European Journal of Biotechnology and Bioscience
ISSN: 2321-9122
Impact Factor: RJIF 5.44
Received: 10-07-2018: Accepted: 13-08-2018
www.biosciencejournals.com
Volume 6; Issue 4; July 2018; Page No. 18-26

Plant growth promotion and inhibitory potential of fluorescence (under UV light) exhibiting root endophytic bacteria isolated from *Abelmoschus esculentus* (Okra) cultivars

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Abstract
Fluorescent Pseudomonads are known for the plant beneficial characters, but their potential is still unexplored. In the present study, root endophytic bacteria of *Abelmoschus esculentus* (Okra) cultivars 'Geetanjali', 'OH-102' and 'Sangur' showing fluorescence property on Kings B Agar (KBA) were isolated. They were further screened for their PGP and seed germination potential. Additionally, they were also tested for their inhibition potential against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Staphylococcus aureus*. The results indicated that, a cultivar effect for Fluorescent Pseudomonads in 'Sangur' cultivar. Isolates from Gitanjali (ORGT12, ORGT06, ORGT05) and OH102 (OROH12, OROH06, OROH05) cultivars were effective in inhibiting the four tested bacterial cultures. Isolate 'ORGT12' was shown to promote significant rate of seed germination (*Abelmoschus esculentus* 71%, *Cyamopsis tetragonoloba* 74%, *Vigna unguiculata* subsp. *unguiculata* 77%) among the tested isolates. Such potential isolates can be explored to reduce the microbial load on plants or their produce and as well as promote the plant growth.

Keywords: endophytes, okra, cultivars, pseudomonas, PGP

Introduction

Plants live in intimate association with a variety of microorganisms that have profound repercussions on plant health. Different crop species were shown to influence soil microbial communities by selecting specific microorganisms in their rhizosphere [1, 2]. Not only plant species but also plant cultivar was shown to influence microbial communities [3–6]. The rhizosphere microorganisms are increasingly used for the commercial purposes as biocontrol and biofertilizer agent [7]. The rhizosphere microorganisms were known to be unsuccessful as a biocontrol or plant growth promotion agents under certain conditions [8]. But endophytic microorganisms were shown to be promising due to its colonisation ability within the plants [9–13]. The knowledge of the different components of microbial communities and their functions in different crops, such as oilseed, rape, potato, strawberry, their cultivars grown in different soil, is fundamental for designing strategies for sustainable plant protection [1, 3, 7, 14].

Pseudomonas species are gram negative rod shaped bacteria that are present in agricultural soils were shown to have plant growth promotion (PGP) and biocontrol properties [15, 16]. These organisms were also shown to be closely associated with various plant species [17-19]. Fluorescent *Pseudomonads* are known for their ability to produce a water soluble yellow-green pigment [20]. They were shown to be one of the most effective *Pseudomonas* spp. that helps in the maintenance of soil health and are metabolically and functionally the most diverse species [20-23]. The populations of fluorescent *Pseudomonas* are known to be present in different crops [24–27]. Okra (*Abelmoschus esculentus*) is chosen as model crop in this study, as this crop has not been explored much with respect to microbial communities. It is locally known as Bhindi, one of the most important vegetable crops of India [28]. It provides vitamin A, B, C, protein, amino acids, minerals and iodine [28]. It is an annual herb and vegetable crop grown throughout the tropical and subtropical parts of the world, although it is believed to have originated from the tropical part of Africa [29–31]. Despite its nutritional value, its optimum yield (2-3 t/ha) in the tropical countries is low partly because of continuous decline in soil fertility [32]. Okra is prone to attack of fungi such as *Macrophomina phaseolina, Rhizoctonia bataticola, R. solani, Fusarium solani, Pythium butleri, Phytophthora palmivora, Cercospora abelmoschii and Erysiphe cichoracearum* [28]. It is affected by diseases such as mosaic, damping off and powdery mildew which are caused by yellow vein mosaic virus, *Phytum phytophlitora* and *Erysiphe cichoracearum* respectively. They have been reported to be either soil or air borne pathogens [33, 34]. Most of these pathogens are also known to be endophytic. These endophytes reside entirely within plant tissues and may grow within roots, stem and/or leaves, emerging to sporulate at plant or host tissue senescence [35, 36] Studies have revealed that endophytic communities are influenced by soil type, cultivar type and pathogen in different crop species [3, 7, 37]. Studies about how the cultivable endophytic bacterial communities, especially fluorescent *Pseudomonas* respond to different Okra cultivars

are scanty [24].

