

RESEARCH ARTICLE



Physio-chemical characterization of cumin and coriander growing soils of semi-arid zones of India and its bioprospecting for plant growth promoting rhizobacteria

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Abstract

Cumin and coriander are indeed vital spices in Indian cuisine and are predominantly cultivated in the arid zones of India like- Rajasthan and Gujarat. The quality and yield of any crop are strongly influenced by soil characteristics and the environmental conditions of the geographical region. In this study, a field survey was carried out for numerous locations of cumin and coriander growing areas to understand the physicochemical and microbial properties of cumin and coriander growing soils of Rajasthan and Gujarat. A total of 31 soil samples were collected and analysed for electric conductivity (EC), pH, organic carbon (OC), nitrogen (N), phosphorus (P), potassium (K) and total microbial counts. A considerable variation was found in soil pH levels of different soil samples which ranged from pH 6.80 to pH 9.03. The EC was found in the range of 0.56 to 0.99 ms/cm. The organic carbon, available nitrogen, phosphorus and potassium were found respectively in the range of 0.29 to 0.92 %, 135-382.8 kg/ ha, 6.16-18.25 kg/ha and 261.0 kg/ha to 412.5 kg/ha. Bacteria were isolated from these soils and based on morphological differences total of 54 bacteria were further studied for plant growth-promoting (PGP) characteristics. The isolates were screened to produce catalase, Indole Acetic Acid (IAA), citrate utilization, phosphate and solubilization. Fifty-one showed IAA production, 18 were found to utilize citrate and 44 were capable of degrading carbohydrates. Isolates that showed higher PGP activities were identified, which may be utilized to improve plant growth and boost crop yield.

Keywords

Bacillus; cumin; coriander; Enterobacter; Micrococcus; rhizosphere

Introduction

Soil ecosystems contain vastly greater microbial diversity than eukaryotic organisms. Approximately 10 billion microbes can be found in one gram of soil, which could indicate thousands of distinct species (1). These organisms create intricate interaction networks that affect soil functions. Microorganisms found in soil are required for processes like the decomposition of organic matter, nutrient cycling and humus development. Within the soil system, the rhizosphere, the area directly surrounding plant roots, is a microbial hotspot and is regarded as one of the planet's most dynamic interfaces. The

rhizosphere, a key interaction zone between plants, soil and microorganisms, hosts a diverse microbial community influenced by plant root exudates and soil properties.

In agro-ecosystems, soil type, environmental conditions and local climate are key factors influencing the types of microorganisms found in the soil. Numerous beneficial microorganisms have been identified by researchers from soil and other natural habitats for specific applications. Plant growth-promoting rhizobacteria (PGPR) are a group of diverse microbes found in plant rhizospheres that are essential for the development and growth of plants. These microbes play an imperative role in plant growth, health and soil fertility, with their abundance and activity being influenced by the root environment. Various microorganisms have been known for specific purposes, such as improving mineral uptake, nitrogen fixation, producing phytohormones, mitigating both biotic and abiotic stress and producing enzymes and volatile organic molecules to combat plant diseases (2).

A large portion of microbes with interactive roles in natural habitats remains uncharacterized owing to the restrictions in culturing them under standard media conditions. This is especially relevant in dynamic biological systems such as the rhizosphere, which supports a complex variety of microbial diversity and metabolic functions. Rhizosphere microorganisms and soil physicochemical properties have a crucial role in the natural restoration of plant communities (3). Gaining a deeper understanding of soil microbial communities is crucial for exploring their roles in ecosystems. The diversity and composition of plant-associated microbiota are vital for uncovering the mechanisms behind their interactions and ecological functions, which can lead to more sustainable agricultural practices and improved plant growth.

The arid and semi-arid parts of India, especially Gujarat and Rajasthan, are famously known as the seed spice bowl of India. These areas contribute to over 80% of the country's total seed spice production. India produces the highest amount of seed spices and is also the largest exporter of seed spices in the world (4). Major seed spice cultivation occurs in states like Gujarat, Rajasthan, Madhya Pradesh, Punjab and Uttar Pradesh (5). Coriander (*Coriandrum sativum L*.) and cumin (*Cuminum cyminum L*.) are important exportearning seed spice crops cultivated in the arid and semi-arid regions of India. Coriander production is predominantly concentrated in Madhya Pradesh, Gujarat and Rajasthan, while cumin is grown in Gujarat and Rajasthan, which together account for ~90% of the total production in the country (6). These ancient crops hold significant economic value as medicinal value.

