Res. Jr. of Agril. Sci. **11**(3): 605-612, May-June 2020

ISSN: 0976-1675

www.rjas.org

Research Journal of Agricultural Sciences

© Centre for Advanced Research in Agricultural Sciences

Research Paper

DI: 5948-0103-115

Serratia marcescens: A Plant Growth Promoting Potent Bacterial Isolate for Eco-friendly and Sustainable Development of Agriculture

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Received: 05 February 2020; Revised accepted: 01 May 2020

A B S T R A C T

The chemical fertilizers and pesticides are most commonly used today in agricultural sector to increase the agricultural yield. The inappropriate use of these agro-chemicals has caused serious damage to environment including human. The fertilizers of biological origin are considered as ecofriendly and their use is gaining importance in sustainable development of agriculture. The digested slurry from biomethanation plant is reported to have manural value and is currently used to increase agricultural yield. The aim of present study was to isolate and identify plant growth promoting bacteria from digester effluent of vegetable waste based biomethanation plant. Seventeen bacteria were isolated from digester effluent were screened for determining their ability to produce plant growth promoting traits. The phosphate solubilizing and IAA producing bacterial isolate was identified as *Serratia marcescens* and was selected further to determine its effect on the growth of crop plants by pot assay. The results showed significant increase in root and shoot development of inoculated crop plants as compared to uninoculated control. The statistical analysis of results revealed significant effects. The study suggests *Serratia marcescens* isolate can serve as candidate for production of efficient bio-inoculants that can serve as substitute to chemical fertilizers & pesticides to prevent environmental pollution.

Key words: Digester effluent, Plant growth promotion, Phosphate solubilization, Indole acetic acid, Siderophores

India is an agricultural country. About 44% million hectare of total land area of our country's surface is cultivable. Because of green revolution farmer was able to get increased agricultural yield in expense of chemical fertilizers and pesticides. The excessive and inappropriate use of these agro-chemicals in recent years has caused negative effects on the environment, nation economy and human health (Bassil *et al*. 2007). The use of biological fertilizers and pesticides are of great importance in this concern. Biofertilizers are the microbial preparations that benefit host plants. Among soil microorganisms, Plant growth promoting rhizobacteria (PGPR) are bacteria producing important plant growth promoting traits (Pattern *et al*. 2002). Plant growth promoting traits includes phosphate solubilization, nitrogen fixation, production of IAA, Siderophore, ammonia catalase, hydrogen cyanide and lytic enzymes, etc. The phosphate solubilizing biofertilizers are environmentally friendly and sustainable (Khan *et al*. 2007). Phosphate solubilizing bacteria (PSB) has been isolated from different environmental sites and their effects on the growth and crop yield has been reported by previous researchers (Jain *et al*. 2010, Jain *et al*. 2012, Hariprasad *et al*. 2009). Indole-3 acetic acid (IAA) is a plant growth hormone produced by plants and bacteria (Zhao 2010). Several bacterial and fungal species are known to be produce IAA(Swain *et al*. 2007). There are several reports on isolation of IAA producing PGPR from rhizosphere and soil (Harikrishnan *et al*. 2014, Pant and Agrawal 2014). Siderophores produced by bacteria create iron limiting conditions and make it unavailable to phytopathogens (Khalid *et al*. 2015). There are several reports on isolation of siderophore producing bacteria from soil (Kannahi and Senbagam 2014, Angel Jenifer *et al*. 2013).

Biomethanation is a widely used microbial technology for waste management. The process converts organic portion of waste into biogas and digester effluent (Owamah *et al*. 2014). The use of digester effluent as a manure is found to

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increase crop yield (Hassan and Abdulsalam 2017). There are very few reports for isolation of plant growth promoting bacteria from digester effluent and studies on their stimulatory effect on the growth of crops and hence the present work was undertaken.

MATERIALS AND METHODS

Biomethanation of vegetable waste

Biomethanation study was performed in 5 litre capacity locally fabricated digesters. The digesters were operated with feeding of the vegetable waste slurry (consisting of equal mixture of potato, onion, tomato, brinjal, cauliflower and cabbage wastes) at organic loading rate 0.320 g volatile solids /l.d, and pH was 7.0 under ambient temperature conditions with two cycles of 20 days hydraulic retention time.

