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Research Article

Diversity of the *Ralstonia Solanacearum* Species of Potato Seeds in Mali

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Abstract

Potato brown rot was classified as a quarantine disease because of the complexity of the pathogen *Ralstonia solanacearum L.*, formerly called *Pseudonomas* or *Burkholderia solanacearum*. This same bacterium causes the disease bacterial wilt. It is a multifaceted bacterium hence the complex name *Ralstonia solanacearum* (CeRs or RASLSSO) or BW (in English). It attacks several plant families, notably nightshades such as potatoes; the tomato; eggplant; chili pepper; tobacco…etc. The potato is the most cultivated plant worldwide. Mali is the second largest producer in West Africa after Nigeria. It imports more than 9,000 to 11,000 tons of seeds each year. Potato cultivation is faced with several biotic and abiotic attacks. Among all these attacks, those caused by bacteria cause the most significant damage. In addition, diseases caused mainly by the *Ralstonia solanacearum* complex lack adequate control solutions. Despite everything, today, very few studies are carried out to identify these bacteria in order to prevent their spread in the soil in Mali. This is why the objective of this study is to identify the bacteria responsible for brown rot in potato seeds in Mali. To achieve our objective, a total of 63 samples were taken from nine varieties of imported and locally multiplied potato seeds. The agent responsible for brown rot was isolated on the Triphenyl-Tetrazolium-chloride culture medium, and several microbiological, biochemical and molecular tests were carried out to identify the different isolates selected. As the main results of this study: twenty-seven bacterial isolates, capable of causing brown rot of potato seeds were isolated, the biochemical tests carried out on these isolates showed that they all of race3 and twenty -four isolates were biovar 3; one from biovar 2 and two from biovar 6. The molecular studies carried out using universal primers confirmed the presence of the species *Ralstonia solanacearum* with two phylotypes (I and II).

Keywords

Seeds, Potato, Brown Rot, *Ralstonia Solanacearum*, PCR

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1. Introduction

The potato (Solanum tuberosum) is an annual plant of the Solanaceae family [1]. It is the main non-cereal food in the world, after corn, wheat, and rice with a global production of 437 million tons on an area of 20.7 million hectares [1]. This culture has developed well in Asia unlike Africa. Speculation gains popularity every year on the African continent. From 2006 to 2018, production increased because of its nutritional importance, employment and source of income [1, 2].

Mali was the second producer in West Africa after Nigeria and first in French-speaking Africa with an average production of 250,000t to 310,000 tons during the 2019 agricultural campaign [3]. Sikasso, the third region of Mali, represents the potato growing area par excellence in Mali and represents the largest production basin. It produces three quarters of national production, or 80% [4]. The potato has been classified as a strategic crop in Mali [5]. From the introduction of the crop in Mali, all seeds were imported until 2018, i.e. 9,000 to 11,000 tons per year. The local multiplication of Elite (E) seeds in class A was implemented from the 2017-2018 agricultural campaign until the present day (2022-2023) by the Potato and Potato Sector Support Program and Milk (PAFA 1) in the Sikasso region in synergy with the Green Innovation Center (CIV/AFC/GIZ). From 2018 to 2023, around 5% of seeds were multiplied locally.

Unfortunately, in recent decades, potato production in Mali has been confronted with several attacks of pathologies, particularly bacteria [6], causing significant economic damage among which, that responsible for the brown rot Ralstonia solanacearum, the most devastating in the world according to [7]. It causes considerable losses of around 50% to 100% on potatoes. [7, 8]. These different pathogens are represented by quarantine bacteria (those responsible for ring rot and brown rot) and quality bacteria (those causing soft rot and common scab diseases). Seeds and soils are the main factors in transmitting the pathogen. Dissemination is favored by water irrigation and work tools [9]. Generally speaking, the pathogen spreads from the mother tuber to the child tubers, up to the stem [10]. For this, strict monitoring of the agent responsible for the disease has been put in place in the nightshade production areas, particularly those of potato seeds throughout the world. In particular, measures to prevent its introduction from one territory to another. It has been classified as a dangerous organism in all continents of the world. It is an organism with obligatory control in many countries where its presence has been declared such as in Mali and quarantine in continents where it is not present in the OPPE.

