Société Ivoirienne de Microbiologie

Identification, characterization and distribution of phytopathogenic bacterial species *Pantoea* associated with rice seed in Africa

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ABSTRACT Rice seeds harbour a variety of microorganisms, including pathogenic fungi, bacteria and nematodes which are capable of causing damage to rice crop. Newly discovered bacterial species belonging to the genus *Pantoea*, have been found to exhibit similar symptoms to *Xanthomonas oryzae* pv. oryzae (*Xoo*) in rice plants. In Sub-Saharan Africa, there are three species of *Pantoea* that are particularly identified as the causative agents for bacterial leaf blight, a disease that causes blighting and discoloration of rice grains. For effective implementation of a *Pantoea* management system, a diagnosis was carried out on rice seeds from 21 African countries. The tests revealed the presence of different *Pantoea* species in 15 African countries including *Pantoea stewartii; Pantoea ananatis; Pantoea deleyi*. Following the identification of the isolated microorganisms two *Pantoea* species were selected for pathogenicity testing in greenhouse using the leaf clipping method on seven elite rice varieties. The pathogenicity tests highlighted the susceptibility of rice variety Gigante to *Pantoea stewartii V11SEN1* a strain identified from Senegal and varieties Moroberekan and Azucena to *Pantoea ananatis IVV6CI1,* a strain isolated from Côte d'Ivoire. From these investigations, it would be important to pay particular attention to these emerging strains of *Pantoea* in Africa.

Keywords : Rice, *Pantoea* species, Pathogenicity testing, Seedborne bacteria

Volume 2, Issue 2 pages 225-238
ISSN 2664-388X
https://doi.org/10.26167/4z72-jg73

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Received: 08th November 2024
Accepted: 28th January 2025
Published online 07th February 2025.

Introduction

Bacterial wilt is the most important bacterial disease (Hasan et al 2024) in many rice-growing regions of the world (Xu et al 2010; Triplett et al 2014) including Africa (Wonni et al 2014; Kini et al 2017). It is considered one of the major threats to rice production due to its wide distribution (Van et al 2006) and its limitation of production worldwide (Wonni et al 2014). Yield losses due to bacterial wilt are estimated at between 20-100% worldwide if conditions are favourable (Kini et al 2017; Wang et al 2023). Known to be caused by *Xanthomonas oryzae pv. oryzae*, it has long been confused with other emerging diseases caused by the genera *Pantoea sp. (*Kini et al 2016; Mondal et al 2011; Vinodhini 2017) and *Sphingomonas (*Kini *et al 2017)*. The *Pantoea* genus belonging to Enterobacteriaceae family, is a ubiquitous microorganism, which can be found on different organisms and environments, such as plants, soils, animals and humans (Walterson et al 2015; Nandarasah et al 2014).

Pantoea species (Pantoea spp.) are a diverse group of bacteria that are rod-shaped and Gram-negative and commonly yellow-pigmented on culture media. They are recognized as pathogens responsible for diseases devastating agriculturally relevant crops such as rice, maize, melon, cotton and onion. Typical crop symptoms include galls, wilting, soft rot and necrosis (Brady et al 2011; Kado 2016). Damage to various plant organs can result in distinct symptoms: leaves may exhibit spots, streaks, burns are often observed; fruits may exhibit internal rot, central rot, soft rot and bulb rot are observed; grains and inflorescences may display seed stalk rots, palea browning and grain discoloration have been reported (Sherafati et al 2014).

In rice, pathologies caused by Pantoea genus can lead to symptoms like palea browning and kernel discoloration, stalk necrosis, stem necrosis and inhibition of seed germination (zizi et al 2020; Yan et al 2010; Cother et al 2004). Several species of Pantoea, including *P. ananatis*, *P. agglomerans*, *P. stewartii*, *P. allii* and *P. wallisii*, have been widely documented as pathogenic bacteria responsible for the aforementioned symptoms (Doni et al 2019; brady et al 2011; Brady et al 2021; Delétoile et al 2009) with specifically the species *P. ananatis*, *P. agglomerans*, *P. stewartii* described, in sub-Saharan Africa, as pathogens on rice plants (Kini et al 2019) and seeds (Dossou and Silue 2018). Pantoea spp. are seed-transmitted and thus pose a significant risk to interstate and continental seed exchanges (Kini 2019).

(Cottyn et al 2001) detected 27 bacterial species from seeds collected in 1995 in the Philippines., five of which cause damage to several crops (Arnold et al 2003; Coutinho and Venter 2009; Cruz et al 2007; De Maayer et al 2014). Three of the five species, namely *P. ananatis*, *P. agglomerans* and *P. stewartii*, are responsible for more than 80% of reported cases of plant diseases. Urgent countermeasures need to be implemented, to limit the spread of this pathogen. This can be achieved through research actions such as disease surveys, diversity analysis, and the identifying sources of resistance to minimize yield losses in regions where the disease is present. Unfortunately, few rice infecting strains have been collected and characterized at the genetic level. This information is needed for the development of broad-spectrum varietal resistance in sub-Saharan Africa. To address these gaps, this study aimed to (1) identify and analyze the diversity of the genus Pantoea present on rice seeds from several African countries, and (2) to test the pathogenicity of different strains identified on seven (07) elites rice varieties (IR24, ADNY 11, Azucena, Moroberekan, Gigante, Sahel 201 and PNA 647 F4-56) collected from West Africa. This effort contributes to improving strategies for controlling this emerging bacterial pathogen.

