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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(8): 673-680

www.thepharmajournal.com
Received: 19-05-2022
Accepted: 22-06-2022

Bio chemical characterization of bacterial microflora from rhizospheric soils of pigeonpea in Telangana

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Abstract
Pigeon pea is the second most important crop in the world and Fusarium wilt is the major disease causing yield loss. Total of forty two rhizosphere soil samples were collected from different pigeon pea growing areas of Telangana state and total of one twenty four bacterial isolates were isolated from 42 soil samples. Diversity of bacterial microflora among all the bacterial isolates were characterized for their morphological, cultural and biochemical characteristics. There was a considerable variation in colony morphology both in type and number among bacterial isolates. Among the 124 rhizosphere isolates evaluated for Methyl red production, 49 isolates tested positive, 32 isolates tested positive for Voges Proskauer test, 103 isolates tested as positive for citrate utilization,86 isolates tested as positive for oxidase test, 98 tested positive for the catalase test only 23 isolates tested positive for the Indole test. Of the total 124 isolates tested, 53 isolates were KOH test positive and had negative Gram reaction while 71 tested negative for the KOH test and gave a positive Gram reaction. Thus, this study suggests that there is a considerable variability among rhizosphere bacterial isolates of pigeon pea.

Keywords: Pigeonpea, biochemicals, bacteria, rhizosphere, *Fusarium udum*

Introduction
Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the most important pulse crops of semi -arid tropic and sub tropic regions *viz.*, Asia and Africa (Maesen, 1990) [10]. It is a valuable multipurpose legume, possessing traits for enhancing the sustainability of dry sub-tropical and tropical farmlands. It is an economically important grain legume crop in the developing countries of the tropical and subtropical regions of the world. India alone accounts for about 80% of total production of pigeonpea. Nowadays, modern agriculture relies on excessive use of chemical fertilizers and pesticides to increase crop production which caused severe adverse effect on soil health and environment. The rhizobacteria facilitate plant growth and development with a wide variety of direct and indirect mechanisms under stress and non -stress conditions (Nadeem *et al.*, 2014) [17]. Interaction between antagonistic micro- organisms and plant pathogens can be used to reduce the fungal diseases of crop plants. PGPRs can be defined as beneficial bacterial strains that colonize the roots of plant for plant growth stimulation and bio control potential. They can affect plant growth by promoting plant-microbe symbiosis, competition for colonization space and nutrients and decreasing the activities of plant pathogens.
In pigeon pea cultivation, *Fusarium udum* incited wilt disease is a major biotic factor that adversely affects crop growth (30–60% of disease incidence occur during flowering and maturity stage) and yield (Sharma *et al.*, 2016) [13]. Management of fusarium infection is often a daunting task mainly due to its soil-borne nature, staying deep in the interior of host tissue, synthesis of persistent dormant structures, and ability to survive and sustain for a longer time without host plants (Boukerma *et al.*, 2017) [1]. In recent, the advancement in biological control and microbial antagonist of phytopathogens has become emerging research areas in Agricultural sciences (Gao *et al*., 2017) [4].
PGPR is a set of plant-associated useful bacterial community that positively influence the plant growth and development (Huang *et al.*, 2016) [6], improve the yield of the crop (Liu *et al.*, 2016) [8], enhances better assimilation of plant nutrients (Calvo *et al.*, 2016) [2], ameliorates crops abiotic stresses (Manjunatha *et al.*, 2019) [11] and that generally portray plant disease biocontrol (Xiang *et al.*, 2017) [15]. So we have conducted bio chemical characterization with 124 rhizosphere isolates to test the variability among isolated rhizospheric bacterial isolates of pigeonpea.

Material and Methods
Collection of soil sample
The soil samples were collected from the rhizosphere soil of pigeonpea from farmer's fields in Telangana State. Total of 42 soil samples were collected up to the depth of 10 to 15cm from the rhizosphere of pigeonpea. The soil intimately adhering to the roots was collected by brushing of root system soil (Yanai *et al*., 2003) [14] and mixed to provide a composite soil sample into small sterilized polythene bags and brought to laboratory for further studies.

