



Isolation and identification of endophytic bacteria from roots of *Capparis decidua* collected from Alwar district

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Abstract

The current study aims to isolate as well as characterize endophytic bacteria residing in the roots of *Capparis decidua* plants collected from the Alwar district of Rajasthan. Thereafter, biochemical and morphological characterization of these endophytic bacteria has been performed to assess plant growth-promoting traits of the bacterial isolates. The results revealed that out of the 12 isolated endophytic bacteria, 100% were found to be motile-rods, with 53.3% of them being gram positive. 100% of the isolated bacterial strains showed catalase activity, indole production, gelatinase activity, urease activity, protease activity, siderophore production and nitrate reduction activity. Furthermore, 58.3% of the endophytic bacteria exhibited amylase activity, lipase activity, VP activity, citrate utilization and phosphate solubilization activity. None of the bacteria showed methyl red test and only 41% of the bacteria produced hydrogen sulfide. All these plant growths promoting attributes exhibited by endophytic bacteria aid in nutrient availability, stress tolerance and development of *Capparis decidua*.

Keywords: *Capparis decidua*, endophytic bacteria, sustainable agriculture, catalase, indole production, siderophore production, plant growth promotion

Introduction

Endophytic bacteria represent a diverse group of microorganisms that inhabit living tissues of plants, asymptotically, and rather facilitate plant growth, development, and stress tolerance. This plant growth promotion attribute of endophytic bacteria is possible owing to production of a variety of bioactive compounds, that augment nutrient availability to the plants, while also improving the host resistance against pathogens as well as tolerance to environmental stresses (Santos *et al.*, 2018) [14]. The last few years have been marked by tremendous exploration of endophytes, especially in the context of sustainable agriculture and ecological resilience. Furthermore, the endophytic bacteria hold special importance for plants growing in arid and semi-arid ecosystems, such as those in Rajasthan, India. These plant species provide special niche for the isolation of stress-tolerant and metabolically versatile endophytes owing to the extreme environmental conditions they tolerate (Liu *et al.*, 2017; Wu *et al.*, 2021) [8, 22].

Capparis decidua, commonly known as "Kair," thrives in the arid and semi-arid regions of the Indian subcontinent and is specially adapted to survive in nutrient-poor, saline, as well as drought-prone soils. However, the ecological resilience of this plant stems not only from its morphological and physiological adaptations, but, presence of endophytic bacteria existing in symbiotic relationships with this plant, contribute a great deal towards sustenance of the plant. Kair offers numerous uses in the form of food source, fodder, as well as medicinal plant in desert ecosystems, however, there is limited information available regarding its microbial endophytic community (Zhang *et al.*, 2021; Marasco *et al.*, 2022) [9, 23].

A number of previous studies have highlighted presence of plant growth promoting endophytic bacteria in a number of desert plants like *Salvadora persica*, *Atriplex halimus*, and *Opuntia spp* (Marasco *et al.*, 2022; Mathur *et al.*, 2024) [9, 10]. In this context, the current study aims to explore the community of endophytic bacteria inhabiting the plant

Capparis decidua, while also exploring the morphological, biochemical and functional traits of the bacterial strains.

The present study aims to isolate and characterize culturable endophytic bacteria from the roots of *Capparis decidua* collected from the Alwar district. Biochemical and morphological characterization of these isolates was conducted to evaluate key plant growth-promoting traits such as catalase activity, IAA production (indole test), phosphate solubilization, siderophore production, nitrate reduction, and enzyme activities like protease, gelatinase, and lipase. It is important to understand these traits in order to explore the potential of endophytes as bio-inoculants for enhancing crop performance under challenging environmental conditions. The findings of the current research will not only contribute to the knowledge of endophytic diversity in arid-zone flora, but also open frontiers for the application of indigenous microbial resources in sustainable agriculture.

Materials and Methods

Sample collection

Sampling of roots of *Capparis decidua* was done from Alwar, Rajasthan state, India. Roots were collected in pre-sterilized polythene bags and were stored at 4°C. Collected samples were subjected for isolation of associated bacterial strains.

Surface sterilization and isolation

Collected root samples were surface sterilized by "immersion in 90% (v/v) ethanol for one minute followed by 1% (v/v) NaOCl for 10 min and then washed six times with sterile distilled water. Sterilized samples were plated on Nutrient Agar (NA) medium and incubated for 24-48 hrs at 30°C. The colonies appeared under were picked up and diluted by dilution series and subsequently streaked on NA agar plates". The sterility check was done by incubating the water of final wash water in NA and no growth of any microorganism was observed, hence proving the sterility check.

Morphological characterization

Gram's staining: To study the morphological features of bacterial isolates, a smear from its suspension was prepared on glass slide; air dried and stained by Hucker's modified Gram's staining method. The air-dried stained slides were examined under compound microscope under high power and oil immersion objectives.

