Isolation and screening of Plant growth promoting *Pseudomonas* sp. from phyllosphere and rhizosphere

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***ABSTRACT*: Use of chemicals as pesticides and fertilizers has been raised in last few decades which increase the yield upto some extant but also resulted in bioaccumulation in living system. Microbes have the ability to support the plant growth without generating toxic residues and act as biofertilizer. In present work, samples were collected from rhizosphere and phyllosphere of *Triticum astivum*. After primary screening total 75 isolates of fluorescent *Pseudomonas* were obtained and further screened for various plant growth promoting (PGP) traits such as Phosphate solubilization, N2 fixation, IAA and biocontrol activity such as siderophore, HCN ammonia production and antifungal activity against selected pathogenic fungi (*Fusarium oxysporum, Rhizoctonia* sp and *Sclerotium* sp). Among these isolates, L14, S23 and Y30 were identified as *Pseudomonas fluorescence*, *Pseudomonas putida* and *Pseudomonas aerofacienss* respectively. Results thus obtained from current work were quite promising as per expected and also suggested the possible substitute for synthetic chemical used in agriculture which ultimately helps in environment conservation.**

***Keywords*: Biocontrol, fluorescence, plant growth promoting rhizobacteria, phyllosphere, rhizosphere.**

INTRODUCTION

India is an agricultural land where more than 65% land is used for agriculture purpose. But to satisfy the need of population, growth in agri-food sector is must but it should be with sustainable approach (Rawat *et al.*, 2011). Generally chemicals are used as biocontrol and biofertilizers which increases the nutrient values of soil essential for the growth of plant but also exposing intense side effects on living system (Sharan, 2011). To avoid harmful impact of chemicals, microbes can be used as substitute to increase the productivity. Microbes with the ability to promoting plant growth influence the plant growth by various direct or indirect mechanisms (Geetha *et al*., 2014). Directly mechanism involved solubilizing phosphate, potassium, oxidizing sulfur, fixing nitrogen, chelating iron and copper, while indirect mechanism involved production of hydrogen cyanide, siderophores, antibiotics and phytohormones. Moreover it also enhances the tolerance of plant towards drought, salinity, metal toxicity (Unni *et al.*, 2015). Earlier many bacterial species from rhizosphere such as *Alcaligens, Acintobacter, Bacillus, Enterobacter, Flavobacterium,* and *Serratia* have been reported with PGPR activity (Glick, 2011; Deepa *et al.*, 2010). These microbes survive on exudates released by plant roots containing amino acids, nucleotides, sugars, lipids etc. (Hermander *et al.*, 2015). Most of these microorganisms play a crucial role in the cycling of elements as saprophytes and in remediating residual pesticides and atmospheric hydrocarbon pollutants, or may act as biofertilizers (Delmotte *et al*., 2009; Innerebner *et al*., 2011; Ali *et al*., 2012).

Among all the PGPR *Pseudomonas* sp. are most promising group of plant growth promoting due to its dual role plant growth promotion and diseases control (Ahmadzadeh *et al.*, 2006). This genus represents most diverse group of bacteria that can be used for the formulation of biofertilizer to replace chemical fertilizers (Sharma *et al.*, 2014). *Pseudomonas* genus is rod shaped, gram-negative, aerobic and non-endospore forming bacteria. Among five classes fluorescent *Pseudomonas* referred as true *Pseudomonas* and found in different environmental conditions (Yadav *et al*., 2013). Fluorescent *Pseudomonas* are also considered to be one of the most promising groups of plant growth promoting bacteria involved in biocontrol of plant diseases (Moeinzadeh *et al.*, 2010; Shivashakti *et al*., 2013). These bacteria can easily be differentiated from other *Pseudomonas* by the production of water soluble yellow-green fluorescent pigment (Pyoveridine and Pyocynine) that fluorescence under UV light (Deniel *et al.*, 1992; Bultreys *et al.*, 2003). Apart from plant growth promotion they also play important role in bioremediation specially in case of metal and oil contamination of soil (Muehe *et al*., 2015). Hence considering all aspects of *Psedomonas* for plant growth promotion (PGP) and biocontrol potential present work was designed for the isolation of bacteria from rhizosphere and phyllosphere of *Triticum astivum* and screening of the isolate for their PGP activity.