Isolation of rhizosphere microflora
Serial dilution was used for the isolation of fungi and bacteria from pigeon pea rhizosphere soil. Ten grams of rhizosphere soil sample was suspended in 90 ml of sterile distilled water blank and kept in a rotary shaker at 160 rpm for 30-45 minutes for constant mixing of soil. Then one ml of 10^{-1} dilution was transferred to a test tube containing 9 ml of sterile distilled water blank using a 1 ml micropipette which gives 10^{-2} dilution. This step was followed repeatedly to obtain concentrations 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} respectively. The isolates were designated using rhizosphere soil sample number followed by serially numbered i.e. (S1RBI-1 (Rhizospheric bacterial isolate number 1from sample-1) to S42RBI-124 (Rhizospheric bacterial isolate number 124 from sample -42)

Cultural characteristics of rhizosphere isolates isolated on King's B, nutrient agar:
Culture characteristics like Colony shape, Colony margin, Colony elevation, Colony appearance, pigmentation of culture were studied for total 124 isolates of rhizospheric bacteria.

Biochemical characterization of rhizosphere isolates
The rhizosphere isolates isolated on King's B, nutrient agar media were studied for their biochemical characteristics and results are presented in Table 1. The biochemical tests including indole production test, methyl red test, Voges Proskauer test, citrate utilization test, oxidase test, catalase test and KOH test along with gram staining were conducted for each of 124 isolates.

Table 1: Culture and morphological characterization of bacterial isolates of rhizobacteria of pigeonpea

S.no	Sample code	Isolate code	Colony Shape	Colony Margin	Colony Elevation	Colony Appearance	Pigmentation of culture
01	S1	S1RBI-1	Circular	Wavy	Slightly raised	Veined	White
02		S1RBI-2	Circular	Entire	Raised	Rough	Creamy white
03		S1RBI-3	Circular	Wavy	Slightly raised	Smooth	White
04	S2	S2RBI-4	Circular	Wavy	Slightly raised	Smooth	Yellowish green
05		S2RBI-5	Irregular	Wavy	Flat	Smooth	Creamy white
06		S2RBI-6	Circular	Entire	Slightly raised	Shiny	Pale white
07		S2RBI-7	Circular	Entire	Flat	Shiny	Yellowish green
08	S3	S3RBI-8	Irregular	Wavy	Raised	Smooth	Peach
09		S3RBI-9	Circular	Wavy	Slightly raised	Rough	Creamy white
10		S3RBI-10	Irregular	Wavy	Flat	Smooth	Greyish white
11	S4	S4RBI-11	Circular	Entire	Flat	Rough	Cream white
12	S5	S5RBI-12	Circular	Entire	Raised	Veined	Pale white
13	S6	S6RBI-13	Circular	Wavy	Slightly raised	Smooth	Creamy white
14		S6RBI-14	Circular	Wavy	Flat	Shiny	Yellowish green
15	S7	S7RBI-15	Irregular	Wavy	Raised	Smooth	Cream
16		S7RBI-16	Circular	Wavy	Flat	Veined	Creamy white
17		S7RBI-17	Irregular	Entire	Slightly raised	Shiny	Pale white
18	S8	S8RBI-18	Circular	Entire	Raised	Rough	Creamy white
19		S8RBI-19	Irregular	Wavy	Flat	Shiny	Pale white
20		S8RBI-20	Circular	Entire	Raised	Smooth	Yellow
21	S9	S9RBI-21	Circular	Wavy	Slightly raised	Rough	Creamy white
22		S9RBI-22	Irregular	Wavy	Flat	Smooth	Creamy white
23		S9RBI-23	Circular	Entire	Slightly raised	Veined	Greyish white
24	S10	S10RBI-24	Circular	Wavy	Flat	Smooth	Cream
25		S10RBI-25	Irregular	Entire	Raised	Smooth	Orange
26		S10RBI-26	Circular	wavy	Flat	Shiny	Yellowish green
27	S11	S11RBI-27	Irregular	Wavy	Flat	Rough	Greyish white
28		S11RBI-28	Circular	Entire	Raised	Smooth	Creamy white
29		S11RBI-29	Circular	Entire	Raised	Smooth	Pale white
30		S11RBI-30	Irregular	Wavy	Flat	Shiny	Creamy white
31	S12	S12RBI-31	Circular	Entire	Slightly raised	Rough	Greyish white
32		S12RBI-32	Circular	Entire	Raised	Smooth	Creamy white
33	S13	S13RBI-33	Circular	Entire	Raised	Smooth	Creamy white
34	S14	S14RBI-34	Irregular	Wavy	Flat	Smooth, shiny	Yellowish green
35		S14RBI-35	Circular	Entire	Raised	Shiny	Pale white
36		S14RBI-36	Circular	Wavy	Flat	Rough	Creamy white
37		S14RBI-37	Irregular	Entire	Raised	Smooth	Pale white
38	S15	S15RBI-38	Circular	Wavy	Flat	Shiny	Yellowish green
39		S15RBI-39	Irregular	Wavy	Flat	Smooth	Pale white
40		S15RBI-40	Circular	Wavy	Raised	Smooth	White
41	S16	S16RBI-41	Circular	Entire	Raised	Shiny	Yellow
42		S16RBI-42	Irregular	Wavy	Flat	Rough	Cream

