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Plant Growth-Promoting Activity of *Pseudomonas aeruginosa* OD13 in Tomato Plant

Anjali Suansia [a*] and Akshaya Kumar Senapati [a]

[a] *Department of Plant Pathology, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar-751003, India.*

Authors' contributions

This work was carried out in collaboration between both authors. Author AKS supervised, reviewed and approved the manuscript.

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ABSTRACT

Aims: The study aimed at assessing the plant growth-promoting properties of the *Pseudomonas aeruginosa* strain OD13.
Place and Duration of Study: The research was conducted at the Department of Plant Pathology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, between September 2019 and March 2022.
Methodology: PGPR (Plant Growth Promoting Rhizobacteria) inoculation can increase crop yield and quality in a range of important crops including cereals, legumes, vegetables, and fruits. In addition to enhancing plant growth, PGPR can also improve soil health by increasing nutrient availability and reducing the need for chemical fertilizers and pesticides. Overall, the use of PGPR is a promising strategy for sustainable agriculture, as it offers a natural and eco-friendly alternative to conventional methods of plant growth enhancement. In the present study, The *Pseudomonas* isolates were obtained from the rhizosphere of solanaceous crops grown in various districts of

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[++] *PhD Student;*
[#] *Professor & Head;*
**Corresponding author: E-mail: anjali.suansia99@gmail.com;*

Odisha. Tomato seeds were used in the experiment, with two groups: one control group and another group treated with the *Pseudomonas aeruginosa* strain OD13. The germination percentage, seedling height, plant height (including root length and shoot length), fresh weight, and dry weight were measured after a 30-day incubation period. The production of indole-3-acetic acid (IAA), Hydrogen cyanide (HCN), siderophore, hydrolytic enzymes, phosphate solubilization ability, heavy metal, and antibiotic sensitivity were also tested. Additionally, biofilm formation was evaluated by motility assay.

Results: *Pseudomonas* isolate significantly enhanced seed germination (90%), root length (6.25cm), shoot length (23.50cm), fresh weight (1.345g), and dry weight (0.085g) of tomato plants. It produced IAA, HCN, siderophore, protease, and lipase and showed phosphate solubilization ability in culture medium. The isolate was found to colonize in and around the tomato root system due to the presence of swimming and twitching motility. Moreover, it was highly resistant to zinc sulphate, ferric chloride, and copper sulphate, as well as conventional antibiotics like tetracycline.

Conclusion: *Pseudomonas aeruginosa* strain OD13 demonstrated great potential as an inoculant for tomato plants and as a suitable model system for studying the genetics of plant growth promotion by beneficial rhizobacteria.

Keywords: Pseudomonas; growth-promoting; IAA; siderophore; germination.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.,) is a widely consumed vegetable globally and holds a dominant position [1]. Its cultivation and production are crucial for the socio-economic development and food security of both local and national populations [2,3]. India has been among the top tomato cultivators globally, second only to China [4]. However, the production of tomatoes in India has been subject to various biotic and abiotic factors that have had an impact on the yield. The scientific community is concerned with finding ways to promote plant growth, which is necessary to solve global socio-economic problems. One potential solution is the use of microorganisms, which is both easy and inexpensive. Microorganisms such as bacteria, fungi, and algae can help plants grow by providing them with nutrients, protection against pests and diseases [5], and improving their ability to absorb water and minerals from the soil. This approach to plant growth promotion using microorganisms is sustainable and environmentally friendly. The rhizosphere is a critical area where plant growth-promoting rhizobacteria (PGPR) play a vital role in responding to soil-borne diseases and acting as biocontrol agents [6,7]. PGPR is a group of bacteria that efficiently colonize plant roots, promoting plant growth and increasing yield [8]. Although the mechanisms underlying the plant growth-promoting effects of PGPR are not fully comprehended, they are thought to involve the production of phytohormones and siderophores, which have antagonistic effects against phytopathogenic microorganisms [9,10], as well as the synthesis of fungicidal compounds, enzymes, and/or antibiotics [11]. Numerous studies have demonstrated a significant increase in growth and yield of various important crops upon inoculation with PGPR [12,13]. *Pseudomonas* strains have also been found to have a positive effect on seed germination and seedling growth [14], and certain strains like *Pseudomonas putida* and *P. fluorescens* have been observed to promote root and shoot elongation in canola [15]. Hence, *Pseudomonas* has the potential for use in agriculture as a biofertilizer [16]. Fluorescent Pseudomonads are widely used due to their ability to employ multiple mechanisms for biocontrol of phytopathogens and promotion of plant growth [17,18]. They produce a range of antibiotics, chitinolytic enzymes, growth-promoting hormones, (Hydrogen cyanide) HCN, siderophores, and catalase. The use of PGPR is now widespread globally and has significant potential for broader use in agriculture as a means of biocontrol of plant pathogens and biofertilization [19-22]. Bacterial strains isolated from the rhizosphere of *Lolium perenne* have been found to act as plant growth-promoting bacteria and exhibit various plant growth-promoting related activities [23]. *Pseudomonas* spp., belonging to the Pseudomonadaceae family, are a highly prevalent group of beneficial rhizobacteria among the heterogeneous and extensive bacterial populations in the rhizosphere. These Gram-negative bacteria are chemoheterotrophs that primarily inhabit the soil and have a wide range of functions. They are capable of colonizing the rhizosphere of various crops, including cereals, pulses, oilseeds, and

