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Original Research Paper

Biochemical characterization of diverse endophytic bacteria isolated from the seed endosperm of wild relatives and cultivated varieties of papaya

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ABSTRACT

Endophytic bacteria associated with papaya seeds play an important role in nutrient mobilization and plant growth promotion. In the present study, endophytic bacteria isolated from seeds of wild papaya relatives (*Vasconcellea cundinamarcensis*, *V. cauliflora*, and *V. goudotiana*) and cultivated papaya varieties (Arka Prabhath and Arka Surya) were characterized for their morphological, biochemical, and plant growth-promoting traits. Molecular identification based on 16S rRNA gene sequencing revealed diverse taxonomic affiliations among the isolates. Functional characterization demonstrated considerable variability in nutrient solubilization, enzymatic activities, and indole-3-acetic acid (IAA) production. Isolates EAP14 and EVCa21 exhibited strong phosphate, potassium, and zinc solubilization abilities, while EVCu15 uniquely showed both cellulase and protease activities. High IAA production was observed in EVG8 and EVCu15, whereas several other isolates displayed moderate to high auxin-producing potential. Antibiotic sensitivity assays revealed diverse resistance patterns among the isolates. Overall, EAP14, EVCa21, and EVCu15 emerged as promising endophytes with multiple plant growth-promoting traits, indicating their potential application in sustainable agriculture and biofertilizer development. These findings highlight the functional diversity of papaya-associated seed endophytes and their possible role in improving nutrient availability and crop productivity.

Keywords: Biochemical characterization, endophytic bacteria, papaya varieties, plant growth-promoting traits, *Vasconcellea* spp.

INTRODUCTION

Papaya (*Carica papaya* L.) is a tropical fruit crop which is important for the economy and is widely grown in tropical and sub-tropical countries including India, the world's largest producer (Altendorf, 2019). In addition to nutritional and industrial applications, papaya productivity and nutritional values can be enhanced by sustainable microbial applications. Endophytic bacteria that live in the plant tissues without causing disease have proven to be beneficial for plant growth by solubilizing nutrients, producing phytohormones, aiding stress tolerance, and suppressing pathogens (Adeleke et al., 2021; Eid et al., 2021). The solubilization of insoluble nutrients (such as phosphorus, potassium and zinc) are the most relevant plant growth promoting properties from an agricultural point of view. Zinc is an essential micronutrient that is required for the activation of enzymes, carbohydrate metabolism, and auxin regulation and deficiency slows down plant growth and productivity (Alloway, 2008). Chemical fertilizers are often applied to correct micronutrient deficiencies but microbial mediated nutrient mobilization provides more sustainable and environmentally friendly approach. In recent years, the ability of endophytic and rhizosphere microbes to improve the availability of zinc and plant growth has been confirmed (Jalal et al., 2024; Shahid et al., 2025). But there is very little information available on the endophytic bacteria associated with seeds of papaya especially the wild plants of the *Vasconcellea* genus.

Endophytic bacteria are also known to promote plant growth by the production of phytohormones like indole-3-acetic acid, secretion of extracellular enzymes, and antagonistic activity against plant pathogens (Compant et al., 2010). A number of bacteria, including *Bacillus* and *Enterobacter* spp., have been identified to have high plant growth promoting and biocontrol potential (Ongena & Jacques, 2008). Thus, the present research work was undertaken to characterize the endophytic bacteria isolated from the seeds of wild papaya relatives (*Vasconcellea cundinamarcensis*, *V. cauliflora* and *V. goudotiana*) and cultivated papaya varieties

Table 1 : Microscopic and macroscopic characterization of endophytes isolated from seeds of wild relatives and cultivated varieties of papaya, on LB agar plate after 24 h

