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EVALUATION OF BENEFICIAL ACTIVITY OF ENDOPHYTIC BACTERIA IN ALOE VERA *(Aloe Barbadensis Miller)*

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ABSTRACT

The present study was carried out to isolate and characterize an endophytic bacterial isolate from Aloe vera and to evaluate its beneficial enzymatic activity. Healthy Aloe vera samples were collected from local gardens and nearby nurseries in Tenkasi, Tamil Nadu, and subjected to surface sterilization before bacterial isolation. Nutrient Agar was selected as the suitable medium based on better bacterial growth and distinct colony development. The purified bacterial isolate was characterized by macroscopic, microscopic, and biochemical methods. The isolate exhibited small, circular, convex, cream to pale-yellow colonies with a smooth and moist texture. Microscopic characterization revealed Gram-positive, rod-shaped bacterial cells, with negative results for acid-fast, capsule, and endospore staining. Biochemical characterization showed negative reactions for indole and methyl red tests and positive reactions for Voges–Proskauer and citrate utilization tests. Qualitative catalase testing showed immediate bubble formation after the addition of hydrogen peroxide, confirming catalase production. Quantitative spectrophotometric analysis based on hydrogen peroxide decomposition showed a catalase concentration of 17.69 mg/mL. The findings indicate that the isolated endophytic bacterium possesses distinct morphological, biochemical, and enzymatic characteristics, with catalase production suggesting its potential role in hydrogen peroxide detoxification and oxidative-stress management. Overall, the study demonstrates the potential of Aloe vera-associated endophytic bacteria as a source of beneficial enzymatic activity.

KEYWORDS: *Aloe vera, endophytic bacteria, bacterial characterization, catalase, hydrogen peroxide, oxidative stress.*

1. INTRODUCTION

Aloe vera *(Aloe barbadensis Miller)* is a succulent medicinal plant commonly known as the **"Plant of immortality."** It has been used for centuries for its therapeutic benefits. Ayurveda, Chinese medicine, and Native American medicine have all acknowledged the medicinal benefits of using Aloe vera. In recent years, the use of Aloe vera as a natural remedy has increased significantly, leading to growing scientific interest in its bioactive components and health benefits. The inner part of the leaf contains different types of compounds such as polysaccharides, anthraquinones, vitamins, minerals, enzymes, and amino acids, which contribute to its wide range of medicinal properties.

Endophytic bacteria are microorganisms that inhabit the internal tissues of plants without causing any visible disease symptoms. They establish a beneficial association with the host plant by improving nutrient uptake, regulating phytohormones, and suppressing plant pathogens. These endophytes enter the plant through the rhizosphere region with the help of natural openings such as root hairs or wounds and colonize plant tissues by motility, attachment, and evasion of plant defence responses. These endophytic bacteria can survive in different types of hosts depending on the plant, bacterial strain, and environmental factors, making them useful bioinoculants in sustainable agriculture.

Endophytic bacteria present in medicinal plants such as Aloe vera contribute to plant growth and show antimicrobial activity. Surface sterilization techniques are used to isolate true endophytes by eliminating epiphytic microorganisms from the plant surface. Studies have shown that endophytic bacteria isolated from various plants, including Azadirachta indica and mangrove species, display morphological and biochemical diversity and produce bioactive compounds with antibacterial properties. These characteristics indicate that plant endophytes can serve as potential sources of novel antimicrobial agents and can be applied in agriculture and medicine.

Enzyme Production

Various enzymes are associated with Aloe vera, including amylase, alkaline phosphatase, oxidase, catalase, cellulase, lipase, peroxidase, and cyclooxygenase. These enzymes are involved in different biological processes and the degradation of fats, proteins, and sugars. Among these enzymes, catalase plays an important role in maintaining cellular protection against oxidative damage.

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Catalase

Catalase is an important intracellular enzyme containing heme and is commonly found in aerobic and facultatively anaerobic microorganisms. It belongs to the oxidoreductase enzyme family and has several industrial, diagnostic, and therapeutic applications. Catalase plays a vital role in maintaining redox balance by removing hydrogen peroxide. In plants, it breaks down hydrogen peroxide into water and oxygen, thereby protecting cells from oxidative damage. It also contributes to plant defence mechanisms and supports normal growth and development. In addition, catalase helps detoxify hydrogen peroxide generated during metabolic processes such as electron transport, photorespiration, and fatty acid oxidation.

