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## Plant Growth Promotion and Biocontrol Potential of *Serratia marcescens* Mediated by IAA, ACC Deaminase, and Chitinase Activities with Emphasis on Antifungal Effects and Seed Studies of Root Crops

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### ABSTRACT

The rhizomicrobiome plays a critical role in agriculture by shaping diverse microbial communities through root exudates and plant-derived organic matter. These microorganisms contribute to nutrient mobilization and uptake, enhance soil structure, and produce a wide range of extracellular compounds, including phytohormones, secondary metabolites, antibiotics, and signalling molecules, which collectively promote plant growth. The microbes and their metabolites act as natural biostimulants and are central to regulating plant stress responses. Studies have shown that the application of plant growth-promoting rhizobacteria (PGPR) or microbial signal compounds is an effective and sustainable approach to enhancing crop productivity. Plant growth-promoting rhizobacteria (PGPR) play a significant role in sustainable agriculture by improving nutrient uptake, modulating plant hormones, and protecting crops against pathogens. The present study investigates the growth-promoting and antifungal capabilities of *S. marcescens* and evaluates its influence on seed germination and early seedling vigour of Carrot (*Daucus carota*), and Radish (*Raphanus sativus*). The strain demonstrated significant IAA production, high ACC deaminase activity, and pronounced chitinolytic activity. Seed studies demonstrated considerable enhancement in germination rate, root elongation, shoot length, and vigour index in seeds treated with the bacterial culture. These findings highlight the potential of *S. marcescens* as an effective bioinoculant for improving root crop establishment and mitigating soil-borne pathogens, offering an ecologically sustainable alternative to chemical inputs.

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### 1. INTRODUCTION:

The expanding global population has increased the demand for food production, a challenge intensified by climate change and limited agricultural land. Consequently, modern agriculture faces the critical task of improving crop productivity while reducing dependence on synthetic chemical fertilizers and pesticides. The rising food demands through

sustainable practices has become a major challenge in recent years. Agricultural productivity is significantly influenced by soil microbial communities, particularly plant growth-promoting rhizobacteria (PGPR), which establish beneficial interactions within the rhizosphere to support plant health and productivity (Bhattacharyya & Jha, 2012). Plant growth-promoting rhizobacteria (PGPR) constitute a diverse assemblage of soil bacteria that actively interact within the rhizosphere, forming complex soil-plant relationships that positively regulate plant growth and productivity. PGPR enhances plant performance through multiple mechanisms, including improved nutrient uptake, phytohormone regulation, mitigation of abiotic stress, and suppression of soilborne pathogens (Glick, 2012). Among these mechanisms, the biosynthesis of auxins such as indole-3-acetic acid (IAA), the

degradation of stress-induced ethylene via ACC deaminase, and the secretion of hydrolytic enzymes like chitinase are particularly important for promoting root growth and enhancing plant resilience.

*Serratia marcescens*, a metabolically versatile Gram-negative bacterium, has emerged as a promising PGPR due to its ability to produce a wide array of plant-beneficial compounds, including IAA, ACC deaminase, siderophores, and chitinases (Khiangam *et al.*, 2014). Its IAA production contributes to root elongation, lateral root formation, and enhanced nutrient and water absorption (Patten & Glick, 2002). Additionally, ACC deaminase activity helps lower plant ethylene levels under stress, leading to improved root architecture and increased tolerance to environmental and biological stressors (Glick, 2014).

Chitinase secretion further enables *S. marcescens* to act as a biocontrol agent by degrading the chitin-rich cell walls of pathogenic fungi such as *Fusarium*, *Rhizoctonia*, and *Sclerotium* species (Ordentlich *et al.*, 1988). These hydrolytic activities collectively strengthen plant defense responses, suppress pathogen colonization, and help reduce the need for synthetic chemical fungicides (Ordentlich *et al.*, 1988; Gooday, 1990; Bhattacharyya & Jha, 2012). Due to its combined plant growth-promoting and biocontrol abilities, *Serratia marcescens* holds considerable promise for sustainable agricultural applications (Glick, 2012; Singh *et al.*, 2019).