43		S16RBI-43	Circular	Entire	Raised	Rough	White
44	S17	S17RBI-44	Circular	Entire	Raised	Smooth	Yellowish green
45		S17RBI-45	Irregular	Wavy	Flat	Smooth	Creamy white
46		S17RBI-46	Circular	Entire	Slightly raised	Rough	Creamy white
47		S17RBI-47	Circular	Entire	Slightly Raised	Shiny	Yellowish green
48	S18	S18RBI-48	Circular	Entire	Raised	Smooth	Pale white
49		S18RBI-49	Circular	Wavy	Slightly raised	Shiny	Yellowish green
50	S19	S19RBI-50	Irregular	Wavy	Flat	Smooth	Greyish white
51		S19RBI-51	Circular	Entire	Raised	Shiny	Pale white
52	S20	S20RBI-52	Circular	Entire	Slightly raised	Smooth	Pale white
53		S20RBI-53	Irregular	Wavy	Flat	Rough	Cream
54	S21	S21RBI-54	Circular	Wavy	Slightly raised	Smooth	White
55		S21RBI-55	Circular	Entire	Raised	Smooth	Creamy white
56	S22	S22RBI-56	Circular	Entire	Raised	Rough	White
57	S23	S23RBI-57	Irregular	Wavy	Flat	Smooth	Pale white
58	S23	S23RBI-58	Circular	Entire	Slightly raised	Smooth	Cream
59		S23RBI-59	Circular	Entire	Slightly raised	Shiny	Yellowish green
60		S23RBI-60	Irregular	Wavy	Flat	Rough	Peach
61	S24	S24RBI-61	Circular	Entire	Raised	Smooth	Cream
62		S24RBI-62	Irregular	Wavy	Flat	Rough	Cream
63	S25	S25RBI-63	Circular	Entire	Raised	Shiny	Yellowish green
64		S25RBI-64	Circular	Entire	Raised	Smooth	Pale white
65	S26	S26RBI-65	Irregular	Wavy	Flat	Rough	Creamy white
66		S26RBI-66	Circular	Entire	Raised	Rough	Peach
67		S26 RBI-67	Irregular	Wavy	Flat	Veined	Greyish white
68	S27	S27 RBI -68	Circular	Entire	Raised	Smooth	Cream
69		S27 RBI-69	Circular	Entire	Flat	Smooth	White
70	S28	S28 RBI -70	Circular	Entire	Slightly raised	Shiny	Yellowish green
71		S28 RBI -71	Irregular	Wavy	Flat	Smooth	Cream
72		S28 RBI -72	Circular	Entire	Raised	Veined	Greyish white
73		S28 RBI -73	Circular	Entire	Slightly Raised	Shiny	Yellowish green
74	S29	S29 RBI -74	Circular	Entire	Raised	Rough	Cream
75		S29 RBI -75	Irregular	Wavy	Flat	Veined	Greyish white
76	S30	S30 RBI -76	Circular	Entire	Slightly raised	Smooth	Creamy white
77		S30 RBI 77	Circular	Wavy	Flat	Rough	Cream
78	S31	S31 RBI -78	Irregular	Wavy	Flat	Smooth	White
79		S31 RBI -79	Circular	Entire	Raised	Smooth	Greenish yellow
80		S31 RBI -80	Circular	Entire	Raised	Smooth	Creamy white
81	S32	S32 RBI -81	Circular	Entire	Slightly raised	Veined	Greyish white
82		S32 RBI 82	Circular	Entire	Raised	Veined	White
83		S32RBI -83	Irregular	Wavy	Flat	Smooth	Greenish yellow
84	S33	S33 RBI -84	Circular	Entire	Raised	Smooth	Creamy white
85		S33 RBI -85	Irregular	Wavy	Flat	Smooth	Pale white
86		S33 RBI -86	Circular	Entire	Raised	Smooth	Pale white
87		S33 RBI 87	Circular	Entire	Raised	Veined	Greyish white
88	S34	S34 RBI -88	Irregular	Wavy	Flat	Smooth	Yellow
89	S34	S34 RBI -89	Circular	Entire	Raised	Smooth	Pale white
90		S34 RBI -90	Irregular	Wavy	Flat	Shiny	Pale white
91		S34 RBI-91	Circular	Wavy	Slightly raised	Smooth	Greyish white
92	S35	S35 RBI -92	Circular	Entire	Raised	Smooth	Creamy white
93		S35 RBI -93	Irregular	Wavy	Flat	Smooth	Pale white
94		S35 RBI -94	Circular	Entire	Raised	Smooth	Creamy white
95		S35 RBI-95	Circular	Wavy	Slightly raised	Smooth	Shiny
96		S35 RBI -96	Irregular	Wavy	Flat	Veined	Orange
97	S36	S36 RBI -97	Circular	Entire	Raised	Smooth	Shiny
98		S36 RBI -98	Circular	Entire	Raised	Smooth	Pale white
99		S36 RBI -99	Circular	Entire	Slightly raised	Rough	Greyish white
100		S36 RBI100	Irregular	Wavy	Flat	Smooth	Yellowish green
101		S36 RBI -101	Circular	Entire	Raised	Shiny	Pale white
102	S37	S37 RBI -102	Circular	Entire	Raised	Rough	White
103		S37 RBI -103	Irregular	Entire	Raised	Smooth	Cream
104		S37 RBI -104	Circular	Entire	Slightly raised	Smooth	White
105		S37 RBI -105	Circular	Entire	Slightly raised	Rough	Creamy white
106	S38	S38 RBI -106	Circular	Entire	Raised	Smooth	Cream
107		S38 RBI -107	Circular	Entire	Slightly raised	Smooth	Yellowish green
108		S38 RBI -108	Circular	Entire	Raised	Rough	Greyish white
109		S38 RBI -109	Irregular	Wavy	Flat	Smooth	Greenish yellow
110		S38 RBI -110	Circular	Entire	Raised	Veined	Greyish white

