Diagnosis and characterization of the bacterial flora of mango (*Mangifera indica* L.) in western Senegal.

Abstract

Fungal and bacterial diseases are one of the major constraints to mango productivity and fruit quality. In Senegal, data on the diversity of pathogenic bacteria associated with mango remain limited. To address this gap, field surveys were conducted in orchards in the Niayes production area, during where symptomatic leaves and twigs were collected. The isolates were subjected to morphological and biochemical characterization, followed by molecular identification through PCR and 16S rDNA sequence analysis.Seven bacterial genera were detected: *Stenotrophomonas, Pseudomonas, Bacillus, Ochrobactrum, Exiguobacterium, Burkholderia* and *Aeromonas*. Several of these genera include known plant pathogens. The most frequent were *Stenotrophomonas* and *Pseudomonas*, representing 35.29% and 23.53% of the isolates, respectively. This study provides the first baseline dataset on the bacterial flora associated with mango in western Senegal, providing essential information for understanding and managing bacterial diseases.

Keywords: Mango, phytopathogenic bacteria, biochemical and molecular characterization, Senegal.

Introduction

The mango (*Mangifera indica* L.), which has been improved through top-grafting and the introduction of American cultivars, makes a significant contribution to local food security and employment in Senegal. It is currently the leading export product in the fruit and vegetable sector, accounting for 47% of national fruit production (IPAR, 2023).

Production is concentrated in the regions of Dakar, Thiès, Saint-Louis, Fatick, Kolda, Ziguinchor and Sédhiou (Diedhiou et al., 2014), with an estimated annual yield of 125,000–150,000 tonnes over a six-month harvest season, the longest in West Africa. However, a decline of 7.03% was recorded in 2020 due to the impact of the pandemic (IPAR, 2023). Prior to this crisis, exports had grown by an average of 20% each year for 16 consecutive seasons, with exports reaching 16,285 tons in 2022 (ASEPEX, 2016; IPAR, 2023). Although exports, mainly from the Niayes and Casamance zones (4%), represent only 10% of national production, Senegal has become the second-largest exporter of West African mangoes after Côte d'Ivoire.

However, mango export potential is limited by the perishable nature of the fruit and quarantine organisms such as fruit flies, fungi and bacteria. Fruit fly (*Bactrocera invadens*) infestations result in losses of 30–50% in the Niayes area and up to 80% in Casamance (Ndiaye et al., 2015). Anthracnose remains responsible for nearly 90% of post-harvest damage in southern Senegal (Diedhiou et al., 2014). In contrast, the contribution of bacterial diseases remains poorly documented in Senegal, with no published studies characterizing their diversity or impact.

Mango bacterial black spot (MBBS) is the most widely recognized bacterial disease of mango. It is associated with several pathogens, including *Bacillus mangiferae* (Doidge, 1915); *Pseudomonas mangiferae-indicae* (Patel et al., 1948; Daniel et al., 1975); *Xanthomonas citri pv. mangiferaeindicae* (Sanahuja et al., 2016; Zombré et al., 2016) and the most widespread pathogen worldwide, *Xanthomonas campestris pv. mangiferaeindicae* (Pruvost et al., 2005). Under favorable climatic conditions, MBBS causes premature leaf and fruit abscission fruit cracking resultingin fruit losses of up to 85% (Prakash & Misra, 1992).

However, MBBS only emerged in West Africa, in the early 2010s. *X. citri pv. mangiferaeindicae* has been reported in several neighboring countries, in Burkina Faso, Côte d'Ivoire, Mali, Ghana, Benin, and Togo (Pruvost et al., 2011, 2012, 2014; Zombré et al., 2015, 2016; Honger et al., 2021). There is therefore concern that the pathogen may have entered the country, especially since bacterial disease symptoms have been observed in local orchards. Regional pest management guides (PIP-COLEACP, 2022) identify MBBS as an emerging risk, underscoring the need for enhanced surveillance. Therefore, effective diagnostic of pathogenic bacteria in mango orchards is essential to mitigate damage and reinforce national surveillance and regional coordination.

This study forms part of ongoing efforts to strengthenmango disease management and specially aims to i) inventory bacterial pathogens associated with mango in the Niayes production area andii) characterize bacteria isolates using morphological, biochemical, and molecular tools, including PCR and 16S rDNA sequencing.

Materials and methods

Sampling:

Field surveys were conducted in sixteen orchards located in Séssène, Notto Diobass, and Taïba Ndiaye in the Thiès region (Figure 1). Symptomatic vegetative organs (leaves and twigs) were collected from ten randomly selected mango trees in each orchard. The samples were then transported to the Laboratory of Phytochemistry and Plant Protection (LPPV) of the Department of Plant Biology at Cheikh Anta Diop University of Dakar (UCAD).

