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Plant Growth Promoting Rhizobacteria for Plant Growth Promotion and Biocontrol Agent against Tomato and Pepper Disease: A review

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ABSTRACT

Tomato and pepper are some of the most important and widely grown vegetable crops in the world. Like many other vegetables and fruits, tomato and pepper are threatened by biotic and abiotic stresses. Conventional agricultural practices frequently rely on synthetic fertilizers and pesticides that have adverse effects on humans, animals and environments. In this situation, the use of Plant Growth Promoting Rhizobacteria (PGPR) can better crop yield, as these are vital components of soil fertility and plant growth promotion, moreover, they can display antagonistic effects against phytopathogens. Under such circumstances, knowledge about the local bacterial populations, their identification, and their implications for improving management practices (vis-a-vis plant growth promotion and destruction of plant diseases) is very important. Recent advances in microbial and molecular techniques have significantly contributed to introducing many different bacterial genera into soils, onto seeds, roots, tubers or other planting materials to control disease and improve productivity. Plant growth promoting rhizobacteria have been generally applied to high value crops like tomato and pepper. Thus, this review is intended to summaries the literature on plant growth promoting rhizobacteria for growth promotion potential and biocontrol agent against tomato and pepper disease.

***Keywords*:** Biocontrol, Phytopathogens, Plant Growth Promotin Rhizobacteria, *Solanum lycopersicum*, *Capsicum annuum*

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1. INTRODUCTION

Fruits and vegetables are important sources of micronutrients and dietary fibres and are components of a healthy diet that helps in preventing major diseases (Amao, 2018). Tomato (*Solanum lycopersicum* L) and pepper (*Capsicum annuum* L) are some of the most important and widely grown fruit/vegetable crops in the world (Rai *et al*., 2017; Kipgen and Bora, 2017). Like many other fruit/vegetables, tomato and pepper are threatened by biotic and abiotic stresses (Bulgari *et al*., 2019).

In the current situation of global climate change, increasing demand for crop production with significant reduction in the use of chemical fertilizers and pesticides, in the limited resource of land, is a great challenge for farmers and agricultural researchers (Singh *et al*., 2019). Still, the demand for food will continue to growth annually and proportionally to the increasing population worldwide (Patel and Minocheherhomji, 2018). Conventional agricultural practices frequently rely on synthetic fertilizers and pesticides that have adverse effects on humans, animals and environments (Aloo *et al*., 2019). In the soil, the continuous use of these chemicals adversely affects the natural microflora present in the rhizosphere and engenders imbalance in the natural ecosystem (Singh *et al*., 2017; Dash *et al*., 2018). To reduce these effects, researchers are now deeply engaged in finding alternative environmentally-friendly means of improving crop production and controlling plant pathogens (Kannojia *et al*., 2019). In this situation, plant growth promoting rhizobacteria (PGPR) has been investigated as these are vital components of soil fertility and plant growth promotion and show antagonistic effects against phytopathogens through a wide variety of mechanisms (Gouda *et al*., 2018; Méndez-Bravo *et al*., 2018).

Rhizobacteria are the bacterial communities abundantly found in the rhizosphere and in the area that is under the influence of the root and its close vicinity. The rhizosphere gives support to many active microbial populations capable of exerting beneficial, neutral, or detrimental effects on plant growth (Agrawal and Agrawal, 2013). Plant Growth Promoting Rhizobacteria (PGPR) are beneficial microorganisms that live in soil and colonize the rhizosphere (Shao *et al*., 2015). PGPR can increase the crop yield and reduce disease occurrence, and, thus, is considered a most promising agent for cash crop production (Huang *et al*., 2017).

In recent years, the use of microbial inoculants as biofertilizers and biocontrol agents in the agriculture industry has increased considerably. Microbial inoculants are favoured to reduce environmental pollution caused by chemicals and pesticides. These microorganisms can promote plant growth and facilitate the absorption and utilization of several mineral nutrients. PGPR can be nitrogen-fixing or have properties that enhance growth of crops (Sarbadhikary and Mandal, 2017).

Moreover, since phosphates are generally present in the form of insoluble phosphates such as tri-calcium phosphate or hydroxy-apatite or rock phosphates as the phosphorus combines with several chemical compounds in the soil, PGPR are also reported to solubilize the insoluble phosphates by producing organic acids (Ghosh *et al*., 2016). Various species of rhizospheric bacteria that enhance plant growth include: *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia*. Of these, endophytic bacteria like *Pseudomonas*, *Bacillus*, *Xanthomonas* and *Erwinia* have been reported to be associated with solanaceous vegetable crops (Agrawal and Agrawal, 2013; Gupta and Kaushal, 2017).

