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Review Article

Impact of Interactions between Rhizosphere and Rhizobacteria: A Review

Shaikh SS*, Wani SJ and Sayyed RZ*
Department of Microbiology, Shri S.I. Patil Arts, G.B. Patel Science & S.T.S.K.V.S. Commerce College, Shahada, Maharashtra, India

***Corresponding author:** Shaikh SS, Department of Microbiology, Shri S.I. Patil Arts, G.B. Patel Science & S.T.S.K.V.S. Commerce College, Shahada, Maharashtra, India; E-mail: sohels7392@gmail.com; sayyedrz@gmail.com

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Abstract

Rhizosphere is supporting area for important and intensive interactions between the plant, soil, microorganisms and soil microfauna, because it is reach source of utilizable carbon source. In fact, biochemical interactions and exchanges of signal molecules between plants and rhizobacteria take place in rhizosphere. The bacteria present in rhizosphere are known as Plant Growth Promoting Rhizobacteria (PGPR) which produces variety of antifungal metabolites (AFM) and plant growth promoting traits which help to reduce the liberal use and doses of agrochemicals. But the inconsistent field performance is issue of concern so we need to focus on the agro compatibility and root colonization potential of these bacteria. Present review focuses on an introduction to rhizosphere and its interactions in rhizosphere, useful microbes present in rhizosphere, root colonization by PGPR, Agro-compatibility of PGPR, and novel prospect of rhizobacteria for bioremediation and commercialization strategies for the advancement of agriculture.

Keywords: Rhizosphere; Agro compatibility; Plant Growth Promoting Rhizobacteria; Root Colonization

Abbreviation

PGPR: Plant; BCA: Biocontrol Agent; ACC: 1-Aminocyclopropane-1-Carboxylate; IPDM: Integrated Plant Disease Management

Rhizosphere

The term “rhizosphere” (Greek rhiza = root, and sphere = field of influence) was first defined by Hiltner [1] as “the zone of soil immediately adjacent to legume roots that supports high levels of bacterial activity. But, over the period of time, rhizosphere has been redefined to include the volume of soil influenced by the root and parts of root tissues as well as the soil surrounding the root in which physical, chemical and biological properties have been changed by root growth and activity [2].

Rhizosphere has been broadly subdivided into the following three zones

a) Endorhizosphere: that consists of the root tissue including the endodermis and cortical layers.

b) Rhizoplane: is the root surface where soil particles and microbes adhere. It consists of epidermis, cortex and mucilaginous polysaccharide layer.

c) Ectorhizosphere: that consists of soil immediately adjacent to the root [3].

Rhizosphere is supporting area for important and intensive interactions between the plant, soil, microorganisms and soil microfauna. In fact, biochemical interactions and exchanges of signal molecules between plants and rhizobacteria take place in rhizosphere [4-5]. Such interactions can significantly influence the plant growth and yields. Rhizobacteria are rhizosphere competent bacteria that aggressively colonize plant roots; they are able to multiply and colonize all the ecological niches found on the roots at all stages of plant growth, presence of such rhizobia in rhizosphere can have beneficial, detrimental or neutral effect on plant [6].

Rhizosphere is rich source of utilizable carbon sources due to rhizodeposition. i.e. organic compounds released by plant roots [3,7]. Different compound released by plant roots in the process of rhizodeposition includes amino acids, fatty acids and sterols, growth factor, organic acids and sugars etc. [7]. Hence, it harbors an extremely complex microbial community and it includes saprophytes, endophytes, epiphytes, pathogens as well as many useful microorganisms [8] like bacteria, fungi, nematodes, protozoa, algae etc. Yadav et al. reported that 1200 × 10^6 bacteria/g dry soil are present in rhizosphere which is very high as compared to fungi (12 × 10^5 fungi/g dry soil), algae (5 × 10^5 algae/g dry soil) and actinomycetes (46 × 10^6 actinomycetes/g dry soil) [7].

