Formulations of Plant Growth-Promoting Microbes for Field Applications

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Abstract

Development of a plant growth-promoting (PGP) microbe needs several steps starting with isolation of a pure culture, screening of its PGP or antagonistic traits by means of different efficacy bioassays performed in vitro, in vivo or in trials under greenhouse and/or field conditions. In order to maximize the potential of an efficient PGP microbe, it is essential to optimize mass multiplication protocols that promote product quality and quantity and a product formulation that enhances bioactivity, preserves shelf life and aids product delivery. Selection of formulation is very crucial as it can determine the success or failure of a PGP microbe. A good carrier material should be able to deliver the right number of viable cells in good physiological conditions, easy to use and economically affordable by the farmers. Several carrier materials have been used in formulation that include peat, talc, charcoal, cellulose powder, farm yard manure, vermicompost and compost, lignite, bagasse and press mud. Each formulation has its advantages and disadvantages but the peat based carrier material is widely used in different part of the world. This chapter gives a comprehensive analysis of different formulations and the quality of inoculants available in the market, with a case study conducted in five-states of India.

Keywords

Formulation • PGP microbes • Peat • Talc • Lignite • Viability

15.1 Introduction

Public health and safety concerns about the environmental impact of chemical fertilizers and pesticides have led to exploration of PGP microbes for sustainable agriculture. Development of PGP

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D.P. Singh et al. (eds.), *Microbial Inoculants in Sustainable Agricultural Productivity*,
DOI 10.1007/978-81-322-2644-4_15

microbes is a multi-step starting with isolation of a pure culture, screening of its PGP or antagonistic traits by means of an array of *in vitro* and *in vivo* bioassays followed by demonstration under greenhouse and field conditions. In order to maximize the potential of an efficient PGP microbe, it is essential to optimize carefully crafted microbial screening procedures, mass multiplication protocols that promote product quality and quantity and a product formulation that enhances bioactivity, preserves shelf life and aids product delivery. Depending on the PGP microbial groups (viruses, bacteria, yeast or fungi and nematodes), the methods used for industrial scale-up varies; for instance, bacteria and yeast are usually produced in liquid fermentation while fungi are produced in a solid state fermentation (Montesinos 2003). PGP microbe that cannot be cultured on synthetic media, such as viruses and nematodes, are usually scaled-up using an alternate host or tissue culture, as done for nuclear polyhedrosis virus (NPV).

Formulation typically consists of an active ingredient either as microbe(s) or as a product of microbe(s) in a suitable carrier material (sterile or non-sterile) with additives, which help in the stabilization and protection of the microbial cells during storage, transport and at the target site. Selection of formulation is very crucial as it can determine the success or failure of a PGP microbe. A sterile carrier has advantages over non-sterile carrier for delivering the right microbe at the precise concentration and thus avoids the unpredictable potential of an indigenous microorganism(s) to suppress cell numbers (Bashan et al. 2014). A good carrier material should be able to deliver the right number of viable cells in good physiological conditions. Some of the additional characteristics of a good carrier material include: (1) it should be easily sterilized, chemically and physically uniform as possible, having high water-holding capacity and suitable for many microbes; (2) should be reasonably priced, easily manufactured and mixed by existing industry; (3) should allow addition of nutrients and adjustment of pH; (4) should be easily handled by the farmers; and (5) should be non-toxic, biodegradable, non-polluting and have sufficient shelf life (at least 1–2 years at room temperature) (Bashan et al. 2014). Several carrier materials are used in formulation that includes peat, talc, charcoal, cellulose powder, farm yard manure, vermin-compost and compost, lignite, bagasse and press mud (Kumar 2014).

Formulations are of many types, which include dry products (such as granules, dusts and wettable powders), liquid products (such as emulsions, oil and water; usually contains one but sometimes two strains of active ingredient) and microencapsulation. The efficacy of microbial inoculants largely depends on the type of formulation and the delivery technology that extends the shelf lives for at least few months and in all cases the PGP/antagonistic activity is retained. The production cost also has to be considered and kept to a minimal while developing a microbial formulation. A good formulation should be easy to handle and apply so that it is delivered at the target site and protects the PGP microbes and enhances its activity from harmful environmental factors under field conditions. A detailed review on different types of formulations, additives used and PGP/antagonistic microbes used on various crops was reported by Nakkeeran et al. (2005) and Bashan et al. (2014). It is understood that the major role of a formulation is to provide more suitable micro-environment that prevents the rapid decline of an introduced PGP microbe in the soil.

15.2 Ingredients of the Formulations

In order to combat the loss of bioactivity of PGP microbes in formulation, certain ingredients are added. Any formulation, be it an experimental or commercial, requires an amendment for multiplication of PGP microbes and/or products for improving the physical, chemical or nutritional properties of the formulated biomass. Some of the ingredients include stickers/binders such as corn flour, gum arabic and carboxymethyl cellulose (CMC); surfactants such as Tween 80; dispersants such as microcrystalline cellulose; thickeners such as xanthan gum; desiccants such

as silica gel and anhydrous sodium sulphate; stabilizers such as lactose and sodium benzoate; and UV protectants (da Costa et al. 1998; Schisler et al. 2004). Irrespective of formulation ingredients and storage conditions used, the PGP microbes will inevitably be exposed to environmental stresses; however, most microbes have intrinsic cellular mechanisms to protect themselves against hostile environments. Hence, there is a need to understand these cellular mechanisms against environmental stress factors and utilize these effects at the time of stabilization. Many reports support the competitive colonizing ability of bacteria and its impact on plant productivity (Dekkers et al. 2000; Fuente et al. 2001; Gopalakrishnan et al. 2014).

15.3 Types of Formulations

Among the various types of formulations available for PGP microbes, the following six are widely used by the researchers:

15.3.1 Liquid-Based Formulations

The PGP microbes are typically formulated in a liquid buffer with or without added protectants such as sugars. For instance, addition of 10 % lactose or 5 % trehalose increased the storage survival of yeast *Pichia anomala* to varying degrees depending on storage temperature and duration compared to non-supplemented control (Torres et al. 2003; Melin et al. 2006). Addition of sucrose or glycerol was also demonstrated to improve survival of rhizobia, phosphate solubilizing bacteria and *Pseudomonas fluorescens* (Taurian et al. 2010). Liquid formulation has been extensively used in enhancing agricultural productivity under field conditions. For instance, inoculation with *Azospirillum brasilense* as liquid formulation enhanced not only vegetative growth but also harvested grains in wheat (Diaz-Zorita and Fernandez-Canigia 2009). The main advantage of liquid formulation is that it is a simple preparation and no cells are killed during the formulation; while the drawback is the actual weight of the products and shorter shelf life, especially when stored at elevated temperatures (Melin et al. 2011).

