CONTRIBUTION TO THE IDENTIFICATION AND PATHOGENICITY OF THE PHYTOPATOGENIC BACTERIA RESPONSIBLE FOR TUBEROUS ROT OF MANIOC IN THE PRODUCTION ZONES OF THE COTE D'IVOIRE

ABSTRACT

Background and Objective : Cassava is an important crop for the Ivorian population. However, yields are relatively low. This low productivity is due to cassava root rots, which are sometimes caused by bacteria. The general objective was to identify the main bacterial diseases responsible for cassava tuberous root rot in production areas in Côte d'Ivoire. Specifically, to identify pathogenic bacteria and then to assess the pathogenicity of the bacterial strains isolated. **Material and Methods:** The plant material consisted of tuberous cassava roots affected by rot. Decay-causing bacteria were isolated on YPGA (Yeast extract Peptone Glucose Agar) culture medium. Strain identification was carried out using the API 20 E and 20NE gallery. Tuberous cassava roots were perforated with a punch and inoculated with bacterial inoculum calibrated to an optical density of 0.2 at a wavelength of 600 nm, corresponding to 108. **Results:** 174 strains were isolated. The highest proportions of strains were obtained in the localities of Ferké and Man, with 19 and 26% respectively. The bacterial species identified were Roualtella planticola, Serratia liquefaciens, Serratia marcescens, Burkholderia cepacia, Chromobacterium violaceum, Erwinia spp, Enterobacter cloacae, Klebseilla pneumoniae spp ozaenae and Raoutellia ornithinolytica. The Erwinia species was the most frequent in five localities, with proportions of 100 and 71.4% respectively in Agboville and Yamoussoukro. Results showed that 91.5% of bacterial strains tested caused rot (soft or dry). On the other hand, 52.4% of bacterial strains induced dry rot and around 39% induced soft rot. **Conclusion:** Various bacterial species were identified in this study. In addition, these species vary in their germinative capacity.

Keywords: Cassava, Rot, Tuberous root, bacteria, API 20 E and 20NE gallery

INTRODUCTION

Cassava is the second most important food crop after yam in terms of production volume. The cassava production zone covers the whole country, with a predominance around major cities such as Abidjan, Bouaké, San-Pedro, Yamoussoukro, Gagnoa, Daloa and Man[1]. In Côte d'Ivoire there are a large number of products derived from cassava processing. In terms of human consumption, attiéké is the main by-product, accounting for 5% of food production[2]. However, yields, which fluctuate between 6 and 15 t/ha, are relatively low. This low productivity is due to damage caused by fungal[3], viral[4] and bacterial diseases caused by Xanthomonas axonopodis pv. manihotis (Xam)[5]. In addition, root rot of cassava is caused by high soil humidity, as well as by telluric pathogens, notably nematodes, fungi and bacteria[6,7]. Recently, severe cassava root rot has been reported by stakeholders in several production zones. Also, the Regional Directorate of Agriculture and the ANADER zone in the ME region, challenged the PRO2M team in 2018 regarding this phenomenon observed in several other regions of the country. Because of the economic consequences that this disease could cause, it is advisable to anticipate its development through effective and

sustainable control strategies in Côte d'Ivoire. This will guarantee sustained cassava production, which in turn will improve producers' incomes and the food security of the Ivorian population. It is in this context that this study was carried out, with the general aim of identifying the main bacterial diseases responsible for cassava tuberous root rot in production areas in Côte d'Ivoire. Specifically, the aim was to identify the phytopathogenic bacteria present in the cassava-growing regions of Côte d'Ivoire, and to assess the pathogenicity of the bacterial strains isolated.

I. MATERIALS AND METHODS

The study was carried out from December 2021 to March 2023.

1.1 Plant material

The plant material consisted essentially of tuberous cassava roots affected by rot. All cassava varieties available to farmers and of production age were evaluated. Root samples of the Yacé variety were used for pathogenicity tests.

1.2 Study area

Cassava production areas cover the whole country, extending around the major towns. The samples analyzed were collected in 13 localities visited, namely: Aboisso, Abengourou, Adzopé, Agboville, Bouaké, Dabou, Toumodi, Yamoussoukro, Daloa, San-Pédro, Man, Katiola and Férké.

13. Methods

1.3.1. Sample collection

In each of the plantations visited, 5 samples of roots showing rot symptoms were collected to isolate the pathogen(s) involved. A total of 871 samples of infected roots were collected in the production areas visited.