Isolation of bacteria from digester effluent

Bacteria were isolated from digester effluent by spread plate technique using Nutrient agar. The bacterial isolates obtained were preserved at 4ºC for further studies.

Screening of bacteria for phosphate solubilization

Qualitative measurement of phosphate solubilization: The phosphate solubilizing potential of bacterial isolates was detected by spot inoculating the loopful of pure cultures on Pikovaskaya's medium (Pikovskaya 1948). These plates were incubated at room temperature for 48 hours and the zones of clearing around the growth were detected. The hydrolysis capacity (HC) was determined as per Hendricks *et al*. (1995).

Quantitative measurement of phosphate solubilization

The selected bacterial isolate grown in 50 ml aliquots of Pikovskaya's broth 28 ± 2°C for 10 days. The amount of released phosphorus in culture broth was estimated by colorimetric method by referring to standard graph of potassium dihydrogen orthophosphate (Rao 1993).

Effect of inoculation of potent PSB isolates on plant growth by pot assay

The healthy plant seeds of maize (*Zea mays* L.), jowar (*Sorghum bicolor*) and wheat (*Triticum aestivum* L.) were surface sterilized by immersing in 95% ethanol for 30 seconds and 0.2% mercury chloride for 3 min. followed by washing 5 times with sterile distilled water. The selected bacterial isolate was grown in nutrient broth on rotary shaker (150 rpm) for 2 days at room temperature. 0.5 ml of culture (OD at 600nm=0.9) was applied on seed surface for seed coating and further dried. Five seeds were sown in each pot used per pot at equal distance and experiment was performed in triplicates for each isolates. The uncoated seeds were used as control. The sterile soil was added with 0.160 g Tri-calcium phosphate (TCP) per kg soil to every pot. The pots were irrigated with sterile plant nutritive solution every day and kept in sunlight. The C-1 labelled pot contained only soil, C-2 contained soil + TCP, B-3 contained soil + TCP + B-3 isolate. After 2 weeks, plants were uprooted and seedlings were measured for shoot and root length. They were separated into root and shoot and their fresh weight was recorded using an electrical digital balance. The fresh plant materials were kept in a hot air oven at 80°C for 24 hrs and then their dry weight were also determined. Statistical analysis of data was carried out to determine the significant effect of bacterial isolate on plant growth as compared to two controls as C-1 and C-2.

Detection of IAA production ability of selected PSB isolate

Qualitative measurement of IAA production: The PSB isolate was inoculated into 20 mL of nutrient broth tubes supplemented with 0.2% (w/v) of L-tryptophan and incubated for 10 days at 28°C. The culture was centrifuged at 3,000 rpm for 20 min. The one mL supernatant was mixed with 2 mL of Salkowski reagent and tubes were incubated in dark for 30 min. The development of the red color was observed as the indication for positive result (Rahman *et al*. 2010, Gordon and Weber 1951). Uninoculated growth medium was used as negative control. The confirmation of IAA was done by extracting aliquots of centrifuged culture broth with ethyl acetate in 1:2 proportions. The organic phase was subjected to dryness by concentration and then diluted with 0.5 ml methanol. This sample along with the standard IAA was applied on silica gel plate and TLC was run by using a solvent system propanol: water (8:2) proportion and developed by using Salkowaski reagent (Chung *et al*. 2003).

Quantitative measurement of IAA production: The selected bacterial isolate was inoculated into 200 mL of nutrient broth supplemented with 0.2% (w/v) of L-tryptophan and incubated for 10 days at 28°C. The cell free extract was collected by centrifugation at 3,000 rpm for 20 min. The one mL supernatant was mixed with 2 mL of Salkowski reagent and tubes were incubated in dark for 30 minutes. The concentration of IAA in the sample was determined by using spectrophotometer using a standard curve of IAA (Rahman *et al*. 2010, Gordon and Weber 1951).

