) Constraints, development and future of the inoculants with special reference to rhizobial inoculants. In: Maheshwari DK, Dubey RC (eds) Innovative approaches in microbiology. Singh and Singh, Dehradun, pp 241–245

Arora NK, Singhal V, Maheshwari DK (2006) Salinity-induced accumulation of poly-β-hydroxybutyrate in rhizobia indicating its role in cell protection. World J Microbiol Biotechnol 22:603–606

Arora NK, Khare E, Naraian R et al (2008) Sawdust as a superior carrier for production of multipurpose bioinoculants using plant growth promoting rhizobial and pseudomonad strain and their impact on productivity of *Trifolium repense*. Curr Sci 95(1):90–94

Arora NK, Khare E, Singh S et al (2009) Effect of Al and heavy metals on enzymes of nitrogen metabolism of fast and slow growing rhizobia under explanta conditions. World J Microbiol Biotechnol 26(5):811–816

Arora NK, Maheshwari DK, Khare E (2010) Plant growth promoting rhizobacteria: constraints in bioformulation, commercialization and future strategies. In: Maheshwari DK (ed) Bacteria and plant health. Springer, Berlin, pp 97–116

Arora NK, Tewari S, Singh S et al (2012) PGPR for protection of plant health under saline conditions. In: Maheshwari DK (ed) Bacteria in agrobiology: stress management. Springer Publication, Dordrecht, pp 239–258

Arora NK, Tiwari S, Singh R (2014) Comparative study of different carriers inoculated with nodule forming and free living plant growth promoting bacteria suitable for sustainable agriculture. J Pharm Chem Biol Sci 2(2):143–149

Arora NK, Verma M, Prakash J et al (2016) Regulation of biopesticides: global concerns and policies. In: Arora NK, Mehnaz S, Balestrini R (eds) Bioformulations: for sustainable agriculture. Springer, New Delhi, pp 283–299

Baldwin IL, Fred EB (1929) Nomenclature of the root nodule bacteria of the Leguminosae. J Bacteriol 17:141–150

Ballard RA, Charman N (2000) Nodulation and growth of pasture legumes with naturalised soil rhizobia 1. Annual *Medicago* spp. Aust J Exp Agric 40:939–948

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16(4):729–770
Bashan Y, Levanony H (1988) Adsorption of the rhizosphere bacterium *Azospirillum brasilense* Cd to soil, sand and peat particles. J Gen Microbiol 134:1811–1820
Bashan Y, de Bashan LE, Prabhu SR et al (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). Plant Soil 378:1–33
Beijerinck MW (1888) Cultur des Bacillus radicicola aus den Kno¨llchen. Bot Ztg 46:740–750
Ben Rebah F, Tyagi RD, Prevost D (2002a) Wastewater sludge as a substrate for growth and carrier for rhizobia: the effect of storage conditions on survival of *Sinorhizobium meliloti*. Bioresour Technol 83:145–151
Ben Rebah F, Tyagi RD, Prevost D et al (2002b) Wastewater sludge as a new medium for rhizobial growth. Water Qual Res J Can 37:353–370
Ben Rebah F, Prévost D, Yezza A et al (2007) Agro-industrial waste materials and wastewater sludge for rhizobial inoculant production: a review. Bioresour Technol 98:3535–3546
Berrada H, Fikri-Benbrahim K (2014) Taxonomy of the rhizobia: current perspectives. Br Microbiol Res J 4(6):616
Bezdicek DF, Kennedy AC (1998) In: Lynch JM, Hobbie JE (eds) Microorganisms in action. Blackwell, Oxford, p 243
Bezdicek DF, Evans DW, Abeda B et al (1978) Evaluation of peat and granular inoculum for soybean yield and N fixation under irrigation. Agron J 70:865–868
Bianco C, Senatore B, Arbucci S et al (2014) Modulation of endogenous indole-3-acetic acid biosynthesis in bacteroids within *Medicago sativa* nodules. Appl Environ Microbiol 80(14):4286–4293
Bioag.novozymes.com (2015) Novozymes BioAg Group. At http://www.bioag.novozymes.com
Bonish PM (1979) Clover rhizobia in soils: assessment of effectiveness using the plant infection count method. N Z J Agric Res 22:89–93
Bouizgarne B, Oufdou K, Ouhdouch Y (2015) Actinorhizal and rhizobial-legume symbioses for alleviation of abiotic stresses. In: Arora NK (ed) Plant microbes symbiosis: applied facets. Springer, New Delhi, pp 273–295
Boussingault J (1838) Recherches chimiques sur la vegetation enterprises dans le but d'examiner si les plantes prennent de I'atmosphere. Ann Chim Phys 67:1–54
Brahmaprakash GP, Sahu PK (2012) Biofertilizers for sustainability. J Indian Inst Sci 92(1):37–62
Brito B, Palacios JM, Imperial J et al (2002) Engineering the *Rhizobium leguminosarum* bv. *viciae* hydrogenase system for expression in free-living microaerobic cells and increased symbiotic hydrogenase activity. Appl Environ Microbiol 68(5):2461–2467
Brockwell J (1982) Inoculation methods for field experimenters and farmers. In: Vincent JM (ed) Nitrogen fixation in legumes. Academic Press, Australia, pp 211–227
Brockwell J (1985) Environmental interactions influencing innovative practices in legume inoculation. In: Shibles R (ed) Proceedings of the world soybean conference III. Westview Press, Boulder, pp 943–950
Brockwell J, Bottomley PJ (1995) Recent advances in inoculant technology and prospects for the future. Soil Biol Biochem 27:683–697
Brockwell J, Gault RR, Chase DL et al (1980) An appraisal of practical alternatives to legume seed inoculation: field experiments on seedbed inoculation with solid and liquid inoculants. Aust J Agric Res 31:47–60
Brockwell J, Holliday RA, Pilka A (1988) Evaluation of root-nodule bacteria introduced into field environment of the symbiotic nitrogen-fixing potential of soils by direct microbiological means. Plant Soil 108:163–170
Brockwell J, Bottomley PJ, Thies JE (1995) Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. Plant Soil 174:143–180
Burges HD, Jones KA (1998) Trends in formulation of microorganisms and future research requirements. In: Burges HD (ed) Formulation of microbial biopesticides, beneficial microorganisms, nematodes and seed treatment. Kluwer Academic Publication, Dordrecht, pp 311–332