15.3.2 Talc-Based Formulation

Talc is composed of minerals in combination with chloride and carbonate and referred as steatite or soapstone or magnesium silicate (Nakkeeran et al. 2005). It is one of the common means of application of bacterial inoculants to soil and is reported effective against plant diseases (Meena et al. 2002; Hassan-El and Gowen 2006). Talc-based formulation of *Streptomyces griseus*, either as single or with chitin, was demonstrated to have stable shelf life of up to 105 days and control *Fusarium oxysporum* f. sp. *lycopersici*, which causes Fusarium wilt in tomato (*Lycopersicon esculentum*) (Anitha and Rabeeth 2009). *Bacillus subtilis* and *P. fluorescens* in talc-based formulations were found to control early blight of tomato caused by *Alternaria solani* and sheath blight of rice caused by *Rhizoctonia solani* (Nandakumar et al. 2001; Sundaramoorthy and Balabaskar 2012). *Ochrobactrum anthropi* TRS-2, a plant growth-promoting bacteria, was found to survive in talc-based formulation up to 9 months and also suppressed brown root rot disease of tea (*Camellia sinensis*) plants (Chakraborty et al. 2009).

15.3.3 Sawdust-Based Formulation

The use of sawdust as carrier is highly recommended where it is easily available, as it contains inherent ability of high organic matter and water-holding capacity compared to other carrier materials (Arora et al. 2001; Kolet 2014). Sawdust was demonstrated as the best carrier among the five tested carriers, viz., alginate beads, charcoal, sand, sugarcane bagasse and sawdust (from *Shorea robusta*), for *P. fluorescens* and *Rhizobium leguminosarum* as both mono-inoculants as well as co-inoculants on *Trifolium repense* (white clover) (Arora et al. 2008). Further, Arora et al.

(2008) also reported that the co-inoculants containing both rhizobial and pseudomonad population proved much better in enhancing the seedling biomass and the nodule number on *T. repense* in addition to increasing the fertility of rhizosphere soil. Recently, Kolet (2014) demonstrated the use of sawdust as carrier material for five cellulolytic bacteria, viz., *Chaetomium globosum*, *C. crispatum*, *C. olivacerum*, *C. nigricolor* and *C. virginicum*. Ambardar and Sood (2010) reported the usefulness of sawdust as carrier material for *P. fluorescens* and *B. cereus*. Chakraborty et al (2013) demonstrated the usefulness of sawdust, talc powder and rice husk as bio-formulations for *Bacillus amyloliquefaciens*, *Serratia marcescens* and *Bacillus pumilus* and reported survivability of up to 9 months of storage.

15.3.4 Fly Ash–Based Formulation

Fly ash, generated in large quantities in thermal power stations, is generally considered as a waste and an environmental hazard. However, it can be used as carrier material as it contains good mineral contents for bio-formulation development of PGP microbes. Fly ash has been reported to promote crop growth and improve soil structure (Kumar et al. 1999). Kumar (2014) noted encouraging results with fly ash as carrier material for *Bacillus* spp., *Azotobacter* spp. and *Pseudomonas* spp. when compared to other formulations. The advantage of using fly ash as bio-formulation is that it increases soil pH and aids in nutrient availability (Dwivedi and Chauhan 2007). Fly ash alone and in combination with other materials was demonstrated in bio-formulation of *Rhizobium* (Kumar and Gupta 2008) and *Trichoderma viride* and *T. harzianum* (Kumar et al. 2012).

15.3.5 Encapsulation-Based Formulation

Encapsulation of PGP microbial cells in polymeric gel (alginate or gluten) is a well-known and established technology where the gel-like matrix allows the cells to remain viable for longer duration (Fravel et al. 1985; Park and Chang, 2000). The main objectives of encapsulation of PGP microbes is to protect them from harsh environment(s) under field conditions, to reduce natural microbial inhabitant competition in soils and to release them gradually to facilitate colonization on host plant roots (Bashan et al. 2002). Immobilization of PGP microbial cells such as *Bacillus megaterium* and *T. viride* using alginate or gluten as the matrix has proved to be advantageous over other methods (Cassidy et al. 1996; Cho and Lee 1999; Sivakumar et al. 2014). Namasivayam et al. (2014) reported enhancement of seedling emergence and PGP of green gram (*Vigna radiata*) and black gram (*Vigna mungo*) upon using encapsulated formulation of *Rhizobium* spp., *Azotobacter* spp. and *Azospirillum* spp. Encapsulation of PGP bacteria, *B. subtilis*, in alginate beads enriched with humic acid effectively protected the bacteria from adverse conditions of the soil for their successful establishment in the rhizosphere (Young et al. 2006). The advantage of using alginate inoculant over peat inoculant is well described (Bashan 1998). It is understood that the use of encapsulation has several advantages over other free cell formulations such as protection from biotic stress (Smit et al. 1996), abiotic stress (Cassidy et al. 1997), inhibitory effect of toxic compounds, enhanced survival and improved physiological activity (Weir et al. 1995) and supply of encapsulated nutritional additives (Trevors et al. 1993).

15.3.6 Peat-Based Formulation

Peat is a carbonized vegetable tissue formed in wet conditions by the slow decay of aquatic and semiaquatic plants such as sedges, rushes, reeds and mosses (Nakkeeran et al. 2005). Peat-based formulation is the most marketed PGP microbial inoculants in developed countries and is most commonly used in rhizobia inoculation industry. In peat-based formulations, bacteria are metabolically active and multiplication continues during the storage period as long as sufficient nutrients, moisture and the optimum temperatures are maintained (Bashan, 1998). The techni-

cal details of production of the peat-based formulations are well described by Catroux et al. 2001; Deaker et al. 2011). Peat-based formulations are coated on seeds or pelleted for sowing in furrows for rhizobia (Toomsan et al. 1984). Of the four formulations (bentonite, talc, rice bran and peat) tested on two different strains of *P. fluorescens*, peat was found more effective as it enhanced the stability and effectiveness of the biocontrol agents (Ardakani et al. 2010). *P. fluorescens* in peat formulation enhanced soybean plant growth under greenhouse conditions when compared to other formulations such as tapioca flour and coconut water in palm oil (Habazar et al. 2014). The main drawback of the peat formulations is its unavailability in many countries.