Papaya variety	Isolate	Shape and growth	Colony form	Margin of colony	Elevation	Colour of colony	Texture	Gram character
V. cauliflora	EVCa1	Cocci, Moderate	Filamentous	Filamentous	Flat	Creamy white	Smooth	Gram negative
V. gaudotiana	EVG2	Cocci, Moderate	Circular	Entire	Raised	Creamy white	Smooth	Gram negative
V. gaudotiana	EVG3	Small rod, Fast	Circular	Entire	Raised	Creamy yellow	Smooth	Gram negative
V. gaudotiana	EVG4	Cocci, Slow	Irregular	Entire	Convex	Creamy	Smooth	Gram positive
V. gaudotiana	EVG5	Small rod, Moderate	Circular	Entire	Convex	Creamy	Smooth	Gram positive
V. cundinamarcensis	EVCu6	Rod shaped, Moderate	Circular	Curled	Convex	Creamy	Smooth	Gram positive
V. cundinamarcensis	EVCu7	Cocci, very slow	Irregular	Entire	Flat	Creamy white	Smooth	Gram positive
V. gaudotiana	EVG8	Cocci, very slow	Circular	Entire	Raised	Light yellow	Smooth	Gram positive
Arka prabhath	EAP9	Small rod, very slow	Circular	Entire	Raised	Creamy orange	Smooth	Gram positive
Arka prabhath	EAP10	-	Irregular	Entire	Raised	Light yellow	Smooth	Gram positive
Arka prabhath	EAP11	Small rod, slow	Circular	Entire	Raised	Light yellow	Smooth	Gram positive
Arka prabhath	EAP12	Cocci, Moderate	Irregular	Entire	Raised	Creamy	Rough	Gram negative
Arka prabhath	EAP13	-	Circular	Curled	Convex	Creamy white	Smooth	Gram positive
Arka prabhath	EAP14	Small rod, slow	Circular	Curled	Raised	Creamy white	Smooth	Gram negative
V. cundinamarcensis	EVCu15	Fast	Circular	Curled	Lobate	White	Rough	Gram positive
Arka prabhath	EAP16	Cocci, slow	Filamentous	Curled	Raised	Creamy yellow	Smooth	Gram positive
Arka surya	EAS17	Slow	Circular	Curled	Flat	Creamy white	Smooth	Gram negative
Arka surya	EAS18	Moderate	Circular	Entire	Raised	Light yellow	Smooth	Gram negative
V. cauliflora	EVCa19	Fast	Circular	Entire	Raised	Creamy yellow	Smooth	Gram positive
V. cauliflora	EVCa20	Fast	Rhizoid	Entire	Raised	Creamy yellow	Mucoid	Gram negative
V. cauliflora	EVCa21	Fast	Circular	Entire	Raised	Creamy yellow	Mucoid	Gram positive
V. cauliflora	EVCa22	Moderate	Circular	Curled	Raised	Creamy yellow	Mucoid	Gram negative

(Arka Prabhath and Arka Surya). The isolates were tested for morphological, biochemical, enzymatic and plant growth promoting characteristics such as solubilization of phosphate, potassium and zinc, production of IAA as well as sensitivity to various antibiotics to identify potential endophyte candidates having agricultural applications.

MATERIALS AND METHODS

Collection of seed samples and isolation of endophytic bacteria

The seeds of three wild relatives of the papaya, namely, *Vasconcellea cauliflora*, *V. goudotiana*, *V. cundinamarcensis* and two cultivated varieties of the papaya, namely Arka Prabhath and Arka Surya were collected from the healthy plants of these plants maintained at the ICAR-Indian Institute of Horticultural Research, Bengaluru, India and except *V. cundinamarcensis*, which was obtained from the Government Botanical Garden, Ooty, India. Surface sterilization was accomplished for seeds by soaking them in 4% sodium hypochlorite, then 70% ethanol, and then rinsing with sterile distilled water. The final rinse water was tested for sterility by plating on Luria-Bertani agar (LBA). For embryo and adherent endosperm tissues, the homogenization was done in sterile distilled water and spread on LBA plates, which were then incubated at 28°C for 24-48 h. Repeated streaking was done on LBA to obtain distinct colonies and kept on LBA plates.

Morphological and molecular characterization

Standard microbiological methods (Holt et al., 1994) were used for morphological characterization of the bacterial isolates, which was done using the Gram reaction and the colony morphology. Pure cultures were used to extract genomic DNA with the Zymo Research Quick-DNA Miniprep Plus Kit. Universal primers 27F and 1492R (Lane, 1991) were used to amplify the 16S rRNA gene. The PCR products were sequenced and taxonomically identified. The sequences obtained were deposited in the NCBI GenBank database with accession numbers shown in Supplementary Table 1.