111	S39	S39 RBI -111	Circular	Entire	Raised	Smooth	White
112		S39 RBI -112	Irregular	Wavy	Flat	Shiny	Yellow
113	S40	S40 RBI -113	Circular	Entire	Raised	Smooth	Creamy white
114		S40 RBI -114	Circular	Entire	Raised	Smooth	Pale white
115		S40 RBI -115	Circular	Entire	Raised	Rough	Creamy white
116		S40 RBI -116	Irregular	Wavy	Flat	Shiny	Yellow
117	S41	S41 RBI -117	Circular	Entire	Slightly raised	Rough	Cream
118		S41 RBI -118	Circular	Entire	Raised	Shiny	Yellow
119		S41 RBI -119	Irregular	Wavy	Flat	Rough	Greyish white
120		S41 RBI -120	Circular	Entire	Raised	Veined	Creamy white
121	S42	S42 RBI -121	Irregular	Wavy	Flat	Shiny	Yellowish green
122		S42 RBI -122	Circular	Entire	Raised	Smooth	Cream
123		S42 RBI -123	Circular	Entire	Raised	Rough	White
124		S42 RBI -124	Irregular	Wavy	Raised	Smooth	Creamy white

Table 2: Bio chemical characteristics of rhizosphere bacterial isolates of pigeon pea from Telangana state