15.4 ICRISAT's Experience in Using Peat Formulation

International Crops Research Institute for the Semi-Arid Tropics (ICIRSAT), based at Patancheru, Hyderabad, India, has been using peat-based formulation for rhizobial inoculants for chickpea (*Cicer arietinum* L), pigeon pea (*Cajanus cajan* L) and groundnut (*Arachis hypogea* L) crops. ICRISAT hypothesized that one of the main reasons why farmers are not using rhizobial inoculants is that they are not getting quality inoculants. Quality of inoculants can be enhanced only if good carrier materials are used for multiplying and maintaining a PGP microbe in it. In order to find a suitable carrier material, a total of 16 rhizobia (six specific for chickpea such as IC-53, IC-59, IC-76, IC-2002, IC-2018 and IC-2099 and five each specific for pigeon pea such as IC-3195, IC-4059, IC-4060, IC-4061 and IC-4062 and groundnut such as IC-7001, IC-7017, IC-7029, IC-7100 and IC-7113) were inoculated on sterilized peat-based carrier material and allowed to multiply at room temperature (28 ± 2 °C) for 2 weeks. At the end of 2-week incubation, formulated peat inoculants were evaluated for rhizobial survival and longevity and this was considered as 0 month. The rhizobial colonies were represented as colony forming units (CFU) and the CFU was enumerated at 1-month interval for a period of 10 months. The results showed that all 16 rhizobia survived and maintained (at least 10^8 CFU/ ml) up to 9 months (except IC-59, IC-2099 and IC-3195; where population started declining from 9th month onwards) in peat formulations. It was concluded that peat-based carrier material is found to be suitable for rhizobia of chickpea, pigeon pea and groundnut (Table 15.1).

15.5 Survival of PGP Microbes in Formulation

The PGP microbe, when inoculated under field conditions, often finds it difficult to establish a niche for survival amongst the predators (such as protozoans) and competitors (such as better adopted native microflora) in addition to unpredictable fluctuating environmental factors. There are also several other factors such as soil type, plant species, type of native bacteria, inoculant density and sunlight that play a key role in declining the inoculated bacterial density and thereby fail to elicit the intended plant response. Sunlight probably is one of the most important factor in reducing bioactivity of aerial PGP microbial agent application to field crops (Slininger et al. 2003) and this has been demonstrated in bacteria (Hughes et al. 1997), virus (Shapiro and Argauer 1997) and fungus (Yu and Brown 1997). Viability of PGP microbe in an appropriate formulation for a certain length of time is essential for commercialization of the technology. For example, *Bacillus*, *Pseudomonas* and *Ochrobactrum* formulations are reported to survive up to 1 year or more in several bio-formulations (Trivedi et al. 2005; El-Hassan and Gowen 2006; Chakraborty et al. 2009). Sawdust, talc powder and rice husk were used as bio-formulations for *B. amyloliquefaciens*, *Serratia marcescens* and *B. Pumilus*, which showed good survivability even up to 9 months of storage (Chakraborty et al. 2013). Hence, it is concluded that survival and establishment of PGP microbe under field conditions in the rhizosphere in competition with native microbial flora is absolutely essential in order to avail the maximum benefits out of it.

Table 15.1 Viability and longevity of 16 rhizobia in peat formulations over 10 months

Rhizobial isolates	Colony forming units (CFU/ml) at different months (values are mean of 3 replications)										
	0	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Chickpea rhizobia											
IC-53	8.5×10^9	8×10^9	6.5×10^9	2.7×10^9	1.2×10^9	2.9×10^8	2.8×10^8	2.8×10^8	2.5×10^8	2×10^8	1.1×10^8
IC-59	3.3×10^9	2.3×10^9	2.2×10^9	1.7×10^9	2.3×10^8	1.5×10^8	1.3×10^8	1.2×10^8	1×10^8	3×10^7	2.0×10^7
IC-76	4.6×10^9	3.5×10^9	2.3×10^9	2×10^9	1.7×10^9	4.2×10^8	4×10^8	3.9×10^8	3.7×10^8	2.65×10^8	1.2×10^8
IC-2002	16×10^9	12×10^9	6.6×10^9	4.3×10^9	1.5×10^9	2.5×10^8	2.2×10^8	2×10^8	1.7×10^8	0.9×10^8	2.3×10^7
IC-2018	7.5×10^9	7.2×10^9	5.6×10^9	4.2×10^9	16×10^8	4.7×10^8	4.2×10^8	3.6×10^8	3.2×10^8	1.8×10^8	1.3×10^8
IC-2099	4.4×10^9	3.8×10^9	2.4×10^9	2.1×10^9	7×10^8	2.1×10^8	2×10^8	1.7×10^8	1.5×10^8	9×10^7	7.0×10^7
Pigeon pea rhizobia											
IC-3195	16×10^9	8.7×10^9	3.4×10^9	2.5×10^9	1.9×10^8	1.1×10^8	9×10^7	8×10^7	4×10^7	1×10^7	4×10^6
IC-4059	8.6×10^9	7.5×10^9	5.8×10^9	3.6×10^9	1×10^9	5.2×10^8	4.9×10^8	3.4×10^8	2.3×10^8	1.1×10^8	4.1×10^7
IC-4060	18×10^9	17×10^9	7.6×10^9	4.1×10^9	1×10^9	4.5×10^8	4.3×10^8	4.1×10^8	3.1×10^8	1.3×10^8	7×10^7
IC-4061	15×10^9	11×10^9	9.7×10^9	4.2×10^9	1.7×10^9	4.4×10^8	4.2×10^8	4.1×10^8	3.6×10^8	2.2×10^8	1.1×10^8
IC-4062	7.7×10^9	6.3×10^9	2.2×10^9	1.9×10^9	2.3×10^8	3.4×10^8	2.6×10^8	2.1×10^8	1.4×10^8	1×10^8	6×10^7
Groundnut rhizobia											
IC-7001	5.2×10^9	4.8×10^9	4×10^9	2×10^9	1.9×10^8	1.2×10^8	2.3×10^8	2.210^8	2×10^8	1×10^8	2.4×10^7
IC-7017	7.6×10^9	6.6×10^9	3×10^9	2.1×10^9	2.2×10^8	1.7×10^8	1.6×10^8	1.3×10^8	1.1×10^8	7×10^8	3.3×10^7
IC-7029	8.2×10^9	6.8×10^9	5.2×10^9	3.6×10^9	2.0×10^9	5.8×10^8	5.5×10^8	5.2×10^8	4.8×10^8	1.3×10^8	1.5×10^7
IC-7100	6.1×10^9	8.2×10^9	6.3×10^9	3.6×10^9	1.7×10^9	3.7×10^8	3.2×10^8	3×10^8	2.7×10^8	1.3×10^8	7×10^7
IC-7113	8.1×10^9	7.5×10^9	5.4×10^9	4.5×10^9	2.1×10^9	5.5×10^8	5.1×10^8	4.5×10^8	3.7×10^8	1.9×10^8	1.2×10^8