Isolation

Leaf and twig samples were surface-sterilizedwith 70% ethanol and then rinsed three times with sterile distilled water. The explantswere macerated in a 0.85% NaCl solution and incubated for 2 hours. Serial dilutions were prepared up to 10^{-4}, and 100µl of each dilution (three replicates) were plated on nutrient agar and incubated at 37°C for 24-28 hrs. Colonies with distinct morphologies were subcultured until pure isolates were obtained.

The isolates obtained in the various orchards are coded by assigning a number preceded by the letter "S". Gram staining was performed to differentiate between Gram-positive (G+) and Gram-negative (G−) bacteria.

Morphological, biochemical, and molecular characterization

The isolates were characterized based on morphological traits (colony shape, color, margin, surface, elevation, etc.) and subjected to biochemical testsfollowing standard references (Tine, 1996; Borkar, 2017). Commercial identification kits (API 20^{E} and API 20^{NE}, BioMérieux) were also used, and the results were interpreted using APIweb software (BioMérieux, 2021).

Isolates DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega Corporation, 2016). Universal primers targeting the 16S rRNA gene (Weisburg et al., 1991) were used for PCR amplification:

fD1: 5’- AGAGTTTGATCCTGGCTCAG - 3’

rD1: 5’AAGCTTAAGGAGGTGATCCAGCC-3’

The composition of the PCR reaction mixture with the volumes of each component is presented in Table1. For each sample, 3 µl of diluted DNA (1/50) was added to the mixture for a final reaction volume of 25 µl per tube. Amplification was performed in a thermocycler under the following program: an initial denaturation at 96°C for 3 min, 30 cycles consisting of denaturation at 95°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 40 s; followed by a final elongation step at 72°C for 3 min.

The amplicons obtained were visualized on agarose gels and then sent for sequencing to Inqaba Biotec (Pretoria, South Africa). The sequences were edited using BioEdit and compared to NCBI (National Centre for Biotechnology Information) reference databases using BLASTn (Basic Local Alignment Search Tool).

Results

The isolates obtained from the surveyed orchards are summarized in Table 2. A total of nineteen isolates were obtained, of which fourteen were from Séssène and five were from Taïba Ndiaye, whileno isolates were recovered from Notto Diobass. Older orchards recorded more isolates than younger ones, withVS2a (8 isolates) and VT1A (3 isolates) recorded the highest number of isolates. Most of the isolates originated from leaves (15), and four (4) were obtained from twigs.

Morphological and Biochemical Identification

The bacterial isolates are bacilli and are immobile, with only isolate S2 forming chains. Gram-negative (G−) bacteria represented 80% of the isolates.
The results of carbohydrate and energy metabolism tests (Table 5) revealed similarities among several isolates regarding both morphology and biochemical traits. Based on these characteristics,the 19 bacterial isolates were classified into 9 groups (G): G1 (S1, S11, S12), G2 (S2), G3 (S3, S7, $S8_1$), G4 (S4, S9), G5 (S5, S10), G6 ($S8_2$), G7 ($S14_1$), G8 ($S14_2$), and G9 (S13, S15, S16, S17).

Among the nine groups, two isolates (S2 and S4) were subjected to identification using the Api 20^E,while seven isolates (S5, S81, S10, S11, S141 and S17) were identified using the Api 20^{NE}. The codes recorded from the Api results were interpreted using the Apiweb identification software. The identified bacteria are presented in Table 3**,** witha percentage of similarity of all species exceeding 85%.Identifications included *Lactobacillus delbrueckii*, *Klebsiella pneumoniae*with Api 20^E, *Ochrobactrum* spp., *Burkholderia* spp., *Stenotrophomonas* spp., *Aeromonas* spp., and *Pseudomonas* spp. with Api 20^{NE}.

Molecular Identification:

After amplification, agarose gel electrophoresis showed bands ranging in size from 1,400 to 1,600 base pairs (Figure 2). No rDNA bands were detected in isolates $S8_2$ and S14.

The sequences obtained from the PCR products were submitted to the NCBI reference database identified for BLASTn analysis. The results revealed various bacterial genera commonly associated with plants and soil, including known plant pathogens, with similaritiesranging from 88% to 98% (Table 4). The genera detected included *Stenotrophomonas* (S1, S3, $S8_1$), S11, S12, and S13), *Bacillus* (S2), *Ochrobactrum* (S5, S6), *Pseudomonas* (S15, S7, $S14_1$, S16, S17), and *Exiguobacterium* ($S14_2$).

Among the 14 isolates obtained from Séssène samples, species identified included*Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Pseudomonas* sp, and *Exiguobacterium* sp. In contrast, the five strains isolated from Taïba Ndiaye were identified as *S. maltophilia*, *Ochrobactrum anthropi*, and *Burkholderia cepacia*. The most prevalent genera in the orchards were *Stenotrophomonas* and *Pseudomonas* with frequencies of 35.29% and 23.53%, respectively.