S. No	Bacterial isolate code	Methyl red test	Voges–proskauer test	Citrate utilization test	Indole production test	Oxidase test	Catalase test	KOH test	Gram staining	Antagonism against *F. udum*
01	S1RBI-1	-	-	+	-	+	+	-	+	-
02	S1RBI-2	-	+	+	-	-	+	-	+	-
03	S1RBI-3	+	-	-	+	+	+	+	-	-
04	S2RBI-4	-	-	+	-	+	+	+	-	+
05	S2RBI-5	+	+	+	-	+	+	-	+	-
06	S2RBI-6	+	-	-	-	+	+	+	-	-
07	S2RBI-7	-	-	+	-	+	-	+	-	+
08	S3RBI-8	+	-	+	-	-	+	-	+	-
09	S3RBI-9	-	+	+	-	+	+	-	+	-
10	S3RBI-10	-	+	+	+	-	+	-	+	-
11	S4RBI-11	+	-	+	-	-	-	-	+	+
12	S5RBI-12	-	-	-	-	+	+	+	-	-
13	S6RBI-13	+	+	+	+	-	+	-	+	-
14	S6RBI-14	-	-	+	-	+	+	+	-	+
15	S7RBI-15	-	+	+	-	-	-	-	+	-
16	S7RBI-16	+	-	+	-	+	+	-	+	+
17	S7RBI-17	-	-	+	-	-	+	+	-	-
18	S8RBI-18	+	+	+	-	+	+	-	+	-
19	S8RBI-19	-	-	-	-	+	+	+	-	-
20	S8RBI-20	-	-	+	-	+	+	+	-	-
21	S9RBI-21	+	+	+	-	+	+	-	+	-
22	S9RBI-22	+	-	-	-	+	+	+	-	-
23	S9RBI-23	-	-	+	-	-	-	-	+	+
24	S10RBI-24	+	+	+	+	-	+	-	+	-
25	S10RBI-25	-	+	+	+	-	+	-	+	-
26	S10RBI-26	-	-	+	-	+	+	+	-	+
27	S11RBI-27	-	-	+	-	+	+	-	+	+
28	S11RBI-28	-	-	+	-	+	+	+	-	-
29	S11RBI-29	+	-	-	-	+	+	+	-	-
30	S11RBI-30	-	+	+	+	-	+	-	+	-
31	S12RBI-31	-	-	+	-	+	-	-	+	+
32	S12RBI-32	-	-	-	-	+	+	+	-	-
33	S13RBI-33	-	+	+	-	-	+	-	+	-
34	S14RBI-34	+	-	+	-	+	+	+	-	+
35	S14RBI-35	-	-	+	-	+	+	+	-	-
36	S14RBI-36	-	-	+	-	-	-	-	+	+
37	S14RBI-37	+	-	+	-	+	+	+	-	-
38	S15RBI-38	+	-	+	+	+	+	+	-	+
39	S15RBI-39	-	+	+	-	+	-	-	+	-
40	S15RBI-40	+	-	+	-	-	+	-	+	-
41	S16RBI-41	-	+	-	-	+	+	+	-	-
42	S16RBI-42	-	-	+	-	-	+	-	+	-
43	S16RBI-43	-	-	+	-	+	-	-	+	+
44	S17RBI-44	+	-	+	-	+	+	+	-	+
45	S17RBI-45	-	+	+	-	-	+	-	+	-
46	S17RBI-46	-	+	+	+	+	-	-	+	-
47	S17RBI-47	+	-	+	-	+	+	+	-	+
48	S18RBI-48	-	-	-	-	+	+	+	-	-
49	S18RBI-49	-	-	+	-	+	+	+	-	+