Catroux G, Hartmann A, Revellin C (2001) Trends in rhizobial inoculant production and use. Plant Soil 230:21–30

Chandra S, Choure K, Dubey RC et al (2007) Rhizosphere competent *Mesorhizobium loti* MP6 induces root hair curling, inhibits *Sclerotinia sclerotiorum* and enhances growth of Indian mustard (*Brassica campestris*). Braz J Microbiol 38:128–130

Chibeba AM, Guimarães MF, Brito OR et al (2015) Co-inoculation of soybean with *Bradyrhizobium* and *Azospirillum* promotes early nodulation. Am J Plant Sci 6:1641–1649

Choudhury ATMA, Kennedy IR (2004) Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. Biol Fertil Soils 39(4):219–227

Ciccillo F, Fiore A, Bevivino A et al (2002) Effects of two different application methods of *Burkholderia ambifaria* MCI 7 on plant growth and rhizospheric bacterial diversity. Environ Microbiol 4:238–245

Clayton GW, Rice WA, Lupwayi NZ et al (2004) Inoculant formulation and fertilizer nitrogen effects on field pea: crop yield and seed quality. Can J Plant Sci 84:89–96

Crawford SL, Berryhill DL (1983) Survival of *Rhizobium phaseoli* in coal-based legume inoculants applied to seeds. Appl Environ Microbiol 45:703–705

Da Silva JG, Serra GE, Moreira JR et al (1978) Energy balance for ethyl alcohol production for crops. Science 210:903–906

Damasceno R, Roggia I, Pereira C et al (2013) Rhizobia survival in seeds coated with polyvinyl alcohol (PVA) electrospun nanofibres. Can J Microbiol 59(11):716–719

Dashti N, Prithiviraj B, Zhou X et al (2000) Combined effects of plant growth promoting rhizobacteria and genistein on nitrogen fixation in soybean at suboptimal root zone temperatures. J Plant Nutr 23:593–604

Dazzo FB, Yanni YG, Rizk R et al (2000) Progress in multinational collaborative studies on the beneficial association between *Rhizobium leguminosarum* bv. *trifolii* and rice. In: Ladha JK, Reddy PM et al (eds) The quest for nitrogen fixation in rice. IRRI, Los Banos, pp 167–189

Deaker R, Roughley RJ, Kennedy IR (2004) Legume seed inoculation technology-a review. Soil Biol Biochem 36:1275–1288

de Bruijn FJ (2016) "Biological Nitrogen Fixation" book summary. Adv Microbiol 6:407–411

de Lajudie P, Laurent-Fulele E, Willems A et al (1998) *Allorhizobium undicola* gen. nov., sp. nov., nitrogen-fixing bacteria that efficiently nodulate *Neptunia natans* in Senegal. Int J Syst Bacteriol 48:1277–1290

Denardin ND, Freire JRJ (2000) Assessment of polymers for the formulation of legume inoculants. World J Microbiol Biotechnol 16:215–217

Deschodt CC, Strijdom WB (1976) Suitability of a coal bentonite base as carrier of rhizobia in inoculants. Phytophylactica 8:1–6

Deshwal VK, Singh SB, Kumar P et al (2013) Rhizobia unique plant growth promoting rhizobacteria: a review. Int J Life Sci 2(2):74–86

Diez-Mendez A, Menéndez E, García-Fraile P et al (2015) *Rhizobium cellulosilyticum* as a co-inoculant enhances *Phaseolus vulgaris* grain yield under greenhouse conditions. Symbiosis 67(1):135–141

Diouf D, Forestier S, Neyra M et al (2003) Optimisation of inoculation of *Leucaena leucocephala* and *Acacia mangium* with *Rhizobium* under greenhouse conditions. Ann For Sci 60:379–384

Dommergues YR, Diem HG, Divies D (1979) Polyacrylamide-entrapped *Rhizobium* as an inoculant for legumes. Appl Environ Microbiol 37:779–781

Dudeja SS, Singh NP, Sharma P et al (2011) Biofertilizer technology and pulse production. In: Singh A, Parmar N, Kuhad RC (eds) Bioaugmentation, biostimulation and biocontrol. Springer-Verlag, Berlin/Heidelberg, pp 43–63

Eckhardt MM, Baldwin IR, Fred EB (1931) Studies on the root-nodule bacteria of *Lupinus*. J Bacteriol 21:273–285

EPA (1997) Environmental protection act, fact sheet: commercialization of *Sinorhizobium* (*Rhizobium*) meliloti, RMBPC-2. At http://www.epa.gov/biotech_rule/pubs/factdft6.html

Ferguson BJ, Indrasumunar A, Hayashi S et al (2010) Molecular analysis of legume nodule development and auto regulation. J Integr Plant Biol 3:61–76