Many studies have examined bacterial communities in various agricultural systems in arid and semi-arid regions, focusing on cumin, coriander and cereal crops (7, 8). Research on edaphic factors such as electrical conductivity (EC), pH, soil temperature, soil colour and porosity has been conducted for seed spice crops like coriander and cumin (9, 10). However, studies on the exploration of arid and semi-arid soils for PGPR bacteria are still unexplored, particularly for the rhizospheric soils of seed spices like cumin and coriander.

Our current study aims to explore the genetic diversity of bacteria in cumin and coriander farmers' fields in Gujarat and Rajasthan. Thus, keeping this view in mind, for the first time, a detailed survey was conducted in the cumin and coriander growing areas of Rajasthan and Gujarat and a detailed analysis of soil samples was done.

Materials and Methods

Site description and sampling

A survey in the field was conducted at various locations of cumin and coriander growing areas of Rajasthan and Gujarat states of India with contrasting agro-climatic conditions (Fig. 1 and 2). The rhizospheric soil sample (soil surrounding plant roots) was collected by uprooting five plants of each species from every site (Table 1). A sterile spade and trowel were used to dig for ~20 cm sideways for uprooting the plants. The



Fig. 1. Sample collection sites of 54 isolates from Rajasthan and Gujarat.

Table 1. Sample collection site and passport data of samples

S. No.	Geographical location	Altitude (m)	Biomaterial Provider	Depth (cm)	Env. biome	Latitude	Longitude	Sample type
NRCSS-1	Thara, Udhanpur	42	Joshi Ragunath Bhai	12-15	Semi arid	23° 53.60 N	71°46.45 E	Cumin soil
NRCSS-2	Radhanpur	29	Champa Lal Rakesh Bhai	10-12	Semi arid	23° 49.46 N	71°34.33 E	Cumin soil
NRCSS-3	Gamdi, Satalpur	24	Laxman Bhai	14-18	Semi arid	23° 42.58 N	71°67.45 E	Cumin soil
NRCSS-4	Fatehgadh, Rapad	39	Savadi Patel	8-10	Semi arid	23° 40.05 N	70°51.19 E	Cumin soil
NRCSS-5	Lodrani, Balasar	34	Vikash Bhai, Mohad ji	12-15	Semi arid	23° 53.58 N	70°26.82 E	Cumin soil
NRCSS-6	Dudhai, Bhuj	42	Parvat Bhai Patel	16-18	Semi arid	23º 18.72 N	70°07.63 E	Coriander soil
NRCSS-7	Songadh, Morbi	13	Sushil Bhai	18-20	Semi arid	23°03.71 N	70°43.92 E	Cumin soil
NRCSS-8	Bhisavada, Porbandar	15	Krishna Bhai Patel	14-18	Semi arid	21º 46.90 N	69°46.45 E	Coriander soil
NRCSS-9	Ratiya, Porbandar	128	Ashok Bhai	8-10	Semi arid	22º 29.16 N	70° 79.32 E	Cumin soil
NRCSS-10	Sukhpur, Junagarh	21	Bika Bhai	12-15	Semi arid	21°71.70 N	70°17.40 E	Coriander soil
NRCSS-11	Sasan, Gir, Somnath	130	Ismale Bhai	16-18	Semi arid	21° 09.15 N	70°35.44 E	Coriander soil
NRCSS-12	Balchhel, Sasan, Junagarh	155	Ram Bhai	18-20	Semi arid	21º 10.59 N	70° 32.85 E	Coriander soil
NRCSS-13	Sakli, Rajkot	90	PurshotamBhai	14-18	Semi arid	21° 41.23 N	70° 33.55 E	Coriander soil
NRCSS-14	Kothariya, Surendranagar	59	Bharat Bhai	8-10	Semi arid	22º 72.71 N	71°64.86 E	Cumin soil
NRCSS-15	Jagudhan	49	College of Horticulture	13-15	Semi arid	23° 30.79 N	72°23.92 E	Cumin soil
NRCSS-16	Muniya, Pratapgarh	274	HansrajDhakad	12-14	Semi arid	21º 02.61 N	75°32.87 E	Coriander soil
NRCSS-17	Polaikalan, Kota	267	GhanshyamDhakad	11-13	Semi arid	25° 10.17 N	75° 54.06 E	Coriander soil
NRCSS-18	Seemalya, Kota	266	Satendra Singh	11-16	Semi arid	25º 19.12 N	76°06.39 E	Coriander soil
NRCSS-19	Deori, Jhalawar	320	Harish Agrwal	12-14	Semi arid	24º 46.58 N	76°03.51 E	Coriander soil
NRCSS-20	Dara, Jhalawar	350	HarphoolMeena	16-18	Semi arid	24°41.40 N	75° 59.79 E	Coriander soil
NRCSS-21	Mandana, Jhalawar	372	Rajesh Oti	10-13	Semi arid	24° 59.16 N	76°00.14 E	Coriander soil
NRCSS-22	Kanwas, Kota	256	Virendra Meena	8-10	Semi arid	24° 92.40 N	76°28.69 E	Coriander soil
NRCSS-23	Ranpur, Kota	279	NemrajBarmuda	16-18	Semi arid	25° 81.70 N	75° 50.20 E	Coriander soil
NRCSS-24	Jalampur, Chittorgarh	312	Tej Singh	9-11	Semi arid	24° 48.09 N	74°38.49 E	Cumin soil
NRCSS-25	Banas, Sirohi	257	BabulalDahur	12-14	Semi arid	24° 42.64 N	72° 58.92 E	Cumin soil
NRCSS-26	Arathwara, Sirohi	218	Panna Chaudhary	12-15	Semi arid	23º 15.06 N	77°25.55 E	Cumin soil
NRCSS-27	Mangliyawas, Ajmer	445	LaxmanPooniya	13-17	Semi arid	26° 16.71 N	74°33.41 E	Cumin soil
NRCSS-28	Sanawara, Barmer	118	Teja Ram	12-14	Semi arid	25° 28.53 N	71°26.80 E	Cumin soil
NRCSS-29	Thaiyat, Jaisalmer	238	Prem Singh	14-18	Semi arid	20° 56.18 N	71°03.46 E	Cumin soil
NRCSS-30	Khara, Phalodi	254	TeiaramBivaii	12-15	Semi arid	27°02.74 N	72°11.06 E	Cumin soil
NRCSS-31	Gotan, Nagour	314	Mohan Choudhary	13-14	Semi arid	26° 40.05 N	73°42.53 E	Cumin soil



