

Isolation, Morphological and Molecular Characterisation of the Endophytic bacterium *Burkholderia contaminans* from leaves of *Irvingia gabonensis*

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DOI: [10.22178/pos.133-10](https://doi.org/10.22178/pos.133-10)

LCC Subject Category: T1-995

Received 25.05.2026

Accepted 28.08.2026

Published online 31.08.2026

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Abstract. Endophytic bacteria constitute an important component of plant-associated microbial communities and occur within healthy plant tissues without necessarily producing visible symptoms of disease. The diversity of culturable bacterial endophytes associated with *Irvingia gabonensis*, an economically and nutritionally important indigenous African tree, remains insufficiently documented. This study aimed to isolate and characterise an endophytic bacterium recovered from healthy *Irvingia gabonensis* leaves. Healthy leaves were subjected to sequential surface sterilisation using ethanol and sodium hypochlorite before internal tissues were aseptically cultured on Nutrient Agar. The recovered isolate, designated PC5, was characterised based on cultural, morphological and biochemical properties. Molecular identification was performed by amplifying and sequencing the bacterial 16S rRNA gene using universal primers 27F and 1492R, followed by sequence comparison using BLASTn against the National Centre for Biotechnology Information nucleotide database. PC5 produced yellow, circular colonies with a smooth-to-mucoid surface. The isolate was Gram-negative, rod-shaped and motile. Biochemical characterisation showed positive reactions for oxidase, catalase and citrate utilisation, while indole production and glucose fermentation were negative. The 16S rRNA sequence from PC5 showed 100% similarity to the closest reference sequence, identified as *Burkholderia contaminans*. The sequence was submitted to the NCBI GenBank database under accession number PV523554, as supplied from the sequence submission record. The findings provide a molecularly characterised record of a culturable endophytic *B. contaminans* isolate associated with healthy *Irvingia gabonensis* leaves collected in Edo State, Nigeria. The study establishes a basis for subsequent investigations of the ecological, physiological and biological characteristics of this isolate.

Keywords: *Burkholderia contaminans*; bacterial endophyte; *Irvingia gabonensis*; 16S rRNA; molecular identification; GenBank; endophytic bacteria.

INTRODUCTION

Endophytic bacteria are microorganisms that inhabit the internal tissues of plants without causing visible disease symptoms under the conditions in which they are detected. They have been recovered from numerous plant species and plant organs, including roots, stems, leaves,

fruits, and seeds. Endophytic bacteria may establish diverse relationships with their hosts, ranging from commensal and mutualistic interactions to associations whose biological significance remains uncertain. Their occurrence within apparently healthy plant tissues makes them important subjects for investigating plant-associated microbial diversity [1].

The occurrence of bacterial endophytes in plants has received increasing attention because members of these communities can differ substantially from bacteria inhabiting the external plant environment. Internal colonisation can provide microorganisms with a relatively protected ecological niche. At the same time, the plant may interact with bacterial communities through processes involving nutrient acquisition, environmental adaptation, and other plant-microbe interactions [1, 2]. Consequently, documenting the bacterial endophytes associated with under-investigated plant species contributes to a better understanding of plant-associated microbial diversity.

The genus *Burkholderia* represents a particularly diverse group of Gram-negative bacteria. Members of the genus have been recovered from soil, water, the rhizosphere, plant tissues and other environments. Some species are associated with beneficial plant interactions, while other members, particularly within the *Burkholderia cepacia* complex, have attracted attention because of their association with human and animal infections [3, 4]. This ecological diversity makes accurate characterisation of *Burkholderia* isolates important when they are recovered from plant tissues.

Plant-associated *Burkholderia* species have been documented in both rhizospheric and internal plant environments. Authors [4] described *Burkholderia* as among the environmentally adaptable plant-associated bacterial groups and highlighted their occurrence within plant tissues. Similarly, authors [3] emphasised the broad ecological distribution of *Burkholderia* and the diversity of members of the *B. cepacia* complex. These observations demonstrate the importance of investigating individual *Burkholderia* isolates in relation to their specific plant hosts and environments.

Burkholderia contaminans is a recognised species belonging to the *B. cepacia* complex. NCBI Taxonomy currently recognises the species as *Burkholderia contaminans* [5], and it is classified within the family *Burkholderiaceae*. The species has been recovered from different environmental and plant-associated sources. Recent work has also demonstrated the continued isolation and genomic characterisation of *B. contaminans* from plant-derived materials, illustrating the ecological breadth of the species [6].