Fernandes-Júnior PI, Rohr TG, de Oliveira PJ et al (2009) Polymers as carriers for rhizobial inoculant formulations. Pesq Agrop Bras 44(9):1184–1190
Ferreira EM, Castro IV (2005) Residues of the cork industry as carriers for the production of legume inoculants. Silva Lusit 13:159–167
Fitouri SD, Trabelsi D, Saïdi S et al (2012) Diversity of rhizobia nodulating sulla (*Hedysarum coronarium* L.) and selection of inoculant strains for semi-arid Tunisia. Ann Microbiol 62:77–84
Flores-Félix JD, Menéndez E, Rivera LP et al (2013) Use of *Rhizobium leguminosarum* as a potential biofertilizer for *Lactuca sativa* and *Daucus carota* crops. J Plant Nutr Soil Sci 176:876–882
Flores-Felix JD, Silva LR, Rivera LP et al (2015) Plants probiotics as a tool to produce highly functional fruits: the case of *Phyllobacterium* and vitamin C in strawberries. PLoS One 10(4):e0122281
Forestier S, Alvarado G, Badjel SB et al (2001) Effect of *Rhizobium* inoculation methodologies on nodulation and growth of *Leucaena leucocephala*. World J Microbiol Biotechnol 17:359–362
Frank B (1889) Ueber die Pilzsymbiose der Leguminosen. Ber Deut Bot Ges 7:332–346
Fred EB, Baldwin IL, McCoy E (1932) Root nodule bacteria and leguminous plants, University of Wisconsin Studies in Science No.5. University of Wisconsin, Madison
Fuchsius L (1542) De historia stirpium commentarii isignes. Michael Isingrin, Basel
Galloway JN, Townsend AR, Erisman JW et al (2008) Transformation of the nitrogen cycle: recent trends, questions and potential solutions. Science 320:889–892
Gamal-Eldin H, Elbanna K (2011) Field evidence for the potential of *Rhodobacter capsulatus* as biofertilizer for flooded rice. Curr Microbiol 62:391–395
Garu G, Yates RJ, Deiana P et al (2009) Novel strains of nodulating *Burkholderia* have a role in nitrogen fixation with papilionoid herbaceous legumes adapted to acid, infertile soils. Soil Biol Biochem 41:125–134
Geetha SJ, Joshi SJ (2013) Engineering rhizobial bioinoculants: a strategy to improve iron nutrition. Sci World J 2013:1–15
Gomez M, Silva N, Hartmen A et al (1997) Evaluation of commercial soybean inoculants from Argentina. World J Microbiol Biotechnol 13:167–173
Granada CE, Strochein M, Vargas LK et al (2014) Genetic diversity and symbiotic compatibility among rhizobial strains and *Desmodium incanum* and *Lotus* spp. plants. Genet Mol Biol 37(2):396–405
Grand View Research (2015) Biofertilizers market analysis by product (nitrogen fixing, phosphate solubilizing), by application (seed treatment, soil treatment) and segment forecasts to 2022. Available at http://www.grandviewresearch.com/
Gu CT, Wang ET, Sui XH et al (2007) Diversity and geographical distribution of rhizobia associated with *Lespedeza* spp. in temperate and subtropical regions of China. Arch Microbiol 188:355–365
Guthrie FB (1896) Inoculation of soil for leguminous crops. Agric Gaz NSW 7:690–694
Gzyl A, Augustynowicz E, Mosiej E et al (2005) Amplified fragment length polymorphism (AFLP) versus randomly amplified polymorphic DNA (RAPD) as new tools for inter-and intra-species differentiation within Bordetella. J Med Microbiol 54(4):333–346
Hamaoui B, Abbadi JM, Burdman S et al (2001) Effects of inoculation with *Azospirillum brasilense* on chickpeas (*Cicer arietinum*) and faba beans (*Vicia faba*) under different growth conditions. Agronomy 21:553–560
Hashem MA (2001) Problems and prospects of cyanobacterial biofertilizer for rice cultivation. Aust J Plant Physiol 28:881–888
Hawkesford M, Horst W, Kichey T et al (2012) Functions of macronutrients. In: Marschner P (ed) Marschner's mineral nutrition of higher plants, 3rd edn. Academic, London, pp 135–189
Hedin PA, McCarty JC (1994) Effects of several commercial plant growth regulator formulations on yield and allelochemicals of cotton (*Gossypium hirsutum* L.) Agric Food Chem 42:1355–1357
Hellriegel H, Wilfarth H (1888) Untersuchungen u¨ ber die Stickstoffnahrung der Gramineon und Leguminosen. Beilageheft zu der Ztschr. Ver. Ru¨ benzucker-Industrie Deutschen Reichs
Herridge D, Gemell G, Hartley E (2002) Legume inoculants and quality control. ACIAR Proc 109:105–115

Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311:1–18

Herrmann L, Atieno M, Brau L et al (2015) Microbial quality of commercial inoculants to increase BNF and nutrient use efficiency. In: de Bruijn FJ (ed) Biological nitrogen fixation volume 2. Wiley, Hoboken, pp 1031–1040

Humphry DR, Andrews M, Santos SR et al (2007) Phylogenetic assignment and mechanism of action of a crop growth promoting *Rhizobium radiobacter* strain used as a biofertiliser on graminaceous crops in Russia. Antonie Van Leeuwenhoek 91(2):105–113

Hungria M, Campo RJ, Souza EM et al (2010) Inoculation with selected strains of *Azospirillum brasilense* and A. *lipoferum* improves yields of maize and wheat in Brazil. Plant Soil 331:413–425

Hungria M, Nogueira MA, Araujo RS (2015) Soybean seed co-inoculation with *Bradyrhizobium* spp and *Azospirillum brasilense*: a new biotechnological tool to improve yield and sustainability. Am J Plant Sci 6:811–817

Hussain I, Arshad M, Zahir ZA et al (1995) Substrate dependent microbial production of auxins and their influence on growth and nodulation of lentil. Pak J Agric Sci 32:149–152

Hynes RK, Jans DC, Bremer E et al (2001) *Rhizobium* population dynamics in the pea rhizosphere of rhizobial inoculant strain applied in different formulations. Can J Microbiol 47:595–600

Jayasinghearachchi HS, Seneviratne G (2004) A Bradyrhizobial-*Penicillium* spp. biofilm with nitrogenase activity improves N_2 fixing symbiosis of soybean. Biol Fertil Soils 40:432–434