Fig. 2. Morphological characterization of cumin and coriander growing area: (A) Rajasthan (B) Gujarat.

clump of soil and root was carefully lifted, placed in a sterile polybag and transferred to the laboratory. The roots were then gently shaken to collect loose soil samples, which were allowed to air dry, ground into a fine powder using a pestle and mortar and sieved through a 2 mm mesh. The physicochemical properties of the soil were determined using the processed samples. The remaining attached soil, known as rhizosphere soil, was collected using sterile brushes and stored in a refrigerator at 4°C until microbial analysis.

Physiochemical analysis of soil samples

The soil pH was measured in a 1:2.5 (10 g soil with 25 ml of distilled water) suspension using a pH meter, following the method described by Piper et al. (11). Electrical conductivity was measured with the help of an EC meter and expressed as dS m⁻¹ (12). Available Nitrogen was determined by the alkaline potassium permanganate method of Subbiah and Asija where permanganate acts as a strong oxidizing agent (13) and available phosphorous content in the soil was extracted by Olsen method (14), The available potassium in the soil was extracted using neutral normal ammonium acetate and measured by aspirating the extract into a flame photometer as given by Jackson (15), organic carbon (% OC) by rapid titration method by using potassium dichromate and sulfuric acid to oxidize organic matter as mentioned by Walkley and Black (16) and organic matter (OM) by Bower (17) in three replicates (Fig. 3).

Isolation of bacteria from rhizospheric soils

Each rhizospheric soil sample was homogenized and diluted in sterilized water. Suspension from appropriate dilution was spread and plated onto nutrient agar (NA) and Luria-Bertani (LB) agar growth medium plates. Then for 24 hours, plates were incubated at 28°C for the isolation of bacteria as per Somasegaran and Hoben (18). Distinguished colonies were picked and streaked on NA plates for their purification and pure isolates were used to prepare Agar stab cultures then stored in refrigerator at 4°C for further studies.

Characterization of bacterial isolates

Using standard microbiological techniques outlined by Somasegaran and Hoben (18), all isolates were analyzed for morphological characteristics, including colony morphology, cell shape, border, mucocity, transparency and color. All 54 isolates were grown on YEMA medium for 48-72 hours at $28^{\circ}C \pm 2^{\circ}C$ in the dark and observed under oil immersion on a compound microscope (Leica Microsystems) for their detailed study of morphology. The gram staining method was done to classify the isolates in gram-positive and gram-negative.