Although the species itself is established, its occurrence in a particular plant host may provide

useful information about the distribution of plant-associated bacteria. For example, a *B. contaminans* strain has previously been characterised as an endophytic bacterium associated with jute, demonstrating that the species can occur within plant tissues [7]. More recent work has also described *B. contaminans* recovered from plant-associated materials and investigated its genomic characteristics [6]. These reports show that isolating *B. contaminans* from a new plant host can be informative, provided the identification and host association are properly documented.

Irvingia gabonensis, commonly known as African wild mango, is an indigenous African tree with considerable nutritional, economic and ethnobotanical importance. Its fruits and seeds are utilised in different communities, while the plant has also received scientific interest because of its nutritional and phytochemical properties. Like other plants, *I. gabonensis* may provide ecological niches for microorganisms associated with its tissues. However, information on culturable bacterial endophytes specifically associated with the leaves of *I. gabonensis* remains limited.

Isolating bacterial endophytes requires adequate removal of microorganisms associated with the external surface of plant tissues. Sequential surface sterilisation using ethanol and sodium hypochlorite is commonly employed for this purpose, followed by a final-rinse control to assess sterilisation effectiveness [8]. An appropriate surface-sterilisation control is particularly important because failure to remove epiphytic microorganisms can lead to their misinterpretation as endophytes.

Phenotypic characterisation remains useful for the preliminary identification of bacterial isolates. Colony morphology, Gram reaction, cellular morphology, motility, and biochemical characteristics can provide an initial indication of an isolate's taxonomic position. However, closely related bacterial species may display overlapping phenotypic properties. Molecular approaches are therefore important for achieving more reliable bacterial identification.

The 16S rRNA gene is one of the most widely used molecular markers for bacterial identification. Its usefulness derives from conserved regions suitable for amplification and variable regions that provide taxonomic information [9, 10]. Comparing obtained sequences with reference sequences in nucleotide databases using BLASTn

can provide evidence for an isolate's taxonomic identity. Nevertheless, 16S rRNA sequencing does not provide equal discriminatory resolution for every bacterial group, and closely related organisms may possess highly similar sequences [9, 10].

The present study therefore focused on isolating and characterising bacterial isolate PC5 recovered from healthy leaves of *I. gabonensis*. The isolate was characterised using cultural, morphological and biochemical methods and subsequently identified using 16S rRNA gene sequencing and BLASTn analysis. The study specifically aimed to establish the molecular identity of PC5 and document its occurrence as a culturable endophytic bacterium associated with *I. gabonensis* leaves.

METHODS

Plant material and study area. Healthy leaves of *Irvingia gabonensis* were collected from Okpella, Etsako East Local Government Area, Edo State, Nigeria. Leaves showing visible disease symptoms, mechanical damage or deterioration were excluded from sampling. The collected leaves were placed in sterile containers and transported to the laboratory for microbiological processing.

The Head of the Botany Laboratory, Faculty of Life Sciences, Edo State University, Uzairue, Edo State, Nigeria, botanically authenticated the plant material. The specimen was assigned identification number ESUI-H-0000221, and a voucher specimen was deposited in the University Herbarium.

Surface sterilisation and isolation of endophytic bacteria. Surface sterilisation was performed using the procedure described by [8], with slight modification. The leaves were thoroughly washed under slow-running tap water with detergent for approximately 30–45 min to remove visible dirt and debris. The leaves were subsequently immersed sequentially in 70% ethanol (v/v) for 1 min and 2.5% sodium hypochlorite for 5 min. Three successive rinses followed this in sterile distilled water for 1 min each.

The effectiveness of surface sterilisation was evaluated using the final-rinse water. The final wash water was inoculated onto Nutrient Agar plates and incubated at 37°C for 48 h. The absence of microbial growth on the control plates was taken as evidence supporting the effectiveness of the surface-sterilisation procedure.

Following sterilisation, leaf tissues were aseptically cut into smaller segments using a sterilised surgical blade across the veins to expose endophytic tissue and inoculated onto Nutrient Agar plates. The plates were incubated and monitored for bacterial growth. Emerging colonies were purified through repeated subculturing until pure cultures were obtained. Five bacterial isolates were recovered during the broader isolation study; the present manuscript focuses exclusively on isolate PC5.

Cultural and morphological characterisation. The cultural characteristics of PC5 were determined from actively growing colonies on Nutrient Agar. Colony colour, shape and surface characteristics were recorded. Gram staining was performed to determine the Gram reaction and cellular morphology of the isolate. Cellular shape was examined microscopically, and motility was determined using standard microbiological procedures.

Biochemical characterisation. PC5 was subjected to selected biochemical tests for preliminary characterisation. The tests included catalase, oxidase, citrate utilisation, indole production and glucose fermentation. The tests were performed using standard microbiological procedures [11].

