

Screening of Amylase- and Cellulase-Producing Endophytic Bacteria from *Cissus quadrangularis*: A short report

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Abstract: Endophytic bacteria are increasingly recognised as valuable biological resources in biotechnology due to their potential to synthesize a wide range of industrially relevant enzymes ability. This study explores the isolation, from *Cissus quadrangularis*, a medicinal vine traditionally used to increase healing process of fractured bone. By combining classical microbiological methods– serial dilution, plating, staining, and biochemical assays characterization, and screening of amylase- and cellulase-producing endophytic bacteria with functional enzyme screening, the study highlights the potential of plant-associated bacteria promising natural sources for environmentally friendly and scalable enzyme production. This research indicates that exploring plant-associated bacteria for relevant enzymes can produce valuable functional enzymes, for industrial use.

Keywords: Endophytic bacteria; *Cissus quadrangularis*; Enzyme production; Amylase; Cellulase; Bioprospection.

1. Introduction:

Plants inherently contain a concealed group of microorganisms within their tissues referred to as endophytes. These microbes exist without inflicting damage. Frequently offer advantages to the host plant, including enhanced stress resilience, nutrient availability and safeguarding plant tissue (Strobel & Daisy 2003).

Among these endophytes, some bacteria possess the ability to produce hydrolytic enzymes, which may help the host plant defend against potential pathogens. In return, endophytes receive nutrients and a stable microenvironment within amylase and cellulase are of great interest. Such enzymes play vital roles in breaking down complex plant polymers like starch and cellulose. Because plants constantly remodel their tissues, the endophytes associated with them often evolve the ability to degrade these compounds efficiently (Rosenblueth M& Martínez-Romero E,2006)

Cissus quadrangularis (commonly called “Hadjod” or “Bone Setter” in India) is a succulent vine widely used in Ayurvedic medicine for fracture healing, anti-inflammatory activity, and swelling reduction. Its ability to survive in harsh conditions suggests that it hosts a highly diverse endophytic flora. Several studies have reported that medicinal plants host more potent enzyme-producing endophytes compared to non-medicinal plants. (Nair, D. N., & Padmavathy, S. J. T. S. W. J. 2014).

2. Aim of the Study:

This experiment aimed to isolate endophytic bacteria from *Cissus quadrangularis* stem using serial dilution techniques, screen the isolates for the production of Amylase (starch-degrading enzyme) and Cellulase (cellulose-degrading enzyme) and identify selected isolates using morphological, biochemical, and enzyme-based assays such as Catalase, Oxidase IMViC reaction, starch breakdown, and cellulose decomposition tests.

3. Materials and Methods:

3.1 Sample collection and Surface Sterilization:

Fresh, healthy leaves and stolon of *Cissus quadrangularis* was collected from Assam Don Bosco University, Tapesia campus, Guwahati, Assam. The collected Plant sample was then transported to the laboratory for isolation and screening for enzyme-producing endophytic bacteria.



Figure 1. Stolon and leaf of the plant, *Cissus quadrangularis*

Plant sample was first washed thoroughly under running tap, cleaned samples were cut into small segments and subjected to a standardized surface-sterilization procedure to eliminate epiphytic microorganisms. Further small segments of plants were soaked in 70% ethanol for 30 seconds, sterilized for 1 minute in sodium hypochlorite (3%), and then washed three times with sterile water.

3.2 Isolation of endophytic bacteria:

Firstly, crush the plant tissues after surface sterilization using a mortar and pestle. Then, diluted the suspension up to 10^{-5} . 100 μ l from 10^{-3} to 10^{-4} dilution were spread onto nutrient agar plates for isolation. The sterility control plate was also incubated to confirm that the surface-sterilization procedure was successful. All plates were incubated at 37 °C for 24 to 48 hours. After the incubation examined the colonies. The selected colonies were restreaked several times to obtain the cultures. The pure cultures were the purified isolates. All selected isolates were subcultured in nutrient agar, and finally, all the purified endophytes were maintained at 4 °C till further used.

3.3 Preliminary characterisation of endophytic bacteria:

The selected colonies from the plate were initially examined through phenotypic characterization, encompassing both morphological and biochemical analyses. Colony features such as form, elevation, margin, pigmentation, and surface texture were observed and documented. Microscopic examination was performed to determine Gram reaction. Further Biochemical characterisation test such as catalase and oxidase, IMVIC tests-Indole, Methyl red, Voges-Proskauer, citrate utilisation was carried out for the selected colonies.