Ralstonia solanacearum is an important soil borne bacterial plant pathogen of worldwide distribution and has a wide host range of more than 200 species in 50 families. It causes severe plant mortality and yield loss up to an extent of 90 to 100 percent (Kipgen and Bora, 2017). Bacterial wilt limits the production of solanaceous crops such as tomato, pepper, eggplant, tobacco and potato, as well as other important crops like peanut, banana, ginger and geranium (Yanti *et al*., 2017b).

It is one of the most serious vascular diseases of Chili pepper (*Capsicum annuum* L.), causing maximum crop losses from 15% to 55% around the world (El-Argawy and Adss, 2016). Moreover, bacterial wilt incited by *Ralstonia solanacearum* has been found the most damaging and widespread diseases of tomato throughout the world, causing substantial production loss varying from 10 to 100% (Radhi *et al*., 2016). Biological control using microbial agents such as rhizobacteria has gained immense traction over the last decade. Among the rhizobacteria, Fluorescent Pseudomonads are aggressive colonizers of rhizospheric bacteria by producing an arsenal of antimicrobial compounds i.e. antibiotics such as 2,4-diacetyl phloroglucinol (DAPG), pyoluteorin, phenazines, pyrrolnitrin, volatile compounds such as hydrogen cyanide (HCN), siderophores and lipopeptides, along with hydrolytic enzymes (Rai *et al*., 2017).

Phytophthora species have devastating effect on a wide range of plants important to agriculture and natural ecosystems. *Phytophthora capsici* causes seed rot and seedling blight in many solanaceous crops (pepper, eggplant, tomato etc.), hence generating multi-billion dollar losses in crop production annually (Kang *et al*., 2016). Fusarium wilt caused by *Fusarium oxysporum* is one of the most prevalent and damaging diseases of tomato, bringing about considerable losses (Boukerma *et al*., 2017).

Most widely grown tomato cultivars are susceptible to soil-borne diseases and especially to *Rhizoctonia* Root Rot caused by *Rhizoctonia solani* and *Sclerotinia* Stem Rot induced by *Sclerotinia sclerotiorum*. These pathogens are responsible for damping-off collar, stem and root rots and eventually plant death, leading to serious yield losses both under greenhouse and open field growing systems (Abdeljalil *et al*., 2016b). In addition to this, infection of pathogenic fungi (particularly *Rhizoctonia solani*) is a significant factor that causes disease and decreases the yield of pepper plant (Calvo *et al*., 2010).

There are some studies on endophytic bacterial suppression of *P. capsici* on pepper at present. *Bacillus megaterium* IISRBP 17, an endophytic bacterial strain that has been isolated from black pepper stem and roots, was found to be effective against *P. capsici* on black pepper, in greenhouse assays (Yang *et al*., 2015). Recently, studies have reported on the effects of *P. capsici* on pepper (*Capsicum annum* L.), and researchers have observed the role of the *Burkholderia* sp. KCTC 11096BP in rescuing pepper growth by mitigating the adverse effects of the pathogen on host. This is due to the production of bioactive secondary metabolites and phytohormones by *Burkholderia* sp. KCTC 11096BP (Afzal *et al*., 2017). Furthermore, several *Bacillus* strains can be used as biological control agents against *Rhizoctonia solani* infecting pepper, tomato and potato plants.

Herein, *Bacillus subtilis* HS93 and *Bacillus licheniformis* LS674 were isolated from roots of pepper plants, and the antagonistic activity of two bacteria may be stimulated by chitin, resulting in significant improvements in their effectiveness against *Rhizoctonia solani* and *Phytophthora capsici* due to their ability to produce antibiotics, lipopeptides, and hydrolytic enzymes (Huang *et al*., 2017). Therefore, the aim of this review is to accentuate the role of plant growth promoting rhizobacteria in the biocontrol of tomato and pepper diseases.

2. PGPR FOR SUSTAINABLE PRODUCTION OF TOMATO AND PEPPER

Plant growth promoting rhizobacteria can directly benefit plant growth through production of growth regulators increasing nitrogen uptake, synthesis of phytohormones, solubilization of minerals, and iron chelation (Abdeljalil *et al*., 2016c). Because of different factors threatening agriculture, scientists are searching for alternatives involving natural and eco-friendly solutions Among this options, the use PGPR in crop production can reduce the agro-chemical use and support ecofriendly sustainable food production (Kang *et al*., 2016; Boukerma *et al*., 2017).

