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Microbiological profile of coriander (*Coriandrum sativum* L.) crop rhizosphere in Rajasthan and screening for auxin producing rhizobacteria

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Abstract

Twenty five coriander plant soil samples have been collected from Ajmer, Baran, Jhalawar and Kota Districts of Rajasthan for isolation of rhizosphere bacteria on selective nutrient agar medium. These soil samples have been analyzed for their electrical conductance (EC), pH, total viable bacterial count, and total viable fungal count. Maximum EC 1.12 dS/m was recorded in Siliya, Kota and minimum EC 0.21 dS/m was recorded in Ummedganj, Kota while maximum pH 8.6 was recorded in Kekari, Ajmer and minimum pH 7.88 was recorded in Bherupur, Jhalawar. The maximum soil bacterial population was observed from soil sample of Mandana locality in district Kota and minimum soil bacterial population was found in that of Tabiji locality in district, Ajmer. Microbial count on nitrogen free Azotobacter medium, Pikovskaya medium containing Tricalcium phosphate and Kings B medium have also been done for prospective microbial analysis of soil samples. Total thirty six cultures were isolated based on their colony morphology. These isolates were screened for Indoleacetic acid (IAA) production and superior bacterial cultures were further screened for their biochemical characteristics to utilize different sources of carbohydrates and production of the specific enzymes.

Key words : Auxin, Coriander (*Coriandrum sativum*), microbial population, rhizosphere

Introduction

Soil bacteria have direct / indirect interaction with plants. By increasing the population of beneficial rhizobacteria in soil, vigorous plant growth can be achieved. Isolation of native strains adapted to the environment and their study may contribute to the formulation of an inoculant to be used in region specific crops. Plant growth promoting rhizobacteria (PGPR) are beneficial soil bacteria, which may facilitate plant growth and development both directly and indirectly.

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and or indirectly. In last few decades a large array of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcalisens, Arthobacter, Burkholderia, Bacillus* and *Serratia* have reported to enhance plant growth (Kloepper *et al* .,11; Glick, 8). The direct promotion by PGPR either providing the plant with a plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. The exact mechanisms by which PGPR promote plant growth are not fully understood, but are thought to include (i) the ability to produce or change the concentration of plant growth regulators like indole acetic acid, gibberellic acid, cytokinines and ethylene (Glick, 8), (ii) asymbiotic N2fixation (Boddey and Dobereiner,4), (iii) antagonism against phytopathogenic microorganisms by production of siderophores, antibiotics and cyanide (iv) solubilization of mineral phosphates and other nutrients (De Freitas *et al*., 6; Gaur, 7). Most popular bacteria studied and exploited as biocontrol agent includes the species of fluorescent *Pseudomonas* and *Bacillus.* To achieve the maximum growth promoting interaction between PGPR and crop seedlings it is important to discover how the rhizobacteria exerting their effects on plant and whether the effects are altered by various environmental factors, including the presence of other micro-organisms (Bent *et al*., 2).Therefore, it is necessary to isolate efficient strains in field conditions. One possible approach is to explore soil microbial diversity for PGPR having combination of PGP activities and well adapted to particular soil environment. So keeping in view the above constrains, the present study was designed for microbial diversity analysis and isolation of plant growth promoting rhizobacteria from major coriander (*Coriandrum sativum)* cultivation areas of Rajasthan.

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Materials and methods

Coriander plant and soil samples have been collected from Baran, Jhalawar and Kota Districts of Rajasthan for isolation rhizospheric and endorhizospheric bacteria on nutrient agar medium and other selective growth media. Soil samples were analyzed for electrical conductance (EC) and pH by using standard protocol. Total viable aerobic bacterial and fungal count was done by using serial dilution method and pour plating on the specific growth media.