Effect of inoculation of potent IAA isolate on plant growth by pot assay

The healthy plant seeds of maize (*Zea mays* L.), jowar (*Sorghum bicolor*) and wheat (*Triticum aestivum* L.) were surface sterilized and coated with the suspension of selected bacterial culture in similar way as explained earlier in effect of PSB on plant growth. The pot experiment was run similarly as explained earlier. The pots were added with 0.1 g of Tryptophan per kg soil and irrigated with sterile plant nutritive solution daily and were placed in sunlight. The C labelled pot was control and contained only soil, B-3 contained soil + B-3 isolate. After 2 weeks, uprooted seedlings were processed similarly and length, fresh mass and dry mass of shoots and roots were recorded using an electrical digital balance. Statistical analysis of data was carried out to determine the significant effect of bacterial isolate on plant growth as compared to uninoculated control.

Screening of potent bacterial isolate for other PGPR traits

The ability of selected bacterial isolate to produce siderophore, ammonia, catalase and hydrogen cyanide was detected by referring to standard methods (Sujatha and Ammani 2013, Cappuccino and Sherman 2010, Alstrom and Burns 1989).

Identification of bacterial isolate

The identification of the selected bacterial isolate upto species level was carried out by morphological, cultural and biochemical characterization as per standard literature (Brenner *et al*. 2005).

RESULTS AND DISCUSSION

Biomethanation of vegetable waste

The biogas yield for mixture of six vegetable wastes at ambient temperature conditions was found to be 510-1340 (mL/d). The average yield was 0.633 L biogas /g VS.d and methane % was found to be 59%.

Isolation of bacteria from digester effluent

The seventeen distinct bacterial isolates obtained were maintained in refrigeration conditions.

Determination of phosphate solubilisation ability of bacterial isolate

The seventeen bacterial isolates obtained from digester effluent were tested for determining their phosphate solubilizing potential. The B-3 isolate showed quantitative phosphate solubilisation in liquid media as 4.60 mg/l.

Effect of inoculation of PSB isolates on plant growth by pot assay

The growth response exhibited by crop plants to the selected PSB isolate B-3 in presence of insoluble phosphorus is represented in (Table 1, Fig 1-3).

Table 1 Effect of selected PSB bacterial isolate B-3 on the growth of crop plants

Treatment	Maize (*Zea mays* L.)		Jowar (*Sorghum bicolor*)		Wheat (*Triticum aestivum* L.)	
	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)
C_1	33.8±2.4587	28.98±2.0290	15.3±1.7847	11.66±1.9243	24.66±3.3193	13.76±2.7346
C_2	33.28±2.8323	28.56±2.6025	14.58±3.2935	15±2.3505	24.14±1.0286	22.62±1.4412
B-3	38.02±2.132	31.88± 1.6453	15.84±1.3759	12.96±1.5340	24.9±3.1193	17.7±1.8855
P value1	0.0014	0.0009	0.0705	0.0483	0.8820	0.0041
P value2	0.0025	0.0053	0.3165	0.0122	0.5818	0.0001

The selected bacterial isolate B-3 (identified as *Serratia marcescens*) showed solubilization of inorganic phosphorus on solid and in liquid media. In case of maize plants, as compared to Control-1 (C_1), *Serratia marcescens* stimulated the shoot length, shoot fresh weight and shoot dry weight by 11.10, 12.59 and 5.47% respectively whereas stimulated root length, root fresh weight and root dry weight by 9.10, 13.04 and 7.39% respectively. As compared to Control-2 (C_2), *Serratia marcescens* stimulated the shoot length, shoot fresh weight and shoot dry weight by 12.47, 15.28 and 9.25% respectively whereas stimulated root length, root fresh weight and root dry weight by 10.41, 17.39 and 9.90% respectively. In case of Jowar plants, as compared to C_1, *Serratia marcescens* stimulated the shoot length, shoot fresh weight and shoot dry weight by 0.97, 10.39 and 14.04% respectively whereas stimulated only root length, root fresh weight and dry weight by 10.03, 34.40 and 2.70% respectively. As compared to C_2, *Serratia marcescens* stimulated the shoot length, shoot fresh weight and shoot dry weight by 7.96, 11.56 and 17.54% respectively whereas stimulated root fresh weight and root dry weight by 66.06 and 27.03% respectively. The notable effect on root length was not observed. In case of wheat plants, as compared to C_1, *Serratia marcescens* stimulated the shoot length and shoot fresh weight by 0.96 and 0.44% respectively whereas stimulated only root length by 22.26%. As compared to C_2, *Serratia marcescens* stimulated the shoot length and shoot fresh weight by 3.05 and 1.33% respectively whereas stimulatory effect was not observed on root length, root fresh weight and root dry weight. The statistical analysis of the results by P test revealed that this isolate stimulate the growth of crop plants significantly as compared to controls. *Serratia marcescens* is also previously reported to be phosphate solubilizing and plant growth promoting bacterium (Wahyudi *et al*. 2011, Lwin *et al*. 2012, Malik and Sindhu 2011). Chakraborty *et al*. (2010) reported that phosphate solubilizing *Serratia marcescens* promote growth in tea seedlings and also reduced brown root rot of tea caused by *Fomes lamaoensis*.