3.4 screening of extracellular enzyme production:

3.4.1 Amylase production:

The amylase-producing bacteria were screened for enzyme activity using the starch agar medium. prepared the medium with 10 g starch, 5 g peptone, 3 g yeast extract, and 15 g agar per liter of distilled water. The selected endophytic bacteria were then spot inoculated onto the medium and were incubated at 32 ± 2 °C for 2-5 days. After the incubation, the plates were filled with the iodine solution for 60 seconds. Then, the excess iodine solution was poured off, and the clear zone, around the colony, was observed. measured the colony diameter (a) and the clear zone diameter (b) three to five times to keep the measurements accurate used the enzyme index (EI) was calculated as b/a , indicating the relative amylase activity of each isolate. Hankin, L., & Anagnostakis, S. L. (1975).

The clear zone is measured by calculating the amylolytic index. The clear zone is measured by calculating the amylolytic index. The formula calculates the amylolytic index according to Eq. (1) Satrimafitrah et al;2020.

$$Amolytic\ Index = \frac{CZ-CD}{CD}(1)$$

CZ means the Clear Zone Diameter (mm). CD means the Colony Diameter (mm).

3.4.2 Cellulase production

To screen isolates for cellulase enzyme production on cellulose agar medium. The cellulose agar medium contained KH_2PO_4 (0.5 g), $MgSO_4$ (0.25 g), cellulose (2.0 g), agar (15 g), gelatin (2 g), and 1 L of distilled water with a pH of 6.8-7.2. poured the cellulose agar medium into the plates, and left the plates for 48 hours. Then filled the plates with 0.1% Congo solution and let the Congo Red solution stain the plates for 15 minutes, at room temperature. washed the plates with a 1 M NaCl solution. Colonies exhibiting Congo Red discoloration were considered positive for cellulase-degrading activity (Demissie, M. S., Legesse, N. H., & Tesema, A. A., 2024).

4. Results:

4.1 Isolation of endophytic fungi from *cissus qudrangularis*.

After serial dilution and incubation for 48 hr, a total of three distinct bacterial isolates were obtained from the dilution plates (10^3 , 10^4 , and 10^5). Each isolate showed clear and separated colony growth and are coded as I1, I2, I3. The sterility control plate showed no microbial growth, confirming that the surface-sterilization procedure was successful. The bacterial isolates obtained from plant's internal endophytic tissues rather than external contamination.



Figure 2. illustrates the distinct isolates obtained from following serial dilution 10^{-3} , 10^{-4} , 10^{-5}

Morphological and Biochemical characterization

Table1. cultural characteristics of Colony morphology

Isolate	Colony size	Colour/pigment	shape	margin	Elevation	surface	opacity
I1	small	yellow	circular	entire	convex	Smooth	Opaque
I2	Medium	cream	circular	entire	Raised/convex	Smooth, creamy	opaque
I3	large	Off-white/pale cream	circular	entire	convex	Smooth shiny	opaque

Gram stain test was carried out for further confirmation of 3 isolates microscopically at 100X magnification. Out of which 1 isolates was gram positive rods and the other 2 isolates were gram negative. results of the gram stain test isolates are shown below:

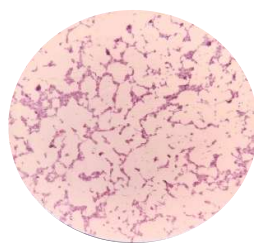


Figure 3. Gram staining of isolate I1 showing purple rod-shaped cells.

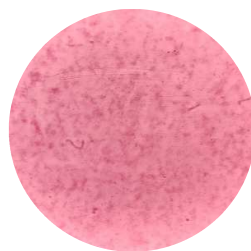


Figure 4. Gram staining of isolate I2 Showing pink – color cells, indicating a gram negative bacteria

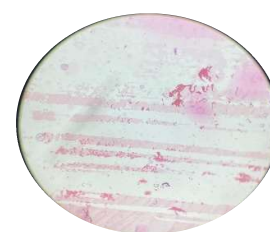


Figure 5. Gram staining of isolate I2 Showing pink – color cells, Indicating a gram negative bacteria

Table 2. Biochemical test of isolates

Biochemical test	I1	I2	I3
catalase	+	+	-
oxidase	-	-	-
Methyl red	-	-	-
Voges – proskauer	-	-	-
indole	-	-	-
Citrate utilization	+	+	-

Screening for extracellular enzymes production

4.1.1 Amylase production



Figure 6. Screening of Amylase producing bacteria

Out of the 3 isolates screened for the amylase activity on starch agar medium, only 1 isolate (I1) showed positive result the calculated amylolytic index was 2.4, indicating significant starch hydrolysis potential; which showed maximum zone of clearance. And the other 2 isolates showed negative result.