John RP, Tyagi RD, Brar SK et al (2011) Bioencapsulation of microbial cells for targeted agricultural delivery. Crit Rev Biotechnol 31:211–226

Jourand P, Giraud E, Béna G et al (2004) *Methylobacterium nodulans* sp. nov., for a group of aerobic, facultatively methylotrophic, legume root-nodule-forming and nitrogen-fixing bacteria. Int J Syst Evol Microbiol 54(6):2269–2273

Jung G, Mugnier J, Diem HG et al (1982) Polymer entrapped *Rhizobium* as an inoculant for legumes. Plant Soil 65:219–231

Kaljeet S, Keyeo F, Amir HG (2011) Influence of carrier materials and storage temperature on survivability of rhizobial inoculant. Asian J Plant Sci 10:331–337

Kalra A, Chandra M, Awasthi A et al (2010) Natural compounds enhancing growth and survival of rhizobial inoculants in vermicompost-based formulations. Biol Fertil Soils 46:521–524

Kandasamy R, Prasad NN (1971) Lignite as a carrier of rhizobia. Curr Sci 40:496

Karaś MA, Turska-Szewczuk A, Trapska D et al (2015) Growth and survival of *Mesorhizobium loti* inside *Acanthamoeba* enhanced its ability to develop more nodules on *Lotus corniculatus*. Microb Ecol 70(2):566–575

Karuppasamy K, Nagaraj S, Kathiresan K (2011) Stress tolerant *Rhizobium* enhances the growth of *Samanea saman* (JECQ) Merr. Afr J Basic Appl Sci 3(6):278–284

Kennedy IR, Choudhury ATMA, Kecskés ML (2004) Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? Soil Biol Biochem 36:1229–1244

Khalid A, Arshad M, Zahir ZA (2004) Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. J Appl Microbiol 96:473–480

Khare E, Arora NK (2011) Dual activity of pyocyanin from *Pseudomonas aeruginosa*- antibiotic against phytopathogen and signal molecule for biofilm development by rhizobia. Can J Microbiol 57(9):708–713

Khavazi K, Rejali F, Seguin P et al (2007) Effects of carrier, sterilization method and incubation on survival of *Bradyrhizobium japonicum* in soybean (*Glycine max* L.) inoculants. Enzym Microb Technol 41:780–784

Kneip C, Lockhart P, Voß C, Maier UG (2007) Nitrogen fixation in eukaryotes–new models for symbiosis. BMC Evol Biol 7(1):55

Kokalis-Burelle N, Vavrina CS, Reddy MS et al (2003) Amendment of muskmelon and watermelon transplant media with plant growth-promoting rhizobacteria: effect on seedling quality, disease and nematode resistance. Hortic Technol 13:476–482

Kostov O, Lynch JM (1998) Composted sawdust as a carrier for *Bradyrhizobium, Rhizobium* and *Azospirillum* in crop inoculation. World J Microbiol Biotechnol 14:389–397
Krapp A, Berthomé R, Orsel M et al (2011) *Arabidopsis* roots and shoots show distinct temporal adaptation patterns toward nitrogen starvation. Plant Physiol 157:1255–1282
Kremer RJ, Peterson HL (1982) Effect of inoculant carrier on survival of *Rhizobium* on inoculated seed. Soil Sci 134:117–124
Kudoyarova GR, Korobova AV, Akhiyarova GR et al (2014) Accumulation of cytokinins in roots and their export to the shoots of durum wheat plants treated with the protonophore carbonyl cyanide m-chlorophenylhydrazone (CCCP). J Exp Bot 65:2287–2294
Kudoyarova GR, Arkhipova TN, Melent'ev AI (2015) Role of bacterial phytohormones in plant growth regulation and their development. In: Bacterial metabolites in sustainable agroecosystem. Springer, International Publishing, pp 69–86
Lachmann J (1858) Über knollen an den wurzeln der leguminosen [About bulbous roots of the legume]. Landwirthschaftliche Mitteilungen. Z Koniglichen
Laranjo M, Alexandre A, Oliveira S (2014) Legume growth-promoting rhizobia: an overview on the *Mesorhizobium* genus. Microbiol Res 169(1):2–17
Leibovitch S, Migner P, Zhang F et al (2001) Evaluation of the effect of SoyaSignal technology on soybean yield (*Glycine max* (L.) Merr.) under field conditions over 6 years in eastern Canada and the northern United States. J Agron Crop Sci 187:281–292
Li J, Zhang S, Shi S et al (2011) Four materials as carriers for phosphate dissolving *Rhizobium* sp. inoculants. Adv Mater Res 156–157:919–928
Lloret L, Ormeño-Orrillo E, Rincón R et al (2007) *Ensifer mexicanus* sp. nov. a new species nodulating *Acacia angustissima* (Mill.) Kuntze in Mexico. Syst Appl Microbiol 30:280–290
Löhis F, Hansen R (1921) Nodulating bacteria of leguminous plant. J Agric Res 20:543–556
López-López A, Rogel MA, Ormeno-Orrillo E et al (2010) *Phaseolus vulgaris* seed-borne endophytic community with novel bacterial species such as *Rhizobium endophyticum* sp. nov. Syst Appl Microbiol 33(6):322–327
Luce MS, Whalen JK, Ziadi N et al (2011) Nitrogen dynamics and indicesto predict soil nitrogen supply in humid temperate soils. Adv Agron 112:55
Lupwayi NZ, Clayton GW, Rice WA (2006) Rhizobial inoculants for legume crops. J Crop Improv 15:289–321
Maheshwari DK, Dubey RC, Aeron A et al (2012) Integrated approach for disease management and growth enhancement of *Sesamum indicum* L. utilizing *Azotobacter chroococcum* TRA2 and chemical fertilizer. World J Microbiol Biotechnol 28(10):3015–3024
Malpighi H (1679) Anatome plantarum. J Martyn Lond 99
Malusá E, Vassilev N (2014) A contribution to set a legal framework for biofertilisers. Appl Microbiol Biotechnol 98(15):6599–6607
Malusa E, Sas-Paszt L, Ciesielska J (2012) Technologies for beneficial microorganisms inocula used as biofertilizers. Sci World J 1–12
Manikandan R, Saravanakumar D, Rajendran L et al (2010) Standardization of liquid formulation of *Pseudomonas fluorescens* Pf1 for its efficacy against *Fusarium* wilt of tomato. Biol Control 54:83–89
Markets and Markets (2015) Biofertilizers market analysis by product (nitrogen fixing, phosphate solubilizing), by application (seed treatment, soil treatment) and segment forecasts to 2022. At http://www.marketsandmarkets.com/Market-Reports/compound-biofertilizers-customizedfertilizers-market-856.html
Marks BB, Megías M, Nogueira MA et al (2013) Biotechnological potential of rhizobial metabolites to enhance the performance of *Bradyrhizobium* spp. and *Azospirillum brasilense* inoculants with soybean and maize. AMB Express 3:21
Marks BB, Megías M, Ollero FJ et al (2015) Maize growth promotion by inoculation with *Azospirillum brasilense* and metabolites of *Rhizobium tropici* enriched on lipochitooligosaccharides (LCOs). AMB Express 5:71

