Multi-Phyto Beneficial Mechanisms and Applications of *Serratia* spp.

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Abstract: Plant diseases generate substantial threat to agriculture. Bacterial species with the ability to prevent these diseases are expected to have promises as natural alternative to chemical control measures. The bacteria of the genus *Serratia* have been reported to have both rhizospheric and endophytic association with protective effect to various plants. Among the various species, *S. marcescens*, *S. plymuthica, S. rubidaea* and *S. liquefaciens* have been extensively studied for their anti-phytopathogenic mechanisms. These involve production of antibiotics such as prodiogisin, pyrrolnitrin and oomycidin and also the production of lytic enzymes such as chitinases. In addition to this, they are also known for their ability for the induction of induced systemic resistance, which indirectly make the plant competent towards various pathogens. *Serratia* spp. are also known for their accumulation of other plant beneficial features like indole-3-acetic acid (IAA) production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity and phosphate solubilization. Hence the multi-beneficial mechanisms of *Serratia* spp. towards plants is highly impressive and is expected to have increasing agricultural applications. Current review is designed to provide insight into plant beneficial promises of *Serratia* spp.

Keywords: Biological control, *Serratia* spp., plant disease, antibiotics, chitinase, induced systemic resistance.

1. INTRODUCTION

Maintenance and enhancement of plant health and yield is of high significance. Among the various methods used for plant disease management, chemical fertilizers or pesticides are known to have serious impact on public health along with its effect in non target organisms. Hence, there is ever increasing demand for natural methods to control plant diseases. Microbial populations with antagonistic property to phyto-pathogens and which protect the plant from diseases are generally considered as biocontrol agent. Bacterial plant growth promoting (PGP) activities are also important to enhance crop yield in changing agro-climatic conditions [1]. Hence, those organisms with either anti-phytopathogenic or PGP or both have very significant application as plant probiotic agents. Several studies have been focused mainly on two genera, *Pseudomonas* and *Bacillus* for the successful implementation as the biocontrol and plant growth-promoting agent because of their abundance in the rhizosphere and spore forming ability respectively. There are numerous other genera that can also expect to have excellent potential for plant growth promotion. Members of *Serratia* like *S. marcescens*, *S. plymuthica*, *S. fonticola, S. rubidae* and *S. liquefaciens* are known for their broad spectrum of plant beneficial features [2-7]. They have a ubiquitous distribution. Importantly, they are present in the rhizosphere or can also occur as endophyte in various parts of plants due to its transport through vascular system.

2. IMPORTANCE OF THE GENUS *SERRATIA*

The genus *Serratia* belongs to the family *Enterobacteriaceae* within the Gamma-proteobacteria subdivision and are gram negative, motile, non-spore forming rod shaped bacteria. Because of its wide distribution, they can be considered to have efficient interaction with plants. These organisms are known to have direct and indirect mechanisms to protect plant from pathogen. Direct mechanisms include competition for nutrients, antibiosis and production of cell wall degrading enzymes. Indirect mechanisms mainly involve phytoimmune modulation through induced systemic resistance [8, 9]. Many members of the *Serratia* also posses PGP traits like mobilization of phosphate, sequestration of iron, phytohormone production and ACC deaminase activity. Even though many other bacteria are also known for these properties, those in *Serratia* are highly remarkable. This is because of its occurrence and wide distribution in the rhizosphere or as endophyte in the various plants. Because of these, they can also be considered to have yet to know phytointeractive chemico adaptations. A detailed insight into such mechanisms have immense applications for their successful applications to make the plant system competitive to manage various factors, which affects its growth and yield.

3. PLANT COLONIZATION BY *SERRATIA*

One of the most important prerequisite for the application of bacteria for the plant beneficial features

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is its colonization ability. This may be considered to be related to a number of both biotic and abiotc factors which affect bacterial phyto interactive competency [10]. This property of *Serratia* spp. have been revealed by visual insight by confocal laser scanning microscopy (CLSM). Here, the colonization preference of the organism in oilseed rape seedlings showed the preference of fluorescent labeled organism to upper parts of the root. The distribution of *Serratia* cells in leaf also proved its colonization in all parts of the plant [11]. Gujral *et al*. [12] have reported the applicability of green fluorescent protein (GFP) to study the colonization in the colonization efficiency of *Serratia* sp. in Sorghum seedlings. in Sorghum seedlings. In the case of rice also endophytic colonization by a diazotrophic *Serratia marcescens* confirmed its successful colonization in the plant tissues [13].