Isolation of Rhizobacteria

All the bacterial strains were isolated on their respective media; phosphobacteria on Pikovskaya's agar medium (Gaur, 7), Pseudomonas on King's B medium (King's *et al*., 10) and Azotobacter on Waksman base No.77 medium (Allen, 1). The bacterial cultures were maintained on the respective slants. Total thirty six cultures were isolated based on their colony morphology. The bacterial isolates were designated as Cor1- Cor-36. These isolates were screened for IAA production, acid production and capacity to solubilise the Tricalcium phosphate. Fourteen bacterial cultures were found superior w.r.t. IAA production and these were further screened for their biochemical characteristics to utilize different sources of carbohydrates and production of the specific enzymes.

In vitro screening of bacterial isolates for their indoleacetic acid (IAA) production

IAA production was assayed by the modified method as described by (Brick *et al.*, 5). Bacterial cultures were grown for 48h on Luria Bertani broth (Hi-media) at 28±2°C. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1ml 0.5 M $FeCl_3$solution). Development of pink colour indicates IAA production. Optical density was taken at 530 nm with the help of spectrophotometer and concentration of IAA produced by cultures was measured with the help of standard graph of IAA (Hi-media) obtained in the range of 10-100 µg/ml.

Result and discussion

Plant rhizosphere is known to be preferred ecological niche for various types of soil microorganisms due to rich nutrient availability. It has been assumed that inoculation with diazotrophic bacteria like *Rhizobium*, *Azotobacter* and *Azospirillum* enhanced the plant growth as a result of their ability to fix nitrogen. Growth promotion may be attributed to other mechanisms such as production of plant growth promoting hormones in the rhizosphere and other PGP activities (Bhashan and Bashan, 3).

Twenty five Coriander plant soil samples have been collected from Ajmer, Baran, Jhalawar and Kota Districts of Rajasthan for isolation of rhizospheric bacteria on selcective nutrient agar medium. These soil samples have been analysed for their electrical conductance (EC), pH, total viable bacterial count, and total viable fungal count. Maximum EC 1.12 dS/m was recorded in Siliya, Kota Minimum EC 0.21 dS/m was recorded in Ummedganj, Kota while Maximum pH 8.6 was recorded in NRCSS,Tabiji, Ajmer and Minimum pH 7.8 was recorded in Bhatawada, Baran (Table 1).

Physicochemical analyses of soil samples from 25 coriander fields of different districts of Rajasthan were examined. Physiochemical properties like pH, EC, are indictors of soil quality in understanding the nutrient status of soil and also its correlation with prevailing microbial population in the area were examined. pH is an important parameter to determine acidic or basic nature of soil. In

Table1. : List of Soil samples collected from different districts of Rajasthan.

S. No.	Village/District	Soil EC (dS/m)	Soil pH
1	Tabiji / Ajmer	0.38	8.32
2	Tabiji / Ajmer	0.38	8.46
3	Tabiji / Ajmer	0.37	8.51
4	Tabiji / Ajmer	0.38	8.51
5	Kekari / Ajmer	0.36	8.48
6	Kekari / Ajmer	0.38	8.67
7	Kekari / Ajmer	0.35	8.53
8	Anta / Baran	0.36	8.55
9	Anta / Baran	0.38	8.46
10	Anta / Baran	0.38	8.67
11	Anta / Baran	0.36	8.67
12	Bamla / Baran	0.36	8.45
13	Bamla / Baran	0.37	8.66
14	Bhatawada / Baran	0.38	8.67
15	Khanpur / Jhalawar	0.82	8.25
16	Khanpur / Jhalawar	1.02	8.22
17	Bherupur / Jhalawar	0.72	7.88
18	Nagunia / Jhalawar	1.04	8.1
19	Ladpura / Kota	0.65	8.0
20	Mandana / Kota	0.55	8.2
21	Mandhaliya / Kota	0.27	8.0
22	Malya / Kota	0.57	7.9
23	Siliya / Kota	1.12	7.9
24	Suwet / Kota	0.55	8.7
25	Ummedganj / Kota	0.21	8.4
Mean		0.51	8.3
CD @0.05		0.09	0.13
SEm		0.04	0.07

