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Harnessing Proficient Rhizobacteria to Minimize the Use of Agrochemicals

Ruchi Sharma*, Anupma Dahiya and Satyavir Singh Sindhu

Department of Microbiology, College of Basic Sciences and Humanities, CCS Haryana
Agricultural University, Hisar - 125 004, India

*Corresponding author

ABSTRACT

Humanity is facing a major challenge in producing enough food from the limited resources for an additional three billion people by 2050. Moreover, the productivity of agricultural crops is adversely affected due to environmental abiotic and biotic stresses. To meet the global food demand, intensive agriculture has resulted in indiscriminate use of chemical fertilizers and pesticides. These agrochemicals and their metabolites have been found to cause pollution in soil, groundwater and atmosphere. To reduce the deleterious effects of these agrochemicals, certain potential microorganisms have been characterized from rhizosphere of different crop plants which can act as biofertilizers and biopesticides. These are considered to be safer alternatives and their application has increased significantly in recent times. Biofertilizers enhance plant growth by a wide variety of mechanisms including phosphate and potassium solubilization, siderophore production, biological nitrogen fixation, production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase and phytohormone production. Biopesticides can control various plant diseases by production of antifungal compounds like antibiotics, siderophores, hydrolytic enzymes, HCN, volatile organic compounds (VOCs) and by induction of systemic resistance. Further, understandings of the different rhizobacteria mediating plant growth promotion mechanisms could be exploited to enhance productivity of crops in sustainable agriculture.

Keywords

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Introduction

The ever-increasing population growth combined with the changing diets, would result in a unpredicted increase in food demand by 2050 (Bruinsma, 2009). On the other hand, agricultural sector is facing burden from many ways via lower soil nutrients, attack of pathogens, pests and weeds, and fluctuating climatic conditions. Severe global economic losses to agricultural crops are encountered annually due to plant diseases caused by pathogens leading to the loss of

30% crop yield (Kumar, 2012). Fertilizers play an axial role in enhancing the food production especially after the introduction of high yielding crop varieties. Moreover, extensive use of agrochemicals in plant protection strategies in many agro-ecosystems to control pests, diseases and weeds resulted in health risks and caused other undesirable effects. The increasing public concerns and growing awareness about the potential adverse environmental effects as well as health hazards associated with the use of synthetic plant protection and other agrochemicals has

prompted search for the sustainable technologies and products which are safer for the end users and the environment. An integrated crop management approach needs to be deployed to counteract degradation of the agro-ecosystem due to the on-going intensive agriculture. This includes the use of biofertilizers and biopesticides, integrated pest management, soil and water conservation practices along with biodiversity conservation.

The policies supporting sustainable agricultural production and extensive research has improved the effectiveness and consistency of microbial inoculants, which has resulted in the characterization of several strains for both biocontrol (Fravel, 2005; Sindhu *et al.*, 2016) and biofertilization (Podile and Kishore, 2006), with mycorrhizal fungi and PGPR (plant growth promoting rhizobacteria) preparations. Natural pesticides are environment friendly and safer than classical chemical pesticides. The rhizosphere zone, supports large and active microbial population of rhizobacteria (root colonizing bacteria) that exert the beneficial effects on the growth of the host plant (Ahmad *et al.*, 2008). Therefore, tremendous efforts are being made recently to develop such microbial inoculants which have beneficial plant growth properties leading to enhanced crop productivity in sustainable agriculture (Barriuso *et al.*, 2008). Such beneficial properties of microbial inoculants could be manifested either by direct promotion of plant growth through increased nutrient availability or phytohormone production or by indirectly protecting plants from phytopathogens, or by fortifying certain abiotic stress tolerance in plants that grow in soils with extremes of high and low temperature, salinity, drought, acidity and presence of heavy metals (Kang *et al.*, 2014; Chaudhary and Sindhu, 2015) (Fig. 1). Application of microbial inoculant approaches to these crops can contribute efficiently to solve or reduce the stress problems.

Successful application of rhizobacteria to crops facing biotic/abiotic constraints requires understanding of the target species and the mechanisms underlying resistance/ tolerance to these stresses (Goswami *et al.*, 2016).