15.6 Regulation and Quality of Commercial Inoculants

An inoculant available in the market should contain sufficient PGP microbe to inoculate plants and produce an economic gain. Many developed countries such as The Netherlands, Thailand, Russia, France, Australia, Canada and the United Kingdom have regulations for inoculant quality which lead to improvements in the quality of commercial inoculants (Bashan et al. 2014). Canada and France has set norms that formulated products should have 10^6 viable rhizobia per seed with no detectable contaminants (Catroux et al. 2001). However, that is not the case in developing countries as most of the inoculants produced are of poor or suboptimal quality. Brockwell and Bottomley (1995) observed that most of the inoculants produced in the world are of relatively poor quality and 90 % of all inoculants have no practical effect on the productivity of crops for which it is used. Upon evaluating 18 different commercial soybean rhizobial inoculants marketed in Argentina, Gomez et al. (1997) found only one liquid inoculant was free of contaminants and carried more than 10^6 *Bradyrhizobium japonicum* while the 17 other inoculants contained between 10^5 and 10^9 contaminants per g product. Olsen et al. (1996) found contaminants in all of the 60 tested commercial inoculants; in addition, the number of rhizobia (5.5×10^5 to 8.1×10^9; per g of product) observed was found to be less than the number of contaminating bacteria (1.8×10^8 to 5.5×10^{10}). The presence and nature of contaminants encountered in inoculants may represent a risk for humans, plants and for the environment, which remains to be assessed.

15.7 Quality of Rhizobial Inoculants Available in the Indian Market – A Case Study

Rhizobia contribute increase in nitrogen fixation and yield in legume crops. Rhizobial inoculants are used where there are no indigenous rhizobia in soil or where the level of the indigenous rhizobia is low. A good quality rhizobial inoculant should be free of contaminants, contains high

number of rhizobia (8.0×10^9 per g of product) and has longer shelf life so that inoculation could be more beneficial for farmers. Even though Bureau of Indian Standards had prescribed certain specifications for rhizobial inoculants to maintain the quality of inoculants (to enable the farmers to obtain certified inoculants), many brands of rhizobial inoculants marketed today in India have been found to vary in quality and reliability. Hence, in order to have a thorough investigation on quality of rhizobial inoculants available in the Indian market, a case study was conducted in 2010–11 by ICRISAT, Patancheru. The major objective of this case study was to check the quality of chickpea rhizobial inoculants available in the market in five states of India.

Rhizobial inoculants of chickpea were purchased from the market in five states of India (Hyderabad in Telangana; Rajanandgoun, Kabirdham and Raipur in Chhattisgarh; Jabalpur, Damoh, Rewa and Satna in Madhya Pradesh; Bhubaneswar in Orissa; and Ranchi in Jharkhand) and stored in refrigerator at 4 °C until processed. A total of 28 samples (14 in May 2010 and another 14 in Nov 2010) were procured and used in this study. All the inoculant samples were analysed for pH, moisture content, purity (plated on yeast extract mannitol [YEM] agar to observe *Rhizobium* like colonies; Log $_{10}$ values), total rhizobial count (Log_{10} values), presence of contamination (Log_{10} values) and further evaluated for their nodulation potential (by plant infection test as per the standard protocol of ICRISAT) in chickpea.

Table 15.2 Identity of the chickpea rhizobial inoculants procured from five states of India

Area	State	Culture type
Batch 1 (May 2010)		
Rajanandgoun	Chhattisgarh	Lignite
Rajanandgoun	Chhattisgarh	Lignite
Rajanandgoun	Chhattisgarh	Lignite
Raipur	Chhattisgarh	Talcum
Bhubaneshwar	Orissa	Liquid
Bhubaneshwar	Orissa	Lignite
Jabalpur	Madhya Pradesh	Lignite
Jabalpur	Madhya Pradesh	Lignite
Satna	Madhya Pradesh	Lignite
Satna	Madhya Pradesh	Lignite
Satna	Madhya Pradesh	Lignite
Satna	Madhya Pradesh	Lignite
Ranchi	Jharkhand	Lignite
Hyderabad	Telangana	Talcum
Batch 2 (Nov 2010)		
Satna	Madhya Pradesh	Lignite
Satna	Madhya Pradesh	Lignite
Satna	Madhya Pradesh	Lignite
Damoh	Madhya Pradesh	Lignite
Jabalpur	Madhya Pradesh	Liquid
Rewa	Madhya Pradesh	Lignite
Rewa	Madhya Pradesh	Lignite
Ranchi	Jharkhand	Lignite
Kabirdham	Chhattisgarh	Lignite
Rajanandgoun	Chhattisgarh	Lignite
Raipur	Chhattisgarh	Talcum
Bhubaneshwar	Orissa	Lignite
Bhubaneshwar	Orissa	Lignite
ICRISAT	Telangana	Peat
Hyderabad	Telangana	Lignite

Of the 28 commercial formulated samples, 23 were made of lignite, three of talcum powder and one of liquid inoculation, whereas the ICRISAT sample was made of peat (Table 15.2). The optimum pH for growing rhizobia is 7.0 while the pH of the rhizobial inoculants from the market varied between 2.1 and 9.4. Among the 28 samples analysed, 13 samples were found highly acidic (pH ranged between 2.1 and 5.8), 7 were alkaline (pH ranged between 8.2 and 9.4) and only 8 samples were found fit for growing *Rhizobium* cultures (Fig. 15.1). The optimum moisture percentage for growing rhizobia in any carrier material is 30 %. Among the 28 rhizobial inoculants, five of them contained less than 15 % moisture while six other sources contained more than 40 % moisture (Fig. 15.2). When the samples were plated on YEM agar to observe *Rhizobium* like colonies, only 15 samples contained *Rhizobium*-like colonies (Fig. 15.3). All but six samples contained contamination and these were found more than the *Rhizobium*-like colonies while the remaining six samples were found to be completely sterile, where neither rhizobia nor any contamination was found (Fig. 15.4). When the 28 samples were analysed for nodulation capability by plant

