Microbial Consortial Products for Sustainable Agriculture: Commercialization and Regulatory Issues in India

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Abstract

Rhizosphere microorganisms directly and indirectly influence the composition and productivity of natural plant communities. Hence, belowground microbial species richness has been proposed as a predictor of aboveground plant diversity and productivity. Though research-based evidences clearly show the advantages of microbial consortia-based products due to their multifunctionality, limited attention is being given to develop quality standards for registration. This chapter focuses on the uses, commercialization, and regulatory issues of various bacterial consortia in sustainable agriculture.

Keywords

Consortia • Sustainable agriculture • Biofertilizers • Biopesticides • Rhizosphere

7.1 Introduction

Microbes are the most diverse communities on Earth that play a pivotal role in Earth's climatic, geological, geochemical, and biological process (Tringe et al. 2005; Xu 2006). The diverse genetic and functional groups of the soil microbial population exert a critical impact on soil function (Barea et al. 2005; Avis et al. 2008), particularly in the root–soil microhabitat referred to as rhizosphere which is considered as the hot spot for interaction between eukaryotes and prokaryotes (Jones and Hinsinger 2008; Hinsinger et al. 2009; Raaijmakers et al. 2009).

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Microbial interaction in the soil can be managed with low biotechnological inputs, to help sustainable and environment-friendly agro-technological practice (Azcón and Barea 2010; Ramos-Solano et al. 2010). The rhizosphere offers a complex microhabitat where root exudates provide a diverse mixture of organic compounds that are used as nutrients or signals by the soil microbial population (Brimecombe et al. 2007; Jones et al. 2009; Dennis et al. 2010; Bulgarelli et al. 2013) which results in a high degree of interaction between microbes, plant, and soil. Thus, understanding the function of microbial communities in the rhizosphere is of current research interest and has been extensively reviewed by many authors (Jones and Hinsinger 2008; Berg and Smalla 2009; Cavaglieri et al. 2009; Keswani et al. 2013; Unno and Shinano 2013; Vacheron et al. 2013; Chaparro et al. 2014; Gupta et al. 2015; Schlaeppi and Bulgarelli 2015; Bisen et al. 2015).

Rhizosphere microorganisms directly and indirectly influence the composition and productivity (i.e., biomass) of natural plant communities (Van der Heijden et al. 1998, 2008; Schnitzer et al. 2011). Hence, belowground microbial species richness has been proposed as a predictor of aboveground plant diversity and productivity (De Deyn et al. 2004; Hooper et al. 2005; van der Heijden et al. 2008; Lau and Lennon 2011). Wagg et al. (2011) further suggested that belowground diversity may act as an insurance for maintaining plant productivity under different environmental conditions.

Microbial groups residing in the rhizosphere include bacteria, fungi, archaea, algae, nematodes, protozoa, viruses, oomycetes, and microarthropods (Lynch 1990; Buée et al. 2009; Mendes et al. 2013). The bacterial groups like *Pseudomonas*, *Azospirillum*, *Methylobacterium*, *Enterobacter*, *Serratia*, *Arthrobacter*, *Azotobacter*, *Bacillus*, etc. lead the microbial population in the rhizosphere soil, followed by fungi, actinomycetes, and other groups (Gray and Smith 2005; Mendes et al. 2013; Nunes da Rocha et al. 2013). The overall interaction of the rhizomicrobiome and its function and impact on plant is represented in Fig. 7.1.

7.2 Plant–Microbe Interactions

Plant–microbe interactions in the rhizosphere depend on the function of the associated microorganisms based on which the microbes are classified as beneficial, deleterious, and neutral groups, and the bacteria that belong to the beneficial group are referred to as "plant growth-promoting rhizobacteria" (PGPR) (Kloepper et al. 1989). The PGPR are reported to enhance plant growth by a multitudinous mechanism which include production of plant growth-regulating substances (Kloepper 1993; Picard et al. 2000; Saravanakumar et al. 2008; Vyas and Gulati 2009; Farajzadeh et al. 2012; Santoyo et al. 2012; Bisen et al. 2016), phytohormones, suppression of plant pathogens through antibiosis (Couillerot et al. 2011; Sayyed and Patel 2011; Singh et al. 2011; Santoyo et al. 2012; Yin et al. 2013; Yokoyama et al. 2013; Sekar and Prabavathy 2014), nitrogen fixation (Franzini et al. 2010; Kathiravan et al. 2013; Mapelli et al. 2013; Sahoo et al. 2013), mineralization of organic phosphorus (Park et al. 2010; Sashidhar and Podile 2010), mediation of abiotic stress