Detection of IAA production ability of selected PSB isolate

The bacterial isolate produced IAA in broth medium. The confirmation of IAA was done by comparing extracted sample with standard IAA on TLC. The pink colored spots were observed on TLC plate at the Rf value 0.9 similar to the standard IAA.

Quantitative measurement of IAA

The amount of IAA produced by B-3 in Tryptophan supplemented nutrient broth was found to be 15 mg/L.

Effect of inoculation of IAA producing bacteria on plant growth

The growth response exhibited by crop plants to the selected IAA producing bacterial isolate is represented in (Table 2, Fig 4-6).

Table 2 Effect of IAA producing bacterial isolate B-3 on the growth of crop plants

Treatment	Maize (*Zea mays* L.)		Jowar (*Sorghum bicolor*)		Wheat (*Triticum aestivum* L.)	
	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)
Control	31.45±2.5252	20.625±1.8875	13.5667±2.2368	8.6±2	24.55±0.9469	15.325±2.4595
B-3	40.375±1.9602	30.975±1.9602	18.5667±2.5813	18.33±2.0502	25.375±1.5586	13.775±2.4998
P value	0.0258	0.00687	0.1881	0.0041	0.3623	0.3057


Fig 1 Effect of PSB activity of B-3 on the growth of maize (from left-C1, C2 and B-3) (left) and effect on the shoot and root development in Maize (from left-C1, C2 and B-3) (right)


Fig 2 Effect of PSB activity of B-3 on the growth of jowar (from left-C1, C2 and B-3) (left) and effect on the shoot and root development in Jowar (from left-C1, C2 and B-3) (right)


Fig 3 Effect of PSB activity of B-3 on the growth of wheat (from left-C1, C2 and B-3)(left) and effect on the shoot and root development in Wheat (from left-C1, C2 and B-3) (right)


Fig 4 Effect of IAA producing bacterial isolate B-3 on the growth of maize (left) and effect on the shoot and root development in maize (right)


Fig 5 Effect of IAA producing bacterial isolate B-3 on the growth of jowar (left) and effect on the shoot and root development in jowar (right)


Fig 6 Effect of IAA producing bacterial isolate B-3 on the growth of wheat (left) and effect on the shoot and root development in wheat (right)