the present study soil pH was found to be alkaline in the range of 7.14 to 8.28 which is in confirmation with earlier findings. The pH of soil plays an important role in the occurrence and dominance of a particular group of microorganism. Soil microorganisms, just like higher plants depends entirely on soil for their nutrition, growth and activity. The major soil factors which influence the microbial population, distribution and their activity in the soil are nutrients, moisture, temperature, aeration pH (H-ion Concentration) and salt concentration (Knight *et al*., 12).

All these factors play a great role in determining not only the number and type of organism but also their activities. Variations in any one or more of these factors may lead to the changes in the activity of the organisms which ultimately affect the soil fertility level.

Electrical conductivity is an important factor in determining the salinity of soil. It represents the availability of salts in the soil. In the present investigation the value of EC ranged between S /cm tos/cm in the studied area. Increase in electrical conductivity of soil, increases the availability of soluble salts to the plants and thus effect on soil fertility status of the soil which in turn may affect plant health.

Microorganism requires a favorable nutritional and physical environment to grow and multiply. Isolation of microorganisms was done by using serial dilution methods followed by purification using gram's staining and repeated streaking on nutrient agar media. These steps are

Table 2. Microbial profile (cfu/g)* of collected rhizosphere soil of coriander from different locations in Rajasthan.

S. No.	District	Locality	Total viable mesophilic bacteria	Total viable mesophilic fungi	Azotobacter	Phosphate Solubilizing Bacteria	Pseudomonas
1	Ajmer	Tabiji	1.32×10^{8}	4.30×10^{6}	0.8×10^{5}	3.8×10^{5}	0.2×10^{5}
2	Ajmer	Tabiji	1.40×10^{8}	4.24×10^{6}	0.3×10^{5}	3.6×10^{5}	0.8×10^{5}
3	Ajmer	Tabiji	1.20×10^{8}	2.8×10^{6}	0.1×10^{5}	4.0×10^{5}	0.7×10^{5}
4	Ajmer	Tabiji	1.45×10^{8}	3.81×10^{6}	0.4×10^{5}	3.5×10^{5}	0.6×10^{5}
5	Ajmer	Kekari	1.27×10^{8}	3.20×10^{6}	0.7×10^{5}	4.2×10^{5}	0.3×10^{5}
6	Ajmer	Kekari	1.31×10^{8}	3.80×10^{6}	0.6×10^{5}	4.5×10^{5}	0.2×10^{5}
7	Ajmer	Kekari	1.31×10^{8}	4.20×10^{6}	0.8×10^{5}	5.4×10^{5}	0.4×10^{5}
8	Baran	Anta	2.20×10^{8}	3.20×10^{6}	0.3×10^{5}	3.2×10^{5}	0.6×10^{5}
9	Baran	Anta	2.30×10^{8}	3.40×10^{6}	0.9×10^{5}	4.8×10^{5}	1.8×10^{5}
10	Baran	Anta	1.87×10^{8}	3.20×10^{6}	0.6×10^{5}	3.3×10^{5}	0.9×10^{5}
11	Baran	Anta	1.98×10^{8}	4.37×10^{6}	0.2×10^{5}	3.6×10^{5}	0.8×10^{5}
12	Baran	Bamla	1.45×10^{8}	2.25×10^{6}	0.7×10^{5}	5.2×10^{5}	0.3×10^{5}
13	Baran	Bamla	1.40×10^{8}	3.25×10^{6}	0.7×10^{5}	4.6×10^{5}	1.6×10^{5}
14	Baran	Bhatawada	1.78×10^{8}	2.47×10^{6}	0.8×10^{5}	3.8×10^{5}	1.2×10^{5}
15	Jhalawar	Khanpur	1.66×10^{8}	4.84×10^{6}	1.8×10^{5}	3.7×10^{5}	0.8×10^{5}
16	Jhalawar	Khanpur	1.80×10^{8}	3.26×10^{6}	0.6×10^{5}	3.8×10^{5}	0.9×10^{5}
17	Jhalawar	Bherupur	1.38×10^{8}	3.58×10^{6}	0.3×10^{5}	4.3×10^{5}	0.3×10^{5}
18	Jhalawar	Nagunia	1.43×10^{8}	2.86×10^{6}	0.8×10^{5}	5.6×10^{5}	0.7×10^{5}
19	Kota	Ladpura	1.37×10^{8}	3.17×10^{6}	0.5×10^{5}	3.3×10^{5}	0.3×10^{5}
20	Kota	Mandana,	2.40×10^{8}	3.42×10^{6}	0.7×10^{5}	4.3×10^{5}	0.9×10^{5}
21	Kota	Mandhaliya	1.33×10^{8}	2.75×10^{6}	2.0×10^{5}	4.1×10^{5}	0.1×10^{5}
22	Kota	Malya	2.38×10^{8}	2.45×10^{6}	0.4×10^{5}	3.2×10^{5}	1.4×10^{5}
23	Kota	Siliya	2.32×10^{8}	2.85×10^{6}	0.9×10^{5}	4.2×10^{5}	0.6×10^{5}
24	Kota	Suwet	1.28×10^{8}	4.16×10^{6}	0.5×10^{5}	3.8×10^{5}	0.5×10^{5}
25	Kota	Ummedganj	1.90×10^{8}	3.28×10^{66}	0.7×10^{5}	3.8×10^{5}	0.7×10^{5}