Various organic compounds are released from the roots by exudation, secretion and deposition, making rhizosphere rich in nutrients as compared to the bulk soil, thus active and enhanced microbial populations in root zone is observed. This phenomenon of establishment of rich microflora in the rhizosphere under the influence of root-secreted nutrients is referred as the rhizosphere effect [3,6,9]. Rhizosphere effect is calculated in terms of rhizosphere ratio, i.e. R: S by dividing the total number of microorganisms in the rhizosphere (R) by the corresponding number in the bulk soil (S) [10]. R: S is the measure of degree of microbial activity, higher the R : S ratio higher is the activity in rhizosphere. R: S ratio higher is also higher in bacteria 23.0 as compared to fungi (12.0), algae (0.2) and actinomycetes (7.0) [7]. Rhizosphere bacteria (Rhizobacteria) which have the capacity to influence the root in a positive way are called as plant growth promoting rhizobacteria (PGPR), these rhizobacteria

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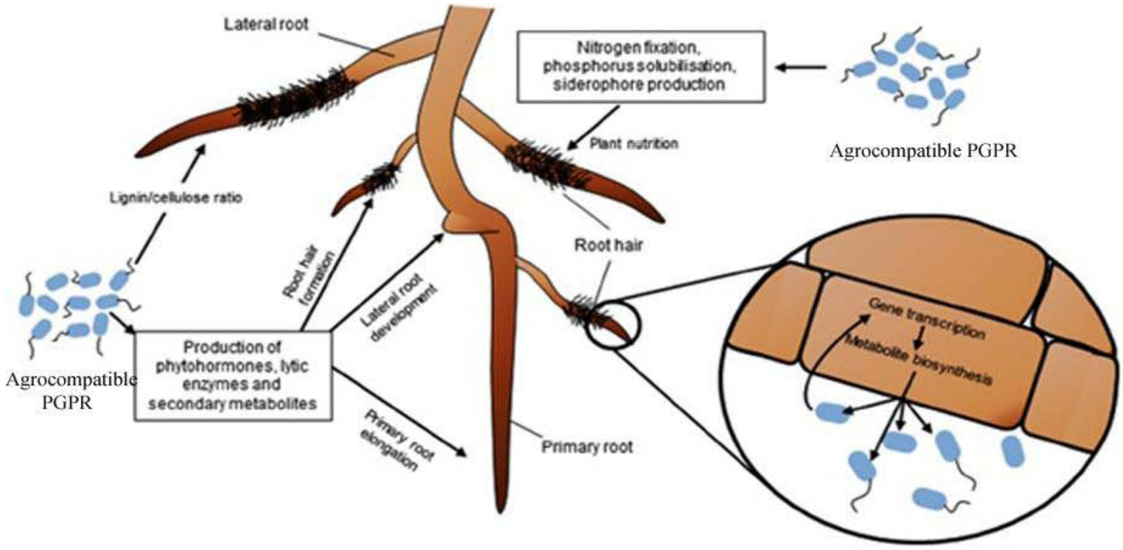

Figure 1: Impact of agrocompatible PGPR on Root system architecture, and functioning (Adopted from labuschagne et al. 2010).

exert a beneficial effect on plant growth [11-21]

Diversity of PGPR in Rhizosphere

PGPR are the microorganism basically present in the rhizosphere which includes the bacterial species including *Alcaligenes, Azospirillum, Arthrobacter, Acinetobacter, Bradyrhizobium, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium Azorhizobium, Bradyrhizobium, Allorhizobium, Sinorhizobium, Frankia* and *Mesorhizobium* [16-23], Chauhan et al. (2015) reported few novel PGPRs like *Pantoea, Methylobacterium, Exiguobacterium, Paenibacillus* and *Azoarcus*, etc. these bacteria associated with the rhizosphere of plant and are able to exert many beneficial effects on plant growth [24]. As above mentioned there is very large quantity of PGPR or rhizobacteria present in rhizospheric region. There are several mechanism by which PGPR can increase the plant growth by various mechanisms of plant growth promotion and biocontrol, these includes nitrogen fixation [25-27] production of phytohormones [13,14,28,29]. Lowering of ethylene concentration by producing ACC deaminase [30-32] and solubilization of phosphorous and various other minerals [33-34]. Siderophore production [21-22, 35-37], hydrolytic enzymes [38-39], and by producing antibiotics etc [40-41].