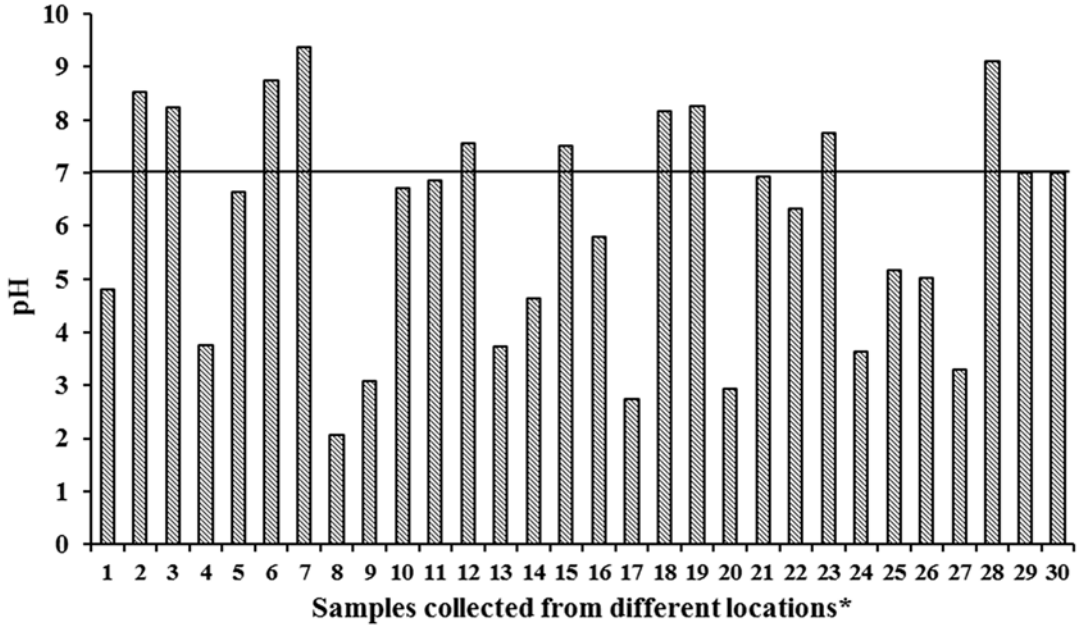

Fig. 15.1 pH of the 28 chickpea rhizobial inoculants procured from five different states of India (Footnote: * = 1–4 from Orissa, 5–11 from Chhattisgarh, 12–13 from Jharkhand, 14–26 from Madhya Pradesh, 27 and 28 from Telangana. Sample numbers 29 and 30 are from ICRISAT)

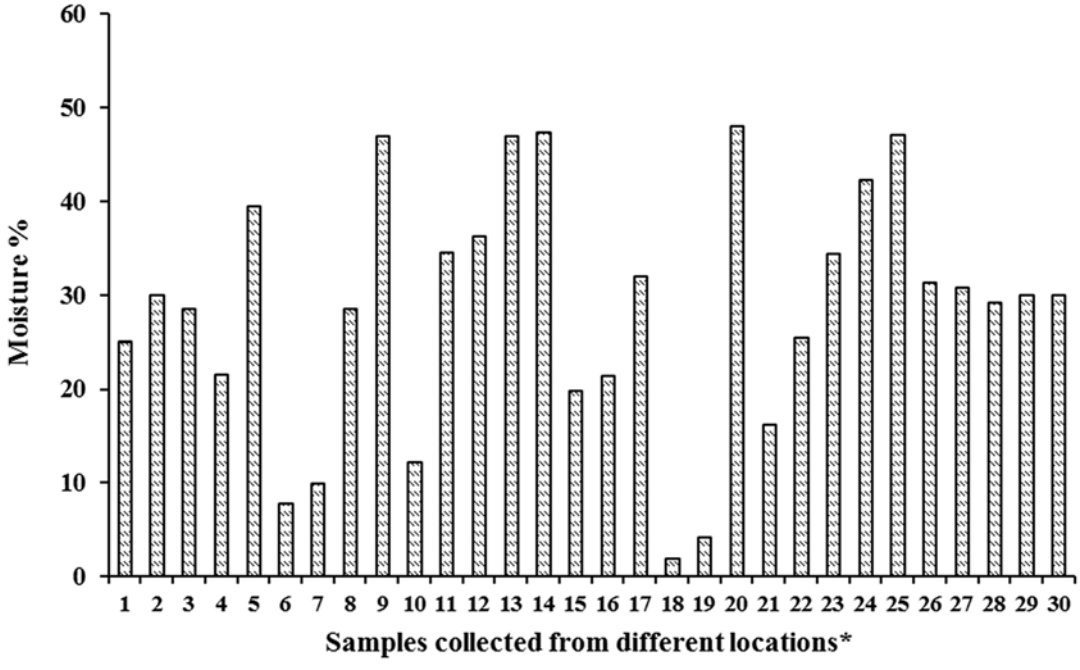

Fig. 15.2 Moisture content of the 28 chickpea rhizobial inoculants procured from five different states of India. (Footnote: * = 1–4 from Orissa, 5–11 from Chhattisgarh, 12–13 from Jharkhand, 14–26 from Madhya Pradesh, 27 and 28 from Telangana. Sample numbers 29 and 30 are from ICRISAT)

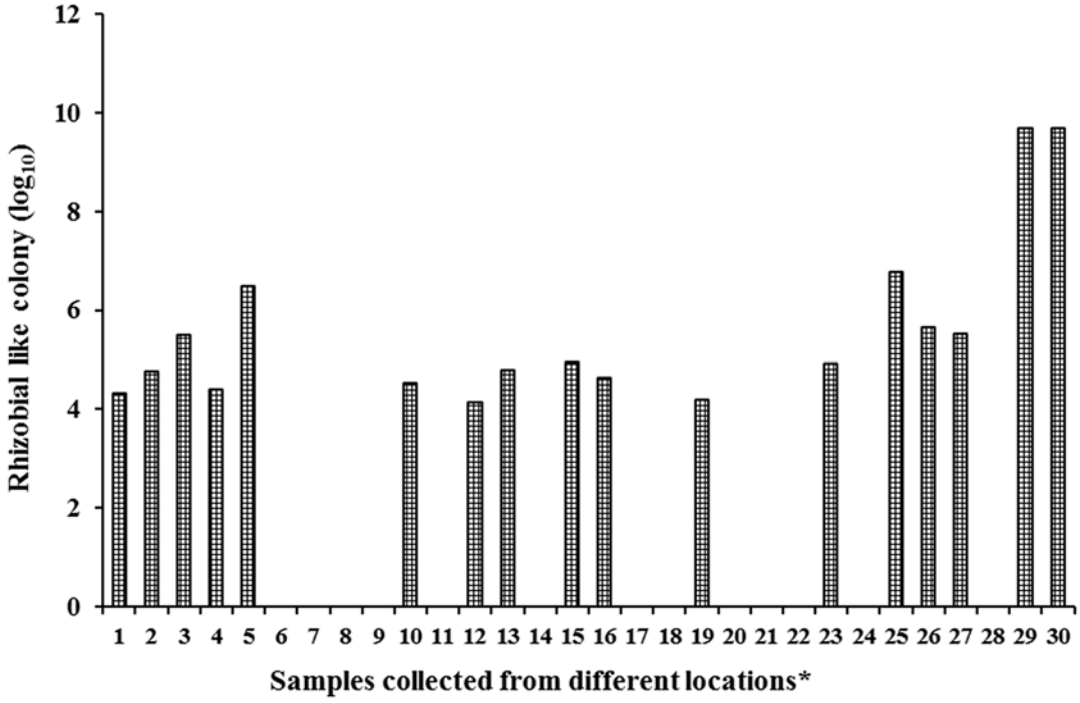

Fig. 15.3 Rhizobia-like colonies present in the 28 chickpea rhizobial inoculants procured from five different states of India. (Footnote: * = 1–4 from Orissa, 5–11 from Chhattisgarh, 12–13 from Jharkhand, 14–26 from Madhya Pradesh, 27 and 28 from Telangana. Sample numbers 29 and 30 are from ICRISAT)