The molecular analysis confirmed the presence of *S. maltophilia, P. aeruginosa*, and *O. anthropi*, *B. cepacia*,identified with the Api 20^{NE}. It also revealed the presence of additional genera: *Bacillus, Burkholderia, Pseudomonas* and *Exiguobacterium*.

Discussion

Mango (*Mangifera indica* L.), cultivated worldwide, ais threatened by several destructive fungal and bacterial diseases that reduce fruit production and quality. In most orchards surveyed in the Niayes area, symptoms similar to bacterial black spot described in previous studies have been observed(Gagnevin and Pruvost, 2001, Ah-you et al., 2007; PIP-COLEACP, 2013).

However, the observed symptoms are nonspecific and may hinder definitive identification of the causal agent. Several bacteria species including *Bacillus mangiferae* (Doidge, 1915), *Pseudomonas mangifera indicae* (Patel et al., 1948; Daniel et al., 1975), *Xanthomonas citri pv. mangiferaeindicae* (Sanahuja et al., 2016; Zombré et al., 2016) and *Xanthomonas campestris pv. mangiferaeindicae* (Pruvost et al., 2005) have been reported to inducesimilar symptoms. Consequently, universal primers were used for 16S rDNA amplification instead of species-specific primers.

Biochemical and molecular analysis identified isolates belongingto the genera *Pseudomonas*, *Stenotrophomonas*, *Bacillus*, *Ochrobactrum*, *Exiguobacterium*, and*Burkholderia*. These findings are consistent with those of Khan et al. (2014), who additionally identified *Erwinia*, *Pantoea*, *Acinetobacter* and *Enterobacter*, asplant pathogenic bacteria.

The molecular analysiscorroborated the Api 20^{NE} identification of several species and revealed the identification of new genera. However, the limitation of 16S rDNA analysis were evident, as several isolates exhibited similarity with multiple strains within the same genus. This is the case for isolate S2, which exhibited 97% similar to *Bacilluscereus*, *B. thuringiensis* and *B. anthracis*; while S15 showed 91% similarity to multiple *Pseudomonas* strains(*Pseudomonas* sp, *P. trivialis*, *P. poae*, *P. fluorescens* and *P. simiae*). In addition, sequences annotated as "uncultured bacterium" reflects the diversity of bacterial taxa associated with mango, many of which remain insufficiently characterized and require further isolation and pathogenicity testing to determine their ability to induce symptoms.

Among the identified genera, *Pseudomonas* and *Burkholderia*are established plant pathogens, suggesting their involvement in the disease symptoms observed in the surveyed orchards.The genus *Pseudomonas* comprises more than 140 species that can be found in water, moist soil, humans, animals and plants, with several species reportedas pathogenic. *Pseudomonas syringae* has been identified as the causal agent of apical necrosis in mango (Cazorla et al., 1998; Golzar and Cother, 2008). *Pseudomonas aeruginosa*, although primarily opportunistic pathogen, can induce soft rot symptoms in crops such as tomato, lettuce, onion and tobacco (Kominos et al., 1972; Abd-Alla et al., 2011). Itis also known to be present on fruits and green plants, where it can persist without causing symptoms (Cho et al., 1975). The presence of *Pseudomonas* sp,*P. aeruginosa* and *Stenotrophomonas maltophilia*and their potential association with symptoms developmentis therefore likely, noting the taxonomic reclassification*S. maltophilia* from *Pseudomonas* to*Xanthomonas* and finally *Stenotrophomonas* (Palleroni and Bradbury, 1993). The isolates identified as *P. aeruginosa*exhibited biochemical characteristics (Table 5) consistent with published description(Richard & Kiredjian, 1995), except for immobility of our isolates.

Species of *Burkholderia* are ecologically distinct from *Pseudomonas* encompassing non-pathogenic (*B. tuberum*), phytopathogen species (*B. cenocepacia, B. gladioli pv. alliicola, B. cepacia*), and pathogens capable of infecting both animals and plants (Compant et al., 2008; Conn et al., 2012). The identification of*B. cepacia*in this study is noteworthy, as this strain has been causing soft rot in onions and infects rice and bananas (Janette et al., 2008), demonstrating its pathogenic nature and justifying its isolation and possible involvement in the development of symptoms observed.The isolate characterized here exhibited traits consistent with previous reports (Richard and Kiredjian, 1995; Seconds et al., 1988), including Gram–,catalase and oxidasepositive and the ability to utilize glucoseor mannitol as sole carbon source.

The isolation of *P. aeruginosa* and *B. cepacia* from mango samples can be explained by their ability to infect plants and induce necrotic or soft rot symptoms. Virulence tests havedemonstrated that *B. cepacia* strain cause necrosis and maceration of 34 to 100% of the onion bulb tissue (Janette et al., 2008), while *P. aeruginosa* induced soft rot sin *Arabidopsis thaliana* and *Lactuca sativa* (Rahme et al., 2000), and mortality in *Arabidopsis* and *Ocimum basilicum* withing 7 days after inoculation (Walker et al., 2004). Their pathogenicity in mango requires further investigation through host specificity and pathogenicity tests.