Materials and Methods

Survey locations and plant material

The survey was carried out during the 2012 and 2013 rainy seasons in 22 rice-growing regions in Africa. We targeted regions in Benin Republic (Central region at Sowe), Burkina Faso (Vallee region), Côte d'Ivoire (Gagnoa), Gambia (Sapu), Liberia (Suakoko), Mali (Sikasso), Niger (Kollo Tillaberi), Nigeria (Sabon Daga), Sierra Leone (Rokupr), Senegal (Ndiaye), Togo (Danyi), Congo (Loudima), Tchad (Mala), Egypt (Sakha), Tanzania (Ifakara), Madagascar (Mahitsy), Cameroun (Garoua), Guinee Bissau (Cabonxanque), Ghana (Nobewam), Rwanda (Rusuli), Burundi (Ndebe), and Guinea Conakry (Kakan). Figure 1 displays the locations where seeds were collected.

The planting materials consisted of 32 rice varieties from three different ecologies (upland, lowland, highland), which were delivered by AfricaRice's breeders to the breeding task force NARS's partners for rice evaluation trials. The seeds were sent to the NARS according to their specific locations in Africa, (refer to Table 1). The seeds were treated in compliance with the import permits requirements of each importing country and then dispatched to partners.

Experimental design and agronomic practices

The seeds from the rice accessions described in Table 1 were grown in 22 African countries under field conditions allowing for natural infection from local pathogens. The experiment was conducted on-farm using a Randomized Complete Block Design with three replications, and was repeated twice in each of the 22 countries. The seeding rate was set at 20cmx50cm and space between blocks were 1.5m. Each plot unit measured 5m2 in size and was planted with a single variety of rice. Seeds were harvested at maturity (3 months after sowing) from each field in 21 countries, the seeds from Guinea Conakry were not available because of rodent attack at Kakan. After threshing and cleaning, the different rice varieties were labelled, and the seeds were placed in newly labelled envelopes (each envelope containing the grains from one plot). These envelops were then delivered to Benin for laboratory analysis. The seeds did not undergo chemical treatment prior to importation.