Understanding of the interactions between naturally-occurring PGPRs and with crop plants could foster the design of agro-systems with decreased fertilizer inputs and improved plant yields (Table 1). The most widely explored plant beneficial trait is the symbiotic biological nitrogen fixation by rhizobia (Udvardi and Poole, 2013; Sindhu *et al.*, 2018), but numerous other nutrient acquisition machineries have been observed which facilitate plant access to macro- and micro-nutrients. Therefore, it is necessary to increase our understanding of the specific aspects of the defence/stress responses in agriculture in order to solve some of the major constraints facing these crops. The relevant advances in microbial inoculant approaches, applications and their functional analysis are discussed to overcome productivity losses and to minimize the environmental pollution.

Biofertilizers: Mechanism of action

Biofertilizers, colonize the rhizosphere or the interior of the plant and promote growth by increasing the supply or availability of nutrients to the host plant. The microorganisms commonly used as biofertilizers may be nitrogen-fixing soil bacteria (*Rhizobium*, *Azotobacter*) and cyanobacteria (*Anabaena*), phosphate solubilizing bacteria (*Pseudomonas putida*) and arbuscular mycorrhizal fungi. A number of rhizobacteria, which stimulated root and shoot growth of different plant species were found to contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase that hydrolysed the ethylene precursor ACC to ammonia and α -ketobutyrate, and as a result, decreased

ethylene biosynthesis by plants (Belimov *et al.*, 2001; Khandelwal and Sindhu, 2013). Another category of PGPM (plant growth promoting microorganisms) contains phytostimulators which are generally auxin-producing bacteria that induce root elongation (Lugtenberg *et al.*, 2002). Mycorrhizal fungi were reported to promote plant growth through P uptake (Koide and Mosse, 2004). Similarly, phytohormone producing bacteria and cellulolytic microorganisms could also be part of biofertilizer formulation. When applied to the field, the beneficial activities such as nitrogen fixation, phosphate solubilization, production of phytohormones resulted in improved growth and productivity (Brar *et al.*, 2012; Sangwan *et al.*, 2012).

Nitrogen fixing biofertilizers fix atmospheric nitrogen into forms which are readily utilizable by plants. These include *Rhizobium*, *Azotobacter* and *Azospirillum* and blue green algae (BGA). While *Rhizobium* requires symbiotic association with the root nodules of legumes to fix nitrogen (Fig. 2), other microorganisms can fix nitrogen independently. Co-inoculation studies with rhizobia and PGPR are becoming a frequent practice in the development of sustainable agriculture (Table 2). PGPR tested as co-inoculants with rhizobia include *B. subtilis*, *Bacillus thuringiensis*, *Azospirillum brasilense*, *Serratia proteomaculans*, *Serratia liquefaciens* and *Pseudomonas aureofaciens*. Cassán *et al.*, (2009) used *A. brasilense*, which produced IAA, GA3 and zeatin and it is a clear example of phyto-stimulation. Phosphate solubilizing microorganisms such as *Bacillus*, *Pseudomonas*, *Aspergillus* etc. secrete organic acids which enhance the uptake of phosphorus by plants by dissolving rock phosphate. Some other bioinoculants include potassium mobilizers and zinc solubilizers (Saravanan *et al.*, 2004; Parmar and Sindhu, 2018; Sindhu *et al.*, 2018). A considerable research has been done to establish the effectiveness of

biofertilizers on various crops, in different agro-climatic regions. However, their effectiveness is found to vary greatly, depending largely on soil conditions, temperature and farming practices. However, biofertilizers are safe alternative to chemical fertilizers to minimize the ecological disturbance and they are cost effective and eco-friendly. They increase crop yield by 10-40% and counteract the negative impact of chemical fertilizers.