Pseudomonas sp. and *Bacillus* sp. as a versatile and most acceptable PGPR and BCA

Among all Gram-negative soil bacteria described earlier which shows PGPR activities, *Pseudomonas* sp. is the most abundant genus in the rhizosphere, these strains has been known for many years for its PGPR activieties, resulting in a broad knowledge of the mechanisms involved [42-43]. The name *Pseudomonas* (derived from the Greek words pseudes "false" and monas "a single unit" or "false unit") comprises one of the most diverse and ecologically fit groups of bacteria on this planet, whose members are collectively referred to by the generic term Pseudomonads. Though, at times the term "Pseudomonad" is also used to refer to former members of the genus [44]. The most effective strains of *Pseudomonas* have been Fluorescent *Pseudomonas* they includes *P. aerogenosa*, *P. fluorescence*, *P. putida* and *P. syringae* [45]. The various modes of action of a *Bacillus subtilis* strain, FZB24 against phytopathogens are examined by Rehman 2016 [46].

The ecological diversity of this genus is enormous, since individual species have been isolated from a number of plant species in different soils throughout the world. *Pseudomonas* strains show high versatility in their metabolic capacity. Most of the PGPR activities/metabolites viz. siderophore production (pyoverdin and pyochelin), antibiotics production (phloroglucinol, phenazines and pyrrolnitrin etc.), ACC deaminase production, phosphate solubilization, phytohormone production, production of lytic enzymes (Cellulase glucanase, chitinase and protease etc.), are generally released by these strains [20,36,42-43,45,47-48] These metabolites produced by *Pseudomonas* strongly affect the environment in positive way, because they inhibit growth of other deleterious microorganisms and because they increase nutrient availability for the plant. Table 1 & 2 illustrate the few examples of mechanism action of PGPR species.

- *Pseudomonas* sp is particularly suited as BCAs because they can use diverse root exudates as nutrient source.

- They are abundantly present in natural soils, especially in the rhizosphere.

- They have high growth rate.

- They directly promote plant growth and have the ability to control diseases by a variety of mechanisms.

- Besides plant growth promotion and disease resistance these strains are also involved in bioremediation, pesticide degradation, and stress management of plants [44].

Although biocontrol strains of fluorescent pseudomonads have contributed greatly to the understanding of the mechanisms that are

Figure 2: Global Pesticide use and market share**.**

Table 1: Partial list of mechanism by which *Pseudomonas* species act as PGPR.

