



Research Article

Isolation, Identification, and Pathogenicity of Bacterial Wilt Pathogens Associated with Ginger in Kano, Nigeria

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ABSTRACT

Bacterial wilt remains a major constraint for ginger (*Zingiber officinale* L.) in most ginger-growing regions of Nigeria, with post-harvest losses exceeding 90%. *Ralstonia solanacearum* is widely recognized as the primary causal agent of the disease. This study set out to isolate, molecularly identify, and confirm the pathogenicity of bacterial pathogens associated with ginger wilt in Kano State, Nigeria. Biochemical tests and carbohydrate oxidation patterns grouped the isolates into typical *R. solanacearum* biovars (I and V). However, 16S rRNA sequencing and maximum-likelihood phylogenetic analysis revealed non-*Ralstonia* pathogens. The causal agents were identified as *Alcaligenes* sp. (CG09, YG018, and YG019; 97.40% similarity for CG09) and a *Brucellaceae*-related taxon (CG011; 91.73% similarity). Pathogenicity tests confirmed these strains as virulent, with 100% wilt incidence ($p=0.42$) in inoculated plants at 21 days post-inoculation and Koch's postulates fulfilled after successful re-isolation. Sequences for CG09 and CG011 have been deposited in GenBank under accession numbers PZ682642 and PZ682641, respectively. This study provides preliminary evidence of *Alcaligenes* sp. and a *Brucellaceae*-related taxon as causal agents of bacterial wilt of ginger in Nigeria. It points out the necessity of using molecular tools to confirm traditional biochemical assays for accurate pathogen identification and effective disease management in Nigerian ginger production.

Keywords: *Alcaligenes* sp.; Bacterial wilt; *Brucellaceae*-related taxon; Molecular diagnostics; *Zingiber officinale*

Citation: Adam, R., Musa, D.D., Kutawa, A.B., Abubakar, K.D., Nura, A., Yaro, A.S., Idris, B., Fatima, S.A., & Yusha'u, M. (2026). Isolation, Identification, and Pathogenicity of Bacterial Wilt Pathogens Associated with Ginger in Kano, Nigeria. *Sahel Journal of Life Sciences FUDMA*, 4(3): 637-648. DOI: <https://doi.org/10.33003/sajols-2026-0403-66a>

INTRODUCTION

Ginger (*Zingiber officinale* L.) is a vital economic and medicinal crop in Nigeria. According to Statista Research Department (2023), Nigeria is a major global producer of ginger with an annual output of approximately 726,000 metric tons as of 2021. In 2023, it exported 85.9 million kg of ginger valued at

\$47.6 million (World Bank, 2023). Currently, Nigeria is ranked 2nd in World's ginger production after India (FAO, 2024), but in terms of export volume, it is lower than top 5 countries (China, Netherlands, Peru, Thailand, and India) (OECD World, 2025). In Africa, Nigeria is the largest producer (Bamigboye, 2023), with Kaduna State serving as the leading production

hub. Ginger cultivation has expanded beyond Kaduna and Nasarawa into southeastern states such as Abia, Anambra, Cross River, Ebonyi, and Imo (Bulus *et al.*, 2020). Kano State, though not a major producer, has emerged as the central market hub, with supplies largely sourced from Kaduna. Preliminary evidence suggests that ginger can adapt to Kano's climatic conditions, opening opportunities for expanded cultivation (Abubakar *et al.*, 2019).

Despite Nigeria's production strength, recent outbreaks of bacterial wilt have caused significant yield losses. Recently, farmers in Kaduna State have reported losses exceeding 90% of harvests due to *R. solanacearum* infection (Mojeed, 2024). Globally, ginger wilt is primarily attributed to the *Ralstonia solanacearum* species complex (RSSC) (Prameela & Suseela, 2020; Hazarika *et al.*, 2019). This pathogen is a threat in tropical and subtropical regions, where it causes severe wilting, stunting, and plant death, leading to yield losses ranging from 20% to 100% (Benti, 2023).