Biopesticides: Mechanism of action

Pests are one of the major problems in crop protection and a major portion of expenditure on pesticides is for protecting the crop in the field. There has been an estimated 67,000 pest species that damage agricultural crops (Ross and Lembi, 1985) and pest management is one of the important activities required to maximise crop production. Plant protection not only requires purchase of chemicals but considerable input is needed on implements and labour required for their repeated applications on the standing crop. The current pest management strategies adopted for the intensive agriculture rely heavily on synthetic chemical pesticides which cause adverse/harmful effects on beneficial organisms and leave toxic residues in food and feed. Increasing demands for residue-free crop produce, growing organic food market and easier registration than chemical pesticides are some of the key drivers of the biopesticide market (Kumar, 2012; Sindhu *et al.*, 2017a).

The available biopesticides may be divided into three major categories: microbial, biochemical (or botanical) and plant-incorporated protectants. Microbial pesticides consist of microorganism (bacteria, fungi, viruses or protozoans) or their derivatives and they have been successfully being used in controlling insect pests. One of the most widely used microbial biopesticides is

Bacillus thuringiensis, popularly known as Bt. The bacterium produces crystalline proteins and specifically kills one or a few related insect species. Success stories of biopesticide in India include control of diamondback moths by *Bacillus thuringiensis*, control of mango hoppers, mealy bugs and coffee pod borers by *Beauveria*, control of *Helicoverpa* on cotton, pigeon pea and tomato by *Bacillus thuringiensis*, control of white fly on cotton by neem products, control of sugarcane borers by *Trichogramma*, and control of rots and wilts in various crops by *Trichoderma*-based products. These biopesticides do not have residue problem which is a matter of significant concern for consumers, particularly for fruits and vegetables. When used as a component of IPM, efficacy of biopesticides can be equal to the conventional pesticides, especially for crops like fruits, vegetables, nuts and flowers. By combining performance and environmental safety, biopesticides perform efficaciously with the flexibility of minimum application restrictions and superior resistance management potential.

The suppression of growth of soil-borne plant pathogens by the use of microorganisms, to reduce the effects of harmful organisms (pests) is referred to as biocontrol. Rhizobacteria inhibit the growth of various pathogenic bacteria and fungi resulting in suppression of the diseases caused by such pathogens (Thomashow and Weller, 1996; Sindhu *et al.*, 2002; Sharma *et al.*, 2018).

Disease suppression by biocontrol agents involves a sustained manifestation of interactions among the plant, the pathogen, the biocontrol agent, the microbial community on and around the plant and the physical environment (Pierson and Weller, 1994). Strains of *Pseudomonas fluorescens*, *P. putida*, *P. aureofaciens*, *P. cepacia* and *P. aeruginosa* have been found to antagonize the growth of pathogens leading to substantial

disease control (Weller, 2007). Jamali *et al.*, (2003) studied effect of antagonistic *B. subtilis* strain Pf-100 on control of Fusarium wilt under greenhouse conditions. Usually general relationship was not observed between the ability of a bacterium to inhibit a pathogen under *in vitro* and *in situ* disease suppression (Schroth and Hancock, 1982; Wong and Baker, 1984). Bacterial strains producing the largest zones of fungal growth inhibition on agar media, do not always make the best biocontrol agents. Therefore, some *in vitro* conditions have been modified to more closely simulate natural conditions (Randhawa and Schaad, 1985). Souagui *et al.*, (2015) isolated actinobacteria (*Streptomyces*) from rhizosphere of *Ononis angustissima* Lam. growing in extreme environment in southern of Algeria (Biskra, Sahara of Algeria). Four isolates i.e., 21, 2A26, 1B10 and 2C34 showed potent antagonism against both pathogenic bacteria and fungi.

Four *Streptomyces* sp. produced extracellular fungal cell-wall degrading enzymes (chitinase and protease), solubilized phosphate and also produced relatively high levels of IAA. *In vivo* biocontrol assays revealed that the *Streptomyces* strains significantly promoted the growth of the chickpea plants and showed greater suppression of chickpea wilt disease caused by *Fusarium oxysporum*. Among the numerous examples of biocontrol agents reported for disease control of soil-borne pathogens, only few studies provide mechanistic information for the activities of these biocontrol agents.

Weeds are another category of agricultural pests, causing great yield loss and labor expense (Schonbeck, 2011). Agricultural weeds can emerge rapidly, resulting in reduction of crop plant growth and quality by competing for nutrients and water provided to crops.