In the recent years human pathogens were shown to be associated with plants which is causing a major concern for human health [38–40]. It is important for us to find a sustainable solution that inhibits such human pathogens and also promotes plant health and nutrition. As a part of this study, endophytic fluorescent *Pseudomonads* were isolated from different Okra cultivars and tested for their plant growth potential; and as well as their antagonistic potential against members of bacterial species that were reported to be pathogenic to humans.

2. Materials and Methods

2.1 Plant material and growth conditions

Three different Okra cultivar (Geetanjali, OH-102 and Sangur) seeds were obtained from Ankur Seeds Private Limited (Nagpur, India) and were sown in field soil filled in poly bags and were maintained in an outdoor net house at Uka Tarsadia University, Tarsadi, Gujarat, India. The soil was collected before the potting for the physiochemical analysis. The plants were regularly watered and were grown until flowering (three weeks).

2.2 Sample collection

The root material was collected by destructive sampling. Each replicate of the three cultivars were processed separately. Three weeks old plants were carefully uprooted and with a sterile scissors all the root material was collected. The collected root sample was transferred to laboratory for further processing. Roots were carefully washed to remove the soil particles attached to the roots. Two grams of the collected root material was subjected to surface sterilization as mentioned by Jan *et al* 2013 [41]. In brief, root materials were washed with tap water for 5-10 minutes. 20 % Tween 20 wash was given to the same for 10 minutes. Distilled water wash for 5 minutes was given to the root materials. For 10 minutes, it was kept in 1.5 % Sodium hypochlorite followed by distilled water wash. It was than sterilized by 70 % Ethyl alcohol for 30 seconds. Final distilled water wash was given to the same for three times. The last wash was inoculated (100µl) onto nutrient agar and potato dextrose agar for conformation of the sterilization procedure. Thereafter, root material was grinded in a sterile mortar and pestle with 20 ml of normal saline. The suspension was subjected to serial dilution in normal saline from which, 0.2 ml of suspension (10^{-6}) was spread onto King's B agar (Himedia, India). The plates were then incubated at room temperature for 24 hours. After incubation, distinct colonies that fluoresced under UV light were purified and were stored in 20% glycerol at -20°C for further studies (except from Sangur cultivar).

2.3 Screening of Isolates for Plant Growth Promoting (PGP) Properties

The following PGP properties were screened for the selected isolates obtained from three varieties.

2.3.1 Siderophore Production

Siderophore (iron chelator) production of the isolates was determined using Chromazurol S (CAS) method in which the nutrient agar medium was supplemented with CAS 60.5 g in 50 ml Iron III soution (1 mM FeCl3. H2O and 10 mM HCl in 10 ml) and Hexa-Decyl Trimethyl Ammonium Bromide (HDTMA) (72.9 mg in 40 ml). Development of yellow-orange halo zone around the culture was considered as positive for siderophore production [42].

2.3.2 Phosphate solubilization

The plates were prepared with Pikovskaya's agar. The culture was streaked on the plates and incubated at room temperature for 7 days. The plates were then examined for clear zone around the microbial colonies in media containing insoluble mineral phosphates (tricalcium phosphate or hydroxyapatite) as sole Phosphate source [43].