1.3.2. Identification of phytopathogenic bacteria

1.3.2.1. Isolation, purification and conservation of strains

Bacteria responsible for rotting were tested on a YPGA (Yeast extract Peptone Glucose Agar) culture medium. The preparation of one litre of YPGA culture medium required 18g agar, 7g glucose 7g yeast and 7g peptone. The medium thus prepared was sterilized autoclaved at 121°C under 1 bar pressure for 30 minutes. After autoclaving, the media were dispensed into Petri dishes in a fume hood. Petri dishes in a laminar flow hood near a flame at a rate of 15 mL per dish.

Bacterial strains were isolated using the Persley[8] method. This involved sterilizing the cassava root fragments collected. These rotten cassava root fragments were soaked in 70% ethanol for 30 seconds, then in 1% sodium hypochlorite for 1 minute, and rinsed three times in sterilized distilled water. The sterilized fragments were individually aseptically diluted in a few drops of sterile water, then left to macerate for 10-15 minutes at 28°C. After incubation, the strains were purified on new YPGA culture media. They were stored at -80°C in Eppendorf tubes containing 25% glycerol.

1.3.2.2. Identification of bacterial germs

A total of 174 isolates were obtained from the 13 localities visited. Isolates were cultured on YPGA media. After 24 hours, colony size was measured, and the description of colony contour and relief, behavior, pigmentation and surface appearance were assessed for each bacterial isolate. Microscopic studies were then carried out. To this end, a fraction of each colony type was introduced into 0.1 ml of distilled water and observed under the microscope. The observations were used to determine the shape and motility of the bacteria. A Gram staining test was applied to the bacteria using the Schaad[9] technique. This determined the type (Gram + or -) of each bacterium. Strain identification was carried out using the API 20 E and 20NE gallery (Fig. 1A). API 20 E and 20NE galleries each comprise 25 micro-tubes containing dehydrated substrates. The micro-tubes were inoculated with a bacterial suspension concentrated to 108 bacteria/mL.

After 24 to 48 hours of incubation at 28°C, the reactions produced, reflected by spontaneous color changes or revealed by the addition of reagents, were observed.. Strains were identified using the Analytical Catalogue or identification software (BIOMERIEUX, REF20100/20160) (Fig. 1B).

1.3.3. Pathogenicity testing of isolated strains

Inoculum was produced from bacterial strains. A total of one hundred and seventy-four (174) strains previously isolated and preserved in glycerol were used for this study. The inoculum was prepared from sterilized distilled water and then calibrated to an optical density of 0.2 at a wavelength of 600 nm, corresponding to 10^8 bacteria/mL.

1.3.4. Evaluation of phytopathological parameters

Phytopathological assessments focused on the incubation period (IP) and the proportion of rot caused by bacterial strains. Symptoms were described for each strain. Root rot rates were determined on a 0 to 4 rating scale. Assessments were made every 2 days for 10 days.

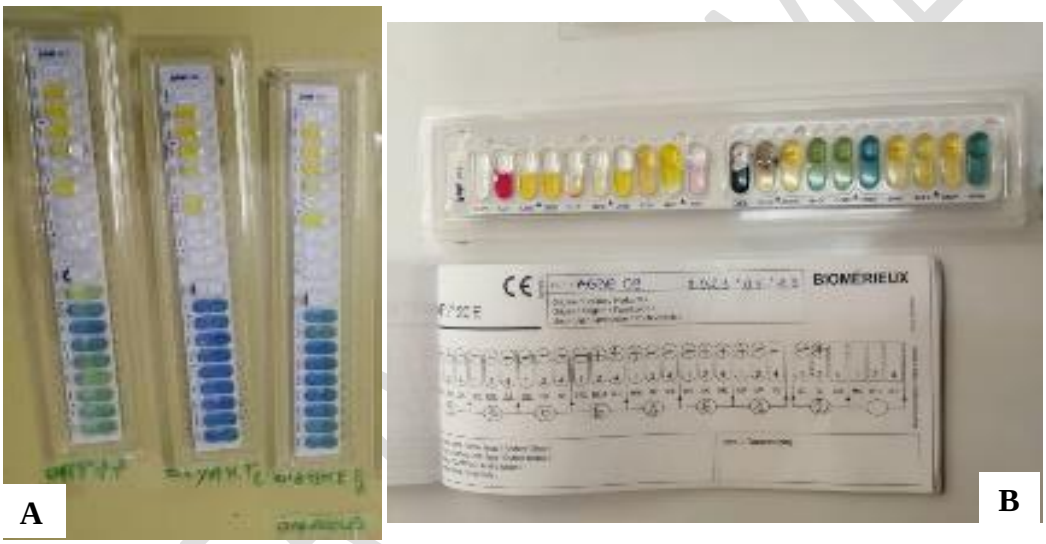


Fig. 1: Steps for identifying bacterial species using the API 20E and 20NE gallery
A : Inoculated cups containing dehydrated substrates

B : Cups after incubation (24 to 48 hours) at 37°C and Analytical Catalogue (BIOMERIEUX, REF20100/20160).