50	S19RBI-50	+	-	+	-	-	-	-	+	-
51	S19RBI-51	-	-	+	-	+	+	+	-	-
52	S20RBI-52	+		-	+	+	+	+	-	-
53	S20RBI-53	-	+	-	-	+	+	-	+	-
54	S21RBI-54	+	-	+	-	+	-	-	+	+
55	S21RBI-55	-	+	+	-	-	+	-	+	-
56	S22RBI-56	+	-	+	-	+	-	-	+	+
57	S23RBI-57	-	-	+	-	+	+	+	-	-
58	S23RBI-58	-	-	+	-	-	+	-	+	-
59	S23RBI-59	+	-	+	-	+	+	+	-	+
60	S23RBI-60	-	+	+	+	+	+	-	+	-
61	S24RBI-61	-	-	+	-	-	+	-	+	-
62	S24RBI-62	+	+	+	+	+	-	-	+	-
63	S25RBI-63	+	-	+	-	+	+	+	-	-
64	S25RBI-64	-	-	-	-	+	+	+	-	-
65	S26RBI-65	-	-	+	-	+	-	-	+	+
66	S26RBI-66	+	+	+	+	-	+	-	+	-
67	S26 RBI-67	-	-	+	-	+	-	-	+	-
68	S27 RBI -68	+	-	+	-	-	+	-	+	-
69	S27 RBI -69	+	-	+	-	+	-	-	+	+
70	S28 RBI -70	-	-	+	+	+	-	+	-	-
71	S28 RBI -71	+	-	+	-	-	+	-	+	-
72	S28 RBI -72	-	-	+	-	-	+	-	+	-
73	S28 RBI -73	-	-	+	-	+	+	+	-	+
74	S29 RBI -74	-	-	-	-	+	+	-	+	-
75	S29 RBI -75	+	+	+	-	-	+	-	+	-
76	S30 RBI -76	-	-	+	-	-	+	-	+	-
77	S30 RBI -77	+	-	+	-	+	-	-	+	+
78	S31 RBI -78	-	-	+	-	+	+	+	+	-
79	S31 RBI -79	+	-	+	-	+	+	+	-	-
80	S31 RBI -80	-	+	+	-	-	+	-	+	-
81	S32 RBI -81	+	-	+	-	-	+	-	+	-
82	S32 RBI -82	-	-	+	+	+	-	+	+	+
83	S32RBI -83	+	-	-	-	+	+	-	+	+
84	S33 RBI -84	-	+	+	-	-	+	-	+	-
85	S33 RBI -85	+	-	-	-	+	+	+	-	-
86	S33 RBI -86	-	-	+	-	+	-	+	-	-
87	S33 RBI -87	-	-	+	-	+	+	-	+	+
88	S34 RBI -88	-	+	+	+	+	+	-	+	-
89	S34 RBI -89	-	-	+	-	+	+	+	-	-
90	S34 RBI -90	-	-	-	-	+	+	+	-	-
91	S34 RBI -91	+	-	+	-	+	-	-	+	+
92	S35 RBI -92	+	-	+	-	-	+	-	+	-
93	S35 RBI -93	-	-	-	-	+	+	+	-	-
94	S35 RBI -94	-	-	+	+	-	+	-	+	-
95	S35 RBI -95	+	-	+	-	+	-	+	-	+
96	S35 RBI -96	-	-	+	-	+	+	-	+	-
97	S36 RBI -97	-	-	+	-	+	+	+	-	+
98	S36 RBI -98	-	-	-	-	+	+	+	-	-
99	S36 RBI -99	+	+	+	+	-	+	-	+	-
100	S36 RBI-100	-	-	+	-	+	+	+	-	+
101	S36 RBI -101	-	-	+	-	+	+	+	-	-
102	S37 RBI -102	-	-	+	+	+	+	-	+	-
103	S37 RBI -103	+	-	+	-	-	+	-	+	-
104	S37 RBI -104	-	-	+	-	+	+	+	-	+
105	S37 RBI -105	-	-	+	+	+	+	-	+	-
106	S38 RBI -106	-	-	-	-	+	+	+	-	-
107	S38 RBI -107	+	-	+	-	+	+	+	-	+
108	S38 RBI -108	-	+	+	+	-	+	-	+	-
109	S38 RBI -109	+	-	+	-	+	+	+	-	-
110	S38 RBI -110	-	+	+	-	-	+	-	+	-
111	S39 RBI -111	+	-	+	-	+	+	-	+	+
112	S39 RBI -112	-	-	+	-	+	+	+	-	-
113	S40 RBI -113	+	+	+	+	-	+	-	+	-
114	S40 RBI -114	-	-	-	-	+	+	+	-	-
115	S40 RBI -115	+	-	+	-	+	-	-	+	+
116	S40 RBI -116	-	-	-	+	+	+	+	-	-
117	S41 RBI -117	+	+	+	-	+	+	-	+	-

118	S41 RBI -118	-	-	+	-	+	+	+	-	-
119	S41 RBI -119	+	-	+	+	+	-	-	+	-
120	S41 RBI -120	-	-	+	-	-	+	-	+	-
121	S42 RBI -121	+	-	+	-	+	-	+	-	+
122	S42 RBI -122	-	+	+	-	-	+	-	+	-
123	S42 RBI -123	+	+	+	+	+	+	-	+	-
124	S42 RBI -124	-	-	+	-	+	-	-	+	-

Methyl red test and Voges–Proskauer test

Methyl red and Voges–Proskauer (MR-VP) broth (Composition of MR-VP medium: Peptone: 7 g, Glucose: 5 g, Potassium phosphate: 5 g, Distilled water: 1000 ml, Final pH: 6.9 ± 0.2 HiMedia MR-VP medium @ 17.00 g in 1000 ml of dH2O) prepared and poured in test tubes then sterilized in autoclave. After that test tubes with 5 ml of sterilized Methyl red and Voges–Proskauer broth were inoculated with bacterial isolates and one test tube was kept as an uninoculated control. These tubes were incubated at 28 ± 2 °C for 48 hours in BOD. After incubation, 2.5 ml of culture was transferred into a new sterile test tube to which five drops of methyl red indicator were added to each tube. Change in colour of broth to red was recorded as Methyl Red positive while yellow colour development was as Methyl Red negative.