vegetables [24]. *Pseudomonas aeruginosa* is known for producing secondary metabolites such as indole acetic acid (IAA) and siderophores and can also solubilize phosphate [25]. This study aimed to investigate the plant growth-promoting activity of a recently isolated strain of *Pseudomonas aeruginosa*, OD13.

2. MATERIALS AND METHODS

2.1 Isolation of Bacteria

For isolation of bacteria, soil samples were randomly collected from rhizosphere of solanaceous crops grown in different districts of Odisha. The samples were isolated on King's B agar plate and the potential strain identified as *P. aeruginosa* strain OD13 (Accession number OQ781265), which was used for testing the growth-promoting activity on tomato plant.

2.2 Plant-growth Promoting (PGP) Activity of *Pseudomonas aeruginosa* Strain OD13 on Tomato plant

2.2.1 Seed germination on germination paper

The *Pseudomonas aeruginosa* strain OD13 was used to pre-treat tomato seeds for a duration of 12 hours. The seeds were then placed on moist germination paper, following the standard procedure of the International Seed Testing Association (ISTA) (2005), with around 25 tomato seeds (Pusa Ruby) per paper. The germination process was carried out at 28 ± 2ºC for 12 days until the seed leaves were fully opened. The control group was treated with distilled water [26]. After the incubation period, the germination percentage was calculated.

2.2.2 Growth promotion of tomato by *Pseudomonas aeruginosa* strain OD13 in green house condition

Two groups of tomato seeds were used in the experiment, one was the control group and the other was treated with *Pseudomonas aeruginosa* strain OD13. The seeds in the treated group were submerged in the *Pseudomonas* solution for eight hours, whereas those in the control group were soaked in sterile water. After this, the seeds were transplanted into separate pots that contained a soil mixture composed of field soil, FYM, and sand in a ratio of 2:1:1. The experiment was conducted for 30 days, and at the end of the incubation period, the root length, shoot length, fresh weight, and dry weight of each experiment were measured [27].

2.3 Characterization of *Pseudomonas aeruginosa* Strain OD13 for Plant Growth Promoting Traits

2.3.1 Production of IAA

The production of IAA was assessed using the method outlined by Patten and Glick [28]. *P. aeruginosa* strain OD13 was grown in minimal medium containing different concentrations of L-tryptophan (0, 50, 100, 200, and 500 ȝg/ml) and incubated for 48 hours at 28 ± 2°C. After incubation, the bacterial cells were removed via centrifugation at 4,000 rpm for 20 minutes at 4°C. A mixture of 1 ml of supernatant and 4 ml of Salkowski's reagent (in a 1:4 ratio) was then incubated at room temperature for 20 minutes. The appearance of a pink color indicated the presence of indoles. The amount of indoles was determined by measuring the absorbance of the supernatant mixture (supernatant+Salkowski's reagent) at 535 nm and comparing it to a standard curve created using an IAA standard graph.