Maróti G, Kondorosi É (2014) Nitrogen-fixing *Rhizobium*-legume symbiosis are polyploidy and host peptide-governed symbiont differentiation general principles of endosymbiosis? Front Microbiol 5:326

Maurice S, Beauclair P, Giraud JJ et al (2001) Survival and change in physiological state of *Bradyrhizobium japonicum* in soybean (*Glycine max* L. Merril) liquid inoculants after long-term storage. World J Microbiol Biotechnol 17:635–643

Mazid M, Khan TA (2014) Future of bio-fertilizers in Indian agriculture: an overview. Int J Agric Food Res 3(3):10–23

Mazid M, Khan TA, Mohammad F (2011) Potential of NO and H_2O_2 as signalling molecules in tolerance to abiotic stress in plants. J Indust Res Technol 1(1):56–68

Meng L, Zhang A, Wang F et al (2015) Arbuscular mycorrhizal fungi and *Rhizobium* facilitate nitrogen uptake and transfer in soybean/maize intercropping system. Front Plant Sci 6:339

Menna P, Pereira AA, Bangel EV et al (2009) Rep-PCR of tropical rhizobia for strain fingerprinting, biodiversity appraisal and as a taxonomic and phylogenetic tool. Symbiosis 48(1–3):120–130

Merwe SP, Strijdom BW, Uys CJ (1974) Groundnut response to seed inoculation under extensive agriculture practices in South African soils. Phytophylactica 6:295–302

Meyer SLF, Roberts DP, Chitwood DJ et al (2001) Application of *Burkholderia cepacia* and *Trichoderma virens*, alone and in combination, against *Meloidogyne incognita* on bell pepper. Nematropica 31:75–86

Mia MAB, Shamsuddin ZH, Zakaria W et al (2007) Associative nitrogen fixation by *Azospirillum* and *Bacillus* spp. in bananas. Infomusa 16(1–2):11–15

Miransari M, Smith D (2009) Rhizobial lipo-chitooligosaccharides and gibberellins enhance barley (*Hordeum vulgare* L.) seed germination. Biotechnology 8:270–275

Mishra J, Arora NK (2016) Bioformulations for plant growth promotion and combating phytopathogens: a sustainable approach. In: Arora NK, Mehnaz S, Balestrini R (eds) Bioformulations: for sustainable agriculture. Springer, New Delhi, pp 3–33

Mmbaga GW, Mtei KM, Ndakidemi PA (2014) Extrapolations on the use of *Rhizobium* inoculants supplemented with phosphorus (P) and potassium (K) on growth and nutrition of legumes. Agric Sci 5(12):1207

Mnasri B, Saïdi S, Chihaoui SA et al (2012) *Sinorhizobium americanum* symbiovar *mediterranense* is a predominant symbiont that nodulates and fixes nitrogen with common bean (*Phaseolus vulgaris* L.) in a Northern Tunisian field. Syst Appl Microbiol 35(4):263–269

Morel MA, Cagide C, Minteguiaga MA et al (2015) The pattern of secreted molecules during the co-inoculation of alfalfa plants with *Sinorhizobium meliloti* and *Delftia* sp. strain JD2: an interaction that improves plant yield. Mol Plant-Microbe Interact 28:134–142

Morel MA, Cagide C, Castro-Sowinski S (2016) The contribution of secondary metabolites in the success of bioformulations. In: Arora NK, Mehnaz S, Balestrini R (eds) Bioformulations: for sustainable agriculture. Springer, New Delhi, pp 235–250

Muñoz N, Soria-Díaz ME, Manyani H et al (2014) Structure and biological activities of lipo-chitooligosaccharide nodulation signals produced by *Bradyrhizobium japonicum* USDA 138 under saline and osmotic stress. Biol Fertil Soils 50:207–215

Nápoles MC, Guevara E, Montero F et al (2009) Role of *Bradyrhizobium japonicum* induced by genistein on soybean stressed by water deficit. Span J Agric Res 7:665–671

Naveed M, Mehboob I, Hussain MB et al (2015) Perspectives of rhizobial inoculation for sustainable crop production. In: Arora NK (ed) Plant microbes symbiosis: applied facets. Springer, New Delhi, pp 209–239

Nelson LM (2004) Plant growth promoting rhizobacteria (PGPR): prospects for new inoculants. Crop Manag 10:301–305

Nobbe F, Hiltner L (1896) Inoculation of the soil for cultivating leguminous plants. U.S. Patent 570 813