In Mali, very few studies have been carried out on potato pathogens in general and the bacteria responsible for brown rot in particular.

It has been declared a compulsory control organism in many countries, such as Mali. Hence this study on the characterization and evaluation of the diversity of the agent responsible for brown rot in potato seeds imported and produced in Mali.

2. Material and Methods

2.1. Biological Material

Twenty tubers from nine potato varieties (Claustar, Evora, El Beïda, Liseta, Mondial, Sahel, Sagitta, Sifra and Spunta) were collected randomly from four local potato seed multipliers and three seed importing companies in the Sikasso and Ségou regions.

2.2. Microbiological Characterization

2.2.1. Sample Preparation and Seeding

Different analyzes were carried out on the sixty tubers taken randomly from boxes of 25 kg of potato seeds. From each case, samples were taken three times. The tubers were washed well with tap water to remove all impurities from the outside of the samples without damaging the seed coat. They were dried at room temperature on sterilized blotting papers. The tubers, previously cleaned, were weighed. Two hundred and fifty grams of tubers from each dried sample were weighed. They were disinfected using chloramine T (2%) and ethanol (70%) for three minutes then rinsed with sterile distilled water. The samples were then cut into two parts on aluminum foil with a clean knife (well disinfected with 96% ethanol) and flambéed under the hood. The tubers were cut into small pieces and inoculated on the Triphenyltetrazolium Chloride (TZC) medium used to distinguish R. solanacearum among other bacteria, then incubated at 28°C [11].

2.2.2. Macroscopic Characterization

After incubation, colonies with the typical characteristics of the bacteria responsible for brown rot were selected and purified. Selection criteria were based on shape, color, consistency, contour, size and odor.

2.2.3. Microscopic Characterization

(i). Gram Stain

Its objective is to be able to make a preliminary selection of purified isolates based on their coloring: gram positive or negative. It was carried out using smears of colonies of different bacterial isolates on a microscopic slide. After carrying out the gram staining technique, a microscopic observation was made to have the shape and color of bacteria present in the colony.

Gram staining makes it possible to differentiate between bacteria according to the properties of the wall. Gram-positive

bacteria stain purple, Gram-negative bacteria pink [12].

(ii). Mobility Test

This test was carried out on the bacterial isolates after 24 hours of incubation at 28°C. A little quantity of each bacterial culture was taken using a sterile Pasteur pipette and placed on a slide covered with a coverslip. The slide thus prepared was directly observed under an optical microscope at 40x magnification [12].

2.3. Biochemical Characterization

The biochemical tests were based on the production of oxidase, catalase, KOH and of fulminous pigments.

2.3.1. Oxidase and Catalase Production Test

The oxidase test consisted of Place 2 to 3 drops of a 1% solution of tetra-methyl-p-phenyl diamine dihydrochloride directly onto a young colony of bacteria on a clean slide. The appearance of a purple color within 1 minute indicated the presence of an oxidase-positive bacteria (oxidase +).

To carry out the catalase test, the different isolates were placed directly on a slide. Then, using the Pasteur pipette, a drop of hydrogen peroxide (H_2O_2) was placed on the bacterial culture. The formation of bubbles corresponds to the production of catalase, therefore to a catalase-positive bacteria (catalase +), [12].

2.3.2. KOH 3% Test

A drop of 3% KOH was placed on a clean glass microscopic slide. Then, a colony of each typical bacteria was emulsified into the drop using a toothpick for 5–10 seconds. The formation of a viscous filament (Agglutination) when lifting the toothpick is referred to as Gram negative (KOH positive).

On the other hand, in the absence of filament, the Gram will be considered positive (KOH negative) [12].