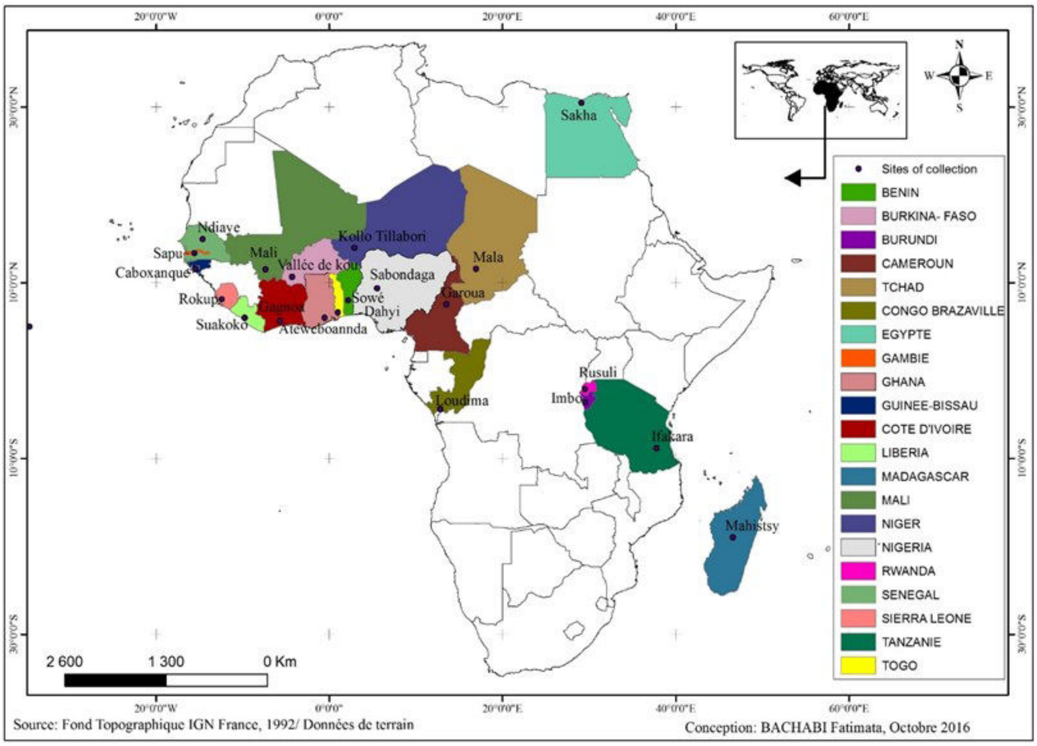


Figure 1. Source of seeds used for diagnosing *Pantoea* spp

Table 1: List of rice accessions collected in Africa

S/ No or Code	Upland (Benin; Congo Brazzaville; Mali Nigeria; Cameroun; Guinee)		Lowland (Côte d'Ivoire; Egypte; Niger; Tchad; Sierra Leone; Burkina Faso; Gambie; Ghana; Liberia; Senegal, Tanzanie)		Highland (Rwanda; Togo; Madagascar; Burundi)	
	AfricaRice varieties					
	Designation	Origin	Designation	Origin	Designation	Origin
1	ART12-9L9P1-2-1-B	AfricaRice	FAROX 508-3-10-F44-2-1	Nigeria/NCRI	82079-TR 489-3-1-1	
2	ART16-4-1-21-3-1-1	AfricaRice	WAB 2076-WAC2-TGR1-B	AfricaRice	scrid006-3-2-3-2	Madagascar
3	ART2-11-L4P3-B-B-1	AfricaRice	WAB 2094-WAC2-TGR4-B	AfricaRice	scrid019-1-1-1-1-2	Madagascar
4	ART2-5L8P2-B-B-2	Afri-	FKR-62N	INERA	88088-TR 1113-4-1-	
5	ART2-9L3P3-B-B-4	AfricaRice	IWA 2	Nigeria, Effissue	89014-TR 1134-2-2-	
6	ART3-5L8P2-B-B-2	AfricaRice	SK-19-38-2 Mali/IER		Exp304	
7	ART3-8L14P3-2-B-2	AfricaRice	WAB 1436-20N-3-B-FKR2-WAC1	AfricaRice	FOFIFA171	Madagascar
8	ART3-9L9P3-1-B-2	AfricaRice	WAB 2056 2- FKR2-5-TGR1-B A	AfricaRice	FOFIFA167	Madagascar
9	BR 49994-13-2-1	Bangladesh	WAB2061-2-FKR1-WAC2-TGR4-B	AfricaRice	HR 17512-11-2-3-1-4-2-3	
10	CNAX3031-78-2-1	Bresil	WITA12		X-JIGNA	
11	CT11891-3-3-3-M-1-3-1-M	CIAT/ Colombie	FAROX 508-3-10-F43-1-1	Nigeria/NCRI	IR68331-R-R-B-34-2-3	
12	F161	AfricaRice	WAB 2060-FKR4-WAC1-TGR5-B	AfricaRice	87020-TR 968-1-1-1	
13	GAMBIAKA	AfricaRice	WAB 2125-WAC B-1-TGR3-WAT B8	AfricaRice	88076-TR 1101-9-2-1	
14	IR68702-1-4-B	IRRI	FKR-19	Burkina/INERA	CHOMRONG DHAN	Laos
15	IRGA318-11-6-9-2B		L-22-26-WAC B-TGR4-B	AfricaRice	FOFIFA 161	Madagascar
16	IWA 2	Nigeria	DKA-M2	Mali/IER	scrid079-1-5-4-2	Madagas-

S/No or Code	Upland (Benin; Congo Brazzaville; Mali Nigeria; Cameroun; Guinee		Lowland (Côte d'Ivoire; Egypte; Niger; Tchad; Sierra Leone; Burkina Faso; Gambie; Ghana; Liberia; Senegal, Tanzanie)		Highland (Rwanda; Togo; Madagascar; Burundi)	
	AfricaRice varieties					
	Designation	Origin	Designation	Origin	Designation	Origin
17	NERICA 1	AfricaRice	TXD 88	Tanzania	FOFIFA172	Madagas-
18	NERICA 11	AfricaRice	WAB 2061-2-FKR1-WAC2-TGR4-B	AfricaRice	NR 11	
19	NERICA13	AfricaRice	WAB 2098-WAC2-1-	AfricaRice	scrid113-3-5-3-5-4	
20	NERICA15	AfricaRice	WAB 2098-WAC3-1-	AfricaRice	SIM 2 SUMADEL	
21	NERICA3	AfricaRice	WAB 2101-WAC4-1-	AfricaRice	SKAU 27	
22	NERICA5	AfricaRice	WAB 2125-WAC B-1-TGR3-WAT B1	AfricaRice	SKAU337	
23	NIRICA12	AfricaRice	WAB 2098 -WAC3-1-TGR2-WAT B5	AfricaRice	scrid094-4-1-4-1	Madagascar
24	OS 6	RDC	WAB 2066-6FKR4-WAC1-TGR1-B-WAT-	AfricaRice	RCPL 3-6	
25	PANAMA 1048	AfricaRice	WAB 2076-WAC1-TGR1-B	AfricaRice	scrid006-2-4-3-4-5	Madagascar
26	scid006-2-4-2-3	Madagascar	WAB 2134-WAC B-TGR1-B	AfricaRice	88024-TR 1049-3-1-1-1	
27	scrid019-1-1-1-1	Madagascar	WAB 2153-TGR3-WAT B5	AfricaRice	WAB56-104 AfricaRice	
28	scrid113-3-5-3-5	Madagascar	WAB 2101-WAC1-1-TGR5-WAT B6	AfricaRice	WAB181-18	AfricaRice
29	SIN EKARI		WAB 2081-WAC2-2-TGR2-WAT B3	AfricaRice	scrid014-1-1-1-1	Madagascar
30	TOX 1779-3-3 -201-1B	Nigeria	WAB 2094-WAC2-TGR2-B	AfricaRice	scrid017-1-4-4-4-1	Madagascar
31	WAB181-18	AfricaRice	NERICA-L19	AfricaRice	V 564-2-7	
32	WAB56-104	AfricaRice	WITA4	AfricaRice	V 1380-4	

