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Microbial Identification by Molecular Characterization and Screening of Phosphate Solubilizing Bacteria of *Coriandrum sativum* Rhizosphere

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ABSTRACT

The present study was conducted to analyze microbial diversity of Coriandrum sativum rhizosphere and isolate phosphate solubilising bacteria from soils of Hadoti, Rajasthan, India. In total fifty six bacteria were isolated from different soil samples collected from Kota, Bundi, Jhalawar and Baran districts of Hadoti region. These soil samples have been analyzed for their organic carbon, available nitrogen, electrical conductance (EC), pH, and temperature. Maximum EC 0.22 dS/m was recorded in Baran district and minimum EC 0.07 dS/m was recorded in Jhalawar district, while maximum pH was recorded 8.4 in Kota district of Rajasthan. Based on their size of halo zone formation on pikovaskaya medium, total ten bacterial isolates were selected as efficient phosphate solubilizing bacterial isolates. The Maximum phosphate solubilization in medium was observed in bacterial isolates COR-61. These isolates were also screened for IAA production, citrate utilization, catalase production, and to utilize different sources of carbohydrates and production of the specific enzymes. On the basis of morphological and biochemical characterization of the isolates we have identified them as members of the following species: Pseudomonas putida, Bacillus megaterium, Bacillus altitudinis, Micrococcus luteus, Bacillus subterraneus, Bacillus aryabhattai, Bacillus tequilensis, Pseudomonas sp. and Planococcus refietoensis. All the ten PSB (phosphate solubilizing bacteria) isolated from coriander seed spice soil of Hadoti region could efficiently solubilize tricalcium phosphate in the medium which could possibly help for future application in sustainable production of agriculture crops.

Key words: *Coriander seed spice crop, Rhizosphere, Plant growth promoting rhizobacteria (PGPR), Phosphate solubilizing bacteria and biochemical and molecular analysis.*

INTRODUCTION

Spices are the most important and widely grown crops in the world. Spices hold prime position in the world trade market and economy. Enormous diversity is present in each spice crop at their geographical level as well as domestic level.

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The climatic conditions of India are most suitable for almost all spices because of its varied agro-climatic regions comprising tropical, subtropical and temperate regions, where people can grow different varieties of spices throughout the year. Rajasthan and Gujarat these both states are well known as "seed spices bowl of India" in worldwide. Rajasthan has achieved considerable victory in the export of seed spices in the last few years. Cumin, Coriander, Fennel, Fenugreek, Ajwain, Caraway, Dill, Nigella, Anise and Celery are the most important seed spices produced in India, out of which four, Cumin, Coriander, Fennel and Fenugreek are considered as major seed spices as they are playing a major role in export and foreign exchange. In Hadoti region Kota district is the major area for production of Coriander seed spice in whole Rajasthan state. Coriander (*Coriandrum sativum* L.*)* is grown as seed spice crop all over the world. Coriander (*Coriandrum sativum* L.) also called cilantro, or dhania (in Hindi) is an annual herbaceous crop. Coriander is belongs to the *Apiaceae* family, formerly known as *Umbeliferaeae.* Coriander plant is a rich reservoir of micronutrients and nutritional elements. Coriander is very little in saturated fat however, contains good amount of linoleic acid which is a good source of α-tocopherol and vitamin K. Plant leaves are rich source of vitamins while seeds are rich in polyphenols and essential oils. Coriander taste is devoted to its essential oil comprising a significant content of linoleic and furanocoumarins (coriandrine and dihydrocoriandrine). Coriander is also well known for its antioxidant, anti-diabetic, anti-mutagenic, anti-anxiety and antimicrobial activity along with analgesic and hormone balancing effect that promotes its use in foods due to numerous health benefits and its protective effect to preserve the food for longer period[19].Phosphorus is one of the major plant nutrients required in optimum amount for proper plant growth. Phosphorus is known to involve many functions in the plant growth and metabolism. Several important cellular, metabolic and reproductive functions rely on sufficient phosphorus supply. Indian soils are characterized by poor and medium status with respect to available phosphorus [2, 12, 16]. Phosphorus in decomposing litter is subject to the same pattern of immobilization and uptake by micro-organisms as found for N[3].The limited bioavailability of phosphorus from the soil combined with the fact that this element is essential for plant growth means that the inability to obtain sufficient phosphorus often limits plant growth[5]. Thus, solubilization and mineralization of phosphorus by phosphate-solubilizing bacteria is an important trait in PGPR as well as plant growth promoting rhizobacteria[7, 18]. The solubilization of inorganic phosphorus occurs as a result of the action of low molecular weight organic acids such as gluconic and citric acid, both of which are synthesized by a variety of soil bacteria[8].

MATERIALS AND METHODS

Coriander soil samples have been collected from Jhalawar, Bundi, Baran, and Kota Districts of Rajasthan for isolation of rhizospheric bacteria and screening of Phosphate solubilizing bacteria. Soil samples were analyzed for electrical conductance (EC), pH, organic carbon, Sodium (Na), Potassium (K), Available nitrogen (N) in soil samples by using standard protocol. Coriander rhizospheric bacterial strains were isolated by using serial dilution method on the specific growth media. All the isolated bacterial strains were analyzed for morphological characters (color, shape, elevation, margin etc.) and for Biochemical characterization and enzyme production assay, Pikovskaya's agar medium, King's B medium, simmons citrate agar, tryptone broth (glucose, sucrose, arabinose) different media and broths were used.