Biochemical and functional characterization

Biochemical characterization of the isolates was done by performing IMViC tests and carbohydrate utilization using HiIMViC biochemical kit (HiMedia, India). The production of cellulase, catalase and gelatinase were assessed according to standard procedures (Singh et al., 2013; Geetha et al., 2014). Phosphate, potassium and zinc solubilization capacities were tested by Pikovskaya's medium, Alexandrov's medium and zinc solubilization medium (Pikovskaya, 1948; Kamran et al., 2017), respectively. The production of ammonia and hydrogen cyanide (HCN) were also assessed according to standard procedures (Geetha et al., 2014). Increased auxin production was qualitatively determined with Salkowski reagent by growing bacterial cultures in tryptic soy broth with L-tryptophan (Ehmann, 1977). The appearance of pink coloration was used as an indicator of indole-3-acetic acid production.

Antibiotic sensitivity assay

The sensitivity of the isolates was tested by using the octa-disc method of antibiotic sensitivity: chloramphenicol, ampicillin, tetracycline, gentamicin, streptomycin, kanamycin, co-trimoxazole and amikacin (HiMedia, India). Zone of inhibition around the discs was noted after 48-72 hours of incubation at 30±2°C.

Statistical analysis

Each sample was tested three times and the results reported as means±standard deviations. One-way ANOVA and Duncan's Multiple Range Test were used to analyze the data at $p<0.05$. The statistical parameters are given in Supplementary Table 3.

RESULTS AND DISCUSSION

In total, 21 isolates of endophytic bacteria were isolated from papaya seeds; two bacteria were isolated from the seeds of *Vasconcellea cundinamarcensis*, five bacteria were isolated from the seeds of *V. cauliflora* and *V. goudotiana* each, seven bacteria were isolated from the seeds of Arka Prabhath, and two bacteria were isolated from the seeds of Arka Surya.

Morphological and molecular characterization of endophytic bacteria

The colony characteristics of the isolates were found to be quite variable with regard to colony form, colony margin, colony elevation, colony colour, colony texture and growth rate (Table 1 and Fig. 1). Colonies varied from circular to irregular and rhizoid; they were creamy white to yellow in colour. The majority of the isolates had smooth colony morphology with EVCa20 and EVCa21 being mucoid. Cocci, short rods and rod-shaped bacteria were found under the microscope. The fast-growing ones were EVG3, EVCa19 and EVCa21 while other isolates EVG8 and EVCu7 showed comparatively slow growth.

Fig. 1 : Colony morphology of bacterial endophytes isolated from seeds of wild Vasconcellea spp. and cultivated papaya varieties (Arka Prabhat and Arka Surya) grown on LB agar after 24 h incubation at 30°C. Colony characteristics including shape, margin, colour, texture, and elevation were recorded

The molecular identification was performed by using 16S rRNA gene sequencing which yielded about 1.5 kb amplicons. The phylogenetic tree showed different clusters of isolates (Fig. 2; Supplementary Table 1). The predominant group of isolates comprised members of Enterobacteriaceae: *Enterobacter*, *Klebsiella*, *Leclercia*, *Pantoea*, *Lelliottia* and *Escherichia*. The isolate EVG3, EAP11, and EAP18 were found to be well grouped with *Siccibacter colletis* with high bootstrap supports. It is noteworthy that the strains of *Bacillus* spp. (EVCu6 and EVCu15) and *Paenibacillus illinoisensis* (EVCu7) were found in a separate clade from Enterobacteriaceae. *Pseudomonas* sp. (EVCa22), *Microbacterium* sp. (EAP12) and *Cellulosimicrobium cellulans* (EAP13) formed separate phylogenetic groups, confirming the taxonomic diversity of papaya-associated endophytes.

Biochemical and enzymatic characterization

The Gram reaction and biochemical profiling showed significant functional diversity of the isolates (Table 1; Supplementary Table 2). Several isolates, such as EVG8, EAP10 and EVCa21, were Gram-positive; while EVCa20, EVCa22 and EAP12 were Gram-negative. The IMViC tests and the carbohydrate utilization tests showed different metabolic capacities of the isolates. EVCa22 was positive in all the IMViC tests, suggesting relatively metabolic versatility, while EVCa1, EVCu7, EAP14 and EVCa19 were less versatile in their biochemical reactions. The enzymatic properties were also determined and EVCu15 was the sole isolate with cellulase and protease activities (Table 2; Fig. 3A, B). EVCa21 and EAP14 had strong catalase activity (+++) while gelatinase activity was negative in all the isolates (Fig. 3C, D).