The selected Indole-3 acetic acid (IAA) producing bacterial isolate was further tested to determine its plant growth promotion ability by pot assay method. The root and shoot development was significantly increased in tested crop plants as compared to control. In case of maize plants, as compared to control, *Serratia marcescens* stimulated the shoot length, shoot fresh weight and shoot dry weight by 22.1, 29.76 and 18.35% respectively whereas stimulated root length, root fresh weight and root dry weight by 33.41, 15.08 and 28.12% respectively. In case of Jowar plants, as compared to control, *Serratia marcescens* stimulated the shoot length, shoot fresh weight and shoot dry weight by 26.93, 21.26 and 22.22% respectively whereas stimulated root length, fresh weight and dry weight by 53.08, 51.16 and 16.67% respectively. In case of Wheat plants, as compared to control, *Serratia marcescens* stimulated the shoot length, shoot fresh weight and shoot dry weight by 3.25, 6.91 and 2.88% respectively whereas no stimulatory effect was seen on root development. Statistical analysis of the results by P test revealed that these isolates stimulate the plant growth significantly than control. Indole-3 acetic acid (IAA) producing bacteria have been reported to have stimulatory effect on plant growth (Montaner and Perez-Tomas 2003). Gram negative bacteria are most common IAA producers as compared to Gram positive (Montaner and Perez-Tomas 2003). The selected phosphate solubilizing bacterial isolate namely *Serratia marcescens* produced quantity of IAA as 15 mg/L. IAA production by rhizobacteria was reported by Mehta and Shah (2015) in the range 15-97.2 mg/L. Shahitha and Poornima (2012) reported the amount of IAA produced varied from 10.2 to 31.2 mg/L in different *Pseudomonas species*. The present result matches with the previous findings. *Serratia marcescens* is also reported to produce Indole-3 acetic acid (IAA) by previous workers. Su *et al.* (2016) evaluated plant growth promoting and biocontrol efficacy of a *Serratia marcescens* strain isolated from tea rhizosphere for the effective management of root rot disease in tea. Devi *et al.* (2016) characterized and identified endophytic bacterium from *Achyranthes aspera* L. as *S. marcescens* and was able to produce siderophores, IAA, ammonia, nitrogen fixing and phosphate solubilizing (Araujo Helvia *et al.* 2019). Pot trial experiments showed significant increase in shoot and root development as compared with uninoculated control. Selvakumar *et al.* (2008) isolated *S. marcescens* strain from the flowers of summer squash plants producing IAA, HCN and siderophore and was promising bioinoculant in cold temperature conditions (Montero-Rodriguez *et al.* 2016).

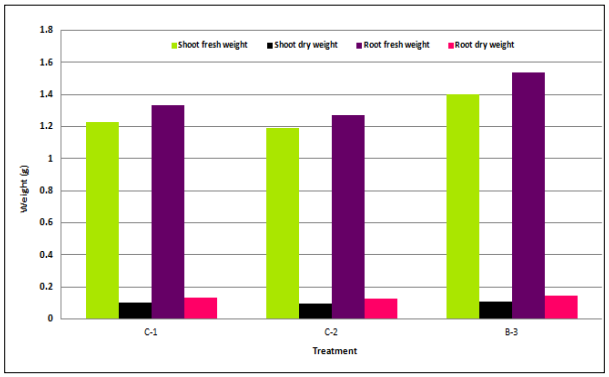

Fig 7 Effect of inoculation of PSB isolate B-3 on shoot and root development in maize

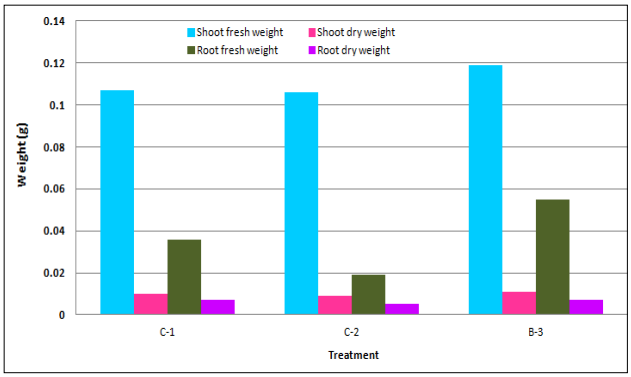

Fig 8 Effect of inoculation of PSB isolate B-3 on shoot and root development in jowar

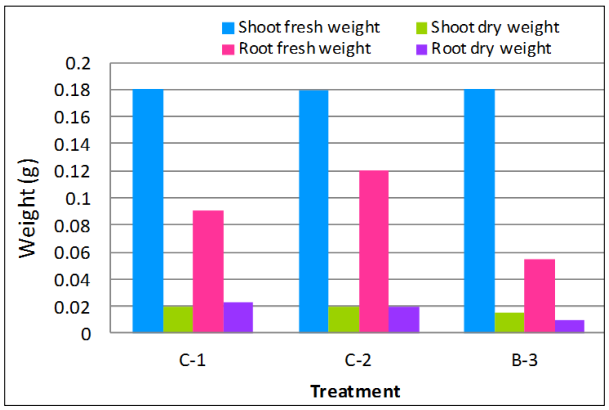

Fig 9 Effect of inoculation of PSB isolate B-3 on shoot and root development in wheat