***Pseudomonas* species**	**Effects/traits**	**Reference**
Pseudomonas sp.	Seed piece inoculation caused a reduction of soft rot disease caused by *Erwinia carotovora*	Kloepper (1983) [49]
P. fluorescens	Prevention of citrus canker caused by *Xanthomonas campestris* pv. *citri* by siderophore production	Unnamalai and Gnanamanickam (1984) [50]
P. putida W4P63	Suppression of soft rot of *Erwinia carotovora* in tuber siderophore production	Xu and Gross (1986) [51]
P. cepacia B5	Suppression of *R. solanacearum* by 2- ketogluconic acid	Aoki et al. (1991) [52]
P. glumae	Suppression of *R. solanacearum* by induction of systemic resistance	Furuya et al. (1991) [53]
P. fluorescens PF59	Suppression of *R. solanacearum* by siderophore production	Hartman et al. (1992) [54]
P. fluorescens A506	Reduction in populations of *Erwinia amylovora* in pear flowers due to competition	Wilson and Lindow (1993) [55]
Pseudomonas sp.	Suppression of the tomato wilt pathogen by siderophore production	Jagadeesh et al. (2001) [56]
Pseudomonas spp.	Increased ability to control bacterial and fungal root pathogens of rice	Lawongsa et al. (2008) [57]
P. lurida	Cold-tolerant phosphate-solubilizing bacterium that promotes wheat seedling growth	Selvakumar et al. (2011) [58]
P. fluorescens SS101 (Pf. SS101)	Protects *Arabidopsis* from the infection of *P. syringae* pv *tomato* through induction of systemic resistance	Judith et al. (2012) [59]
P. brassicacearum J12	Suppresses *R. solanacearum* by 2,4-diacetylphloroglucinol (2,4-DAPG)	Zhou et al. (2012) [60]
P. fluorescens	Protects groundnut plants from salt stress under by producing ACC deaminase	Saravanakumar and Samiyappan (2007) [61]
P. chlororaphis O6	Induced systemic drought and salt tolerance in asparagus plants	Cho et al. (2008) [62]
P. putida	Tolerates high temperature (45°C) and salinity stress (1M NaCl) through involvement of heat stress responsive molecular chaperones and membrane proteins during heat stress	Rangeshwaran et al. (2013) [63]
P. chlororaphis	Confers salt stress tolerance in gladiolus in sodic soil	Damodaran et al. (2014) [64]
Pseudomonas Sp	Tolerates osmotic stress	Kumari et al. (2016) [65]

involved in disease suppression.

Similar to fluorescent pseudomonads, specific strains of *Bacillus* spp. can provide plant protection by antibiosis and induced systemic resistance. Commercially available biocontrol rhizobacteria include *Bacillus subtilis* strains GB03 (Kodiak; Gustafson), MBI 600 (Subtilex; Becker Underwood) and QST 713 (Serenade; Agra Quest), *Bacillus pumilus* strain GB34 (Yield Shield; Gustafson), *Bacillus Licheniformis* strain SB3086 (Eco Guard; Novozymes), a mixture of B. subtilis strain GB122 and *Bacillus amyloliquefaciens* strain GB99 (BioYield; Gustafson), several *Bacillus* sp. and a few strains of *Pseudomonas fluorescens*, *Pseudomonas putida* and *Pseudomonas chlororaphis* (Cedomon; BioAgri). These biocontrol bacteria can be applied as dry products (granules or powders), cell suspensions (with or without microencapsulation) or seed coatings [45].

Compatibility of PGPR with agro-chemicals

Since the PGPR are most acceptable alternative to traditional agrochemical but the survival and shelf life and productivity is topic of concern. so the PGPR who perform well in lab condition are fail to do so in field because, in modern agricultural production, application of chemical based herbicide/pesticides/fertilizers/insecticides has become a regular practice. These pesticides are adsorbed on cell surface of organism, affecting ion transport, and metabolism inside the cell by binding to amino and sulfide groups. In the course of this processes, changes take place in the oxidoreduction level of soil, and depending on the chemical composition and dose of herbicide, the microorganism concerned may be killed [78]. Abundant use of chemical fertilizers and pesticides has also contaminated the soil environment, so this may be the reason for inconsistent

Table 2: Partial list of mechanism by which *Bacillus* species act as PGPR.

