Colonization and plant growth promotion of Sorghum seedlings by endorhizospheric *Serratia* sp.

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ABSTRACT

Plant Growth Promoting Rhizobacteria (PGPR) are a heterogenous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly or indirectly. In last few decades, a large array of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus and Serratia* have been reported to enhance plant growth. Growth promotion by PGPR can result from one or more mechanisms including, biological control through competition, production of siderophores or antibiotics, induced disease resistance, direct growth promotion through phytohormone production, increased nutrient availability through nitrogen fixation or organic and inorganic phosphate (P)-solubilization and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production. In addition to these traits plant growth promoting bacterial strains must be rhizospheric competent, able to survive and colonize in the rhizospheric soil. PGPR are able to exert a beneficial effect upon plant growth. Beneficial plant–microbe interactions in the rhizosphere are the determinants of plant health and soil fertility. An endophytic bacterial strain isolated from Sunflower endorhizosphere with multiple plant growth promoting attributes has been identified and characterized. Plant growth promoting traits were analyzed by determining growth in nitrogen free medium, mineral phosphate solublization (MPS), indole acetic acid (IAA) and hydrogen cyanide (HCN) production. The ability to colonize roots for a rhizobacteria, is very important trait to be considered a true PGPR. Hence, *Serratia* is a promising plant growth promoting isolate shows multiple PGPR attributes that can significantly influence Sorghum seedling growth. The result of this study provides a strong basis for further development of this strain as a bioinoculants to attain the desired plant growth promotion activity in Sorghum plant.

Keywords: PGPR, IAA, nitrogen fixation, MPS activity, endorhizosphere, biofertilizer, colonization, gfp tagging

INTRODUCTION

The green revolution brought impressive gains in food production but with insufficient concern for sustainability. Dependence on chemical fertilizers for future agricultural growth would mean further loss in soil quality, possibilities of water contamination and unsustainable burden of the fiscal system. Microorganisms are important in agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers as much as possible. In the era of sustainable agricultural production, the interactions in the rhizosphere play a pivotal role in transformation, mobilization, solubilization etc., from a limited nutrient pool in the soil and subsequent uptake of essential plant nutrients by the crop plants to realize full genetic potential of the crop. Although the

presence of bacterial endophytes in plants is variable and occasionally, transient, they are often capable of elicit drastic physiological changes that modulate the growth and development of the plant. Plant microbe interaction can be studied in detail with the help of tracking methods that can facilitate the visualization and localization of microbes in plant tissues.

The present work indicates that several plant growth promoting traits can be present in a single organism and can be used as better inoculants as compared to introduction of consortium or combination of microorganisms each with single trait. P-solublization and IAA production in *Serratia* sp. is in accordance with previous studies showing that members of *Serratia* genus displays P-solublization and IAA production [7,8].The process of root colonization can be applied for beneficial purposes such as biocontrol, biofertilization, bioremediation and phytostimulation . Root colonization is often the limiting step in the use of rhizobacteria as biological control agents [10]. Hence, the plant-microbe interaction study provides promising strategy to improve overall production by the use of isolated PGPR. Multiple plant growth promoting activities among PGPR have been already reported but such as an investigations on indigenous isolates from India are fewerly explored. Sorghum is the fifth most economically important cereal crop in the world after wheat, maize, rice, and barley.

MATERIALS AND METHODS

Isolation and characterization

Collection of bacterial strains from Helianthus annus (Sunflower) roots and enrichment in nitrogen fixing bacterium (NFb) medium

Bacterial isolates were isolated from endorhizosphere of *Helianthus annus* (sunflower). Roots of Sunflower were taken and weighed; 1 gm roots were surface sterilized with chloramine T (1%) for 15 minutes. The roots were then washed with sterile distilled water twice. After washing, the root pieces were crushed in PBS. The suspension was inoculated in semi solid nitrogen fixing bacterium (NFb) medium for enrichment of nitrogen fixing microorganisms. After 3 subsequent culturing of the microorganisms from pellicle region after serial dilutions, were streaked on nutrient agar medium. The plates were incubated at 30°C for 24-48 hrs and the colony growth was observed. Colonies with differentiable morphologies from all the plates were individually streaked for isolation on LA and Kings B medium, Jensens medium, methyl red plates and incubated for 24 hrs. The red coloured colony (putative *Serratia sp.*) was chosen for further study.