IAA based Screening

Standard procedures were followed to screen isolates for PGP -associated attributes. Screening comprised the production of indole-acetic acid (IAA); isolates showed the development of pink colour considered as IAA producers (19).

Phosphate solubilization activity

It was assessed using Pikovskaya's medium. Isolates were aseptically spot inoculated at the centre of the agar plates. Each plate was incubated at $28 \pm 2^{\circ}$ C for a duration of five to six days. Phosphate solubilization was shown by a distinct zone surrounding a colony that was expanding (20). Around a colony that was expanding, a distinct zone showed phosphate solubilization.

Catalase production, Carbohydrate degradation and citrate utilization test

Catalase production was measured by the method described by *Purushottam et al.* (21), in these pure isolates (24 h old) were taken on glass slides and one drop of hydrogen peroxide (H₂O₂) (30%) was added. The catalase enzyme was present when a gas bubble appeared (21). Carbohydrate degradation and citrate utilization tests were performed using Bergey's manual (22). The catalase test was used to determine whether the catalase enzyme was present in bacterial colonies. One drop of 30% H₂O₂was applied to pure isolates that had been left for 24 hours on glass slides.



Fig. 3. Physicochemical properties of the collected soil samples : (A) pH; (B) EC; (C) Av N (Available Nitrogen), Av P (Available phosphorus) and Av K (Available Potassium); (D) % OC (Organic Carbon) and % OM (Organic Matter).

Microbial Identification using 16S rRNA amplicon sequencing

DNA was isolated from the culture of five selected isolates (23). DNA quality was evaluated on 0.8% Agarose Gel For, the identification of bacterial isolates, the DNA was amplified using 16S rRNA (8F and 1492R) primer (24). There was only one distinct 1500 bp PCR amplicon band found. The purified PCR amplicon was then processed further for sequencing. Using aligner software, a consensus sequence of 1489 bp 16S rRNA was produced from forward and reverse sequence data. The NCBI GenBank database's BLAST alignment search tool was utilized with the 16S rRNA sequence. The top fifteen sequences were chosen based on their maximum identity score and Clustal W, a multiple alignment algorithm, was used to align them (25).

Result and Discussion

Soil pH

Considerable variations in soil pH were observed among the analyzed samples, ranging from 6.80 to 9.03. The soil samples having less than 7.0 pH were rich in organic carbon and organic matter (Table 2). The soil samples NRCSS-2, NRCSS-6 and NRCSS-11 were slightly acidic and less preferred by cumin and coriander crops due to a reduction in the solubility of minerals. Most of the analyzed soil samples fall in the level of pH 7-8 and found the most suitable soil for the cultivation of the crops (Table 2). Soil sample NRCSS-31 collected from Gotan, Nagour with the highest pH level (9.03) was not ideal for the cultivation of cumin and coriander. The pH in the neutral range (6.5 to 7.5) is appropriate for the presence of the majority of primary, secondary and micronutrients in the soil and assists in improved crop growth. An increase in pH above 8.0 reduces the readiness of P and micronutrients such as B, Zn, Mn, Fe and Cu. The decrease in availability as pH rises influences the growth and yield of coriander and cumin in the research area. Zinc, copper and manganese concentrations fall 100 times with the increase of individual units in pH (26).

EC of the Soil

Electrical conductivity is frequently used as a measure of salinity and to estimate the quantities of soluble salts in soil. The soils under analysis ranged from 0.56 to 0.99 mS/cm (Table 2) and were considered normal soil. The electrical conductivity of soil (EC) is the indicator of its salinity and an important indicator of soil health (27). It will have an impact on soil microbial activity, crop quality and yield and the availability of plant nutrients-all of which are linked to essential soil processes. Electrical conductivity (EC) does not directly affect plant growth, but it does reflect nutrient availability and salinity levels in the soils. The ideal EC value normally ranges from 0.8 to 1.8, while values exceeding 2.5 can adversely affect plant growth and development (28). Elevations of EC have been linked to increases in nitrate and other soil nutrients (P, K, Ca, Mg, Mn, Zn and Cu). Most bacteria are salt-sensitive (high EC) and actinomycetes and fungi are less sensitive than bacteria, except for salt-tolerant halophytes (29).