2. 1. Role of PGPR as plant growth enhancer

Plant growth promoting rhizobacteria plays an important role in enhancing plant growth through a wide variety of mechanisms. According to Agrawal and Agrawal (2013) report the influence of the most promising isolates of *Bacillus* on plant growth bacterized tomato seed were planted in paper towel method. All rhizobacterial isolates produced Indole-3-acetic acid (IAA) in vitro by the addition of L-tryptophan, in the culture medium. All the strains, HBS-VIII, FAR-IIIb, HBR-II, GAR-III and HBR-VII significantly improved seed germination when compared to the uninoculated control (UIC).

The isolate HBRVII showed significantly increased seed germination (97.5%) and also shoot and root length as well as enhanced vigour index of 115.50, 714.35 after 6 and 16 days respectively. In other recent study on field application of two plant growth promoting rhizobacteria on tomato, *Bacillus subtilis* VBLR10 and *Cellulosimicrobium cellulans* VBLR39 in tomato crops were found to be efficient in plant growth promoting activities as they exhibited good phosphate solubilization and IAA production. The strains showed excellent plant growth promoting activities in small field trials. Both strains survived for more than one year in alluvial soil sample at 28 °C and at 37 °C. The efficiency and applicability of the strains under field conditions were also established conclusively as they improve growth and yield parameters of tomato plants both qualitatively and quantitatively (Sarbadhikary and Mandal, 2017).

2. 2. PGPR against bacterial pathogens of tomato and pepper

Biological control of plant pathogens through other microorganisms has emerged in the recent years (Majid *et al*., 2016 and Huang *et al*., 2017). Several bacterial strains have been widely used as biological agents for the management of bacterial wilt of tomato and pepper plants. Nguyen and Ranamukhaarachchi (2010) evaluated the potential biocontrol against *R. solanarearum* in tomato and pepper plants found that three antagonists isolated from soil (*Bacillus megaterium*, *Enterobacter cloacae* and *Picbia guillermondii*) showed high potential for disease suppression and also increased fruit weight, biomass and plant height of tomato and pepper.

In addition to this, Rai *et al*. (2017) assessed the biocontrol potential of *Pseudomonas protegens* RS-9 against *R. solanacearum* in tomato. In the in vitro evaluation, the strains produce antimicrobial metabolites viz. 2,4-diacetylphloroglucinol (2,4-DAPG), hydrogen cyanide (HCN), pyrrolnitrin and pyoluteorin. Inoculation of field soil in pots with RS-9 in greenhouse led to significant increase in plant height and dry weight of tomato plant and incidence of bacterial wilt reduced by 65.6%. Consequently, the study revealed *Pseudomonas protegens* RS-9 to be the promising strain for biocontrol of bacterial wilts of tomato.

Biocontrol of bacterial wilt disease in pepper crops have been reported in the recent study. The study on screening of rhizobacteria from rhizosphere of healthy chili to control bacterial wilt disease and to promote growth and yield of chili found that *R. solanacearum* were inoculated on the 6 weeks chili plants using two strains of rhizobacterial isolates from chili rhizosphere (RZ.2.1.AG1 and RZ.1.3.AP1) showed high potential for disease suppression and also increased growth and yield of chili (Yanti *et al*., 2017b). The other study found that six indigenous strains of *Bacillus* spp., controlled 100% of *R. solanacearum* on pepper with no wilt symptom and improve the growth of *R. solanacearum*-inoculated chili plants. Out of the tested *Bacillus* species, only *Bacillus subtilis* CIFT-MFB-4158A produced siderophore and antibiotic for the growth inhibition of *R. solanacearum* (Yanti *et al*., 2017a).

2. 3. PGPR against fungal pathogens of tomato and pepper

Recent advances in microbial and molecular techniques have significantly contributed to introducing many different bacterial genera into soils, onto seeds, roots, tubers or other planting materials to control disease and improve productivity (Zohora *et al*., 2016). Recent study on rhizobacterial isolates was assessed for its antifungal potential against *Rhizoctonia solani* mycelial growth and its capacity to suppress Rhizoctonia root rot disease and to enhance growth of infected tomato plants.