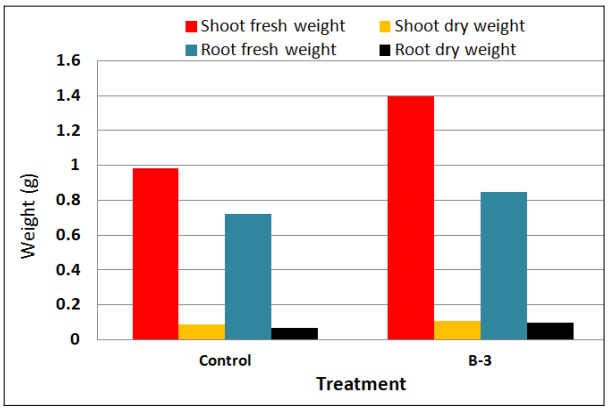

Fig 10 Effect of inoculation of IAA producing bacteria B-3 on shoot and root development in maize

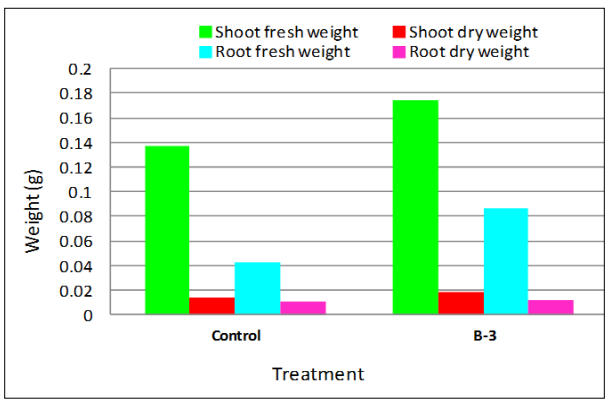

Fig 11 Effect of inoculation of IAA producing bacteria B-3 on shoot and root development in jowar

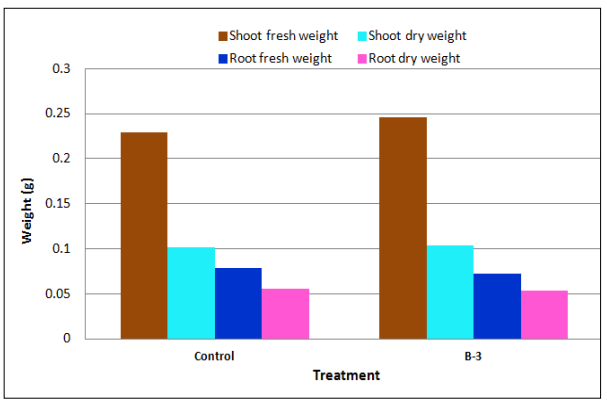

Fig 12 Effect of inoculation of IAA producing bacteria B-3 on shoot and root development in wheat

Screening of selected bacterial isolate for other plant growth promoting traits

The selected PSB and IAA producing isolate B-3 showed production of siderophore, ammonia, HCN and catalase also.

Identification of bacterial isolate

The selected potent bacterial isolate B-3 was identified as *Serratia marcescens* based on its morphological, cultural and biochemical characterization (Fig 13, Table 3).