Isolation of the bacteria

Bacteria were isolated from naturally infected seeds. One hundred (100) seeds per variety per country, were randomly taken from each envelop, cleaned with 5% sodium hypochlorite, rinsed with sterile distilled water and dried on Whatman paper. Bacterial isolation was conducted using Nutrient Agar and Nutrient Broth Yeast extract (NBY) media following standard procedures (ISTA). The seeds were cultured on NBY with 8 g/L nutrient broth, 2 g/L yeast extract, 0.5 g/L KH2PO4, 2 g/L KHPO4, 2 g/L glucose, 15 g/L agar powder, and 1000 ml of sterile distilled water. The seeds were divided into 4 replicates of 25 seeds per Petri dish and incubated in an oven (LAB-LINE L.C) at 28°C for 3 days. The bacteria colony growing around each seed was isolated using a sterile loop and then purified on a semi-selective Peptone

Sucrose-Agar (PSA) medium containing 10 g/L peptone, 10 g/L sucrose, 1 g/L Na-glutamate, 15 g/L Agar, and 1000 ml of sterile distilled water (Poulin et al 2014). Physicochemical catalase and gram tests were performed to select gram negative bacteria using the method of (Schaad 2001).

Identification of bacteria by biochemical tests

The standard biochemical Gram test as described by Lelliott and Stead 1987 were used for the identification of bacteria after purification. A sterile single-use toothpick was used to collect the 24-48 h pure colony of bacteria to be tested. The collected bacteria were rapidly immersed in one drop aqueous solution of 3% KOH on a microscope slide for 15 sec. A positive KOH test is characterized by the formation of a viscous suspension, while for a negative KOH test the suspension remain at the initial state. (Paray et al 2023)

DNA extraction, PCR and sequencing

Total genomic DNA was extracted from isolated bacteria using a commercial kit (Wizard Genomic DNA Purification kit, Promega Co.) following the manufacture's protocol. The DNA was quantified spectrophotometrically (PerkinElmer, Singapore) and DNA quality was checked by electrophoresis on 0.8 % agarose gel. DNA gyrase subunit B, gene (gyrB) was amplified using primers gyrB 07-F 5' GTV CGT TTC TGG CCV AG and gyrB 08-R CTT TAC GRC GKG TCA TWT CAC 3' for Pantoea species (700 bp), (Brady et al 2009) (Table 2). PCR conditions consisted of an initial denaturing step at 94 °C-3 mn, 35 amplification cycles (94 °C-30 s/60 °C-30s/72 °C-1 mn) and a final extension step of 72 °C-10 min. The PCR mixture contained nanopure water, 10 mM dNTPs mix, 25mM MgCl2, Taq buffer 5X, 2 µl Taq polymerase and 10 mM of each primer. The amplification products were separated by electrophoresis on a 1% agarose gel. The amplification products were purified with a QIA quick PCR purification kit (QIAGEN QIQamp DNA Mini Kit). The PCR product was sequenced directly from the PCR reactions and from recombinant plasmids, using the primers gyrB 07 and gyrB 08. DNA fragments were sequenced using the BIG Dye Terminator v3.1(ABI PRISM® 377 Bioanalyzer DNA Sequencer) cycle sequencing kit, with 96-Lane Upgrade instruments. The sequenced products were analyzed using MEGA 7.

Pathogenicity test

The pathogenicity test was conducted at AfricaRice Plant Pathology Unit's greenhouse (confined room). The experimental setup consisted of a randomized block design with three (03) repetitions. Two bacteria isolated from seeds obtained from Côte d'Ivoire and Senegal were plated on PSA medium, purified through subculturing on the same medium, and then quantified using a spectrophotometer (600nm). Inoculation was carried out on 35-day-old plants from 07 elite rice varieties (IR24, ADNY 11, Azucena, Moroberekan, Gigante, Sahel 201 and PNA 647 F4-56), using the leaf-clipping method described by (Kauffman et al 1973). Scoring was carried out 15 or 21days after inoculation by measuring the length of the lesions on ten leaves per variety, isolate and repetition. An inoculated variety was considered susceptible to the disease when the length of the lesions is greater than 5 cm to 4 cm or 3 cm, as shown for bacterial blight (Jeung et al 2006; Djèdatin et al 2012). To meet the requirements of Koch's postulate, the bacteria were re-isolated and confirmed by polymerase chain reaction (PCR) and sequencing (Janse and Roodt 2005). The data collected (length of lesions) were analyzed using analysis of variance (ANOVA).

Results

Morphological identification of isolated bacteria

Visual examination revealed three distinct morphological groups of bacterial colonies B1, B2, and B3 based on their color. Group B1 exhibited light-yellow appearance colonies, while B2 consisted of egg-yolk colonies, and B3 appeared whitish and pasty (Figure 2) Only light-yellow (B1) colonies and the egg-yolk colored (B2) were isolated and purified on PSA medium without antibiotics because that it was reported that Pantoea species are yellow color.

