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RESEARCH ARTICLE

DIAGNOSIS AND CHARACTERIZATION OF THE BACTERIAL FLORA OF MANGO (MANGIFERA INDICA L.) IN WESTERN SENEGAL

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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.

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 Sedhiou (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

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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 (Bactrocerainvadens) 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 citripv. mangiferaeindicae (Sanahuja et al., 2016; Zombre 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. citripv. mangiferaeindicaehas been reported in several neighboring countries, in Burkina Faso, Côte d'Ivoire, Mali, Ghana, Benin, and Togo (Pruvost et al., 2011, 2012, 2014; Zombre 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 Sessène, NottoDiobass, 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).

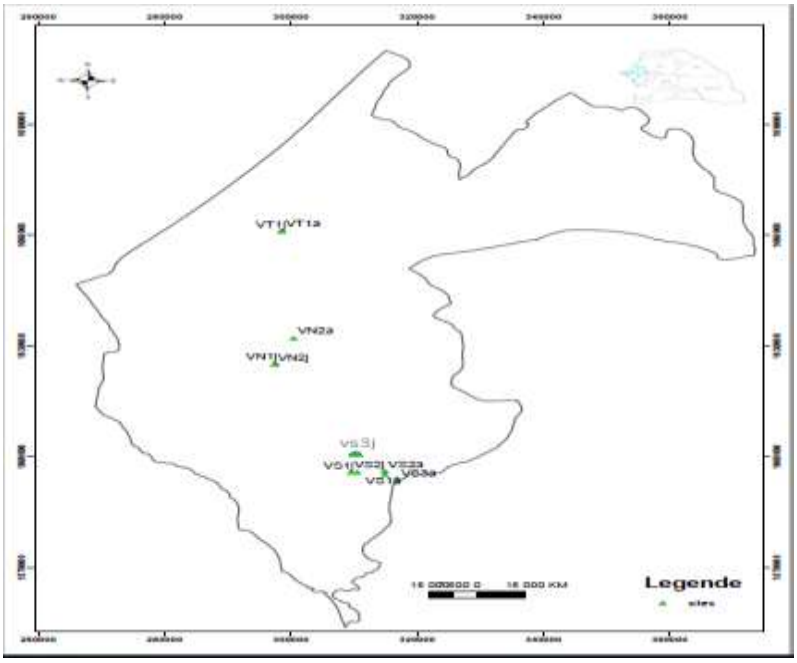

Figure 1: Geographic location of the surveyed mango orchards within the Niayes production area of Senegal.

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}, BioMerieux) were also used, and the results were interpreted using APIweb software (BioMerieux, 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 Table 1. 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 InqabaBiotec (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).

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

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 Sessène and five were from Taïba Ndiaye, whileno isolates were recovered from NottoDiobass. 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 pneumoniaewith Api20^{E},

Ochrobactrum spp., Burkholderia spp., Stenotrophomonas spp., Aeromonas spp., and Pseudomonas spp. with Api 20^{NE}.

Tableau 2: Isolates obtained from Mango orchards

Site	Orchard ID	Isolate ID	No of isolates
Sessène	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 = Sessène; T = Taïba Ndiaye; a = old orchard; j = young orchard; F = leaf; R = twig.

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 Sessène samples, species identified includedStenotrophomonas maltophilia, Pseudomonas aeruginosa, Bacillus cereus, Pseudomonassp, and Exiguobacterium sp.

In contrast, the five strains isolated from Taïba Ndiaye were identified as S. maltophilia, Ochrobactrumanthropi, and Burkholderiacepacia. The most prevalent genera in the orchards were Stenotrophomonas and Pseudomonaswith 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, Pseudomonasand Exiguobacterium.