Pellicle formation

The ability of the isolate to fix atmospheric nitrogen was studied by pellicle formation in the semisolid nitrogen free media. Results showed that the isolates was able to grow in nitrogen free media successfully and increased the total N in the growth culture. The putative *Serratia* sp. was investigated for the ability to fix nitrogen. A thin sub-surface pellicle was observed within 24 hrs, became denser at 48 hrs and moved closer to the surface. The semisolid medium containing pellicle was vortexes and used to inoculate fresh semisolid medium.

Carbon source utilization

Biochemical characterization of the isolate was carried out on the basis of carbon source utilization ability. Dextrosee, sucrose, mannitol, acetate, lactate, citrate and glycerol were used for identification.

Plant growth promotion activities

Phosphate solubilization

Qualitative estimation of the P-solubilizing activity of the isolate was carried out on Pikovskaya agar. Overnight grown cultures were inoculated on Pikovskay's Medium plates and incubated for 5-7 days. The occurrence of zone of clearance around the colony was considered as positive for phosphate solubilization [12].

Production of IAA

Nitrogen fixing bacterium (Nfb) medium (5ml) supplemented with 100 µg/ml tryptophan was taken in test tubes. The cultures were inoculated into the test tubes and keep in shaker incubator for 48 hrs at 150 rpm, 30°C. Culture grown 48 hrs was then centrifuged and 1ml supernatant was collected. 5 ml Salkowasky reagent (50 ml, 35% of perchloric acid,1 ml 0.5 M FeCl3 solution)was added to each and the absorbance was taken at 530 nm [13]. Protein estimation was done by Bradford method [14].

Production of HCN

The *Serratia* sp. was screened for the production of hydrogen cyanide by method of Bakker and Schinner, 1997[15]. Nutrient broth was amended with 4.4 g glycine/l and *Serratia* strain was streaked on modified agar plate. A whatmann filter paper no 1 soaked in 2%sodium carbonate in 0.5% picric acid solution was placed at the top of the plate were sealed with parafilm and incubated at 360C for 4 days. Development of orange to red colour indicates HCN production.

Catalase production

The *Serratia* strain was grown in a nutrient agar medium for 18-24 hrs at 37°C.The cultures were mixed with appropriate amount of H_2O_2 on a glass slide to observe the evolution of oxygen.

Production of Hydrolytic Enzymes

Pectinase assay: Nutrient agar plates having 0.5% pectin were prepared. The cultures were inoculated and incubated at 30°C for 24 hrs. Then the plates were flooded with 2% cetyltrimethylammonium bromide (CTAB) solution and kept for 30 min with shaking at regular intervals. The CTAB solution was decanted and the culture was washed with 1N NaCl [16]. The zone of clearance formed showed the presence of pectinase activity.

Cellulase assay: Cellulase activity for the isolate was assayed on the indicator plates. For the cellulase assay NFb plates supplemented with 0.2% carboxymethyl cellulose and 0.5% tryptone were spotted with bacterial cells. After incubating for 48 hrs at 30°C the plates were over layer with congo red (1mg ml^{-1}) solution for 30 minutes. Congo red solution was then poured off followed by washing the surface of the plate with 1M NaCl solution [17]. The zone of clearance formed after washing suggested the presence of cellulose activity.

Bacterial colonization, survival and persistence
Genetic tagging of Serratia sp. *with gfp gene*

An *E. coli* strain containing gfp gene (Genei kit) was used for conjugative mobilization of gfp gene to *Serratia* sp. and colonies were selected NA+ Rif+ Amp. The transformed colonies were selected by observing them under UV light and appeared to show fluorescence.

Rhiospheric colonization of Sorghum by Serratia sp. containing gfp gene

Surface sterilized sorghum seeds were germinated on wet cotton and 0.3% agar. After 4 days, the seedlings were transferred into the culture tubes containing Robins and Shive medium supplemented with 0.3% agar(Fig1). The tubes containing seedlings were kept in plant growth chamber with a 14 hrs light/10 hrs dark regimens. Three days after the transfer of the seedlings into the growth medium gfp tagged bacteria were inoculated into the growth medium to a density of 10^5 cells/ml. Roots of 7, 15 and 21 days after inoculation (DAI) were examined for colonization by bacterial cell count after maceration of the root pieces after 7, 15 and 21 DAI. The *gfp* tagged culture of *Serratia* was inoculated in LB and was grown overnight. The absorbance was adjusted to 0.3-0.4 at 600 nm. Seedling was inoculated with tagged bacteria and the same seedlings were taken out and studied in triplicate for colonization by keeping root pieces under UV to see fluorescence. Bacterial presence and persistence was studied by observing colony forming units as well and were recorded by dilution plating in phosphate buffer saline (PBS).Growth promotion of root /shoot and dry weight was also recorded after 7, 15 and 21 DAI.