MATERIAL & METHODS

Isolation and preliminary screening of bacterial isolates

For the isolation of *Pseudomonas* from rhizosphere and phyllosphere, soil and leaf samples were collected in sterile plastic bags from Kandaghat, Kalighat from, Solan (H.P.). Isolation was done on king's B medium. The plates were incubated at 30˚C for 24 h. Among the obtained isolates, fluorescent *Pseudomonas* was detected by exposing them to UV light, and fluorescent colonies were considered as fluorescent *Pseudomonas.*

Screening for Plant growth promotion and biocontrol activity

All the fluorescent *Pseudomonas* isolates were screened for plant growth promoting and biocontrol activities viz. IAA solubilization, phosphate solubilization, HCN production, siderophores production, antifungal activity.

IAA production: IAA production by the isolates was estimated by using Salkowaskis reagent. Appearance of pink color was indicating IAA production which can be read at 535nm. For quantitative estimation IAA prepared in 50% ethanol was used as standard (Glickmann and Dessaux, 1995; Kumar *et al.*, 2011).

Phosphate solubilization activity: All bacterial isolates were then screened for inorganic phosphate solubilization. Qualitative estimation was done by using Pikovskaye medium containing tri-calcium phosphate, iron phosphate. Positive results can be recorded by formation of clear halo zone around the culture (Pikovskaye, 1948). For quantitative estimation of inorganic phosphate solubilization ability was done as per methodology described by Jackson (Jackson *et al.*, 1973) and Nautiyal (Nautiyal *et al.*, 2001).

Siderophores production

Siderophore production ability of isolates was determined by using Chrome Azurol dye and CAS agar plate. Iron consumption and siderophores formation ability was determined by production of orange color and formation of orange halos around the colonies (Schwyn and Neilands, 1987).

Hydrogen cyanide production

The production of HCN was detected by spreading 1 ml of 24 h old broth culture on the King's B medium supplemented with 4.4g/l glycine and incubated with the whatmann filter paper flooded with the solution containing 0.5% picric acid in 2% sodium carbonate. After 24-48 h, yellow to brown change in the color of the filter paper was observed (Castric, 1975).

Nitrogen fixation ability

Nitrogen fixation ability if the isolates were tested using a nitrogen free medium (Jensen's media). Formation of water droplets showed nitrogen fixation by the isolates.

Ammonia production

All the bacterial isolates were tested for the production of ammonia using Nessler's reagent. Production of ammonia can be detected by formation of faint yellow to dark brown color (Cappuccino and Sherman, 1992).

***In vitro* antifungal activity**

A 10 mm disk of a pure culture of *Fusarium* sp., *Rhizoctina* sp. and *Sclerotinia* sp. were placed at the center of a Petri dish containing PDA (Potato Dextrose Agar). A loopful bacterial isolates were streaked on PDA 1.5cm from the edge of each plate. Plate was cultured for 72 h at 30°C and then the percent of radial growth inhibition (PIRG) were recorded by the following formula (Naureen *et al.*, 2009):

$$PIRG = (C-T/\ C) \times 100$$

C= Radial growth of fungal pathogen

T = Growth of test.

Biochemical identification:

The most efficient bacterial isolate was identified on the basis of its morphological and biochemical characteristics as described by Bergey's Manual of Systematic Bacteriology (Brenner *et al*., 2005).

RESULTS & DISCUSSION

Rhizosphere and phyllosphere samples of wheat plant were collected from Kandaghat, Kalighat of Solan district Himachal Pradesh (figure 1).

Isolation and preliminary screening of bacteria

Bacterial isolates were obtained on King's B media from sample collected from rhizosphere and phyllosphere of wheat plants (figure 2). All the isolates were visualized under UV light to select fluorescent *Pseudomonas* (figure 3). After primary screening total 75 fluorescent bacteria were obtained among them 30 isolates from rhizosphere showed yellow fluorescence (designated as Y1toY30) while 45 isolates (20 from phyllosphere and 25 from rhizosphere; showing green fluorescence and designated as L1 - L20 and S1-S25.

Hashidoko *et al*., (2002) and Singh *et al*., (2013) studied microflora of rhizosphere and phyllosphere of different plants. Majeed *et al*., (2015) have screened these isolates for their nitrogen fixation and growth promoting ability. O'sullivan *et al*., (1992) identify fluorescent *Pseudomonas* by the production of water soluble yellow-green pigment. Recently Li and his team (2017) worked on *Pseudomonas* sp for ther plant growth promotion ability and also studied genetic diversity in them. During their work they also found that *P. koreensis and P. entomophila* is suitable for production of bio-fertilizers.