2.3.2 HCN production

The HCN production ability of *P. aeruginosa* OD13 was evaluated using the method described by Wei et al. [29]. Filter paper pads (Whatman no.1) were placed on the lids of sterilized petri plates, and sterilized tryptic soya agar medium (TSA) amended with glycine (4.4 g/L) was poured into the plates. 24 hours old *P. aeruginosa* culture was streaked onto the medium. Two milliliters of sterile picric acid solution (Picric acid 2.5g; Na_2CO_3 12.5 g and distilled water1 litre) (Miller and Higgins, 1970) were then added to the filter paper padding in each plate. The plates were sealed with parafilm to contain the gaseous metabolite produced by *P. aeruginosa* and to allow for a chemical reaction with picric acid on the top to occur. The filter paper was examined for color changes after incubating the plates for one week at 30°C, and the HCN production potential of the rhizobacteria was assessed using the following scoring system.

- No colour change : No HCN production
- Brownish colouration : Weak HCN production
- Brownish to orange : Moderate HCN production

- Orange to reddish brown : Strong HCN production

2.3.3 Siderophore production

The production of siderophores was determined by spotting 5 µl of an overnight culture on Chrom Azurol S (CAS) agar plates and incubating them for 16 hours at 28°C. The CAS-iron complex on the plates changes from blue to an orange halo around the colony, indicating qualitative analysis of siderophore production and iron (III) chelating activity. This method was first described by Schwyn and Neilands in 1987 [30].

2.3.4 Phosphate solubilizing activity

Under aseptic conditions, *P. aeruginosa* was inoculated on Pikovskaya's agar plates with tricalcium phosphate as a substrate and incubated at 30 °C for 5 days. After incubation period, the plates were observed for the presence of a solubilization zone around the colony. If a solubilization zone was present, the strain was considered to be a phosphate solubilizer. This method was first described by Pikovskaya in 1948 [31].

2.3.5 Production of hydrolytic enzymes

The ability of the *P. aeruginosa* strain OD13 to produce protease was evaluated by observing the clear zones on skim milk agar after a 5-day incubation period at 30 °C. To assess lipase production, the lipase medium was used, and the presence of clear zones surrounding the colonies was considered as positive [32]. For detecting pectinase production, the strain was assessed as per the method previously described by Andro et al. [33], and positive results for cellulase and pectinase production were indicated by the presence of clear halos around the colonies. To assess chitinolytic and cellulase activity, the isolate was plated on chitin agar and CMC agar, respectively, following the method of Cattelan et al. [34].

2.4 Biofilm Formation by *Pseudomonas*

2.4.1 Motility assay

2.4.1.1 Swimming motility

The swimming motility assay was conducted following the protocol outlined by Rasamiravaka et al. [35]. LB medium containing 0.3% (w/v) agar was used for the assay plates. A spot of freshly grown *Pseudomonas* at a concentration of 10^8 cfu/ml was injected onto the plate with a volume of 3µl. The plates were sealed with parafilm to prevent dehydration and were incubated at 30°C for 48 hours.

2.4.1.2 Swarming motility

To assess swarming motility, the method described by Rasamiravaka et al. [35] was used. LB agar plates with 0.5% (w/v) agar were prepared and spot inoculated with 3 µl of a freshly grown bacterial culture (10^8 CFU/ml). The plates were sealed with parafilm to prevent dehydration and incubated at 28°C for 48 hours. The diameter of the swarm was measured in mm.

2.4.1.3 Twitching motility

The measurement of twitching motility was carried out using the method described by Rasamiravaka et al. [35]. A 5 µl droplet of freshly grown *Pseudomonas* culture (10^8 cfu/ml) was inoculated on LBA medium plates containing 1% (w/v) agar for assessing twitching motility. The plates were sealed with parafilm to prevent dehydration and incubated at 28°C for 48 hours.

2.5 Heavy Metal Resistance / Sensitivity test of *P. aeruginosa* Strain OD13

The well diffusion method was used to test the sensitivity/resistance of OD13 to different heavy metals, including Lead acetate, Cadmium sulphate, Nickel cholride, Selenium dioxide, Zinc chloride, Ferric chloride, Copper sulphate, and Mercuric chloride with different concentrations. A 24-hour-old culture of OD13 was swabbed onto nutrient agar (NA) and allowed to dry completely before making the wells using a sterile cork borer. Heavy metals of different concentrations (1, 2, 4, 6, 8, and 10 mM/ml) were then added into the wells, and the plates were incubated for 24 hours at 30°C. The diameter (mm) of each zone of inhibition was measured and recorded at the end of incubation, and the results were compared against a standard map to determine the sensitivity and resistance patterns of OD13 to each heavy metal [36].

