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Isolation of Plant Growth Promoting Bacteria from Wheat (*Lok-1*) rhizosphere

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Abstract: ***Interactions between plants and microorganisms in the rhizosphere (rhizobacteria) affects crop yields likewise Plant growth promoting rhizobacteria (PGPR) influence the growth of plant by mean of various direct or indirect mechanisms. In current report the present investigations were undertaken to isolated and screen the PGPR from its natural growing zones of Wheat (Lok-1) rhizosphere. In present study, out of 50 different isolates, only 6 strains showed all the traits of plant growth promotion The isolated PGPR belong to gram positive genera and, were highly motile in nature. The study reviled that, all the isolates exhibited production of in dole acetic acid, Gibberellic acid, indol-3 butyric acid, Siderophore, Ammonia, HCN and solubilized inorganic phosphate. The investigated result supports possible utilization of the selected isolates in wheat Varity(Lok-1);growth promotion with respect to increase in agro productivity.***

Keywords: PGP bacteria, Rhizosphere, WheatVarity(*Lok-1*); IBA, rhizosphere.,

1. Introduction

The rhizosphere, representing the thin layer of soil surrounding plant roots and the soil occupied by the roots,Supports large active groups of bacteria (Villacieros*et al.,* 2003)known as plant growth promoting rhizobacteria (PGPR).(Kloepper*et al.,1980*)PGPR canStimulate plant growth indirectly by inhibiting other deleterious microbes or root pathogens(Lemanceau;1992,Kloepper;1993)The plant-microbe interactions in the rhizosphere are responsible for increasing plant health and soil fertility.(Khan;2006)Indirect mechanisms involves the biological control of plant pathogens and ;deleterious microbes, through the production of hydrogen cyanide, catalase or through competition for nutrients and space can improve significantly plant health and promote growth, as evidenced by increases in seedling emergence, vigour, and yield.(Khan;2006) After N2 fixation, Phosphate (P) solubilization is very important plant growth promoting activity.

Plant growth-promoting rhizobacteria (PGPR) could play a significant role in the development of sustainable agriculture. These are rhizobacteria that are beneficial to plants (Kloepper*et al.,* 1989) and affect plant growth directly or indirectly through various mechanisms of action (Glick *et al.,* 1998; Mantlelin and Touraine, 2004). Indirect promotion occurs when PGPR improved plant by preventing growth restricting conditions (Glick *et al.,* 1999). The direct promotion by PGPR entails either providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of certain nutrients from the environment. Among the plant hormones whose concentrations are most likely to be altered by the PGPR include ethylene, auxin, gibberellins and cytokinin (Xie*et al.,* 1996; Zahir*et al.,* 2005).In plants such as wheat, increased ethylene production shortens grain filling period, decreases in grain weight, and hastens maturity, and triggers senescence and 5premature death (Bais*et al*., 2002).

To the depth of our knowledge and the review indicated that the use of bio inoculant is an eco-friendly and affordable way for promoting the agro productivity.Also, the significant increases in crop yields have been reported and advocated by applying PGPR microbial inoculants.(Salamon;2000) Hence, the present study was carried out to isolate the various plant growth promoting bacteria from the wheat rhizospheric soils.

2. Materials and Methods

Collection of Sample

For present investigation, the ten different rhizospheric soil samples were collected from wheat farms. For the collection and preservation of samples, each sample was collected from 15cm depth and it was packed in a sterile polythene bag and labelled properly. The collected soil samples were enriched in nutrient broth and incubated for 24hrs at 37°C.All the enriched samples were further sub cultured on nutrient agar plates adopting streak plate method. The well isolated colonies were again sub cultured on nutrient agar slant and further screened its characterization for marker biochemical ofPGPR

Screening of Plant Growth Promoting Bacteria

Production of Indole acetic acid (IAA)
Indole acetic acid (IAA) production was detected as described by (Brick *et al.,1991*)The supernatant 2ml ,of each enriched culture was separately mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl3 solution). Development of pink colour indicates IAA production.

Estimation of indol-3 butyric acid (IBA)
The indol-3 butyric acid production by Plant growth promoting rhizobacteria was determined by development of brownish yellow spot on the paper chromatographic set up (Blommaert*et al;1954).*

Estimation of Gibberellic acid (GA)
Gibberellic acid production was estimated by colorimetric method of (Holbrook *et al,*.1961) One ml bacterial cultures was inoculated in 100 ml nutrient broth and allowed to grow

for 72 h at 28°C ± 2°C at 150 rpm. The medium was then centrifuged at 10,000 rpm for 15 min. The culture supernatant was acidified (pH 2.5 using 2.0 N HCl) and extracted by adding equal volume of ethyl acetate in three stages to obtain 45 ml extract. Extract was evaporated to 5.0 ml and gibberellic acid was detected by colorimetric method with reference solution.