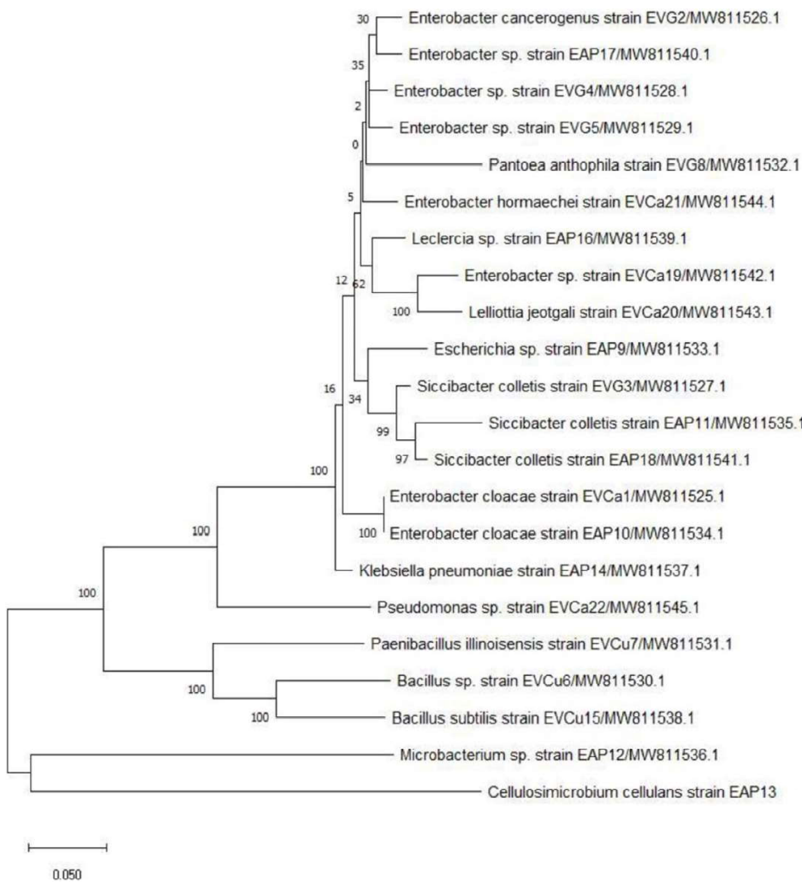

Fig. 2 : Phylogenetic analysis of bacterial endophytes based on 16S rRNA gene sequences

Sequences were aligned using ClustalW in MEGA version 11, and the phylogenetic tree was constructed using the maximum likelihood method. Bootstrap analysis was performed with 1000 replications and values ≤50% are indicated at branch nodes. GenBank accession numbers precede species names. The scale bar represents substitutions per nucleotide position

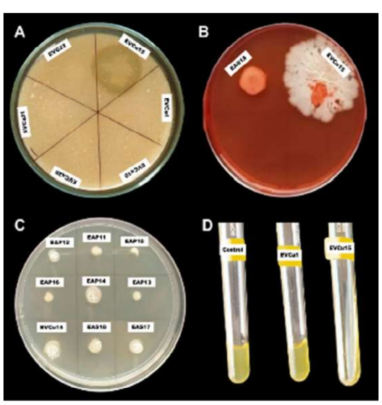

Fig. 3 : Enzymatic activities of endophytic bacteria isolated from papaya seeds

(A) Protease activity on skim milk agar. (B) Cellulase activity on CMC agar following Congo red staining. (C) Catalase activity based on bubble formation after exposure to 3% H, O, . (D) Gelatinase activity assessed by gelatin hydrolysis. Data represent mean ± standard deviation of three technical replicates (n=3)