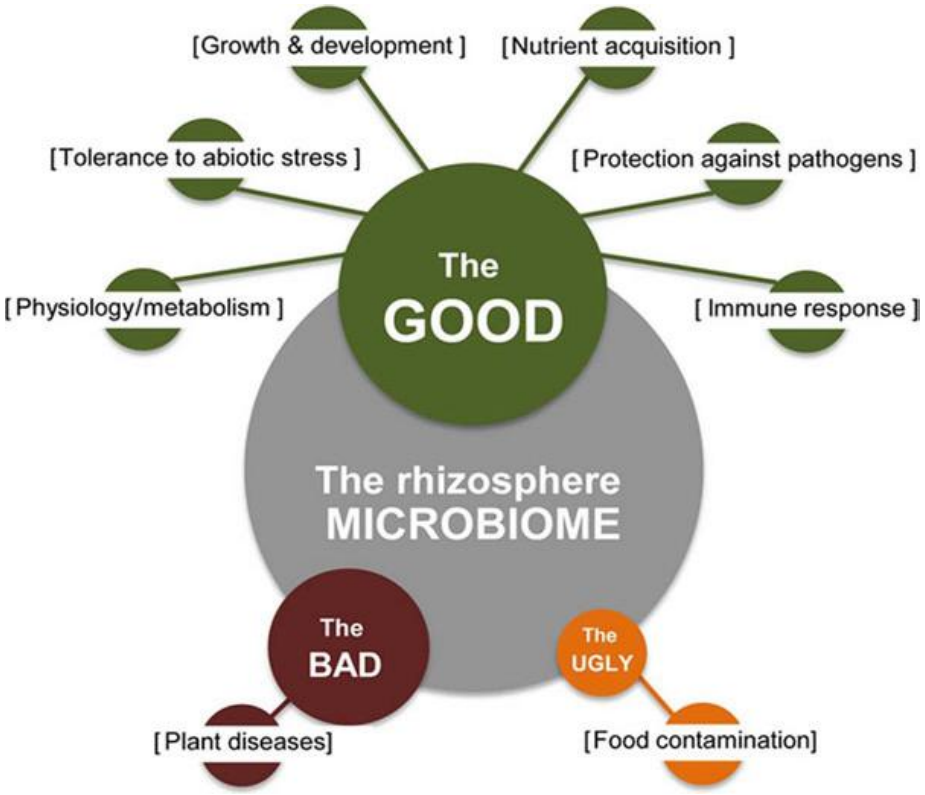

Fig. 7.1 Schematic representation of the functions and interactions of the rhizomicrobiome (Source: Mendes et al. 2013)

tolerance (Tringe et al. 2005; Zahir et al. 2009; Palaniyandi et al. 2013; Parihar et al. 2015; Shrivastava and Kumar 2015), production of phytoalexins/flavonoid-like compounds, and enhancement of mineral uptake (Parmar and Dadarwal 1999). The microbial community in the rhizosphere harbors members of few groups that adversely affect plant growth and health, viz., pathogenic fungi, oomycetes, bacteria, and nematodes (Raaijmakers et al. 2009; Damiani et al. 2012; Weller et al. 2012; Sekar and Prabavathy 2014).

Rhizosphere-associated copious beneficial microbial groups with multi-beneficial plant growth-promoting traits have been reported by many researchers (Raupach and Kloepper 1998; Picard and Bosco 2008; Ryan et al. 2008; Hartmann et al. 2009; Sekar and Prabavathy 2014; Viswanath et al. 2015; Krishnan et al. 2016; Raju et al. 2016). Bacterial groups secrete signaling molecules that influence bacterial gene expression and physiological behavior in a density-dependent manner termed quorum sensing (QS) (Zhang and Pierson 2001; Schuhegger et al. 2006; Liu et al. 2007; Viswanath et al. 2015); especially the rhizosphere regions were reported to harbor high N-acyl homoserine lactone (AHL) population (Elasri et al. 2001; DeAngelis et al. 2008; Viswanath et al. 2015). The QS-controlled phenotypes play a vital role for successful inter-/intra-gene and host interactions, whether symbiotic or pathogenic (Boyer and Wisniewski-Dyé 2009), and also influence interaction

with plants such as root colonization and induction of systemic resistance (Pang et al. 2008; Hartmann et al. 2014).

During the past few decades, the interaction between rhizobacteria and plants has been well explored and has resulted in the application of microbial products as crop inoculants (biofertilizers/biopesticides), for increased crop biomass and disease suppression. Combined application of potential PGPR strains is termed as microbial consortium (MC) which offers multi-beneficial plant growth-promoting traits and provides solution to underpinning problems like drought, salinity, increasing temperature, pest, and phytopathogenic infections in the agricultural system leading to global food safety and security. Microbial consortia are inoculants in a synergistic mixture which fulfill diverse functions in the rhizosphere and are the most promising contenders for solving challenges linked to sustainable eco-friendly agriculture (Jain et al. 2013).

7.3 Microbial Consortium as Biofertilizer and Biocontrol Agents

Currently agriculture is heavily dependent on mineral fertilizers and inorganic pesticides, and impacts of the continuous application are reflected in deteriorating soil health and increased resistance to pest and pathogens (Kumar et al. 2010; Cai et al. 2016). In the past 40 years, usage of nitrogen fertilizer has increased by sevenfold and pesticide usage by threefold. In the future these trends will continue unabated, as application of both inorganic fertilizer and pesticides is expected to increase by an additional threefold by 2050 which would cause unprecedented damage to the agroecosystem (Tilman et al. 2001).

Engineering the plant rhizomicrobiome is an alternative approach to increase soil health and enhance plant productivity (Jia et al. 2004; Wagg et al. 2011; Chaparro et al. 2012; Pindi and Satyanarayana 2012). Microbial interaction in the rhizosphere provides plants with multiple plant growth-benefiting traits and stress-tolerant traits apart from enhancing their own population and functions (Roesti et al. 2006; Jain et al. 2012; Wang et al. 2012; Jain et al. 2013; Singh et al. 2013; Thijs et al. 2014; Keswani et al. 2014; Armada et al. 2015). The inconsistency in performance of single microbial products in field application has emphasized the need for co-inoculation or consortia of microbial products (Bashan and de-Bashan 2005).

7.4 Bacteria–Bacteria Consortium for Plant Growth Promotion

Rhizobia and other PGPR share a common microhabitat, the root–soil interface, where interaction between different microbial groups was reported during root colonization. Co-inoculation of rhizobia with other PGPR enhanced nodulation and nitrogen fixation through the production of plant hormone, flavonoids, Nod factor, or enzymes in pigeon pea and other legumes (Tilak et al. 2006; Dardanelli et al.