Root crops such as Carrot (*Daucus carota*) and Radish (*Raphanus sativus*) are economically valuable vegetables cultivated worldwide. However, their production is frequently challenged by poor seedling establishment, slow early growth, and soilborne fungal infections, all of which can negatively impact yield and crop quality (Pandey & Gupta, 2020; Fahsi *et al.*, 2021). Enhancing early root development is particularly crucial in these crops, and PGPR-based seed treatments offer an eco-friendly approach to improve germination, vigor, and resilience during the initial stages of growth (Bhattacharyya & Jha, 2012; Glick, 2014).

## 2. MATERIALS AND METHODS:

### 2.1 Culture preparation:

The strain used in the present study was isolated from rhizosphere soil during an earlier investigation focused on screening of beneficial soil bacteria. The isolation was performed using serial dilution and was performed on yeast extract malt extract agar (YEMA) medium, followed by purification through repeated streaking. The strain

was maintained under laboratory conditions and preserved at -4°C for further study. Morphological characteristics were confirmed through Gram staining and biochemical properties, while molecular identification was performed using the 16S rRNA gene sequencing method.

For the current study, the previously identified strain was retrieved from storage and subcultured on yeast extract malt extract agar (YEMA) medium plates to obtain fresh, active culture.

### 2.2 Quantitative estimation of Indole-3-Acetic Acid (IAA) production

The quantitative estimation of indole-3-acetic acid (IAA) production by the isolate was carried out following the colorimetric method (Hartmann *et al.*, 1983) with minor modifications. The bacterial strain was inoculated into 50 mL of yeast extract malt extract (YEMA) broth supplemented with 0.1% (w/v) L-tryptophan, the precursor for auxin synthesis, and incubated at 30 °C for 48 h under shaking conditions (120 rpm). After incubation, the cultures were centrifuged at 10,000 rpm for 10 min at 4°C to obtain cell-free supernatants. For IAA quantification, 2 mL of the supernatant was mixed with 2 mL of Salkowski reagent (1 mL of 0.5 M FeCl<sub>3</sub> in 50 mL of 35% HClO<sub>4</sub>) and incubated in the dark for 30 min to allow development of a pink chromophore. The absorbance of the reaction mixture was measured at 530 nm using a UV-VIS spectrophotometer. IAA concentration was determined using a standard curve prepared with analytical-grade indole-3-acetic acid (0–100 µg mL<sup>-1</sup>), and results were expressed as µg mL<sup>-1</sup> of culture filtrate.

### 2.3 Phosphate solubilization assay (Pikovskaya Agar)

The phosphate-solubilizing ability of the isolate was evaluated qualitatively using Pikovskaya agar medium containing insoluble tricalcium phosphate as the primary phosphorus source. Actively growing bacterial culture was spot-inoculated onto freshly prepared Pikovskaya agar plates and incubated at 28–30 °C for 3–4 days. Phosphate solubilization was confirmed by the formation of clear halo zones surrounding the colonies, indicating the dissolution of tricalcium phosphate through organic acid production. The phosphate solubilization index (PSI) was calculated using equation 1.

$$\text{PSI/ SPI/ CI} = (\text{colony diameter} + \text{halo diameter}) \div \text{colony diameter}$$
 Eq.1

### 2.4 Siderophore production assay (CAS agar plate method)

Siderophore production of the isolate on Chrome Azurol S (CAS) agar following the method of

Schwyn and Neilands (1987) with minor modifications. The CAS indicator solution was prepared by dissolving 60.5 mg of Chrome Azurol S in 50 mL of distilled water, 27.2 mg of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in 10 mL of 10 mM HCl, and 72.9 mg of hexadecyltrimethylammonium bromide (HDTMA) in 40 mL of distilled water. The CAS and  $\text{FeCl}_3$  solutions were mixed with stirring, and HDTMA was added dropwise until a stable blue complex formed. To prepare CAS agar, an iron-limited basal agar medium was autoclaved, cooled to  $\sim 50$  °C, and supplemented with the CAS indicator solution at a 1:10 (v/v) ratio under sterile conditions. Plates were poured and allowed to solidify. Test cultures were spot-inoculated (5  $\mu\text{L}$  of  $10^8$  CFU  $\text{mL}^{-1}$  suspension) onto CAS agar plates, and incubated at 28–30 °C for 10 days. Siderophore production was indicated by the formation of an orange/yellow halo around colonies due to the removal of iron from the CAS–Fe (III) complex. Halo and colony diameters were measured using a standard scale, and siderophore production was expressed qualitatively and quantitatively as the siderophore production index (SPI) using equation 1.