Beyond these genera, mango microbiome also includes *Acetobacter senegalensis*, a thermotolerant acetic acid bacterium previously isolated from mango fruit (Ndoye et al., 2007). However, *Xanthomonas citri pv. mangiferaeindicae*, the causal agent of MBBS and widely reported in neighboring countries (Pruvost et al., 2011, 2012, 2014; Zombré et al., 2015, 2016; Honger et al., 2021) has not been isolated from our samples. Nevertheless, MBBS has been identified as an emerging risk in West Africa, underscores the need for targeted surveillance (PIP-COLEACP, 2022). Therefore, future studies should employ pathovar-specific primers targeting*X. citri pv. mangiferaeindicae*and perform PCR directly on symptomatic plant material, coupled with or bypassing the isolation on solid media,to confirm or exclude its presence in Senegalese orchards.

Conclusion

This study provides the first baseline characterization of bacteria flora associated with mango trees in Niayes production zone. Seven bacteria genera were identified through morphological, biochemical and molecular analyses, with *Pseudomonas* and *Stenotrophomonas*being the most prevalent.The absence of *Xanthomonas citri pv. Mangiferaeindicae* in the sampleshighlights the need for continued surveillance and expended surveys across other mango production area.

References

Abd-Alla, M. H., Bashandy, S. R., Ratering, S., & Shnell, S. (2011). First report of soft rot of onion bulbs in storage caused by *Pseudomonas aeruginosa* in Egypt. *Journal of Plant Interactions, 6*(4), 229–238. https://doi.org/10.1080/17429145.2010.535618

Agence Sénégalaise de Promotion des Exportations (ASEPEX). (2016**).***Guide export - Mangue du Sénégal*. Dakar, Sénégal : ASEPEX / Projet d'Amélioration de la Compétitivité de la Mangue Sénégalaise (PACMS). Retrieved from https://www.senegal-export.com/IMG/pdf/guide20sur20d0d4.pdf

Ah-You, N., Gagnevin, L., Chiroleu, F., Jouen, F., Rodrigues Neto, J., & Pruvost, O. (2007). Pathological variations within *Xanthomonas campestris* pv. *mangiferaeindicae* support its separation into three distinct pathovars that can be distinguished by AFLP. *Journal of Plant Pathology, 89*(1), 43–45. https://doi.org/10.1094/PHYTO-97-12-1568

BioMérieux. (2021). API® Reference Guide. Marcy-l'Étoile, France : bioMérieux SA. https://www.biomerieux-industry.com/sites/default/files/2021-08/API%20Reference%20Guide.pdf

Borkar, S. G. (2017). *Laboratory techniques in plant bacteriology*. CRC Press. https://doi.org/10.1201/9781315206882

Cazorla, F. M., Tores, J. A., Olalla, L., Pérez-García, A., Farré, J. M., & de Vicente, A. (1998). Bacterial apical necrosis of mango in southern Spain: A disease caused by *Pseudomonas syringae* pv. *syringae*. *Phytopathology, 88*(7), 614–620. https://doi.org/10.1094/PHYTO.1998.88.7.614

Cho, J. J., Schroth, M. N., Kominos, S. D., & Green, S. K. (1975). Ornamental plants as carriers of *Pseudomonas aeruginosa*. *Phytopathology, 65*(4), 425–431. https://doi.org/10.1094/Phyto-65-425

Compant, S., Nowak, J., Coenye, T., Clément, C., & Barka, E. A. (2008). Diversity and occurrence of *Burkholderia* spp. in the natural environment. *FEMS Microbiology Reviews, 32*(4), 607–626. https://doi.org/10.1111/j.1574-6976.2008.00113.x

Daniel, J. F., Gardan, L., Luisetti, J., & Pruner, J. P. (1975). Identification de l'agent responsable de la maladie des taches noires de la mangue (*Mangifera indicae*). *Fruits, 30*(10), 625–630https://agritrop.cirad.fr/414615/

Diédhiou, P. M., Mbaye, N., Faye, R., & Samb, P. I. (2014). Field tests of fungicides against post-harvest rot of mangoes in Senegal. *International Journal of Science, Environment and Technology, 3*(2), 597–606. https://doi.org/10.4236/ajps.2014.515236

Doidge, E. M. (1915). A bacterial disease of the mango *Bacillus mangiferae* n. sp. *Annals of Biology, 2*, 1–45. https://doi.org/10.1111/j.1744-7348.1915.tb05424.x

Gagnevin, L., & Pruvost, O. (2001). Epidemiology and control of mango bacterial black spot. *Plant Disease*, *85*(9), 928–935. https://doi.org/10.1094/PDIS.2001.85.9.928