2008; Remans et al. 2008; Medeot et al. 2010; Bansal and Srivastava 2012; Gupta et al. 2015). *Azospirillum*, a free-living diazotroph, *Azotobacter*, *Bacillus*, *Pseudomonas*, *Serratia*, and *Enterobacter* are a few genera that have been successfully used with rhizobium as co-inoculants (Gaind et al. 2007; Remans et al. 2008; Cassán et al. 2009; Ahmad et al. 2011; Dashadi et al. 2011; Tajini et al. 2012; Ahemad and Kibret 2014; Gopalakrishnan et al. 2014). Besides the indigenous rhizobia community, inoculated diazotrophs like *Azospirillum* enhanced growth and yield in leguminous crops upon inoculation and increased fixed nitrogen quantity (Remans et al. 2008). Co-inoculation of *A. lipoferum* and *R. leguminosarum* bv. *trifolii* improved nodulation in white clovers, pigeon pea, and chickpea (Deanand et al. 2002). Most of the studies showed co-inoculation of *Azospirillum*, and *Rhizobium* significantly increased both the upper and total nodule number, acetylene reduction activities, faster 15 N dilution, and the total macro- and micronutrient mineral content as compared to other inoculants (Rodelas et al. 1996; German et al. 2000; Dardanelli et al. 2008; Askary et al. 2009; Cassán et al. 2009; Dashadi et al. 2011). Mehboob et al. (2013) extensively reviewed the effects of co-inoculation of rhizobia with various rhizospheric bacteria. *Azotobacter* was found to be a potential co-inoculant with rhizobium and enhanced the production of phytohormones and vitamins (Chandra and Pareek 2002; Qureshi et al. 2009; Dashadi et al. 2011; Akhtar et al. 2012). Co-inoculation of *G. intraradices*, *Pseudomonas striata*, and *Rhizobium* showed significant increase in plant growth, number of pods, and chlorophyll content in chickpea root rot (Akhtar and Siddiqui 2008).

Combination of *Rhizobium* with *Bacillus* strains was reported to improve root structure and nodule formation in bean, pigeon pea, and soybean (Halverson and Handelsman 1991; Srinivasan et al. 1997; Rajendran et al. 2008; Schwartz et al. 2013). Significant increase in root weight and seed yield of chickpea was reported upon inoculation of *Rhizobium* with *B. subtilis* OSU-142 and *B. megaterium* M-3 (Elkoca et al. 2010). Interaction of *Paenibacillus lentimorbus* NRRL B-30488 and *Piriformospora indica* DSM 11827 and their consortia with native rhizobia population in the rhizosphere of *Cicer arietinum* enhanced nodulation, thereby increasing plant growth (Nautiyal et al. 2010). When *R. tropici* CIAT899 was co-inoculated with *Chryseobacterium balustinum* Aur9, it resulted in increased root hair formation and infection sites leading to early nodule development and increased nodule formation (Estevez et al. 2009). A mixture of *Bacillus atrophaeus* and *Burkholderia cepacia* significantly reduced vascular wilt and corm rot in gladiolus diseases and enhanced plant growth by the elicitation of defense enzymes under field and greenhouse condition (Shanmugam et al. 2011).

Combined application of IAA-producing *Pseudomonas* sp. and *Mesorhizobium* sp. increased nodule formation and plant dry weight compared to *Mesorhizobium* alone inoculated and uninoculated (Malik and Sindhu 2011) plants. Similar effects were observed in chickpea upon co-inoculation with *Mesorhizobium* sp. and *P. aeruginosa* (Verma et al. 2013; Verma et al. 2014). Comparable plant growth-promoting effects along with antagonistic activities against *F. oxysporum* and *R. solani* were observed in chickpea by co-inoculation of *Mesorhizobium*, *Azotobacter chroococcum*, *P. aeruginosa*, and *T. harzianum*.

Consortia of *Burkholderia* sp. MSSP and *Sinorhizobium meliloti* PP3 showed improved yield of pigeon pea compared to treatment with individual isolates (Pandey and Maheshwari 2007). *Enterobacter* increased the nodule numbers in green gram when co-inoculated with *Bradyrhizobium* sp. (Gupta et al. 1998). Similar result was obtained when *Medicago truncatula* cv. *Caliph* was co-inoculated with *Pseudomonas fluorescens* WSM3457 and *Ensifer* (*Sinorhizobium*) *medicae* WSM419 (Fox et al. 2011).

Tomato plants inoculated with consortia of *Pseudomonas*, *Azotobacter*, and *Azospirillum* showed a maximum uptake of K by the shoots' (~7.97 %) enhanced fruit lycopene content and antioxidant properties (Ordookhani et al. 2010). Combined and individual application of *P. fluorescens* Pf1 and *B. subtilis* TRC 54 for the management of *Fusarium* wilt under greenhouse and field conditions improved defense-related enzymes peroxidase (PO) and polyphenol oxidase (PPO) and significantly reduced wilt incidence under greenhouse (64 %) and field (75 %) conditions (Akila et al. 2011). Application of the mixture of phloroglucinol-producing *P. fluorescens* F113 and a proteolytic rhizobacterium suppressed sugar beet damping-off (Dunne et al. 1998). Combination of different strains of *Pseudomonas* with iron-chelating and iron-inducing systemic resistance suppressed *Fusarium* wilt of radish compared to individual strain application (de Boer et al. 2003). Many strains of fluorescent pseudomonads and *Bacillus* sp. stimulated seed germination as well as root and shoot development in several crops (Rudresh et al. 2005). Root-nodulating *Sinorhizobium fredii* KCC5 and *P. fluorescens* LPK2e isolated from nodules of *Cajanus cajan* and disease-suppressive soil of tomato rhizosphere led to protocooperation as evidenced by synergism, aggressive colonization of the roots, and enhanced growth, suggesting potential biocontrol efficacy against *Fusarium* wilt in *C. cajan* (Kumar et al. 2010).