1.3.5. Statistical analysis

An analysis of variance was applied to the recorded data, and in the event of a significant effect of the factor studied, the Newman-Keuls statistical test, with a threshold of 5%, was used to separate the means into homogeneous groups.

II. RESULTS AND DISCUSSION

2.1. Results

2.1.1. Bacteria associated with cassava root rot

2.1.1.1. Distribution of isolated bacterial populations by locality

A total of 174 bacterial strains associated with cassava root rot were isolated from the samples collected. The highest proportions of strains were obtained in the localities of Ferké and Man, with 19 and 26% respectively. In Adzopé, Dabou, Katiola and San-Pedro, the bacterial populations obtained were low, ranging from 1 to 5%. Intermediate rates of between 7 and 13% were recorded in Agboville, Bouaké, Daloa and Yamoussoukro (Fig. 2).

2.1.1.2. Distribution of identified bacterial species

Fig. 3 shows the distribution of the most aggressive strains by locality. The nine species identified are unevenly distributed across the locations surveyed. Roualtella planticola was identified as the pathogen responsible for cassava rot in Daloa, Man and Ferké, with proportions of 33.3, 60 and 66.7% respectively. Bacterial colonies are shown in Fig 4. The two bacterial species Serratia liquefaciens (Fig. 5) and Serratia marcescens (Fig. 6) were identified in the Yamoussoukro locality, and to a greater extent in Katiola and Daloa respectively. Burkholderia cepacia (Fig. 7) and Chromobacterium violaceum (Fig. 8) were identified in Katiola. Erwinia spp (Fig. 3) was the most frequent species. It was found in five localities: Agboville, Yamoussoukro, Man Férké and Bouaké, with proportions of 100, 71.4, 40, 33.3 and 33.3% respectively. Erwinia spp colonies are shown in Fig 9. Klebsiella pneumoniae spp ozaenae and Raoutellia ornithinolytica (Fig. 10) recorded in Bouaké. Enterobacter cloacae (Fig. 11) was only observed in Daloa.