Odee DW, Indieka SA, Lesueur D (2002) Evaluation of inoculation procedures for *Calliandra calothyrsus* Meisn. grown in tree nurseries. Biol Fertil Soils 36:124–128

Ogasawara M, Suzuki T, Mutoh I et al (2003) *Sinorhizobium indiaense* sp. nov., and *Sinorhizobium abri* sp. nov., isolated from tropical legumes, *Sesbania rostrata* and *Abrus precatorius*, respectively. Symbiosis 34:53–68

Olah B, Briere C, Becard G et al (2005) Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the DMI1/DMI2 signalling pathway. Plant J 44:195–207

Olsen PE, Rice WA, Bordeleau LM et al (1994) Analysis and regulation of legume inoculants in Canada: the need for an increase in standards. Plant Soil 161:127–134

Olsen PE, Rice WA, Bordeleau LM et al (1996) Levels and identities of nonrhizobial microorganisms found in commercial legume inoculant made with nonsterile peat carrier. Can J Microbiol 42:72–75

Olufajo OO, Adu JK (1993) Nodulation of soyabeans grown under field conditions and inoculated with *Bradyrhizobium japonicum* strains. In: Mulongoy K, Gueye M, Spencer DSC (eds) Biological nitrogen fixation and sustainability of tropical agriculture. Wiley, New York, pp 147–154

Ophir T, Gutnick DL (1994) A role for exopolysaccharides in the protection of microorganisms from desiccation. Appl Environ Microbiol 60(2):740–745

Paau AS, Graham LL, Bennett M (1991) Progress in formulation research for PGPR and biocontrol inoculants. In: Keel C, Koller B, Défago G (eds) Plant growth-promoting rhizobacteria-progress and prospects. IOBC/WPRS Bulletin, Zurich, pp 399–403

Pan B, Smith DL (2000) Pre-incubation of *Bradyrhizobium japonicum* cells with genistein reduces the inhibitory effects of mineral nitrogen on soybean nodulation and nitrogen fixation under field conditions. Plant Soil 223:235–242

Parra-Cota FI, Peña-Cabriales JJ, de los Santos-Villalobos S et al (2014) *Burkholderia ambifaria* and *B. caribensis* promote growth and increase yield in grain amaranth (*Amaranthus cruentus* and *A. hypochondriacus*) by improving plant nitrogen uptake. PLoS One 9(2):88–94

Peix A, Rivas-Boyero AA, Mateos PF et al (2001) Growth promotion of chickpea and barley by a phosphate solubilizing strain of *Mesorhizobium mediterraneum* under growth chamber conditions. Soil Biol Biochem 33:103–110

Philpotts H (1976) Filter mud as a carrier for *Rhizobium* inoculants. J Appl Bacteriol 41:277–281

Pindi PK, Satyanarayana SDV (2013) Liquid microbial consortium-a potential tool for sustainable soil health. J Biofertil Biopestic 3:124

Pongslip N (2012) Phenotypic and genotypic diversity of rhizobia. Bentham Science Publishers, Thailand, p 201

Prithiviraj B, Zhou X, Souleimanov A et al (2003) A host specific bacteria-to-plant signal molecule (Nod factor) enhances germination and early growth of diverse crop plants. Planta 216:437–445

Pugashetti BK, Gopalgowda HS, Patil RB (1971) Cellulose powder as legume inoculant base. Curr Sci 40:494–495

Qiu M, Li S, Zhou X et al (2014) De-coupling of root-microbiome associations followed by antagonist inoculation improves rhizosphere soil suppressiveness. Biol Fertil Soils 50(2):217–224

Qurashi AW, Sabri AN (2012) Bacterial exopolysaccharide and biofilm formation stimulate chickpea growth and soil aggregation under salt stress. Braz J Microbiol 43(3):1183–1191

Qureshi MA, Ahmad ZA, Akhtar N et al (2012) Role of phosphate solubilizing bacteria (PSB) in enhancing P-availability and promoting cotton growth. J Anim Plant Sci 22:204–210

Qureshi MA, Shahzad H, Imran Z et al (2013) Potential of *Rhizobium* species to enhance growth and fodder yield of maize in the presence and absence of L-tryptophan. J Anim Plant Sci 23(5):1448–1454

Raja Sekar K, Karmegam N (2010) Earthworm casts as an alternate carrier material for biofertilizers: assessment of endurance and viability of *Azotobacter chroococcum*, *Bacillus megaterium* and *Rhizobium leguminosarum*. Sci Hortic 124:286–289

Rajwar A, Sahgal M, Johri BN (2013) Legume-rhizobia symbiosis and interactions in agroecosystems. In: Arora NK (ed) Plant microbe symbiosis-fundamentals and advances. Springer, New Delhi, pp 233–265

Ramalingam A, Kudapa H, Pazhamala LT et al (2015) Proteomics and metabolomics: two emerging areas for legume improvement. Front Plant Sci 6:1116

Rao DLN (2014) Recent advances in biological nitrogen fixation in agricultural systems. Proc Indian Natl Sci Acad 80(2):359–378

Revellin C, Meunier G, Giraud JJ et al (2000) Changes in the physiological and agricultural characteristics of peat-based *Bradyrhizobium japonicum* inoculants after long-term storage. Appl Microbiol Biotechnol 54:206–211

Rivas R, Willems A, Subba-Rao NS et al (2003) Description of *Devosia neptuniae* sp. nov., that nodulates and fixes nitrogen in symbiosis with *Neptunia natans*, an aquatic legume from India. Syst Appl Microbiol 26(1):47–53

Rivera D, Obando M, Barbosa H et al (2014) Evaluation of polymers for the liquid rhizobial formulation and their influence in the *Rhizobium*-Cowpea interaction. Univ Sci 19(3):265–275

Roberto SR, Marinho de Assis A, Yamamoto LY et al (2012) Application timing and concentration of abscisic acid improve color of "Benitak" table grape. Sci Hortic 142:44–48