Due to limited molecular characterization of ginger wilt pathogens in Nigeria, existing studies have focused mainly on tomato and other solanaceous crops (Adebayo & Ekpo, 2007; Popoola *et al.*, 2015). *Ralstonia solanacearum* has historically been assumed to be the primary pathogen in affected ginger fields (Nair, 2013). However, recent reports from China have demonstrated that ginger wilt can be induced by taxonomically diverse non-*Ralstonia* pathogens, including *Enterobacter*, *Pseudomonas*, *Achromobacter*, and *Serratia* species (Liu *et al.*, 2021; Hua *et al.*, 2024; Wu *et al.*, 2025; Zhang *et al.*, 2025c). Research shows that other pathogens can also cause wilt-like symptoms in ginger, forming disease complexes with *Ralstonia*; for example, *Fusarium* species and other fungal pathogens like *Phyllosticta zingiberi* have also been detected alongside *Ralstonia* in wilt-affected ginger samples, forming complex diseases involving multiple pathogens (Seid *et al.*, 2020), hence making purely biochemical identification unreliable.

Accurate pathogen identification is the first step for effective management of bacterial wilt in ginger. In Nigeria, and in Kano as its central market hub, where ginger wilt causes significant agricultural losses, the causative agents remain poorly characterized at the molecular level, and most diagnoses rely solely on phenotypic traits under the assumption that *Ralstonia solanacearum* is the major causal agent. However, recent studies have revealed that ginger wilt can involve taxonomically diverse non-*Ralstonia* pathogens, making biochemical identification

unreliable. This research investigated bacterial wilt pathogens associated with wilt symptoms in ginger in Kano by characterizing isolates phenotypically, assigning them to biovars, identifying them molecularly via *16S rRNA* gene sequencing, and confirming their pathogenicity.

MATERIALS AND METHODS

Plant material and sampling

Mature symptomatic ginger rhizomes of two cultivars, 'Yar China Ginger' (CG) and Yellow Ginger (YG), were collected from Yankaba market, Kano State, and Kagarko farm, Kaduna, between March and June 2024. Rhizomes were authenticated at the Herbarium Department of Plant Science and Biotechnology, Federal University Dutsinma, with voucher number FUDMA/PSB/00099. Sixty (60) rhizomes were purposively selected, comprising 30 specimens from each site, exhibiting typical wilt symptoms; specifically, vascular discoloration and soft rot. The samples were transported to the Department of Microbiology laboratory at Bayero University, Kano, in sterile polythene bags and analyzed immediately after collection.

Isolation of bacterial wilt pathogen

Rhizome pieces (4-5 mm) were excised from the region between healthy and diseased vascular tissues using a sterile scalpel. The pieces were surface-sterilized by immersion in 0.1% sodium hypochlorite (NaOCl) solution for 30s, rinsed three times in sterile distilled water, and air-dried under sterile conditions as described by Schaad *et al.* (2001). Because this study specifically aimed to isolate and characterize *Ralstonia* spp., the sterilized tissues were directly plated in triphenyltetrazolium chloride (TZC) medium manufactured by British Drug Houses Lab England, a semi-selective agar indicative for this pathogen, prepared as follows (per liter): peptone 10 g, casein hydrolysate 1g, dextrose 5 g, agar 15 g, and 5 mL of a 1% aqueous solution of 2,3,5-triphenyltetrazolium chloride, as described by Sharma and Singh (2019). Plates were incubated at 32 °C for 48 h. Typical virulent *Ralstonia*-like colonies characterized by fluid, irregular, pink-red centers with white, irregular margins were selected and sub-cultured onto fresh nutrient agar plates to obtain pure cultures.

Morphological and Biochemical characterization

The sixty symptomatic rhizomes plated on TZC yielded different bacterial colonies. These colonies were categorized into five distinct morphotypes based on their cultural characteristics on the TZC agar, depending on colony size, color intensity,

fluidity, and margin shape. A representative isolate from each of the five morphotypes was selected. Five of these isolates were then subcultured for subsequent biochemical, biovar, and molecular identification. Gram reaction was determined by standard Gram staining. Catalase activity was measured using 3% H₂O₂, and oxidase activity was measured using Kovacs reagent (1% tetramethyl-p-phenylenediamine dihydrochloride), as described by Shweta *et al.* (2018). Starch hydrolysis was determined on nutrient agar containing 1% starch, which was flooded with Lugol's iodine after incubation, as described by Pawaskar *et al.* (2014).