DNA extraction and 16S rRNA gene amplification. Genomic DNA was extracted from a pure culture of PC5 using a bacterial DNA extraction procedure. The extracted DNA served as a template for PCR amplification of the bacterial 16S rRNA gene.

The 16S rRNA gene was amplified using the universal bacterial primers 27F and 1492R. The PCR products were examined by agarose gel electrophoresis. A band of approximately 1,500 bp was obtained, consistent with the expected size of the amplified bacterial 16S rRNA gene region.

Sequencing and molecular identification. The amplified PCR product was purified and sequenced. The resulting sequence was quality-checked before comparison with nucleotide sequences available in the NCBI nucleotide database.

Sequence similarity was determined using BLASTn. Taxonomic identification was based on comparison of the obtained sequence with closely related reference sequences. PC5 showed 100% sequence similarity to the closest reference sequence identified as *Burkholderia contaminans*.

GenBank submission. The high-quality 16S rRNA gene sequence obtained from PC5 was submitted to the NCBI GenBank database. The accession number supplied for the isolate was PV523554.

RESULTS AND DISCUSSION

Isolation and phenotypic characterisation of PC5. After surface sterilisation and culture of internal leaf tissues, bacterial growth was obtained, and isolate PC5 was selected for detailed characterisation. No microbial growth was observed on the final-rinse control plate. This observation supports the effectiveness of the surface-sterilisation procedure and reduces the likelihood that the recovered isolate represented a microorganism remaining on the external leaf surface. Similar final-wash controls have been used in studies of culturable plant endophytes [8].

PC5 produced yellow, circular colonies with a smooth-to-mucoid surface on Nutrient Agar. Microscopically, the cells were Gram-negative rods, and the isolate was motile. The biochemical profile showed positive reactions for oxidase, catalase and citrate utilisation, whereas indole production and glucose fermentation were negative.

The phenotypic characteristics provided preliminary evidence consistent with a Gram-negative, oxidase-positive *Burkholderia*-like bacterium. However, these characteristics were not consid-

ered sufficient for definitive species identification. This is important because phenotypic overlap among related bacterial taxa can make species-level identification based exclusively on conventional microbiological tests uncertain [9, 10].

Table 1 – Cultural, morphological and biochemical characteristics of isolate PC5

Characteristic	PC5
Colony colour	Yellow
Colony shape	Circular
Colony surface	Smooth to mucoid
Gram reaction	Negative
Cell shape	Rod
Motility	Positive
Oxidase	Positive
Catalase	Positive
Citrate utilisation	Positive
Indole production	Negative
Glucose fermentation	Negative
Presumptive identification	<i>Burkholderia</i> spp.

Molecular identification of PC5. PCR amplification of the 16S rRNA gene generated an approximately 1,500-bp product from PC5. Sequence analysis followed by BLASTn comparison identified the isolate as *Burkholderia contaminans*. The sequence showed 100% similarity with the closest reference sequence used for identification.

Table 2 – Molecular identification of isolate PC5 based on 16S rRNA gene sequence analysis

Isolate	Closest NCBI match	Reference accession	Similarity, %	GenBank accession
PC5 (M5)	<i>Burkholderia contaminans</i>	CP172007	100	PV523554

The molecular result was consistent with the preliminary phenotypic characterisation. 16S rRNA sequencing provided a stronger basis for identification than phenotypic characteristics alone. The bacterial 16S rRNA gene is widely used for taxonomic identification because of its conserved and variable regions and its broad representation in sequence databases [9, 10].

The reference sequence reported in the BLAST comparison, CP172007, should be interpreted as the reference record against which PC5 was compared and not as the GenBank accession of the present isolate. CP172007 is associated with a *B. contaminans* genomic record reported by [6]. The distinction between the reference sequence

and the sequence generated in the present study is therefore important.

The 100% sequence similarity observed in the present analysis strongly supports identifying PC5 as *B. contaminans*. However, 16S rRNA sequence identity should not be interpreted as absolute proof of strain-level identity. Previous studies have highlighted the limitations of 16S rRNA sequencing, particularly when closely related bacterial species have highly similar sequences [9, 10]. Consequently, this study describes PC5 as a 16S rRNA-identified *B. contaminans* isolate.

Burkholderia contaminans is a plant-associated bacterium. The identification of PC5 as *B. contaminans* is consistent with the genus's established

ecological diversity. *Burkholderia* species occur in soil, rhizosphere and plant-associated environments, and some members can colonise internal plant tissues [3, 4]. The recovery of PC5 from apparently healthy leaf tissue therefore supports the presence of a *Burkholderia* member within the culturable endophytic bacterial community associated with *I. gabonensis*.