Phosphate solubilization:

After primary screening all the isolates were spotted on pikovskaya agar to check their phosphate solubilizing activity. Almost all isolates were able to solubilizing phosphate and give clear zone (figure 4a) but only 20 isolates gave the zone more than 5mm and there were further quantified byusing NBRIP broth (Figure 4b). Parani K and his colleagues (2012) have studied phosphate solubilizing ability of bacterial isolates using media containing insoluble mineral phosphate such as tri calcium phosphate by formation of clear zone. In year 2013, Lavania and Nautiyal screened 15 different substrates for estimation of phosphate solubilization. The concentration of phosphate (mg/ml) was calculated (Table 1) by using standard graph shown in figure 5. On comparing all three substrate maximum solubilization was recorded using Tricalcium phosphate as substrate.

IAA production

Substantial production of IAA by *Pseudomonas* isolates were estimated by using nutrient broth with or without tryptophan. In presence of IAA in presence of tryptophan and gave pink color (figure 6). As the concentration of tryptophan in the medium increases, the amount of IAA produced increased. It was possible that tryptophan induced the synthesis of enzymes which function in the synthesis of IAA (Joshi *et al*., 2015).

As also reported earlier, similar results were obtained as in the presence of tryptophan the maximum increase in production of IAA is 35 folds than that in absence of tryptophan in the media which suggested that tryptophan may as precursor for IAA.

Siderophore production:

Siderophore are commonly referred to as microbial Fe-chelating low molecular weight compounds. The presence of siderophore-producing PGPR in rhizosphere increases the rate of Fe^{3+} supply to plants and therefore enhances the plant growth and productivity of crop (Singh, 2013).

In present study to check the siderophore activity all the 75 strains were spotted on blue-CAS agar plates. All the isolated strains were having Fe- chelating ability and hence give orange hole around the colony as shown in figure 8 and table 3. It was studied that strains of *Pseudomonas* could grow on CAS agar and were considered as siderophore producers because of formation of orange holes around colonies. Production of siderophore may indirectly influence the plant growth (Parani K *et al*., 2012)

Ammonia production

Isolates were screened for ammonia production by Nessler's reagent which forms orange to brown color (figure 9; Table 3). Utilization of PGPR in order to increase the productivity may be a viable alternative to organic fertilizers which also helps in reducing the pollution and preserving the environment (Walpola and Yoon, 2013).

HCN production:

HCN production by rhizobacteria has been postulated to play an important role in the biological control of pathogens. Glycine acts as precursor for HCN production from which HCN is oxidized by two microbial enzymes, glycineoxygen oxidoreductase and glycine dehydrogenase for the production of HCN and CO_2 (Peter, 1975). During screening all the isolates were found to have potential of HCN production and resulted in change the color of filter paper from white to brown (Figure 10 and Table 3). Wani and co-workers in 2007 and Kannapiran and co-workers in 2011 also tested the rhizosphere isolates for HCN production by same method using filter paper.

Nitrogen-Fixing activity:

Nitrogen fixation ability of rhizobacteria is an important feature for plant growth promotion as it made nitrogen available to plants in form of ammonia (NH_3) and enhance plant growth (Chaiharm *et al.*, 2008). All the isolates showed the droplet formation after 24 h of incubation which indicated their nitrogen fixing ability (Figure 11). Tariq and co- workers (2015) also found that *Pseudomonas* sp. having ability to fix atmospheric nitrogen.

Antifungal activity:

The basis of antibiosis (activity of biocontrol), based on secretion of molecules that kill or reduce the growth of the target pathogen. Several substances produced by antagonistic bacteria have been related to pathogen control and indirect promotion of growth in many plants (Glick *et al*., 2011). In present study all the isolated strains were screened for antifungal activity by testing against plant pathogenic fungi *Fusarium* sp., *Rhizoctonia* sp. and *Sclerotium* sp. Among all isolates Y2, Y3, Y4, Y5, Y8, Y12, Y14, Y15, Y17 strains have antifungal activity against *Rhizoctonia* sp. (Figure 12), Y3, Y6, Y17, Y19, Y30, S19, S21, S23, S25 having antifungal activity against *Sclerotium* sp. (Figure 13) and S13, S22, S24, S25, Y2, Y6, Y7, Y10, Y17, Y19, L14, L15 strains having antifungal activity against *Fusarium* sp. Similar work was also carried out by Parani *et al*. (2012) that the strains of *Pseudomonas* sp. also showed antimicrobial activity against *Fusarium oxysporum* and many other phytopathogen filamentous fungi. Percentage radial growth inhibition was calculated (Table 4) by formula:

C-T/C*100 where C= growth of control and T= growth of test.