Biovar Classification

Biovar determination was performed using the carbohydrate/sugar alcohol oxidation protocol described by Setiawan *et al.* (2024), following the standard EPPO (2004) classification scheme for *Ralstonia solanacearum*. Sterile basal medium (pH 7.0) was prepared containing 1% NH₄H₂PO₄, 0.2% KCl, 0.2% MgSO₄ · 7H₂O, 1% peptone, and 0.3% bromothymol blue as a pH indicator. The basal medium was supplemented individually with filter-sterilized carbohydrate solutions to a final concentration of 2% (w/v). The six test carbohydrates were: maltose, lactose, cellobiose, mannitol, sorbitol, and dulcitol. The medium was dispensed into sterile tubes, inoculated with a loopful of a 24-hour bacterial suspension, and incubated at 28 °C. Tubes were examined for color change at 4, 5, and 7 days post-inoculation. Oxidation of disaccharides was indicated by a color change from green to yellow; oxidation of sugar alcohols was indicated by a change from blue to yellow. Biovars were assigned based on the oxidation patterns as defined by EPPO (2004).

16S rRNA gene amplification and Sequencing

Genomic DNA was extracted using a hybrid approach that combined a commercial DNA extraction kit (ZymoBiomix miniprep kit) for lysis with the traditional phenol-chloroform purification method, as described by Mahuku (2004). Freshly cultured bacterial cells were suspended in 200 µL of lysis buffer, followed by the addition of 20 µL of proteinase K. The mixture was incubated at 65 °C for 2 hours to ensure efficient cell lysis. Subsequently, DNA purification was performed using phenol-chloroform extraction. A 200 µL volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added to the lysate, and the mixture was centrifuged at 12,000 × g for 10 minutes to separate the aqueous and organic phases. The upper aqueous phase, containing the DNA, was carefully transferred to a new 1.5 mL microcentrifuge tube. DNA was

precipitated by adding 100 µL of cold 70% ethanol, followed by centrifugation at 12,000 × g for 10 minutes, resulting in a visible DNA pellet. The pellet was washed with ethanol to remove residual impurities, air-dried to eliminate traces of ethanol, and finally resuspended in distilled water. The purified DNA was stored at –20 °C. Amplification of the 16S rRNA gene was performed using the extracted DNA as template and Taq Mix Master Mix with the universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTGTTACGACTT-3') following the protocol originally described by Lane (1991). The PCR reaction was performed in a total volume of 25 µL containing 1× PCR buffer, 2.5 mM MgCl₂, 0.2 mM each dNTP, 0.5 µM each primer, 1.25 U *Taq* DNA polymerase, and approximately 50 ng template DNA. The thermal cycling conditions were: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s; and a final extension at 72 °C for 5 min. The amplified products (~1500 bp) were resolved on a 1.5% agarose gel stained with ethidium bromide, visualized under ultraviolet light, and documented using a gel documentation system. The PCR products were purified using the Zymo miniprep purification kit and sequenced using Sanger sequencing technology (Inqaba Biotechnical Industries, South Africa).

The consensus sequences were compared against the NCBI GenBank database using the BLASTn algorithm (<https://blast.ncbi.nlm.nih.gov>). The 16S rRNA sequences for isolates CG09 and CG011 were submitted to GenBank and given accession numbers PZ682642 and PZ682641, respectively. The sequence for isolate YG06 was found to be chimeric and therefore excluded from phylogenetic analysis and GenBank deposition.

Phylogenetic Analysis

A phylogenetic tree was constructed to determine the evolutionary relationships of the bacterial isolates based on partial 16S rRNA gene sequences. The evolutionary history was inferred in MEGA12 using the Maximum Likelihood method based on the Jukes-Cantor (1969) model to account for nucleotide substitution patterns. Evolutionary rate differences among sites were modeled using a discrete Gamma distribution across five categories (+G, parameter = 0.3019). The initial tree for the heuristic search was selected by choosing the topology with the superior log-likelihood (-1,343.86) between a Neighbor-Joining (NJ) tree and a Maximum Parsimony (MP) tree. The NJ tree was generated using a matrix of

pairwise distances computed via the p-distance method. To establish statistical robustness, node support and tree reliability were evaluated using 1,000 bootstrap replicates. All positions with less than 95% site coverage were removed using the partial deletion option, resulting in a robust final dataset comprising 408 positions across 12 nucleotide sequences.