2.3.3 Indole Acetic Acid Production

IAA production by the isolated rhizospheric bacteria was detected by colorimetric quantification. Culture were grown in 5 ml Tryptone Broth, followed by incubation at room temperature for 24 hours. The cultures were then centrifuged at 10,000 rpm for 15 minutes and 3 ml of supernatant was taken from each culture and 2-3 drops of Ortho-phosphoric acid was added. Then 4 ml of Salkowski reagent (1 ml of 0.5 M FeCl3 in 50 ml of 35 % $HClO_4$) was added to each aliquot. The sample were then incubated for 25 minutes at room temperature in dark. The absorbance was then read at 530 nm. The obtained quantification values were recorded by preparing standard curve made using IAA as standard (10-100 µg/ml) [44].

2.3.4 Hydrogen Cyanide Production

Suspension culture were spread on nutrient agar medium containing 4.4 g per liter of glycine with a Whatman filter paper No. 1 soaked in 0.5% picric acid solution. The plates were sealed with parafilm and incubated at room temperature for 4 days. Change of colour from light yellow to light brown or dark brown on the filter paper was considered positive for HCN Production [45].

2.3.5 Potassium solubilisation

Bacterial culture was streaked onto nutrient agar plates with mica powder and were incubated at 28°C for seven days. After the addition of Lugal's iodine, the plates were observed for clear zones around the isolates for potassium solubilizing ability.

2.4 Morphological Characterization

Morphological characterization of the potential bacteria was done by Gram's Staining method. The cells were studied on the basis of their shape, size, arrangement and colour. A heat fixed thin smear of culture was prepared on a clean glass slide, which was then flooded with crystal violet and after one minute, it was washed with water and flooded mordant Gram's Iodine. The smear was decolorized with 95% ethyl alcohol, washed with water and then counter stained with safranin for 45 seconds. After washing with water, the smear was dried and examined under microscope.

2.5 Screening for pigment production and fluorescence

The isolates were once again tested for their pigment production and fluorescence capacity in nutrient broth

(Himedia, India). After 24 hrs after the inoculation, the isolates were tested for fluorescence under UV lamp. The pigment production was checked after 48 hrs. The colouration was compared with the uninoculated broth. The broth was centrifuged at 13000 RPM and the supernatant was also exposed to UV light.

2.6 Biochemical tests

A. Sugar Utilization Test-A loopful culture of bacteria was inoculated into the sugar broth and was incubated at 37°C overnight. Acid production change the colour of medium to pink and gas production was concluded from a small bubble in Durham's tube. Results were recorded as (A) for acid production and (G) for gas production.
B. Indole Production Test-A loopful culture of bacteria was inoculated into the 1% Tryptone broth and was incubated at 37°C overnight. After incubation, 1 ml of Ehrlich's reagent was added after the addition of 3-4 drops of xylene in the medium. Formation of pink coloured ring indicates positive result.
C. Methyl Red Test-A loopful culture of bacteria was inoculated into Glucose phosphate broth and was incubated at 37°C overnight. After incubation, 4-5 drops of Methyl red indicator was added. Red colour formation leads to positive result.
D. Voges - Proskauer Test-A loopful culture of bacteria was inoculated into Glucose phosphate broth and was incubated at 37°C overnight. After incubation, 0.6 ml of α – naphthol and 0.2 ml Potassium hydroxide solution were added. Red colour formation leads to positive result.
E. Citrate Utilization Test-A loopful culture of bacteria was streaked onto Simmons's Citrate Agar slant and was incubated at 37°C overnight. Deep blue colour formation leads to positive result.
F. Gelatin Hydrolysis Test-A loopful culture of bacteria was streaked onto the Nutrient Gelatin Agar plate and was incubated at 37°C overnight. After incubation, plate was flooded by Frazier's Reagent. Formation of clear zone around the colony leads to positive result.
G. Starch Hydrolysis Test-A loopful culture of bacteria was streaked onto the Starch Agar plate and was incubated at 37°C overnight. After incubation, plate was flooded by Lugol's Iodine. Formation of clear zone around the colony leads to positive result.
H. Nitrate Reduction Test-A loopful culture of bacteria was inoculated into the Peptone Nitrate broth and was incubated at 37°C overnight. After incubation, 0.5 ml of α – napthylamine and Sulphanilic acid reagent were added. Development of red colour leads to positive result.
I. Catalase Test-A loopful culture of bacteria was inoculated into the nutrient broth and incubated at 37°C overnight. After incubation 1 ml of 3% Hydrogen peroxide was added. Gas bubble formation indicates positive result.
J. Dehydrogenase Test-A loopful culture of bacteria was inoculated into the nutrient broth containing 1 ml of sterile 1 % Methylene Blue and was incubated at 37°C
K. Overnight. After incubation, disappearance of blue colour leads to positive result. (Bergey's Manual of Systematic Bacteriology).