Catalase Test

For the characterization of aerobic (need oxygen) or facultative anaerobes (can live with or without oxygen) small amount of a bacterial culture (18 to 24 hours old) was placed by flame sterilized inoculating loop on a clean grease free glass slide then added one to two drops of 3% H_2O_2. Observations were recorded[9].

Indole Test

Isolated bacterial culture was inoculated in pre sterilized tryptone broth, which contains amino acid tryptophan. Culture tubes were incubated overnight at 37^{o}C. After incubation few drops of Kovac's reagent were added to the broth containing bacteria. Culture tubes were observed for the appearance of cherry red color. This indicated positive test for production of indole[9].

Citrate Utilization Test

Bacterial colonies were picked up with a straight wire and inoculated into slant of Simmon's citrate agar medium containing sodium citrate and a pH indicator bromothymol blue. Inoculated culture tubes were incubated overnight at 37^{o}C. Utilization of citrate involves the enzyme citrase, which breaks down citrate to oxaloacetate & acetate. Oxaloacetate was further broken down to pyruvate and CO_2. Production of Na_2CO_3 as well as NH_3 from utilization of sodium citrate and ammonium salt respectively results in alkaline pH. This results in change of medium's color from green to blue[9].

Starch Hydrolysis Test

Aseptically bacterial culture was inoculated on the surface of Starch -Agar medium either through a single streak or spotting by micropipette. Overnight (16 -18 hours) grown bacterial culture was incubated the plate for 24-48 hours at 35±2°C. The agar surface was flooded with Gram's iodine and looked for clear halo around the bacterial growth. Clear zone indicates positive result for starch hydrolysis[9].

Urease Test (Christensen's urea agar method)

An 18 to 24 hours old culture is used to streak the entire slant surface. Inoculated slants were incubated at 35°C and observed the color change after 6 hours, 24 hours and everyday for up to 6 days. Ammonia production will be indicated by bright pink color on the slant that may be penetrated into the butt[9].

Carbohydrate Fermentation Test

Phenol red broth was prepared in test tubes and sterilized it well in autoclave at 15psi pressure, 121°C temperature. Isolated bacterial cultures were inoculated in phenol red broth containing different sugars (Glucose, sucrose, arabinose etc.) and incubated at 37°C for 48 hours. Tubes were observed for change in color. Phenol red broth was indicated yellow for positive carbohydrate fermentation or no change in color indicated negative test for carbohydrate fermentation[9].

Screening of PSB Isolation and enumeration of PSB was carried out following dilution plate technique using pikovaskaya and NBRIP broth. For the isolation of PSB, the soil samples were serially diluted up to 10^{-6} dilution, plated on Petri dishes and incubated at 35 ± 2 ºC for seven days. At the end of incubation, PSB colonies were visually identified from the surrounding zone of clearance[15].

RESULT AND DISCUSSION

Coriander rhizospheric soil samples were collected from different locations of Hadoti region (Jhalipura, Anta, Ladpura, Mandola, Bundi, Kota, Modak, Sawan Bhado, Madawar, and Jhalawar) (Table 1). Maximum EC (Electrical conductivity) 0.22 dS/m was recorded in Baran district and minimum EC 0.07 dS/m was recorded in Mandawar, Jhalawar district (Fig 2), while maximum pH was recorded 8.4 in Jhalipura, Kota district and 7.5 pH were recorded which is minimum in Anta, Baran of Rajasthan (Table 1) (Fig 1). Electrical conductivity is a main aspect in determining the salinity of soil. It represents the availability of salts in the soil. Increase in electrical conductivity of soil, increases the availability of soluble salts to the plants and thus effect on soil fertility of the soil which in turn may affect plant health and productivity. Mishra *et al.*[13] analyzed in fennel soil samples the EC ranged between 1.02 to 0.15 dS/m whereas pH of collected fennel field soil samples of Rajasthan ranged from 8.8 to 7.6. Maximum EC (1.02 dS/m) was recorded for fennel soil samples collected from Khanpura locality in District Jhalawar while minimum EC (0.15 dS/m) was observed with samples of KVK Pali-A.The Na availability in soil samples were ranged between 137.53 Kg/hq to