Nutrient solubilization and plant growth-promoting traits

There was significant variation in the phosphate, potassium and zinc solubilization ability of the isolates (Supplementary Table 3; Fig. 4 and 5). EAP14 had the highest activity for phosphate solubilization (1.4 cm) followed by EVCa21 (1.3 cm) and EVG8 (1.0 cm). Likewise, potassium solubilization was highest for EAP14 (2.5 cm) and EVCa21 (2.4 cm) and next for EVCa19 and EVCa20. EAP14 was the most effective with the highest zone of clearance (2.6 cm) and EVCa21 and EVCa22 recorded 1.4 cm each for zinc oxide solubilization. The EAP14 and EVCa22 and EVCa21 had the highest (2.9 cm) and next highest (1.5 cm, 1.4 cm) zinc phosphate solubilization, respectively. There were no nutrient-solubilization activities in several isolates such as EVCu6, EVCu7, EAP13 and EVCu15. All nutrient-solubilization assays

Table 2 : Biochemical and functional traits of endophytes isolated from seeds of wild relatives and cultivated varieties of papaya

Bacteria	Catalase production	Ammonia Production	HCN production	Gelatinase production	Protease production	Cellulase production
EVCa1	-	-	-	-	-	-
EVG2	+	-	-	-	-	-
EVG3	-	-	-	-	-	-
EVG4	-	-	-	-	-	-
EVG5	-	-	-	-	-	-
EVCu6	-	-	-	-	-	-
EVCu7	-	-	-	-	-	-
EVG8	+	-	-	-	-	-
EAP9	-	-	-	-	-	-
EAP10	-	-	-	-	-	-
EAP11	-	-	-	-	-	-
EAP12	+	-	-	-	-	-
EAP13	-	-	-	-	-	-
EAP14	++	-	-	-	-	-
EVCu15	-	-	-	-	+	+
EAP16	-	-	-	-	-	-
EAS17	+	-	-	-	-	-
EAS18	+	-	-	-	-	-
EVCa19	++	-	-	-	-	-
EVCa20	++	-	-	-	-	-
EVCa21	+++	-	-	-	-	-
EVCa22	-	-	-	-	-	-

showed significant difference among the isolates ($P<0.05$) confirmed by the statistical data analysis. Overall, EAP14 and EVCa21 were the most effective multifunctional nutrient solubilizers. All the isolates failed to produce ammonia and hydrogen cyanide (HCN) (Table 2; Fig. 6A, B). However, there was inter-isolate variability in the qualitative production of IAA (Fig. 6C; Supplementary Fig. 1). High production levels of IAA were recorded in EVG8 and EVCu15 while in the other cultivars, high levels of IAA production potential were recorded, from EAP10, EAP11, EAP16, EAS17, EVCa19, to EVCa20. EVCa1, EVG2, EVCu6, EAP12, EAP13, EAP14, EVCu7 and EAS18 produced moderate amounts of IAA, with very low amounts produced in EVCu7.

Antibiotic sensitivity profile

There was significant variation in the antibiotic sensitivity of the isolates when tested by antibiotic

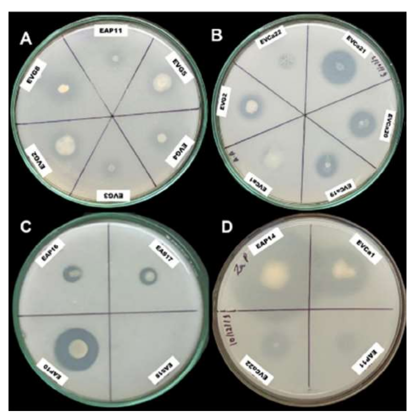

Fig. 4 : Mineral-solubilization activities of endophytic bacteria isolated from papaya seeds

(A) Phosphate solubilization on Pikovskaya agar, (B) Potassium solubilization on Alexandrov agar, (C) Zinc oxide (ZnO) solubilization, (D) Zinc phosphate (ZnP) solubilization. Solubilization efficiency was assessed based on halo-zone formation around bacterial colonies. Data represent mean±standard deviation of three technical replicates (n=3)