Golzar, H., & Cother, E. J. (2008). First report of bacterial necrosis of mango caused by *Pseudomonas syringae* pv. *syringae* in Australia. *Australasian Plant Disease Notes, 3*(1), 107–109. https://doi.org/10.1007/BF03211259

Honger, J. O., Osabutey, S., Nyaku, S. T., & Lambon, J. B. (2021). Molecular characterization, pathogenicity and copper sensitivity of *Xanthomonas citri* pv. *mangiferaeindicae*, the causal agent of mango bacterial black spot disease in Ghana. *Archives of Phytopathology and Plant Protection*, *54*(19–20), 1703–1721. https://doi.org/10.1080/03235408.2021.1936995

Initiative Prospective Agricole et Rurale (IPAR). (2023). Étude sur le profilage d'opportunités de la chaîne de valeur mangue en Casamance. Feed the Future Senegal Dooleel Mbay / USAID. Dakar, Sénégal.https://www.ipar.sn/

Jacobs, J. L., Fasi, A. C., Ramette, A., Smith, J. J., Hammerschmidt, R., & Sundin, G. W. (2008). Identification and onion pathogenicity of *Burkholderia cepacia* complex isolates from the onion rhizosphere and onion field soil. *Applied and Environmental Microbiology, 74*(10), 3121–3129. https://doi.org/10.1128/AEM.01941-07

Khan, A. I., Khan, A., Asif, H., Jiskani, M. M., Mühlbach, H.-P., & Azim, M. K. (2014). Isolation and 16S rDNA sequence analysis of bacteria from dieback-affected mango orchards in southern Pakistan. *Pakistan Journal of Botany*, *46*(4), 1431–1435.

Conn, K. E., Lutton, J. S., Rosenberger, S. A., Black, L., Gabor, B., Kao, J., & Lutton, J. (2012). *Onion disease guide: A practical guide for seedsmen, growers and agricultural advisors.* Seminis® Plant Health, 72 pp.

Kominos, S. D., Copeland, C., Grosiak, B., & Postic, B. (1972). Introduction of *Pseudomonas aeruginosa* into a hospital via vegetables. *Applied Microbiology, 24*(4), 567–570. https://doi.org/10.1128/am.24.4.567-570.1972

Ndiaye, O., Ndiaye, S., Djiba, S., Ba, C. T., Vaughan, L., Rey, J.-Y., & Vayssières, J.-F. (2015). Preliminary surveys after release of the fruit fly parasitoid *Fopius arisanus* Sonan (Hymenoptera: Braconidae) in mango production systems in Casamance (Senegal). *Fruits, 70*(2), 91–99. https://doi.org/10.1051/fruits/2015001

Ndoye, B., Cleenwerck, I., Engelbeen, K., Dubois-Dauphin, R., Guiro, A. T., Trappen, S. V., Willems, A., & Thonart, P. (2007). *Acetobacter senegalensis* sp. nov., a thermotolerant acetic acid bacterium isolated in Senegal (sub-Saharan Africa) from mango fruit (*Mangifera indica* L.). *International Journal of Systematic and Evolutionary Microbiology, 57*(7), 1576–1581.https://doi.org/10.1099/ijs.0.64678-0

Palleroni, N. J., & Bradbury, J. F. (1993). *Stenotrophomonas*, a new bacterial genus for *Xanthomonas maltophilia* (Hugh 1980) Swings et al. 1983. *International Journal of Systematic Bacteriology, 43*(3), 606–609. https://doi.org/10.1099/00207713-43-3-606

Patel, M. K., Kulkarni, Y. S., & Moniz, L. (1948). *Pseudomonas mangiferae-indicae,* pathogenic on mango. *Indian Phytopathology, 1*(2), 147–152.

PIP-COLEACP. (2013).*Bactériose du manguier provoquée par Xanthomonas citri pv. mangiferaeindicae.* Programme PIP, COLEACP. Bruxelles, Belgique. 13 p. https://www.coleacp.org/pip

PIP-COLEACP. (2022). Guide de production COLEACP pour la mangue issue de la production biologique (*Mangifera indica*): Guide de bonnes pratiques phytosanitaires. https://www.coleacp.org

Prakash, O., & Misra, A. K. (1992). Important diseases of mango and their effect on production. Biol. Mem, 18, 39–55.