Biochemical characterization of most efficient isolate:

After analyzing PGP and biocontrol activity, out of 75 strains of fluorescent *Pseudomonas* most efficient isolate L14, S23 and Y29 were selected for biochemical characterization (Table 5). All the three isolates were found to be gram negative, motile rods. From the biochemical tests and according to Burgey's manual of systematic bacteriology it was confirmed that isolate L14 was *Pseudomonas fluorescence*, isolate S23 was *Pseudomonas putida* and isolate Y30 was *Pseudomonas aerofaciens* as shown in figure 14 (Benner *et al*., 2005).

SUMMARY AND CONCLUSION

In present scenario, chemical fertilizers are generally used to supply essential nutrients to the soil–plant system throughout the world. However, the prices and the environmental concerns of chemical fertilizers are real issues of today's agriculture. Therefore, biological systems such as microbes can be used as substitute for chemicals upto some extent for enhancing the yield without leaving any hazardous impact on environment. Therefore, the study was focused on searching some plant growth promoting bacteria such as fluorescent *Pseudomonas* from the phyllosphere and Rhizospheric soil of *Triticum astivum.* After primary screening of obtained isolates 75 isolates were found to be fluorescent *Pseudomonas* which were further screened for their plant growth and biocontrol potential. The most efficient isolates were then characterized according to Bergey's manual of systematic bacteriology by biochemical analysis and identified as *Pseudomonas fluorescence, Pseudomonas putida* and *Pseudomonas aerofaciens.* From the present study it was concluded that fluorescent *Pseudomonas* were most prominent bacteria in rhizosphere as well as on phyllosphere of the wheat plant. Along with rhizosphere, phyllospheric bacteria also show positive result to maximum PGPR traits and can be used as biofertilizer which helps in plant growth promotion and protection of plant form pathogenic fungus and act as biocontrol agent. Among all the isolated strains of fluorescent *Pseudomonas* i.e. *Pseudomonas fluorescence, Pseudomonas putida* and *Pseudomonas aerofaciens* the best acting PGPR was *Pseudomonas putida* and can be most preferred biofertilizer because it is not an opportunistic organism like *Pseudomonas fluorescence* and beneficial to both plant and mankind.

Table:

Table 1: Concentration of phosphate solubilized by isolated strains of bacteria:

Isolates	Diameter of zone (mm)	Conc. of Phosphate solubilization (mg/ml) when *Tricalcium* phosphate is substrate	Conc. of Phosphate solubilization (mg/ml) when Ammonium dihydrogen phosphate is substrate	Conc. of Phosphate solubilization (mg/ml) when Phosphorus dihydrogen phosphate is substrate
S9	6mm	3.4	0	0
S10	5mm	4.51	0	0.5
S11	11mm	7.6	0.1	0.7
S15	10mm	3.2	0	0
S16	8mm	3	2	0.9
S19	6mm	3.5	0	0.7
S21	5mm	4	0	0
S23	6mm	6	0.2	0.2
Y11	11mm	4.52	0	3
Y13	5mm	4.55	0	3.9
Y15	6mm	4	0	0
Y16	5mm	3.2	0.5	1
Y17	17mm	7.4	1.4	1.8
Y26	8mm	5.6	0.9	3.2
Y28	12mm	6.1	0	3.1
Y30	9mm	5.1	0	3.5
L6	5mm	3.9	0	0
L14	6mm	2.8	0	3.7
L19	8mm	3.44	0	1
L20	5mm	3.5	1.1	0.2

Table 2: Concentration of IAA produced by isolated strains of bacteria:

Isolates	Conc. Of IAA in tryptophan free media mg/ml	Conc. Of IAA in presence of tryptophan as substrate mg/ml	Increase in conc. (folds)
S1	Require tryptophan	1	10
S2	Require tryptophan	1	10
S3	0.5	2	4
S4	0.2	1.1	5.5
S5	0.25	2.3	9.2
S6	Require tryptophan	0.55	5
S7	0.54	0.7	1.2
S8	Require tryptophan	1	10
S9	0.3	0.52	1.7
S10	Require tryptophan	0.56	5
S11	0.54	1.45	2.6
S12	0.29	1.2	4.1
S13	Require tryptophan	1.25	12
S14	Require tryptophan	0.6	6
S15	0.29	2.3	7.9
S16	Require tryptophan	0.32	3.2
S17	0.48	1.1	2.2
S18	0.22	0.7	3.5
S19	Require tryptophan	1.46	1.4
S20	0.2	0.9	4.5
S21	Require tryptophan	1.5	1.5
S22	0.3	2.4	8
S23	Require tryptophan	1.59	15
S24	0.46	2.2	4.7
S25	0.51	1.84	3.6
L1	1.4	2.9	2
L2	Require tryptophan	1.9	19
L3	1.5	4	2.6
L4	Require tryptophan	0.7	7
L5	Require tryptophan	0.68	6

L6	1.6	4.8	3
L7	0.85	1.45	1.7
L8	2.2	5.7	2.5
L9	1	5.4	5.4
L10	Require tryptophan	1.9	19
L11	Require tryptophan	1.2	12
L12	Require tryptophan	2.5	25
L13	Require tryptophan	1.1	11
L14	0.7	1.5	2
L15	Require tryptophan	0.7	7
L16	0.2	1.4	7
L17	Require tryptophan	1.2	12
L18	1.1	1.2	1
L19	0.21	3.7	17
L20	Require tryptophan	0.9	9
Y1	0.98	1.6	1.6
Y2	0.8	1.2	1.5
Y3	1.3	2.84	2
Y4	2	3.4	1.7
Y5	Require tryptophan	3.5	35
Y6	Require tryptophan	1.8	18
Y7	1.24	2	1.6
Y8	0.4	0.7	1.7
Y9	0.4	1.1	2.7
Y10	0.3	1.21	4
Y11	Require tryptophan	0.8	8
Y12	0.9	2.6	2.8
Y13	0.2	2.65	13
Y14	0.7	1.5	2
Y15	Require tryptophan	2.9	29
Y16	1.1	5.7	5
Y17	1.2	3.3	2.7
Y18	3.5	5.4	1.5
Y19	3.1	5.3	1.7
Y20	3.7	5.4	1.4
Y21	1.2	4.6	3.8
Y22	0.4	3	7.5
Y23	0.6	4.2	7
Y24	0.6	4	6.6
Y25	1.3	4.6	3.5
Y26	Require tryptophan	2.2	22
Y27	Require tryptophan	2	20
Y28	Require tryptophan	2	20
Y29	Require tryptophan	2	20

Y30	0.5	2.8	5.6

Table 3: HCN, Ammonia and Siderophore production activity

S. No	Isolates	Ammonia production	HCN production	Siderophore production
01.	S1	++	+	+
02.	S2	+++	+	+
03.	S3	++	++	+
04.	S4	+++	+++	+
05.	S5	+	++	+
06.	S6	+	+++	++
07.	S7	+	++	+
08.	S8	++	++	+
09.	S9	+++	+++	++
10.	S10	+	+	+++
11.	S11	++	+	+
12.	S12	++	++	+
13.	S13	+++	+	+
14.	S14	++	++	++
15.	S15	++	++	++
16.	S16	+	+	++
17.	S17	+++	+++	+
18.	S18	++	++	+
19.	S19	+++	++	+
20.	S20	+++	++	+
21.	S21	+++	++	+
22.	S22	+++	+++	++
23.	S23	++	+++	+++
24.	S24	++	++	+
25.	S25	++	++	+
26.	L1	+	+ +	++
27.	L2	++	++	+
28.	L3	++	+	+
29.	L4	+	++	+
30.	L5	+	++	+
31.	L6	+	++	++
32.	L7	+	++	++
33.	L8	+	++	+
34.	L9	+	+++	+
35.	L10	+	+++	+
36.	L11	+	+++	+
37.	L12	+++	+++	++
38.	L13	+	++	+
39.	L14	+	+++	+++
40.	L15	+++	+++	+
41.	L16	+	++	+
42.	L17	+	++	+
43.	L18	+	+	+
44.	L19	++	++	++
45.	L20	+++	+++	+
46.	Y1	++	+	+
47.	Y2	+	+	++
48.	Y3	++	++	++
49.	Y4	+	++	+
50.	Y5	++	+++	++
51.	Y6	+	+	++
52.	Y7	+	+	++
53.	Y8	+	+	+
54.	Y9	++	+++	+++
55.	Y10	+	+	+