Table 2. Characteristics of primers used for *Pantoea* diagnosis

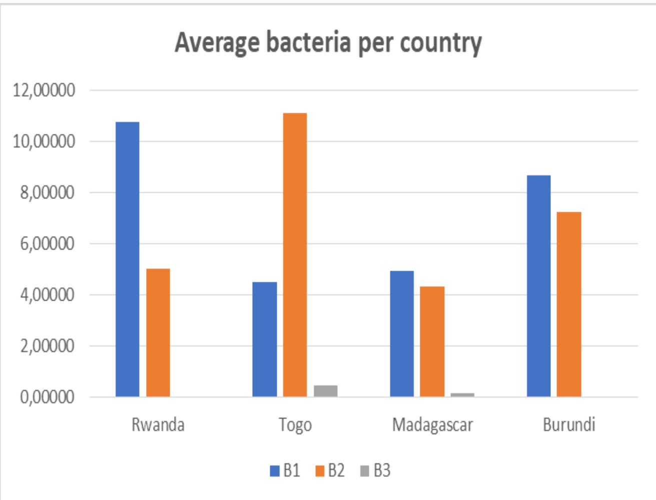
Bacteria	Primers	Sequences 5'-3'	size	Amplified region	PCR Conditions
Pantoea spp.	gyrB 07-F	GTV CGT TTC TGG CCV AG	700	GYRB	94 °C/3 mn, 35 X (94 °C 30 s/60 °C 30s /72 °C 1 mn),t 72 °C/ 10 min
Pantoea spp.	gyrB 08-R	CTT TAC GRC GKG TCA TWT CAC			

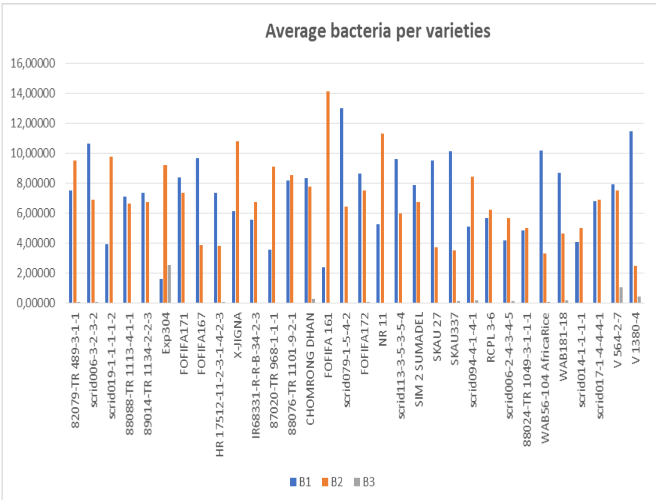
A total of 366 bacteria with B1 and B2 morphology were isolated from samples across 21 countries. Gram tests revealed that all bacteria with B1 and B2 aspects are Gram-negative. Subsequently, all of them were subjected to a catalase test. The observed effervescence in all colonies confirmed the presence of catalase, suggesting these bacteria are likely aerobic. Then, the average of B1, B2 and B3 bacteria was identified according to the varieties used in each test, and according to the countries was done (Figure 3).

The prevalence of group B1 bacteria, morphologically similar to *Pantoea sp*, was evaluated in each country, of each type of ecology. It is observed in the various tests that, in the lowland ecology zones, the bacteria of group B1 are higher in Niger, and more prevalent on the genotype WAB 2125-WAC B-1-TGR3-WAT B8 (code 13 / AfricaRice). In the Upland zone, the bacteria of this group are superior in Guinea Bissau, and on the variety CT11891-3-3-3-M-1-3-1-M (code 11 CIAT/Colombia). Finally, in the Highland ecology, the B1 are more numerous in Rwanda, and prevail over the variety scrid079-1-5-4-2 (code 16 /Madagascar)

Figure 2: Rice seeds plated on NBY medium, photographed three days after the appearance of B1, B2 and B3 bacteria