Promega Corporation. (2016). Wizard® Genomic DNA Purification Kit technical manual (Technical Manual No. TM050). Madison, WI: Promega Corporation. https://www.promega.com/resources/protocols/technical-manuals/0/wizard-genomic-dna-purification-kit-protocol/

Pruvost, O., Roumagnac, P., Gaube, C., Chiroleu, F., & Gagnevin, L. (2005). New media for the semiselective isolation and enumeration of *Xanthomonas campestris pv. mangiferaeindicae*, the causal agent of mango bacterial black spot. Journal of Applied Microbiology, 99(4), 803–815. https://doi.org/10.1111/j.1365-2672.2005.02681.x

Pruvost, O., Boyer, C., Grygiel, P., Boyer, K., Vernière, C., Gagnevin, L., Soro, S., N'Guessan, C., & Koné, D. (2014). First report of *Xanthomonas citri pv. mangiferaeindicae* causing mango bacterial canker on *Mangifera indica* in Ivory Coast. Plant Disease, 98(12), 1740. https://doi.org/10.1094/PDIS-07-14-0669-PDN

Pruvost, O., Boyer, C., Vital, K., Vernière, C., Gagnevin, L., & Somda, I. (2011). First report in Burkina Faso of *Xanthomonas citri pv. mangiferaeindicae* causing bacterial canker on *Mangifera indica*. Plant Disease, 95(10), 1312. https://doi.org/10.1094/PDIS-04-11-0324

Pruvost, O., Boyer, C., Vital, K., Vernière, C., Gagnevin, L., & Traoré, Y. N. (2012). First report in Mali of *Xanthomonas citri pv. mangiferaeindicae* causing mango bacterial canker on *Mangifera indica*. Plant Disease, 96(4), 581. https://doi.org/10.1094/PDIS-01-12-0001-PDN

Richard, C., & Kiredjian, M. (1995). Méthode de laboratoire pour l'identification des bacilles à Gram négatif non aérobies stricts : Pseudomonas, Alcaligenes, Flavobacterium, Acinetobacter, Brucella, Bordetella (2e éd., pp. 22–26). Institut Pasteur.

Rahme, L. G., Ausubel, F. M., Cao, H., Drenkard, E., Goumnerov, B. C., Lau, G. W., Mahajan-Miklos, S., Plotnikova, J., Tan, M. W., Tsongalis, J., Walendziewicz, C. L., & Tompkins, R. G. (2000). Plants and animals share functionally common bacterial virulence factors. Proceedings of the National Academy of Sciences of the United States of America, 97(16), 8815–8821. https://doi.org/10.1073/pnas.97.16.8815

Sanahuja, G., Ploetz, R. C., Lopez, P., Konkol, J. L., & Palmateer, A. J. (2016). Bacterial canker of mango, *Mangifera indica*, caused by *Xanthomonas citri pv. mangiferaeindicae*, confirmed for the first time in the Americas. Plant Disease, 100(12), 2522.https://doi.org/10.1094/PDIS-03-16-0412-PDN

Seconds, C., Marty, N., Dournes, J. L., & Chabanon, G. (1998). *Burkholderia cepacia* dans tous ses états. Médecine et Maladies Infectieuses, 28(Suppl.), 72–78.

Tine, E. (1996). Travaux pratiques de microbiologie générale : Guide des tests biochimiques d'identification des bactéries. Analyses Biologiques – Industries Alimentaires, 1ère année. No

Walker, T. S., Bais, H. P., Déziel, E., Schweizer, H. P., Rahme, L. G., Fall, R., & Vivanco, J. M. (2004). *Pseudomonas aeruginosa*-plant root interactions: Pathogenicity, biofilm formation, and root exudation. Plant Physiology, 134(1), 320–331.https://doi.org/10.1104/pp.103.027888

Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology, 173(2), 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991

Zombré, C., Sankara, P., Ouédraogo, S. L., Wonni, I., Pruvost, O., Boyer, C., Vernière, C., Adandonon, A., Vayssières, J. F., & Ahohuendo, B. C. (2015). First report of *Xanthomonas citri pv. mangiferaeindicae* causing mango bacterial canker on *Mangifera indica* L. in Benin. Plant Disease, 99(12), 1854. https://doi.org/10.1094/PDIS-04-15-0392-PDN

Zombré, C., Wonni, I., Ouédraogo, S. L., Kpemoua, K. E., Assignon, K., Sankara, P., Vernière, C., Boyer, C., Boyer, K., Javegny, S., & Pruvost, O. (2016). First report of *Xanthomonas citri pv. mangiferaeindicae* causing mango bacterial canker on *Mangifera indica* in Togo. Plant Disease, 101(3), 503. https://doi.org/10.1094/PDIS-09-16-1259-PDN