Pathogenicity Test

The pathogenicity test was conducted at the Greenhouse Department of Plant Science and Biotechnology, Bayero University, Kano, from June to September 2024. The area is located at latitude 11°58'50"N and longitude 8°28'46"E, with an elevation of 486.5 m above sea level. It features a Sudan-Sahel climate marked by distinct wet and dry seasons, which significantly impact plant growth and agricultural yields. Healthy ginger rhizomes ('Yar China Ginger and Yellow Ginger (also known as Taffin giwa)) were raised for three months in the greenhouse under controlled conditions and watered regularly. Before planting, rhizomes were surface-sterilized by immersion in 0.1% sodium hypochlorite (NaOCl) solution for 5 min, and sandy-loam soil of 1,000 g per plant was autoclaved at 120 °C for 20 min (RAU-530D) and then placed in sterile polythene bags. When the plants reached three months old, bacterial inoculation was done using the leaf-clip method, a standardized protocol for rapid virulence assessment in *Ralstonia* spp. (Sharma and Singh, 2019; Kabyashree *et al.*, 2021; Iiyama *et al.*, 2021). For each inoculum (YG018, YG019, YG06, CG09, and CG011), a 24-h bacterial suspension was prepared by suspending in sterile distilled water and adjusting the concentration to 10⁸ CFU/mL using a 0.5 McFarland standard (CLSI, 2024). Sterile scissors were dipped into the bacterial suspension and used to snip the tips of 2–3 leaves per plant, directly exposing the cut vascular tissues. Control plants underwent the exact clipping method, but sterile distilled water was used instead. After inoculation, all plants were monitored daily for 21 days for the onset of wilting, leaf yellowing, and vascular discoloration. At 21 days post-inoculation (dpi), samples were taken from symptomatic stems and leaves to fulfill Koch's postulates. These symptomatic vascular tissues were cut and surface sterilized. The tissue was crushed and plated onto fresh TZC medium. The recovered colonies were compared to the original inocula to confirm identity.

RESULTS

Isolation and Phenotypic Characteristics

Mature symptomatic ginger rhizomes collected for isolation exhibited clear vascular discoloration and rotting (Plate 1). The 60 collected samples yielded bacterial growths that were categorized into five distinct morphotypes on TZC agar: (1) highly fluidal, irregular colonies with pink centers, (2) pure white mucoid colonies, (3) uniformly dark red colonies, (4) small pinkish creamy irregular colonies, and (5) flat translucent colonies.

Morphological and Biochemical Characterization

Among the 60 samples that yielded bacterial growths of various morphotypes, five isolates exhibited identical cultural characteristics on TZC agar, forming fluidal, irregular colonies with distinct pink centers typical of virulent *Ralstonia* (Plate 2A). Microscopic examination revealed all five isolates as Gram-negative bacilli, catalase- and oxidase-positive, and negative for starch hydrolysis (Table 1). These morphological and biochemical traits are consistent with several *Ralstonia* species.

Biovar test

Carbohydrate oxidation tests showed that YG06 oxidized maltose, lactose, cellobiose, mannitol, and sorbitol (but not dulcitol), a pattern matching Biovar V of *Ralstonia solanacearum* (Table 2). The other four isolates failed to oxidize any of the tested carbohydrates, corresponding to Biovar I.

Amplification and Sequencing of 16S rRNA gene.

PCR amplification of the 16S rRNA gene yielded a single band of ~1500 bp for the representative isolates (Plate 3). BLASTn analysis of the resulting sequences (Table 3) linked isolate CG011 to its closest match, a *Brucellaceae* member (with 91.73% similarity). Isolates CG09, YG018, and YG019 were linked to *Alcaligenes* (with 97.40% similarity). Because these three isolates shared identical sequences and phenotypic characteristics, only isolate CG09 was deposited in GenBank (accession number PZ682642) and used for phylogenetic reconstruction as a representative of the group. Isolate CG011 was deposited under the accession number PZ682641. Isolate YG06 was inconclusive due to sequence chimerism. None of the sequences matched *Ralstonia* spp. All top BLAST hits belonged to non-*Ralstonia* genera.

Evolutionary analysis by the Maximum Likelihood method

The phylogenetic tree (Figure 1) showed isolate CG09 formed a tight monophyletic group with the *Alcaligenes* species with 99% bootstrap support.

Likewise, isolate CG011 formed a well-supported clade with the *Brucellaceae* family (100% bootstrap support). The outgroup *Escherichia coli* separated clearly from the main clades, and neither of our study isolates clustered with the *Ralstonia solanacearum* species complex.

Pathogenicity Confirmation

The inoculation tests confirmed isolates YG018, YG019, YG06, CG09, and CG011 as virulent. All strains induced typical wilt symptoms, achieving 100% wilt incidence across the inoculated ginger plants at 21 days post-inoculation ($p = 0.42$ ns). Symptoms began appearing from day 4 post-inoculation and were

monitored for 21 days post-inoculation. It started with leaf yellowing and drooping before progressing to vascular discoloration (Plate 4B). Koch's postulates were successfully fulfilled through symptom development and re-isolation of bacteria from the affected vascular tissues of the stem and leaves. Upon culturing, these isolates exhibited morphological and biochemical characteristics identical to the original strains, confirming them as the true causal agents of the observed wilt. The control plants treated with sterile water remained healthy throughout the experimental period, showing no symptoms of the disease (Plate 4A).