RESULTS AND DISCUSSION

Attraction towards oxygen (aerotaxis), first demonstrated by Beijerinck [18], seems to exist in a wide variety of bacteria [19]. All bacteria isolated after several cycles of enrichments in semi solid N-free medium suggested the presence of nitrogen fixing ability in the isolate. The pure culture of *Serratia* sp. showed significant pellicle formation indicating aerotaxis movement of bacteria towards microaerobic zone in the NFb semisolid medium. Oxygen concentration plays a central role in the physiology of diazotrophs. It has to be in a range which permits energy generation by respiration, but does not inhibit oxygen sensitive nitrogen fixation process. Energy taxis has a significant ecological role in vertical stratification of microorganisms in microbial mats and water columns. It plays a central role in the behavior of magnetotactic bacteria and also appears to be important in plant microbe interactions. Nevertheless, in contrast to extensive studies of chemotaxis, relatively few reports on aerotaxis have been published since the turn of the century [20,21].

The ability of the isolates to fix atmospheric nitrogen was studied by pellicle formation in the semisolid nitrogen free media. Results showed that *Serratia* sp. was able to grow in nitrogen free media successfully and increased the total N in the growth culture. Cell suspension of bacterial culture was investigated for the occurrence of nitrogen fixation. A thin sub-surface pellicle was observed within 24 hrs, became denser at 48 hrs and moved closer to the surface. The semisolid medium containing pellicle was vortexed and used to inoculate fresh semisolid medium. This process of subculturing was repeated 3 times to ensure exhaustion of any source of fixed N [22].

Microorganisms are critical for the transfer of phosphorous from poorly available forms and are important to maintain phosphorous in readily available pools. The present studies proved that, potential isolate of *Serratia* mediated P-solubilization and thereby enhanced uptake by the plants, which resulted in increased root proliferation. *Serratia* strain was able to solubilize the complex forms of phosphorous to plant available form as evidenced on the basis of zone of hydrolysis formed. Observation of zone of clearance around the colonies showed positive results. Therefore, the ability of rhizobacteria to solublize precipitated phosphates and enhance phosphate ability to the plant represents a possible mechanism of plant growth promotion under field condition. Therefore, the phosphate solublization activity of *Serratia* isolate was examined by detecting extracellular

solublization of precipitated tricalcium phosphate with glucose as carbon source. Gluconic acid mediated solublization of calcium phosphate has been shown in *Erwinia herbicola* and *Burholderia cepacia* . P-Solubilization activity in *Serratia* sp. was in accordance with previous studies showing that members of the *Serratia* genus display high P-solubilization activities [23-25].

IAA production was estimated according to the Hartmann *et al.,* (1983) [13]. Among different isolates *Serratia* sp. strain produced maximum IAA after 48 hrs of incubation. It produced 0.52 µg IAA/ug protein. Cynogenesis from glycine results in the production of HCN, which is volatile in nature. Reaction of HCN with picric acid in the presence of Na_2CO_3, results in the colour change of the filter paper from deep yellow to orange and finally to orange brown to dark brown. Production of HCN and catalase activity was detected in *Serratia* strain and confers advantage to strain. Gene tagging with gfp gene was carried out with *Serratia* to observe its presence on the root and inside the roots. The presence was confirmed by flourscence shown by transformed bacterial cells having *gfp* gene.

In this study the *Serratia* strain was checked for the production of plant polymer hydrolyzing enzymes pectinases and cellulases. The *Serratia* strain was seen to produce pectinases and cellulases which were visible by formation of zone of clearance . Although pectinases might play an important role in plant microbe interactions, intercellular colonization of roots, their role in endodophytic colonization has not yet been studied well [27]. Besides gaining entry inside the plant through natural openings and wounds, endophytic bacteria actively penetrate plant tissues using hydrolytic enzymes like cellulases and pectinases.

Criteria to recognize true endophytic bacteria have been published and this requires not only the isolation from surface disinfected tissues but also microscopic evidence to visualize tagged bacteria inside plant tissues. The latter criterion is not always fulfilled. Use of the term putative endophytes has been recommended for those not validated microscopically. Surface sterilized seeds were grown gnotobiotically on 0.3% agar and transferred to culture tubes (fig.1).True endophytes may also be recognized by their capacity to re infect disinfected seedlings [28]. Observation of root after inoculation with gfp tagged bacteria after different interval of time suggested significant fluorescence on the root tip and at the emergence of lateral roots (fig 2). These are the sites for maximum colonization by the bacterium. Examining roots after inoculation with PGPR showed appreciable growth in root and shoot length and dry weight (Table 1) respectively.