2.3.3. Fluorescent Pigment Production Test

The selected isolates, cultured on King B medium in Petri dishes, then incubated for 48 hours at 30°C, were illuminated with an ultraviolet ray lamp, in a dark enclosure. The petri dish exhibiting bright luminosity in the dark is fluorescent [13].

2.3.4. Pot Pathogenicity Test on Potato Plants

It makes it possible to identify among the isolates tested those which can cause wilting of plants. The variety used was Naïma, resulting from local propagation. Its growing cycle is 80 days. Young bacterial colonies aged up to 48 hours were inoculated in bottles containing 100ml of Kelman's liquid agar medium, without Tetrazolium. The bacterial solutions were stirred for 48 hours. Infiltration was carried out using a sterile syringe with three repetitions per isolate. The roots were wounded before deliberately inoculating them with a disinfected knife to allow direct contact between the inoculum and the sap-conducting vessels. A total of 78 plants aged 30 days maximum were inoculated with the 27 bacterial isolates and the liquid medium without inoculum was used as a control. The plants were stressed at least 16 hours before and 24 hours after inoculation during the cool period. [14]. Observations were conducted 72 hours up to 30 days after inoculation. They were carried out on the number of withered plants (figure 2).

2.3.5. Identification of Biovars

The technique which makes it possible to distinguish the different biovars of *Ralstonia solanacearum* within the different isolates was based on biochemical tests relating to the abilities of the isolates to use and/or to oxidize three disaccharides, in particular maltose, lactose and cellobiose as well as three hexose alcohols mannitol, sorbitol and dulcitol (table 1), [15, 16].

The basic medium used for this purpose consists of this 1.0 g Bacto-peptone, 1.0 g $NH_4H_2PO_4$, 0.2 g KCl, 0.2 g $MgSO_4$, $7H_2O$, 0.03 g Bromothymol Blue, 3. 0 g of Agar in a liter of distilled water 1,000 ml [15-17].

The tubes containing the different sugars were inoculated by taking colonies of pure isolates using sterile toothpicks. Each of the seeded isolates was replicated three times per sugar and per alcohol. The tubes were incubated at 28°C and examined at 48 h and 72 h. A color change from olive green to yellow indicating a change in acidic pH, the test result would be positive, [18, 19].

For sugar alcohols, the reaction took place between 3 and 5 days and a little longer for disaccharides. The type of Biovar was determined according to its ability to oxidize each substrate (Table 1).

Table 1. *Biovar determination of Ralstonia solanacearum isolates based on acid production through the use of various carbohydrates.*

Substrates	Biovars						
	1	2	3	4	5	6	3b
Cellobiose	-	+	+	-	+	+	+
Maltose	-	+	+	-	+	+	+

Substrates	Biovars						
	1	2	3	4	5	6	3b
Lactose	-	+	+	-	+	+	-
Dulcitol	-	-	+	+	-	-	-
Sorbitol	-	-	+	+	-	+	+
Mannitol	-	-	+	+	+	+	+

+: Use of the substrate; -: Substrate not used
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2.3.6. Determination of Breeds by Tobacco Hypersensitivity Test

The tobacco hypersensitivity test was developed by [21] and used to distinguish between races 1, 2 and 3 of the species *Ralstonia solanacearum*. A density of a bacterial suspension of approximately 108 CFU/ml, aged (24 to 48 hours) was injected between the epidermis and the palisade parenchyma of young tobacco leaves aged 22 days. Infiltration of the isolates was done using a needleless syringe, by carefully pressing the tip of the syringe on the lower part of the leaves. Observations were made 24,48,72 and 192 hours after infiltration according to the scheme of [21]. The test was considered positive if a necrotic area corresponding to the infiltrated area appeared after 7-8 days, indicating race 1. Isolates showing a reaction between 12 -24 hours, indicating race 2 and race 3 a been indicated by a reaction between 2-8 days. The test is negative if no reaction is observed.