2.6 Antibiotic Resistance / Sensitivity test of *P. aeruginosa* Strain OD13

A sensitivity/resistance test was conducted on the isolate OD13 using commercially available

antibiotics, including Ampicillin (10µg), Azithromycin (15µg), Augmentin (30µg), Cefaclor (30µg), Cephotaxime (30µg), Cefaperazone (75µg), Cefuroxime (30µg), Erythromycin (15µg), Penicillin (10µg), Ciprofloxacin (5µg), Clarithromycin (15µg), Cephadroxil (30µg), Streptomycin (10µg), Rifampicin (5µg), Cefpodoxime (10µg), Levofloxacin (5µg), Amikacin (30µg), Vancomycin (30µg), Tetracycline (30µg), Cefixime (5µg), Chloramphenicol (30µg), Ceftriaxone (30µg), and Clindamycin (2µg). The well diffusion method was used by swabbing the OD13 culture on nutrient agar and allowing it to dry for 10 minutes before making wells with a sterile cork borer. Antibiotics were added to the wells, and the plates were incubated for 24 hours at 30°C. The diameter of each zone was measured, and the sensitivity and resistance profiles were determined based on the diameter of the clearance zone and evaluated according to a standard chart [36].

3. RESULTS AND DISCUSSION

3.1 Plant-Growth Promoting (PGP) Activity of *Pseudomonas aeruginosa* Strain OD13 on Tomato Plant

The *Pseudomonas* isolate had a significant effect on the growth of tomato seedlings. The study found a marked increase in seed germination (90%), root length (6.25cm), and shoot length (23.50cm) when compared to the control group (Table 1). Moreover, the isolate OD13 showed a remarkable enhancement in the crop plant's biomass, including fresh weight (1.345g) and dry weight (0.085g). The vigor index of treated plant (2679) was approximately twice of the untreated (control) plant (1383). These results suggest that the *Pseudomonas* isolate could be a potential candidate for enhancing the growth and yield of tomato plants.

3.2 Characterization of *Pseudomonas aeruginosa* Strain OD13 for Plant Growth Promoting traits

In the culture medium, strain OD13 was found to produce several substances, including indole acetic acid-like compounds, hydrogen cyanide (HCN), iron-chelating siderophores, hydrolytic enzymes (protease and lipase), and a solubilization zone around the colony (Table 2).

This growth-enhancing effect can help improve plant growth, protection, and yield. Soil rhizosphere bacteria act as a PGPR system for tomato and other solanaceous plants, influencing plant growth mechanisms by growing around plant roots and tissues. Rhizobacteria can act as biofertilizers and support nutrient utilization by forming a symbiotic relationship with PGPR in favorable plant growth conditions. It is highly desirable to have a single strain with both biocontrol and growth-enhancing properties, as this can be effectively utilized in sustainable agriculture to improve crop yields [37]. In a study by Gharineh et al. [38], the vigour of wheat cultivar was determined based on germination of *Psedomonas* treated seed. Microorganisms promote plant growth by releasing plant growth hormones, especially auxins, as reported by several studies [39-42]. PGPR strains have been reported to stimulate plant growth by producing plant growth promoters such as gibberellins, cytokinins, and indole acetic acid [11,43-46]. These promoters can either directly or indirectly affect plant growth and development [47,48]. In previous research, fluorescent *Pseudomonas* strains RBT13 improved seed germination, shoot length, and root length in chickpea and soybean [49]. Indole acetic acid (IAA) is a plant hormone that is involved in various aspects of plant growth and development, including cell division, cell growth, and root initiation [50]. Many plants growth-promoting rhizobacteria (PGPR) are capable of producing IAA, such as *Pseudomonas fluorescens*, which has played a significant role in plant growth and development [43,48,51]. Other *Pseudomonas* strains, such as *P. plecoglossicida* and *P. aeruginosa*, have also been found to produce IAA and promote plant growth [52,53,51]. Inoculation with *P. aeruginosa* PGPR2 was found to significantly improve the growth of mungbean seedlings, including plant height, root length, fresh and dry plant weight [54]. The increase in root length was likely due to the production of IAA by *Pseudomonas* sp., which led to a larger surface area for nutrient uptake and improved growth and yield of the plants that were inoculated. The *Pseudomonas* strains exhibited several plant growths promoting traits, including the production of IAA, HCN, siderophore pyoverdine, protease, and phosphate solubilization ability [54-56].