56.	Y11	+	+	++
57.	Y12	++	+	++
58.	Y13	+	+	+
59.	Y14	+	+	+
60.	Y15	++	+	+
61.	Y16	+++	++	+
62.	Y17	+	++	+
63.	Y18	+	+	++
64.	Y19	++	+	+++
65.	Y20	++	++	+
66.	Y21	++	++	+
67.	Y22	+	++	++
68.	Y23	+	+	++
69.	Y24	+	++	+
70.	Y25	++	++	+
71.	Y26	++	+	+
72.	Y27	+	+	+
73.	Y28	+	++	++
74.	Y29	+	++	+
75.	Y30	++	+++	++

Interpretations: +=less activity; ++=moderate activity; +++=high activity.

Table 4: Percentage Radial growth inhibition of isolates against pathogenic fungi:

Sr. No.	Isolates having antifungal activity against *Rhizoctonia*	Growth of control (mm)	Growth of test (mm)	% radial growth inhibition= C-T/C*100
1.	Y2	60	54	10%
2.	Y3	60	55	8.3%
3.	Y4	60	50	16.6%
4.	Y5	60	58	3.33%
5.	Y8	60	52	13.3%
6.	Y12	60	54	10%
7.	Y14	60	57	5%
8.	Y15	60	50	16.6%
9.	Y30	60	54	10%
Isolates having antifungal activity against *Fusarium*				
1.	S13	40	35	8.3%
2.	S23	40	36	6.6%
3.	S24	40	35	8.3%
4.	S25	40	34	10%
5.	L14	40	32	13.3%
6.	L15	40	37	5%
7.	Y2	40	30	16.6%
8.	Y6	40	34	10%
9.	Y7	40	32	13.3%
10.	Y10	40	33	11.6%
11.	Y17	40	33	11.6%
12.	Y19	40	34	10%
Isolates having antifungal activity against *Sclerotium*				
1.	S19	80	77	5%
2.	S21	80	76	6.6%
3.	S23	80	72	13.3%
4.	S25	80	75	8.3%
5.	Y3	80	73	11.6%
6.	Y6	80	73	11.6%
7.	Y17	80	75	10%
8.	Y19	80	77	5%
9.	Y30	80	76	6.6%

Y29 (*Psuedomonas aerofaciens*)

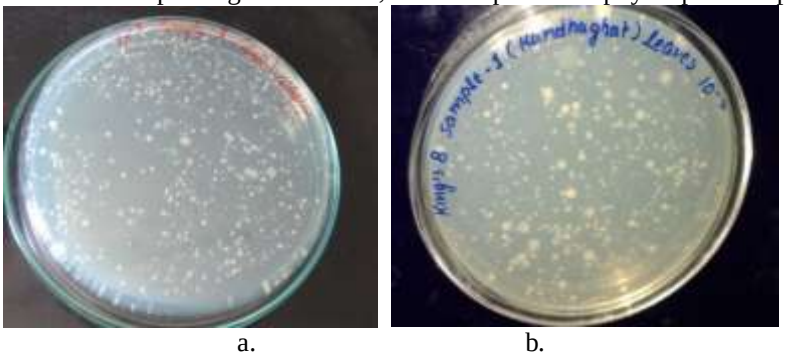
Table 5: Morphological and biochemical characterization of isolates:

Sr. No.	Methods	Isolate L14	Isolate S23	Isolate Y30
1.	**Colony morphology on King's B media**	Round, mucoid, creamish color colonies of intermediate size with greenish pigmentation	Oval shaped, small, creamish color colonies with greenish pigmentation	Small, round creamish color colonies with orange pigmentation
2.	**Appearance under U.V. light**	Green fluorescence	Green fluorescence	Yellow fluorescence
3.	**Gram staining**	Gram –ve, rods	Gram –ve, rods	Gram –ve, rods
4.	**Biochemical analysis**			
	Indole	-ve	-ve	-ve
	Methyl Red (MR)	-ve	-ve	-ve
	Voges Proskauer (VP)	-ve	-ve	-ve
	Citrate	+ve	+ve	+ve
	Nitrate	-ve	-ve	+ve
	Oxidase	+ve	+ve	-ve
	Urease	-ve	-ve	-ve
	Triple Sugar Iron (TSI)	K/A	K/A	A/A
	Motility	+ve	+ve	+ve
	Catalase	+ve	+ve	+ve
	Gleatine hydrolysis	+ve	-ve	
	Glucose fermentation	+ve	+ve	-ve
	Lactose fermentation	-ve	-ve	+ve
	Sucrose fermentation	+ve	+ve	+ve
	Mannitol fermentation	-ve	-ve	+ve
5.	**Species**	*Pseudomonas fluorescence*	*Pseudomonas putida*	*Pseudomonas aerofaciens*