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	Ochrobactrumanthropi	24h: 1641344 48h: 1643755	98,8
	$S8_1$	Burkholderiacepacia	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
	$S14_2$	Burkholderiacepacia	24h: 1557577 48h: 1557577	95,6
	S17	Pseudomonas aeruginosa	24h: 5554575 48h: 5757775	97,5

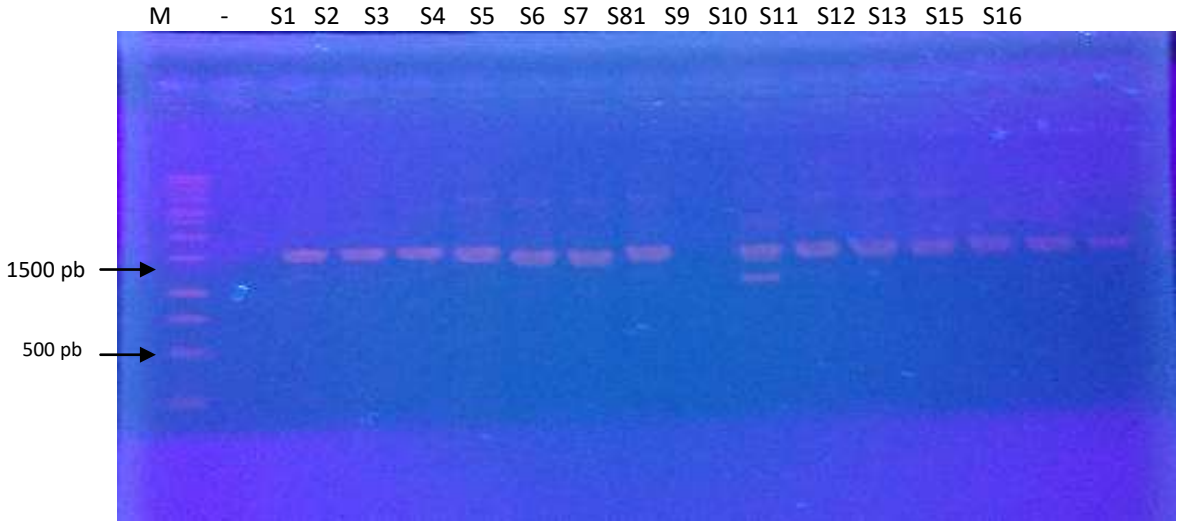

Figure 2: Visualization of 16S rDNA from bacterial isolates on agarose gel (M: marker; –: negative control; S: strain)

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	Ochrobactrumanthropi 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
$S14_2$	Exiguobacteriumsp MG819389	98

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

Tests	*Stenotrophomonas maltophilia*	*Bacillus cereus*	*Ochrobactrum anthropi*	*Pseudomonas aeruginosa*	*Exiguobacterium sp*	*Ochrobactrum sp*
Shape	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	-	-	+	-	-	-

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

Discussion:-

Mango (Mangifera indica L.), cultivated worldwide, is 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 mangiferaindicae (Patel et al., 1948; Daniel et al., 1975), Xanthomonas citripv. mangiferaeindicae (Sanahuja et al., 2016; Zombre et al., 2016) and Xanthomonas campestris pv. mangiferaeindicae(Pruvost et al., 2005) have been reported to induces similar 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, andBurkholderia. 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 multiplePseudomonas 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 Burkholderiaare 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 Pseudomonassp,P. aeruginosa and Stenotrophomonas maltophiliaand their potential association with symptoms developmentis therefore likely, noting the taxonomic reclassificationS. maltophilia from PseudomonastoXanthomonas and finally Stenotrophomonas (Palleroni and Bradbury, 1993). The isolates identified as P. aeruginosaexhibited 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 ofB. cepaciain 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 Ocimumbasilicum 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 citripv. mangiferaeindicae, the causal agent of MBBS and widely reported in neighboring countries (Pruvost et al., 2011, 2012, 2014; Zombre 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 targetingX. citripv. Mangiferaeindicaeand 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 Stenotrophomonasbeing the most prevalent.The absence of Xanthomonas citripv. mangiferaeindicaein the sampleshighlights the need for continued surveillance and expended surveys across other mango production area.

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