Co-inoculation of *B. subtilis* and *R. tropici* significantly reduced disease severity of bean root rot caused by *F. solani* f. sp. *phaseoli* and enhanced yield compared to control (de Jensen et al. 2002). *P. aeruginosa* PJHU15, *T. harzianum* TNHU27, and *B. subtilis* BHHU100 from rhizospheric soils triggered defense responses against *Sclerotinia* rot through elicitation of host defense response (Jain et al. 2012). Microbial consortium comprising of *P. fluorescens* (PHU094), *Trichoderma* (THU0816), and *Rhizobium* (RL091) activated physiological defense response in chickpea against collar rot pathogen *Sclerotium rolfsii* (Singh et al. 2013). Chickpea treated with consortium showed maximum activity of phenylalanine ammonia lyase and polyphenol oxidase and accumulation of total phenol content in chickpea than other treatments. Consortium of *B. subtilis*, *T. harzianum*, and *P. aeruginosa* showed improved yield along with disease reduction compared to either single or two microbe interaction upon challenge with the pathogen (Jain et al. 2015).

Interaction between *Streptomyces lydicus* WYEC 108 and *Rhizobium* was shown to promote growth in pea probably by nodule colonization of *Streptomyces* (Tokala et al. 2002). Nadeem et al. (2013) pointed out that the use of multi-strain microbial consortia is a better alternative for efficient performance, survival, and competence of the inoculum in natural environment and field conditions.

7.5 Arbuscular Mycorrhizal Fungi (AMF) and Bacterial Consortium for Plant Growth Promotion

Synergistic interaction between PGPR and AMF has been reported to increase yield and biomass in several plants under nursery and field conditions (Jia et al. 2004; Singh et al. 2008; Adesemoye et al. 2009; Singh et al. 2009; Wang et al. 2011; Tajini et al. 2012). Rhizosphere microorganisms either interfere or benefit mycorrhiza establishment (Pivato et al. 2009; Bonfante and Genre 2010; Miransari 2011; Tajini et al. 2012; Aroca et al. 2013). The beneficial effects exerted by the so-called mycorrhiza helper bacteria (MHB), a term referring to bacteria which enhance mycorrhiza formation, were reported by Frey-Klett et al. (2007). AMF and PGPR mycorrhiza helper bacteria interaction has beneficial implication in agriculture (Rabie et al. 2005; Aliasgharzad et al. 2006; Gamalero et al. 2008; Miransari 2011; Wang et al. 2011; Armada et al. 2015).

Co-inoculation of AMF with one or more PGPR has been reported to enhance growth and productivity in different crops (Dutta and Podile 2010; Reddy and Saravanan 2013). Several studies have reported the positive interactions between AMF and a wide range of PGPR, including phosphate-solubilizing bacteria, nodule-forming N_2-fixing rhizobia, and free-living *Azospirillum* spp., *Bacillus* sp., and *Pseudomonas* sp. (Gamalero et al. 2008; Singh et al. 2009). Co-inoculation of AMF and PGPR was reported to have a synergistic effect on plant growth especially under growth-limited conditions (Vivas et al. 2003a, b). Among the microbial groups, PGPR and AMF promote activities which improve agricultural development (Barea et al. 2005). The bioinoculants AMF and PGPR had a significant effect on grain quality, for instance, the phosphorus content doubled in the bioinoculant-applied rainfed wheat, both in greenhouse and field experiments (Roesti et al. 2006). Co-inoculation of AM fungi and biocontrol agents resulted in the suppression of soilborne pathogens such as *Fusarium* and *Rhizoctonia*. Enhanced bioprotection results by the combination of mechanism exhibited by individual organisms, such as competition, altered root exudates, morphological changes in the root system, antibiosis, and activation of plant defense response (Saldajeno et al. 2008).

The AM symbiosis in legumes and its role in improving nodulation and nitrogen fixation by legume–rhizobia association either at the colonization or symbiotic functional stage have been reported (Lesueur et al. 2001; Lesueur and Sarr 2008; Azcón and Barea 2010). Positive effects of the combination of mycorrhizal fungi and/or PGPR on plant growth and plant health as biostimulators, biofertilizers, and bioprotectants have been described by many authors (Barea et al. 2002; Azcón and Barea 2010; Sharma et al. 2016). Arbuscular mycorrhizal fungi (AMF) and rhizobia are the most important symbionts for the plant to acquire nutrients efficiently and to promote growth. Tajini et al. (2012) used *Glomus intraradices*, a potential P mobilizer, and *R. tropici* CIAT899, a nitrogen fixer, to increase the phosphorus-use efficiency for symbiotic nitrogen fixation in common bean (*Phaseolus vulgaris* L.). Co-inoculation of rhizobia and arbuscular mycorrhizal fungi (AMF) promoted growth of soybean under low phosphorus and nitrogen conditions, indicated by increased shoot dry weight (Wang et al. 2011).

Boby and Bagyaraj (2003) reported the effect of *G. mosseae*, *P. fluorescens*, and *T. viride* consortium against soilborne root-rot wilt caused by *Fusarium chlamydosporum* in *Coleus forskohlii*. Consortia of *T. viride* and *G. mosseae* decreased the disease severity and enhanced maximum growth compared to other combinations. Another study by Singh et al. (2009) reported the most effective suppression of root-rot wilt in *C. forskohlii* by a consortium of AM fungus *G. fasciculatum* and *P. fluorescens*. Though in both the reports consortium showed enhanced biocontrol activity against root-rot wilt, the combination of efficient compatible strains in the consortium contributes to more efficient control of the pathogen.