408.20 Kg/hq whereas K of collected coriander field soil samples of Rajasthan ranged from 164.19 Kg/hq to 409.69 Kg/hq (Fig 3), Maximum Organic carbon (1.15 %) was recorded for coriander soil samples collected from Mandawar locality in District Jhalawar while minimum Organic carbon (0.14%) was observed with samples of Mandola, Baran district (Fig 4). Maximum available Nitrogen (0.065%) was recorded for coriander soil samples collected from Anta locality in District Baran (Table 2).Total fifty six cultures were isolated based on their morphological characterization on selective growth media. These isolates were screened for solubilization of phosphate in defined growth media and the maximum phosphate solubilization Index (350) was observed with isolate COR-61 whereas minimum (83.33) was recorded for isolate COR-83 (Fig 5). Total ten bacterial isolates (COR-3, COR-5, COR-37, COR-55, COR-61, COR-65, COR-72, COR-73, COR-83 and COR-96) were found PSB (Phosphate solubilizing bactera) (Table 3). N. Tenzing *et al.*[14] isolated total ten PSB isolates and six strains were identified as *Bacillus megaterium*, two strains as *Pseudomonas putida* and CP2 and CTP2 as *P. fluorescence* from different crop soils such as Okra, Chilli, tomato, Cotton and Egg plant.Gaur *et al.*[6] studied the bacterial cultures morphological, cultural and physiological and biochemical characteristics using the manual of microbiological methods and identified the organism Bacillus sp., using Bergey's manual of Determinative Bacteriology. In our study, based on the Morphological characterization and biochemical tests, the COR strains were identified up to species level. The results of various biochemical tests for ten COR isolates were showed in Table 4 and table 5. All selected COR isolates were characterized. Two isolates COR-3 and COR-83 was Gram negative whereas COR-5, COR-37, COR-55, COR-61, COR-65, COR-72, COR-73 and COR-96 were Gram positive. All were found rod shaped and motile except COR-55 and COR-72 isolates. Five isolates were found to be endospore formers, *viz.*, COR-5, COR-37, COR-65, COR-73 and COR-83

The selected ten COR isolates viz., COR-3, COR-5, COR-37, COR-55, COR-61, COR-65, COR-72, COR-73, COR-83 and COR-96 showed positive tests for catalase, phosphate solubilization and glucose fermentation. Five isolates, viz., COR-3, COR-5, COR-65, COR-73 and COR-96 showed positive oxidase test; six isolates: COR-5, COR-61, COR-65, COR-72, COR-83 and COR-96 showed positive methyl red test; four isolates, viz., COR-55, COR-65, COR-72, COR-73 and COR-96 showed positive Vogus Proskauer test. Indole acetic acid production was shown by isolates COR-3, COR-37, COR-65, COR-73, COR-83 and COR-96. Five isolates, viz., COR-3, COR-55, COR-65, COR-73 and COR-83 showed positive citrate utilization test. Total Six out of ten isolates, viz., COR-3, COR-5, COR-37, COR-61, COR-65 and COR-73 found positive for starch hydrolysis test. Seven isolates, viz., COR-5, COR-37, COR-55, COR-61, COR-65, COR-73 and COR-83 showed positive nitrate reduction test; six isolates: COR-3, COR-37, COR-55, COR-65, COR-72 and COR-96 showed positive urease production test; only one isolates, viz., COR-65 showed positive HCN production test.

All the isolates were found to ferment glucose. Sucrose fermentation was shown by isolates COR-3, COR-5, COR-55, COR-61, COR-72, COR-73 and COR-96. Lactose fermentation was shown by isolates COR-5, COR-37, COR-61, COR-65 and COR-73. Shobha *et al.*[23] reported seven *B. megaterium* isolates from rhizosphere soils of various plants (beans, brinjal, chilly, lady's finger, mango, marigold, paddy, ragi and tomato). All the isolates were Gram positive, rod shaped, endospore forming, positive for casein hydrolysis, catalase, citrate, gelatin, organic acids, oxidase, starch and negative for indole, Vogus Proskauer, H_2S production, lipid utilization. Colonies on nutrient agar media become visible to be cream in color, irregular in shape with entire margins. Carbohydrate utilization test was

found to be positive for glucose, lactose and mannitol (Table 4 and table 5).

Antibiotic sensitivity of selected PSB isolates from Hadoti region of Rajasthan

On the basis of the pattern of antibiotic response of all the bacterial isolates were distinguishable from each other. COR-3, COR-5, COR-37, COR-61 and COR-73 observed sensitive with Colistin (CL) except COR-55, while COR-37, COR-61 and COR-96 showed resistant ability towards Rifampicin (RIF) (Table 6). COR-72 observed resistance with Ampicillin (AMP), Chloramphenicol (C), Streptomycin (S) and Penicillin (P); while sensitive with Sulphatriad (S3) and Tetracyclin (TE). COR-83 showed resistance with Ampicillin (AMP), Chloramphenicol (C), and Penicillin (P); while sensitive with Streptomycin (S), Sulphatriad (S3) and Tetracyclin (TE).