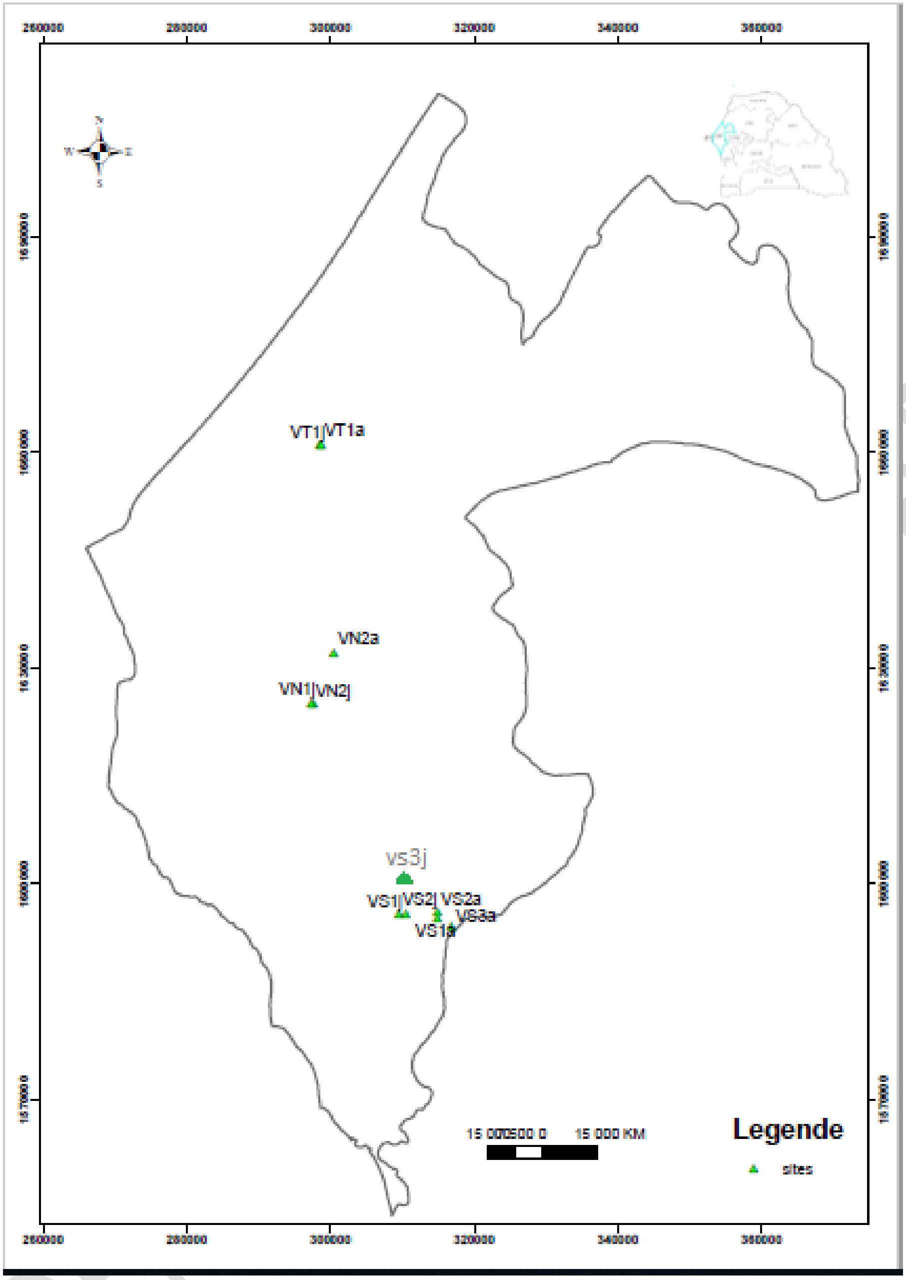

Figure 1: Geographic location of the surveyed mango orchards within the Niayes production area of Senegal.

Tableau 1: Composition of the PCR reaction mixture

Component	Volume/Sample (µl)
Water	14.05
PCR Buffer	5x
dNTPs (10 mM)	0.2
Primer FD1 (10 µM)	1.25
Primer RD1 (10 µM)	1.25
GoTag Flexi DNA polymerase	0.25

Tableau 2: Isolates obtained from Mango orchards

Site	Orchard ID	**Isolate ID**	**No of isolates**
Séssène	VS2aF3	S1	14
	VS2j F3	S2	
	VS3a F10 (1)	S3	
	VS2aF10 (2)	S4	
	VS1jF22	S7	
	VS3a F10 (2)	S9	
	VS2a F10 (1)	S11	
	VS3a F9	S12	
	VS2a F11	S13	
	VS2aF10 (3)	$S14_1$	
	VS2aF10 (3)	$S14_2$	
	VS2jF8	S15	
	VS2aR2	S16	
	VS2aF4	S17	
Taïba Ndiaye	VT1jR1	S5	5
	VT1aR6	S6	
	VT1aR3	$S8_1$	
	VT1aR3	$S8_2$	
	VTaF	S10	

V = orchard; S = Séssène; T = Taïba Ndiaye; a = old orchard; j = young orchard; F = leaf; R = twig.