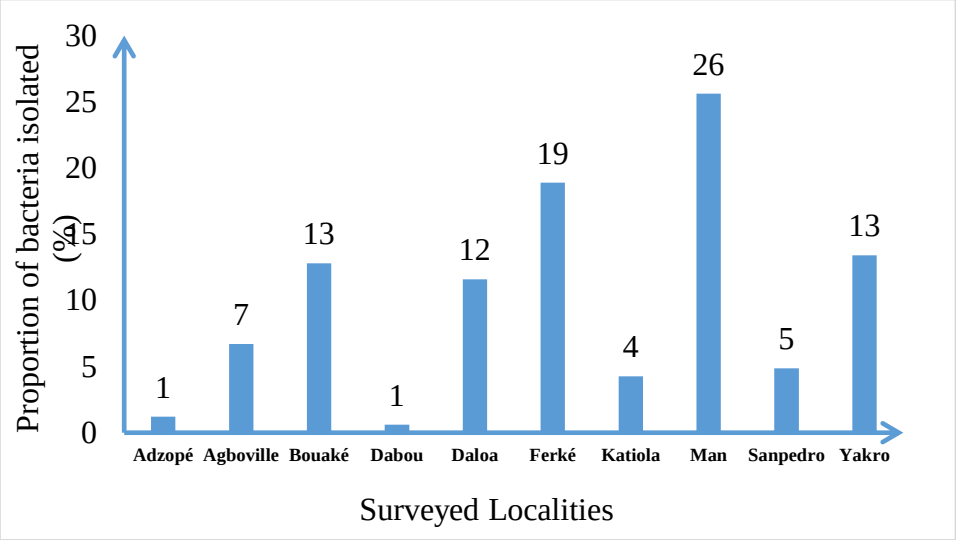


Figure 2 : Distribution of bacterial strains by Localities

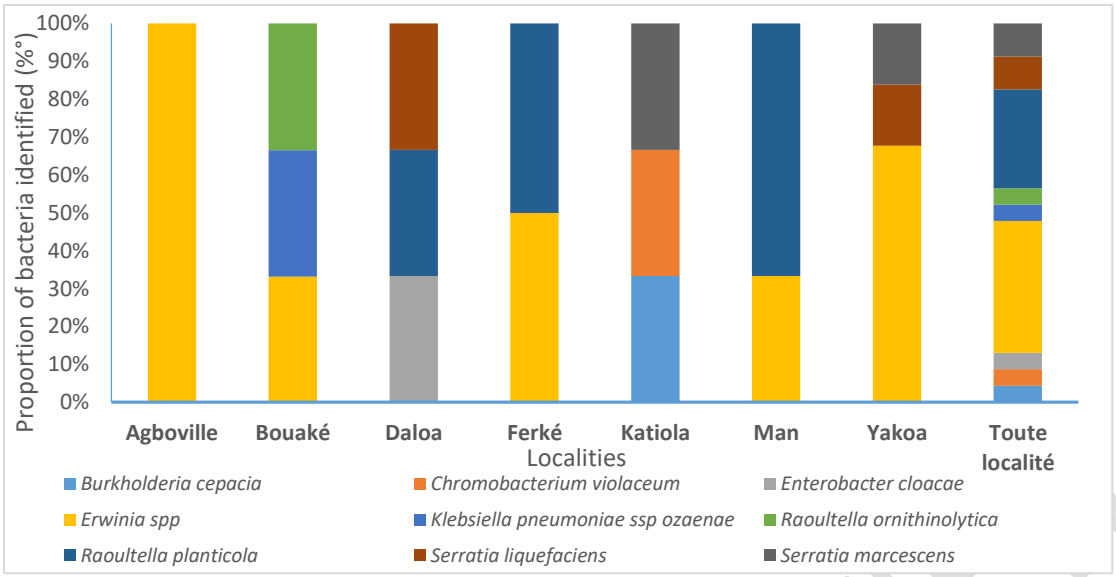


Fig. 3: Bacterial species associated with cassava rots according to locality

Fig. 4: Macroscopic aspects of bacterial colonies of *Raoultella planticola*

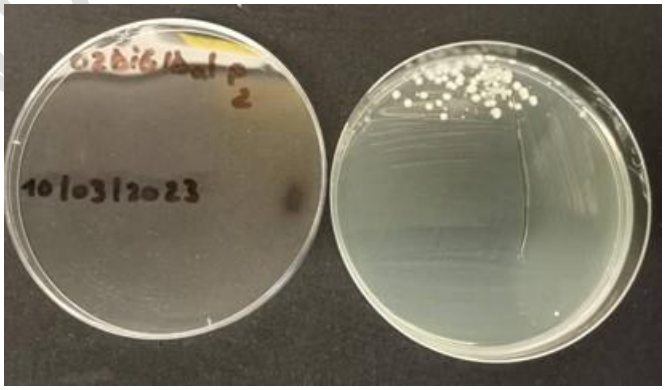


Fig. 5: Macroscopic aspects of bacterial colonies of Serratia liquefaciens

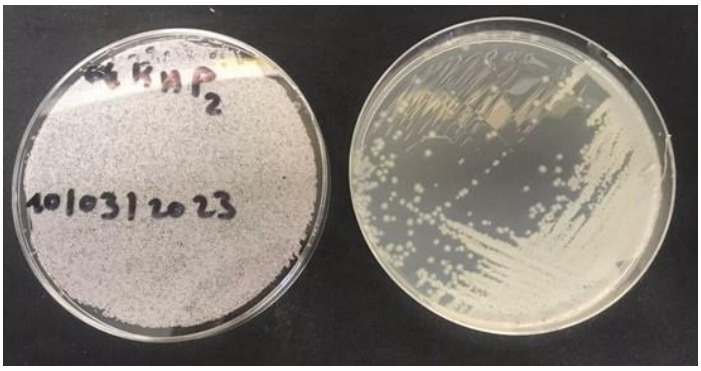


Fig. 1: Macroscopic aspects of bacterial colonies of *Serratia marcescens*

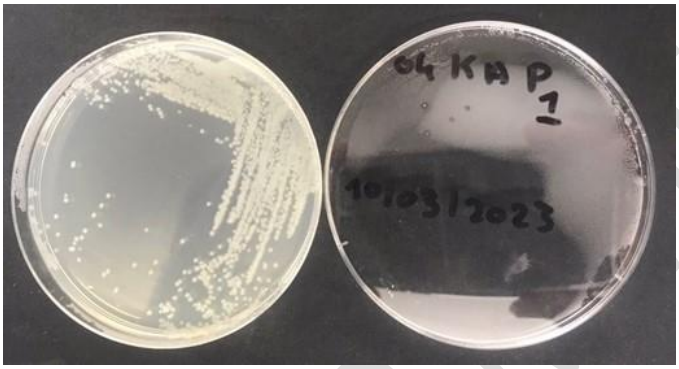


Fig. 7: Macroscopic aspects of *Burkholderia cepacia* bacterial colonies

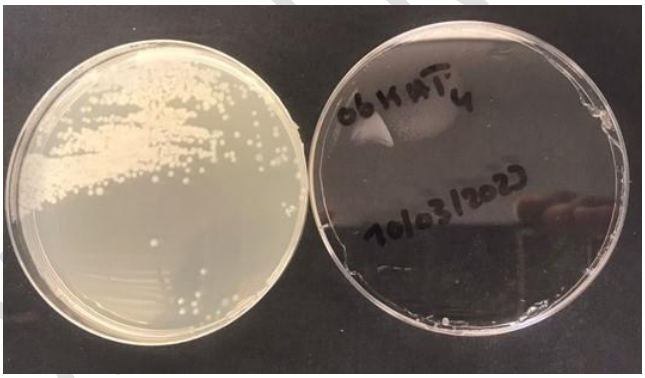


Fig. 8: Macroscopic aspects of bacterial colonies of *Chromobacterium violaceum*

Fig. 9: Macroscopic aspects of bacterial colonies of *Erwinia spp*.

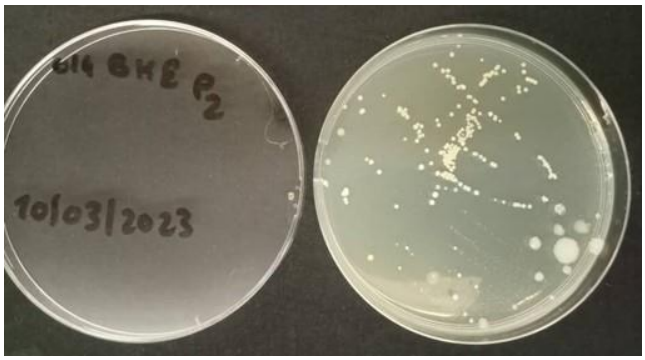


Fig. 10: Macroscopic aspects of colonies of *Raoultella ornithinolytica* bacterial

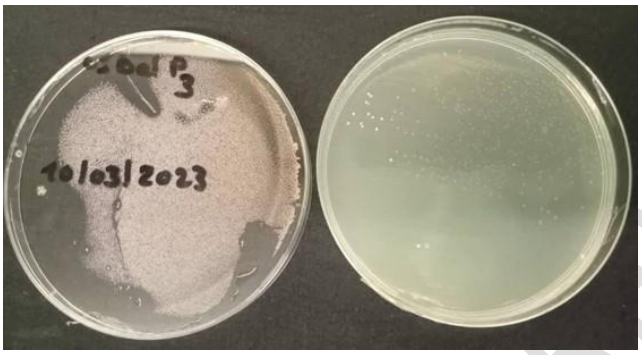


Fig. 11: Macroscopic aspects of *Enterobacter cloacae* colonies

2.2. Pathogenicity of isolated strains

2.2.1. Types of rot caused by bacteria in different localities