Available N, P, K

The results of the sample analysis indicated that the nitrogen

levels of the soil samples under examination varied significantly. The nitrogen content of the soil varied from 135.0 kg/ha (NRCSS-30) to 395.9 kg/ha (NRCSS-6) in Dudhai, Bhuj (Table 2). The low nitrogen content in the soil may be due to sandy texture. Available phosphorus in the studied soil samples varied from 6.16 kg/ha (NRCSS-20) to 21.62 kg/ha (NRCSS-9) (Table 2). The lower level of phosphorus in the soil is due to the low application of phosphorus fertilizers in the cumin and coriander. Phosphorus (P) is required to keep the other plant nutrients in balance and to ensure that the crop grows normally. As the soil dries out and the potassium bonds to the layers of clay, potassium fixing takes place. Under certain conditions, an initial amount of exchangeable potassium depends on the soil texture. From the analyzed soil sample potassium ranged from 261.0 kg/ha (NRCSS-14) to 412.5 kg/ha (NRCSS-19) (Table 2) indicating that sufficient potassium is present in the studied cumin and coriander growing soils. Crops generally absorb nitrate because microbial nitrification, the rapid conversion of NH4⁺ to nitrate occurs efficiently at a near-neutral pH 7. Nitrification occurs slowly in acidic soils (pH <6), giving an advantage to plants that are capable of absorbing NH₄⁺. The pH also affects the type of phosphorus (P), which is essential for plant growth and its availability in soil. Consequently, to maximize the effectiveness of P fertilizers, the pH of the soil must be adjusted to an ideal level that is particular to the kind of soil (30).

Organic Carbon

The organic carbon (%) ranged from 0.29 to 0.92 in the analyzed samples (Table 2). The lowest OC was found in the soil samples NRCSS-18 and NRCSS-29. Climate conditions like high temperatures and little rainfall, as well as human activity through crop cultivation techniques, were thought to have an impact on the amounts of organic matter content in the soils (31). High erosion in these samples may be the cause of the low amount of OC. While highest OC was found in NRCSS-6 which was collected from Dudhai and Bhuj. Organic matter is directly proportional to the organic carbon of the soil. The arid zone's soil samples have low organic carbon content (<0.50) because of high temperatures, little rainfall, sparse scrub vegetation and sandy soil textures that encourage severe oxidation (32). One such possible cause could be the summertime tillage operation and the minimal yearly addition of organic matter. Earlier workers (33, 34, 35) also reported low OC content in soils of arid and semi-arid regions of India. Globally, arid and semi-arid regions store around 27% of global soil organic carbon (SOC) (35).

Correlation between soil parameters

Pearson's correlation coefficient was estimated to determine associations between the soil physicochemical properties (Table 3). There was a significant positive correlation between EC with pH (0.370*), Av K with pH (0.595*), OC with Av N (0.767*) and OM with Av N (0.766*). Similarly, a significant negative correlation was found for Av N with pH (-0.577*), Av K with Av N (-0.658*), OC with pH (-0.459*), OC with Av K (-0.569*), OM with pH (-0.461*), OM with Av K (-0.568*) and OM with OC (1.00*). A stronger positive correlation between Av K and pH suggests that higher levels of available potassium (Av K) are associated with higher soil pH. This is also supported by previous studies where limed soils often hold more