Table 3 Identification of B-3 isolate

Test	Result
Colony characters on nutrient agar	Colony size 2mm, circular, regular margin, raised, smooth consistency, red colored, transparent, Gram negative, motile and straight short rods
Physiological and biochemical characteristics	
Indole production	-

Methyl red	-
Voges Proskauer	+
Citrate utilization	+
H_2S production	-
Catalase	+
Oxidase	-
Nitrate reduction	+
Casein hydrolysis	-
Starch hydrolysis	+
Gelatin hydrolysis	+
Urea hydrolysis	-
Egg yolk reaction	+
Arginine hydrolysis	-
Lysine decarboxylation	+
Acid from Glucose, Fructose, Sucrose, Mannose, Maltose, Trehlose	+
Acid from Galactose, Lactose, D-Arabinose, D-Xylose	-
Identity of bacterial isolate B-3	*S.marcescens*

(+) = positive test; (-) = Negative test

Fig 13 Growth of bacterial isolate B-3 (*Serratia marcescens*) on Nutrient agar plate at R.T. after 48 hrs incubation

The digester effluent from biomethanation plant is reported to increase the fertility of soil and in turn increases agricultural crop yield (Hassan and Abdulsalam 2017, Alfa *et al*. 2014). This bacterium isolated from digester effluent of vegetable waste based biomethanation plant was found to produce other plant growth promoting traits like siderophores, ammonia, catalase and hydrogen cyanide. *Serratia marcescens* is also previously reported to be siderophore producing (Araujo Helvia *et al*. 2019, Montero-Rodriguez *et al*. 2016). Raghavan *et al*. (2015) reported that *S. marcescens* could be good alternative to chemicals for growth promotion and management of soft rot disease in ginger (*Zingiber officinale* Rosc.). Wang *et al*. (2013) suggested that *S. marcescens* JPP1 strain could potentially be utilized for the biological control of phytopathogenic fungi and aflatoxin in Chinese peanut (Li *et al*. 2013). *Serratia marcescens* is also reported to produce chitinase that inhibits several fungal phytopathogens (Oteino *et al*. 2015).

Serratia marcescens, a potent plant growth promoting bacterium isolated from the digester effluent of vegetable waste based biomethanation plant is ubiquitous in nature and is reported to be opportunistic pathogen from Enterobacteriaceae family, commonly involved in nosocomial infections (Bhattacharyya and Jha 2012). Besides pathogenecity of *S. marcescens* was reported to have greater industrial applications. It produces red pigment called Prodigiosin, a secondary metabolite that shows antibacterial, antifungal, anti-malarial, antioxidant, anti-proliferative, immunosuppressive and anticancer properties (Gururani *et al*. 2013). The pigment can be used as colourant in candle, dye in textile and other various industries (Chakraborty *et al*. 2010, Dhar *et al*. 2018). *Serratia marcescens* produces biosurfactant namely Serrawetins that has wide applications in pharmaceutical industry (Devi *et al*. 2016). This bacterium is reported to produce several hydrolytic enzymes and plant growth promoting metabolites and hence finds application in agriculture and environmental processes (Selvakumar *et al*. 2018). Montero-Rodriguez *et al*. (2016) demonstrated the potential of *S. marcescens* to produce biodiesel as it possesses the ability to accumulate lipids using agro-industrial wastes (Raghavan *et al*. 2015). *Serratia marcescens* was reported to degrade several types of pesticides and has potential application in bioremediation of pesticide-contaminated environments (Wang *et al*. 2013, Frankowski *et al*. 2001).

Excess and inappropriate use of chemical fertilizers and pesticides from a period of green revolution has caused serious damage to agriculture and environment. In the present study, *Serratia marcescens* isolated from the digester effluent of vegetable waste based biomethanation plant was found to have ability to solubilize inorganic phosphorus, to produce IAA, siderophores, ammonia, catalase and hydrogen cyanide. The effect of phosphate solubilizing activity and IAA producing ability of *Serratia marcescens* was tested on crop plants by separate pot trial experiments and found that this isolate exerted positive effect on the shoot and root development of crop plants. The study suggests that multiple plant growth promoting traits producing *Serratia marcescens* isolate can serve as suitable candidate for development of efficient bio-inoculants in ecofriendly and sustainable development of agriculture and will play important role in prevention of environmental pollution by avoiding excessive applications of or substitute to chemical fertilizers and pesticides.

Acknowledgement

The author is thankful to Principal, Lal Bahadur Shastri College of Arts, Science and Commerce, Satara for providing laboratory facilities for this work.

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