Rodrigues AC, Silveira JAG, Bonifacio A et al (2013) Metabolism of nitrogen and carbon: optimization of biological nitrogen fixation and cowpea development. Soil Biol Biochem 67:226–234

Rodrigues AC, Vendruscolo CT, Moreira AS et al (2015) *Rhizobium tropici* exopolysaccharides as carriers improve the symbiosis of cowpea-*Bradyrhizobium-Paenibacillus*. Afr J Microbiol Res 9(37):2037–2050

Rodriguez-Navarro DN, Temprano F, Orive R (1991) Survival of *Rhizobium* sp. (*Hedysarum coronarium* L.) on peat-based inoculants and inoculated seeds. Soil Biol Biochem 23:375–379

Rogers C, Oldroyd GE (2014) Synthetic biology approaches to engineering the nitrogen symbiosis in cereals. J Exp Bot 65(8):1939–1946

Rohr TG (2007) Rheological study of the mixture carboxymethylcellulose/starch and its use as a vehicle for the bacterial inoculation. Dissertation, Rural Federal University of Rio de Janeiro

Rokhzadi A, Asgharzadeh A, Darvish F et al (2008) Influence of plant growth promoting rhizobacteria on dry matter accumulation and yield of chickpea (*Cicer arietinum* L.) under field conditions. Am Eurasian J Agric Environ Sci 3(2):253–257

Rubiya E (2006) Coaggregated diazotrophic cultures- a novel delivery system of bioinoculation for lowland rice (*Oryza sativa* L.). Dissertation, Aannamalai University

Ruíz-Valdiviezo VM, Canseco LMCV, Suárez LAC et al (2015) Symbiotic potential and survival of native rhizobia kept on different carriers. Braz J Microbiol 46(3):735–742

Santi C, Bogusz D, Franche C (2013) Biological nitrogen fixation in non-legume plants. Ann Bot 111(5):743–767

Sassi-Aydi S, Aydi S, Abdelly C (2012) Inoculation with the native *Rhizobium gallicum* 8a3 improves osmotic stress tolerance in common bean drought-sensitive cultivar. Acta Agric Scand B-S P 62(2):179–187

Sheu SY, Chou JH, Bontemps C et al (2012) *Burkholderia symbiotica* sp nov., isolated from root nodules of *Mimosa* spp. native to north-east Brazil source. Int J Syst Evol Microbiol 62:2272–2278

Singleton P, Keyser H, Sande E (2002) Development and evaluation of liquid inoculants. In: Herridge D (ed) Inoculants and nitrogen fixation of legumes in Vietnam, vol 109e. ACIAR Proceedings, Canberra, pp 52–66

Sivakumar PK, Parthasarthi R, Lakshmipriya VP (2014) Encapsulation of plant growth promoting inoculant in bacterial alginate beads enriched with humic acid. Int J Curr Microbiol Appl Sci 3:415–422

Sivasakthivelan P, Saranraj P (2013) *Azospirillum* and its formulations: a review. Int J Microbiol Res 4(3):275–287

Smil V (2001) Enriching the earth: Fritz Haber, Carl Bosch and the transformation of world food production. The MIT Press, Cambridge, MA, p 338

Smith R (1992) Legume inoculant formulation and application. Can J Microbiol 38:485–492

Smith RS (1995) Inoculant formulations and applications to meet changing needs. In: Tikhonovich IA, Provorov NA, Romanov VI, Newton WE (eds) Nitrogen fixation: fundamentals and applications. Kluwer Academic Publishers, Dordrecht, pp 653–657

Smith D, Zhang F (1999) Composition for enhancing grain yield and protein yield of legumes grown under environmental conditions that inhibit or delay nodulation thereof, US Patent No. 5.922.316. US Patent and Trademark Office, Washington, DC

Smith RS, Ellis MA, Smith RE (1981) Effect of *Rhizobium japonicum* inoculant rates on soybean nodulation in a tropical soil. Agron J 73:505–508

Soares ALL, Ferreira PAA, Pereira JPAR et al (2006) Selected rhizobia of agronomic efficiency and diversity of native nodulating populations pardons (MG).II – bean. R Bras Ci Solo 30:803–811

Soe KM, Yamakawa T (2013) Low-density coinoculation of Myanmar *Bradyrhizobium yuanmingense* MAS34 and *Streptomyces griseoflavus* P4 to enhance symbiosis and seed yield in soybean varieties. Am J Plant Sci 4(09):1879

Somasegaran P (1991) Inoculant production with emphasis on choice of carriers, methods of production and reliability testing/quality assurance guidelines. In: Thompson JA (ed) Report on the expert consultation on legume inoculant production and quality control. FAO, Rome, pp 87–106

Somasegaran P, Hoben HJ (1994) Handbook for rhizobia. Springer, New York, p 450

Sparrow SD, Ham GE (1983) Survival of *Rhizobium phaseoli* in six carrier materials. Agron J 75:181–184

Strijdom BW, van Rensburg HJ (1981) Effect of steam sterilization and gamma irradiation of peat on quality of *Rhizobium* inoculants. Appl Environ Microbiol 41(6):1344–1347

Suman A, Verma P, Yadav AN et al (2016) Development of hydrogel based bio-inoculant formulations and their impact on plant biometric parameters of wheat (*Triticum aestivum* L.) Int J Curr Microbiol App Sci 5(3):890–901

Tairo EV, Ndakidemi PA (2013) Possible benefits of rhizobial inoculation and phosphorus supplementation on nutrition, growth and economic sustainability in grain legumes. Am J Res Commun 1(12):532–556

Tajini F, Trabelsi M, Drevon J (2011) Co-inoculation with *Glomus intraradices* and *Rhizobium tropici* CIAT899 increases P use efficiency for N_2 fixation in the common bean (*Phaseolus vulgaris* L.) under P deficiency in hydroaeroponic culture. Symbiosis 53:123–129