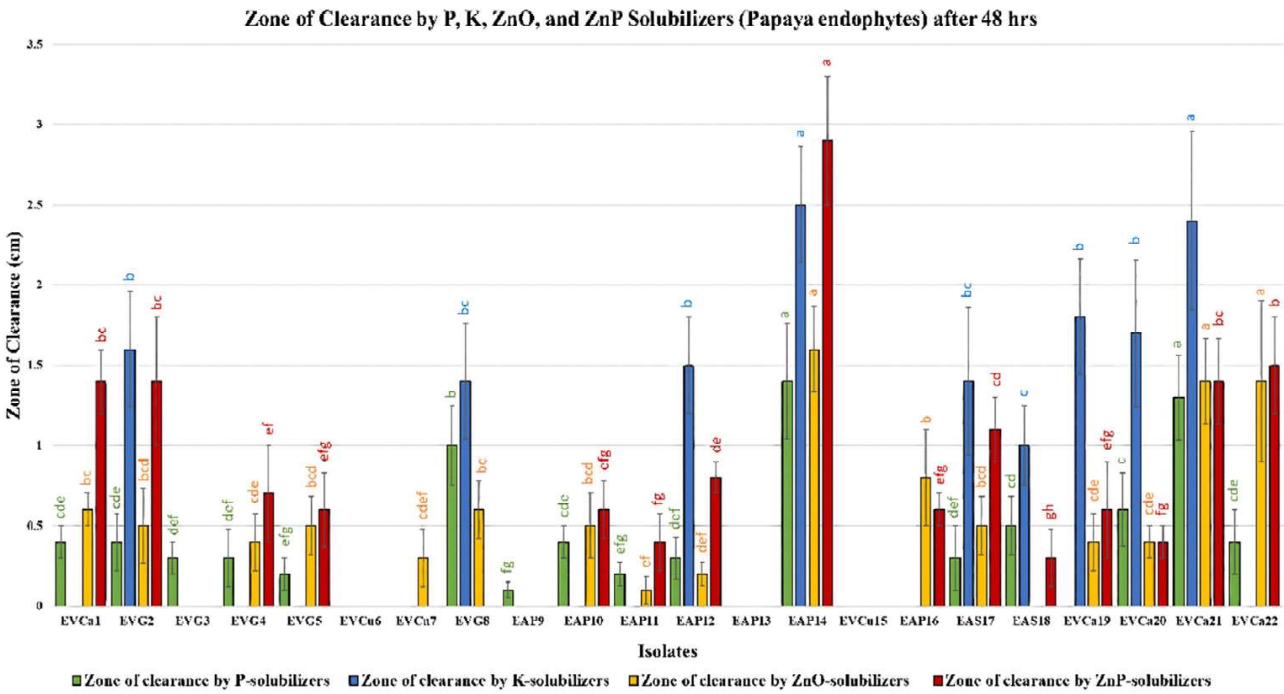

Fig. 5 : Zone of clearance produced by phosphorus (P), zinc oxide (ZnO) and zinc phosphate (ZnP)-solubilizing endophytic bacteria isolated from papaya seeds. Clearance zones were measured to assess nutrient-solubilization potential. Bars represent mean ± standard deviation of three technical replicates (n=3). Different lowercase letters indicate significant differences among isolates according to Duncan's Multiple Range Test at P<0.05

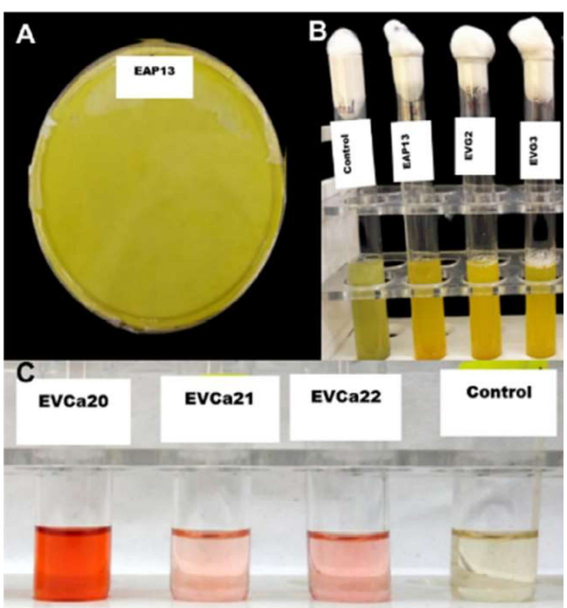

Fig. 6 : Plant growth-promoting traits of endophytic bacteria isolated from papaya seeds

(A) Hydrogen cyanide (HCN) production, (B) Ammonia production, (C) Indole-3-acetic acid (IAA) production detected using Salkowski reagent following growth in L-tryptophan-supplemented medium. Data represent mean±standard deviation of three technical replicates (n=3)