2.7 Antibacterial Activity

This was performed in triplicates by agar diffusion method in which various test microorganisms (*Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, and Staphylococcus aureus* procured from IMTECH, Chandigarh, India) were spread onto nutrient Agar plate and then ditches were made on them. The bacterial isolate suspension was inoculated into the ditch. The plates were then examined after 34 hours at 37°C for clear zone around the ditch whose diameter was measured against the control of distilled Water and Nutrient Broth [46]. As a control antibiotics (Amikacin (30µg), Imipenem (10µg), and Ceftazidime (30µg)) were also tested against these test bacteria by disc susceptibility technique (the disks were procured from HIMEDIA, Mumbai, India).

2.8 Seed Germination Assay

The seeds of different varieties (Black Eye Peas, Okra, Cluster Beans) were surface sterilized using 5% Sodium hypochlorite with one wash of sterile distilled water followed by 70 % Ethanol and three consecutive sterile distilled water washes. The third wash was plated onto nutrient agar and potato dextrose agar petri plates for checking the sterility. Seeds were then soaked in bacterial suspension of overnight cultures for 60 min and latter placed on an autoclaved petri plates containing sterilized filter paper. It was performed in triplicates with each plate having 60 seeds. It was given moisture at regular intervals [47]. The rate of germination of seeds were studied against the germination of seeds coated with sterile distilled water and nutrient broth as a control.

2.9 Identification of the isolates

Based upon their PGP and other properties tested, eleven isolates were sent for identification to Gujarat State Biotechnology Mission (GSBTM, Gandhinagar, India). They were identified using universal primers (27F-1492R).

3 Results

3.1 Soil Analysis

The detailed soil analysis was performed at Mahuva Sugar Factory, Bamaniya, and Gujarat. The soil has a pH 7.7 with chemical constituents of Phosphorus 84.6 mg/kg, Sulphur 69.5 mg/kg, Potassium 4.2 mg/kg, Calcium 29.8 mg/kg, Magnesium 36.0 mg/kg, Nitrogen 3.6 mg/kg and EC 0.25dS/m.

3.2 Isolation of bacteria

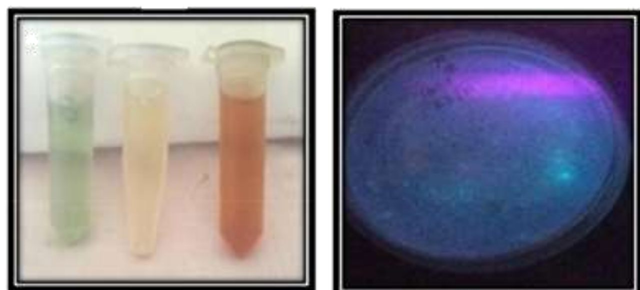
In total, 76 colonies were isolated (KBA), from all the three cultivars. Out of 76 isolates 37 were randomly selected for their further characterization. These isolates were assessed for their colony morphology (Table 1).

Table 1: Colony Morphology of the isolates.