Plate 1: Symptomatic ginger rhizomes showing vascular discoloration and soft rot

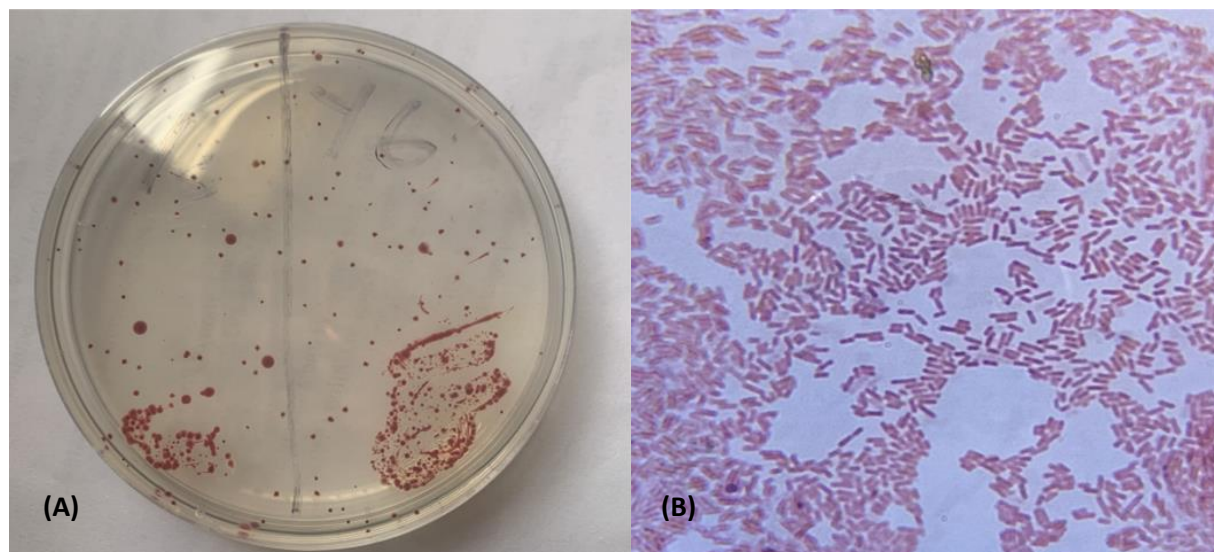


Plate 2: (A) Cultural appearance of isolate YG018 on TZC medium, representative of the selected morphotype 1
The plate shows the fluidal, pink-centred, irregular colonies typical of virulent *Ralstonia* spp. (B) Rod-shaped cells after microscopic examination of isolate YG018 (1000 $\times$ magnification)

Table 1: Morphological and biochemical profiles of bacterial isolates associated with ginger wilt

Isolate ID	Gram Reaction	Shape	Catalase	Oxidase	Starch Hydrolysis
YG018	-	Bacilli	+	+	-
YG019	-	Bacilli	+	+	-
YG06	-	Bacilli	+	+	-
CG011	-	Bacilli	+	+	-
CG09	-	Bacilli	+	+	-

Table 2: Carbohydrate oxidation pattern and assigned biovars of the bacterial isolates

Isolate ID	Maltose	Lactose	Cellobiose	Mannitol	Sorbitol	Dulcitol	Biovar
YG018	-	-	-	-	-	-	I
YG019	-	-	-	-	-	-	I
YG06	+	+	+	+	+	-	V
CG011	-	-	-	-	-	-	I
CG09	-	-	-	-	-	-	I