Molecular Characterization

In the present investigation, based on the plant growth promoting activities exhibited by the isolates, total ten isolates were selected for molecular characterization. The 16s rDNA gene was sequenced with primers 16SF Universal and 16 SR Universal and the sequence obtained was analyzed using Blast search tool (NCBI). The evolutionary history was inferred using the Neighbor-Joining method[21]. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed[4]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Jukes-Cantor method[10]. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 16-20 nucleotide sequences. The codon positions included were $1^{st}+2^{nd}+3^{rd}$+Noncoding. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA5[24]. The phylogenetic analysis was done on CLUSTAL W and dendogram was prepared. The obtained sequences (1172 and 1444 bp) of the two Isolates COR-3 and COR-83 showed high identity (97 to 99%) with *Pseudomonas* genus. The phylogenetic position of the isolate indicates that these isolates are clustering with the *Pseudomonas* genus, especially homology shared with species of *Pseudomonas putida* strain TDRl3 (97% similar) and *Pseudomonas* sp. NBFPALD_RAS138 (99% similar), respectively. The sequences of both isolates COR-3 and COR-83 were submitted to NCBI. The Genbank accession no. KY810614 for isolate COR-3 and KX231809 for isolate COR-83 are available under National centre for biotechnology information (NCBI).The isolates COR-5, COR-37, COR-61, COR-65 and COR-73 were showed high identity (93 to 100%) with *Bacillus* genus. The phylogenetic position of the isolates indicates that these isolates are clustering with the *Bacillus* genus, especially homology shared with species of *Bacillus megatarium* strain MO29 (96% identical), *Bacillus altitudinis* strain EH19 (93% identical), *Bacillus subterraneus* strain CES-M15/10 (97% identical), *Bacillus aryabhattai* strain IHBB 7164 (100% identical) and *Bacillus tequilensis* strain IHBB 9348 (99% identical), respectively. The sequences of five isolates COR-5, COR-37, COR-61, COR-65 and COR-73 were submitted to NCBI. The Genbank accession no. of isolate COR-5, COR-65 and COR-73 are KX228234, KX231810 and KX231808 respectively, are available under National centre for biotechnology information (NCBI). Isolate COR- 55 and COR-72 showed high identity with *Micrococcus* genus, 99% and 96% identical, respectively. The phylogenetic position of the isolate indicates that these isolates are clustering with the *Micrococcus* genus, especially homology shared with species of *Micrococcus luteus* strain INBI-1 (99% similar) and *Micrococcus luteus* Strain CHN10 (96% similar). The sequences of both isolates COR-55 and COR-72 were submitted to NCBI. The Genbank accession no. KK223364 for isolate COR-55 are available under National centre for biotechnology information (NCBI) (Table 7). COR-96 was showed 94% identical to *Planococcus* genus

which is nearly closed to *Micrococcus* genus. The sequence of 1247 bp of isolates COR-96 was obtained. The evolutionary position of the COR-96 isolate indicates that the isolate COR-96 is clustering with the *Planococcus refietoensis* Strain YJST4. The nucleotide sequence of isolate COR-96 was submitted to NCBI. Rana *et al.*[17] selected 10 bacterial isolates (AW1 to AW10) from wheat rhizosphere. On the basis of 16S rDNA sequencing data, rhizobacteria isolates AW1, AW6 and AW3 showed 99% homology with the *Bacillus* genus. AW4 and AW5 showed 99% and 96% homology with *Providencia* sp., AW8 showed 98% homology with *Pseudomonas aeruginosa*, AW2 and AW10 showed 99% homology with *Alcaligenes* sp., AW7 and AW9 revealed 99% homology with *Brevundimonas* sp.Saharan *et al.*[20] selected five bacterial strains as plant growth promoting rhizobacteria for molecular characterization among the 266 rhizobacterial isolates from 24 rhizospheric soil samples of Ocimum sp. were collected from different vicinities of Delhi, Kurukshetra and Haridwar (India). 16S rDNA gene sequencing of CHII(II)K7 and DDI(I)1 showed similarities with the *Pseudomonas* sp. while CHIII(I)Y6, UHI(II)7 and CHII(I)NA4 showed homology with *Bacillus* sp. The obtained sequences (852 to 1452 bp) of the five isolates CHII(II)K7, DDI(I)1, CHIII(I)Y6, UHI(II)7 and CHII(I)NA4 showed homology with the 16S rDNA sequence of *Pseudomonas* sp. PcFRB039 (99%), *Pseudomonas fluorescens* strain CB32 (100%), *Bacillus cereus strain* F198_B10 (99%), *Bacillus licheniformis* strain AK02 (91%) and *Bacillus* sp. JSG1 (98%).Almoneafy *et al.*[1] reported 4 strains (AM1, D16, D29 H8) exhibiting strong antagonistic activity from 200 *Bacillus* isolates obtained from tomato and potato rhizosphere and molecularly characterized them by 16SrDNA gene sequencing. The obtained sequences were analysed using BLAST to match the most identical sequence for determination of the source microbe. The evolutionary study revealed that the strains D16 as *Bacillus subtilis*, AM1 as *Bacillus amyloliquefaciens* and D29 as *Bacillus amyloliquefaciens* and H8 as *B. methylotrophicus*. The phylogenetic tree was made using MEGA5 (version 5.03) (Kumar *et al.*[10]). Further, Sang *et al.*[22]. isolated 576 endophytic bacteria from the leaves, stems, and roots of 10 rice cultivars and identified 12 of them as diazotrophic bacteria using a specific primer set of *nif* gene. Through 16S rDNA sequence analysis, *nifH* genes were confirmed in the two species of *Penibacillus*, three species of *Microbacterium*, three *Bacillus* species, and four species of *Klebsiella*. Rice seeds treated with these plant growth promoting bacteria (PGPB) showed improved plant growth, increased height and dry weight and antagonistic effects against fungal pathogens.