Plant variety	Name of the Isolates	Size	Shape	Edge	Pigment
Gitanjali	ORGT01	Small	Round	Entire	Orange
	ORGT02	Small	Round	Entire	White
	ORGT03	Small	Round	Entire	White
	ORGT04	Small	Round	Entire	White
	ORGT05	Small	Round	Entire	Orange
	ORGT06	Small	Round	Entire	White
	ORGT07	Small	Round	Entire	White
	ORGT08	Small	Round	Entire	Orange
	ORGT09	Small	Round	Entire	Orange
	ORGT10	Small	Round	Entire	Orange
	ORGT11	Small	Round	Entire	White
	ORGT12	Small	Round	Entire	Orange
	ORGT13	Small	Round	Entire	Orange
	ORGT14	Small	Round	Entire	Orange
OH-102	OROH01	Small	Round	Entire	Orange
	OROH02	Small	Round	Entire	White
	OROH03	Small	Round	Entire	Orange
	OROH04	Small	Round	Entire	Orange
	OROH05	Small	Round	Entire	White
	OROH06	Small	Round	Entire	White
	OROH07	Small	Round	Entire	Orange
	OROH08	Small	Round	Entire	Orange
	OROH09	Small	Round	Entire	Orange
	OROH10	Small	Round	Entire	Orange
	OROH11	Small	Round	Entire	Orange
	OROH12	Small	Round	Entire	White
	OROH13	Small	Round	Entire	Orange
	OROH14	Small	Round	Entire	Orange
	OROH15	Small	Round	Entire	Orange
Sangur	ORSG01	Small	Round	Entire	White
	ORSG02	Small	Round	Entire	White
	ORSG03	Small	Round	Entire	White
	ORSG04	Small	Round	Entire	Orange
	ORSG05	Small	Round	Entire	White
	ORSG06	Small	Round	Entire	White
	ORSG07	Small	Round	Entire	White

3.3 Biochemical characterization and other properties of bacterial isolates

For the selected 37 isolates biochemical, PGP, pigment and fluorescence properties were tested (Figure 1). From ‘Gitanjali’ out of the 14 isolates only three showed positive for twelve properties, in ‘OH-102’ out of 15 isolates only two isolates showed positive for ten properties, followed by ‘Sangur’, out of seven only one showed positive for ten properties. The isolates from Sangur did not show neither fluorescence nor pigment production property.



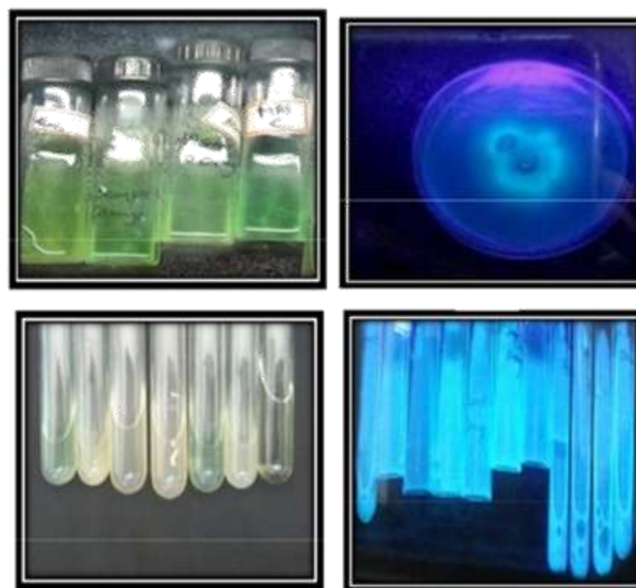


Fig 1: Bacterial isolates exhibiting fluorescence under UV light (above). Pigment production by bacterial isolate (a) and their fluorescence in UV light (b).

These isolates produced three different colours of pigmentation namely green, yellow and brown. All these pigmented broths (supernatant) were exhibiting fluorescence when exposed to UV light. The selected isolates were also tested for their various biochemical properties and plant growth promotion (PGP) (figure 2). All the tested isolates were shown positive for IAA production, catalase and dehydrogenase tests. The tested isolates were shown negative towards potassium solubilization property. IAA production of the isolates ranged from nearly 10-160 µg/ml.