*mean of three replications at appropriate dilution.

essential to obtain well separated discrete colonies in different selective media. Total viable mesophilic bacterial count of the samples ranged from $1.2x10^8$ to $2.4x10^8$ cfu/g soil while the mesophilic aerobic fungal population varied from $2.25x10^6$ to $4.48x10^6$ cfu/g soil. The maximum soil bacterial population was observed from soil sample of Mandana locality in district Kota and minimum soil bacterial population was found in that of Tabiji locality in district, Ajmer. Similarly, maximum fungal population was recorded from soil samples of Khanpur locality in Jhalawar district while minimum was observed from Bamla locality in Baran district (Table2) Microbial count on nitrogen free Azotobacter medium revealed the population of Azotobacter in soil samples which varied from $0.1x10^5$ to $2.0x10^5$ cfu/g. The Pikovskaya medium containing Tricalcium Phosphate as sole source of phosphorus for bacterial growth provided the rough estimate of phosphate solubilizing bacterial population and it ranged from $3.2x10^5$ to $5.8x10^5$ cfu/g and Pseudomonads population on Kings B medium varied from 0.1x105 to 1.8x105 cfu/g soil among the collected soil samples of coriander crop (Table 2).

Sakthivel and Karthikeyan (14) had studied thirty rhizospheric soil samples collected from commercially grown *Coleus forskohlii* from Perambalur and Salem districts of Tamil Nadu. The results obtained showed that among the 30 isolates of Perambalur and Salem districts of ranged from (4.00-9.22x10^6 and 4.66-10.00x10^6) of *Azospirillum* spp., (3.00-7.66x10^6 and 3.88-8.00x10^6) of *Bacillus* spp, (4.66-12.00 x10^6 and 4.88-13.00 x10^6) of *Pseudomonas* spp., and (2.22-8.00 x10^6 and 3.66-9.00 x10^6) of *Azotobacter* spp. respectively for the two districts. In present investigation, the population of plant growth promoting rhizobacteria was found lower than reported by Sakthivel and Karthikeyan (14) which may be due to different agro-ecological condition prevailing in the Rajasthan as compared to Tamilnadu. In addition to plant growth promoting traits, these bacterial strains must be rhizospheric competent, able to survive and colonize in the rhizosphere soil (Grover, 9)