Table 1: Soil samples collected from different districts of Rajasthan

S. No.	Location	Soil EC (dS/m)	Soil pH
1.	Jhalipura, Kota	0.09	8.4
2.	Anta, Baran	0.19	7.5
3.	Ladpura, Kota	0.20	7.7
4.	Anta, Baran	0.17	7.5
5.	Mandola, Baran	0.22	8.2
6.	Bundi	0.10	7.6
7.	Modak, Kota	0.13	8.3
8.	Sawan bhado, Kota	0.16	7.8
9.	Mandawar,Jhalawar	0.07	8.2

Table 2: Sodium (Na), Potassium (K), Organic carbon (%) and available nitrogen (%) analysis in collected soil samples

S. No.	Location	Na (Kg/hq)	K (Kg/ hq)	Organic Carbon (%)	Available Nitrogen (%)
1.	Jhalipura, Kota	347.53	246.96	0.44	0.040
2.	Anta, Baran	374.86	344.51	0.26	0.001
3.	Ladpura, Kota	398.83	279.10	0.315	0.034
4.	Anta, Baran	137.53	409.69	0.49	0.065
5.	Mandola, Baran	408.80	197.34	0.14	0.037
6.	Bundi	317.74	324.24	0.87	0.024
7.	Modak, Kota	312.36	359.96	1.05	0.060
8.	Sawan bhado, Kota	368. 40	164.19	0.75	0.021
9.	Mandawar,Jhalawar	268.24	196.89	1.15	0.031

Table 3: Screening of selected bacterial isolates for Phosphate solubilization

Isolate (s)	Diameter of Zone (mm)	Solubilization Index
COR-3	30	100.0
COR-5	18	157.14
COR-37	22	120.0
COR-55	15	200.00
COR-61	36	350.0
COR-65	40	233.33
COR-72	29	222.22
COR-73	29	314.28
COR-83	33	83.33
COR-96	25	127.27

Table 4: Morphological characterization of selected bacterial isolates for Phosphate solubilization

S. No.	Isolate (s)	Gram's staining	Endospore staining	Shape	Arrangements	Motility
1	COR-3	-	-	Rod	Chain	+
2	COR-5	+	+	Rod	Chain	+
3	COR-37	+	+	Long rods	Chain	+
4	COR-55	+	-	Cocci	Tetrad	-
5	COR-61	+	-	Rods	Chain	+
6	COR-65	+	+	Rod	Chain	+
7	COR-72	+	-	Cocci	Tetrad	-
8	COR-73	+	+	Rods	Single	+
9	COR-83	-	+	Rods	Single	+
10	COR-96	+	-	Rod	Single	+

Table 5: Biochemical characterization of selected bacterial isolates for Phosphate solubilization

Tests	COR-3	COR-5	COR-37	COR-55	COR-61	COR-65	COR-72	COR-73	COR-83	COR-96
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	-	-	-	+	-	+	-	+
Methyl Red	-	+	-	-	+	+	+	-	+	+
Vogus Proskauer	-	-	-	+	-	+	+	+	-	+
Citrase	+	-	-	+	-	+	-	+	+	-
Indole	+	-	+	-	-	+	-	+	+	+
Amylase	+	+	+	-	+	+	-	+	-	-
Nitrate reduction	-	+	+	+	+	+	-	+	+	-
Urease	+	-	+	+	-	+	+	-	-	+
Phosphate solubilization	+	+	+	+	+	+	+	+	+	+
HCN	-	-	-	-	-	+	-	-	-	-
Glucose Fermentation	+	+	+	+	+	+	+	+	+	+
Sucrose fermentation	+	+	-	+	+	-	+	+	-	+
Lactose Fermentation	-	+	+	-	+	+	-	+	-	-

Table 6: Antibiotic assay of COR isolates

Isolates	**Antibiotics**											
	AMP	**RIF**	**C**	**VA**	**S**	**PG**	**S3**	**E**	**P**	**CL**	**TE**	**K**
	10 mcg	**15 mcg**	**25 mcg**	**5 mcg**	**10 mcg**	**2 unit**	**300 mcg**	**60 mcg**	**1 unit**	**10 mcg**	**25 mcg**	**1000 mcg**
COR-3		S		S		R		S		S		S
COR-5		S		S		S		S		S		S
COR-37		R		S		R		R		S		S
COR-55			S							R		
COR-61		R		R		R		R		S		S
COR-65	S		S		R		S		S		S	
COR-72	R		R		R		S		R		S	
COR-73		S		R		R		R		S		S
COR-83	R		R		S		S		R		S	
COR-96		R	S									R

Ampicillin (AMP), Rifampicin (RIF), Chloramphenicol (C), Vancomycin (VA), Streptomycin (S), Penicillin G (PG), Sulphatriad (S3), Erythromycin (E), Penicillin (P), Colistin (CL), Tetracyclin (TE) and Kanamycin (K).
S= Sensitive, R= Resistance