***Bacillus* species**	**Effects/traits**	**Reference**
Bacillus amyloliquefaciens strain 1 N 937a	Shows biocontrol to Tomato mottle virus in tomato plant	Murphy et al. (2000) [66]
Bacillus licheniformis	Biocontrol over *Myzus persicae* in Tomato and pepper plant	Lucas et al. (2004) [67]
Bacillus cereus BS 03	Biocontrol over pegeonpea by ISR	Dutta et al. (2008) [68]
Bacillus amyloliquefaciens	Enhancement of plant growth by root colonization and Indol acetic acid production	Idris et al. (2007) [69]
Bacillus amylliquefaciens	Production of antibiotics like iturin against *R. solani*	Yu et al. (2002) [70]
Bacillus subtilis AF1	Biocontrol in *Aspergillus niger* in ground nut	Sailaja et al. (1997) [71]
Bacillus thuringiensis	Insecticidal activity against *Erwinia carotovora* virulance	Dong et al. (2004) [72]
Bacillus pumilus and *Bacillus licheniformis*	Plant growth promotion by production of Gibberline.	Gutierrez-Manero et al. (2001) [73]
Bacillus subtilis	Suppression of Fusarium wilt of cotton	Brannen et al. (1994) [74]
Bacillus amyloliquefaciens FZB45	Phytase activity contributes to its plant growth-Promoting effect.	Idris et al. (2002) [75]
Bacillus spp	Induced systemic resistance and promotion of plant growth	Kloepper et al. (2004) [76]
Bacillus spp	Plant growth promotion in wheat	Panwar and Singh (2000) [77]

performance of PGPR in field condition. Some workers have shown that mixing of PGPR with other agrochemicals such as fertilizers and insecticides is an important strategies involved in integrated plant disease management (IPDM). Another aspect of this is to know the compatibility of PGPRs with agrochemicals. Surendran et al. (2012) studied the compatibility of *Pseudomonas fluorescens* with 15 fungicides, 9 insecticides and 10 weedicides tested under laboratory condition. Most of the insecticides, herbicides and 12 fungicides except saaf, kocide (copper hydroxide) and zineb were found compatible with P. fluorescens. This study indicated that most of the pesticides can be mixed with P. fluorescens for use in agriculture [79]. Chennakesavulu et al. (2013) studied in vitro compatibility of six fungicides against Fusarium udum with potential antagonist P. fluorescens CPF4 at different concentrations [80].

Mathew (2003) has demonstrated the compatibility of P. fluorescens with pesticides and also antagonism by the isolate [81]. *P. fluorescens* (PF-9), *Bacillus* sp. (B-44), and chitinolytic bacteria (Chb-1) are compatible with carbendazim at 500 and 1,000 ppm concentrations. Of these, PF-9 was most effective in reducing/severity of Sheath blight in rice caused by *Rhizoctonia solani* either alone or in combination with one spray of 0.1% carbendazim, followed by combination of PF-9 and B-44 [82]. Chen et al (2003) showed that effective root colonization by *Bacillus* sp. when applied with the jinggangmycin [83]. Kumar et al. (2011) showed the compatibility of *Bacillus subitilis* MBI 600 with various fungicides such as benomyl, validamycin, carbendazim, azoxystrobin, tricyclazole, mancozeb, propiconazole, and hexaconazole up to 1,000ppm level, among which all fungicides showed the compatibility up to 600ppm [84]. Combined exposure of rhizosphere-competent bacteria with chemical fertilizer has been a novel integrated nutrient management strategy to maintain and increase soil fertility by optimising all possible sources of plant nutrients required for crop growth and quality [85]. Chemical fertilisers $(N_5+_5P_{15}+_{15}K_{15}S_{10}+_{10}+_{10})$ with *Bacillus subtilis* BSK17 and BSK5 enhanced the *Cicer arietinum* yield by 90 and 81%, respectively, on the other hand further increase in dose of chemical fertilizers resulted in decrease in yield so it was shown that *Bacillus subtilis* BSK17 strain plays a more effective role in improving yield quality of *Cicer arietinum* and effectively controlled *Fusarium oxysporum* along with combined exposure of chemical fertiliser i.e. N, P, K and S [38]. Patil et al. (2014) reported the compatibility of *P. fluorescens* Pf4 against five insecticides namely imidacloprid, chloropyriphos, carbofuron and endosulfon, were as indaxocarb was incompatible with pf5 [86]. *P. fluorescens* also showed sensitivity towards fungicides, carboxin, chlorothalonil, carbendazim and captan [87]. Laha and Venkatraraman (2001) also studied the compatibility of *P. fluorescens* with carbendazim while studying sheath blight management in rice [82]. Enhanced growth of *P. fluorescens* by carbendazim at 100ppm was also reported while studying the role of BCA in red rot disease of sugarcane [88].