Identification by culture characteristics: The bacterial colonies were examined by naked eyes as well as under stereo-binocular microscope for the external features such as size, shape, margins, elevation, optical characteristics and pigmentation. The characteristics of the bacterial isolates were noted by standard protocol.

Biochemical and physiological characterization

On the basis of morphological and cultural characteristics of colonies, the isolates were differentiated and subjected to other biochemical tests:

Protease test

The isolates were initially screened by conducting a proteolytic activity experiment on Milk Agar plates. The composition of the agar plates included 3g/L yeast extract, 5g/L peptone, 12g/L agar, and 100ml/L sterile UHT non-fat milk. The plates were injected with bacteria and then incubated for a duration of 24 hours. A clear zone of skim hydrolysis gave an indication of protease producing organisms.

Gelatinase assay

The pre-selected isolates were introduced into 10 mL of Nutrient Gelatin (NG) solution, which consisted of 13g/L of dehydrated Nutrient Broth and 120g/L of gelatin, using a stab inoculation method. A negative control was prepared with uninoculated NG. The tubes were placed in an environment with a constant temperature of around 20°C for a duration of 48 hours. After incubation, the tubes were placed in the freezer for a duration of 15 minutes to allow them to harden. Cultures that remained liquefied produced gelatinase and demonstrate rapid gelatin hydrolyses.

Catalase test

The enzyme catalase test was performed by inoculating and incubating the isolate in LB broths for 24 to 48 hours at 37°C. A loop of bacterial culture was spreaded on slide and few drops of 3% H₂O₂ was added. The presence of effervescence indicated positive result.

H₂S production

The ability of organism to produce H₂S from sulfur containing amino acid or inorganic sulfur compounds is done by using lead acetate paper strips from HiMedia. Bacteria were inoculated in peptone water. A lead acetate paper strip was inserted between the plug and inner wall of culture tube and incubated at 30°C for 18-24 hours. Change in the color of the tip or the entire strip to black indicates the production of H₂S.

Indole production

The development of indole was identified by applying Kovac's reagent and examining the forming of a red circle. New isolates were inoculated in tryptone broth with 0.2 ml Kovac's reagent (conc. HCl, 25 ml; amyl alcohol, 75 ml; p-

dimethyl amino benzaldehyde, 5g) and thoroughly combined.

Amylase

"Starch hydrolysis test was done to study the activity of amylase. The endophytic isolates were streaked on nutrient agar plates containing 2 per cent insoluble starch and incubated at room temperature. Hydrolysis of starch was tested by flooding with iodine solution and the plates were observed for the presence of clear zones surrounding the colonies and considered for positive reaction".

Urease activity

Inoculated cultures were in urea broth with pH indicator phenol red. The organisms producing ammonia raises the pH of the broth. Within 4-5 days of developing, extreme change in color from yellow to deep pink of the broth was observed as positive result.

Indole Production test

This test determines the ability of microbes to degrade the amino acid tryptophan with the help of an enzyme tryptophanase. In this SIM agar, containing substrate tryptophan is used. After inoculation and incubation Kovacs reagent is added and agitated. Production of cherry red colored layer indicates the production of indole.

Methyl Red and Voges Proskauer Test

"The Methyl red and Voges-Proskauer (MR VP) broth prepared in two sets was inoculated with the endophytic isolates and incubated for 48 hours at 30°C. To the first set of tubes, few drops of an alcoholic solution of methyl red were added. The development of distinct red color was indicative of positive reaction for MR test. α -naphthol solution (5 per cent solution in 70 per cent ethyl alcohol) was added to the second set of tubes and shaken gently for 15 min". The positive reaction of acetyl methyl carbinol production was indicated by development of red color. This indicates positive result for the VP test.

Citrate utilization

Simmon's Citrate Agar was used to verify citrate use. Plates were incubated and observed for the change in colour of the medium from greenish to yellow.

Phosphate solubilization

Phosphate is one of the most vital nutrients next to nitrogen for microbes. Several species of bacteria and fungi degrade and solubilize the insoluble phosphates into soluble forms through the mechanism of secretion of some organic acids. Pikovskaya's agar medium, was used to determine the ability of bacteria to solubilize phosphate. Spot inoculation was done on Pikovskaya's agar medium. After incubation of 48-72 hours formation of clear zone around the colonies were considered as positive.

Siderophore production

Siderophore are the iron chelator that complexes with iron and make them readily available to the plant root surfaces. In soil a competition exists among the microorganism for iron uptake. Siderophore production increases with the number of organic substrates added to the soil. Production of siderophore was determined by using Chrome Azurol S agar medium given by Schwyn and Neilands, 1987. Bacteria

culture was spotted on CAS plate. After incubation at 28°C for 3 days, siderophore production was assessed on the basis of development of orange halo against a dark blue background.