2.4. Molecular Characterization

2.4.1. DNA Extraction

A sample of young bacterial colonies aged 24 hours was placed in 1.5ml Eppendorf tubes containing 500ul of NaCL. The mixtures were centrifuged for 2 min at a speed of 10,000 rpm and then the supernatants were discarded. Seven hundred and fifty microliters (750 µl) of Cetyltrimethylammonium Bromide (TAB 3%) buffer solution were added to the pellet. The new mixture was incubated in a water bath for 30 minutes at 65°C then 400 µl of chloroform was added and centrifuged for 10 minutes at 10,000 rpm. The supernatants were transferred to a new tube containing 1 ml of 100% ethanol, then cooled on ice for 10 min for DNA precipitation. After centrifugation for 5 minutes, the pellet was collected then washed with 500 µl of 70% ethanol. The whole was centrifuged for 1 min. The samples were dried at room temperature for 1 hour. Then 150 µl of Nuclease Free water was added to the DNA pellets and vortexed to dissolve the pellet in order to obtain a homogeneous DNA suspension for carrying out the PCR. The DNA suspensions of the samples were stored at 4°C overnight.

2.4.2. Bacterial DNA Amplification

The DNAs were amplified with four pairs of primers specific to the different phylotypes of the species *Ralstonia solanacearum*. The primer names and sequences are listed in the table below.

Table 2. *Specific primers used in multiplex PCR to identify the species Ralstonia solanacearum and the Phylotypes belonging to it.*

Primer names		Sequences 5'-3'	Amplified fragment size	Phylotypes
Nmult	21:1F	CGTTGATGAGGCGCGCAATTT	144 bp	I (Asiaticum)
Nmult	21:2F	AAGTTATGGACGGTGGAAGTC	372 bp	II (Americanum)
Nmult	23:AF	ATTACSAGAGCAATCGAAAGATT	91 bp	III (African)
Nmult	22:InF	ATTGCCAAGACGAGAGAAGTA	213pb	IV (Tropical)
Nmult	22:RR	TCGCTTGACCCTATAACGAGTA	Reverse primer	Any phylotype
759	R	GTCGCCGTCAGCAATGCGGAATCG	280 bp (Internal marker)	*Ralstonia*
760	F	GTCGCCGTCAGCAATGCGGAATCG		

(Source: FEGAN and PRIOR, 2005)

A total reaction volume of 15 µl was formed for each DNA sample in the PCR tubes (trips of 8 PCR Tubes), consisting of 7.5 µl Go Taq G2 Hot Start Green Master Mix 2X (Promema), 1 µl (100pmol /µl) of the forward primer, 1 µl (100pmol/µl) of the reverse primer, 2µl (20ng/µl) of the DNA suspension and 3.5 µl of Nuclease-Free Water. Amplification was carried out using the 2720 applied System thermal cycler programmed as follows: initial denaturation: 94°C for 5 mins, denaturation: 94° for 1 min, hybridization: 55°C for 45 seconds, elongation: 72°C for 1 min 30, final elongation: 72°C for 10 minutes and conservation of the DNA: 4°C forever in order to multiply the target sequence(s) in large quantities. The denaturation, hybridization and elongation steps are repeated 35 times. The PCR was carried out in two phases; The first was the identification of the species using Oligo nucleotide primers 759R/760F according to the conventional protocol of [22], markers internal to the ceRs, for the diagnosis of *Ralstonia solanacearum* molecule. The second phase was based on the identification of the phylotype using multiplex PCR.

2.4.3. Electrophoresis and Visualization of PCR Products

6µl of the PCR product were loaded and migrated on a D1LOW EEO 1.5% (weight/volume) agarose gel which is recommended for the separation of DNA fragments. The migration was carried out for 1 h at 90V. After migration, the gel was prepared with 0.5X TBE buffer solution (Tris-acetic acid-EDTA). 30µl of 10% ethidium bromide, visualized, the gel was photographed under UV using the Gel DOC device. The size of the bands is estimated by comparison with the bands of the 100 bp molecular weight marker (Ladder).