Highland Ecology





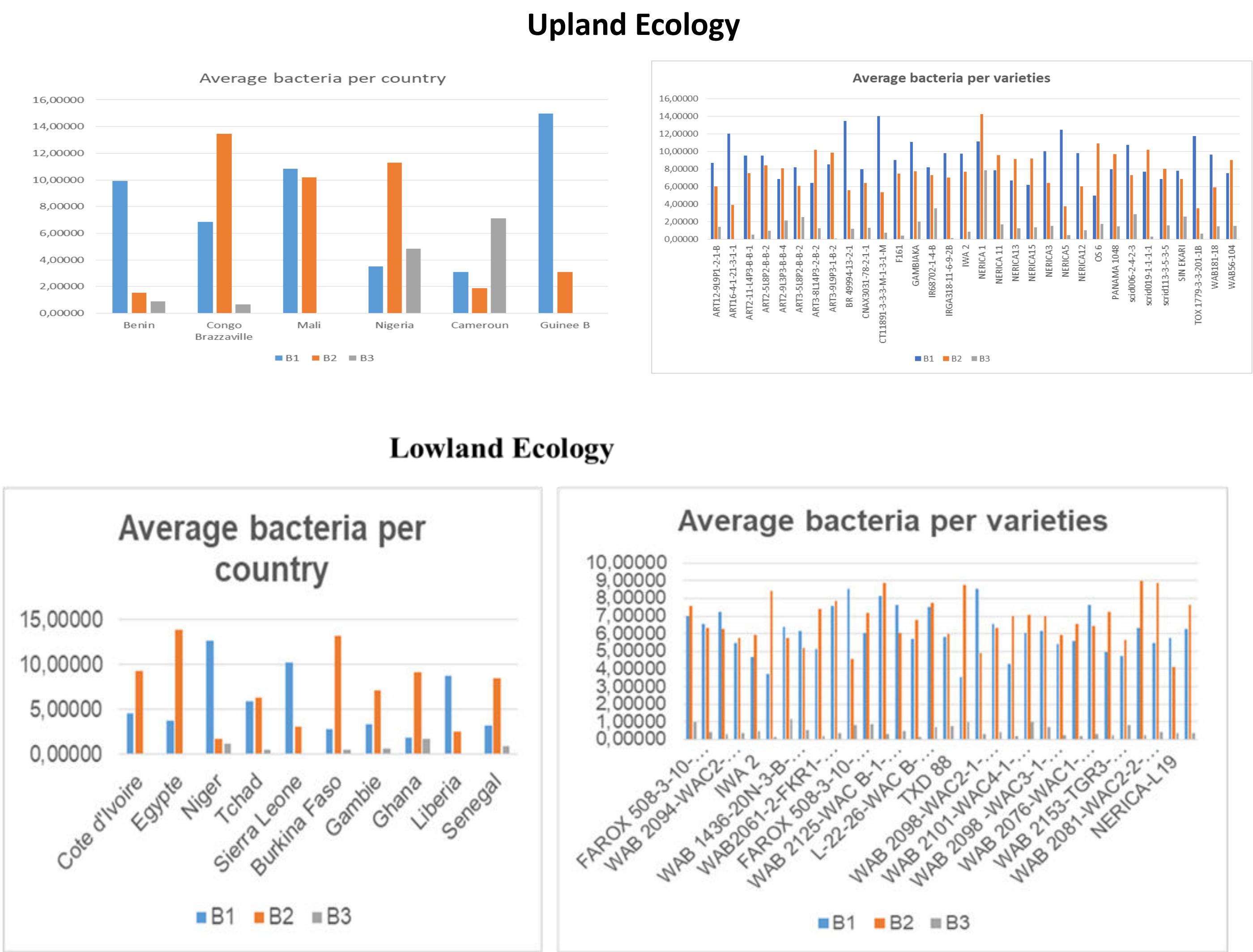


Figure 3: Averages of purified bacteria by country and rice variety, according to Highland, Upland and Lowland ecologies.

Identification of different Pantoea species in seed samples based on PCR techniques

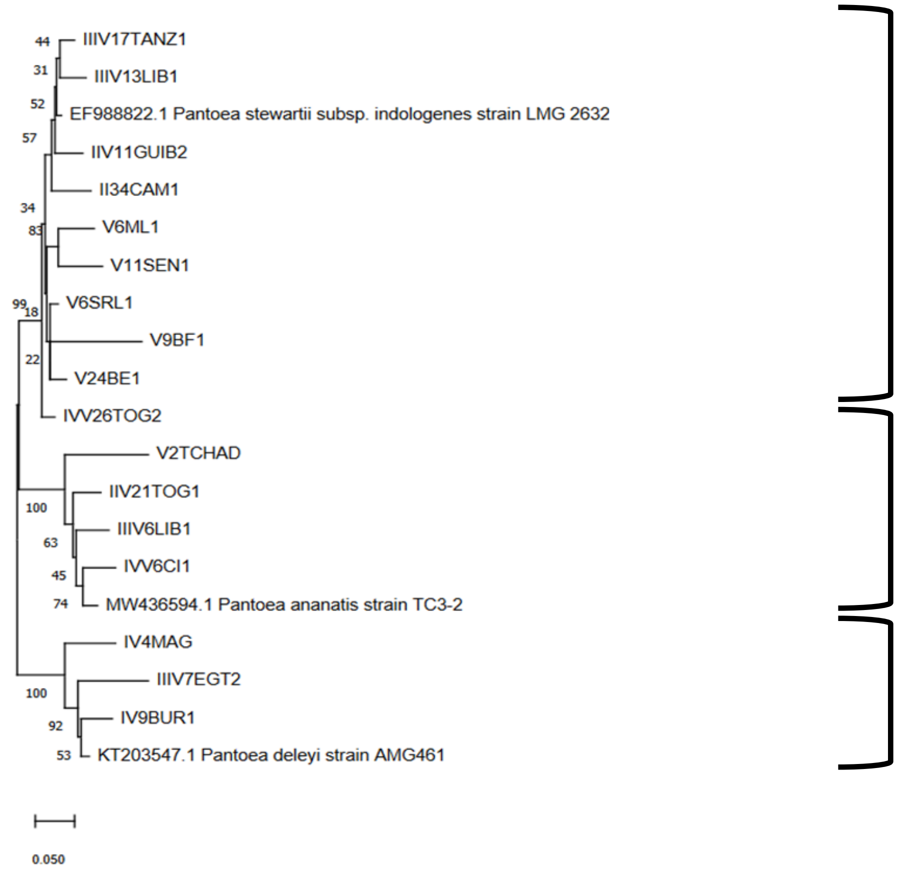
Amplification of gyrB was successful for 322 out of 366 samples. Two positive samples per country were sequenced (total n= 42). From the 42 sequences, BLAST revealed that several species of bacteria identified from this samples are belonging to the Enterobacteriaceae family (Table 3) and (Figure 4). 03 species of Pantoea have been identified: Pantoea stewartii was the most prevalent group of bacteria, found on seeds from 11 of the 21 countries. Pantoea ananatis were found on seeds from Togo, Tchad, Côte d'Ivoire and Liberia. and Pantoea deleyi in Egypt, Madagascar and Burundi.