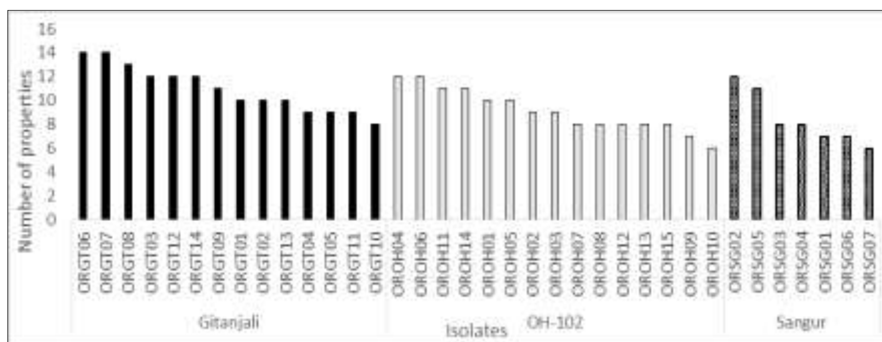


Fig 2: Properties exhibited by the selected bacterial isolates from Gitanjali, 'OH-102' and 'Sangur' Okra cultivars.

3.4 Seed germination assay

The isolates were examined for their seed germination potential (figure 3 and 4). Their potential was tested against three different crop species (*Vigna unguiculata* subsp. *unguiculata* (Black eye peas), *Abelmoschus esculentus* (Okra), *Cyamopsis tetragonoloba* (Cluster Beans)). Based upon the plant growth promotion properties that were tested in this study, three isolates ORGT09, ORGT12 and OROH05 were identified for seed germination study. As a control nutrient broth and distilled water were used. In Black eyed pea, the seeds started germinating from second day onwards; whereas other two crop species from the fourth day onwards the germination of seeds occurred. 'ORGT12' was shown to promote significant rate of seed germination (okra 71%, cluster bean 74%, and black eye peas 77%) among the tested. Lowest rate of seed germination was in 'ORGT09' at 33% in both the crop species 'black eye peas' and 'okra' which was much lesser than the nutrient broth control (38%) in 'cluster beans'.

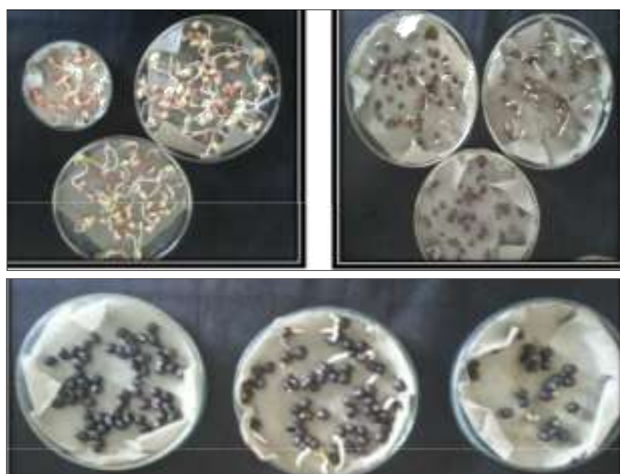


Fig 3: Seed germination assay was performed against three crop species (A-*Vigna unguiculata* subsp. *unguiculata* (Black eye peas), B-*Abelmoschus esculentus* (Okra), C-*Cyamopsis tetragonoloba* (Cluster Beans)) using bacterial isolates (II) and with two controls (distilled water (III) and nutrient broth (I)).