2.5. Data Analysis

The size of the fragments (bands) obtained was determined using the ECap software. The presence of the 280 bp fragment, amplified by specific primer pair 759R/760F, indicates the presence of the species *Ralstonia solanacearum*. For the identification of phylotypes by multiplex PCR, the 144 bp fragment indicates the presence of phylotype I (Asiaticum), 372 bp indicates the presence of phylotype II (Americanum), 91 bp indicates the presence of phylotype III (African) and 213 bp indicates the presence of phylotype IV (Tropical) [22-24].

3. Results

3.1. Microbiological Characteristics

3.1.1. Colonies Isolated from Samples Collected

Thirty-one isolate withs characteristic colonies of the species *Ralstonia solanacearum* were retained and purified. Among them, seven were isolated from five imported potato varieties and fourteen from three locally produced varieties. The distribution of the isolates obtained according to origin and host varieties is recorded in Table 3.

Table 3. *Distribution of isolates according to origin and host varieties.*

Source of seeds	Potato host varieties	Name of isolates	Total number
Imported	Claustar	RIC1	13
	Mondial	RIM1, RIM2	
	Liseta	RIL1, RIL2	
	Sagitta	RISa1, RISa2	
	Sifra	RISi1	
	Spunta	RISp1	
	Evora	RIE1, RIE2	
	El Beida	RIEL1, RIEL2	
	Sahel	RLSh1	
Local	Claustar	RLC1, RLC2, RC3, RLC4, RLC5, RLC6, RLC7, RLC8, RLC9, RLC10	18
	Spunta	RLSp1, RLSp2, RLSp3, RLSp4 RLSp5, RLSp6 RLSp7	
Total			31

R.L.: Ralstonia from local production seed; RI: Ralstonia from imported seed; C-Claustar; Sp-Spunta; Si-Sifra; Sa-Sagita; El-Elbeïda; E-Evora; Sh-Sahel; M-World

3.1.2. Macroscopic Characters

Ofs mucous, pinkish-red and elliptical colonies, characteristic of the species *Ralstonia solanacearum*, were retained and purified (figure 1).

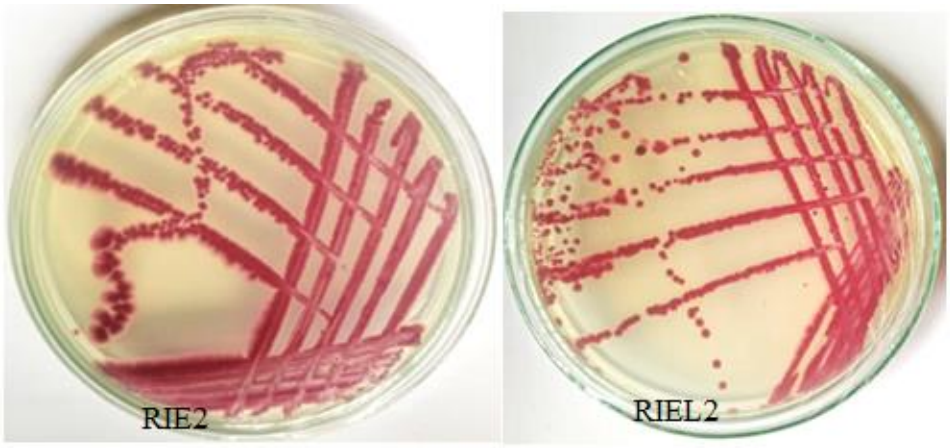

Figure 1. *Macroscopic appearance of some isolates on TZC solid media.*

3.2. Microscopic and Biochemical Characters

Among the thirty-one isolates, twenty-seven were characteristic of *Ralstonia solanacearum* by presenting themselves as Gram negative bacilli, KOH 3% positive andmobile thanks to their flagellum. The four other non-characteristic *Ralstonia* isolates were excluded from the list.

All isolates were positive for catalase and negative for fluorescent pigment production. Thirteen and fourteen isolates were positive and negative for catalase and oxidase, respectively. The KOH, catalase and Oxidase positive, Gram and pigment production negative isolates are characteristic of the species and were retained for further identification. The test results are reported in Table 4.