Total thirty six cultures were isolated based on their difference colony morphology on selective growth media. These isolates were screened for auxin (IAA) production in defined growth media and the maximum IAA production (7.26ppm) was observed with isolate Cor-19 whereas minimum (0.8ppm) was recorded for isolate Cor-36. The IAA Fourteen bacterial cultures were found superior with respected to IAA production based on mean value (3.27ppm) of auxin production of all the rhizobacterial isolates (Table 3). Mishra *et al.,* (13) have reported that plant growth promoting rhizobacteria (PGPR) found in the soil have significant role in plant nutrition. These efficient phytohormone producer bacterial isolates were further screened for their biochemical characteristics to utilize different sources of carbohydrates and production of the

Table 3. Screeing of rhizobacterial isolates for auxin production in synthetic medium.

S.No.	Culture	Auxin (ppm)
1	COR-1	1.16
2	COR-2	4.28
3	COR-3	1.10
4	COR-4	2.75
5	COR-5	2.83
6	COR-6	2.00
7	COR-7	1.34
8	COR-8	5.08
9	COR-9	2.13
10	COR-10	4.87
11	COR-11	4.74
12	COR-12	1.30
13	COR-13	1.40
14	COR-14	2.90
15	COR-15	5.26
16	COR-16	0.80
17	COR-17	3. 94
18	COR-18	5.19
19	COR-19	7.23
20	COR-20	2.03
21	COR-21	5.95
22	COR-22	5.89
23	COR-23	5. 20
24	COR-24	3.04
25	COR-25	3.12
26	COR-26	3.88
27	COR-27	4.94
28	COR-28	1.09
29	COR-29	2.23
30	COR-30	2.92
31	COR-31	2.95
32	COR-32	5.19
33	COR-33	1.90
34	COR-34	5.01
35	COR-35	3.10
36	COR-36	0.84
	Mean	3.27
	CD@0.05	0.35
	Sem±	0.11

Table 4: Biochemical screening of selected bacterial isolates.

Culture No.	Citrate	Lys-ine	Ornit hine	Ure ase	Phenylalanine deamination	Nitrate reduction	H_2S production	Glucose	Adonitol	Lactose	Arabinose	Sorbitol	Genus identifed
COR-2	+	+	+	-	-	+	-	+	-	-	+	-	Bacillus
COR-8	+	+	+	-	+	-	-	+	-	-	+	-	Bacillus
COR-10	+	+	+	-	+	-	-	-	-	-	-	-	Azotobacter
COR-11	+	+	+	-	+	+	+	-	-	-	-	-	Azotobacter
COR-15	+	+	+	-	-	+	-	-	-	-	+	-	Azotobacter
COR-17	+	+	+	-	-	+	-	-	-	+	+	-	Azotobacter
COR-18	+	V	+	V	Nd	V	V	-	-	-	-	-	Pseudomonas
COR-19	+	+	+	-	Nd	+	V	-	-	-	+	-	Pseudomonas
COR-21	Nd	V	V	-	-	+	-	+	-	-	-	-	Pseudomonas
COR-22	V	+	+	-	-	+	-	+	+	-	-	-	Pseudomonas
COR-23	-	+	+	-	-	+	-	+	+	+	-	-	Bacillus
COR-27	+	+	+	-	-	+	-	-	-	-	+	-	Bacillus
COR-32	Nd	V	V	-	-	+	-	+	-	-	-	-	Bacillus
COR-34	+	V	+	V	Nd	V	V	-	-	-	-	-	Azotobacter

+ = Positive (more than 90%)

- = Negative (more than 90%)

V =11-89% Positive

Nd = No data available

specific enzymes (Table 4). These cultures have been identified as *Azotobacter* spp., *Bacillus* spp. and Pseudomonads based on the biochemical screening results and their ability to grow on selective growth media. Biochemical Test Kit identification system utilizing seven conventional biochemical tests and five carbohydrate utilization tests are based on the principle of pH change and substrate utilization. On incubation organisms undergo metabolic changes which are indicated by a colour change in the media that can be either interpreted visually or after addition of the reagent.