Previous studies demonstrate that *B. contaminans* is not restricted to a single environmental niche. Authors [7], for example, characterised an endophytic *B. contaminans* strain associated with jute seed. More recently, authors [6] reported a *B. contaminans* strain isolated from *Pericarpium citri reticulatae* and provided genomic characterisation. These reports demonstrate that *B. contaminans* can be associated with plant-derived environments, although the biological characteristics of individual isolates may differ.

The present finding is therefore significant primarily because of the host association and geographical context. To our knowledge, the present study provides a molecularly characterised record of *B. contaminans* isolated as a culturable endophyte from *I. gabonensis* leaves collected in Edo State, Nigeria. The observation expands the documented range of plant hosts from which culturable *B. contaminans* has been recovered.

Importantly, isolating *B. contaminans* from *I. gabonensis* does not, by itself, establish a beneficial, pathogenic, or biotechnological role for PC5. Although some *Burkholderia* strains have demonstrated plant-associated functions, such characteristics are strain-dependent and require direct experimental evaluation [3, 4]. The present study therefore deliberately limits its interpretation to isolation and molecular characterisation.

Significance of the *B. contaminans*-*I. gabonensis* association. Documenting bacterial endophytes from indigenous plants helps us understand the microbial diversity associated with economically important plant species. *I. gabonensis* is widely utilised in parts of Africa, but its associated bacterial communities remain less extensively characterised than those of several intensively studied agricultural plants.

The present isolation of PC5 demonstrates that healthy *I. gabonensis* leaf tissues can harbour culturable bacteria that are not readily distinguishable to species level using morphology and conventional biochemical tests alone. The subsequent molecular identification demonstrates the

value of combining classical microbiological characterisation with DNA-based identification.

The GenBank deposition of the PC5 sequence under accession number PV523554, as supplied for this study, further provides a molecular record for future comparative investigations. Such sequence records are useful for building knowledge of bacterial diversity associated with indigenous plant species.

The current study also establishes a baseline for future investigations of PC5. Additional analyses may be required to determine its genomic characteristics, ecological functions and other biological properties. Such investigations should be treated as separate questions rather than inferred from the 16S rRNA identification alone.

Limitations of the present identification approach. Although 16S rRNA sequencing is a widely accepted approach for bacterial identification, it has recognised limitations. Closely related organisms can possess highly similar or identical 16S rRNA sequences, and database annotations may occasionally contain errors or incomplete taxonomic information [9, 10]. Therefore, the 100% similarity observed for PC5 should be regarded as strong evidence supporting the assigned identity but not as definitive evidence of strain-level genomic identity.

Higher-resolution approaches, including multi-locus sequence analysis, additional housekeeping genes or whole-genome sequencing, could provide further taxonomic resolution in future work. Such approaches may also clarify the evolutionary relationship between PC5 and other *B. contaminans* strains. However, these analyses are outside the scope of the present investigation.

CONCLUSIONS

A culturable bacterial endophyte, PC5, was successfully isolated from healthy, surface-sterilised leaves of *Irvingia gabonensis* collected from Okpella, Etsako East Local Government Area, Edo State, Nigeria. PC5 produced yellow, circular colonies with a smooth-to-mucoid surface and was characterised as a Gram-negative, rod-shaped and motile bacterium. The isolate was positive for oxidase, catalase and citrate utilisation but negative for indole production and glucose fermentation.

Molecular characterisation based on 16S rRNA gene sequencing and BLASTn analysis identified

PC5 as *Burkholderia contaminans*, showing 100% sequence similarity with the closest reference sequence. The sequence generated for the isolate was submitted under GenBank accession number PV523554, as supplied for the study.

The findings provide a molecularly characterised record of *B. contaminans* associated with healthy surface-sterilised *I. gabonensis* leaves and contribute to the documentation of culturable endophytic bacteria associated with this indigenous African plant. Further studies are warranted to investigate the genomic and biological characteristics of PC5.

Acknowledgements

The authors acknowledge the Faculty of Life Sciences, Edo State University Uzairue, Edo State, Nigeria, for providing laboratory facilities and institutional support for this study. The authors also acknowledge the laboratory personnel who assisted with microbiological and molecular analyses.

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Conflict of Interest

The authors declare no conflict of interest associated with this study.

Funding

This research received no external funding.

Data Availability

The 16S rRNA gene sequence generated from isolate PC5 was submitted to the NCBI GenBank database under accession number PV523554, as supplied by the authors. Additional experimental data are available from the corresponding author upon reasonable request.

Ethical Statement

This present study involved plant material and bacterial isolation and did not involve human participants or experimental animals. Therefore, ethical approval for human or animal experimentation was not applicable.

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