Table 4. *Microscopic and biochemical characteristics of the isolates according to the tests.*

Isolates tested	Testing				
	KOH	Gram	Catalase	Oxidase	Fluorescent pigment production
RISa1; RIE1; RIM1; RIEL1; RLSh1; RLSp1; RLSp2; RLSp3; RLSp4; RLC3; RLC6; RLC7; RLC8; RLC9; RLC10; RLSp7	+	-	+	+	-
RISi1; RISp1; RIE2; RISa2; RIEL2; RLC1; RLC2; RLC4; RLC5; RLSp5; RLSp6	+	-	+	-	-

R.L.: *Ralstonia* from locally produced seeds; RI: *Ralstonia* from imported seeds.

3.2.1. Pathogenicity of Isolates Retained on Potato Plants

After thirty days of observations, twenty-one isolates caused wilting on six-week-old potato plants of the "Naïma" variety (figure 2). The RLC4 and RLC6 isolates were more pathogenic showing symptoms from the first week after inoculation. The isolatesRIE2; RIM1; RLC3; RLC9; RLSp6 did not cause wilting on plants and was considered nonpathogenic (Table 5).

Table 5. *Reaction of bacterial isolates tested on potato plants in pots showing the number of plants infiltrated by the isolates and dead plants.*

Isolates tested	Number of plants infiltrated per isolate	Number of wilted plants per infiltrated isolate
RISa1; RISi1; RISa2; RISp1; RIE1; RIEL1; RIEL2; RLSh1, RLC1; RLC2; RLC4; RLC5; RLC6; RLC7; RLC8; RLC10; RLSp1; RLSp2; RLSp3; RLSp4; RLSp5; RLSp7	3	3
RIE2; RIM1; RLC3; RLC9; RLSp6	3	0

R.L.: *Ralstonia* from Local seed; RI: *Ralstonia* from imported seed; C-Claustar; Sp-Spunta; Si-Sifra; Sa-Sagita; El-Elbeïda; E-Evora; Sh-Sahel; M-World

Figure 2. *Potato plants of the Naïma variety after inoculation with bacterial isolates. A: Overview of wilted plants 15 days after planting without infiltration, LaboSem-Sotuba, 2020; B: Wilting of plants of the Naïma variety 9 days after inoculation with the bacterial isolate RIEL2, LaboSem-Sotuba, 2020; C: Wilting of plants of the Naïma variety 22 days after inoculation with the bacterial isolate RLSp7 LaboSem-Sotuba, 2020.*

3.2.2. Biovars

The test for the degradation of disaccharides and hexose alcohols was carried out on the twenty-seven isolates, the results are recorded in Table 6.

Table 6. *Distribution of isolates according to biovars after the test on disaccharides and hexose alcohols.*

Isolates tested	Disaccharides			Hexose alcohols			Biovars
	Cellobiose	Lactose	Maltose	Mannitol	Dulcitol	Sorbitol	
RLC6	+	+	+	-	-	-	2
RISa1; RISi1; RISp1; RIE1; RIE2; RISa2; RIEL1; RIEL2; RLSh1; RLSp1; RLSp2; RLC1; RLSp3; RLSp4; RLC2; RLC3; RLC4; RLC5; RLC7; RLSp5; RLSp6; RLC8; RLC9; RLSp7	+	+	+	+	+	+	3
RIM1; RLC10	+	+	+	+	-	+	6

RL: *Ralstonia* from Local seed; RI: *Ralstonia* from imported seed

3.2.3. Breed of Isolates

The results of testing the tobacco isolates showed that all isolates are race 3.

3.3. Molecular Characters

PCR to identify the species and its phylotypes showed that the isolates are of the species *Ralstonia solanacearum* divided into two phylotypes I and II (Table 7, Figure 3).