ОМ

Table 2. Physiochemical properties of the collected soil samples

S. No.	рН	EC (mS/cm)	Available Nitrogen (kg/ha)	Available phosphorus (kg/ha	Available) Potassium (kg/ha)	Organic Carbon (%OC)	Organic Matter (%OM)		
NRCSS-1	7.42	0.96	315.2	7.62	276.5	0.55	0.94		
NRCSS-2	6.89	0.92	345.3	12.53	289.6	0.75	1.29		
NRCSS-3	7.13	0.68	370.1	16.50	270.9	0.68	1.17		
NRCSS-4	7.67	0.56	305.0	11.62	310.2	0.51	0.87		
NRCSS-5	7.63	0.98	335.6	8.33	305.6	0.85	1.46		
NRCSS-6	6.98	0.83	395.9	7.25	299.7	0.92	1.58		
NRCSS-7	7.20	0.76	370.8	12.50	291.2	0.71	1.22		
NRCSS-8	7.94	0.91	335.6	17.29	317.4	0.55	0.94		
NRCSS-9	8.01	0.84	350.7	21.62	321.0	0.74	1.27		
NRCSS-10	7.52	0.90	374.8	17.23	325.1	0.90	1.55		
NRCSS-11	6.80	0.59	347.5	15.76	275.2	0.65	1.12		
NRCSS-12	7.05	0.68	339.7	13.89	289.4	0.68	1.17		
NRCSS-13	7.96	0.63	307.4	9.74	278.4	0.77	1.32		
NRCSS-14	7.63	0.65	327.2	10.06	261.0	0.82	1.41		
NRCSS-15	8.26	0.85	382.8	7.23	304.8	0.62	1.06		
NRCSS-16	8.04	0.89	280.6	6.29	325.2	0.45	0.77		
NRCSS-17	8.32	0.88	295.7	8.21	389.0	0.55	0.94		
NRCSS-18	7.57	0.70	265.2	9.58	270.4	0.29	0.49		
NRCSS-19	7.85	0.88	287.5	7.55	412.5	0.37	0.63		
NRCSS-20	7.63	0.63	266.6	6.16	395.6	0.45	0.77		
NRCSS-21	7.65	0.70	257.0	11.15	302.6	0.32	0.55		
NRCSS-22	7.54	0.63	289.7	18.25	298.1	0.62	1.06		
NRCSS-23	8.24	0.92	291.9	9.82	328.3	0.51	0.87		
NRCSS-24	8.13	0.82	299.1	14.55	348.6	0.48	0.82		
NRCSS-25	7.95	0.78	273.3	15.45	365.3	0.49	0.84		
NRCSS-26	7.77	0.81	278.7	10.55	377.4	0.42	0.72		
NRCSS-27	7.87	0.68	280.3	7.95	387.5	0.49	0.84		
NRCSS-28	7.95	0.86	189.8	9.23	375.6	0.41	0.70		
NRCSS-29	7.86	0.87	150.9	6.24	388.6	0.29	0.49		
NRCSS-30	8.23	0.74	135.0	11.50	410.0	0.35	0.60		
NRCSS-31	9.03	0.99	145.3	9.85	390.6	0.42	0.72		
Table 3. Correlation between physicochemical properties of soil samples									
		рН	EC	Av N	Av P	Av K C	ос ом		
рН		1							
EC		0.370*	1						
Av N		-0.577*	-0.087	1					
Av P		-0.206	-0.190	0.312	1				
AV K		U.595° -0 459*	0.267	-U.658^ 0.767*	-U.295	⊥) 560*	1		
		-0.733	0.003	0.101	0.313 -(1 1		

0.766*

potassium than acidic soils (36). Both, OC and OM exhibit strong positive correlations with Av N. This suggests that there is typically more accessible nitrogen in soils that have a higher organic content. When soil organic matter breaks down, nitrogen and other nutrients are released into the soil (37). Thus, soil with a higher OC content will have higher N content to the soil than soil with a lower OC content (37). A moderate negative correlation between OM and Av K indicates that a higher concentration of it is associated with lower Av K. A perfect positive correlation (1.00^{*}) indicates that OM and OC are perfectly correlated, which is expected since organic matter largely consists of organic carbon.

-0.461*

0.007

Isolation and Morpho-cultural Characteristics of bacterial isolates

Fifty-four distinct cultures were obtained from the rhizospheric soil samples of semi-arid and arid zones of Rajasthan and Gujarat. Isolated bacteria were named CRB 1 to CRB 54 and their morpho-cultural and biochemical characterization was done. The colony colors varied, exhibiting creamy, white, milky and mucoid shades, along with round-shaped, yellow and watery colonies. These colonies were either opaque or translucent and displayed either a firm, gummy texture or a smooth, mucoid texture, with entire colony margins (Plate 1). Forty-six isolates were found as rod-shaped bacteria and observed as gram-negative bacteria whereas eight isolates *viz.*, CRB 9, CRB 11, CRB 21, CRB 35, CRB 43, CRB 45, CRB 51 and CRB 53 were gram-positive bacteria (Table 1). Shetta et al (38) isolated rhizobacteria growing on Congo Red dye-containing medium on YEMA were shown to grow quickly and not absorb indicator solutions. On the other hand, all of the isolates in the YEMA medium containing Brom Thymol Blue belonged to the fast-growing rhizobacteria group, which was distinguished by color changes to yellow as a result of the media's formation of acids or bases. Based on the formation of acids and bases in the medium, bacteria with fast growth (yellow) and slow growth (blue) are classified using YEMA + BTB media (39).

