



Isolation and identification of endophytic bacteria from stem 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 stem 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 9 isolated endophytic bacteria, 100% were found to be motile-rods, with 44.4% 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, 44.4% 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 55% of the bacteria produced hydrogen sulfide. All this plant growth promoting attributes exhibited by endophytic bacteria aid in nutrient availability, stress tolerance and development of *Capparis decidua*.

Keywords: *Capparis decidua*, stem endophytic bacteria, sustainable agriculture, plant growth promotion attributes

Introduction

The past few decades have been marked by increased exploration in the field of endophytic bacteria owing to their beneficial roles in plant growth, stress tolerance, and biotic defense mechanisms. Endophytes colonize internal plant tissues without causing any apparent disease symptoms and are considered as hidden extension of the plant's own physiology, offering support in nutrient acquisition, hormonal regulation, and environmental adaptation. Among bacterial endophytes, plant growth-promoting endophytic bacteria (PGPEB) are especially significant, as they can enhance plant fitness through a variety of direct and indirect mechanisms, including phytohormone production, nitrogen fixation, phosphate solubilization, siderophore production, enzyme secretion, and resistance induction against pathogens.

The desert plant *Capparis decidua*, commonly known as "Kaira" is a hardy, leafless, spiny shrub native to arid and semi-arid regions of Rajasthan and other parts of northwest India. This plant thrives in extreme environmental conditions, including high temperatures, drought, poor soil fertility, and has remarkable ability to survive and grow in such inhospitable environments (Singh *et al.*, 2011^[21]; Rathee *et al.*, 2010^[20]; Gull *et al.*, 2015)^[10]. While most of the already published studies on endophytes focus on agriculturally important crops, there is a growing interest in understanding the microbiota associated with desert plants, as they may harbor unique, stress-tolerant microbial communities with biotechnological potential. In this regard, the stem tissues of xerophytic plants represent an underexplored niche harboring endophytes with specialized traits necessary for survival in extreme climates.

Several previous studies have shown crucial enzymatic and metabolic properties that enable endophytic bacteria to not only adapt to the harsh environment but also support their host plants. For instance, bacterial endophytes from desert species such as *Zygophyllum*, *Tephrosia*, and *Prosopis* have been reported to produce catalase, protease, and indole acetic acid, all of which contribute to oxidative stress

mitigation, protein degradation, and root development, respectively (Yadav *et al.*, 2021^[22]; Elsheikh *et al.*, 2021)^[9]. However, very few studies have systematically documented the biochemical and functional diversity of stem-associated endophytic bacteria in *Capparis decidua*. The stem of desert plants represent a relatively stable internal environment where endophytes may contribute to internal nutrient cycling, resistance to desiccation, and systemic growth promotion (Zhang *et al.*, 2021)^[24]. Therefore, deeper characterization of the bacteria inhabiting the stems of *C. decidua* can provide valuable insights into plant-microbe interactions in xeric habitats and reveal potential microbial candidates for sustainable agriculture, especially in degraded or marginal soils.

Considering this, the present study aims to isolate and characterize endophytic bacterial strains from the stems of *Capparis decidua* collected from arid regions of Rajasthan. Specifically, this work focuses on evaluating the morphological features (Gram staining, shape, motility) and biochemical properties of these isolates, including enzymatic activities (e.g., catalase, amylase, protease, gelatinase, urease), nitrogen metabolism (e.g., nitrate reduction, urease activity), plant-growth-promoting traits (e.g., IAA production, phosphate solubilization, siderophore production), and stress adaptation traits (e.g., catalase, lipase, citrate utilization). By exploring these bacterial traits in detail, this study contributes not only to our understanding of plant-microbe symbioses in extreme environments but also lays the groundwork for potential applications in sustainable agriculture, ecological restoration, and microbial biotechnology.

Materials and methods

Sample collection

Sampling of stem of *Capparis decidua* was done from Alwar, Rajasthan state, India. stem 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 stem 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 amount 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

The morphological and biochemical analysis of endophytes isolated from the stems of *Capparis decidua*, as shown in Table 1, provides further insights into their potential plant growth-promoting activities. The analysis includes various characteristics such as Gram staining results, shape, and specific biochemical activities that highlight the functional capabilities of these isolates.