### 2.5 ACC deaminase activity

ACC deaminase activity was quantified following the method of Penrose and Glick (2003) with minor modifications. Bacterial cells were grown in YEMA broth to late log phase, harvested by centrifugation at 8,000 rpm for 10 min, washed twice with 0.1 M Tris–HCl (pH 7.5), and resuspended in the same buffer to an OD600 of  $\sim 0.6$ . Cells were permeabilized by adding 10% (v/v) toluene and vortexing for 1 min, then equilibrated at room temperature for 15 min. The enzyme assay was initiated by adding 0.5 mL of permeabilized cell suspension to 0.5 mL of 0.1 M Tris–HCl (pH 8.5) containing 10 mM 1-aminocyclopropane-1-carboxylate (ACC) and incubating at 30 °C for 30 min. The reaction was stopped by the addition of 0.5 mL 0.56 M HCl, and the amount of  $\alpha$ -ketobutyrate produced was determined calorimetrically after reaction with 2,4-dinitrophenylhydrazine (DNPH) and subsequent addition of 2 N NaOH; absorbance was read at 540 nm.  $\alpha$ -Ketobutyrate concentrations were calculated from a standard curve generated with authentic  $\alpha$ -ketobutyrate. Protein content of the cell suspension was measured by the Bradford assay, and ACC deaminase activity was expressed as nmol  $\alpha$ -ketobutyrate  $\text{mg}^{-1}$  protein  $\text{h}^{-1}$  using equation 2.

$$\text{ACC deaminase activity} = \frac{\text{nmol } \alpha\text{-ketobutyrate}}{\text{mg protein} \times \text{reaction time (h)}} \quad \text{Eq.2}$$

### 2.6 Chitinolytic activity assay

The chitinase-producing ability of *Serratia*

*marcescens* was evaluated using both qualitative and quantitative methods. For qualitative screening, colloidal chitin agar plates were prepared by suspending 1% (w/v) colloidal chitin in minimal salts agar medium. Bacterial cultures (10  $\mu\text{L}$  of actively growing suspension) were spot-inoculated onto the plates and incubated at 28–30 °C for 5–7 days. Chitinolytic activity was indicated by the formation of clear halo zones around the colonies due to the hydrolysis of colloidal chitin. The chitinolytic index (CI) was calculated using equation 1.

### 2.7 Antifungal Activity

The antifungal potential of the isolate was evaluated using the agar well diffusion method. The fungal cultures were procured from the National Fungal Culture Collection of India, Pune. The organisms include *Fusarium oxysporum* NFCCI 708, *Aspergillus flavus* NFCCI 384, *Pythium* NFCCI 3842, and *Rhizoctonia solani* NFCCI 252. Actively growing fungal cultures were uniformly spread onto sterile potato dextrose agar (PDA) plates using a sterile cotton swab to obtain a confluent lawn. Wells of 6 mm diameter were aseptically punched into the agar using a sterile cork borer. Each plate contained four wells, loaded with standard control as nystatin (100  $\mu\text{g mL}^{-1}$ ), Culture broth containing pigment and bacterial cells, cell-free supernatant obtained after centrifugation of the culture broth, and pigment extract obtained after centrifugation and solvent extraction. For the preparation of cell-free supernatant, the bacterial culture was centrifuged at 10,000 rpm for 10 min, and the clear supernatant was collected. Pigment was extracted from the cell pellet using methanol solvent, concentrated, and re-dissolved to a defined volume. Equal volumes (100  $\mu\text{L}$ ) of each preparation were loaded into the respective wells. The plates were incubated at 28  $\pm$  2 °C for 48–72 h, after which antifungal activity was assessed by measuring the diameter of the zone of inhibition (mm) around each well.