Pathogenicity tests showed the existence of two types of rot induced by the bacteria isolated in the different localities visited. Of all the bacterial strains tested, 91.5% caused rot (soft or dry). The distribution of rot types showed that more than half (52.4%) of the bacterial strains induced dry rot and around 39% induced soft rot. Soft rot was strongly induced by bacterial strains isolated in Bouaké (42.9%), Daloa (68.4%), San-Pedro (62.5%) and Yamoussoukro (68.2%), while in Adzopé and Dabou, this type of rot was absent. In Adzopé, Agboville, Bouaké, Dabou, Ferké, Katiola and Man, 50 to 100% of the bacterial strains isolated caused dry rot (Table 1).

Table 1: Types of induced rot according to bacterial strain origin

Origin of bacterial strains	Proportion of bacterial populations (%)			
	No rot	Soft rot	Rot dry	Total
Adzopé	50.0	0.0	50.0	100
Agboville	0.0	9.1	90.9	100
Bouaké	0.0	42.9	57.1	100
Dabou	0.0	0.0	100.0	100
Daloa	0.0	68.4	31.6	100
Ferké	12.9	32.3	54.8	100
Katiola	0.0	42.9	57.1	100
Man	21.4	19.0	59.5	100
San-Pedro	0.0	62.5	37.5	100
Yakro	0.0	68.2	31.8	100
Overall total	8.5	39.0	52.4	100

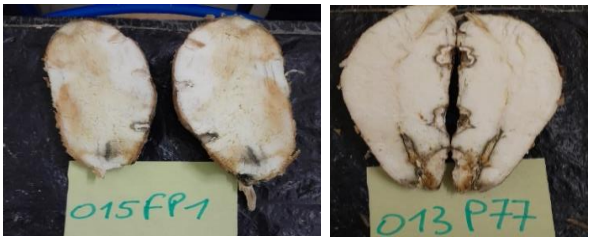
2.2.2. Bacterial species associated with cassava root rot