Among the nine strains analyzed, there is a mix of Gram-positive and Gram-negative isolates, with a predominance of rod-shaped bacteria. Catalase activity is present in all strains, which is crucial for protecting plant tissues from oxidative damage. The ability to produce indole, an indicator of auxin production, is observed in seven of the isolates (ACdS1, ACdS2, ACdS3, ACdS4, ACdS5, ACdS6, ACdS7). This suggests that these strains have the potential to promote root elongation and overall plant growth through

auxin synthesis. Amylase activity, which facilitates the breakdown of starch into sugars, is present in five strains (ACdS2, ACdS4, ACdS5, ACdS6). Lipase activity, found in three isolates (ACdS2, ACdS4, ACdS5), indicates the potential to degrade lipids, enhancing nutrient availability for the plant. Gelatinase production is noted in five strains (ACdS1, ACdS2, ACdS4, ACdS5, ACdS6), which supports protein degradation, while urease activity, contributing to nitrogen availability, is also observed in five isolates (ACdS2, ACdS4, ACdS5, ACdS6, ACdS7).

Phosphate solubilization, which is critical for mobilizing phosphorus, is demonstrated by all strains except ACdS1 and ACdS3. This capacity is significant as phosphorus is a vital nutrient for plant growth. The ability to produce siderophores, observed in seven isolates (ACdS1, ACdS2, ACdS4, ACdS5, ACdS6, ACdS7, ACdS8), indicates their potential to sequester iron, making it more accessible to the host plant. Furthermore, nitrate reduction capability is present in three strains (ACdS1, ACdS4, ACdS5), which may enhance nitrogen availability for plant growth.

In summary, the biochemical characterization of the endophytes isolated from the stems of *Capparis decidua* indicates that many of these strains possess significant plant growth-promoting activities. Specifically, all strains exhibit catalase activity, while seven strains show indole production and phosphate solubilization is evident in six. These properties underscore the potential of these endophytes to enhance the growth and health of *Capparis decidua*, suggesting valuable applications in sustainable agriculture and plant health management. The diverse biochemical capabilities of these endophytes reflect their ecological significance and potential to improve soil health and plant productivity.

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

Strain code	Gram staining	shape	catalase	amylase	MR	lipase	Indole	VP	gelatinase	Urease	Citrate	Motility	protease	H2S	Phosphate solubilization	Siderophore test	Nitrate reduction
ACdS1	-	Rod	+	-	-	-	+	-	+	+	-	+	+	+	-	+	+
ACdS2	+	Rod	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
ACdS3	-	Rod	+	-	-	-	+	-	+	+	-	+	+	+	-	+	+
ACdS4	+	Rod	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
ACdS5	+	Rod	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
ACdS6	+	Rod	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
ACdS7	-	Rod	+	-	-	-	+	-	+	+	-	+	+	+	-	+	+
ACdS8	-	Rod	+	-	-	-	+	-	+	+	-	+	+	+	-	+	+
ACdS9	-	Rod	+	-	-	-	+	-	+	+	-	+	+	+	-	+	+

Discussion

The current study aimed to characterize the morphological as well as biochemical attributes of nine endophytic bacterial strains isolated from the stem tissues of *Capparis decidua*, growing in Alwar district of Rajasthan. All the isolated bacterial were found to be uniformly rod-shaped consistent with previous research on desert plant endophytes (Marasco *et al.*, 2018 ^[16]; Marasco *et al.*, 2022) ^[15]. Rod shapes are often more prevalent and favoured in endophytic bacteria owing to their ease of tissue penetration and mobility within host structures. Rod-shaped bacteria possess efficient nutrient uptake as well as effective colonization abilities, offering an ecological advantage over other bacterial strains in nutrient-limited desert conditions. Furthermore, the results showed that 44.4% of the isolated bacteria were Gram-positive, which is somewhat lower than

in studies on root-associated endophytes. Nonetheless, this finding still aligns with previously published reports suggesting that Gram-positive bacteria, particularly Firmicutes and Actinobacteria, are well-suited for survival in extreme environments. The thick peptidoglycan walls of gram positive bacteria are beneficial in resisting desiccation and prevent them from osmotic stress, which are commonly encountered conditions in arid ecosystems (Zeidler *et al.*, 2019; Misra *et al.*, 2013) ^[17].