Table 3: Amylase activity and amylolytic index of isolate I1

Parameter	Value
Clear Zone 1 (mm)	43
Clear Zone 2 (mm)	39
Clear Zone 3 (mm)	35
Clear Zone 4 (mm)	19
Mean Clear Zone (mm)	34
Colony Diameter (mm)	10
Amylolytic Index (AI)	2.4
Activity Classification	Strong amylase activity

4.1.2 Cellulase production:

Out of the 3 isolates screened for the cellulase activity on cellulose agar medium, only 1 isolate (I1) showed positive result; which showed zone of clearance. And the other 2 isolates showed negative result.



Figure. 7 shows zone of clearance

5. Discussion:

This research successfully isolated bacteria from *Cissus quadrangularis* and assessed their capability to produce two key industrial enzymes—amylase and cellulase. Following surface sterilization and serial dilution three bacterial isolates (I1, I2 and I3) were recovered, demonstrating that the plant contains microbial populations. The lack of growth, in the sterility control verified that the isolation method effectively eliminated surface contaminants confirming that the isolates were endophytes.

Microscopic and biochemical analysis revealed differences, among the isolates. Isolate I1 was classified as a Gram- rod whereas isolates I2 and I3 were Gram-negative. This variation aligns with studies indicating that medicinal plants typically host a diverse array of endophytic bacteria often comprising genera like *Bacillus*, *Pseudomonas*, *Enterobacter* and *Micrococcus* (Anwar et al, 2020 & Nair, D. N., & Padmavathy S. J. T. S. W. J. 2014). Numerous of T

these bacterial clusters are recognized as producers of enzymes which corresponds closely with the current results.

Testing for enzyme activity showed that isolate I1 demonstrated amylolytic and cellulolytic actions while isolates I2 and I3 failed to show any enzyme zones within the tested parameters. The amylase index of 2.4 indicates a capacity for starch breakdown and the visible clear zone on cellulose agar verifies effective cellulase synthesis. The absence of activity in the isolates could be attributed to metabolic variations, substrate preference or requirements, for alternate environmental factors. Comparable patterns have been noted in research showing that merely a small portion of isolated endophytes exhibited notable enzyme production (Strobel, G., & Daisy, B. 2003)

The dual enzymatic activity of isolates I1 may reflect its ecological role inside the plant. Since cellulose and starch are natural components of plant tissues, the ability to break down these polymers may help the bacterium survive, adapt, and coexist without harming the host. Such interactions are often mutualistic, with endophytes participating in nutrient turnover, plant defense, and physiological support (Rosenblueth M, Martínez-Romero E.2006)

Considering the enzyme-producing potential observed among the isolates, I1 stands out as particularly noteworthy. Amylases and cellulases are enzymes worldwide utilized in food production, paper and textile manufacturing, fermentation processes and biofuel creation. Endophytic bacteria frequently generate enzymes that're more stable, environmentally friendly and effective compared to synthetic ones making them appealing for sustainable industrial uses. Their function, in eco- waste breakdown and bioprocessing also supports the objectives of green biotechnology.

In conclusion the findings emphasize *Cissus quadrangularis* as a reservoir of endophytic microbes with noteworthy biochemical potential. Among the strains isolated I1 emerges as a prospect for continued investigation and potential biotechnological application. Subsequent studies should encompass characterization, enzyme isolation, enhancement of production and analysis, in varying cultivation environments. Investigating plant specimens or tissues might also uncover further enzyme-generating strains

6. Conclusion:

In the investigation we effectively isolated endophytic bacteria from *Cissus quadrangularis* to assess their potential to generate amylase and cellulase enzymes. Of the three isolates obtained isolate I1 exhibited promising amylase and cellulase activities. This indicates that I1 is proficient in degrading plant polysaccharides and might contribute to the internal nutrient processing, within plants.

Our research suggests that *Cissus quadrangularis* may be a more effective source of beneficial microbes. The dual enzyme-producing capability of isolate I1 makes it a potential microbial resource suitable for biofuel development as well as food and textile sector applications.

Further research is needed for molecular identification and enzyme production optimization to better understand and use this isolate. Overall, the study highlights the value of investigating endophytes from traditional medicinal plants, as they may serve as sustainable and novel sources of enzymes for future biotechnological applications.

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