Table 7. *Distribution of isolates according to phylotypes obtained by PCR.*

Isolates	Phylotypes
RLSp6; RLC9	I
RISa1; RISi1; RISp1; RIE1; RIE2; RIM1; RISa2; RIEL1; RIEL2; RLSh1; RLSp1; RLSp2; RLC1; RLSp3; RLSp4; RLC2; RLC3; RLC4; RLC5; RLC6; RLC7; RLSp5; RLC8; RLC10; RLSp7	II

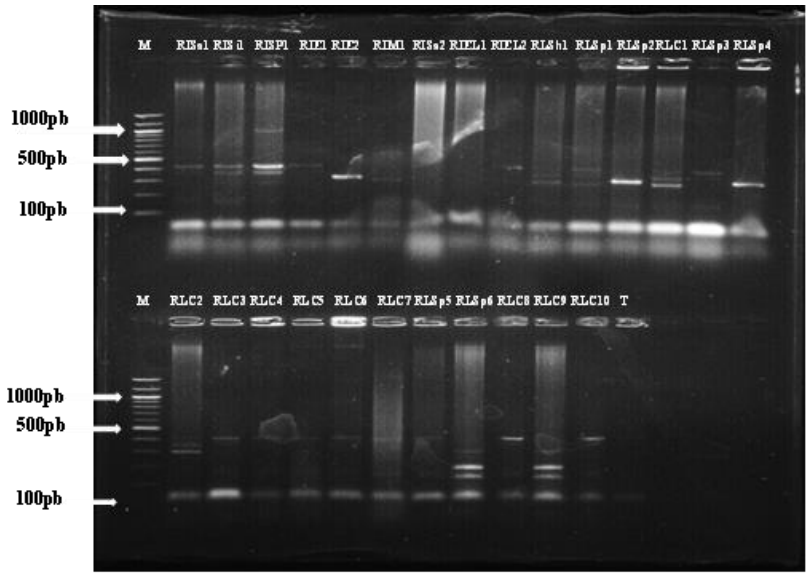

Figure 3. *Migration profiles of Ralstonia solanacearum isolates amplified with the multiplex primers Nmult:21:1F (phylotype I), Nmult:21:2F (phylotype II), Nmult:23:AF (phylotype III), Nmult:22: InF (phylotype IV); Nmult:22:RR on AgaroseD1LOW EEO at 1.5%, migrated to 90V for 1 hour. After migration, the gel was photographed under UV using the Gel Doc device. RLSp6, RLC9 (phylotype I); RISa1; RISi1; RISp1; RIE1; RIE2;; RISa2; RIEL1; RIEL2; RLSp1; RLSp2; RLC1; RLSp3; RLSp4; RLC2; RLC3; RLC4; RLC5; RLC7; RLSp5; RLC8; RLSp7; RLC6; RIM1; RLC10 (phylotype II).*

4. Discussion

Microbiological and biochemical tests made it possible to identify twenty-seven isolates of *Ralstonia solanacearum* in potato seed samples imported and locally produced in Mali from the 2017-2018 agricultural season. The number of isolated and purified bacterial isolates was higher with locally produced Claustar and Spunta varieties than imported ones. These results confirm those [25, 26] who reported the presence of *Ralstonia* strains on infected plants, potato tubers and other nightshades in Niger and Mali respectively. Furthermore, [27] identified eight *Ralstonia solanacearum* in samples of potato tubers collected from four districts of the Vakinankaratra region in Madagascar on Cassamino acid peptone glucose (CPG) culture medium. But first, [28] identified 24 strains of *Ralstonia solanacearum* during the integrated control of bacteriosis caused by the species on potatoes in small farms in the Vakinankaratra Region. Our results revealed that of the 27 *Ralstonia solanacearum* isolates identified, 40.74% were oxidase positive and 100% catalase positive. These results were different from those obtained by [29] who found 87% of his isolates catalase positive and 81% oxidase positive. These strains were isolated from infected tomato plants and soil in the Baguinéda Irrigated Perimeter

Office area in Mali. They agree with those of [27] of which out of eight isolates identified as *Ralstonia solanacearum,* 38% were positive for catalase and oxidase in four districts of the Vakinankaratra region.