The root surface and surrounding rhizosphere are significant carbon sinks [29]. Photosynthetic allocation to this zone can be as high as 40% [30]. Thus, along root surfaces there are various suitable nutrient-rich niches attracting a great diversity of microorganisms including phytopathogens.

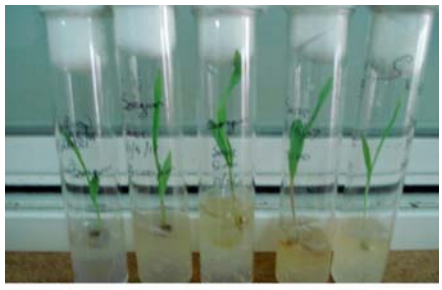

Figure 1. Hydroponic growth of Sorghum seedlings.

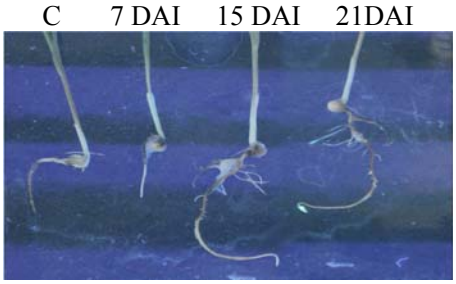

Fig 2. Fluorescence seen against UV in the roots 7, 15 and 21 DAI.

Table1. Showing the promotion of root/ shoot length and dry weight of Sorghum seedlings after inoculation with *Serratia* sp.

	Length of root (mm)			Length of shoot(mm)		
Sorghum seedlings inoculated with PGPR	7DAI	15D AI	21D AI	7DAI	15D AI	21D AI
	6.4	7.4	9.1	8.2	9.2	10.5
	7.4	8.5	10.2	8.2	9.6	11.0
	10.8	11.3	12.1	9.1	10.7	11.5
Average length	8.2	9.1	10.4	8.5	9.8	11.0
Control (Sorghum seedlings without PGPR)	3.5	4.2	5.0	6.0	6.5	7.2
	2.9	3.5	4.1	5.2	5.9	7.1
	3.7	4.2	4.8	5.8	6.3	6.3
Average length	3.43	3.85	4.97	6.0	4.57	6.87
	Root dry weight(mg)			Shoot dry weight(mg)		
Sorghum seedlings inoculated with PGPR	7 DAI	14D AI	21D AI	7DAI	14D AI	21D AI
	0.31	0.45	0.61	1.29	1.71	2.52
	0.29	0.46	0.50	1.30	1.83	2.31
	0.30	0.48	0.72	1.32	1.92	2.41
Average	0.23	0.47	0.61	1.31	1.82	2.3
Sorghum seedlings not inoculated with PGPR	0.1	0.28	0.41	1.21	1.22	1.51
	0.2	0.30	0.43	1.22	1.43	1.60
	0.1	0.29	0.44	1.23	1.54	1.71
Average	0.11	0.29	0.42	1.20	1.40	1.60

A number of different nitrogen fixing and phosphate solublizing bacteria may be considered to be PGPR like Azoto*bacter, Azospirillum, Rhizobium, Arthrobacter, Bacillus, Burkholderia, Enterobacter, Klebsiella, Pseudomonas and Serratia* are reported as PGPR. These can be isolated on their respective media. Plant growth promoting rhizobacteria (PGPR) colonizing the surface or inner part of roots play an important positive role that directly or indirectly influences plant growth

and development. Although *S. marcescens* is generally regarded as a potential human and animal pathogen. Various species of *Serratia* have been isolated from cultures (*Gossypium hirsutui*) and sweet corn (*Zea mays*) as well as rice and rice seeds. Indeed non clinical isolates of *S. marcescens* have been used as agricultural biocontrol agents due to the chitinase activity [31] and ability to induce systemic resistance in plants [32]. Although first report of diazotrophy by *S. marcescens* [33] by transferring *Klebsiella* genes involved in N_2 fixation, *S. marcescens* showed that they were functional. The only other report of diazotrophy in the genus *Serratia* is that of an *S. rubidea* strain isolated from the endorhizosphere of wheat (*Triticum aestivum*) and *Ammophila anenaria* [34].