### 2.8 Seed germination studies

Seed germination tests were carried out following the method of Pandey and Gupta (2020) with minor modifications appropriate for root crops. Seeds (carrot and radish) were surface-sterilized by immersion in 70% (v/v) ethanol for 1 min followed by 1% (v/v) sodium hypochlorite (NaOCl) for 2 min, then rinsed five times with sterile distilled water to remove residual disinfectant. Sterilized seeds were air-dried aseptically and then soaked in the bacterial suspension adjusted to OD600 = 1.0 ( $\approx 10^7$  CFU  $\text{mL}^{-1}$ ) for 1 h (seed bacterization). Control seeds were similarly soaked in sterile distilled water. After treatment, seeds were placed (10 seeds per plate) on Whatman No. 1 filter paper

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moistened with sterile distilled water in sterile Petri dishes (three replicates per treatment) and incubated in a growth chamber at  $20 \pm 2$  °C under a 12 h light/12 h dark photoperiod for 7 days.

At the end of the incubation period, germination was recorded based on radicle emergence ( $>1$  mm). Germination percentage was determined using the method outlined by Fahsi *et al.* (2021), calculated as:

$$\text{Germination Percentage (\%)} = \left( \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \right) \times 100$$

Additionally, the effect of bacterial treatment relative to the control was assessed by determining relative seed germination, expressed as:

$$\text{Relative Seed Germination (\%)} = \left( \frac{\text{Germinated seeds in treatment}}{\text{Germinated seeds in control}} \right) \times 100$$

Root and shoot lengths of germinated seedlings were measured on day 7, and the seedling vigor index (SVI) was calculated as

$$\text{SVI} = \text{Germination (\%)} \times (\text{mean root length} + \text{mean shoot length}).$$

## 3. RESULTS:

### 3.1. Culture Preparation

The bacterial strain used in this study was successfully revived from subculturing on yeast extract malt extract agar (YEMA) media plates (**Figure 1**). The culture exhibited uniform, well-defined colonies with consistent morphology, indicating good preservation stability during storage at  $-4$  °C. Microscopic examination (**Figure 2**) reconfirmed the strain's Gram reaction and cellular characteristics, which matched observations recorded during the initial isolation study. Biochemical attributes also remained consistent with earlier findings, suggesting that the strain retained its physiological properties after storage. The molecular identity through the 16S rRNA sequencing method confirms the rhizosphere bacterial isolate as *Serratia marcescens*.



Figure 1: Pure Culture of *Serratia marcescens*.

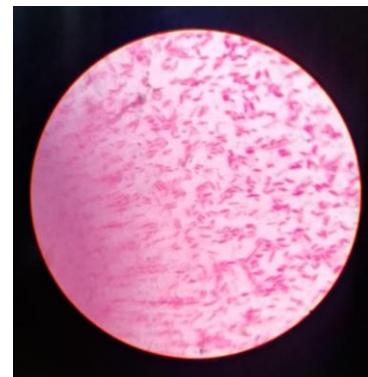


Figure 2: Gram staining showing -ve Rods

### 3.2 Quantitative estimation of Indole-3-Acetic Acid (IAA) production

The rhizosphere bacterial isolate exhibited a strong ability to synthesize indole-3-acetic acid in tryptophan-supplemented medium. Quantitative estimation revealed that the culture produced significantly higher IAA levels compared to the tryptophan-free control. The absorbance values obtained at 530 nm showed a consistent and reproducible increase across biological replicates, demonstrating stable IAA biosynthesis. Peak production was recorded after 48 h, indicating active auxin biosynthesis during the exponential growth phase. The characteristic pink/reddish brown coloration with Salkowski reagent confirmed the presence of IAA (**Figure 3**). These results suggest that the isolate has the potential to influence root elongation and early seedling vigour through phytohormone production. The culture supernatant of the isolate exhibited an absorbance (OD) of 0.76 at 530 nm, corresponding to an estimated IAA concentration of approximately 97.5  $\mu\text{g mL}^{-1}$  (**Figure 4**).



Figure 3: A. Control with Salkowski reagent.