The study showed potent catalase activity in 100% of the isolated bacterial strains, suggesting a strong capacity to detoxify reactive oxygen species such as hydrogen peroxide. This trait is particularly useful for microbes inhabiting oxidative environments within plant tissues, and it also contributes to the host plant's defense against abiotic stresses such as high temperature and drought. However,

unlike the catalase activity, amylase and lipase activity was detected in only 44.4% of the identified bacterial isolates. Amylase plays a crucial role in starch degradation as well as carbohydrate mobilization. Lipase on the other end aids in lipid breakdown and in plant-endophyte systems, such enzymatic activity not only supports bacterial metabolism but may also aids in making energy sources available during periods of environmental stress. Lipases may also help bacteria in the utilization of lipid substrates available in host tissues (Lestari *et al.*, 2018 ^[14]; Katoch *et al.*, 2017) ^[12]. None of the isolates showed methyl red (MR) test positivity, indicating absence of excessive acidification within plant tissues, which is favourable for plant systems since excess acid can lead to cellular damage, especially under stress conditions.

The Voges-Proskauer (VP) test was positive in four strains, indicating acetoin production, which acts as a neutral metabolite, and aids in induction of systemic resistance mechanisms in plants, offering added defense against environmental stressors and pathogens (Das *et al.*, 2013 ^[8]; Abdallah *et al.*, 2016) ^[1]. The study showed that 100% of the isolated bacterial endophytes produced indole-3-acetic acid (IAA), a key plant hormone that enhances root development, cell elongation, and nutrient uptake. Although these bacteria were isolated from stems, their IAA-producing ability may play a systemic role in plant growth regulation. Production of IAA by endophytic bacteria acts as a hallmark of plant-growth-promoting microbes, particularly in stress-prone environments (Herlina *et al.*, 2017 ^[11]; Bhutani *et al.*, 2018) ^[3]. All the isolated bacteria were positive for gelatinase, urease and protease activity. Gelatinase enables the breakdown of complex proteins into simpler forms, contributing to nutrient cycling. Urease activity facilitates nitrogen availability by converting urea into ammonium. Urease activity is a very beneficial trait in desert soils where nitrogen sources are often scarce. Protease is useful in nutrient acquisition and aids in suppressing pathogens by degrading their cell wall proteins, suggesting a possible biocontrol function (Bezerr *et al.*, 2021; Castro *et al.*, 2014) ^[4]. Previous studies have reported beneficial activity of gelatinase, urease and protease in host plant growth and development (Kushwaha *et al.*, 2020 ^[13]; Rasool *et al.*, 2021) ^[19].

Citrate utilization efficacy was observed in 44.4% of the bacteria and shows metabolic versatility as well as ability of bacteria to thrive in diverse carbon conditions. This trait is beneficial under nutrient-scarce environments such as those faced by *C. decidua* in arid habitats. 100% of the isolated bacteria from stems were motile and showed siderophore production as well as nitrate reduction activity. On one hand, motility enhances the bacteria's ability to migrate through plant tissues, aiding in its ability to colonize internal niches effectively. On the other hand, siderophore production and nitrate reduction ensure that plants acquire iron in iron-deficient soils, while also limiting pathogen growth by depriving them of essential nutrients. Nitrate reduction ensures proper nitrogen cycling and supports plant nitrogen nutrition. This trait is of particular ecological relevance in arid environments, where nitrate availability is often constrained. Several previous studies have also shown siderophore production and nitrate reduction ability in endophytic bacteria (Misra *et al.*, 2024 ^[18]; Da Silveira *et al.*, 2019 ^[7]; Cui *et al.*, 2022) ^[6].

Interestingly, 55.5% of the isolates were capable of producing hydrogen sulfide (H₂S). While H₂S is typically viewed as toxic, recent research shows that, in small concentrations, it plays a signaling role in plant stress responses and may regulate physiological processes such as stomatal closure (Chaudhary *et al.*, 2022). Phosphate solubilization was observed in four strains, indicating their ability to convert insoluble forms of phosphate into bioavailable forms. This function is especially vital in arid ecosystems where phosphorus is often present in non-labile forms, limiting plant uptake.

Conclusion

The current study reveals a robust set of plant growth-promoting traits among endophytic bacterial strains isolated from the stem of *Capparis decidua*. The presence of traits such as catalase, IAA production, protease, siderophore, nitrate reduction, and urease activity demonstrates the adaptive capabilities of these bacteria in supporting plant growth under desert conditions. These results are largely consistent with previously published reports on endophytes from arid plants, thereby highlighting the potential of stem-associated endophytes in sustainable agriculture and desert ecosystem management.

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