Results

Total 12 endophytic bacteria were isolated which were encoded as ACdR1-ACdR12.

The morphological and biochemical analysis of endophytes isolated from the roots of *Capparis decidua*, as presented in Table 1, reveals important insights into their potential plant growth-promoting activities. Each strain is characterized by its Gram staining results, shape, and various biochemical tests, indicating their functional capabilities. Notably, all twelve strains are Gram-positive rods, demonstrating a consistent morphological characteristic among the isolates. The results indicate that a significant number of strains exhibit various plant growth-promoting activities. For instance, all twelve isolates show catalase activity, which is crucial for protecting plant tissues from oxidative stress. Additionally, nine strains (ACdR1, ACdR2, ACdR3, ACdR6, ACdR8, ACdR11, ACdR12) possess amylase activity, suggesting their ability to break down starch into simpler sugars, thus providing readily available energy for the plant.

The production of indole, which is associated with auxin synthesis, is observed in eleven strains (ACdR1, ACdR2, ACdR3, ACdR4, ACdR5, ACdR6, ACdR8, ACdR10, ACdR11, ACdR12). This indicates their potential to promote root elongation and overall growth. Lipase activity is found in six strains (ACdR1, ACdR2, ACdR3, ACdR6,

ACdR8, ACdR11), pointing to their ability to degrade lipids and enhance nutrient availability.

Moreover, gelatinase production, which aids in protein degradation, is observed in eight isolates (ACdR1, ACdR3, ACdR4, ACdR5, ACdR6, ACdR8, ACdR11, ACdR12). Urease activity, contributing to nitrogen availability, is present in seven strains (ACdR1, ACdR4, ACdR5, ACdR6, ACdR8, ACdR11, ACdR12). Additionally, phosphate solubilization is demonstrated by nine isolates (ACdR1, ACdR2, ACdR3, ACdR6, ACdR8, ACdR11, ACdR12), highlighting their ability to mobilize phosphorus, an essential nutrient for plant growth.

The presence of siderophores in eight strains (ACdR1, ACdR2, ACdR3, ACdR6, ACdR8, ACdR11, ACdR12) enhances their capability to sequester iron, making it more accessible to plants. Furthermore, nitrate reduction capabilities are observed in six isolates (ACdR1, ACdR2, ACdR6, ACdR11, ACdR12), indicating their potential role in nitrogen fixation or as nitrogen sources.

In summary, the analysis reveals that a majority of the isolated endophytes exhibit significant plant growth-promoting activities. Specifically, all strains demonstrate catalase activity, while eleven show indole production and nine exhibit phosphate solubilization. These characteristics underline the potential of these endophytes to enhance the growth and health of *Capparis decidua*, offering valuable avenues for research and applications in sustainable agriculture and plant health management. The diverse functional capabilities of these endophytes underscore their ecological significance and potential utility in improving soil health and plant productivity.

Table 1: Morphological and biochemical analysis of endophytes isolated from root of *C. decidua* collected from Alwar district

Strain code	Gram staining	shape	catalase	amylase	M R	lipase	Indole	V P	gelatinase	Urease	Citrate	Motility	protease	H ₂ S	Phosphate solubilization	Siderophore test	Nitrate reduction
ACdR1	+	Rod	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
ACdR2	+	Rod	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
ACdR3	+	Rod	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
ACdR4	-	Rod	+	-	-	-	+	-	+	+	-	+	+	+	-	+	+
ACdR5	-	Rod	+	-	-	-	+	-	+	+	-	+	+	+	-	+	+
ACdR6	+	Rod	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
ACdR7	-	Rod	+	-	-	-	+	-	+	+	-	+	+	+	-	+	+
ACdR8	+	Rod	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
ACdR9	-	Rod	+	-	-	-	+	-	+	+	-	+	+	+	-	+	+
ACdR10	-	Rod	+	-	-	-	+	-	+	+	-	+	+	+	-	+	+
ACdR11	+	Rod	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
ACdR12	+	Rod	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+