Pathogenicity of P. Stewartii isolate V11SEN1 and P. ananatis isolate IVV6CI1 on IR24, ADNY 11, Azucena, Moroberekan, Gigante, Sahel 201 and PNA 647 F4-56

Pathogenicity assays demonstrated that P. stewartii and P. ananatis have statistically identical pathogenic potential. Both were virulent against all seven varieties, with highly significant differences (P<0.001) observed between the varieties (Table 4). Lesions longer than 3 cm were frequently noted (Fig 5). The Student Newman-Keuls (SNK) test revealed that Moroberekan and Gigante exhibited the highest severity values.

Table 3: Identification of the bacterial isolates

Isolate	Country of origin	Sampling area	Length (bp)	Sequence coverage	e-value	Percent Identity	Closest organism after BLAST com-
V24BE1	Benin republic	Central re-gion at	700	91%	0.0	99%	***Pantoea stewartii*** EF988822.1
V9BF1	Burkina Faso	Vallee re-	700	95%	0.0	97%	
V6ML1	Mali	Sikasso	700	92%	0.0	98%	
IIIV13LIB1	Liberia	Suakoko	700	91%	0.0	99%	
IV2NGA1	Nigeria	Sabon Daga	700	90%	0.0	99%	
V11SEN1	Senegal	Ndiaye	700	95%	0.0	99%	
V6SRL1	Sierra Leone	Rokupr	700	96%	0.0	98%	
IVV26TOG	Togo	Danyi	700	95%	0.0	98%	
IIIV17TAN	Tanzania	Ifakara	700	97%	0.0	99%	
II34CAM1	Cameroun	Garoua	700	95%	0.0	99%	
IIV11GUIB	Guinee Bissau	Cabonxanq	700	97%	0.0	96%	
IVV6CI1	Côte d'Ivoire	Gagnoa	700	96%	0.0	98%	***Pantoea ananatis*** MW436594.1
IIIV6LIB1	Liberia	Suakoko	700	92%	0.0	98%	
IIV21TOG1	Togo	Danyi	700	95%	0.0	99%	
V2TCHAD	Tchad	Mala	700	90%	0.0	95%	
IIIV7EGT2	Egypt	Sakha	700	97%	0.0	94%	***Pantoea deleyi*** KT203547.1
IV4MAG	Madagascar	Mahitsy	700	97%	0.0	96%	
IV9BUR1	Burundi	Ndebe	700	94%	0.0	98%	



Group 01: ***Pantoea stewartii***:

Identified in seed from Tanzania, Liberia, Guinée Bissau, Cameroun, Mali, Senegal, Sierra Leone, Togo, Burkina-Faso, Benin

Group 02: ***Pantoea ananatis***:

Identified in seed from Togo,

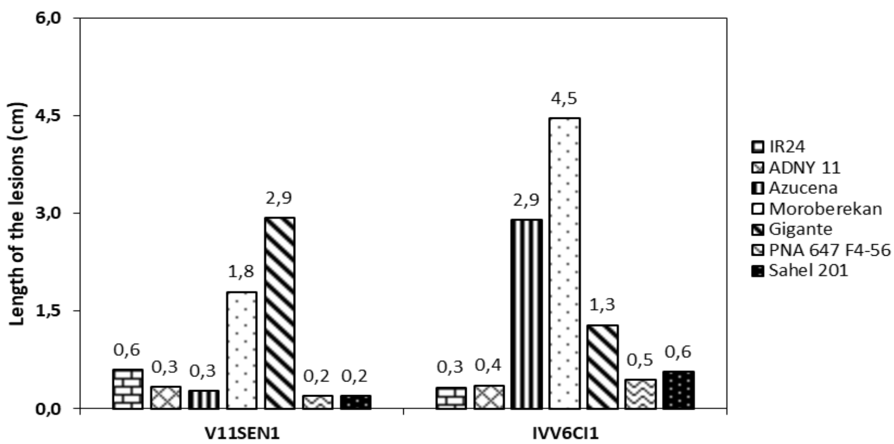
Group 03: ***Pantoea deleyi***:

Identified in seed from Burundi,

Figure 4: Phylogenetic neighbor joining tree based on the concatenated partial sequences of gyrB showing the phylogenetic relationships between type strains of the genera *Pantoea*