2. MATERIALS AND METHODS

2.1 Sample Collection

Healthy Aloe vera plant samples were collected from local gardens Tenkasi, Tamil Nadu. The collected samples were placed in sterile polythene bags and transported to the laboratory for further microbiological analysis.

2.2 Surface Sterilization

The collected plant material was thoroughly washed with distilled water for approximately 5 min to remove adhering debris. The samples were then immersed in 3% hydrogen peroxide for 5 min to eliminate surface microorganisms. After treatment, the samples were washed three times with sterile distilled water to remove residual hydrogen peroxide.

2.3 Isolation of Endophytic Bacteria

Nutrient Agar (NA) was used as the primary isolation medium. Small portions of surface-sterilized Aloe vera gel were aseptically placed onto sterile Nutrient Agar plates and incubated at 28–37°C for 24–48 h. Bacterial colonies showing distinct morphological characteristics were selected and subcultured onto fresh Nutrient Agar to obtain pure isolates.

2.4 Purification of Bacterial Isolate

A single well-isolated colony was transferred onto fresh Nutrient Agar using the streak plate method and incubated for 24–48 h. The streaking procedure was repeated, when necessary, until a pure colony was obtained. The purified isolate was maintained on Nutrient Agar slants for further analysis.

2.5 Macroscopic Characterization

The purified bacterial isolate was characterized based on colony morphology, including size, shape, elevation, margin, colour, and texture after growth on Nutrient Agar.

2.6 Microscopic Characterization

The bacterial isolate was subjected to simple staining, Gram staining, acid-fast staining, endospore staining, and capsule staining. The stained preparations were examined microscopically to determine cell morphology, Gram reaction, acid-fastness, endospore formation, and capsule production.

2.7 Biochemical Characterization

The purified isolate was subjected to indole, methyl red, Voges–Proskauer, and citrate utilization tests. The respective reactions were recorded based on the colour changes observed after incubation and addition of the required reagents.

2.8 Qualitative Catalase Test

Catalase activity was determined by placing a loopful of the bacterial culture on a clean glass slide followed by the addition of 3% hydrogen peroxide. Immediate formation of oxygen bubbles was considered a positive catalase reaction.

2.9 Quantitative Determination of Catalase

For quantitative catalase analysis, the bacterial isolate was grown in nutrient broth containing yeast extract (3 g/L), peptone (10 g/L), and sodium chloride (1 g/L), adjusted to pH 7.2. An overnight culture adjusted to OD_{600} = 0.6 was inoculated into fresh nutrient broth and incubated at 30°C for 24 h with shaking. The culture was centrifuged at 4500 rpm for 10 min to obtain the crude enzyme extract.

For the assay, 0.1 mL of crude enzyme extract was mixed with 2.7 mL of 50 mM sodium phosphate buffer, followed by the addition of 1.5 mL of 30 mM hydrogen peroxide. The decrease in absorbance was measured at 240 nm using a UV–Visible spectrophotometer. Catalase concentration was calculated using the Beer–Lambert equation with a molar absorptivity of 3121 M^{-1} cm^{-1} and a 1 cm path length. The calculated catalase concentration was further converted from mol/L to mg/mL using the molecular weight of catalase (240,000 g/mol), and the final concentration was expressed as mg/mL.”

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3. RESULTS

3.1 Media Selection

Aloe vera gel samples were inoculated on Nutrient Agar and Nutrient Broth and incubated under the same conditions. Better bacterial growth with distinct colonies was observed on Nutrient Agar compared with Nutrient Broth. Therefore, Nutrient Agar was selected as the suitable medium for the isolation and further characterization of the endophytic bacterial isolate.

3.2 Isolation of Endophytic Bacteria

Surface-sterilized Aloe vera gel samples were inoculated onto the selected medium and incubated at 37°C for 24–48 h. Bacterial growth was observed, and the isolate showing distinct colony characteristics was selected for further purification and characterization.

3.3 Macroscopic Characterization

The purified endophytic bacterial isolate produced small, circular, convex colonies with entire margins and cream to pale-yellow colouration. The colonies showed a smooth and moist surface on Nutrient Agar. The macroscopic characteristics are summarized in Table 1.

Table 1. Macroscopic characterization of the endophytic bacterial isolate

CHARACTERISTIC	OBSERVATION
Size	Small
Shape	Circular
Elevation	Convex (slightly raised)
Margin	Entire (smooth)
Colour	Cream to pale yellow
Texture	Smooth moist

3.4 Microscopic Characterization

Microscopic characterization showed that the isolate consisted of rod-shaped bacterial cells. Gram staining revealed purple-coloured rod-shaped cells, indicating a Gram-positive reaction. The isolate was negative for acid-fast staining, endospore formation, and capsule production. The observations are summarized in Table 2.