Rhizosphere competence and root colonization by PGPR

Rhizosphere is the major soil ecological environment wherein different kinds of plant-microbe interactions can be observed. As a result of microbial colonization in and around the growing plant roots various kinds of relationships such as associative, symbiotic, neutralistic or parasitic, may develop, depending upon factors like nutrient status of the soil, overall soil environment, plant defense mechanism and certainly the proliferating microorganism itself [89].

Most of rhizobacteria perform well under in vitro assay conditions but when subjected to natural conditions under soil environment they fail to do so. Only those rhizobacteria which are efficient in colonizing the roots of plant will perform well and will promote the plant growth. Therefore, root or seed colonization of rhizobacteria should be considered as important parameter of plant growth promotion. Root colonization is an important prerequisite for bacteria to be considered as true PGPRs, and it is commonly believed that a BCA should colonize the rhizosphere and the surface of the plant it protects. Therefore, any given PGPR is often ineffective as a BCA against root disease if it does not colonize the roots efficiently [90]. To exhibit their plant growth-promotion and protection capabilities, the foremost requirement for the PGPR is to colonize the suitable sites in the rhizosphere (Figure 1). The effectiveness of PGPR mediated processes is strongly influenced by factors such as the competence and persistence of the particular strain in the rhizosphere, its root colonizing capacity, synthesis and release of various metabolites, plant species and plant genotypes within a species and the competing microflora in the rhizosphere [3].

Root colonization is also considered to be a crucial step in the application of microorganisms for beneficial purposes such as biofertilization, phytostimulation, bicontrol and phytoremediation [91]. Root colonization, which is a complex process, is under the influence of various parameters such as bacterial traits, root exudates, biotic and abiotic factors [92]. Among all the PGPR reported so far *Pseudomonas* and *Bacillus* sp. are the most important root colonizers in various crops in agriculture. Several members of this group have widespread distribution in the soil, are efficient colonizers of the rhizosphere, and produce various types of metabolites inhibitory which cause antifungal effect to phytopathogens [90,93]. Motility and chemotaxis can serve as suitable traits for the selection of efficient root colonizing strains, non motile mutant not able to colonize the root [44].

New prospect of rhizobacteria for bioremediation

Besides the role of PGPR in Plant growth promotion and disease resistance in last few years PGPR are widely used for bioremediation of heavy metal contamination in soil [94-95]. Contaminated sites are often nutrient poor or hard to provide nutrient because unsuitable conditions. Such soils can be nutrient enriched by applying metal-tolerant microbes that provide key needed plant nutrients. Applying metal-tolerant microbes therefore may be vital in enhancing the detoxification of heavy-metal-contaminated soils [95]. Recently our group have demonstrated role of rhizobacteria in bioremediation of heavy metal contaminated soil and plant growth promotion in heavy metal contaminated soil. Siderophore producing *Alcaligenes feacalis* RZS2 and *Pseudomonas aeruginosa* RZS3 strains chelated various heavy metal ions like $MnC_{12}.4H_2O$, $NiC_{12}.6H_2O$, ZnC_{12}, CuC_{12} and CoC_{12} other than $FeCl_3.6H_2O$ proves their bioremediation potential [35]. Gray and Smith (2005) rhizobacteria have been reported as beneficial for their host plants in metal contaminated soils [96]. Some rhizobacteria are also involved in phytoremediation to extract, detoxify, or sequester pollutants from shallow soil and water [97].