B. Pink/reddish brown coloration with Salkowski reagent.

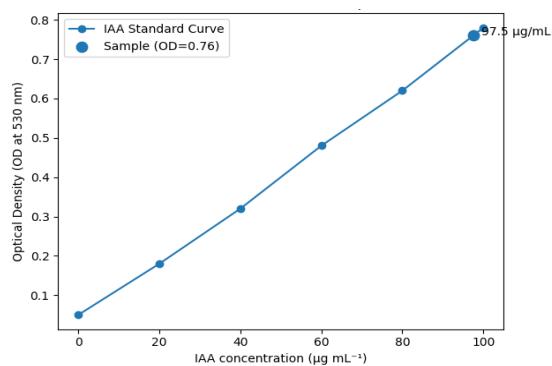


Figure 4: Standard curve for indole-3-acetic acid (IAA) quantification with isolate estimation.

### 3.3 Phosphate Solubilization (Pikovskaya Agar)

The isolate formed a prominent, clear solubilization zone on Pikovskaya's agar, indicating its ability to solubilize inorganic phosphate (Figure 5). The dissolution zones were consistently observed across replicates, indicating secretion of organic acids or phosphatases that mobilize insoluble forms of phosphate. The development of transparent halos around colonies suggests the secretion of organic acids that convert tricalcium phosphate into plant-available forms. This trait is significant for improving nutrient acquisition, especially for root crops that rely heavily on phosphorus during early growth. The colony diameter of the isolate was  $0.26 \pm 0.03$  cm, and the zone diameter was  $1.36 \pm 0.09$  cm. The phosphate solubilization index (PSI) was calculated using equation 1. The phosphate solubilization index (PSI) of  $6.14 \pm 0.21$  indicates strong phosphate solubilization activity.

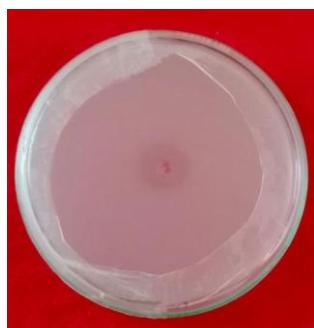


Figure 5: Clear zone formation in the plate around the colony showing phosphate solubilization on Pikovskaya's agar medium.

### 3.4 Siderophore Production (CAS Agar Assay)

The isolated strain produced prominent siderophores as evidenced by distinct orange halos formed around the colonies on chrome azurol S (CAS) agar plates (Figure 6). The conversion of the blue CAS medium into an orange zone indicated strong iron-chelating ability. The halo formation was uniform, well-defined, and significantly larger than the control plates without bacterial inoculation. The colony diameter of the isolate was  $0.53 \pm 0.08$  cm, and the zone diameter

was  $4.56 \pm 0.29$  cm. The siderophore production index (SPI) was calculated using equation 1. The siderophore production index (SPI) is  $9.60 \pm 0.88$ . The intensity and diameter of halo zones confirmed active secretion of siderophores capable of sequestering ferric ions. This trait suggests that the strain can improve iron acquisition in plants while simultaneously inhibiting pathogenic microbes through iron competition.

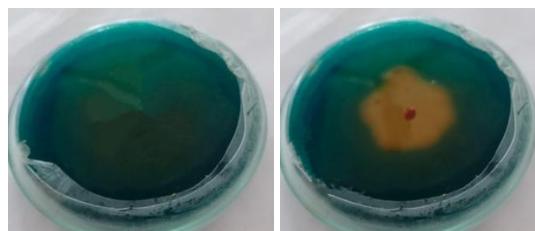


Figure 6: A. Control plate B. Orange halo formation around the colony, showing the siderophore production on CAS medium

### 3.5 ACC Deaminase Activity

Quantitative assessment of ACC deaminase activity revealed that the isolate was capable of efficiently utilizing ACC as a sole nitrogen source. The absorbance values obtained at 540 nm after incubation showed an OD value of 0.43. Bacterial growth in the minimal medium containing ACC was significantly higher compared to the nitrogen-free control, indicating enzymatic breakdown of ACC into  $\alpha$ -ketobutyrate and ammonia. The measurable concentration of  $\alpha$ -ketobutyrate generated during the assay demonstrated a clear, positive ACC deaminase reaction. The bacterial culture produced 82.2 nmol  $\alpha$ -ketobutyrate within 30 min. Based on protein normalization, ACC deaminase activity was calculated as 432.6 nmol  $\alpha$ -ketobutyrate  $\text{mg}^{-1}$  protein  $\text{h}^{-1}$ , indicating strong ethylene-modulating potential. The  $\alpha$ -ketobutyrate concentration was determined using a standard graph of  $\alpha$ -Keto butyrate (0–100 nmol) (Figure 7). This activity reflects the strain's ability to reduce stress-associated ethylene levels in plants, thereby potentially improving seed germination, root elongation, and stress tolerance in host crops.