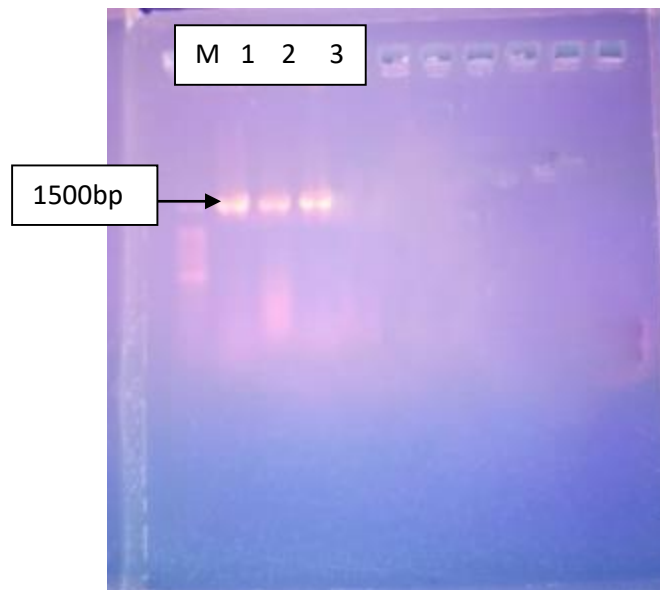


Plate 3: Agarose gel electrophoresis of 16S rRNA PCR products showing a ~1500 bp band for representative isolates

Lane M is the DNA ladder; Lanes 1, 2, and 3 represent isolates CG011, CG09, and YG06, respectively. (Note: PCR negative control yielded no amplification product)

Table 3: BLASTn Alignment, Percentage Similarity and GenBank Accession Numbers for 16S rRNA sequences of Bacterial isolates

Sample ID	Sequence bp	Closest match in GenBank	Percentage Similarity	Accession No. of the closest homolog	AccessionNo (for this study)
CG011	426	<i>Brucellaceae</i> family	91.73	NR_042600.1	PZ682641
CG09	539	<i>Alcaligenes</i> sp	97.40	NR_180910.1	PZ682642

Note: The 16S rRNA sequence for isolate YG06 was excluded due to chimerism and was not deposited. Isolates YG018 and YG019 were also sequenced and identified as *Alcaligenes* sp., but were not deposited; isolate CG09 serves as their representative accession.

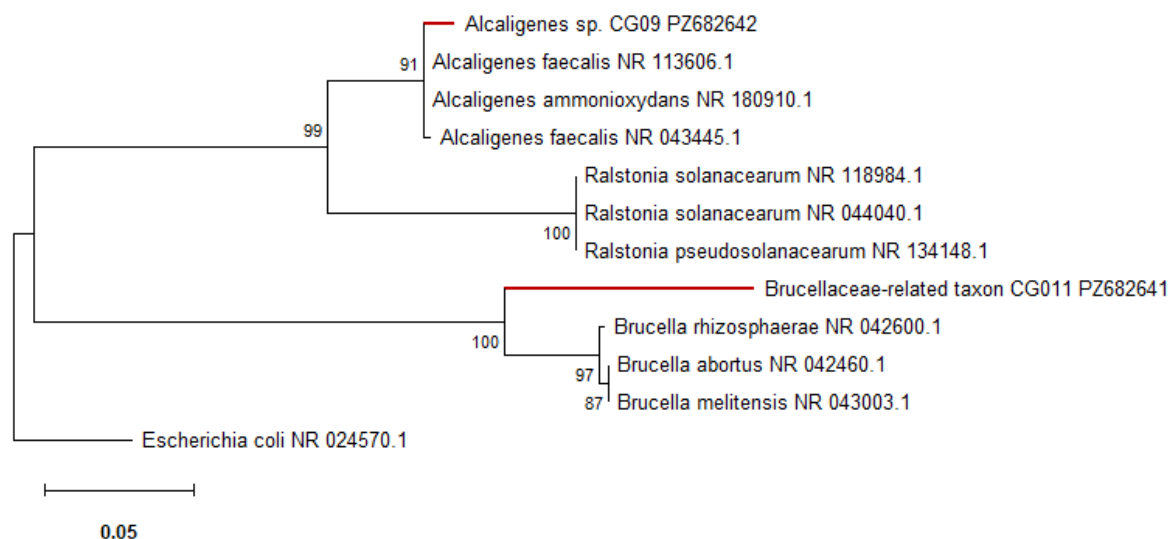


Figure 1: Maximum-likelihood phylogenetic tree based on partial 16S rRNA gene sequences, illustrating the evolutionary relationship between the bacterial isolates CG09 and CG11

Isolate CG09 was used as a representative strain for isolates YG018 and YG019. *Escherichia coli* was used as the outgroup to root the tree.