Colonization and pot trial experiments were conducted on *A. aspera* L. plant by using an endophytic *Serratia* sp. AL2-16. This AL2-16 was reported to have an increased colony forming unit from a range of 16.2×10^6 to 11.2×10^8 CFU/g between third and fifth days of inoculation. It was also found to cause significant increase in shoot length by 95.52%, fresh shoot weight by 602.38%, fresh root weight by 438%, and area of leaves by 127.2% when compared with uninoculated control [14].

4. BIOCONTROL APPLICATIONS OF *SERRATIA* spp.

Serratia species are well known for their activity against broad spectrum of phytopathogens. Several studies demonstrated its use to inhibit pathogens including foliar fungal pathogens (Table **1**). *Serratia plymuthica* 3Rp8 which was isolated as an indigenous colonizer of *Brassica napus* rhizosphere was found to have *in vitro* antagonist activity against fungal phytopathogens *Verticillium dahliae*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* [15]. *Serratia plymuthica* 3Re4-18, an endophyte from *Solanum tuberosum* also reported to have effective antagonistic activity against plant pathogenic fungi. Kurze *et al*. [16] have proposed promises of *Serratia plymuthica* HRO-C48 for controlling verticillium wilt pathogen of strawberry and other fungi as a soil amendment in fields. Different methods of application like bio-priming, pelleting or seed coating were shown to reduce the degree of verticillium wilt under greenhouse conditions. A study on the effect of ethanol extract prepared from *Serratia marcescens* N4-5 on cucumber seed treatment revealed that it has the ability to effectively suppress the damping-off of cucumber caused by *Pythium ultimum* [17]. These are explanatory to the potential of *Serratia* spp. in agriculture application. The biodiversity rich areas can expect to have more promising strains of *Serratia* spp. with heavy deposition of broad spectrum antifungal properties.

Table 1: Antagonistic Activity of *Serratia* spp. Against Various Pathogens

Biocontrol Agent	Plant Disease	Pathogen	References
S.marcescens	Damping off Bean	*Rhizoctonia solani*	[45]
S.marcescens 90-166	Fusarium wilt of cucumber	*Fusarium oxysporum*	[46]
	Anthracnose (Cucumber)	*Colletotrichum orbiculare*	[46]
S. plymuthica C1	Phytophthora blight of pepper	*Phytophthora capsici*	[45]
S. plymuthica HRO-C48	Damping off cucumber	*Pythium aphanidermatum*	[45]
	Brassica napus L . spp oleifera	*Verticillium longisporum* and *Phoma lingam*	[47]
Serratia plymuthica A30	Blackleg of potato	*Dickeya* sp	[48]
Serratia marcescens	Black scurf diseases of potato	*Rhizoctonia solani*	[49]
Serratia marcescens CFFSUR-B2	Black Sigatoka in banana (Musa spp.)	*Mycosphaerella fijiensis*	[50]
Serratia marcescens	Damping-off of Cucumber	*Pythium ultimum*	[51]
Serratia nematodiphila CT-78	Rice bacterial leaf blight	*Xanthomonas oryzae* pv. *oryzae*	[52]
Serratia plymuthica	Dry rot of potato	*Fusarium sambucinum*	[52]

Generally, plant beneficial bacteria can have biosynthetic potential to produce range of antiobiotics and enzymes. A variety of antibiotics have been identified from the *Serratia* spp. which include pyrrolnitrin (Prn), prodigiosin and haterumalides [18-21].

Pyrrolnitrin [3-chloro-4-(2-nitro-3-chlorophenyl) pyrrole] is a tryptophan-derived secondary metabolite that has been reported to have broad spectrum antifungal activity against various fungal and bacterial pathogens [22]. It is the major antagonistic pyrrole compound produced by the strains of *Serratia* sp. which is commercially used as biocontrol agents. Several pyrrolnitrin producing strains of genera *Pseudomonas* [23] and *Burkholderia* [24] have already been reported. This indicates the potential of this compound in biocontrol activity shown by diverse organisms. Pyrrolnitrin production was assumed to be a key factor of *S. plymuthica* IC1270-mediated biocontrol of several fungal post-harvest pathogens of peaches and apples [25].