Figure:

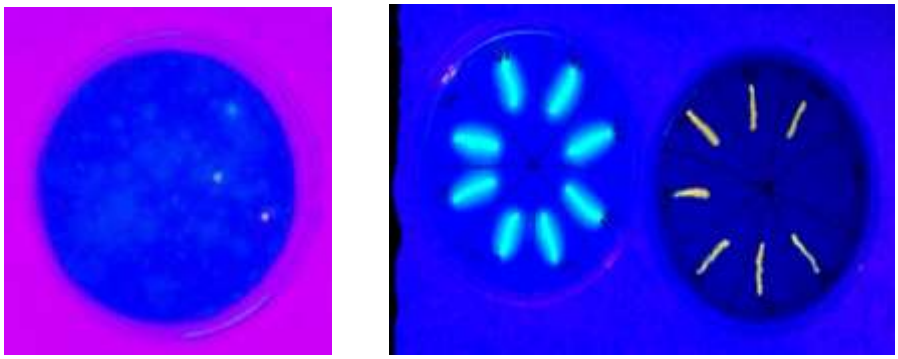
a b

Figure 1: a. Wheat plants grown in field, b. Rhizosphere and phyllosphere of plant



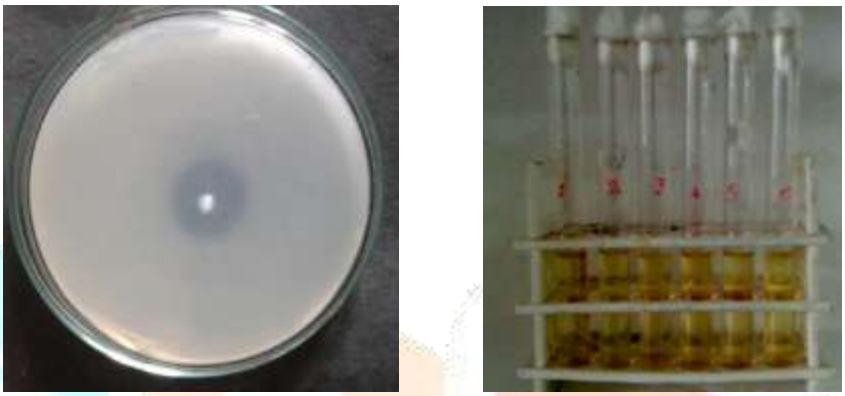
a. b.

Figure 2: Bacterial isolates on King's B media from a. Rhizosphere and b. phyllosphere



a. b.

Figure 3: Fluorescent *Pseudomonas* isolates under U.V. light

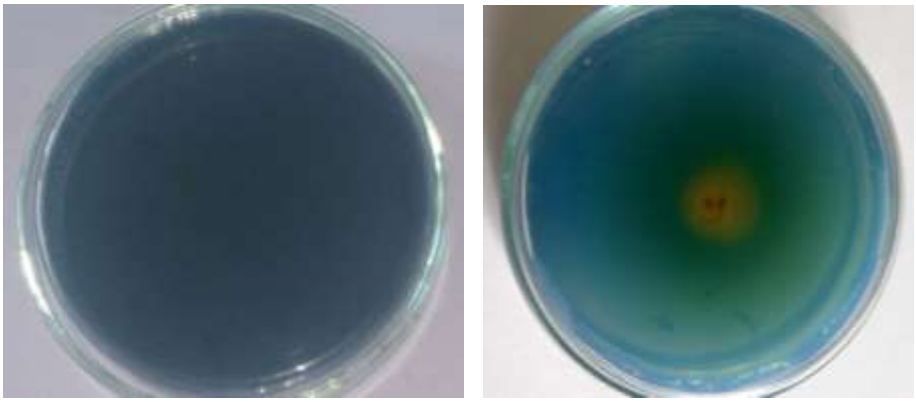


a. b.