Consortium of *Bradyrhizobium* sp. BXYD3 and *G. mosseae* significantly decreased the severity of *Cylindrocladium parasiticum* incidence in soybean by altering the pathogen defense-related (PR) genes *PR2*, *PR3*, *PR4*, and *PR10* expression level (Gao et al. 2012). A combined bio-inoculation of 2,4-diacetylphlorogluci nol-producing PGPR strains and AMF synergistically improved the nutritional quality of the grain in three Indian rainfed wheat without negatively affecting mycorrhizal growth (Roesti et al. 2006), and in addition it stimulated both mycelial development and spore germination in *G. mosseae* and enhanced root colonization in tomato (Barea et al. 1998). Combined application of AM fungus *F. mosseae* with *Paenibacillus* and *Pantoea* spp. enhanced all the biometric parameters in French bean especially the total shoot dry biomass and fruit yield.

Rhizobium and AMF co-inoculation increased leaf area and biomass production in broad bean (*Vicia faba*), AMF colonization increased the supply of P, and *Rhizobium* facilitated N accumulation (Jia et al. 2004). The application of a consortium of microbial inoculants such as mycorrhiza and *Azospirillum brasilense* effectively increased plant growth and enhanced the ability of plants to alleviate drought and nutrient stress (Azcón and Barea 2010). AM fungus *G. intraradices* enhanced growth, photosynthetic efficiency, and antioxidative response in rice against drought stress (Ruiz-Sanchez et al. 2010).

Kamal et al. (2016) evaluated the impact of *Streptomyces labedae* (SB-9), *Streptomyces flavofuscus* (SA-11), *Pseudomonas poae* (KA-5), *P. fluorescens* (KB-7), and *G. intraradices* consortium combination which showed pronounced increase in the finger millet plant growth under drought condition.

Seed priming with consortia of *T. harzianum* and fluorescent pseudomonas decreased the *Fusarium* wilt incidence, increased seed germination by 22–48 %, and reduced the germination period (Srivastava et al. 2010). The enhanced performance of microbial consortia compared to single inoculation is reported in several crops including legumes (Antoun et al. 1998; Valdenegro et al. 2001; Ane et al. 2004; Bagyaraj and Kehri 2012; Bagyaraj 2014). Consortium product "Shu Dekang" showed significant control of several phytopathogenic infestations like leaf speck disease, banana wilt, and root-knot disease (Zheng et al. 2010). Thus, PGPR consortia with multiple functions provide multiple growth-promoting and stress-tolerant benefits in plants.

7.6 Microbial Consortium for Abiotic Stress Alleviation

The global climate is a great challenge for the agricultural sector, as predicted increases in salinity, drought, and rising temperature cause abiotic stress in the plant which reduce crop productivity (Grover et al. 2011; Larson 2013). About 60 % of the global geographical area faces soil degradation either by waterlogging or salinity or alkalinity, which threatens food security, the situation being worse in higher rainfall areas where waterlogging follows shortly after the rains (Singh 2000).

Plant-associated microbial communities have received considerable attention for their ability to confer many of the same benefits to crop productivity and stress resistance as have been achieved through plant breeding programs (Mayak et al. 2004; Barrow et al. 2008; Marulanda et al. 2009; Mapelli et al. 2013). Microbial symbionts are capable of conferring multiple stress tolerance against both abiotic and biotic stress (Mayak et al. 2004; Rodriguez et al. 2008) benefits in both monocot and dicot crop species (Timmusk and Wagner 1999; Redman et al. 2002; Zhang et al. 2008).

Application of microbial inoculants specially consortia will be one of the solutions to alleviate plant abiotic stress and enhance plant growth and productivity under stress conditions (Yang et al. 2009; Jain et al. 2013). Multiple beneficial PGP and abiotic stress-resistant strains, efficient 2,4-DNT-degrading consortia composed of *Burkholderia*, *Variovorax*, *Bacillus*, *Pseudomonas*, and *Ralstonia* spp., have been reported (Shirley et al. 2000; Snellinx et al. 2003) to enhance the root length of *Arabidopsis* under 2,4-DNT stress, by doubling the root length within 9 days (Thijs et al. 2014).

Co-inoculation of *A. brasilense* with *R. tropici* on bean relieved negative effects of salt stress and nod gene transcription (Dardanelli et al. 2008). Microbial consortium comprising of *P. fluorescens* (PHU094), *Trichoderma* (THU0816), and *Rhizobium* (RL091) enhanced the expressions of defense systems like antioxidant enzymes superoxide dismutase and peroxidase activities (Singh et al. 2013) under stress. The response of rice plants to inoculation with an AMF and *A. brasilense* consortia under drought stress conditions was due to enhanced ascorbate accumulation. The effect of *A. brasilense* was pronounced only when mycorrhizal colonization was established; thus, the bacterial and fungal consortia were responsible for the protection of plant against plant pathogens (Ruiz-Sanchez et al. 2011). PGPR consortium of endophytic bacterium *P. pseudoalcaligenes* in combination with *B. pumilus*-treated plants showed increased concentrations of NPK and reduced concentrations of Na and Ca in paddy under saline conditions (Jha and Subramanian 2013). Co-inoculation of *P. fluorescens* Aur6 and *Chryseobacterium balustinum* Aur9 in three field experiments induced systemic resistance in rice against rice blast and increased rice productivity and grain quality under saline conditions (Lucas et al. 2009).