DISCUSSION

For many years, researchers worldwide have focused almost exclusively on *Ralstonia solanacearum* as the cause of bacterial wilt of ginger. Studies from major ginger-producing regions, including India, Indonesia, Myanmar, and the continental United States, consistently identify the *Ralstonia solanacearum*

species complex (RSSC) as the primary causal agent (Chiu *et al.*, 2024; Prameela & Suseela, 2020; Prasetyo *et al.*, 2020). The disease is now recognized as a global threat, spreading through soil and causing devastating impacts on agriculture, particularly in tropical and subtropical regions (Prameela & Suseela, 2020). This focus on *Ralstonia* has influenced how

laboratories diagnose the disease, how scientists breed resistant plants, and how farmers manage the problem worldwide. Standard diagnosis heavily relies on Triphenyl Tetrazolium Chloride (TZC) agar to distinguish *R. solanacearum* from other bacteria during isolation, which typically presents as fluidal colonies with characteristic pink centers (Kelman, 1954). In this study, when the five isolates (YG018, YG019, YG06, CG09, and CG011) were examined under the microscope, their biochemical properties tested, and their carbohydrate and sugar alcohol utilization analyzed, they appeared to be characteristic of *Ralstonia* spp. All isolates were Gram-negative bacilli, positive for catalase and oxidase, and produced fluidal, pink-centered irregular colonies on TZC medium, a morphology indicative of virulent species of *R. solanacearum*. The carbohydrate/sugar alcohol oxidation was indicated by a change in color from green to yellow, which assigned YG06 to biovar V and the remaining four isolates to biovar I, both profiles consistent with *R. solanacearum* under the conventional European and Mediterranean Plant Protection Organization (2004) classification scheme. However, through 16S rRNA sequencing, non-*Ralstonia* genera: *Alcaligenes* sp. (CG09) and a *Brucellaceae*-related taxon (CG011) were identified. The identification of these genera in this study indicates that reliance solely on colony morphology and biochemical tests risks severe diagnostic misclassification, which can obscure taxonomic diversity within the ginger pathosystems. Hence, molecular confirmation is required not only through 16S rRNA but also at the species level using Multi-locus sequence typing (MLST) or Whole-genome sequencing (WGS) for exact species resolution.

In the Phylogenetic analysis, isolates CG09 and CG011 clustered within the *Alcaligenes* and *Brucella* clades with high bootstrap values of 99% and 100%, respectively, placing the causal agents outside *Ralstonia* and confirming non-*Ralstonia* bacteria as responsible for ginger wilt in this study. This divergence stresses the insufficiency of phenotypic methods and hence suggests that local ginger wilt in Kano, Nigeria, differs from the traditional *Ralstonia* model. Recent reports from China have identified *Achromobacter xylosoxidans* (Hua *et al.*, 2024), *Enterobacter kobei*, *Enterobacter mori* (Wu *et al.*, 2025), *Pseudomonas asiatica* (Zhang *et al.*, 2025a), *Pseudomonas aeruginosa* (Zhang *et al.*, 2025b), and *Serratia marcescens* (Zhang *et al.*, 2025c) as causal agents of ginger wilt disease. In Africa, Mulusew *et al.* (2026) recently reported the first case of *Enterobacter*

mori and *Serratia marcescens* also as the causal agents of bacterial wilt in ginger in Ethiopia, showing that non-*Ralstonia* bacteria can independently drive wilt symptoms in ginger across geographically distinct regions. Together, these reports from China and Ethiopia in Africa, and this present study in Kano, expand this pattern in the African continent to Nigeria, with preliminary findings suggesting that non-*Ralstonia* bacteria are associated with ginger wilt, though further studies are needed to confirm causation.

In this study, the identification of the genus *Alcaligenes* as a pathogenic agent capable of inducing wilt symptoms expands the known range of pathogens to consider when diagnosing and managing ginger wilt disease. *Alcaligenes faecalis* has been documented as an opportunistic plant pathogen causing wilt in tomato and pepper (Tanaka *et al.*, 2025), and various *Alcaligenes* species have been found enriched in diseased ginger microbiomes (Wang *et al.*, 2024). Although previous studies mainly suggested the role of this genus as secondary or opportunistic components, the pathogenicity test here confirms isolate CG09 and its representatives (YG018, YG019) as pathogenic, with 100% wilt incidence ($p=0.42$) in inoculated ginger plants. The BLASTn results showed that this isolate (CG09) shares a 97.40% similarity with *Alcaligenes* sp. However, because this study uses 16S rRNA sequencing, it can reliably establish phylogenetic placement and genus-level identification but not species-level resolution (Sharma *et al.*, 2022). Confirming its species identity and specific virulence factors would require additional data such as Whole-genome sequencing or Multi-locus sequence typing (MLST).