Table.1 Categories of beneficial rhizobacteria and their mechanism of action involved in stimulation of plant growth

Bioinoculant types	Definition	Mechanism of action	References
Biofertilizer	A substance that contains live microorganisms which, when applied on the seed, plant surface or soil, colonizes the rhizosphere and promote plant growth through increased supply of primary nutrients for the host plant	Biological nitrogen fixation Utilization of insoluble phosphorus Solubilization of bound potassium and zinc	Vessey (2003), Chaudhary and Sindhu (2016), Somers <i>et al.</i> , (2004), Sravanan <i>et al.</i> , (2004), Sindhu <i>et al.</i> , (2016)
Phytostimulator	Microorganism, having ability to produce phytohormones such as indoleacetic acid, gibberellic acid, cytokinins and ethylene	Production of phytohormones	Lugtenberg <i>et al.</i> , (2002), Somers <i>et al.</i> , (2004) Malik and Sindhu (2011)
Biopesticide	Microorganisms that promote plant growth by controlling phytopathogenic fungi, insects or weeds	Production of antibiotics, HCN, siderophores, hydrolytic enzymes, and induced systemic resistance, Phytotoxins and IAA	Sindhu <i>et al.</i> , (2016) Somers <i>et al.</i> , (2004) Yasuda <i>et al.</i> , (2008) Sindhu and Sehrawat (2017)

Table.2 Different rhizobacteria used as bioinoculant for various crop types

PGPR	Crop	Conditions tested	Observed effects	Reference
<i>Pseudomonas</i> sp. PS1	Green gram (<i>Vigna radiata</i> (L.) wilczek)	Pots	Significantly increased plant dry weight, nodule numbers, total chlorophyll content, leghaemoglobin, root N, shoot N, root P, shoot P, seed yield and seed protein	Ahemad and Khan (2012)
<i>Rhizobium</i> strain MRP1	Pea (<i>Pisum sativum</i>)	Pots	Significantly increased the growth, symbiotic properties (nodulation and leghaemoglobin content), amount of N and P nutrients in plant organs, seed yield and seed protein of pea plants	Ahemad and Khan (2011)
<i>Rhizobium</i> strain GSA6, GSA11 or GSA110	Clusterbean (<i>Cyamopsis tetragonoloba</i> L.)	Chillum jar	Increased nodulation efficiency, plant biomass	Khandelwal and Sindhu (2012)
<i>Azorhizobium caulinodans</i> strains Sb3, S78	<i>Sesbania bispinosa</i>	Pots	Enhanced nodule mass, nitrogenase activity and plant dry weight	Saini <i>et al.</i> , (2003)
<i>Pseudomonas</i> sp.	Soybean, mungbean, wheat	Pots	Promoted growth of plants	Gupta <i>et al.</i> , (2002); Sindhu <i>et al.</i> , (1999)
<i>Mezorhizobium</i> strain MBD26	Chickpea	Chillum jar	Salinity tolerance and improved plant growth	Chaudhary and Sindhu (2015)
<i>Pseudomonas</i> isolate HCS36	Clusterbean (<i>Cyamopsis tetragonoloba</i> L.)	Pots	Control of root rot disease, increased nodulation, plant biomass	Chaudhary and Sindhu (2015)

Table.3 List of some commercially available bioinoculants

Trade name	Rhizobacteria	Crops tested
Galtrol, Diegal, No gall	<i>Agrobacterium radiobacter</i>	Several crops
Epic, HiStick N/T, Kodiak, Rhizo-Plus, Serenade, Subtilex	<i>Bacillus subtilis</i>	Barley, beans, cotton, peanut, pea, rice and soybean
System	<i>Bacillus subtilis</i>	Bean, barley, cotton and peanut
Blue Circle, Deny, Intercept	<i>Burkholderia cepacia</i>	Alfalfa, barley, beans, clover, cotton, maize, peas, sorghum, vegetables and wheat

Fig.1 Schematic illustration of important mechanisms for plant growth promotion by PGPR