Siderophore production (SDP)
It was assayed according to Schwyne and Neilands (1982), Isolates producing an orange halo zone around growth on Chromeazurol S agar (CAS) after 48-72 hours of incubation were considered as positive.

Production ofHydrogen cyanide(HCN)
Hydrogen cyanide (HCN) production was evaluated by streaking the bacterial isolates on King's B agar medium amended with glycine. Whatman No.1 filter paper soaked in picric acid (0.05% solution in 2% sodium carbonate) was placed in the lid of each Petri plate. Theplates were then sealed air-tight with Paraffin and incubated at 30°C for 48 h. A colour change of the filter paper from deep yellow to reddish-brown colour was considered as an indication of HCN production. (Baker *et.al.*,1987).

Phosphate Solubilization (PS)
Phosphate Solubilization Bacterial isolates was evaluated from the ability to solubilize inorganic phosphate. Pikovskaya's agar medium (HiMedia, Mumbai) containing calcium phosphate as the inorganic form of phosphatewas used in this assay. A loopful of bacterial culture were placed on the plates and kept for incubation at 28°C for 7 days. The presence of clear zone around the bacterial colonies indicates the solubilization of phosphate. (Yogendra Singh *et al; 2013*)

Production of Ammonia (AP)
Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube separately and incubated for 48-72 h at 28 ± 2°C. Nesseler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production. (Cappucino*et.al,*.1992).

3. Results and Discussion

Total 50 isolates with different colony characters were observed fromallten soil samples. Only six isolates showed all the traits to secrets the PGP marker biochemical, the result showed that all six isolates were belonging to Gram positive category and are bacillus and motile in nature.(Table no.1&2). The study revealed that, all the isolates exhibited production of indole acetic acid, indol-3 butyric acid,Gibberellic acid and HCN, ammonia production and produced Siderophoreas well as solubilized inorganic phosphate. Keeping the view of Eco physiology the study enlightened the predominance of Gram positive, motile, rod shape bacteria as predominant community among the PGPR community in wheat rhizosphere of Varity(*Lok-1*).,which may be due to the similarities in nutritional requirements between thePGPR bacterial isolates and high compatibility with the type of available nutrients in wheat rhizosphere is possible. Very few reports could be traced with the findings of isolates with the potential to secrete the multiple PGPR markers. Hence, wheat rhizosphere may be the good source and reservoir for PGPR isolation. The investigated result supports the possible utilization of the selected isolates in wheat growth promotion of Varity(*Lok-1*) with respect to increase in agro productivity.

Table 1: Characterisation of wheat rhizospheric isolates

S.No	Isolates No	Size (mm)	Colour	Colony shape	Motility	Grams status	Morphological shape
1	KP-1	1	White	circular	Motile	-	Short rod
2	KP-2	2	White	irregular	Motile	+	Short rod
3	KP-3	2	White	circular	Motile	-	Bacilli
4	KP-4	1	White	irregular	Motile	+	Bacilli
5	**KP-5**	**1**	**White**	**circular**	**Motile**	**+**	**Bacilli**
6	**KP-6**	**1**	**White**	**irregular**	**Motile**	**+**	**Bacilli**
7	KP-7	2	White	irregular	Motile	-	Bacilli
8	KP-8	4	White	irregular	Motile	+	cocci
9	KP-9	2	White	circular	Motile	-	Short rod
10	KP-10	1	White	circular	Motile	+	Bacilli
11	**KP-11**	**2**	**cream**	**circular**	**Motile**	**+**	**Bacilli**
12	KP-12	2	White	circular	Motile	-	Bacilli
13	KP-13	3	White	circular	Motile	+	Short rod
14	**KP-14**	**2**	**yellow**	**circular**	**Motile**	**+**	**Bacilli**
15	KP-15	1	White	circular	Motile	-	Bacilli
16	KP-16	5	White	irregular	Motile	+	cocci
17	KP-17	2	White	circular	Motile	-	Bacilli
18	KP-18	3	White	irregular	Motile	+	Short rod
19	KP-19	4	White	circular	Motile	-	Bacilli
20	KP-20	2	White	irregular	Motile	+	Short rod
21	KP-21	3	White	circular	Motile	-	Bacilli
22	**KP-22**	**2**	**orange**	**circular**	**Motile**	**+**	**Bacilli**
23	KP-23	1	White	circular	Motile	-	Bacilli
24	KP-24	2	White	irregular	Motile	+	Short rod
25	KP-25	2	White	circular	Motile	-	Bacilli
26	KP-26	1	White	irregular	Motile	+	cocci