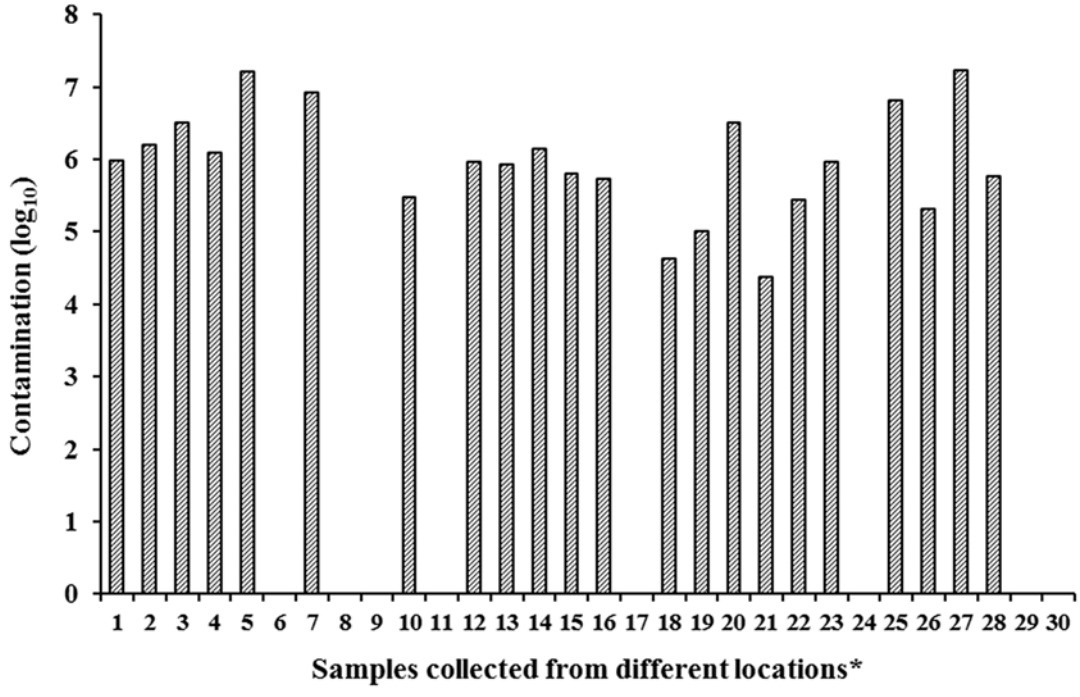

Fig. 15.4 Microbial contaminants present in the 28 chickpea rhizobial inoculants procured from five different states of India. (Footnote: * = 1–4 from Orissa, 5–11 from Chhattisgarh, 12–13 from Jharkhand, 14–26 from Madhya Pradesh, 27 and 28 from Telangana. Sample numbers 29 and 30 are from ICRISAT)

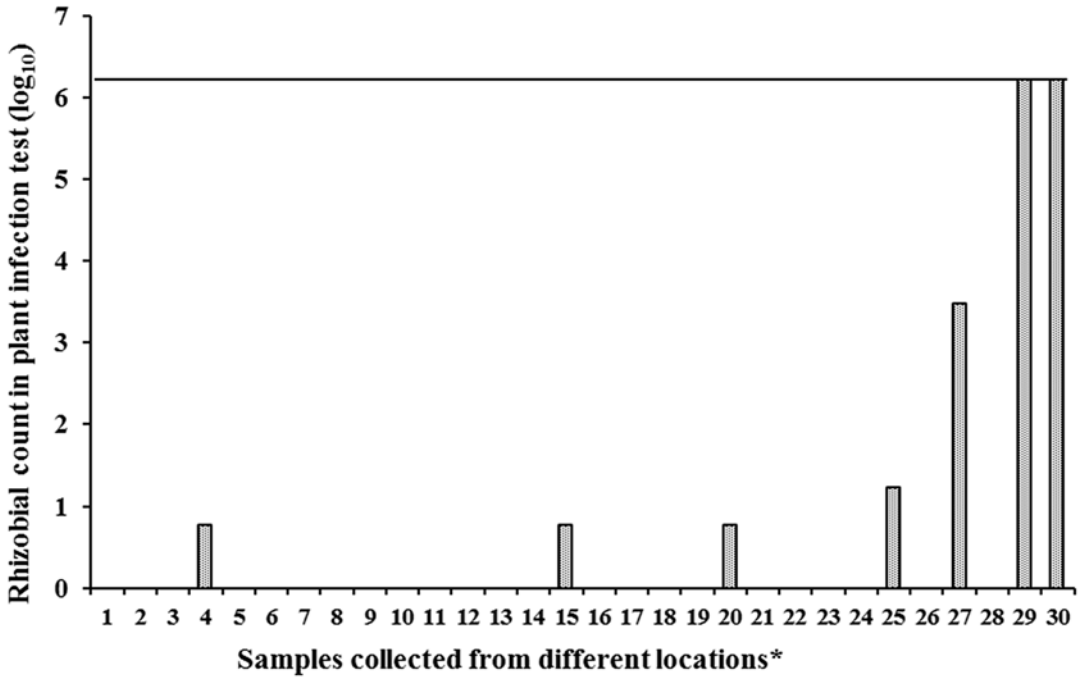

Fig. 15.5 Rhizobial counts in the 28 chickpea rhizobial inoculants procured from five different states of India (Footnote: * = 1–4 from Orissa, 5–11 from Chhattisgarh, 12–13 from Jharkhand, 14–26 from Madhya Pradesh, 27 and 28 from Telangana. Sample numbers 29 and 30 are from ICRISAT)

infection test in chickpea (which tells whether the rhizobia is capable of producing nodules or not), only five rhizobial inoculants were able to produce nodules. Of the five nodulated inoculant samples, rhizobia were found very less (log values 0.78–3.49) compared to positive control (Log values 6.23; where ICRISAT rhizobial inoculants were used (Fig. 15.5). Thus, it was concluded that rhizobial inoculants available in the Indian market contained no or very little rhizobia.