Analysis of the results identified 9 bacterial species (Table 2). *Erwinia spp* (Fig. 12) and Raoultella planticola (Fig. 13 A, B) were the most represented species, at 40% and 24% respectively. *Burkholderia cepacia* (Fig. 14), *Chromobacterium violaceum*, *Enterobacter cloacae* (Fig. 15), *Klebsiella pneumoniae ssp ozaenae* ((Fig. 16) and *Raoultella ornithinolytica* (Fig. 17) were poorly represented at 4%, while *Serratia liquefaciens* and *Serratia marcescens* (Fig. 18) accounted for 8% of bacterial populations. Pathogenicity tests revealed that *Burkholderia cepacia*, *Klebsiella pneumoniae* ssp ozaenae and *Serratia liquefaciens* typically caused dry rot, while *Chromobacterium violaceum*, *Enterobacter cloacae*, *Raoultella ornithinolytica* (Fig. 19) and *Serratia marcescens* induced soft rot. Both types of rot (soft and dry) were induced by *Erwinia spp* and *Raoultella planticola*. In terms of decay rates (root volume destroyed), *Raoultella planticola, Chromobacterium violaceum, Erwinia spp, Enterobacter cloacae, Raoultella ornithinolytica*, and *Serratia marcescens* induced soft rot with severity levels ranging from 50 to 100% root volume destruction. *Burkholderia cepacia, Klebsiella pneumoniae ssp ozaenae, Raoultella planticola* and *Serratia liquefaciens* were identified as agents responsible for dry root rot, with average rates ranging from 30% to 100%.

Table 2: Species of rot-causing bacteria identified

Species of bacteria	Bacterial populations (%)	Soft rot (%)	Dry rot (%)	Average rot rate (%)
Burkholderia cepacia	4	-	100	70
Chromobacterium violaceum	4	100	-	60
Enterobacter cloacae	4	100	-	90
Erwinia spp	**40**	100	0	56
Klebsiella pneumoniae ssp ozaenae	4	-	100	60
Raoultella ornithinolytica	4	100	-	70
Raoultella planticola	**24**	50	50	33.3
Serratia liquefaciens	8	-	100	55
Serratia marcescens	8	100	-	40
Total général	100	60	40	-

Fig. 12: Soft root rots caused by *Erwinia spp*.



A : Soft rot **B :** Dry rot

Fig. 13: Root rots caused by *Raoultella planticola*

Fig. 14: Rot caused by *Burkholderia cepacia*

Fig. 15: Dry rot caused by *Enterobacter cloacae*

Fig. 16: Dry rot caused by *Klebsiella pneumoniae ssp ozaenae*

Fig. 17: Dry root rot caused by *Serratia liquefaciens*

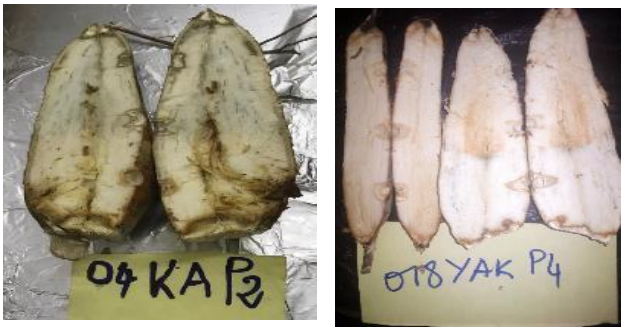


Fig. 18: Soft root rot caused by *Serratia marcescens*

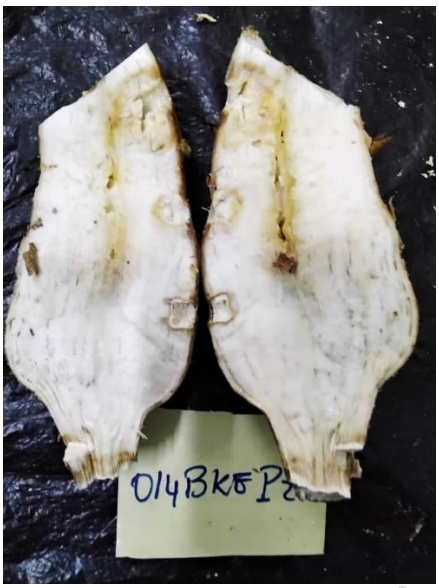


Fig. 19: Soft root rot caused by *Raoultella ornithinolytica*

2.2. DISCUSSION

All laboratory observations on isolates from cassava root rot confirm the involvement of several bacterial pathogens.