-0.568*

1.00*

1

Biochemical characterization

0.315

The IAA assay was conducted in the presence of L-tryptophan in the medium, with fifty-one isolates showing positive results indicated by the development of pink colour. However, three isolates-CRB 2, CRB 9 and CRB 42-displayed negative results. Bacteria with plant growth-promoting (PGP) activities, such as IAA synthesis, phosphate solubilization, carbohydrate degradation and citrate utilization, have been reported with enhanced crop growth and yield (40). It has been observed that numerous PGPR from the genera Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Pantoea, Pseudomonas and Serratia may produce IAA when given the right precursor, like tryptophan. Tryptophan is abundant in the root exudates of many plants and bacteria use it to synthesize auxins, which are released into the rhizosphere as secondary metabolites (41). This also leads to enhancement in the capability of the plants for drought tolerance by different mechanisms. IAA is secreted during plant-microbe interaction by a variety of microbes in the rhizosphere and is known for regulating plant growth and development have an imperative role in the mitigation of drought stress in different crops. Rhizobacteria actively invade roots through biochemical signaling between plants and rhizobacteria and IAA plays a major part in this interaction between microorganisms and plants. These bacteria that invade roots release a variety of compounds and enzymes that can aid plants in becoming more droughttolerant (42). The primary endogenous regulators of many aspects of plant growth and development are plant hormones. One of the hormones that has been studied the most, auxin, controls the division, elongation, differentiation and pattern development of plant cells (43).

All fifty-four isolates were found p-solubilizers as they were shown an evident size of a halo zone on Pikovskaya's media. Based on our observations, 18 bacterial isolates were identified as citrate utilizers and 44 isolates were carbohydrate degraders. Additionally, all bacterial isolates (CRB1-CRB54) tested positive for the catalase test. Microorganisms having carbohydrate degradation capability play a key role in the turnover of organic matter by producing enzymes that can degrade complex polysaccharides and facilitate the recycling of nutrients. Carbohydrate-degrading bacteria are globally distributed and enrichment of soil with these could be important for accelerated carbon cycling (44). Bacteria which secrete various types of acids like citrate have an important role in nutrient cycling by making them available to the plants (45). Catalases are important in the growth of bacteria as they catalyze the decomposition of

H₂O₂ and play an important role in the defense against oxidative stress (46). Cluster analysis of all isolates based on morpho-biochemical traits categorized the 54 isolates into two major clusters: Cluster A and Cluster B (Supplementary Table S2 and Fig. 4). Cluster B was further divided into two subclusters: B1 and B2. Cluster B1 included 45 isolates, while Cluster B2 comprised 7 isolates, as depicted in Fig. 4. Cluster A



Fig. 4. Dendrogram depicting relationship between 54 isolates based on morphobiochemical traits.

included 2 isolates, cluster B1 included 45 isolates and cluster B2 comprised 7 isolates, as depicted in dendrogram. Phenol red dextrose broth inoculated with bacterial isolates was observed for yellow color indicating carbohydrate fermentation. The carbohydrates that have been fermented and produce acid, wastes turn the phenol red to yellow, thereby indicating the production of acids. Similarly, the citrate consumption test shows that certain microbes can use citrate as a carbon source for energy when fermentable glucose or lactose is not present. This is dependent upon the enzyme citrate permease being present, which aids in the transfer of citrate into the bacterial cell (47).

Microbial Identification and their role in plant growth

Based on morphology and different PGPR activities a total of five isolates (CRB-20, CRB-23, CRB-41, CRB-52, CRB-54) were selected for identification using 16S rRNA sequencing. NCBI Blast analysis indicated that CRB-20, CRB-23, CRB-41, CRB-52 and CRB-54 revealed similarity with Micrococcus luteus, Bacillus megaterium, Bacillus tequilensis, Chryseobacterium indologenes and Bacillus aryabhattai and accession number assigned as EU438932.1. AY553116.1. KR085788.1, JF894157.1 and KJ767333.1 respectively. These comparisons were based on nucleotide homology as referenced in Table 4 and Supplementary Fig. S1. Dastager et al. (48) have isolated a potential Micrococcus strain NII-0909 from Western ghat forest soil of India which possessed multiple plant growth traits, like P -solubilization, IAA and siderophore production and was useful for cowpea seedling growth promotion. The strain showed the activities of 1-aminocyclopropane-1-carboxylate deaminase, auxin synthesis, phosphate solubilization and siderophore

Table 4. Selected Microbial strains, crops, location with PGPR properties and identification NCBI accession number

Sample ID	Crop rhizosphere	Agro ecological zone*	Color	Shape	IAA production so	P lubilization	Similarity	NCBI accession number
CRB-20	Cumin	Zone II a Transition plain of Inland drainage	Yellow	Cocci	+	+	Micrococcus luteus	EU438932.1
CRB-23	Coriander		White	Rod	+	+	Bacillus megaterium	AY553116.1
CRB-41	Coriander	Zone V Humid	White	Short rod	+	+	Bacillus tequilensis	KR085788.1
CRB-52	Coriander	south Eastern Plain	Creamy white	Rod	+	+	Chryseobacterium indologenes	JF894157.1
CRB- 54	Coriander		Creamy white	Rod	+	+	Bacillus aryabhattai	KJ767333.1