Tableau 3:Bacteria species Identified from mango isolates in Niayes area Using API systems

Api	Isolate ID	Identified species	Identification code	Probability (%)
20^{E}	S2	*Lactobacillus delbrueckii*	24h : 23261373	//
	S4	*Klebsiella pneumoniae*	24h : 52357733	97,5
20^{NE}	S5	*Ochrobactrum anthropi*	24h : 1641344 48h : 1643755	98,8
	$S8_1$	*Burkholderia cepacia*	24h : 1473775 48h : 1473355	95,6
	S11	*Stenotrophomonas maltophilia*	24h : 1472345 48h : 1472355	99,5
	$S14_1$	*Aeromonas salmonicida*	24h :5450004 48h :5454204	85,6
	$S14_2$	*Burkholderia cepacia*	24h : 1557577 48h : 1557577	95,6
	S17	*Pseudomonas aeruginosa*	24h : 5554575 48h : 5757775	97,5

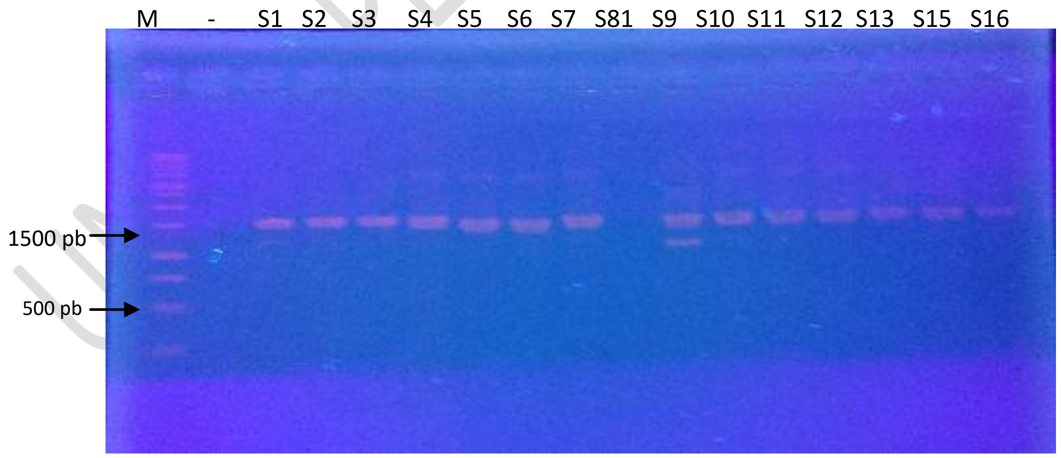

Figure 2: Visualization of 16S rDNA from bacterial isolates on agarose gel (M: marker; –: negative control; S: strain)

Tableau 4: BLASTn Sequence identification bacteria isolatedfrom mango orchard in Niayes area

Isolate ID	Reference species identified (GenBank accession)	Similarity (%)
S1	*Stenotrophomonas maltophilia* CP022053.2	92
S3		97
$S8_1$		92
S11		94
S12		91
S13		88
S2	*Bacillus cereus* MF767513.1	97
S5	*Ochrobactrum anthropi* KY570296.1	96
S6		95
S15	*Pseudomonas sp* KT890304.1	91
S7	*Pseudomonas aeruginosa* JQ659891.1, FM997073.1, EF062514.1, FJ859913.1	93
$S14_1$		96
S16		93
S17		91
$S14_2$	*Exiguobacterium sp* MG819389	98

Tests	*Stenotrophomonas maltophilia*	*Bacillus cereus*	*Ochrobactrum anthropi*	*Pseudomonas aeruginosa*	*Exiguobacterium sp*	*Ochrobactrum sp*	*Burkholderia cepacia*	*Aeromonas salmonicida*
Shape	bacillus	bacillus	bacillus	bacillus	bacillus	bacillus	bacillus	bacillus
Gram reaction	-	-	+	-	-	-	-	-
Oxidase	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+
Motility	-	-	-	-	-	-	-	-
Nitrate reductase	+/-	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Oxidative	+	-	+	+	+	-	-	-
Fermentative	-	+	-	-	-	+	+	+
Lactose utilization	-	-	-	-	-	+	-	+
Glucose utilization	-	+	-	-	-	+	+	+
ADH	-	//	-	+	+	-	-	-
PNPG	+	//	-	-	-	-	+	-
RM	-	+	-	-	-	-	-	+
VP	-	-	-	-	-	+	-	-
Citrate utilization	-	-	-	+	-	+	-	-
Mannitol	+	+	+	-	+	+	+	+
Urease	-	-	+	-	-	-	-	-
Kovacs	-	-	-	-	-	-	-	-
TDA	-	-	+	-	-	-	-	-

Tableau 5:Biochemical characteristics of identified bacterialisolated from mango orchard in Niayes area

(+) positive reaction; (–) negative reaction; (+/–) variable or weak reaction; (//) not determined. ADH = arginine dihydrolase; PNPG = p-nitrophenyl-β-D-galactopyranoside; MR = methyl red; VP = VogesProskauer; TDA = tryptophan deaminase.