Table 7: Molecular identification of chosen bacterial isolates

S. No.	**Isolate**	**Bacteria identified**	**Homology shared**	**Accession number generated by NCBI**
1.	COR-3	*Pseudomonas putida*	*Pseudomonas putida* strain TDRl3	KY810614
2.	COR-5	*Bacillus megaterium*	*Bacillus megaterium* strain MO29	KX228234
3.	COR-37	*Bacillus altitudinis*	*Bacillus altitudinis* strain EH19	-
4.	COR-55	*Micrococcus luteus*	*Micrococcus luteus* strain INBI-1	KK223364
5.	COR-61	*Bacillus subterraneus*	*Bacillus subterraneus* strain CES M15/102	-
6.	COR-65	*Bacillus aryabhattai*	*Bacillus aryabhattai* strain IHB B 7164	KX231810
7.	COR-72	*Micrococcus luteus*	*Micrococcus luteus* Strain CHN10	-
8.	COR-73	*Bacillus tequilensis*	*Bacillus tequilensis* strain IHBB 9348	KX231808
9.	COR-83	*Pseudomonas sp.*	*Pseudomonas* sp. NBFPALD_RAS138	KX231809
10.	COR-96	*Planococcus refietoensis*	*Planococcus refietoensis* Strain YJST4	-