Trivedi et al. (2007) reported the Enhancing plant growth by *Rhodococcus erythropolis* in presence of Cr^6 and reduction of Cr^6 to Cr^{3+} [98]. By considering the sequestration ability and Due to the sensitivity of the microbial communities to heavy metals, microbes have been applied for the bioremediation [94,99-101]. PGPR have been successfully used to reduce plant stress in metal-contaminated soils, rhizospheric microbes establish a synergistic relationship with plant roots which enhances nutrient absorption and improves plant performance, as well as the quality of soils [95].

Commercial scenario of rhizobacteria

With increasing interest in ecofriendly biological control of soil borne phytopathogens, several companies now have developed biocontrol agents, Biofungicides, Biofertilizers as commercial products under various trade names. Recently Bashan et al. (2016) have described the some criteria for the for these organism for formulation and application as biofertilizer/ Biopesticide [102]. Shaikh et al. (2016) have shown the consumption of N, P, K fertilizers and NPDB project by government of India for the exploitation of Biofertilizers for the replacement of chemical fertilizers [19]. Calvo et al. (2014) global market for plant biostimulants (Biofertilizers) has been projected to reach $2,241 million by 2018 and to it should leads to annual growth rate of 12.5 % from 2013 to 2018 [103]. Not only bacterial but fungal, viral and other agents are also being used for the preparation of biopesticides. Market share includes bacterial (60%), fungal (27%), viral (10%) and other agents for eg. nematodes (3%) [104]. It seems that USA is having most share of biopesticide used all over the world, Europe is second and then asia (Figure 2).

Future Perspectives

Among rhizobacteria genus *Pseudomonas* and *Bacillus* has attracted commercial attention because of its functional potential as PGPR and BCA. To make biocontrol more successful, it has been proposed to improve the BCA on genetic level in such a way that single strain becomes multiple edge weapons. According previous reports following approaches can be applied for this purpose- i) modifying the regulation of expression of traits important to biocontrol and ii) enhancing the stability and activity of biocontrol. To understand the rhizosphere competence and root colonization, use of green fluorescent protein (gfp) and in situ monitoring based on confocal laser scanning microscope (CLSM) has become necessary. Modification of the genes involved in the ability of PGPR also plays a key role in improving the potential of biocontrol agent. The rhizosphere competence, as well as antifungal activity of *P. fluorescence* carrying phenazine-1-carboxylic acid (PCA) coding mini-Tn5 vector was enhanced by introducing carboxamide (PCN) producing *phzH* gene from *Pseudomonas chlororaphis* PCL1391. Other such biocontrol enhancing and plant growth promoting genes include, cry-toxin-encoding cryAc7 gene of *Bacillus thuringiensis* chitinase encoding *chiA* gene of the *Serratia marcescens* and ACC deaminase gene from *enterobacter cloacae*. Genetically engeneered rhizobacteria having multiple genes are the topic to be focused in which the modified rhizobacteria can be used for multiple purposes mentioned in chapter.

Conclusion

Rhizobacteria are attracting researchers for their various applications. Rhizobacteria is an eco-friendly alternative to chemical fertilizers and pesticides, the use of which is regulated and sometimes forbidden. The performance of the plant under natural soil is inexorably linked to the activity of their microbial associates. The BCA capable of showing agrocompatility under different soil condition can lead to new horizons in the field of biocontrol. Such type of a biocontrol agent can be co-cultured with other compatible PGPR for plant benefaction. These rhizobacteria are also be utilized for the removal of heavy metal contamination this aspect is useful in the waste water management or bioremediation of soil.

Competing Interest

All authors declare that we do not have competing interest.

Authors' Contributions

Shaikh SS: Experimental work and Drafting and Preparation of Manuscript.

Wani SJ: Experimental work and Drafting and Preparation of Manuscript.

Sayyed RZ: Guidance during work & Drafting of Manuscript.

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