27	KP-27	2	White	circular	Motile	-	Bacilli
28	KP-28	4	White	irregular	Motile	+	Short rod
29	KP-29	2	White	circular	Motile	-	Bacilli
30	KP-30	2	White	circular	Motile	+	Bacilli
31	**KP-31**	**2**	**brown**	**irregular**	**Motile**	**+**	**Bacilli**
32	KP-32	4	White	circular	Motile	-	Bacilli
33	KP-33	3	White	irregular	Motile	+	Bacilli
34	KP-34	4	White	circular	Motile	-	Bacilli
35	KP-35	2	White	irregular	Motile	+	Bacilli
36	KP-36	2	White	circular	Motile	-	Bacilli
37	KP-37	2	White	irregular	Motile	+	cocci
38	KP-38	1	White	circular	Motile	-	Short rod
39	KP-39	2	White	circular	Motile	+	Bacilli
40	KP-40	2	White	irregular	Motile	+	Bacilli
41	KP-41	1	White	circular	Motile	-	Short rod
42	KP-42	2	White	irregular	Motile	+	cocci
43	KP-43	4	White	circular	Motile	-	Bacilli
44	KP-44	2	White	irregular	Motile	+	cocci
45	KP-45	4	White	circular	Motile	-	Short rod
46	KP-46	2	White	irregular	Motile	+	Bacilli
47	KP-47	1	White	circular	Motile	-	cocci
48	KP-48	4	White	circular	Motile	+	Bacilli
49	KP-49	2	White	irregular	Motile	-	Short rod
50	KP-50	2	White	circular	Motile	+	Bacilli

Positive(+),Negative(-) .

Table 2: characterisation of PGP marker biochemical in wheat rhizospheric isolates

	Isolates	IAA	IBA	GA	SDP	HCN	PS	AP
1	**KP-1**	+	-	-	+	-	-	+
2	**KP-2**	-	-	+	-	+	-	-
3	**KP-3**	-	+	-	-	-	+	-
4	**KP-4**	-	-	+	-	-	-	+
5	**KP-5**	+	+	+	+	+	+	+
6	**KP-6**	+	+	+	+	+	+	+
7	**KP-7**	-	-	-	+	-	-	--
8	**KP-8**	-	-	+	-	-	-	-
9	**KP-9**	-	+	--	-	-	+	-
10	**KP-10**	+	-	-	-	-	+	-
11	**KP-11**	+	+	+	+	+	+	+
12	**KP-12**	-	-	-	+	-	+	-
13	**KP-13**	-	+	-	-	-	-	-
14	**KP-14**	+	+	+	+	+	+	+
15	**KP-15**	+	-	-	-	-	-	+
16	**KP-16**	+	-	-	-	-	-	+
17	**KP-17**	-	-	+	-	-	-	+
18	**KP-18**	-	-	+	+	-	-	-
19	**KP-19**	-	-	+	-	-	-	-
20	**KP-20**	-	-	+	+	-	-	-
21	**KP-21**	+	-	-	-	-	-	-
22	**KP-22**	+	+	+	+	+	+	+
23	**KP-23**	+	-	-	+	-	-	+
24	**KP-24**	-	-	+	-	-	-	+
25	**KP-25**	-	+	-	-	-	+	-
26	**KP-26**	+	-	-	+	-	-	+
27	**KP-27**	+	-	-	-	+	-	-
28	**KP-28**	-	-	+	-	-	-	-
29	**KP-29**	-	-	-	+	-	-	-
30	**KP-30**	+	-	-	-	-	+	-
31	**KP-31**	+	+	+	+	+	+	+
32	**KP-32**	+	-	-	-	-	-	+
33	**KP-33**	-	+	-	-	-	-	-
34	**KP-34**	-	-	+	-	-	-	+
35	**KP-35**	-	-	+	+	-	-	-
36	**KP-36**	-	-	+	-	-	-	-
37	**KP-37**	-	-	+	+	-	-	-

38	**KP-38**	+	-	-	-	-	-	-
39	**KP-39**	+	-	-	-	-	-	+
40	**KP-40**	+	-	-	-	-	-	+
41	**KP-41**	-	-	+	-	-	-	-
42	**KP-42**	-	-	+	-	-	-	+
43	**KP-43**	-	-	+	+	-	-	-
44	**KP-44**	-	-	+	-	-	-	-
45	**KP-45**	-	-	+	+	-	-	-
46	**KP-46**	+	-	-	-	-	-	-
47	**KP-47**	+	-	-	+	-	-	+
48	**KP-48**	-	+	-	-	-	-	-
49	**KP-49**	-	+	-	-	-	+	-
50	**KP-50**	+	-	-	+	-	-	+

Present(+), Absent (-) .

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