Finally, it is concluded that isolation of native strains adapted to the environment and their study may contribute to the formulation of an inoculant to be used in region specific crops. The isolated native strains of rhizobacteria from coriander crops shall be further evaluated assess their potential as biofertilizers in integrated nutrient management system for sustaible production of this major seed spices crop of Rajasthan and by increasing the population of beneficial rhizobacteria in soil, vigorous plant growth can be achieved.

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References

1. Allen, O.N. 1953. Experiments in Soil Bacteriology. Burgess Publ. Co., Minneapology Minnesota, U.S.A., pp. 69-70.
2. Bent, E., Tuzun S., Chanway C.D. and Enebak S. 2001. Alternations in plant growth and in root hormone levels of lodge pole pines inoculated with rhizobacteria. *Com. J. Microbiol.* **47**:793-800.
3. Bhasan Y. and Bashan de L. E. 2005. Bacteria. **In**: Encyclopedia of Soils in ihe Environment, Elsevier,V. K. **1**:103-115.
4. Boddey, R.M., and Dobereiner J. 1995. Nitrogen fixation associated with grasses and cereals: recent progress and perspectives for the future. *Fert. Res*. **42**:241-250.
5. Brick,J.M., Bostock, R.M and Silverstones, S.E. 1991. Rapid *in situ* assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. *Appl. Environ. Microbiol.* **57**:535-538.
6. De Freitas, J.R., Banerjee M.R. and Germida J.J. 1997. Phosphate solubilizing rhizobacteria enhance the growth and yield but not Phosphorous uptake of canola (*Brassica napus* L.). *Biol. Fertil. Soil* **24**:358-364.
7. Gaur, A.C. 1990. Physiological functions of phosphate solubilizing microorganisms. **In**: (Gaur, A.C. Ed.), Phosphate Solubilizing Microorganisms As Biofertilizers. Omega Scientific Publishers, New Delhi, pp.16-72.
8. Glick, B.R. 1995. The enhancement of plant growth by free living bacteria. *Can J. Microbiol.* **41**:109-114.
9. Grover M., Pandey A. K., Mishra B. K., Lata and Roy R. C., 2009. Sugarcane crops: plant growth promoting bacteria in growth, yield and productivity. **In**: (Hertsburg Claus T. Ed). Sugar Beet Crops: Growth, fertilization & Yield. Nova Science Publishers Inc., NewYork, pp.135-151.
10. King's, E.O., Ward M.K. and Rency D.E. 1954. Two sample media for the demonstration of pyocyanin and fluorescin. *J. Lab. Clin. Med*. **44**: 301-307.
11. Kloepper, J.W., R. Lifshitz and R.M. Zablotowicz, 1989.Free-living bacterial inocula for enhancing crop productivity. *Trends Biotechnol*. **7**:39-43.
12. Knight B P, McGrath S P and Chaudri A M. 1997. Biomass carbon measurements and substrate utilization patterns of microbial populations from soils amended with cadmium, copper, or zinc. *Appl. Environ. Microbiol.* **63**:39-43.
13. Mishra B. K., Khandelwal S. K. and Rajamani G. 2010. Plant growth promoting rhizobacteria (PGPR) associated with plant nutrition. **In**: (Singh A. Ed). Organic Farming- An Overview. Pointer Publication, Jaipur, pp.84-91.
14. Sakthivel U. and Karthikeyan B. 2012. Isolation and characterization of plant growth promoting rhizobacteria (pgpr) from the rhizosphere of *Coleus forskohlii* grown soil. *International J Recent Scientific Res.,* **3(5**):288-296**.**

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