Fig. 7 : Antibiotic sensitivity profiles of bacterial endophytes isolated from papaya seeds determined by the disk diffusion method

Antibiotics used: (1) Chloramphenicol (30 µg), (2) Ampicillin (10 µg), (3) Tetracycline (30 µg), (4) Gentamicin (10 µg), (5) Streptomycin (10 µg), (6) Kanamycin (30 µg), (7) Co-trimoxazole (25 µg), and (8) Amikacin (30 µg). Data represent mean ± standard deviation of three technical replicates (n=3)

sensitivity assays (Table 3; Fig. 7; Supplementary Fig. 2). Broad sensitivity was seen in EVCu6 and EVCu15 for most of the tested antibiotics such as chloramphenicol, tetracycline, gentamicin, streptomycin, kanamycin, amikacin and co-trimoxazole. The sensitivity of EVG3 and EVG8 was also high, while EVG2 and EVG4 were moderately susceptible. The papaya-associated endophytes showed varying antibiotic resistance profiles with EAP13, EAS17 and EVCa22 being resistant to several antibiotics.

Table 3 : Antibiotic sensitivity test of endophytes isolated from seeds of wild relatives and cultivated varieties of papaya

Isolate	C	AmP	TE	GEN	S	K	coT	AK
EVCa1	++	-	++	++	++	++	++	+++
EVG2	+	+	+	++	++	++	++	+++
EVG3	++	+++	++	++	+	+	+++	++
EVG4	+	+	++	+++	+++	+++	+++	+++
EVG5	+	-	+	++	++	+++	++	+++
EVCu6	+++	+++	+++	+++	+++	+++	+++	+++
EVCu7	++	+	-	++	-	+	++	++
EVG8	+++	+++	+++	++	++	++	+++	++
EAP9	++	+++	++	+++	+++	+	-	+
EAP10	++	+++	+++	+++	+++	++	+++	+
EAP11	++	+++	+++	++	+	++	+++	++
EAP12	+	-	+	+	++	++	++	++
EAP13	++	+	+++	+++	++	-	-	-
EAP14	++	-	++	++	+	++	+++	++
EVCu15	+++	+++	+++	+++	+++	+++	+++	+++
EAP16	++	+	++	++	++	++	+++	+++
EAS17	+	-	+	++	++	++	++	++
EAS18	++	+++	++	++	+++	++	+++	++
EVCa19	++	+++	+++	+++	++	++	+++	++
EVCa20	+	++	++	+++	++	++	+++	++
EVCa21	++	-	++	+++	++	++	+++	++
EVCa22	-	-	++	+++	++	+++	-	+++

C: chloramphenicol, Amp: ampicillin, TE: tetracycline, GEN: gentamycin, S: streptomycin, K: kanamycin, CoT: Co-trimoxazole, AK: amikacin

The present study showed high diversity of the endophytic bacteria isolated from the seeds of wild species of *Vasconcellea* and cultivated species of papaya. The diversity found in the wild relatives compared to cultivated ones was found to be in accordance with previous reports that state that host genotype, environmental conditions and agricultural practices affect the endophytic microbial composition (Patil et al., 2021). The isolates recovered in this study are not truly endosperm-specific bacteria as they are associated with the embryo and residual endosperm tissues of mature papaya seeds.

Morphological and molecular characterization revealed that the isolates were highly diverse and belonged to genera including *Enterobacter*, *Bacillus*, *Pseudomonas*, *Microbacterium*, *Paenibacillus*, and others. The bacterial communities observed in papaya have been described previously in other plant tissues like papaya, potato, and carrot (Krishnan et al., 2012; Sarjono et al., 2020). The abundance of Enterobacteriaceae that was found in this study could be in relation with their rapid growth, metabolic adaptability and efficient colonization of the internal tissues of plants. Functional and evolutionary diversification of endophytes associated with papaya is further highlighted by the distinct clustering of the *Bacillus*, *Pseudomonas* and *Microbacterium* groups.

The biochemical tests showed high functional variation among the isolates. EVCa22 was shown to have a wide range of metabolic functions while EVCu15 was uniquely identified as having cellulase and protease activity, which would be important for nutrient cycling and degradation of organic matter. The same kind of enzymatic activities have been found in endophytes of crop and medicinal plants helping the plants to colonize, adapt to stress and mobilize nutrients

(Walitang et al., 2017; Vijayalakshmi et al., 2016). The catalase activity was also found to be high in EVCa21 and EAP14, suggesting their potential role in reducing oxidative stress in plant tissues, which is crucial for stress tolerance and plant-microbe interactions.