Figure 4: Phosphate solubilizing ability of *Pseudomonas* a. Halo zone showing P solublization b. Quantitative estimation

a. b.

Figure 6: Qualitative and quantitative estimation of IAA production a. 5 days old broth culture b. pink color indicates IAA production



a. b.

Figure 8: Siderophore production activity by fluorescent *Pseudomonas* isolate a. control b siderophore production

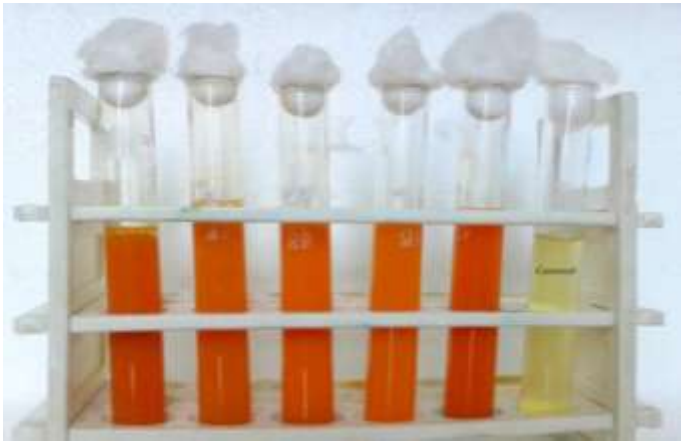


Figure 9: Ammonia production activity by isolated strains of fluorescent *Pseudomonas*

a. b.

Figure 10: Isolated strains showing HCN production a. control plate b. Brown color indicates HCN production

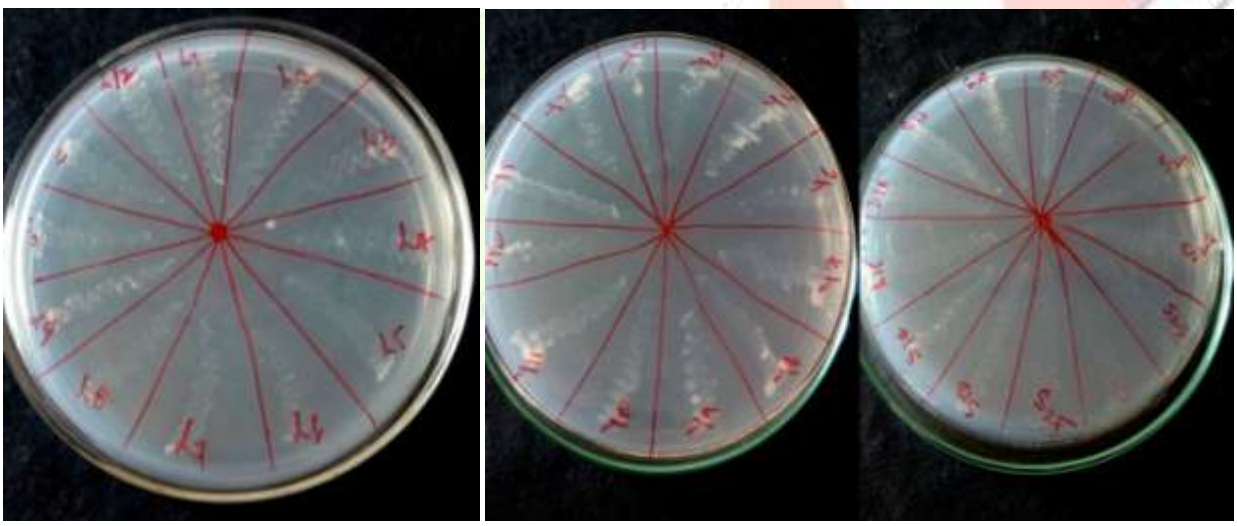


Figure 11: Nitrogen fixation activity of isolated strains of fluorescent *Pseudomonas*

a: Control b. Test

Figure 12: Antifungal activity of strains against *Rhizoctonia* sp.

a: Control b. Test

Figure 13: Antifungal activity of bacterial strains against *Sclerotium* sp.

a.

b.

c.

Figure 14 Various biochemical test a. isolate L14 (*Pseudomonas fluorescence*), b. isolate S23 (*Pseudomonas putida*) and c. isolate

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