Talbi C, Argandoña M, Salvador M et al (2013) *Burkholderia phymatum* improves salt tolerance of symbiotic nitrogen fixation in *Phaseolus vulgaris*. Plant Soil 367:673–685

Tank N, Saraf M (2010) Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. J Plant Interact 5:51–58

Temprano FJ, Albareda M, Camacho M et al (2002) Survival of several *Rhizobium/Bradyrhizobium* strains on different inoculant formulations and inoculated seeds. Int Microbiol 5:81–86

Tewari S, Arora NK (2014) Talc based exopolysaccharides formulation enhancing growth and production of *Hellianthus annuus* under saline conditions. Cell Mol Biol 60(5):73–81

Thakare CS, Rasal PH (2000) Evaluation of efficient *Rhizobium* strains for groundnut. Legum Res 23(4):259–261

Thenmozhi P, Dinakar S (2014) Exopolysaccharides (EPS) mediated induction of systemic resistance (ISR) in *Bacillus-Fusarium oxysporum* f. sp. *lycopersici* pathosystem in tomato (var. PKM-1). Int J Curr Microbiol App Sci 3(9):839–846

Thies JE, Singleton PW, Bohlool BB (1991) Modeling symbiotic performance of introduced rhizobia in the field by uses of indices of population size and nitrogen status of the soil. Appl Environ Microbiol 57:29–37

Tian CF, Wang ET, Wu LJ et al (2008) *Rhizobium fabae* sp. nov., a bacterium that nodulates *Vicia faba*. Int J Syst Evol Microbiol 58(12):2871–2875

Tilak KVBR, Subba Rao NS (1978) Carriers for legume (*Rhizobium*) inoculants. Fert News 23:25–28

Trujillo ME, Willems A, Abril A et al (2005) Nodulation of *Lupinus albus* by strains of *Ochrobactrum lupini* sp. nov. Appl Environ Microbiol 71:1318–1327

Van Berkum P, Beyene D, Baa G et al (1998) *Rhizobium mongolense* sp. nov., is one of three rhizobial genotypes identified which nodulate and form nitrogen-fixing symbioses with *Medicago ruthenica* [(L.) Ledebour]. Int J Syst Bacteriol 48:13–22

Van Schreven DA, Harmsen GW, Lindenbergh DT et al (1953) Experiments on the cultivation of *Rhizobium* in liquid media for use on zindderzee polders. Antonie van Leeuwenhoek 19:300–308

Vandergheynst JS, Scher HB, Gou HY et al (2007) Water in oil emulsions that improve the storage and delivery of the biolarvacide *Lagenidium giganteum*. BioControl 52:207–229

Vassileva V, Ignatov G (2002) Relationship between bacteroid poly-β-hydroxybutyrate accumulation and nodule functioning in the *Galega orientalis-Rhizobium galegae* symbiosis under diamine treatment. Physiol Plant 114(1):27–32

Verma JP, Yadav J, Tiwari KN et al (2014) Evaluation of plant growth promoting activities of microbial strains and their effect on growth and yield of chickpea (*Cicer arietinum* L.) in India. Soil Biol Biochem 70:33–37

Waelkens F, Voets T, Vlassak K et al (1995) The *nodS* gene of *Rhizobium tropici* strain CIAT899 is necessary for nodulation on *Phaseolus vulgaris* and on *Leucaena leucocephala*. Mol Plant-Microbe Interact 8:147–154

Wang ET, Martínez-Romero E (2000) *Sesbania herbacea–Rhizobium huautlense* nodulation in flooded soils and comparative characterization of *S. herbacea*-nodulating rhizobia in different environments. Microb Ecol 40(1):25–32

Wani PA, Khan MS, Zaidi A (2007) Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp. (*Vigna*) on growth, symbiosis, seed yield and metal uptake by greengram plants. Chemosphere 70:36–45

Waswa MN (2013) Identifying elite rhizobia for commercial soybean (*Glycine max*) inoculants. Dissertation, Agricultural Technology, University of Nairobi

Woronin MS (1866) U¨ ber die bei der Schwarzerle (*Alnus glutinosa*) und bei der gewo¨hnlichen Gartenlupine (*Lupinus mutabilis*) auftretenden Wurzelanschwellungen. Me'moires de l'Academie Impe'riale des Sciences de St. Pe'tersbourg, VII Series, vol. X

Xu S, Liu Y, Wang J et al (2015) Isolation and potential of *Ochrobactrum* sp. NW-3 to increase the growth of cucumber. Int J Agric Policy Res 3(9):341–350

Zahir ZA, Kashif Shah M, Naveed M et al (2010) Substrate-dependent auxin production by *Rhizobium phaseoli* improves the growth and yield of *Vigna radiata* L. under salt stress conditions. J Microbiol Biotechnol 20(9):1288–1294

Zehr JP, Jenkins BD, Short SM et al (2003) Nitrogenase gene diversity and microbial community structure: a cross-system comparison. Environ Microbiol 5:539–554

Zerpa M, Mayz J, Méndez J (2013) Effects of *Bradyrhizobium japonicum* inoculants on soybean (*Glycine max* (L.) Merr.) growth and nodulation. Ann Biol Res 4(7):193–199

Zhang F, Smith DL (2002) Inter-organismal signalling in suboptimal environments: the legume-rhizobia symbiosis. Adv Agron 76:125–161

Zhao LF, Xu YJ, Ma ZQ et al (2013) Colonization and plant growth promoting characterization of endophytic *Pseudomonas chlororaphis* strain Zong1 isolated from *Sophora alopecuroides* root nodules. Braz J Microbiol 44(2):623–631

Zhu R, Tang F, Liu J et al (2016) Co-inoculation of arbuscular mycorrhizae and nitrogen fixing bacteria enhance alfalfa yield under saline conditions. Pak J Bot 48(2):763–769