Table 2. Microscopic characterization of the endophytic bacterial isolate

STAINING METHOD	OBSERVATION	INTERPRETATION
Simple staining	Rod shaped cells observed	Bacilli
Gram staining	Purple coloured rod shaped cells	Gram-positive
Acid fast staining	Blue coloured cells	Acid-fast negative
Endospore staining	Pink coloured cells observed	Endospore negative
Capsule staining	No clear halo observed	Capsule Negative

3.5 Biochemical Characterization

The biochemical characterization showed that the isolate was negative for indole and methyl red tests, while positive reactions were observed for Voges–Proskauer and citrate utilization tests. The results are presented in Table 3.

Table 3. Biochemical characteristics of the endophytic bacterial isolate

TEST	OBSERVATION	INTERPRETATION
Indole test	No red ring	Negative
Methyl red test	yellow/orange colour	Negative
Voges Proskauer test	Pink to red colour	Positive
Citrate utilization test	Green to blue with growth	Positive

3.6 Qualitative Catalase Activity

The isolated endophytic bacterium showed immediate bubble formation following the addition of hydrogen peroxide, indicating a positive catalase reaction and confirming catalase production.

3.7 Quantitative Determination of Catalase Concentration

Quantitative catalase activity was evaluated using a spectrophotometric assay based on the decomposition of hydrogen peroxide. The bacterial culture was standardized to $OD_{600} = 0.6$, and the crude enzyme extract was obtained by centrifugation. The decrease in absorbance was measured at 240 nm using a UV–Visible spectrophotometer. The initial absorbance was 0.50 and the final absorbance was 0.27, giving a decrease in absorbance (ΔA) of 0.23. Using the Beer–Lambert equation, the calculated concentration was 7.37×10^{-5} mol/L. This value was further converted to mg/mL using the molecular weight of catalase (240,000 g/mol), giving a final catalase concentration of 17.69 mg/ml.

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Table 4. Quantitative determination of catalase concentration

PARAMETER	OBSERVATION
Molar absorptivity	3121 M^{-1} cm^{-1}
Molecular weight	240000g/mol
Path length	1cm
Initial absorbance (before adding H_20_2)	0.50
Final absorbance (after adding H_20_2)	0.27
Decrease in absorbance (ΔA)	0.23
Catalase concentration	17.69mg/ml

FIGURES

Fig.1 (Macroscopic characterization)

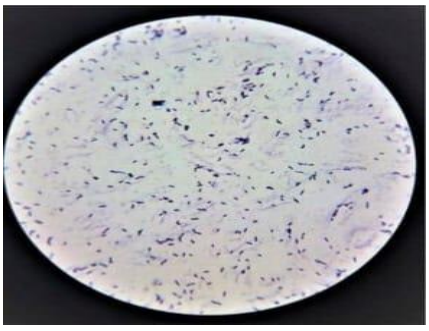

Fig.2 (Simple staining)

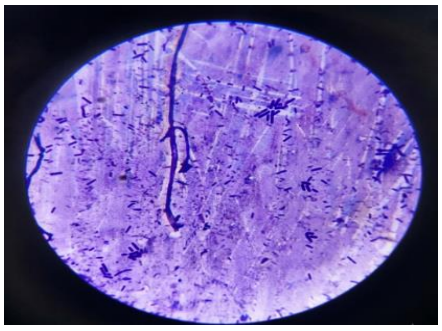

Fig.3 (Gram staining)

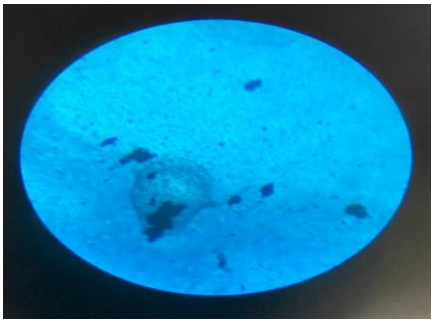

Fig. 4 (Acid fast staining)

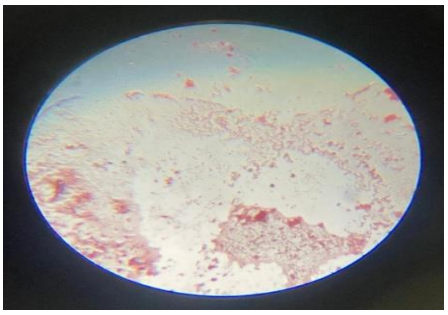

Fig.5 (Endospore staining)