In addition, bacterial identification using API 20E and 20NE galleries revealed the following nine (09) bacterial species: *Burkholderia cepacia, Chromobacterium violaceum, Enterobacter cloacae, Erwinia spp, Klebsiella pneumoniae ssp ozaenae, Raoultella ornithinolytica, Raoultella planticola, Serratia liquefaciens and Serratia marcescens*.

According to Kéléké *et al.*[10], the amylotic flora composed of Enterobacter is essentially due to the fact that cassava is an energy food containing up to 86% starch. The genera Klebsiella and Enterobacter (Enterobacteriaceae) have been isolated at fairly high frequencies from rotting cassava roots in the Republic of Congo. The Klebsiella genus, with its fermentative role in cassava degradation, is fairly well known[11]. As for the Enterobacter genera, typical of animals and humans, their presence suggests contamination by their faecal matter, which would be transported by run-off water[12,13].

The *Erwinia genus* is known as the causal agent of both wet and dry root rot of cassava, which would explain its strong presence in root rot in Côte d'Ivoire. It is responsible for fire blight and attacks a wide range of economically important host plants such as cassava, apples, carrots and reservoir plants[14]. Erwinia has been identified in several cassava-growing areas with high rot rates, and represents a threat to cassava production in Côte d'Ivoire.

Al-Hajjar *et al*[15] stated that Raoultella ornithinolytica and Raoultella planticola species are telluric agents infecting tuberous cassava roots. Grimon and Grimon[16] showed that species of the Serratia genus are facultative anaerobic, chemoorganotrophic bacteria with low nutritional requirements, belonging to the Enterobacteriaceae family. The various species of the Serratia genus can be hosted by plants and animals (including humans). They are also likely to be involved in cassava root rot.

CONCLUSION

This study identified the pathogenic bacteria present in the cassava-growing regions of Côte d'Ivoire, and then assessed the pathogenicity of these strains.

The Man locality recorded the highest proportion of strains, estimated at 26%. Nine bacterial species were identified: *Roualtella planticola, Serratia liquefaciens, Serratia marcescens, Burkholderia cepacia, Chromobacterium violaceum, Erwinia spp, Klebsiella pneumoniae spp ozaenae, Raoutellia ornithinolytica* and *Enterobacter cloacae*. These species are unevenly distributed across the surveyed localities. On the other hand, Erwinia spp was the most frequent species, with higher proportions of 100 and 71.4% observed in Agboville and Yamoussoukro respectively.

Assessment of the pathogenicity of the bacterial strains revealed that 91.5% induced either soft or dry rot. Thus, 52.4% of bacterial strains induced dry rot, while 39% induced soft rot. Yamoussoukro recorded the highest proportion of soft rot (68.2%). Dry rot was caused by 100% bacterial strains in the Man locality.

In order to find a lasting solution to the control of pathogenic bacteria of cassava tuberous root rot in Côte d'Ivoire, it will be necessary in the continuation of this study :

- Test the antibacterial activity of different products *in vitro* and under semi-controlled conditions ;

- Evaluate in the field the effect of products on the incidence and severity of disease induced by these strains.

Significance statement : The aim of this study was to identify the main bacterial

diseases responsible for cassava tuberous root rot in production areas in Côte d'Ivoire. More specifically, the aim was to identify pathogenic bacteria and assess the pathogenicity of the bacterial strains isolated. The results revealed the isolation of 174 bacterial strains. The highest proportions of strains were found in Ferké and Man, with 19% and 26% respectively. The bacterial species identified were Roualtella planticola, Serratia liquefaciens, Serratia marcescens, Burkholderia cepacia, Chromobacterium violaceum, Erwinia spp, Enterobacter cloacae, Klebseilla pneumoniae spp ozaenae and Raoutellia ornithinolytica. The Erwinia species was the most frequent in five localities, with proportions of 100 and 71.4% respectively in Agboville and Yamoussoukro. The results show that 91.5% of the bacterial strains tested caused rot (soft or dry). On the other hand, 52.4% of bacterial strains induced dry rot and around 39% induced soft rot. The identification of these bacterial species could lead to the development of effective control methods against cassava tuberous root rot in Côte d'Ivoire.