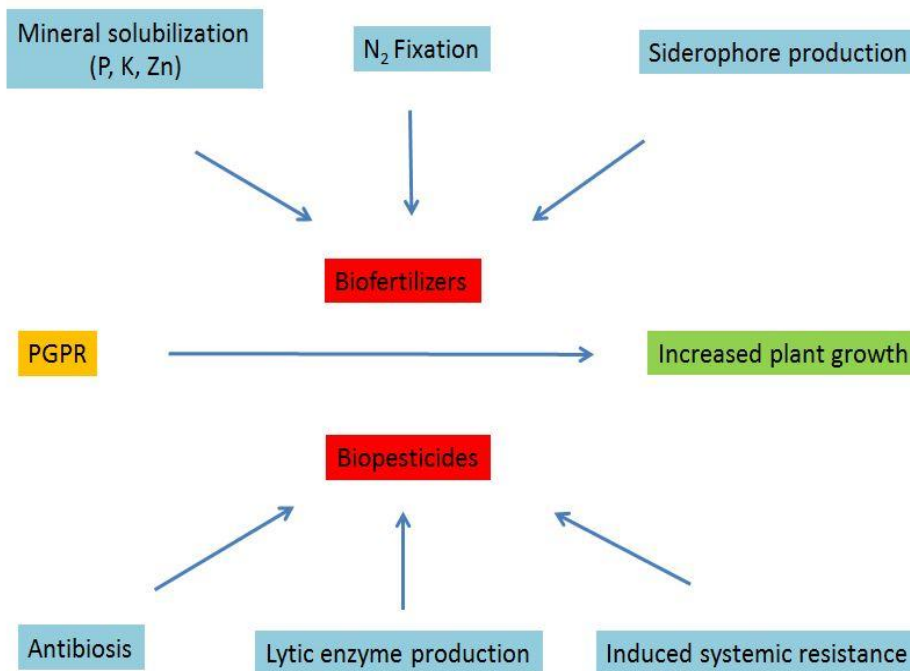


Fig.2 Increase in nodulation efficiency in chickpea (b) inoculated by rhizobacteria as compared to (a) uninoculated control



Annual weeds reproduce through prolific seed production and they germinate in response to light, increased fluctuations in soil temperature and moisture, improved aeration and accelerated nutrient release, while perennial weeds regenerate new plants from small fragments of roots, rhizomes, stolons and other underground structures. Severe weed problems present a serious threat to horticultural crop production with favorable environmental conditions in the soil. There have been many microbial agents under evaluation for their potential as bioherbicides with horticultural crops, turf and forest trees, including obligate fungal parasites, soil-borne fungal pathogens, non-phytopathogenic fungi, bacteria and nematodes (Sindhu *et al.*, 2017b).

One of the first bioherbicides registered was DeVine (Encore Technologies, Plymouth, MN, USA) with the active ingredient *Phytophthora palmivora*, which was developed to control strangler vine (*Morrenia odorata*) on citrus in Florida (Charudattan, 2005). The integration of biological control into current pest management systems may be an effective alternative for organic agricultural production. Biopesticide technology could be used as a component in integrated pest management strategies to help avoid pest resistance, reduce production costs and increased crop yield in organic farming. While there have been significant efforts to develop biopesticides, few have been registered for use. Future research should focus on the development of more cost-effective and efficient biopesticides as well as the optimization of their use in agri-production systems.

Diverse PGPR molecules that elicit plant defense

Various proteins and small molecules from PGPRs have been characterized that act by