Table 4. Analysis of variance (ANOVA) of the effect of the two isolates of *Pantoea* on the seven varieties of rice

Source of variation	Df	Mean square	F value	Pr
Varieties (V)	6	0.2467	8.021	<0.001
Isolates (I)	1	0.1146	2.899	0.0909
V x I	6	0.7308	4.73	<0.001



(R): Resistant; (MR): Moderately Resistant; (MS): Moderately Susceptible

Figure 5: Lesion length on seven varieties of rice caused by isolates of *Pantoae stewartii* (**V11SEN1)** and *Pantoea ananatis (***I*VV6CI1)***

Discussion

The Enterobacteriaceae bacterial family contains some of the most devastating human and animal pathogens. Enterobacteriaceae also include important plant pathogens, such as Pantoea which were dectected in the current study. Several studies have reported the harmful effects of *P. stewartii*; *P. ananatis* and *P. deleyi*, in agriculture. Several species of Pantoea have already been described as causative agents of devastating rice diseases, leading to significant economic losses in rice production worldwide (Azizi et al 2019; Azizi et al 2020; Berinson et al 2020). A recent study has shown that rice bacterial blight, a devastating disease that threatens rice production worldwide, and traditionally caused by *Xanthomonas oryzae* pv. oryzae can also be caused by the two different Enterobacteriaceae, specifically *Enterobacter asburiae* and *Pantoea ananatis* (Xue et al 2021).

This study identified several Pantoea species associated with rice seeds from various African countries. This finding aligns with previous research demonstrating the widespread presence of Pantoea across diverse environments like water, soil, plants, and even animals. Similar to (Dossou and Silue 2018) where Pantoea species were previously reported to be associated with seeds of rice collected commonly with our study in ten African countries, namely Benin, Togo, Burundi, Mali, Cameroon, Senegal, Central African Republic, Nigeria, Cote d'Ivoire and Tanzania. With additional 7 countries detected Pantoea in rice seeds from bring in total 17 African countries including Liberia, Guinea Bissau, Sierra Leone, Burkina Faso, Tchad, Egypt and Madagascar.

The findings of this study indicated that *Pantoea stewartii* and *P. ananatis* may be pathogenic and virulent to rice seedlings. This corroborates with the findings of other authors who reported a diverse range of bacterial species from African countries capable of infecting rice including *P. ananatis* and *P. stewartii* (Kini et al 2016). The virulence of those species could be explained by the fact they Gram-. Gram-negative are virulent and they produce a range of virulence factors that enable them to cause infection. Gram-negative bacteria, with their thin peptidoglycan layer and outer membrane, may be more virulent than Gram-positive bacteria, which have a thicker peptidoglycan layer but lack an outer membrane. Variations in response to *P. ananatis* and *P. stewartii* infection were evident among rice varieties where Moroberekan and Gigante emerged as the most susceptible varieties. Moroberekan and Gigante varieties may possess inherent genetic weaknesses that make them more susceptible to *P. ananatis* and *P. stewartii* infection compared to other tested varieties. These weaknesses could be related to lack of specific resistance genes, thinner cell walls or weaker physical barriers or less efficient defense signaling pathways. It was reported that some varieties may have genes coding for resistance factors that can directly attack or inhibit the growth of the bacteria or that allow the plant to detect and respond to pathogen invasion, triggering defense mechanisms. Increased survey efforts to examine the prevalence of the bacteria *P. stewartii* and *P. ananatis* is merited as varieties such as Moroberekan, Gigante and Azucena are highly sensitive even though they are known to be resistant to other important rice diseases in Africa. For example, Moroberekan is resistant to blast (Séré et al 2013), while Gigante and Azucena are resistant to Rice Yellow Mottle Virus (Ndjiondjop 1999). These varieties are widely used by breeders in the creation of varieties resistant to blast and RYMV

These bacteria seem to represent a new threat in agriculture, and in rice cultivation for Africa. Moreover, because of their association with seeds, it is important to address plant quarantine issues for the continent (Ashura et al 1999).

To prevent the spread of these new pathologies, selecting for host resistance is a commonly used means, particularly in rice, where several varieties containing genes for resistance against the RYMV virus have been popularized (Ndjiondjop et al 1999; Pidon et al 2020). If further tests with more isolates of *P. stewartii* and *P. ananatis* confirm the susceptibility of important rice varieties, this information should be taken into account in the varietal improvement schemes for rice in Africa.

Conclusion

The pathogenicity of *P. ananatis* and *P. Stewartii* isolated from rice seeds in Africa on seven elite varieties showed and that they are seedborne transmitted pathogens. This indicates they were the most abundant and contagious among the bacteria species identified. They showed highest pathogenic potential on Moroberekan and Gigante that were the most susceptible varieties. Given their presence, incidence and virulence in many varieties grown in African countries, it is important to develop efficient pathogen surveillance and management methods against these seedborne pathogens.

Acknowledgments

We extend special thanks to: IITA-Ibadan Biosciences (Nigeria), IITA (Benin), CIMMYT GHU (Mexico), IRRI GHU & Plant Pathology (Philippines), AfricaRice Biotechnology & GHU Laboratory, ESA/Togo, Nangui Abrogoua University, who made this work possible.

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