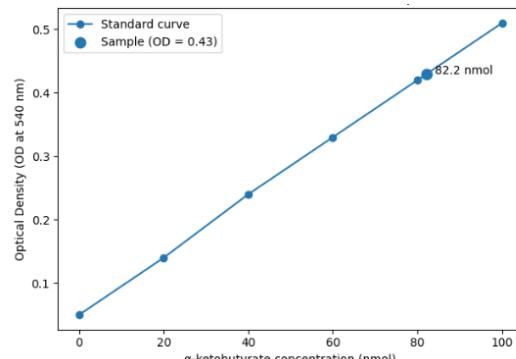


Figure 7: Standard curve for  $\alpha$ -ketobutyrate quantification with isolate estimation.

### 3.6 Chitinolytic Activity

The strain showed strong chitinase activity, evidenced by a clear hydrolytic zone around the colony on colloidal chitin agar plate (**Figure 8**). The formation of these transparent halos indicated enzymatic degradation of chitin, demonstrating the production of extracellular chitinases capable of breaking down fungal cell wall polymers. The consistency of halo formation across replicates suggests robust enzymatic secretion. The colony diameter of the isolate was  $0.23 \pm 0.05$  cm, and the zone diameter was  $1.32 \pm 0.04$  cm. The chitinolytic index (CI) was calculated using equation 1. The chitinolytic index (CI) is  $6.74 \pm 0.65$ . This confirms the strain's potential as an effective biocontrol agent against chitin-containing fungal pathogens commonly affecting root crops.



Figure 8: Clear zone formation in the plate around the colony showing chitinolytic activity on colloidal chitin agar medium.

### 3.7 Anti-fungal activity

The isolate showed distinct antifungal activity among the tested pathogens. The standard antifungal agent, nystatin, produced a clear zone of inhibition, validating the assay conditions. The culture broth containing pigment and bacterial cells exhibited notable antifungal activity, indicating the combined effect of extracellular metabolites and pigment-associated compounds. The crude pigment extract also produced a visible inhibition zone, suggesting that the pigment itself contributes significantly to antifungal activity. These results indicate that antifungal activity is likely mediated by both pigment-associated and extracellular bioactive metabolites, and that the presence of live cells may enhance antagonistic effects through synergistic mechanisms. **Table 1** represents the tested pathogens and the zone of inhibition(mm).

Table 1: Antifungal activity of *Serratia marcescens*

Pathogens	Zone of Inhibition (mm)			
	Standard	Broth containing cells and pigment	Cell free supernatant	Pigment
<i>Fusarium oxysporum</i>	25mm	18mm	9mm	12mm
<i>Rhizoctonia solani</i>	20mm	12mm	5mm	8mm
<i>Aspergillus flavus</i>	28mm	20mm	12mm	15mm
<i>Pythium</i>	18mm	10mm	4mm	7mm

### 3.8 Seed Germination and Early Growth Promotion

Seed germination assays demonstrated a significant improvement in germination performance for carrot, beetroot, and radish seeds treated with the bacterial inoculum compared to untreated controls (**Figures 9 and 10**). The radish seeds germinated rapidly compared to the carrot seeds, after 7 days of incubation (**Figure 11**). No germination was observed in control carrot seeds, while treated seeds germinated successfully. After 9 days, the radish control seeds' growth declined, while the treated seeds' growth rate was improved. The treated seeds exhibited earlier emergence, higher germination percentage, and improved uniformity. The germination index (GI) values were consistently higher for the inoculated treatments, reflecting better metabolic activity and early seedling vigour. The Germination Index (GI) and seedling vigour index (SVI) values were depicted in **Table 2**. The relative seed germination percentage of radish was 62.5%, while for carrot could not be calculated due to zero germination in the control.