promoting plant defense pathways. Several uncharacterized proteins such as from *Bacillus amyloliquefaciens* NC6 (protein 'PeBA1') and the other from *Brevibacillus laterosporus* strain A60 (protein 'PeBL1') activate ISR responses in tobacco against tobacco mosaic virus (TMV) and *P. syringae* pv. *tabaci* and *B. cinerea* (Wang *et al.*, 2015; 2016). The small molecule phenylacetic acid (PAA) is classified as an antimicrobial, but PAA isolated from *Azospirillum brasilense* was found to be similar to auxin (Somers *et al.*, 2004). More recently, PAA from *Bacillus fortis* strain IAGS162 was shown to be the elicitor of ISR in tomato against *F. oxysporum* f.sp. *lycopersici* (Akram *et al.*, 2016). Through metabolomics techniques, *Pseudomonas aeruginosa* strain PM12 was found to promote ISR in tomato challenged with *F. oxysporum* via 3-hydroxy-5-methoxy benzene methanol (HMB). A soil drench of 1.0mM and 10.0mM HMB significantly reduced the disease index (Fatima and Anjum, 2017). The bacteriocin peptide Thuricin 17 (Th17) from the PGPR *Bacillus thuringiensis* strain NEB17, has long been known to have antimicrobial activities (Gray *et al.*, 2006), but recent proteomic analysis of salt-stress *Arabidopsis* suggested that it may alleviate the deleterious effect of the abiotic stress on photosystems I and II through upregulation of chloroplast proteins (Subramanian *et al.*, 2016).

Commercialization of biofertilizers and biopesticides

Several bacterial strains are commercially available in the form of formulated products which are used as biofertilizers and biocontrol agents (Sethi *et al.*, 2014; Jha and Saraf, 2015) (Table 3). Since PGPRs have its own potentiality in controlling plant diseases and pest management, these commercial products such as Diegall, Galltrol-A, Zea-Nit, Epic, Quantum 4000, Victus, Mycostop etc. have

therefore, been registered for the practical use of farming community. Besides, the potentiality of PGPR inoculants in improvement of agricultural plants in developing countries can never be ignored. In India, more than 40 stakeholders from different provinces have registered themselves for the mass production of PGPRs with Central Insecticide Board, Faridabad, Haryana through collaboration with Tamil Nadu Agricultural University, Coimbatore, India.

Conclusion and future prospects

Indian agriculture has undergone dynamic change since the “Green Revolution,” which provided self-sufficiency and ushered in an era of rural prosperity. While the production of food grains increased fourfold, soil and environment health have been affected adversely by the application of 250 times more chemical fertilizers and 400 times higher applications of pesticides than needed (Teng, 2007). Biopesticides and biofertilizers have been reported to enhance the crop productivity by control of pests and providing nutrients in ecofriendly manner. They have been used in various forms and there is a large market potential for biofertilizer and biopesticide products that can only be tapped through a better understanding of rural markets and product/marketing constraints. To achieve these objectives, an extensive research and development efforts are needed in areas pertaining to production, quality assurance, field application and knowledge transmission of biocontrol and biofertilizer products.

In addition to the continuous search for new biomolecules and improving efficiency of the known biopesticides, recombinant DNA technology is being used for enhancing efficacy of biopesticides. Fusion proteins are being designed to develop next-generation

biopesticides. The technology allows selected toxins to be combined with a carrier protein which makes them toxic to insect pests when consumed orally, while they were effective only when injected into a prey organism by a predator (Kumar, 2013). The fusion protein may be produced as a recombinant protein in microbial system, which can be scaled up for industrial production and commercial formulations. Several other innovative approaches are being applied to develop biopesticides as effective, efficient and acceptable pest control measure among the farmers. Deployed appropriately, biofertilizers and biopesticides have the potential to bring sustainability to global agriculture for food security.

Recently, the ability to ‘engineer’ the rhizosphere (Dessaux *et al.*, 2016) may prove more effective as we move away from the current paradigm of species-based consortia and microbiome cataloguing (Maymon *et al.*, 2015; Schlaeppli and Bulgarelli, 2015). The functions conferred by the changes in plant expression induced by the microbiome as a whole may prove to be more relevant than the specific microbe species enacting those changes, and the mechanisms by which the microbes act (Sindhu *et al.*, 2017b). Thus, concomitant explorations of microbial genetic manipulation of the plant and chemical genomics approaches (Stokes and McCourt, 2014) to evaluate key microbial molecular components inducing plant beneficial responses could allow the understanding of PGPR action in the field. Sustainable PGPR applications may be developed for agricultural and horticultural management practices by identifying and elucidating the specific mechanisms of microbial-plant beneficial activity. Because crops are grown under a multiplicity of climatic and environmental conditions which change from farm to farm or even within one field, and such variations causes disparity in the

potentiality of microbial inoculants based biofertilizers and biopesticides.

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