Prodigiosin (methyl- 3-pentyl-6 methoxyprodigiosin), is a linear tripyrrole red pigment antibiotic characterized by a common pyrrolylpyrromethane skeleton, produced by various bacteria such as *Serratia marcescens*, *S. rubidaea, Pseudomonas magneslorubra*, *Vibrio psychroerythrous, Vibrio gazogenes*, *Alteromonas rubra* and *Rugamonas rubra* [26-27] The chlorinated macrolides, haterumalide are polyketide substances with antifungal properties, which have been purified from cell free supernatant of *Serratia plymuthica* strain A153 [19, 20]. Haterumalides NA, B and NE were found to inhibit apothecial formation in sclerotia and ascospore germination of *Sclerotinia sclerotiorum*, and spore germination of several other filamentous fungi as well as oomycetes. Oocydin A is a chlorinated macrocyclic lactone with potent biological activity purified from *Serratia marcescens*. This was found to have the inhibitory effect against *Phtyophthora* spp. [28].

Growth enhancement through the production of cell wall degrading enzymes is another mechanism used by plant growth promoting bacteria to control soil borne pathogens [29]. Chitinases, the most studied lytic enzyme produced by biocontrol bacteria, have immense applications for the management of plant diseases [30]. Chitin, the major structural component of most fungal cell walls are hydrolysed by chitinases produced by several chitinolytic bacteria such as *Serratia* [31], *Bacillus* [32], *Enterobacter* and *Pseudomonas* [33]. *Serratia* is one of the most competent chitin-degrading bacteria and there are several reports on the isolation of chitinases from different *Serratia* spp. [34-36]. Vaikuntapu *et al*. [37] have discovered a new chitinase D from plant growth promoting *S. marcescenece* GPS5 involved in the enzymatic conversion of chitin. Chitinolytic enzymes purified from *Serratia marcescens* B2 have shown to have inhibitory effects on the spore germination of pathogenic fungus *Botrytis cinerea* [38]. Frankowskii *et al*. [39] have purified two chitinolytic enzymes CHIT60 and CHIT100 from *S. plymuthica* HRO-C48 and both the enzyme inhibited spore germination and germ tube elongation of the phytopathogenic fungus *Botrytis cinerea*. Exogenous addition of chitin in foliar sprays was found to increase the efficiency of chitinolytic *Serratia marcescens* GPS5 in controlling late leaf spot of groundnut caused by *Phaeoisariopsis personata* [40]. Mehmood *et al*. [36] characterized a 60 kDa chitinase from *Serratia proteamaculans* 18A1 which demonstrated the antifungal activity against the pathogenic fungi *Fusarium oxysporum* and *Aspergillus niger*.

Microorganisms and plants require a higher level of iron which make the plant, bacteria and fungi to compete for iron [41]. Some bacteria manage that by synthesizing low-molecular weight compounds (400–1500 Da) called siderophores. These bind with ferric ion and make siderophore-ferric ion complex, which subsequently binds with membrane receptors at the bacterial cell surface and facilitates the uptake of iron by microorganisms. Plant growth enhancement with the help of bacterial siderophores have been studied extensively and it showed effect of siderophore producing microorganisms on increased iron inside plant tissues leading to improved plant growth. There are over 500 known types of siderophores with different chemical structures and are classified into 3 main groups like catecholates (phenolates), hydroxamates and carboxylates. *Serratia marcescens* 90-166 strain was found to induce resistance in cucumber through the production of siderophores. Hence this mechanism provides biocontrol features to *Serratia* by restricting the iron availability to pathogen and enhancing its uptake in plants.

Induced systemic resistance is a physiological state of enhanced defensive capacity elicited in the plant in response to specific environmental stimuli or by the subsequent biotic stresses. Priming of plants with potential organisms can enhance innate defense against a broad range of plant pathogens. Many bacterial components can affect induced systemic resistance such as lipopolysaccharides (LPS), flagella, siderophores, cyclic lipopeptides, 2, 4-diacetylphloro-glucinol, homoserine lactones, and volatiles like, acetoin and 2, 3-butanediol.

Someya *et al*. [42] have revealed the ability of *S. marcescens* strain B2 to manage rice blast through increased production of Lipoxygenase (LOX) after bacterial inoculations. The chitin amendment with the *S. marcescens* GPS5 culture was also found to have increased production of defense related genes such as beta 1, 3-glucanase, peroxidase and phenylalanine ammonia lyase [40]. *S. plymuthica* R1GC4 was also reported to modulate induced systemic resistance in cucumber against *Pythium ultimum.* This was evidenced by the occurrence of deposition of enlarged callose-enriched wall appositions of callose, pectin, and cellulose at sites of pathogen entry and penetration to prevent the plant from the pathogen attack [43]. *S. plymuthica* HRO-C48 was also found to be capable of inducing resistance in bean and tomato against *Botrytis cinerea* [44].