Under drought stress cucumber seedlings treated with consortium product of Shu Dekang containing *B. cereus* AR156, *B. subtilis* SM21, and *Serratia* sp. XY21 showed enhanced photosynthetic efficiency, less wilt symptoms, decreased leaf monodehydroascorbate (MDA), increased leaf proline content, enhanced induced

systemic tolerance, and superoxide dismutase activity. Downregulation of the expression of the genes *cAPX*, *rbcL*, and *rbcS* encoding cytosolic ascorbate peroxidase and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) large and small subunits was observed (Wang et al. 2012).

Consortium of *P. polymyxa* and *R. tropici* increased plant growth, nitrogen content, and nodulation of common bean (Figueiredo et al. 2008). The co-inoculation of *G. intraradices* and *R. leguminosarum* protected bean plants under drought conditions in semiarid region by increase in plant biomass, grain yield, and several antioxidant enzyme activities in the host plants (Zahran 1999; Valdenegro et al. 2001; Aroca et al. 2007).

The consortium of *Pseudomonas mendocina* and *G. intraradices* protected and enhanced plant growth in *Lactuca sativa* L. cv. by the production of antioxidant enzymes such as superoxide dismutase, catalase and total peroxidase, phosphatase, and nitrate reductase in leaves (Kohler et al. 2008). Under salinity stress inoculated plants showed significantly higher shoot biomass and glomalin-related soil protein (GRSP) compared to uninoculated plants (Kohler and Caravaca 2010). *A. brasilense–Rhizobium* combination enhanced the growth of *P. vulgaris* under salt stress by increasing nodulation, flavonoid, and lipochitooligosaccharide production (Dardanelli et al. 2008; Smith et al. 2015). Gamalero et al. (2008) showed the impact of ACC deaminase in cucumber treated with PGPR *P. putida* UW4 and *Gigaspora rosea*, where synergistic action was reflected on plant biomass, root length, total leaf area, and increased photosynthetic performance index. *Zea mays* co-inoculated with *Rhizobium* and *Pseudomonas* under salinity conditions showed increased production of proline and maintenance of relative water content of leaves, reduction in electrolyte leakage, and selective uptake of K ions (Bano and Fatima 2009).

Consortium of *B. thuringiensis* and AMF reduced the oxidative damage to lipids and increased drought-induced proline in *Zea mays* under stress. *B. thuringiensis* increased plant nutrition, and AMF enhanced the stress tolerance/homeostatic mechanisms, by regulation of plant aquaporins with many putative physiological functions (Armada et al. 2015). *B. subtilis* and *Arthrobacter* sp. co-inoculation alleviated adverse effects of 8 % soil salinity on wheat and enhanced the dry biomass, total soluble sugars, proline content, and antioxidant enzymes in wheat leaves which decreased under salinity stress (Upadhyay et al. 2012). Prasanna et al. (2015) used cyanobacterial inoculants *Anabaena–Azotobacter* biofilm and *Anabaena* sp.*–Providencia* sp. to enhance the Zn mobilization in maize hybrids and elicit plant defense response. Both consortia were found to enhance the activity of defense enzymes such as polyphenol oxidase (PPO), peroxidase (POD), and phenylalanine ammonia lyase (PAL) in roots, with a positive correlation of Zn concentration in the flag leaf.

7.7 Commercialization and Registration of Biofertilizers in the World

Unlike in microbial biopesticide category, microbial consortia are acknowledged and promoted in the case of biofertilizers in many countries. In the USA and EU, currently there is no specific legal definition for biofertilizers. In EU, all microorganisms irrespective of its principle action are included as possible products for

organic production as per the European Commission Regulation No. 889/2008 (Malusa and Vassilev 2014). But, India has a comprehensive legal framework on biofertilizers. The Ministry of Agriculture issued an order in 2006 (subsequently amended in 2009) categorizing the biofertilizers under Essential Commodities Act of 1966 and brought under Fertilizer Control Act 1985. Under this act production and marketing standards were specified for different biofertilizers. As per the definition of biofertilizer under the Indian act, it does not specify any microbial consortia, while the proposed concept of microbial consortium under the legal provision regulating the production and marketing of biofertilizer in EU was specified in the definition itself (Malusa and Vassilev 2014).

7.8 Biofertilizer Commercialization and Regulatory Issues in India

Biofertilizer commercialization began with the rhizobia product in the year 1895 by Nobbe and Hiltner under the brand of "Nitragin." In India, N. V. Joshi first started the commercialization of rhizobium for the growth promotion of leguminous plant (Rivas et al. 2015). During its ninth five-year plan, the Ministry of Agriculture initiated the popularization and promotion of biofertilizer production, developing standards for different biofertilizers, training, and utilization by launching National Project on Development and Use of Biofertilizers (NPDB), and a National Biofertilizer Development Centre was established, with six regional centers (Ghosh 2004). The government of India and state governments took several measures for promoting the production of biofertilizers by providing grants and subsidies at different levels.

The Ministry of Agriculture passed a new decree on the control of biofertilizer production and marketing standards with regard to different kinds of microorganisms. The product should fulfill seven quality parameters like physical form, minimum count of viable cells, contamination level, pH, particle size in the case of carrier-based materials, maximum moisture percent by weight of carrier-based products, and efficiency character. In bacterial bioproducts the minimum viable cells to be maintained is 5×10^7 CFU g^{-1} for solid carrier or 1×10^8 CFU ml^{-1} for liquid carrier. For products containing mycorrhizal fungi, at least 100 viable propagules must be present per gram of product. Nitrogen-fixing efficiency of biofertilizer product should be capable of fixing at least 10 mg N g^{-1} of sucrose consumed and for phosphate solubilization product a zone of solubilization at least 5 mm in a media. AMF products should provide 80 infection points in roots g^{-1} of inoculum (Ministry of Agriculture 2009).