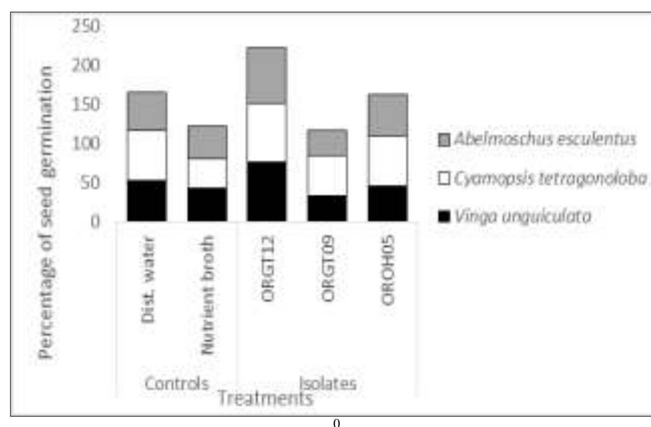


Fig 4: Seed germination assay was performed against three crop species (*Vigna unguiculata* subsp. *unguiculata* (Black eye peas), *Abelmoschus esculentus* (Okra), *Cyamopsis tetragonoloba* (Cluster Beans)) using three bacterial isolates (ORGT12, ORGT09 and OROH05) with two controls (distilled water and nutrient broth).

3.5 Antibacterial Activity

The isolates were tested for their antibacterial activity against different test bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Staphylococcus aureus* (figure 5). The diameters of zones were measured for each of isolate upon lawn growth of the tested organisms as depicted in Figure 5b (for e.g. *E.coli* was tested in the picture depicted below). As a control test microorganisms were also tested with the known concentrations of the antibiotics. The tested microorganisms were shown to be susceptible to the tested antibiotics (≥ 25mm). The top three isolates from each cultivar against the tested organism were identified (figure 5a). The controls sterile distilled water and nutrient broth showed no inhibition or '0 mm'. The isolates from Gitanjali and OH102 cultivars were effective in inhibiting the four tested bacterial cultures than from Sangur cultivar.

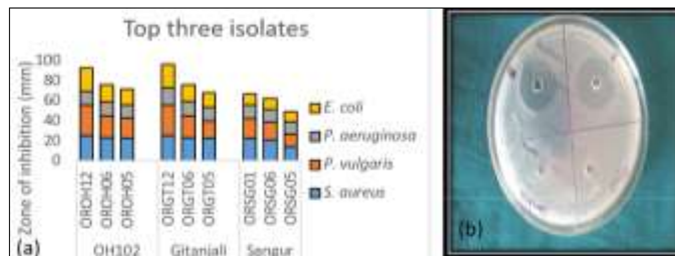


Fig 5: a & b. (a) Zone of inhibition of the top three isolates that were isolated from the cultivars (OH102, Gitanjali and Sangur) against the four tested organisms (*E. coli, P. aeruginosa, P. vulgaris and S. aureus*). (b) Testing of supernatant from the bacterial cultures (6 and 7 in the picture) by ditch method against the growth of *E.coli*, as controls sterile distilled water (D/W) and nutrient broth (control) were added.

Over all more than 50 % of the isolates tested showed inhibition against the tested organisms except for *E.coli* the isolates that were varied upon the culture tested). Of the 15 isolates that were isolated from OH102 all showed inhibition towards *S. aureus* followed by 10 with *P. vulgaris*, eight with *P. aeruginosa* and six with *E.coli*. From Gitanjali cultivar out of 14 isolates 12 showed inhibition against *S. aureus* followed by seven with *P. vulgaris*, eight with *P. aeruginosa* and six with *E.coli*. But from 'Sangur' cultivar only four isolates consistently showed inhibition against all the four tested organisms. The maximum zone of inhibition was 30 mm was shown by 'ORGT12' & 'OROH12' isolates against *Proteus vulgaris* and minimum of 11 mm was shown by 'ORSG 01' against *E.coli*.

3.6 Identification of the isolates

Out of 11 isolates only seven were identified and remaining isolates could not be amplified.