5. PLANT GROWTH PROMOTING FEATURES OF *SERRATIA* spp.

Plant growth promoting bacteria enhance plant growth directly by solubilization of phosphorous, production of plant growth hormones and ACC deaminase activity, which reduce the level of ethylene [53]. Inorganic phosphate solubilization is one of the major mechanisms for plant growth promotion carried out by the plant-associated bacteria. Microorganisms, especially bacteria, play a vital role in the release and cycling of immobilized phosphorous. They convert insoluble phosphate compounds into available forms for plants *via* the process of acidification, chelation, exchange reaction and by the secretion of organic acids [54]. The bacterial genera perform this property include *Azospirillum, Beijerinckia, Azotobacter, Bacillus, Burkholderia, Erwinia, Microbacterium, Pseudomonas, Enterobacter, Rhizobium, Flavobacterium* and *Serratia* [55, 56]. The distribution of this property in *Serratia* also make it as a favorable partner to the plant. Microbial synthesis of the phytohormone auxin (indole-3-acetic acid) is another important feature of plant growth promotion this was further supported with a recent study on the inoculation of IAA-producing *Serratia marcescens* into soybean. This greatly improved physiological parameters such as shoot length, root length, fresh weight, dry weight and chlorophyll content along with a significant increase in the endogenous abscisic acid production and the total gibberellin content [57].

Ethylene is an important metabolite endogenously produced by all plants for the normal growth and development. It is also a stress hormone because it is associated with stress conditions like salinity, drought, water logging, heavy metals and pathogenicity. However, the presence of increased level of ethylene negatively affects the overall plant growth and development that may lead to reduced crop yield.

The enzyme ACC deaminase of microbial origin has the potential to uptake ACC secreted by the plant root and convert it into α-ketobutyrate and ammonia. This results in the decrease of ACC levels and thereby decreasing ethylene levels to minimise plant stress. Inoculation of plants with ACC deaminase producing bacteria can protect plants against stress conditions. Generally the bacterial strains exhibiting ACC deaminase activity have been identified to belong to the genera such as *Acinetobacter*, *Achromobacter*, *Agrobacterium*,*Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia* and *Rhizobium*. The presence of this PGP in *Serratia* is also indicative of its multi-beneficial adaptations to support plant growth.

Application of biocontrol agents with plant growth promoting traits is an excellent approach for the enhanced production of high quality foods [58]. Very interestingly, *Serratia* spp. which have been reported to have broad antifungal properties are also found to secrete various phytohormones especially Indole 3-acetic acid. Indole-3-acetic acid (IAA) is a phytohormone, which is known to be involved in root initiation, plant cell division, extension, and differentiation. IAA also affects plant growth development by stimulating seed and tuber germination, increasing the rate of xylem and root development; controlling the processes of vegetative growth, initiating lateral and adventitious root formation, mediating responses to light, gravity and fluorescence, affecting photosynthesis, pigment formation, biosynthesis of various metabolites, and providing resistance to stressful conditions. Moreover, IAA producing bacteria promote shoot and root elongation which provide host plant to a greater access to nutrient absorption in soil [59]. One of the main reasons for the use of *Serratia plymuthica* HRO-C48 as biocontrol agent is due to their ability for the production of IAA [15, 31, 60]. Similar studies on plant growth promoting properties and biocontrol mechanisms of *Serratia marcescens* KiSII isolated from the rhizosphere of coconut palms were found to have the ability for phosphate solubilization, indole acetic acid production and 1 aminocyclopropane-1-carboxylate-deaminase activity. At the same time, production of chitinase, β-1, 3 glucanase and siderophores were identified as the basis of their biocontrol efficiencies [61]. Singh and Jha [62] have studied *Serratia marcescens* CDP-13 from *Capparis deciduas* plant, which was also found to be positive for ACC deaminase activity, phosphate solubilization, production of siderophore, indole acetic acid production, nitrogen fixation, and ammonia production.

CONCLUSION

Biological control measures have been known to have prime importance in the recent years as it replace or minimise the use of chemical fertilizer in agricultural system. Biological methods are safer, environment friendly and cost effective method for the enhanced production of crops. Remarkably, *Serratia* spp. with both biocontrol and plant growth promoting properties have promises to novel trends in plant science to utilize plant associated bacteria to enhance plant growth and disease resistance. Thus, development of *Serratia* based formulations may expect to have a promising field applications.

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