Markets and Markets (2015) report shows that the biofertilizer market is projected to grow at a CAGR of 14.0 % from 2015 to 2020 and is expected to reach US $1.88 billion by 2020. Leading players in the biofertilizer market include Gujarat State Fertilizers & Chemicals Ltd. (India), Novozymes A/S (Denmark), Rizobacter Argentina S.A. (Argentina), Camson Bio Technologies Limited (India), and Lallemand, Inc. (Canada) (RNR Market Research 2014). Biofertilizer market in

Asia is strongly influenced by the government and its policies to promote sustainable and green agriculture. Around US $1.5 billion has been spent on the development of biofertilizer and biopesticide products (Rivas et al. 2015). Currently there is an increase in organic agriculture practice in the country with around 1,000,000 ha under organic cultivation (Keshri 2016).

In India, around 100 public and private companies are involved in biofertilizer production, and the list of a few companies and their consortial products are listed in Table 7.1 (Rivas et al. 2015). Biofertilizer production and consumption have gained importance in the recent times in India (Pindi and Satyanarayana 2012). The average consumption in the country is about 45,000 t per annum, while its production is less than half of the consumption. The maximum production capacity lies in Agro Industries Corporation followed by state agriculture departments, National Biofertilizers Development Centres, State Agricultural Universities, and private sectors (Mazid and Khan 2014).

7.9 Commercialization and Registration of Biopesticides in the World, Asia, and India

Worldwide the use and demand for biopesticides are rising due to the increased awareness of pesticide residue-free crops. The global-level estimate for microbial products in 2014 was US $ 2,183 million which is projected to double by US $ 4556 million in 2019 with a CAGR of 15.3 %. Of the several microbial types, the bacterial segment accounted for the largest share (US $1.6 billion). Similar to biopesticides, market for biofertilizers at global level is projected to reach US $1.88 billion by 2020 at a CAGR of 14.0 % from 2015 to 2020 (Markets and Markets 2015).

Globally, more than 200 biopesticide active ingredients are registered, and 700 products are available in the market. In the case of India, 15 biopesticides were registered as on 2008 under IA (1968), and its market share is only 4.2 % of the overall pesticide market; however, it is predicted to increase at an annual growth rate of 10 % (Suresh 2012). While its growth was multifold during the past years, NAAS (2013) reported around 400 registered biopesticide active ingredients and over 1250 actively registered biopesticide products in Indian markets. It shows the awareness among farmers as well as policy support of the government to use the ecologically safe products for pest management. However, there is no specific mention about microbial consortium among 400 registered biopesticides individually.

At the international level, the regulatory frameworks differ widely among different countries. In the USA, biopesticide production is institutionalized under a separate division as “Biopesticides and Pollution” within the Environmental Protection Agency (EPA). To maintain the quality, it specified good laboratory practices regulatory testing for microbial biopesticides in 1983 as EPA M guidelines. Following the line, in 1996 the Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF) harmonized its system with guidelines of EPA. Similarly in Europe, biopesticides are evaluated through the European Pesticide Regulation EC No. 1107/2009 which promotes the production of less harmful substances, and it has

Table 7.1 List of few representative commercial consortial products

S. no	Product	Consortia	Company	Country
1.	Life®	PGPR consortia	Biomax	India
2.	Biomix®	PGPR consortia	Biomax	India
3.	Biozink®	PGPR consortia	Biomax	India
4.	Biodine®	PGPR consortia	Biomax	India
5.	Jet 9	PGPR consortia	Sivashakthi Bio Planttec Ltd.	India
6.	Calosphere	PGPR consortia	Camson Bio Technologies Ltd.	India
7.	Calspiral	*Azospirillum* + PGPR	Camson Bio Technologies Ltd.	India
8.	Symbion-N	*Azospirillum* + *Rhizobium* + *Acetobacter* + *Azotobacter*	T. Stanes & Company Ltd.	India
9.	Bio Power	*Azospirillum* + *Azotobacter* + PSB + VAM	SKS Bioproducts Pvt Ltd.	India
10.	Bio Super	*Pseudomonas* + *Cellulomonas* + *Bacillus* + *Rhodococcus*	SKS Bioproducts Pvt Ltd.	India
11.	Premium EMC	PGPR consortia	International Panaacea Ltd.	India
12.	Nodulator® N/T	*B. subtilis* MBI 600 + *B. japonicum*	BASF Canada, Inc.	Canada
13.	Nodulator® PRO	*B. subtilis* + *B. japonicum*	BASF Canada, Inc.	Canada
14.	BioBoots®	*Delftia acidovorans* + *Bradyrhizobium* sp.	Brett-Young Seeds	Canada
15.	EVL Coating®	PGPR consortia	EVL, Inc.	Canada
16.	BioAtivo®	PGPR consortia	Instituto de Fosfato Biológico (IFB) Ltda.	Brazil
17.	BioJet®	*Pseudomonas* sp. + *Azospirillum* sp.	Eco Soil Systems, Inc., San Diego, CA	USA
18.	BioYield	*B. subtilis* + *B. amyloliquefaciens*	Gustafson, Inc., Dallas	USA
19.	TagTeam®	Rhizobia + *Penicillium bilaii*	Novozymes	USA
20.	VitaSoil®	PGPR consortia	Symborg	Spain