Figure 9: A. Seeds soaked in inoculum for treatment, B. Seeds soaked in ddH<sub>2</sub>O for control.

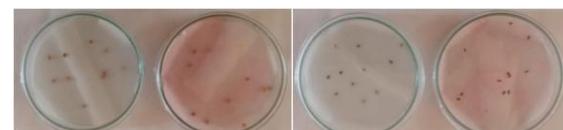


Figure 10: A. Radish seeds after seed treatment and control B. Carrot seeds after seed treatment and control



Figure 11: A. Radish seeds germination after incubation for 7 days, B. Carrot seeds germination after incubation for 7 days

Table 2: Germination percentage and seedling vigour index (SVI) of Radish and carrot seeds

Crop	Treatment	Germination index (GI) %	Seedling vigour index (SVI)
Radish	Treated	80	308
Carrot	Treated	50	55
Radish	Control	50	192.5

\*Note: Carrot seeds control, GI, and SVI were 0, as

no control seeds were germinated

#### 4. DISCUSSION

The present study demonstrates that the rhizosphere-derived *Serratia marcescens* strain possesses multiple plant growth-promoting and biocontrol traits that collectively contribute to enhanced seed germination and early seedling development in root crops. The observed combination of indole-3-acetic acid (IAA) production, ACC deaminase activity, siderophore secretion, phosphate solubilization, and chitinolytic potential highlights the multifunctional nature of the strain and supports its suitability as a plant growth-promoting rhizobacterium (PGPR).

IAA production is one of the most critical mechanisms by which PGPR influence plant growth, particularly during the early stages of development. In the present study, the strain exhibited substantial IAA synthesis, as confirmed through quantitative estimation using Salkowski's reagent. Elevated IAA levels are known to stimulate cell elongation, lateral root initiation, and root hair formation, thereby enhancing nutrient and water uptake (Patten & Glick, 2002; Spaepen *et al.*, 2007). The improved root and shoot growth observed in treated radish and carrot seedlings can be partially attributed to bacterial auxin production, which likely facilitated rapid radicle emergence and early seedling vigour.

In addition to auxin production, the strain exhibited high ACC deaminase activity, expressed as nmol  $\alpha$ -ketobutyrate  $\text{mg}^{-1}$  protein  $\text{h}^{-1}$ , following the method of Penrose and Glick (2003). ACC deaminase plays a vital role in lowering plant ethylene levels by degrading its precursor, ACC. Ethylene, while essential at low concentrations, inhibits root elongation and seed germination when present at elevated levels, particularly under stress conditions (Glick, 2014). The strong ACC deaminase activity observed in this study suggests that the strain can alleviate ethylene-induced growth inhibition, thereby promoting better seed germination and seedling establishment. This mechanism likely contributed to the improved germination percentage and seedling vigour index recorded for treated radish and carrot seeds.

Iron acquisition is another crucial factor influencing plant growth, especially in calcareous or iron-deficient soils. The formation of prominent orange halos on CAS agar confirmed the strain's ability to produce siderophores. Siderophores chelate ferric iron and make it available to plants while simultaneously depriving competing phytopathogens of this essential micronutrient (Neilands, 1995; Alexander & Zuberer, 1991). This

dual role enhances plant nutrition and suppresses pathogen proliferation, reinforcing the biocontrol potential of the strain.

Phosphorus solubilization is a well-recognized PGPR trait that improves plant access to insoluble phosphate pools in soil. The clear halo zones observed on Pikovskaya agar indicated efficient solubilization of tricalcium phosphate, likely mediated through the secretion of organic acids that lower pH and release soluble phosphate (Pikovskaya, 1948; Nautiyal, 1999). Enhanced phosphorus availability is particularly important during early seedling growth, supporting energy metabolism and root development. This trait likely complemented the auxin-mediated root growth observed in treated seedlings.