15.8 Conclusion

Application of PGP microbial agents to rhizosphere, phyllosphere and spermosphere particularly under field conditions is less effective or at times totally ineffective. This is mainly due to the type of carrier material used and variation in climatic conditions that suppress growth and survival of PGP microbial agents (Guetsky et al. 2001). Therefore, the efficacy of PGP microbes needs to be improved through the usage of compatible mixed inoculum of PGP microbial agents rather than using a monoculture. Also, for the commercial delivery of a PGP microbe, the beneficial microorganism must be manufactured at industrial scale (in large fermenters), preserved for storage and formulated by means of biocompatible additives in order to increase its survival and stability and to improve the application. The future of PGP microbes depends not only in developing an efficient strain of PGP microbe but also in developing new active ingredients (secondary metabolites from potential PGP microbes). It is not important what formulation is used in developing a PGP microbe but it is important that the formulation has a product shelf life with retained biological activity for up to a year preferably at ambient temperatures. The development of new formulation(s) for PGP microbes is a challenging task as it requires greater effort in terms of funding and research. However, continued research may lead to improvements in for-

mulations for the best PGP microbes/ products. Also, conducting formulation research in the private sector will greatly expedite progress in this critical area for advancing the successful incorporation of PGP microbes and/or their products. Finally, the acceptance of PGP microbes as nutrient/pest management tools is dependent on the development of low-cost bio-agents/products which provide consistent efficacy.

References

Ambardar VK, Sood AK (2010) Suitability of different growth substrate for mass multiplication of bacterial antagonists. Indian Phytopathol 63:380–383

Anitha A, Rabeeth M (2009) Control of *Fusarium* wilt of tomato by bio-formulation of *Streptomyces griseus* in greenhouse condition. Afr J Basic Appl Sci 1:9–14

Ardakani SS, Heydari A, Khorasani N, Arjmandi R (2010) Development of new bio-formulations of *Pseudomonas fluorescens* and evaluation of these products against damping-off of cotton seedlings. J Plant Pathol 92:83–88

Arora NK, Khare E, Naraian R, Maheswari DK (2008) Saw-dust as a superior carrier for production of multipurpose bio-inoculant using plant growth promoting rhizobial and Pseudonmonad strains and their impact on productivity of *Trifolium repnse*. Curr Sci 95:90–94

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

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16:729–770

Bashan Y, de-Bashan LE, Prabhu SR, Hernandez JP (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998─2013). Plant Soil 378:1–33

Bashan Y, Hernandez JP, Levya LA, Bacilio M (2002) Alginate micro-beads as inoculant carriers for plant growth-promoting bacteria. Biol Fertil Soils 35: 359–368

Brockwell J, Bottomley PJ (1995) Recent advances in inoculant technology and prospectus for the future. Soil Biol Biochem 27:683–687

Cassidy MB, Lee H, Trevors JT (1996) Environmental applications of immobilized microbial cells: A review. J Ind Microbiol 16:17–101

Cassidy MB, Lee H, Trevors JT (1997) Survival and activity of *lac*-lux marked *Pseudomonas aeruginosa* UG2Lr cells in encapsulated K-caragreenan over 4 years at 4 ºÇ. J Microbiol Methodol 30:167–170

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

Chakraborty U, Chakraborty BN, Basnet M, Chakraborty AP (2009) Evaluation of *Orchrobactrum anthropi* TRS-2 and its talc-based formulation for enhancement of growth of tea plants and management of brown root rot disease. J Appl Microbiol 107:625–634

Chakraborty U, Chakraborty BN, Chakraborty AP, Sunar K, Dey PL (2013) Plant growth-promoting rhizobacteria mediated improvement of health status of tea plants. Indian J Biotechnol 12:20–31

Cho C, Lee W (1999) Formulation of a biocontrol agent by entrapping biomass of *Trichoderma viridi* in gluten matrix. J Biosci Bioeng 87:822–824

da Costa MS, Santos H, Galinski EA (1998) An overview of the role and diversity of compatible solutes in bacteria and archaea. Adv Biochem Eng Biotechnol 61:117–153

Dekkers LC, Mulders IH, Phoelich CC, Chin-A-Woeng TFC, Wijfjes AH, Lugtenberg BJJ (2000) The *sss* colonization gene of the tomato *Fusarium oxysporum* f. sp. *radices-lycopersici* biocontrol strain *Pseudomonas fluorescens* WCS365 can improve root colonization of other wild type *Pseudomonas* spp. bacteria. Mol Plant Microbe Interact 13:1177–1183

Deaker R, Kecskés ML, Rose MT, Amprayn K, Ganisan K, Tran TKC, Vu TN, Phan TC, Hien NT and Kennedy IR (2011) Practical methods for the quality control of inoculant bio-fertilisers. Monograph Series No.147. Australian Centre for International Agricultural Research, Canberra, p 101

Diaz-Zorita M, Fernandez-Canigia M (2009) Field performance of a liquid formulation of *Azospirillum brasilense* on dryland wheat productivity. Eur J Soil Biol 45:3–11

Dwivedi J and Chauhan R (2007) In: Proceedings of the 44th annual convention of chemists by Indian Chemical Society, Kolkata at MGIAS, Jaipur, p. D10

El-Hassan SA, Gowen SR (2006) Formulation and delivery of the bacterial antagonist *Bacillus subtilis* for management of lentil vascular wilt caused by *Fusarium oxysporum* f. sp. *lentis*. J Phytopathol 154:148–155

Fravel DR, Marois JJ, Lumsden RD, Connick WJ Jr (1985) Encapsulation of potential biocontrol agents in an alginate-clay matrix. Phytopathology 75:774–777

Fuente AB, De La L, Leticia Q, Natalia B, Elena F, Nora A, Alicia A (2001) Inoculation with *Pseudomonas fluorescens* biocontrol strains does not affect the symbiosis between rhizobia and forage legumes. Soil Biol Biochem 34:545–548

Gomez M, Silva N, Hartmann A, Sagardoy M, Catroux G (1997) Evaluation of commercial soybean inoculants from Argentina. World J Microbiol Biotechnol 13:167–173

Gopalakrishnan S, Vadlamudi S, Bandikinda P, Sathya A, Vijayabharathi R, Rupela O, Kudapa B, Katta K, Varshney RK (2014) Evaluation of *Streptomyces* strains isolated from herbal vermicompost for their plant growth-promotion traits in rice. Microbiol Res 169:40–48