been promoting the registration of low-risk products for pest control through (2009/128/EC) simple and transparent registration protocols (Villaverde et al. 2014). Canada follows only the safety test and the rest of the countries need data of both safety and efficacy tests. The EPA, JMAFF, and EC regulations toward biopesticides are developed in such a way that it requires less data when compared to chemical products and reduced the time to process the registration applications. In this context, the International Organization for Biological Control of Noxious Animals and Plants (2010) carried out a global-level review on the use of biopesticides and regulatory measures. It stressed the need for streamlining the registration process through harmonizing data requirements and protocols for risk assessments. In India, any microorganisms used for pest and disease management require registration for both production and sale with the Central Insecticides Board (CIB) of the Ministry of Agriculture as per the Insecticides Act (IA), 1968, of the Government of India (GOI) and Insecticides Rules, 1971, which were recently replaced by the Pesticides Management Bill 2008. The biopesticides are considered as generally regarded as safe (GRAS) under this act, and to promote its production and use, it provides the benefit of priority in processing of registration as well as provisional registration. Thus, the producers can register the product either for regular registration under section 9 (3) or for provisional registration under section 9 (3B) of the IA. While applying for registration, the data on product characterization, safety, toxicology, efficacy, and labeling are necessary. In addition to the priority and provisional registration for biopesticides in the Act, the registration protocols are made easier and accept generic data for any new products containing strains which are already registered. Such affirmative clauses are inbuilt in the Act which shows the interest of the government in promoting the safe products for pest management similar to other countries. In order to regulate the commercial production of these products, the Government of India established four different bodies to regulate the biopesticide production. The Central Insecticides Board (CIB) is involved in developing appropriate policies, and the Registration Committee (RC) is responsible to register the products for production. Whereas the Central Insecticides Laboratory (CIL) is in charge to monitor the quality of the products available in the market, finally the State Department for Agriculture (SDA) issues the manufacturing license and performs quality check. However, coordination among the four bodies plays a vital part in ensuring the registration and availability of quality products in the market. Recently, efforts were taken to harmonize the IA of 1968 with the Organization for Economic Cooperation and Development (OECD) during 2000s on the methods and approaches to assess biological pesticides. On this basis, CIB has rationalized the guidelines and data requirements for registration and infrastructure necessary for production of the biopesticides (NAAS 2013). However, research studies on how the harmonization eased the process of registration are yet unavailable. On the other side, as per the notification dated March 26, 1999, of the Central Insecticides Board, Ministry of Agriculture, biopesticide was put under the Insecticide Schedule Act 1968, and hence, the generation of toxicological data became a prerequisite for the registration of biopesticide. In spite of the relatively abundant number of patents for microbial pesticides, the number of commercial applications has not been as

dramatic as expected due to the high cost involved in toxicologic analysis, biosafety, and environmental concerns (Montesinos 2003).

7.10 Registration and Regulations for Microbial Consortia

Though research-based evidences clearly show the advantages of microbial consortia-based products due to their multifunctionality, limited attention is being given to develop quality standards for registration (Jain et al. 2013). NAAS (2013) reiterated that microbial consortium-based products require meticulous calibration in terms of cultural methods and their microbial composition in the product cycle. It is well understood that the evaluation of the efficacy of biofertilizer-based microbial consortia is complex due to its multiple mechanisms of action, viz., plant growth protection, stimulation, etc. However, farmers and market agencies prefer microbial consortia-based products due to its practical easiness in use, economic reasons, and multifunctional properties. Hence, initiatives have been taken to address the concern at different levels. The overall matter appears even more complex as some microorganisms either as single or as member of the microbial consortia can have both effects as biofertilizers/bioeffectors and plant protectants. The study of Malusa and Vassilev (2014) suggested that the principal function of the product can be taken for classification and labeling considering its potential environmental risks and study of its ecotoxicology and impact on environment when other products such as additives or nanomaterials are included in the formulations.

7.11 Conclusion

Though the performance of the PGPR and its consortia has been proved to promote plant growth and enhance productivity in the field conditions by several strains in different crops, the use of these products has not been popular among farmers due to several reasons such as (1) lack of awareness among farmers and (2) availability and supply of quality bioproducts. A survey conducted by Srinivas and Bhalekar (2013) reported the communication gap that exists between farmers and manufactures, miscommunication about the quality of the product, and sustainability of biofertilizer as the major hurdle. In natural conditions and in disease-suppressive soil, the existence of mixture of microbial antagonists (Lemanceau and Alabouvette 1991) has been reported. Hence, augmentation of compatible strains of PGPR to infection court will mimic the natural environment and could broaden the spectrum of biocontrol against different plant pathogens. Efficiency of biocontrol agents could be increased by the development of compatible strain mixtures of different biocontrol organisms by considering the following norms (Raupach and Kloepper 1998). While developing a consortial formulation, the following needs to be addressed: (1) compatible strain combination that differs in the pattern of plant colonization, (2) compatible strain combination with broad spectrum of action against different plant pathogens, (3) compatible strain combination with different modes

of action, and (4) compatible strain combination of genetically diverse group to adapt to different pH, moisture, temperature, and relative humidity. The use of microbial inoculants must take into account the importance of retaining microbial diversity in the rhizosphere and in achieving realistic and effective biotechnological applications. Molecular biology-based approaches by developing molecular markers to analyze the impact of the introduced isolate on the microbial diversity and community structure and to predict responses to microbial inoculation/processes in the environment (ecological engineering) are essential. Further studies must address the consequences of the cooperation between microbes in the rhizosphere under field conditions to assess their ecological impacts and biotechnological applications. In this context further research and efforts are needed to promote the use of microbial consortia considering its multifunctional characteristics; at the same time, quality standards for the crop-specific/soil property-specific potential combination of microbes have to be generated to ease the registration process. While developing such standards, harmonization at global level would help to speed up the process and reduce the time and resources which are vital to promote quality products.

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