Table 1. Growth promoting activity of *Pseudomonas aeruginosa* on Tomato plants

Growth parameters	*P. aeruginosa* strain OD13	Control
Germination %	90.00±4.00[a]	66.00±4.01[b]
Shoot Length (cm)	23.50±1.04[a]	3.75±0.57[b]
Root Length (cm)	6.25±0.5[a]	3.75±0.5[b]
Total length (cm)	29.75	21.00
Fresh Weight (g)	1.345±0.12[a]	0.918±0.005[b]
Dry Weight (g)	0.085±0.001[a]	0.056±0.002[b]
Vigor Index	2679	1383

**Data represent means ± standard deviations of six replicates (p = 0.05), the treatment means followed by same letter did not differ significantly by DMRT*

Table 2. Characterization of plant growth promoting *Pseudomonas aeruginosa* strain OD13

Plant growth promoting traits	*Pseudomonas aeruginosa* strain OD13
IAA production (µg/ml)	7
Siderophore production	+
HCN production	+
Phosphate solubilization	+
Chitinase production	-
Protease production	+
Lipase production	+
Cellulase production	-
Pectinase production	-

3.3 Biofilm Formation by *Pseudomonas aeruginosa* Strain OD13

3.3.1 Motility test

The *Pseudomonas aeruginosa* strain OD13 was able to effectively colonize the root system of tomato plants due to its ability to move through the environment using both swimming and twitching motility. The isolate showed a high degree of swimming motility on LBA medium after 48 hours, with a diameter of up to 52 mm. In contrast, its twitching motility was only minimally observed after 48 hours, with a zone diameter of 22 mm on LBA medium when compared to the control.

Biofilms are important for microorganisms as they provide protection from external stressors such as antibiotics, disinfectants, and host defenses, making them difficult to eradicate. Microorganisms produce biofilms that adhere to both living and non-living surfaces, and are surrounded by a matrix of organic material that provides structure and stability to the microbial population [57]. These biofilms are highly organized and contain microbial cells and extracellular polysaccharides that offer protection to the community and also facilitate the exchange of genetic material between bacterial species [58]. The ability to form biofilms is a well-studied feature of *Pseudomonas aeruginosa*, and it has been shown to be critical for its survival in various environments. The formation of biofilms is a complex process involving several factors, including cell surface appendages, extracellular matrix production, and quorum sensing. In addition to aiding in colonization, biofilms can also enhance nutrient acquisition, promote cooperative behaviors among microbial populations, and facilitate survival in hostile environments [58]. Previous studies have demonstrated that *Pseudomonas fluorescens* produces the most biofilm in Potato dextrose broth, Pikovskaya and King's B broth, followed by Nutrient broth and Jensen's medium [59]. Similarly, the current study found that the highest level of biofilm produced by the isolate OD13 was observed after 48 hours of growth in LB medium. Recently, Haney et al. [60] showed that a strong biofilm formed on the glass surface of tubes when *Pseudomonas aeruginosa* (PA14) was grown under static conditions with the aid of the growth medium.

Table 3. Heavy metal tolerance exhibited by *Pseudomonas aeruginosa* strain OD13

Name of Heavy Metal	Concentration (mM)					
	1	2	4	6	8	10
Copper sulphate	+++	+++	+++	+++	++	++
Cadmium sulphate	++	++	++	++	+	+
Ferric chloride	+++	+++	+++	++	++	++
Lead acetate	-	-	-	-	-	-
Mercuric chloride	+	-	-	-	-	-
Nickel chloride	+++	+++	++	++	+	-
Selenium dioxide	+++	+++	++	++	++	+
Zinc sulphate	+++	+++	+++	+++	+++	++

** +++ Good Growth, ++ Average Growth, + Poor Growth, - No Growth*

Table 4. Evaluation of antibiotic resistance of *Pseudomonas aeruginosa* strain OD13