The screening of disease-suppressive and plant growth-promoting abilities of *Bacillus* spp., *Enterobacter cloacae*, *Chryseobacterium jejuense*, and *Klebsiella pneumonia* showed that 45-100% disease in disease severity and significant increments in plant height by 62-76%, roots fresh weight by 53-86%, and aerial part fresh weight by 34-67%. *B. thuringiensis* B2 (KU158884), *B. subtilis* B10 (KT921327) and *E. cloacae* B16 (KT921429) were found to be the most efficient isolates in decreasing *R. solani* radial growth, suppressing disease severity, enhance plant growth (Abdeljalil *et al*., 2016a). Furthermore, *Bacillus subtilis* RB14 was used as an antagonist against fungal pathogen *R. solani* K1 to control damping-off diseases in tomato plants. Treated tomato seeds showed 99% germination index similar to the untreated seeds. *B. subtilis* RB14 treated seed showed 80% reduction in disease incidence during in vivo plant experiments. *B. subtilis* RB14 produces lipopeptide antifungal antibiotic iturin A which could suppress *R. solani* K1 (Zohora *et al*., 2016). In addition to this Fusarium wilt caused by *Fusarium oxysporum* on tomato reorted that cell free supernatant of Bacillus subtilis and Cellulosimicrobium cellulans could reduce radial mycelial growth of *F. oxysporum* on the growth medium (Sarbadhikary and Mandal, 2017).

Many *Bacillus* strains have been proved to be effective against *R. solani*. An in vitro study was conducted to determine the antagonistic effectiveness of *B. thuringiensis* against damping-off and root and stem rot chili pepper caused by *R. solani*. Out of the tested strains, majority of the antagonistic isolates, GM-23, GM-11and GM-121, were effective in the reduction of *R. solani* infection. These results suggest that the *B. thuringiensis* strains have an excellent potential to be used as bio-control agents of *R. solani* in chili pepper (Mojica-Marín *et al*., 2008). In addition , *B. subtilis* SL-44 to promote pepper (*Capsicum annuum*) growth and control *R. solani* under pot experiment indicated that the dry and fresh weights of pepper in SL-44 and *R. solani* (S-R) treatment were 45.5% and 54.2% higher than those in *R. solani* (R) treatment and 18.2% and 31.8% higher than those in CK (control, noninoculation) treatment. The plant height in S-R treatment increased by 14.2% and 9.0% compared with those in the R and CK treatments, respectively. *B. subtilis* SL-44 has a great potential as biocontrol agent against *R. solani* on pepper plants (Huang *et al*., 2017).

Phytophthora blight by *Phytophthora capsici* causes severe yield loss on pepper plants worldwide (Kim *et al*., 2009). Kime *et al*. (2012) reported that *Chryseobacterium wanjuense* KJ9C8 produced protease and HCN with swarming activity on pepper roots and in rhizosphere soil might confer effective biocontrol activity. PGPR are being tried as consortia and have been found to be more effective than single inoculation.

For instance, two endophytic bacterial strains, Fy11 and Zy44 (*Bacillus amyloliquefaciens*) were tested as a single application and in combination for their abilities to suppress pepper phytophthora blight under greenhouse conditions. When both strains were applied in a mixture, the disease severity was more reduced. Both endophytic strains were efficient colonizers of pepper, and could vertically transfer from roots to shoots and leaves. The crude lipopeptides produced by strain Zy44 effectively reduced disease index, and the control efficacy reached 84.8% and 67.2% in 7 days and 15 days after inoculation with the pathogen, respectively (Yang *et al*., 2015). Moreover, *Burkholderia* sp. KCTC 11096BP exhibits maximum growth inhibition of the pathogen *P. capsici*.

The bacterium inoculation to pepper plants significantly enhanced growth attributes of pepper in infected and control treatments. The systemic acquired resistance (SAR) of the host plant was up-regulated by *Burkholderia* sp. KCTC, as endogenous salicylic acid (235.5 ng/g) and jasmonic acid (22.8 ng/g) levels were found higher in such treatments. *Burkholderia* sp. KCTC 11096BP mitigates the adverse effects of *P. capsici* on pepper crop and can improve crop productivity at the field level (Kang *et al*., 2016).

3. INDUCED SYSTEMIC RESISTANCE

Table 1. Plant growth promoting rhizobacteria for plant growth promotion and as a biocontrol of bacterial and fungal pathogens on tomato and pepper crops.