RÉFÉRENCES

3. Affery A M., Abo K., N Aya C., Wonni I., Bomisso E L., Kouamé K D., Tuo S., Bolou Bi B A., Koné M et Koné D. 20218. Pathogenic diversity of *Xanthomonas axonopodis* pv. manihotis strains in Côte d'Ivoire, susceptibility of cassava varieties and control approach. Journal of Applied Biosciences 130: 13187 - 13198 ISSN 1997-5902. https://doi.org/10.35759/JABs.130.6

2. Edith, K.K.N., K.K. Felix, K.Y. Francis, G. Theophile and T. Kablan, 2016. Characterization of waste from Attieke factory: Case of Azito village (Abidjan, Cote d'Ivoire). Eur. Scient. J., 12: 73-81. DOI : **10.19044/esj.2016.v12n35p73**

3. Yeo F. E., K. J. N. Ehui, L-N'. D. G. E. Amari., A. S-P N'Guetta and D. Kone. 2017. Distribution and incidence of cassava anthracnose in côte D'ivoire and pathogenic characteristics of Colletotrichum gloeosporioides Penz Manihotis isolates. Plant Pathol. J., 16 : 73 – 81. DOI: **10.3923/ppj.2017.73.81**

4. Mamba-Mbayi G., P. Tshilenge-Djim, K.K. Nkongolo and A. Kalonji-Mbuyi. 2014. Characterization of Congolese Strains of Xanthomonas axonopodis pv. Manihotis Associated with Cassava Bacterial Blight. American Journal of Plant Sciences 5 : 1191 - 1201. DOI: **10.4236/ajps.2014.59132**.

5. Monde G., J. Walangululu, S. Winter and C. Bragard. 2010. Dual infection by cassava begomoviruses in two leguminous species (Fabaceae) in Yangambi, North-Eastern Democratic Republic of Congo. Arch Virol,155 : 1865 – 1869. DOI: **10.1007/s00705-010-0772-3**

6. Msikita W., James B. D., Bissang B., Baimey H., Wilkinson H. T. et Ahounou M., R. (2005). Prevalence and Severity of *Nattrassia mangiferae* Root and Stem Rot Pathogen of Cassava in Bénin. Plant dis, 89 : (1) 12-16. DOI: **10.1094/PD-89-0012**

7. Bandyopadhyay R., Aigbe S. O. and J. F. Leslie. (2006). Fusarium species from the Cassava root rot complex in West Africa. Pathology 59 : 673-676.

8. Persley G J. 1973. Pathogenic Variation in *Xanthomonas Albilineans* (Ashby) Dowson, the Causal Agent of Leaf-Scald Disease of Sugar Cane. *Australian Journal of Biological Sciences* 26 (4) 781-786.https://doi.org/10.1071/BI9730781

9. Schaad N W.,1988. *Plant Pathogenic Bacteria*. 2nd edn. St Paul, MN, USA : American Phytopathological Society.

10. Kéléké S., Kimpolo-Kimfolo L et Brauman Alain. (1995). Origin and importance of amylase activity in lactic acid fermentation of cassava roots. Pp : 320-330. Language of publication: French. https://www.researchgate.net/publication/32972748_Origine_et_importance_de_l%27activite_amylasique_dans_la_fermentation_lactique_des_racines_de_manioc_Origin_and_importance_of_amylase_activity_in_cassava_roots_during_lactic_acid_fermentation

11. Oyewole et Odunfa S A. 1988. Microbiological studies on cassava fermentation for 'lafun' production. Volume 5, Issue 3, Pages 125-133. https://doi.org/10.1016/0740-0020(88)90010-X

12. Ryan, R. M et Deci, E. L. 2004. Avoiding Death or Engaging Life as Accounts of Meaning and Culture : Comment on Pyszczynski *et al*. (2004). *Psychological Bulletin, 130*(3), 473–477. https://doi.org/10.1037/0033-2909.130.3.473

13. Assanvo, J. B., Agbo, G. N., Behi, Y. E. N., Coulin, P., et Farah, Z. (2006). Microflora of traditional starter made from cassava for attiéké production in Dabou (Côte d'Ivoire). Food Control, 17: 37- 41.

14. Momol M.T and H.S. Aldwinckle. (2000). Genetic diversity and host range of Erwinia amylovora. In: Fire blight: the disease and its causative agent, Erwinia amylovora. (Vanneste J.L., ed.). CABI publishing, Wallingford, UK, 55–72.

15. **Al-Hajjar S., Ibrahim L., Kurdi W., Tulbah M., Alnemer M., Bin Jabr M., Elsaidawi W., Binmanee A., Ali M., Bukhari H., Altuwaijri L., Allaboon Raneem., Alghamdi Reem., Saeed Bashayer., Adi Yasser et Alhamlan F. 2022.** Observational cohort study of perinatal outcomes of women with COVID-19.
DOI: 10.1016/j.jiph.2022.11.007

16. Grimom, F. and Grimont, P.A.D. 1991. DNA fingerprinting, in "Nucleic acid techniques in bacterial systelnat ics. pp. 249-279. https://books.google.com/books/about/Nucleic_Acid_Techniques_in_Bacterial_Sys.html?id=89PwAAAAMAAJ