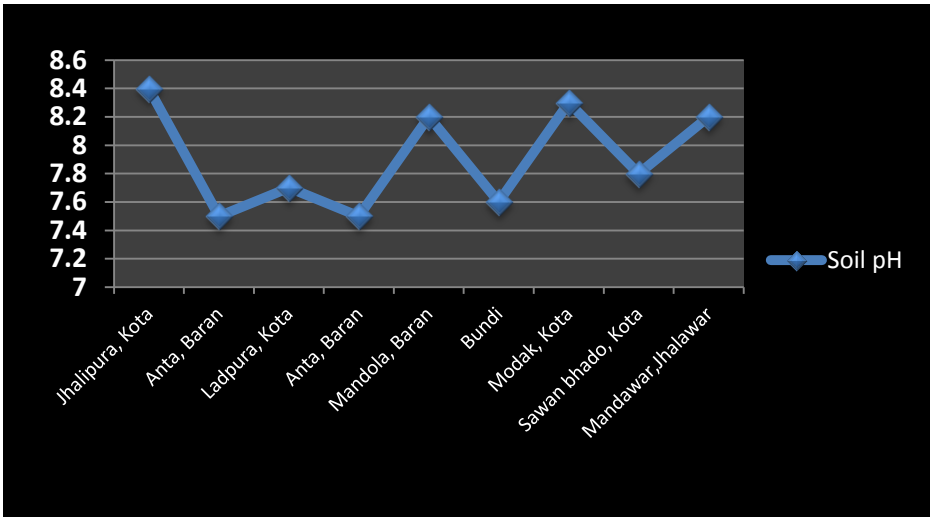

Fig. 1: pH analysis of collected soil samples of Coriander Rhizosphere

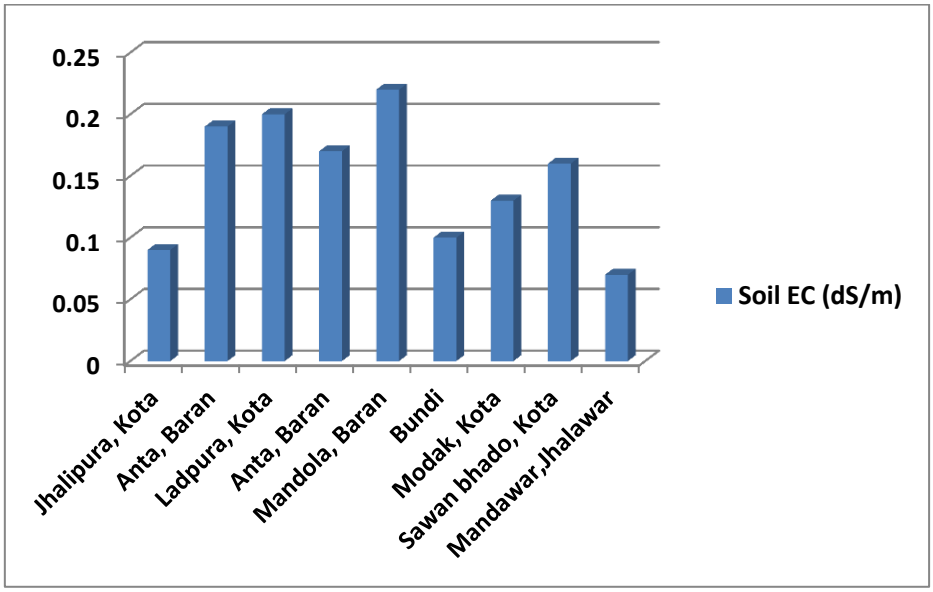

Fig. 2: EC analysis of soil samples of Coriander Rhizosphere

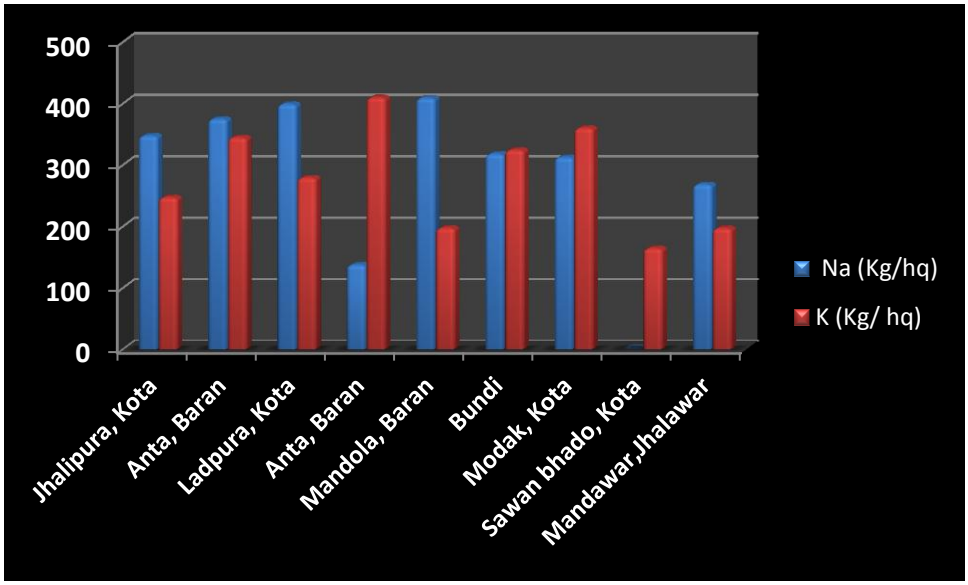

Fig 3: Comparative analysis of Na and K (Kg/hq) nutrients available in soil samples of Coriander Rhizosphere

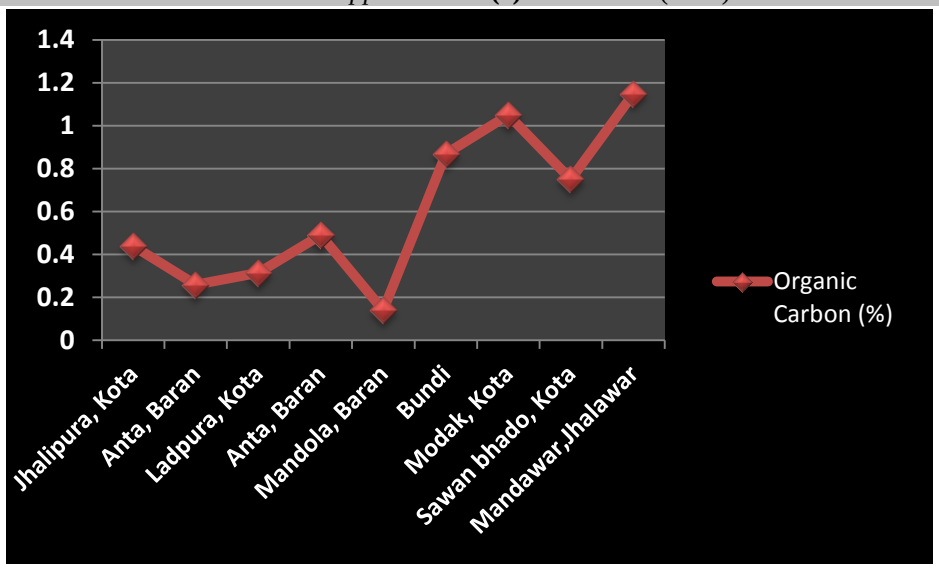

Fig. 4: Organic Carbon (%) present in soil samples of Coriander Rhizosphere

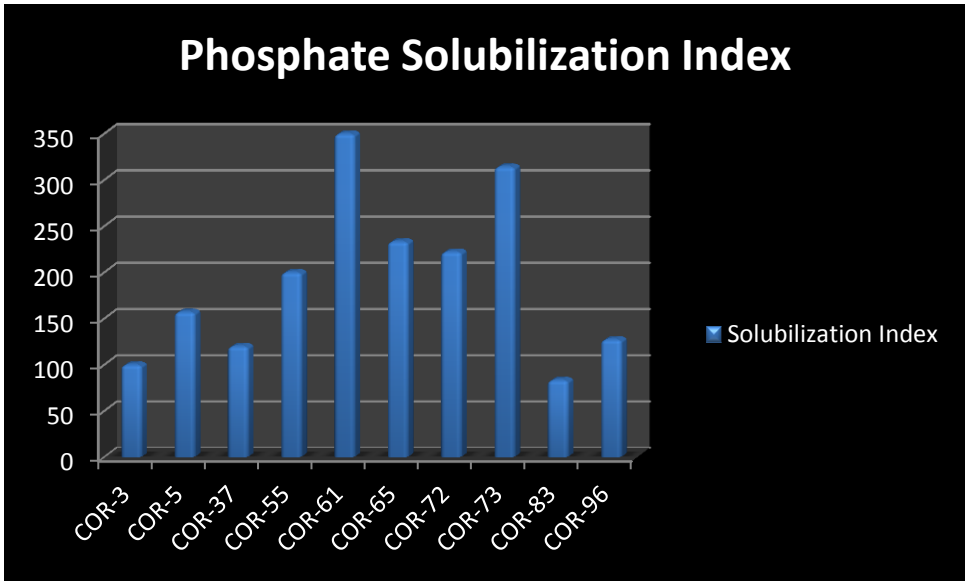

Fig. 5: Phosphate solubilizing index of isolated COR bacterial strains from coriander Rhizosphere of Hadoti region

CONCLUSION

This study has revealed that the phosphate solubilizing efficacy of the isolated COR strains could be used to solublize higher amount of phosphates in the soils and provide higher production in coriander seed spice crop. All isolated ten bacterial strains belongs to these genus were found to be potent candidates to be developed as inoculants as they exhibited multiple Plant growth promoting traits for crop improvement.