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generation that promote plant growth. It was able to generate IAA (109 μ g/ml) and solubilize (122.4 μ g of Ca₃PO₄ ml⁻¹) at 30 °C. A decrease in the pH of the NBRIP medium and the release of organic acids were linked to the strain NII-0909's P-solubilizing activity. The micrococcus strain NII-0909 has the potential to be used as a biofertilizer to improve soil fertility and encourage plant development, as these studies showed. Dubey et al. (49) isolated Micrococcus luteus Strain AKAD 3-5 and studied its growth-promoting traits such as IAA, phosphate solubilization, ammonia, Exopolysaccharide (EPS), biofilm and enzyme production activity. Similarly, the growth promoting potential of the Micrococcus luteus in soybean and tomato (49, 50). Likewise, the isolate of M. luteus (CRB-20) identified in the present investigation may be utilized as a potential microbial inoculant for cumin and coriander crop cultivation. Bacillus megaterium is known to promote plant growth as Qili Zhu (51) reported that in maize *B. megaterium* promotes growth by altering rhizosphere microbial communities and organic phosphorus utilization in saline soils. Bacillus megaterium has demonstrated significant plant growth-promoting potential in tomato (52). Similarly, Bacillus tequilensis strain 'UPMRB9' collected and identified from coriander growing area is reported to improve biochemical attributes and nutrient accumulation in rice (53), the reported strain may be a potential PGPR to improve coriander quality and yield in saline soils of Rajasthan and Gujarat. Moreover, studies on B. tequilensis in rice and soybean, have shown its capacity to enhance salinity and temperature tolerance (53, 54). Chryseobacterium sp. is known as efficient PGPR in the rooting induction of olive can be a good natural source to improve the rooting and yield in cumin and coriander (55). Bacillus aryabhattai is another well-known PGPR, particularly effective in promoting growth in soybean, enhancing the production of butanoic acid and other nutrients (56). It has also shown growth-promoting potential in maize and cucumber (57, 58). Given these findings, it is anticipated that the isolates from this experiment will similarly improve the nutritional value of both spice crops.

The findings in the present study are also in line with the earlier reports where PGPRs, such as *B. megaterium* and *B. tequilensis* were isolated from the soil samples of these regions with similar crops (14) . It might be due to agroclimatic influence on these spicy herbaceous plants which in turn exert control on root rhizosphere microflora. These five rhizobacteria had two important traits i.e. phosphate solubilization and IAA production for their plant growth promotion potential isolates were Gram-positive which are considered to be spore formers and thus able to tolerate the edaphic stresses of semi-arid and sub-humid saline soils.

Conclusion

In this study, the physico-chemical characteristics of soils from cumin and coriander-growing areas across various agroecological regions of Rajasthan and Gujarat were examined. Significant variability in the agronomical properties of the soil was observed, which directly correlates with soil fertility and crop productivity. These findings underscore the importance of diverse soil conditions in influencing the growth of both cumin and coriander. To further understand the potential of soil microbial communities in enhancing crop growth, soil and plant samples were collected for microbial isolation, with a focus on strains that exhibit plant growthpromoting properties. Representative samples were selected for molecular identification, leading to the identification of rhizobacterial strains (M. luteus, B. megaterium, B. tequilensis, C. indologenes and B. aryabhattai) with promising attributes. These identified rhizobacterial strains offer significant potential for developing microbial consortia that could improve the growth and yield of cumin and coriander, especially in semiarid regions where environmental stress factors often limit agricultural productivity. The use of these microbial isolates could be crucial for optimizing soil fertility and enhancing crop resilience in such areas. Supporting literature reinforces the beneficial role of rhizobacterial strains in promoting crop growth and further studies are warranted to validate their specific utility in the growth enhancement of cumin and coriander, crops that are highly valued for their agricultural and economic significance.

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Authors' contributions

SC, RS and BKM carried out conceptualization, methodology, investigation, data curation and writing-original draft. RS, RSS and RG carried out visualization and validation. SK, MS, PKS and RK carried out the analysis. AKV, RY, KKV, RC and MS participated in the writing review and editing. All authors read and approved the final manuscript.

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During the preparation of this work the author(s) used grammarly and QuillBot (free version) in order to improve the language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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