Nutrient-solubilization assays showed that EAP14 and EVCa21 were very effective for the solubilization of phosphate, potassium and zinc compounds. The results confirm that phosphate- and potassium-solubilizing bacteria have a significant role in enhancing nutrient availability and plant growth as reported in previous studies (Rodriguez & Fraga, 1999; Meena et al. 2018). The zinc solubilization endophytes are also significant since the zinc deficiency is a primary agricultural and nutritional problem in the tropical soils. It has been known that bacterial zinc mobilization via production of organic acids/ siderophores is a promising approach in sustainable micronutrient biofortification (Saravanan et al., 2007; Upadhayay et al., 2022). These properties of strong zinc-solubilizing ability of EAP14, EVCa21 and EVCa22 therefore suggest their potential application as bioinoculants to enhance nutrient acquisition in papaya.

None of the isolates were capable of ammonia or hydrogen cyanide (HCN) production, but this does not imply that they may not have plant growth-promoting ability as many fungal endophytes possess other traits that promote plant growth including the production of phytohormones and nutrient solubilization. The differences in IAA production in different isolates also highlight the diversity of their functions as endophytes of papaya. Isolates EVG8 and EVCu15 had good IAA production potential suggesting their potential role in root development and plant growth promotion. Other examples of endophytes from various crops that produce IAA which plays a crucial role in the growth enhancement of the host have been reported (Bhuvaneshwari et al., 2019; Nath et al., 2022). The isolates showed significant difference in their antibiotic sensitivity profile with EVCu6 and EVCu15 being broad spectrum sensitive and EAP13, EAS17 and EVCa22 being multi drug resistant. The variability was also observed in endophytes isolated from other medicinal and crop plants and occurs due to ecological adaptation and microbial competition inside the plant tissues (Kumar et al., 2015; Singh et al., 2022).

This study focused exclusively on culturable bacterial endophytes, and therefore may not represent the complete endophytic diversity associated with papaya seeds. The use of a single culture medium likely favoured fast-growing bacteria, particularly Enterobacteriaceae and *Bacillus* spp., while slow-growing or unculturable taxa may have remained undetected. Furthermore, only *in vitro* conditions were used to assess all the plant growth promoting properties. Hence, more experiments involving in planta validation and culture-independent methods should be conducted in the future to elucidate the ecological and agricultural role of these endophytes. Significantly, our earlier study found *Bacillus subtilis* strain EVCu15 to be quite functional with plant growth promoting and antagonistic properties (Patil et al., 2025). Overall, the various biochemical and functional traits identified in the papaya seed associated endophytes underscore their potential as bioinoculants in sustainable agriculture, nutrient mobilization, and crop improvement.

CONCLUSION

This study showed significant biochemical and functional differences between the endophytic bacteria associated with the seeds of wild members of the genus *Vasconcellea* and cultivated varieties of papaya. The taxonomic diversity of the isolates was confirmed morphologically and molecularly, including species of *Enterobacter*, *Bacillus*, *Pseudomonas*, *Microbacterium*, and other genera. The functional screening revealed that the nutrient-solubilisation and plant growth promoting characteristics varied considerably among the isolates. EAP14 and EVCa21 demonstrated good phosphate, potassium and zinc-solubilization capacities, whereas EVG8 and EVCu15 had high IAA-producing potential. EVCu15 exhibited both cellulase and protease activity while strong catalase activity was exhibited by EVCa21 and EAP14. The antibiotic sensitivity assays also revealed some variable adaptive features among the isolates. In general, plant growth-promoting traits of isolates like EAP14, EVCa21 and EVCu15 proved to be good candidates for sustainable agricultural applications. The results obtained can contribute to the understanding of papaya endophytes, and serve as a foundation for further research into their potential use in nutrient mobilization, biofertilization, and crop improvement.

Data availability statement

The 16S rRNA gene sequences generated in this study have been deposited in the NCBI GenBank database under the accession numbers listed in Supplementary Table 1.

Note: Shanmugaraj C., Dubey S., Pooja M.N. contributed equally

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