The chitinolytic activity demonstrated by the strain further strengthens its role as a biocontrol agent. Chitinases degrade chitin, a major structural component of fungal cell walls, thereby inhibiting fungal growth and colonization (Ordentlich *et al.*, 1988; Gooday, 1990). The formation of clear hydrolysis zones on colloidal chitin agar suggests active secretion of extracellular chitinases, which may protect germinating seeds and young seedlings from soil-borne fungal pathogens. This enzymatic defense mechanism is particularly relevant for root crops, which are highly susceptible to damping-off and root rot diseases during early growth stages. The antifungal activity observed in the agar well diffusion assay is strongly supported by the chitinolytic and plant growth-promoting traits of the *Serratia marcescens*. Chitin, a major structural component of fungal cell walls, is a primary target of bacterial chitinases, and degradation of this polymer results in loss of cell wall integrity and inhibition of fungal growth. The appreciable chitinolytic index recorded for the isolate provides a mechanistic basis for the inhibition zones observed against fungal pathogens. In addition, the antifungal activity detected in the culture supernatant and pigment extract indicates the involvement of extracellular metabolites and pigment-associated bioactive compounds, which are known to act synergistically with hydrolytic enzymes. PGPR traits such as siderophore production further contribute to antifungal efficacy by limiting iron availability to competing fungi and enhancing plant defense responses. Collectively, these results demonstrate that the antifungal activity of *Serratia marcescens* is a combined outcome of its chitin-degrading ability and multifunctional PGPR attributes, highlighting its potential as an effective biocontrol agent (Chet & Inbar, 1994; Compant *et al.*, 2005; Raaijmakers *et al.*, 2009).

Seed germination assays revealed crop-specific responses to bacterial inoculation. Radish exhibited relatively high germination in the control, with moderate enhancement in treated seeds, whereas carrot showed no germination in the control but a marked improvement following bacterial treatment. This difference may reflect inherent variations in seed physiology and sensitivity to microbial inoculation. The substantial increase in carrot germination and seedling vigour index upon treatment indicates that the strain effectively mitigated germination constraints, possibly by reducing ethylene stress and enhancing nutrient availability. Similar improvements in seed germination and vigour following PGPR inoculation have been reported in earlier studies (Pandey & Gupta, 2020; Fahsi *et al.*, 2021).

Overall, the synergistic action of multiple PGPR traits exhibited by *Serratia marcescens* explains its effectiveness in enhancing seed germination and early seedling growth. Rather than relying on a single mechanism, the strain integrates hormonal regulation, nutrient mobilization, stress mitigation, and pathogen suppression, resulting in improved plant performance. These findings support previous reports highlighting the importance of multifunctional PGPR for sustainable agriculture (Bhattacharyya & Jha, 2012; Glick, 2012). The strain evaluated in this study, therefore, represents a promising candidate for development as a biofertilizer and biocontrol agent for root crop cultivation.

## 5. CONCLUSION:

The present study demonstrates that the rhizosphere-derived *Serratia marcescens* strain possesses multiple plant growth-promoting and biocontrol attributes that collectively enhance seed germination and early seedling development in root crops. The strain exhibited significant indole-3-acetic acid production, strong ACC deaminase activity, effective siderophore secretion, phosphate solubilization ability, and pronounced chitinolytic activity. These traits highlight its multifunctional role in promoting plant growth, improving nutrient availability, mitigating ethylene-mediated stress, and suppressing soilborne fungal pathogens.

Seed germination and seedling vigour studies revealed that bacterial treatment markedly improved germination percentage, relative seed germination, and seedling vigour index in radish and carrot compared to untreated controls. The enhanced root and shoot growth observed in treated seedlings can be attributed to the synergistic action of bacterial phytohormone production, stress alleviation mechanisms, and nutrient-mobilizing capabilities. The absence of germination in

untreated carrot seeds further emphasizes the beneficial impact of microbial inoculation during early developmental stages.

Overall, the findings support the potential application of *Serratia marcescens* as an eco-friendly bioinoculant for sustainable agriculture, particularly for root crop cultivation. The integration of growth promotion and biocontrol functions within a single bacterial strain underscores its suitability as an alternative to chemical fertilizers and fungicides. Future studies include greenhouse and field conditions to validate its efficacy across diverse soil environments and to explore its formulation as a commercially viable biofertilizer.

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## CONFLICTS OF INTEREST:

Authors disclose no conflict of interest.

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