PGPR Involved	Crop	Pathogen	PGPR Properties	References
Bacillus spp.	Tomato	-	Produced IAA	Agrawal and Agrawal (2013)
Bacillus subtilis & *Cellulosimicrobium cellulans*	Tomato	*Fusarium oxysporum*	Phosphate solubilization, IAA production and antifungal Activities	Sarbadhikary and Mandal, (2017)
Pseudomonas putida & *Pseudomonas fluorescens*	Tomato	*Fusarium oxysporum*	Inhibition of mycelial growth, ISR	Boukerma *et al*. (2017)
Bacillus megaterium, Enterobacter cloacae & *Picbia guillermondii*	Tomato & Pepper	*Ralstonia solanacearum*	- Not specified	Nguyen and Ranamukhaarachchi (2010)

Pseudomonas protegens	Tomato	*Ralstonia solanacearum*	Produce 2,4-DAPG, hydrogen cyanide, pyrrolnitrin & pyoluteorin	Rai *et al*. (2017)
Bacillus thuringiensis, *Bacillus subtilis*, & *Enterobacter cloacae*,	Tomato	*Rhizoctonia solani*	Diffusible & volatile metabolite	Abdeljalil *et al*. (2016a)
Bacillus subtilis	Tomato	*Rhizoctonia solani*	Antibiotic iturin A	Zohora *et al*. (2016)
Bacillus subtilis	Pepper	*Rhizoctonia solani*	siderophore production, synthesized IAA	Huang *et al*. (2017)
Bacillus spp.	Pepper	*Ralstonia solanacearum*	produce IAA, & siderophore (only by *B. Subtilis* only)	Yanti *et al*. (2017a)
Chryseobacterium wanjuense	Pepper	*Phytophthora capsici*	produced protease and hydrogen cyanide	Kim *et al*., 2012
Burkholderia sp	Pepper	*Phytophthora capsici*	Promote ISR by procducing: salicylic acid & jasmonic acid	Kang *et al*., (2016)
Bacillus amyloliquefaciens	Pepper	*Phytophthora capsici*	Production antifungal lipopeptides, Promote ISR by producing: *Capsicum annuum* pathogenesis-protein 4 and *C. annuum* b-1,3-glucanase	Yang *et al*. (2015)

Rhizobacteria can produce siderophores, antibiotics or promote induced systemic resistance (ISR) in plants which is a key defense pathway. Elicitation of plant's defense by PGPR has received increasing attention in recent years (Sarbadhikary and Mandal, 2017). As the study indicated Yang *et al*. (2015) to find out the inhibitory mechanisms of synergistic activity against pepper phytophthora blight through a mixture of *Bacillus amyloliquefaciens* Fy11 and Zy44 strain.

The ISR mediated by strains Fy11 and Zy44 on pepper were analyzed using priming effect of the defense-related genes. The expressions of CaPR4 (*Capsicum annuum* pathogenesis-protein 4) and CaBGLU (*C. annuum* b-1,3-glucanase) were strongly induced in the plants treated with strain Fy11 in 24 h after pathogen challenge. Consequently, the synergistic interactions were due to the summation of biocontrol mechanisms of both biocontrol agents. Recently, Boukerma *et al*. (2017) evaluated the potential of *Pseudomonas fluorescens* PF15 and *Pseudomonas putida* PP27 to protect tomato plants against Fusarium wilt caused by *Fusarium oxysporum* under greenhouse conditions. Fluorescent *Pseudomonas* revealed a delay in the onset of symptoms and slower kinetics of disease progression compared to the pathogen control. McKinney's index, which measures the severity of the disease, was reduced by 37–72% and the levels of infection (incidence) by 7–36%.

4. CONCLUSIONS

Vegetables constitute an important part of human healthy foods. Out of this, tomato and pepper are one the most important and widely grown vegetable crops in the world. Like many other vegetable, tomato and pepper are threatened by biotic and abiotic stresses. Thus, scientists and vegetable growers are working hard to develop different strategies to overcome these problems. Among the options, the use of PGPR in agricultural practices has received greater attention. PGPR benefit the plant growth and development through various direct and indirect mechanisms like the production of secondary metabolites, i.e. plant growth substances, phosphate solubilization, siderophores production, antagonism to fungal and bacterial pathogens and promote induced systemic resistance. PGPR inoculants have been attracted much attention throughout the world. Detection of tomato and pepper specific PGPR and understanding the interactive relationship between PGPR and vegetable require special attention so that vegetable-specific inoculant is developed. Therefore, considerable attention should be taken by scientists and farmers to use PGPR for enhancing tomato and pepper production in different agro-ecological areas.

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