Name of Antibiotic	Sensitivity
Ampicillin (A)	+
Azithromycin (At)	+
Augmentin (Au)	+
Cefaclor (Cj)	+
Cephotaxime (Ce)	+
Cefaperazone (Cs)	+
Cefuroxime (Cu)	+
Erythromycin (E)	+
Penicillin (P)	+
Ciprofloxacin (Cf)	-
Clarithromycin (Cw)	+
Cephadroxil (Cq)	+
Streptomycin (S)	-
Rifampicin (R)	+
Cefpodoxime (Cep)	+
Levofloxacin (Le)	-
Amikacin (Ak)	-
Vancomycin (Va)	+
Tetracycline (T)	+
Cefixime (Cfx)	+
Chloramphenicol (C)	+
Ceftriaxone (Ci)	+
Clindamycin (Cd)	+

**-: Sensitive and +: resistance*

3.4 Resistance / Sensitivity of Heavy Metal by *Pseudomonas aeruginosa* Strain OD13

The study found that *Pseudomonas aeruginosa* strain OD13 demonstrated good resistance to zinc sulphate, ferric chloride, and copper sulphate up to a concentration of 10 mM. However, it had the lowest resistance to Nickel chloride, cadmium sulphate and selenium dioxide. The isolate was found to be more sensitive to other metals, including mercuric chloride and nickel chloride, compared to the control (Table 3). The results suggest that OD13 has the potential to tolerate certain heavy metals commonly found in contaminated environments, which can be useful for bioremediation purposes. However, it may not be suitable for environments with high concentrations of cadmium sulphate and selenium dioxide. Overall, the metal tolerance profile of OD13 indicates its potential use as a bioinoculant for enhancing plant growth in heavy metal contaminated soils.

3.5 Antibiotic Resistance / Sensitivity of *Pseudomonas aeruginosa* Strain OD13

The antibiotic sensitivity of *Pseudomonas aeruginosa* strain OD13 was evaluated using

twenty-three commercially available antibiotics. The study found that the isolate was resistant to 19 of the antibiotics but was sensitive to Ciprofloxacin, Streptomycin, Levofloxacin, and Amikacin (Table 4). It is important to consider the antibiotic sensitivity of *P. aeruginosa* when selecting antibiotics for managing bacterial wilt in the Integrated Disease Management (IDM) program. These results can be beneficial in developing more effective management strategies for bacterial wilt and other bacterial disease of tomato.

These are significant because these heavy metal salts and antibiotics are commonly used in agriculture as fertilizers and antibiotics. The strain could potentially be used as biofertilizer in crop systems, but appropriate fertilizer and antibiotic treatment must be considered. Other studies also found high resistance in rhizosphere isolates of *Pseudomonas* sp. 4036 and *Pseudomonas stutzeri* ST6 to zinc, iron, and various antibiotics, indicating it can be used as biofertilizer and biocontrol agents for plant pathogens [61,36].

4. CONCLUSION

The results of the study clearly showed that the inoculation of *Pseudomonas aeruginosa* strain OD13 had a significant positive effect on the growth and development of tomato plants. The seed germination, seedling growth, and biomass of both fresh and dry weight, as well as root and shoot length, and the vigour index of the tomato plants, were all markedly improved. These results indicate that OD13 has strong potential as a plant growth promoter and bioinoculant for tomato plants, and that it could be a valuable addition to field trials and future agricultural development. Moreover, the effective plant growth promotion potential of *Pseudomonas aeruginosa* strain OD13 also makes it an excellent candidate for further study into the genetics of plant growth promotion by beneficial rhizobacteria. The ability of OD13 to improve the growth and development of tomato plants is likely due to its production of various plant growth-promoting traits, such as the production of indole-3-acetic acid, siderophore pyoverdine, protease, and phosphate solubilization ability. These traits enable the bacterium to facilitate nutrient uptake by the plant, leading to increased growth and yield. Overall, the findings of this study suggest that *Pseudomonas aeruginosa* strain OD13 has the potential to be an effective tool for enhancing the growth and yield of tomato plants, and could also contribute to a greater understanding of the genetic basis of plant growth promotion by beneficial rhizobacteria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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