The remaining 2.5 ml culture in Methyl red and Voges–Proskauer (MR-VP) broth was taken, to this 0.6 ml of Barritt's reagent A and 0.2 ml of Barritt's reagent B was added. Development of crimson to ruby pink (red) colour, which may be most intense on the surface, was recorded as indicative of positive Voges–Proskauer test while no change in colour was recorded as negative if Voges–Proskauer test. (Barritt's reagent A α-Naphthol (1-Naphthol):5 g Absolute ethanol: 100 ml Barritt's reagent B Potassium hydroxide: 5 g Distilled water: 100 ml)

Citrate utilization test

Simmons ‘citrate agarlants were prepared in test tubes. Tested bacterial isolates were streaked on those slants and one slant was kept as an uninoculated control. These slants were incubated at 28 ± 2 °C for 48 hours. The colour of slants turns to Prussian blue from green and growth was visible on the surface then the isolate was confirmed as citrate positive and those slants which were not shown any colour change recorded as citrate negative. (Composition of Simmons' citrate agar (SCA) medium Magnesium sulfate: 0.2 g Ammonium dihydrogen phosphate: 1 g Dipotassium phosphate: 1 g Sodium citrate: 2 g Sodium chloride 5 g Bromothymol blue 0.080 g Agar 20 g Distilled water: 1000 ml Final pH: 6.8 ± 0.2 HiMedia SCA @ 24.28 g in 1000 ml of dH2O.)

Indole production

Tryptone broth (Composition of tryptone broth (Tryptone water) Tryptone: 10 g Sodium chloride: 5 g Distilled water: 1000 ml Final Ph: 7.5 ± 0.2 HiMedia Tryptone Broth @ 15.00 g in 1000 ml of dH2O. Kovac's indole reagent p-dimethylamino benzaldehyde: 5 ml Amyl alcohol: 75 ml Concentrated Hydrochloric acid: 25 ml) was prepared and poured 5ml of it in each test tube then they were sterilized in autoclave (at 15 psi pressure (121.6 °C) for 20 minutes.). Test tubes with 5 ml of sterilized tryptone broth were inoculated with test bacterial isolates and one tube without bacterial inoculation was kept as an uninoculated control. These tubes were incubated at 28 ± 2 °C for 48 hours. After incubation, five drops of Kovac's indole reagent was added to each test tube. Development of cherry red colour in the top layer of the tube was indicated as indole positive otherwise indole negative.

Oxidase test

Oxidase test was done by spreading a loopful of overnight bacterial culture on the oxidase disc (from HiMedia). The reaction was observed within 5-10 seconds at 25-30 °C. Bacterial isolates which showed a change of disc colour to blue was as oxidase positive. A change observed after 10 seconds or no change at all was recorded as a negative reaction.

Catalase test

One day old pure cultures of tested bacterial isolates were placed on a clean, dry glass slide and one drop of 30 per cent hydrogen peroxide was added to it. The presence of catalase enzyme in bacterial isolates was observed by the appearance of bubbles of oxygen within 1 minute after the addition of hydrogen peroxide.

KOH test

This test was performed by placing two drops of 30 per cent potassium hydroxide solution on a clean, dry glass slide, to which a loopful of 24 hour old pure culture of bacterial test isolate was mixed by using an inoculation loop till the culture was dissolved in the KOH solution or a mucoid suspension was observed. Then, inoculation loop was lifted along with mucoid material and the reaction was recorded. Those isolates which formed a thread like slime after vigorous mixing and pulling of the loop they were noted as a Gram-negative bacterium. Those isolates which were not formed a thread like slime while lifting the needle; the culture was recorded as a Gram-positive bacterium**.**

Results and Discussion

The results revealed that among the 124 rhizosphere isolates evaluated for Methyl red production, 49 isolates were tested positive as the colour of broth was changed to red due to production of stable acid by bacteria in (MR-VP) broth recorded as while 75 isolates were tested negative as there is no colour change to red. Indole is produced by the decomposition of tryptophan by the bacteria. For this test 23 isolates were positive and 101 isolates were negative. Whereas 32 of the total 124 isolates were shown positive reaction with Voges Proskauer testas acetylmethyl carbonil is converted to diacetyl, while 92 isolates were negative. More number of isolates (103) was positively shown citrate utilization, as positive bacteria produces citrate permease enzyme and converted the citrate to pyruvate.