The pathogenicity test of our strains on potato plants in pots showed great variability within the species with a variable degree of virulence between the strains. Strains RLC4 and RLC6 showed virulence by causing the death of plants after 7 days of inoculation. The RIEL1, RIEL2 and RLSp7 strains caused the death of the plants after 15 days of inoculation. On the other hand, 81.48% of the strains caused the death of the inoculated plants after 30 days of observation. These results allow us to explain that there is variability in virulence within the species *Ralstonia solanacearum*. [25] obtained 42.31% of its pathogenic strains on inoculated potato and tomato plants. From the first, the second week and the fourth week, variability was observed at the level of 80.77% of the strains causing the death of the inoculated plants. The same observation was made by [25] from the fourteenth, sixteenth and eighteenth day of observation. This author confirms the variability of the pathogenic power of the species *Ralstonia solanacearum* from samples of imported or locally produced potato seeds.

Three biovars were identified in this study: biovar 2, 3 and 6. The most dominant was biovar 3 with 88.5%, followed by biovar 6 with 7.7%. These results are different from those obtained by [26-29] who obtained only biovar 3. [25] obtained biovar 2 and 3 with a dominance of biovar 2 on potato plants and tubers grown from seeds imported from Europe. He had also identified two variables within biovar 2 (biovar 2 biotype 2A and biovar 2 biotype 2T). Biovar 2 biotypes 2A was the most dominant at 59.1%. Similarly, [27] obtained two biovars isolated from potato tubers in the four districts of the Vakinankarata region of Madagascar. Biovar 2 was also the most dominant with 25%. [30] had isolated and identified thirty-six strains of *Ralstonia solanacearum* on nightshade plants (eggplant, tomato, chilly, pepper) and other families (gourd and marigold) in five agro-climatic zones in India. The authors identified two biovars, biovar 3 and 6 with a dominance of biovar 3. These results are in line with ours where biovar 3 was the most dominant.

The leaves of tobacco plants aged twenty-one days after transplanting were inoculated with twenty-seven strains of *Ralstonia solanacearum* in the pots. Dark brown lesions, surrounded by a yellow halo, were observed after 24 hours of infiltration and developed from 48 hours to 72 hours, up to a week without the plants being wilted: this is race 3 Our results agree with those of [29] who also obtained race 3 using the same method. On the other hand, they are different from those of [26] who had obtained race 1. This race 1 is known for its broad spectrum of hosts including nightshades, notably potatoes and other weed plants. It is called the “African” race, [24-32]. Race 3 has been described as the pathogen of potato and tomato. It is specific to temperate zones and more widespread on the five continents [24-33]. Only race 1 was identified by [30] on nightshade plants (eggplant, tomato, chili, pepper) and other families (gourd and marigold). Our results agree with those of [34]. They were able to identify race 1 and race 3 in soil samples collected from thirty-four sites in seven major tomato bacterial wilt endemic areas in Nigeria. Race 1 was more dominant with 88.24% of strains.

5. Conclusion

The twenty-seven isolates were confirmed as *Ralstonia solanacearum* (ceRs.) strains by PCR with the use of the specific primer 759R/760F. Multiplex PCR confirmed the presence of phylotypes I and II in this study. According to them, Phylotype I has a wider host range than the other three [35-37]. [30] had 100% of its strains isolated as Phylotype I. Our results are in line with those [25] who obtained three phylotypes (I, II and III) with a dominance of phylotype II (61.54%).

The microscopic, biochemical and molecular characterization of the thirty-one isolates made it possible to select and isolate twenty-seven bacterial isolates of *Ralstonia solanacearum* strains from imported and locally produced potato seeds.

Strains were characterized as *Ralstonia solanacearum,* with diversity within the species. Two biovars and two phylotypes were identified within the diversity.

Abbreviations

PCR	Polymerization Chain Reaction
UV	Ultraviolet
Pb	Pair of Bands
Labosem-Sotuba	Plant Seed Certification Laboratory

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Conflicts of Interest

The author declares no conflicts of interest.

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