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REFERENCES

1. Almoneafy, A. A., Xie, G. L., Tian, W. X., Xu, L. H., Zhang, G. Q. and Ibrahim, M. Characterization and evaluation of *Bacillus* isolates for their potential plant growth and biocontrol activities against tomato bacterial wilt. *African Journal of Biotechnology*, **11(28):** 7193-7201(2012).
2. Baby, U. I. Biofertilizers in tea. *Planters' Chronicle* **98:** 395-396 (2002).

3. Bargali, S. S., Kiran Shukla, Lalji Singh, Lekha Ghosh & M. L. Lakhera. Leaf litter decomposition and nutrient dynamics in four tree species of dry deciduous forest. *Tropical Ecology* **56:** 191-200 (2015).
4. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**:783-791(1958).
5. Feng K., Lu H. M., Sheng H. J., Wang X. L., and Mao J., Effect of organic ligands on biological availability of inorganic phosphorus in soils," *Pedosphere*, **14(1)**, 85–92 (2004).
6. Gaur, A. C., M. Madan & K. P. Ostwal. Solubilization of P compounds by native micro flora of rock phosphate. *Indian Journal of Experimental Biology* **11:** 427-429 (1973).
7. H. Rodríguez and R. Fraga. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances*, **17(4-5);** 319–339 (1999).
8. H. Rodriguez, T. Gonzalez, I. Goire, and Y. Bashan, Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium Azospirillum spp. *Naturwissenschaften*, **91(11)**: 552–555 (2004).
9. Hillol Chakdar, Premlal Kashyap, Alok K. Shirvastava and K. Pandiyan. *Laboratory manual of National training on advances in plant growth promoting rhizobacteria: diversity to utility*. ICAR- National Bureau of Agriculturally Important Microorganisms (NBAIM). pp: 1-87 (2016).
10. Kimura M. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16:**111-120 (1980).
11. Kumar, S., Tamura, K. and Nei, M., Mega3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. *Briefings in Bioinformatics*, **5(2):** 150-163(2004).
12. Li, S. T., J. I. Zhou, H. Y. Uang, X. Q. Chen & C. W. Du., Characteristics of fixation and release of phosphorus in three soils. *Acta Pedologzca Sznica* **40:** 908-914 (2003).
13. Mishra B. K., B. Singh, P. Singh, S. S. Rathore, O. P. Aishwath, K. Kant and P. N. Dubey., Isolation and evaluation of phosphate solubilizing microorganisms from fennel (*Foeniculum vulgare* Mill.) rhizospheric soils of Rajasthan. *International J. Seed Spices* **5(1)**:71-75(2015).
14. N. Tenzing Baliah, G. Pandiarajan & B. Makesh Kumar. Isolation, identification and characterization of phosphate solubilizing bacteria from different crop soils of Srivilliputtur Taluk, Virudhunagar District, Tamil Nadu. *Tropical Ecology*. **57(3):** 465-474 (2016).
15. Pikovaskaya, R.I., Mobilization of phosphorous in soil in connection with vital activity of some microbial species. *Mikrobiologiya*, **17**: 362-370 (1948).
16. Ramanathan, S., K. Natarajan & P. Stalin.,Effect of foliar nutrition on grain yield of rice fallow black gram. *Madras Agriculture* **91:** 160-163 (2004).
17. Rana, A., Saharan, B., Joshi, M., Prasanna, R., Kumar, K. and Nain, L., Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. *Annals of Microbiology*, **61:** 893-900 (2011).
18. Richardson A. E. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Functional Plant Biology*. **28(9):** 897–906 (2001).
19. S. Bhat, P. Kaushal, M. Kaur and H. K. Sharma., Coriander (*Coriandrum sativum* L.): Processing, nutritional and functional aspects. *African journal of Plant sciences*. Vol. **8 (1)**: 25-33 (2014).
20. Saharan, B. S. and Verma, S., Evaluation of rhizospheric bacteria from *Ocimum* sp. as potential PGPR. *Int. J. Microbial Res. Technol.*, 2(3): 22-27 (2015).
21. Saitou N. and Nei M., The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution.* **4**:406-425 (1987).

22. Sang Hye Jia, Mayank Anand Gururanib, Se-Chul Chun., Isolation and characterization of plant growth promoting endophyticdiazotrophic bacteria from Korean rice cultivars. *Microbiological Research* **169:** 83– 98 (2014).
23. Shobha, G. and Kumudini, B. S., Antagonistic effect of the newly isolated PGPR Bacillus sp. on Fusarium oxysporum. *International Journal of Applied Science Engineering Research*, **1(3):** 463-474 (2012).
24. Tamura K., Peterson D., Peterson N., Stecher G., Nei M., and Kumar S., MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* **28:** 2731-2739 (2011).