Phosphorous is second to nitrogen in mineral nutrients most commonly limiting the growth of crops. Phosphorous is an essential element for plant development and growth making up about 0.2% of plant dry weight and forms a major factor for maximum agricultural yield. There are two components of P in soil, organic and inorganic phosphates. A large proportion is present in insoluble forms, and therefore, not available for plant nutrition. Inorganic P occurs in soil, mostly in insoluble mineral complexes. These precipitated forms cannot be absorbed by plants. To convert insoluble phosphates to a form accessible to plants is an important trait for a PGPB for increasing plant yields. Plants acquire P from soil solution as phosphate anions. Phosphate anions are extremely reactive and may be immobilized through precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Al^{3+} depending on particular properties of a soil. In these forms, P is highly insoluble and unavailable to plants .As a result, the amount available to plants is usually a small proportion of this total. MPS ability by phosphate solubilizing microorganisms, caused by lowering of pH of the medium either by H+ extrusion [37]or by the secretion of organic acids and chelating metabolites releases phosphates from insoluble phosphate compounds. Endorhizosphere bacteria also show mineral phosphate solubilization (MPS) activity. Bacterial ability to solubilize mineral phosphate has been of interest to agricultural microbiologists as it can enhance the availability of Pi for microbial and/or plant growth.

Therefore the ability of endorhizobacteria to solubilize precipitated phosphates and enhance phosphate availability to sorghum, represents a possible mechanism of plant growth promotion under field conditions. Detection and estimation of the phosphate solubilization ability of microorganisms have been possible using plate screening methods. Phosphate solubilizers produce clearing zones around the microbial colonies in media. Insoluble mineral phosphate ssuch as tricalcium phosphate or hydroxyapatite are combined in the media. Pikovskay's medium is a general medium for selection of phosphate solubilizer [39,40]. Soil microorganisms are very important in the biogeochemical cycles of both inorganic and organic nutrients in the soil and in the maintenance of soil health and quality [41,42].

The capacity to synthesize IAA is widespread among soil and plant associated bacteria. It is also considered responsible for plant growthpromotion by beneficial bacteria like *Azospirillum, Alcaligens faecalis, Klebsiella, Enterobactercloace, Herbasipillum seropediace,* symbiotic *Rhizobium and Bradyrhizobium* [43]. Plant growth promotion is brought about by causing root elongation and proliferation which leads to enhanced water and mineral uptake by the host plant. Most root promoting bacteria synthesize IAA and this has been clearly demonstrated in many cropping systems. While low levels of IAA stimulate root elongation, high levels of bacterial IAA, whether from IAA over producing mutants or strains that naturally secrete high levels or from high-density inocula, stimulate the formation of lateral and adventitious roots [44,45]. To date approximately 85 bacterial species are known to produce indole. Recently, indole has been identified as signaling molecule in diverse functions [46]. HCN production by rhizospheric bacterium has been invariably viewed while it is considered effective from the biocontrol point of view. Bacterial strains showing catalase activity must be highly resistant to environmental, mechanical and chemical stress.

The use of gfp reporter protein shows great promise for localization of bacterial gene expression inside the host. Putative pathways of entry into roots have been studied in aseptically grown seedlings of rice and Kallar grass inoculated with strain of *Azoarcus* [28]. One site of primary colonization is the root tip at the zone of elongation and differentiation where the bacteria can invade inter and intra cellularly and can penetrate into central tissues that will later differentiate into the stele. Another route of entry appears to be the points of emergence of lateral roots, where bacterial cells have been detected between the cell layers of the lateral root and the cortex of the main root. Similar patterns of invasion have also been described for *A. diazotrophicus* [47] and *Herbaspirillum spp* [48] as well as non–diazotrophic endophytic bacteria in dicotyledons plants. Entry near lateral roots has also been observed for *Azospirillum* sp. suggesting that taxonomically unrelated bacteria might share similar mechanisms of interactions with both monocotyledons and dicotyledons plants [49]. Although other portals of entry into the plant exist e.g. wounds caused by microbial nematode phytopathogens, or the stomata found in leaf tissues [50], root cracks are recognized as the main hot spot for bacterial colonization.

GFP has proved to be a valuable tool for studying a variety of biological questions with living systems. The extremely stable plasmid carries a bright mutant of GFP which allows easy detection. The strength of GFP as a marker lies in the detection of individual cells in a nondestructive manner. The results indicate that endophytic PGP *Serratia* sp inoculation significantly increased the sorghum seedling root and shoot growth. Probably plant growth promoting *Serratia* sp. by production of growth stimulating phytohormones, mobilisation of phosphate, antibiotic production contribute to the overall growth of Sorghum seedlings.