Table 2: Identity of the isolates

Sr. No.	Sample ID	BAB ID	Organisms name	% Identity
1	OROH01	BAB 6001	*Pseudomonas aeruginosa*	99%
2	OROH04	BAB 6002	*Enterobacter cloacae*	99%
3	OROH05	BAB 6003	*Enterobacter cloacae*	99%
4	ORGT02	BAB 6004	*Pseudomonas aeruginosa*	99%
5	ORGT05	BAB 6005	*Enterobacter cloacae*	100%
6	ORGT06	BAB 6006	*Enterobacter spp.*	100%
7	ORGT07	BAB 6007	*Pseudomonas mendocina*	99%

4. Discussion

Soil bacteria are known to colonize and increase plant health and yield are known as Plant Growth Promoting Rhizobacteria, and members belonging to phylum Proteobacteria and Firmicutes (for e.g. *Rhizobium*, *Azotobacter*, *Bacillus*, *Pseudomonas* and other genera) are being applied as plant growth promotors and biocontrol agents [7, 48, 49]. Studies have shown that endophytic microorganisms isolated from plant roots were also known have plant growth promotion and biocontrol capacity [7, 9, 17, 20, 50]. Of these organisms, certain members of fluorescent *Pseudomonas* were reported to have plant growth and biocontrol potential [20, 24, 26, 51]. These organisms are known to employ various mechanisms for root colonization, antagonistic effects, enzyme production and chelation of iron for the betterment of plan health [18, 20, 26, 26, 52–54]. Not only beneficial but also harmful effects especially pathogenic, were also reported of *Pseudomonas* [27, 55–57]. Studies involving *Pseudomonas* with crop species such as wheat, tomato, potato and others are many [25, 58–63] but such studies using *Abelmoschus esculentus* (Okra) as a model crop species and its associated endophytic fluorescent *Pseudomonas* communities are scanty. Wheat cultivars exerted effect on culturable Pseudomonas populations in their rhizosphere [61]. Similarly out of the three cultivars 'OH102' and 'Gitanjali' showed fluorescence exhibiting isolates except 'Sangur' cultivar. But Vacheron *et al* [51] showed no such maize cultivar effects on fluorescent *Pseudomonas*. Soil also was shown to influence the cultivable *Pseudomonas* populations, in the present study soil also may have influenced *Pseudomonas* populations with respect to the cultivars [61]. The isolates in the present study showed plant growth promotion properties (IAA, Siderophore production) and HCN production. *Pseudomonas* isolated from Strawberry were shown positive for HCN production [25]. *Pseudomonas* species were also shown to be IAA producers, Siderophore, HCN, and catalase producers [64]. Increased seed germination was shown by the present tested isolates, this property may be attributed to the IAA production. Pseudomonas species were reported to be IAA producers and showed an increase in seed germination potential [17, 65, 66]. The present isolates were able to inhibit the growth of some bacterial species. It was reported that Pyocyanin pigment from *Pseudomonas aeruginosa* was able to inhibit the drug resistant isolates of *Staphylococcus aureus* and *Candida albicans* than *E. coli, Klebsiella* spp., *Shigella* spp. and *Salmonella typhi* [67]. This inhibition was also shown to be dependent on the concentration of Pyocyanin [67]. Antimicrobial Activity of Pyocyanin produced by *Pseudomonas aeruginosa* was shown to inhibit gram positive bacteria also [68]. Bacteria isolated from wound (Pseudomonas aeruginosa, *Staphylococcus spp.*, E. coli) and urinary tract infections (*Pseudomonas aeruginosa*, *Staphylococcus spp.*, *E. coli* and *Bacillus spp.*) were also shown to be inhibited by pyocyanin pigment [69]. A phosphate solubilizing *Pseudomonas* isolate was shown to inhibit gram positive (*Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus*) more than gram negative (*Escherichia coli*, *Vibrio cholerae* and *Shigella flexneri*) organisms [70].

5. Conclusions

The present study indicated that cultivar specificity exists with respect to cultivable *Pseudomonas* populations. The isolates showed PGP properties and as well as inhibition of bacteria, whose members are known to be pathogenic in nature. Such isolates may help us in inhibiting pathogens on fresh vegetables that are consumed directly (e.g. salads) and as well as promote the plant growth. But such studies needs to be further validated using molecular methods and field studies.

6. Acknowledgements

We would like to thank Uka Tarsadia University for providing resources to support this work.

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