Isolate CG011 showed a low 16S rRNA sequence similarity of 91.73% to its closest GenBank match within the family *Brucellaceae*. The genus *Brucella* is primarily known for mammalian pathogens such as *B. abortus* and *B. melitensis* (Whatmore *et al.*, 2014). Its association with plant or environmental relatives is rare within the family *Brucellaceae*. A 16S similarity score falls far below the 98.7% threshold that Stackebrandt and Ebers (2006) and Kim *et al.* (2014) established for species delineation and even stretches to genus-level boundaries. This divergence indicates that isolate CG011 represents a distinct phylogenetic lineage. It is premature to classify it as a novel species or an established plant pathogen based on a partial 16S fragment. Short-read 16S sequences can sometimes be influenced by amplification artifacts, low read lengths, or environmental co-extracted DNA. Although CG011 was virulent in the

pathogenicity assay, its exact taxonomic status must be treated as provisional until whole-genome Average Nucleotide Identity (ANI) and sequencing of housekeeping genes such as *rpoB*, *gyrB* is carried out.

The sequence for isolate YG06 was found to be chimeric, a sequencing artifact in which pieces of different DNA templates are incorrectly joined (Ashelford *et al.*, 2006). This chimerism made YG06 unsuitable for phylogenetic analysis. Although YG06 tested positive for pathogenicity and displayed a biovar V oxidation profile, its molecular identity remains inconclusive. This finding emphasizes the need for rigorous quality control, sequence verification, and the use of specific molecular markers like *rpoB* and *gyrB* in plant pathology to prevent misdiagnosis and obtain a reliable reference sequence for future diagnostic development.

These findings also imply that biovar classification is unreliable for identifying ginger wilt pathogens. The Carbohydrate oxidation test assigned isolates to biovars I and V. *Alcaligenes* and *Brucellaceae*-related isolates failed to oxidize any of the carbohydrates, while YG06, provisionally identified, oxidized enough to be assigned to biovar V. Under the EPPO (2004) diagnostic scheme, the biovar V-like carbohydrate oxidation profile of YG06 and the biovar I-like profiles of the other isolates would have been mistaken for clear evidence of *R. solanacearum* infection. This would have led farmers and plant health authorities to apply *Ralstonia*-specific preventive measures such as resistant cultivars strictly bred against RSSC strains. Previous research has shown that phenotypic classification struggles even within the *Ralstonia* species complex (Prior *et al.*, 2016). Prameela and Suseela Bhai (2020) reported that morphological and biochemical tests can miss important taxonomic differences, while sequencing-based approaches provide more reliable resolution. Venbrux *et al.* (2023) demonstrated that traditional diagnostic methods are insufficient for accurate pathogen identification in complex pathosystems. Misidentification in disease diagnosis has devastating effects. Breeding programs designed to select for *Ralstonia* resistance would fail if the true pathogens are non-*Ralstonia* or other uncharacterized species. Management practices, chemical treatments, and biological control measures developed for *Ralstonia* would be ineffective against these pathogens. During the 2022–2023 Kaduna outbreaks, tens of thousands of smallholder farmers lost their livelihoods, with losses exceeding ₦15 billion and yield reductions of

over 90% in some communities (AFEX, 2024; Mojeed, 2024).

In the Nigerian agricultural system, molecular DNA testing is often inaccessible, limiting accurate pathogen identification. This research supports a practical two-step diagnostic approach: first, using traditional biochemical tests for initial screening and sorting of samples, then confirming results with targeted molecular sequencing for critical cases. Developing simple, affordable DNA-based tools, such as PCR assays, for non-*Ralstonia* pathogens would improve disease detection and management. As Umesha and Raghava (2021) and Venbrux *et al.* (2023) emphasize, molecular confirmation is no longer a luxury but a necessity for accurate pathogen diagnosis in modern agriculture.

CONCLUSION

Ralstonia solanacearum has long been blamed for the bacterial wilt of ginger. This study provides a preliminary evidence that in Kano, Nigeria, non-*Ralstonia* bacteria, specifically *Alcaligenes* spp. (CG09, YG018, YG019) and a *Brucellaceae*-related isolate (CG011) are causal agents of this disease. Colony morphology and biochemical tests misclassified these strains as *Ralstonia solanacearum*, but 16S *rRNA* gene sequencing and pathogenicity assay confirmed their virulence as wilt-causing pathogens. This study shows that phenotypic tests are no longer reliable. Hence, ginger production in Nigeria requires integration of robust molecular diagnostics as a routine component of local disease surveillance.

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