Guetsky R, Elad Y, Shtienberg D, Dinoor A (2001) Combining biocontrol agents to reduce variability of biological control. Phytopathology 91:261–267

Hassan-El SA, Gowen SR (2006) Formulation and delivery of the bacterial antagonist *Bacillus subtilis* for management of lentil vascular wilt caused by *Fusarium oxysporum* f. sp. *lentis*. J Phytopathol 154:148–155

Habazar T, Yanti Y, Ritonga C (2014) Formulation of indigenous rhizobacterial isolates from healthy soybean's root, which ability to promote growth and yield of soybean. Int J Adv Sci Eng Inform Tech 4:5

Hughes PR, Wood HA, Breen JP, Simpson SF, Duggan AJ, Dybas JA (1997) Enhanced bioactivity of recombinant baculoviruses expressing insect specific spider toxins in lepidopteran crop pests. J Invertebr Pathol 69:112–118

Kolet M (2014) Assessment of sawdust as carrier material for fungal inoculum intended for faster composting. Int J Curr Microbiol Appl Sci 3:608–613

Kumar V (2014) Characterization, bio-formulation development and shelf-life studies of locally isolated biofertilizer strains. Octa J Environ Res 2:32–37

Kumar V, Goswami G, Zacharia KA (1999) Issues and concern. In: International conference on fly-ash disposal and utilization. Ind Soc Soil Sc. October, Calcutta, pp 18–21

Kumar V, Gupta P (2008) Efficacy of fly-ash based *Rhizobium* on growth and incidence of powdery mildew in pea. Ann Plant Protect Sci 16:248–249

Kumar V, Gupta P, Dwivedi S (2012) Bio-efficacy of fly-ash based *Trichoderma* formulations against damping-off and root-rot diseases in tomato. Indian Phytopathol 65:404–405

Meena B, Radhajeyalakshmi R, Marimuthu T, Vidhyasekaran P, Velazhahan R (2002) Biological control of groundnut late leaf spot and rust by seed and foliar application of a powder formulation of *Pseudomonas fluorescens*. Biocontrol Sci Technol 12:195–204

Melin P, Hakansson S, Eberhard TH, Schnurer J (2006) Survival of the biocontrol yeast *Pichia anomala* after long-term storage in liquid formulation and different temperature, assessed by flow cytometry. J Appl Microbiol 100:264–271

Melin P, Schnurer J, Hakansson S (2011) Formulation and stabilization of the biocontrol yeast *Pichia anomala*. Antonie Van Leeuwenhoek 99:107–112

Montesinos E (2003) Development, registration and commercialization of microbial pesticides for plant protection. Int Microbiol 6:245–252

Nakkeeran S, Fernando WGD, Siddiqui ZA (2005) Plant growth-promoting rhizobacteria formulations and its scope in commercialization for the management of pests and diseases. In: Siddiqui ZA (ed) PGPR: Biocontrol and Biofertilization. Springer, Dordrecht, pp 257–296

Namasivayam SKR, Saikia SL, Bharani RSA (2014) Evaluation of persistence and plant growth-promoting effect of bio-encapsulated formulation of suitable bacterial bio-fertilizers. Biosci Biotechnol Res Asia 11:407–415

Nandakumar R, Babu S, Viswanathan R, Sheela J, Raguchander T, Samiyappan R (2001) A new bioformulation containing plant growth-promoting rhizobacterial mixture for the management of sheath blight and enhanced grain yield in rice. Bio Control 46:493–510

Olsen PE, Rice WA, Bordeleau LM, Denudiff AH, Collins MM (1996) Levels and identities of non-rhizobial microorganisms found in commercial legume inoculant made with non-sterile peat carrier. Can J Microbiol 42:72–75

Park JK, Chang HN (2000) Microencapsulation of microbial cells. Biotechnol Adv 18:303–319

Schisler DA, Slininger PJ, Behle RW, Jackson MA (2004) Formulation of *Bacillus* spp. for biological control of plant diseases. Phytopathology 94:1267–1271

Shapiro M, Argauer R (1997) Components of the stilbene optical brightener Tinopal LPW as enhancers of the gypsy moth (Lepidoptera: Lymatridae) baculovirus. J Eco Entomol 90:899–904

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

Slininger PJ, Behle RW, Jackson MA, Schisler DA (2003) Discovery and development of biocontrol agents to control crop pests. Neotropical Entomol 32:183–195

Smit E, Wolters AC, Lee H, Trevors JT, van Elsas JD (1996) Interaction between a genetically marked *Pseudomonas fluorescens* strain and bacteriophage ØR2f in soil: Effects of nutrients, alginate encapsulation and the wheat rhizosphere. Microbiol Ecol 31:125–140

Sundaramoorthy S, Balabaskar P (2012) Consortial effect of endophytic and plant growth-promoting rhizobacteria for the management of early blight of tomato incited by *Alternaria solani*. J Plant Pathol Microbiol 3:7

Taurian T, Anzuay MS, Angelini JG, Tonelli ML, Ludueña L, Pena D, Ibáñez F, Fabra A (2010) Phosphate-solubilizing peanut associated bacteria: screening for plant growth-promoting activities. Plant Soil 329:421–431

Trevors JT, van Elsas JD, Lee H, Wolters AC (1993) Survival of alginate encapsulated *Pseudomonas fluorescens* cells in soil. Appl Microbiol Biotechnol 39:637–643

Trivedi P, Pandey A, Palni LMS (2005) Carrier-based preparations of PGP bacterial inoculants suitable for use in cooler regions. World J Microbiol Biotehnol 21:941–945

Toomsan B, Rupela OP, Mittal S, Dart P, Clark KW (1984) Counting Cicer Rhizobium using a plant infection technique. Soil Biol Biochem 16:503–507

Torres R, Usall J, Teixido N, Abadias M, Vinas I (2003) Liquid formulation of the biocontrol agent *Candida sake* by modifying water activity or adding protectants. J Appl Microbiol 94:330–339

Weir SC, Dupuis SP, Providenti MA, Lee H, Trevors JT (1995) Nutrient enhanced survival of and phenanthrene mineralization by alginate encapsulated and free *Pseudomonas* spp. UG14Lr cells in creosote contaminated soil slurries. Appl Microbiol Biotechnol 43:946–951

Young CC, Rekha PD, Lai WA, Arun AB (2006) Encapsulation of plant growth-promoting bacteria in alginate beads enriched with humic acid. Biotechnol Bioeng 95:76–83

Yu Z, Brown GC (1997) Auto-dissemination of a beet army worm (Leptidoptera: Noctuidae) baculovirus under laboratory conditions. J Econ Entomol 90:1187–1194