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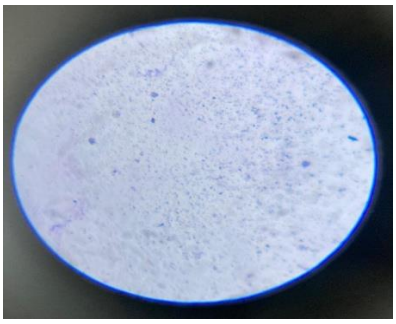

Fig.6 (Capsule staining)

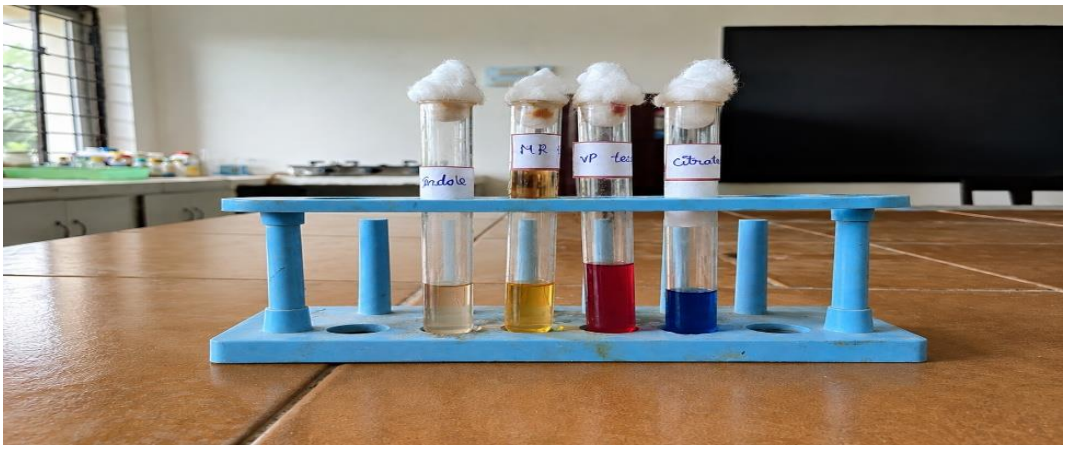

Fig.7 (Biochemical tests)

Fig.8 (catalase test)

4. DISCUSSION

The present study demonstrated the successful isolation and characterization of an endophytic bacterial isolate from Aloe vera. Nutrient Agar supported better bacterial growth and distinct colony development compared with Nutrient Broth, indicating its suitability for the isolation and maintenance of the bacterial isolate. The successful recovery of bacteria after surface sterilization suggests that the isolated microorganism was associated with the internal plant tissue rather than being a surface-associated microorganism.

The isolated bacterium showed distinct macroscopic characteristics, including small, circular, convex colonies with an entire margin and cream to pale-yellow colouration. Microscopically, the isolate exhibited Gram-positive, rod-shaped cells. Negative results for acid-fast, capsule, and endospore staining further supported its observed morphological characteristics. These morphological features provided useful preliminary information for the characterization of the isolated endophytic bacterium.

Biochemical characterization showed negative reactions for indole and methyl red tests, whereas Voges–Proskauer and citrate utilization tests were positive. The combination of morphological and biochemical characteristics indicates that the isolate possesses distinct physiological properties that can be useful for its preliminary identification. However, species-level identification would require additional approaches such as molecular characterization.

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The isolate showed immediate bubble formation in the qualitative catalase test following exposure to hydrogen peroxide, confirming catalase production. Catalase activity is associated with the breakdown of hydrogen peroxide and can contribute to protection against oxidative stress. The positive catalase reaction therefore demonstrates the enzymatic capability of the isolated endophytic bacterium to decompose hydrogen peroxide.

Quantitative analysis further demonstrated catalase production by the isolate. The decrease in absorbance from 0.50 to 0.27 indicated the consumption of hydrogen peroxide during the assay. Based on the Beer–Lambert calculation, the catalase concentration was determined as 17.69 mg/mL. This quantitative finding supports the qualitative catalase-positive result and indicates appreciable catalase production by the isolated endophytic bacterium.