Infection and invasion of roots by bacteria requires degradation of plant cell walls, which might be an active process involving plant polymer degrading enzymes. Cellulase and pectinases are often produced by phytopathogenic bacteria such as *Pseudomonas solanacearum* [51] and *Erwinia chrysanththemi* which hydrolyses the major component of the cell wall i.e. cellulose and pectin, in order to penetrate the plant tissues. The transition from intercellular to the intracellular status of rhizobia inside rice has been suggested to occur via local thinning and solublization of the plant fibrillar wall due to the action of hydrolytic enzymes secreted by bacteria [53]. The cellulase and pectinase activities of the inoculated strain did not affect the growth or health of the seedlings, as there was no noticeable difference between the growth of inoculated and uninoculated seedlings. The fluorescence indicates the presence of bacteria around root tips and at the lateral root emergence. As soon as the cells are inside the plant, competent endophytes respond to plant cues to enable further induction of cellular processes necessary for entering the endophytic life stage and spreading to other tissues of the root cortex and beyond. At this point competent endophytes can quickly multiply inside the plant, often reaching high cell no. e.g 10^8 cells g^{-1} dry weight root tissues [54].

The middle lamellae which connects plant cells with each other, consists mainly of pectin, whereas primary or secondary (in mature cells) cell walls contain ~30% or 70% cellulose, respectively. Intercellular spreading of bacteria may thus be mediated by pectic enzymes breaking down the middle lamellae, as proposed for*Azosprillum* or phytopathgens, however this has not been studied in detail in diazotrophic endophytes. For intercellular colonization, the barrier of the 10 or secondary wall has to be overcome, suggesting the involvement of cellulolytic enzymes. Their enzymatic digestion might facilitate vertical spreading of endophytes *Azoarcus sp. BH72* expresses two cellulolytic enzymes. One of which is an endogluconase that cleaves internal β-1,4 glycosidic bonds of cellulose fibres. As these bacteria do not use these enzymes for metabolizing cellulose or cellobiose, then their role might be to assist in invasion of the plant.

In the present study the gfp gene tagged *Serratia* strain showed colonization of the *Sorghum* seedling roots. Initial colonization is restricted to the root cap region while later the presence of bacteria can be seen on lateral root hair region and rhizoplane region of the seedling . After 21 days

of inoculated seedling showed almost stable colonization pattern. The reason could be that after initial colonization the bacteria having moderate level of plant polymer hydrolyzing activity might move inside the roots and from their they might get distributed to other parts of the plant through xylem. The colony forming units increase for 21 DAI and reaches up to 10^7cfu/ml . This shows their persistence and survival inside the plant tissue. The *Serratia* strain isolated has been shown to form clear zone of hydrolysis suggesting presence of hydrolytic enzymes e.g. pectinases and cellulases. However it would be interesting to determine the presence of other hydrolytic enzymes such as chitinase, lipase and ligninase.

CONCLUSION

Plant growth promoting bacteria (PGPB) are soil and rhizosphere bacteria that can benefit plant growth by different mechanisms [2]. The negative impact of chemical fertilizers and their increasing costs, is detrimental for agricultural practices. Therefore, the use of PGPB as natural fertilizers is advantageous for the development of sustainable agriculture. Endophytic *Serratia* sp. strain isolated from sunflower was characterized for plant growth promoting activities. The endophytic *Serratia* sp. showed significant IAA production, MPS, HCN and plant polymer hydrolysing i.e. cellulase and pectinase activity.

The results presented here show that *Serratia* sp. could become endophytically established in roots and could also increase the root/shoot length and dry weights of the inoculated seedlings. Although endophytes have been confirmed and localized in various plants and are postulated to play an important role in sustainable crop production, the mechanism of the endophytic establishment are not well known. It has been suggested that the bacteria could enter through the fissures created by the emergence of lateral roots or could actively dissolve the cell wall components to gain entry. Inoculation with rhizobacteria could be efficiently used to improve root, shoot and biomass of sorghum. New findings concerning the role of rhizospheric microbes in plant diversity and carbon sinks have stimulated ecologists to further our understanding of plant microbe interactions. Worldwide there is a profound need to explore varied agro-ecological niches for the presence of native beneficial microorganisms. Many studies have been undertaken to understand the nature and properties of these unique microbes which harbor potential plant growth promoting traits with increasing awareness about the chemical fertilizers based agricultural practices [55].

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