Discussion

The current study aims to investigate the morphological as well as biochemical characteristics of 12 different endophytic bacterial strains collected from the roots of the plant *Capparis decidua*, growing in Alwar district of Rajasthan. The results of the study showed that all the isolated bacterial strains were rod-shaped, which is consistent with previously published studies on desert plants where too, the researchers reported presence of rod-shaped bacteria as frequently reported dominant endophytes, owing to their adaptability as well as efficient colonization ability inside the desert plants (Shah *et al.*, 2021) [15]. Furthermore, the study showed that the isolated bacterial strains were found to be 58.3% as gram-positive. This pattern of predominantly gram-positive bacteria appears to be somewhat similar to previous observations in studies of

arid-land plants, where too the researchers reported that firmicutes as well as actinobacteria predominate exist in roots of desert plants, owing to their resilience to harsh soil as well as sturdy climatic conditions. Thereafter, the results showed presence of catalase activity in all the isolates (100%), which is in perfect concordance with previously published studies on endophytes from arid ecosystems where too, the researchers have reported presence of catalase activity in endophytes. Catalase production by endophytic bacteria in plants helps in detoxification of reactive oxygen species (ROS), which aid in both protection of microbial cells as well as plant tissues under oxidative stress (Ullah *et al.*, 2019) [19].

The study further showed amylase production, observed in 58.3% of isolates, the enzyme amylase facilitates the hydrolysis of complex carbohydrates into simpler sugars.

Therefore, presence of amylase activity in endophytic bacteria in plants supports the microbial energy needs and may also improve carbon availability in the rhizosphere, indirectly benefiting plant metabolism. These findings are in perfect alignment with previously reported studies, where too the researchers showed that amylase-producing endophytes aid in starch mobilization (Barman *et al.*, 2015; Mesquita *et al.*, 2021; Dowarah *et al.*, 2021)^[1, 5, 11]. None of the bacterial isolates were positive for the methyl red test, indicating no strong acid production from glucose fermentation. This attribute is favorable for plant health, since excess of acidity in root tissues is detrimental for plant health. Lipase activity was found to be present in nearly 58.3% of strains. Lipase enzyme aids in degradation of lipid components, which plays a crucial role in microbial colonization as well as membrane interaction with plant cells. Furthermore, all the isolated bacterial strains were found to be positive for indole production, indicative of their potential to synthesize indole-3-acetic acid (IAA). IAA production by endophytic bacteria enables the host plant in root development, cell elongation, as well as stress adaptation. This finding is in perfect agreement with results presented by (Samarina *et al.*, 2017; Egorshina *et al.*, 2012)^[6, 13], where too, the researchers emphasized on the role of IAA -producing endophytic bacteria in stimulating host plant root growth.

VP (voges-proskauer) test was found to be positive in nearly 58.3% of the bacteria isolates, suggesting the production of acetoin, a neutral metabolite known to induce systemic resistance and promote plant vigor, as demonstrated in previously published studies by (Sigian *et al.*, 2022; Shastri *et al.*, 2020)^[16]. Thereafter, the results showed gelatinase and urease activity in nearly 100% of the bacterial isolates. Gelatinase aids in proteolytic cleavage of structural proteins for nutrient recycling within the host plant whereas urease contributes to nitrogen cycling within the plant, by converting urea into ammonium, essential in nutrient-deficient desert soils (Van Phuong *et al.*, 2021)^[20]. 58.3% of the isolated bacterial strains showed citrate production, aiding in carbon uptake under low-nutrient conditions. Citrate production by bacterial endophytes enables in supporting plant growth in poor soils and has been reported in several previously published studies (Li *et al.*, 2017; Singh *et al.*, 2022)^[18]. Survival of host plant is highly promoted by presence of motile endophytic bacteria within the host plants. In this regard, the current study showed that 100% of the endophytic bacteria reported in the study exhibited motility, which aids in root colonization, enabling navigation of bacteria towards root exudates and establish effective symbiosis (Santos *et al.*, 2018; Castro *et al.*, 2014)^[3, 14]. Other traits reported in 100% of the bacterial strains included, protease activity, siderophore production and nitrate reduction. Protease activity by endophytic bacteria enables host defence by suppression of pathogenic fungi by degrading their proteins while also contributing to nutrient mobilization within the plants (Vijayalakshmi *et al.*, 2016)^[21]. Siderophore production and phosphate solubilization on the other hand contribute towards fulfilling nutritional needs of the host plant by aiding in increased availability of iron and phosphorus to the host plant. Both these attributes are extremely important in promoting survival of plants in nutrient deficient soils. This observation is in perfect agreement with previously reported studies, where too, the researchers reported improvement of

plant growth by endophytic bacteria owing to their siderophore production and phosphate solubilization efficacy (Cui *et al.*, 2022; Berza *et al.*, 2022)^[2, 4].

Conclusion

Overall, the study showed traits multifaceted role of endophytic bacteria in promoting the growth as well as resilience of *Capparis decidua*, as evident from several plant growth promoting attributes exhibited by these bacterial strains. Furthermore, these findings are consistent with previously published global reports on desert-adapted endophytes and unveil the numerous potential applications of endophytic bacteria in achieving sustainable agriculture goals and ecological restoration of arid and semi-arid ecosystems.

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