In the oxidase test, 86 isolates tested positive as the colour of oxidase disc changed to blue and 38 tested negative. Catalase enzyme decomposes hydrogen peroxide to water and oxygen. Among 124 isolates, 98 tested positive for the catalase test and 26 tested negative for catalase test. Of the total 124 isolates tested, 53 isolates were KOH tested positive as they produced thread like slime and they had a negative Gram reaction while 71tested negative for the KOH test and gave a positive Gram reaction.

The results suggested that the majority of pigeonpea rhizosphere isolates studied were indole production negative, methyl red negative, Voges Proskauer positive, citrate utilization positive, catalase positive and oxidase negative. Gram-positive dominate the isolates studied indicating most of the isolates present in the pigeonpea rhizosphere soil come under the Bacillus genus followed by others.

Kaur and Sharma (2013) [9] isolated a total of 35 isolates of rhizobacteria were isolated from 25 soil samples collected from healthy chickpea rhizospheric locations of Punjab (India). Ten isolates of rhizobacteria were characterized as *Pseudomonas* sp. on the basis of morphological, biochemical and growth promotion activities.

Similarly, Joseph *et al*. (2007) [7] reported that the population of *Pseudomonas* spp. (1.1-2.1 X 106 cfu/g of soil) dominated in chickpea rhizosphere. These results are also in close agreement where out of 121 isolates from mungbean rhizosphere, 65% were gram negative represented by Pseudomonas, Bacillus, Enterobacter, Proteus and Klebsiella (Gupta, 1995) [5]. Similarly, Cattlen *et al.* (1998) [3] reported *Pseudomonas*, *Burkholderia*, *Bacillus* and *Alcaligenes* as predominant genera in rhizosphere of soybean.

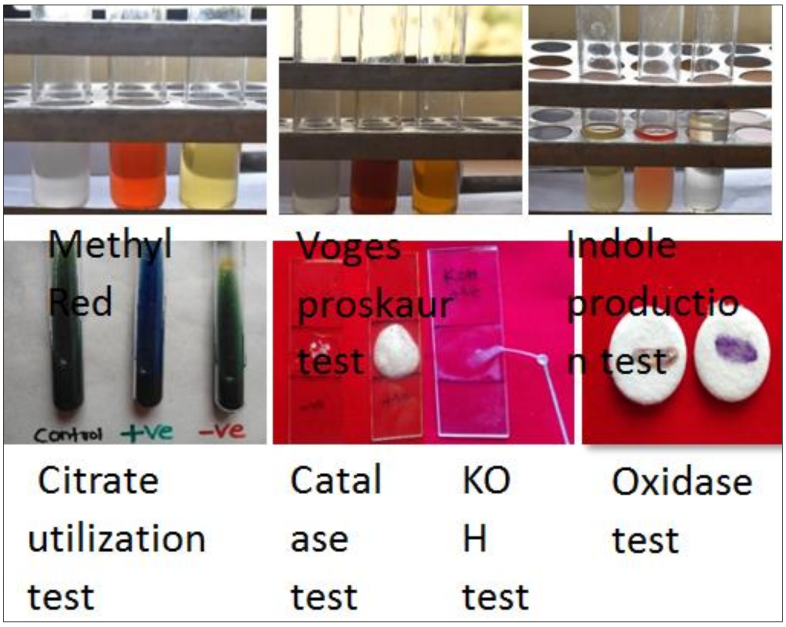

Fig 1: Biochemical Characterization of Rhizospheric bacteria of Pigeonpea

Conclusion

Among all the isolates 34 isolates showed antagonism for pathogen *F. udum*. Future study can be conducted to evaluate Plant growth promoting characters aiming potential isolates. They can be used as bio control agents. Thus, this study suggests the use of these 34 isolates as bio control agents which might be beneficial for pigeon pea cultivation as they showed antagonism for pathogen *F. udum*. Further they can be used in pot cultures and followed by field experiments know their efficiency in improving plant parameters.

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