Overall, the findings indicate that the isolated Aloe vera-associated endophytic bacterium possesses distinct morphological, biochemical, and catalase-producing characteristics. The observed catalase activity suggests that the isolate may have a role in hydrogen peroxide detoxification and oxidative-stress management, supporting its potential as a source of beneficial enzymatic activity. Further molecular identification and additional functional studies would be required to establish its species identity and broader biological potential.

5.Conclusion

The isolate showed positive catalase activity with a concentration of 17.69 mg/mL.
Catalase activity helps in oxidative-stress management in the plant-associated environment.
This supports favourable conditions for secondary metabolite synthesis.

REFERENCES

1. *Wasim A, Saif A, Pratap SA. A Comprehensive Review of Aloe vera: Health Benefits, Mechanisms of Action and Future Perspectives. Int. J. Res. Dev. Technol. 2023; 1:1-8.*
2. *Afzal I, Shinwari ZK, Sikandar S, Shahzad S. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. Microbiological research. 2019 Apr1;221:36-49.*
3. *Lodewyckx C, Vangronsveld J, Porteous F, Moore ER, Taghavi S, Mezgeay M, der Lelie DV. Endophytic bacteria and their potential applications. Critical reviews in plant sciences. 2002 Nov 1;21(6):583-606.*
4. *Mitra A, Singh M, Banga A, Pandey J, Tripathi SS, Singh D. Bioactive compounds and therapeutic properties of Aloe vera-a review.*
5. *Wang X, Li S, Liu Y, Ma C. Redox regulated peroxisome homeostasis. Redox biology. 2015 Apr 1; 4:104-8.*
6. *Kumar KS, Debjit Bhowmik DB, Chiranjib C, Biswajit B. Aloe vera: a potential herb and its medicinal importance.*
7. *Nemani R, Sinha R, Shobha M, Sahu AK, Kumari S. Isolation and characterization of endophytic bacteria from Aloe vera plant and screening of its antibiotic activity. Int J Innov Res Sci Eng Technol.2019;8(2).*
8. *Isleem RS, Eid AM, Hassan SE, Aboshanab KM, El-Housseiny GS. Deciphering the nature and statistical optimization of antimicrobial metabolites of two endophytic bacilli. AMB Express. 2025 Jan 13;15(1):10.*
9. *Azzahra F, Prasetyawati ET, Lestari SR. Isolation and characterization of endophytic bacteria from the roots of Avicennia sp. in the mangrove area of Gunung Anyar, Surabaya. Jurnal Pembelajaran Dan Biologi Nukleus. 2025 Mar 31;11(1):219-32.*
10. *Miller E. Microbiology laboratory manual. Oregon (US): Open Oregon Educational Resources;2024*
11. *Moyes RB, Reynolds J, Breakwell DP. Preliminary staining of bacteria: simple stains. Curr Protoc Microbiol. 2009 Nov;15.*
12. *Tripathi N, Zubair M, Sapra A. Gram staining. In StatPearls [Internet] 2025 Mar 28. StatPearls Publishing.*
13. *Prakoeswa FR, Rumondor BB, Prakoeswa CR. Acid-fast staining revisited, a dated but versatile means of diagnosis. The Open Microbiology Journal. 2022 Aug 17;16(1).*
14. *Schaeffer AB, Fulton MD. A simplified method of staining endospores. Science. 1933 Feb 17;77(1990):194-.*
15. *Acharya T. Capsule Stain: Principle, Procedure, Results. Microbe Online. 2025*
16. *Vasugi N, Subha TS, Elizabeth Jacquline L. Isolation, characterization, and identification of endophytic bacteria from Pichavaram mangrove forest with haemolytic, antibacterial, and antioxidant activity. Int J Adv Res. 2025;13(6):1421-1440.*
17. *Aryal S. Citrate utilization test principle media procedure and result [Internet]. 2018*
18. *Azzahra F, Prasetyawati ET, Lestari SR. Isolation and characterization of endophytic bacteria from the roots of Avicennia sp. in the mangrove area of Gunung Anyar, Surabaya. Jurnal Pembelajaran Dan Biologi Nukleus. 2025 Mar 31;11(1):219-32.*
19. *Saeed SW, Naseer I, Zahir ZA, Hilger T, Shahid S, Iqbal Z, Ahmad M. Bacillus strains with catalase enzyme improve the physiology and growth of rice (Oryza sativa L.). Stresses. 2023 Oct 20;3(4):736-48.*
20. *Darnell-Middleton SL. The relationship between eggshell quality and bone biology in the laying hen. University of Glasgow (United Kingdom); 1999.*