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Isolation and Characterization of *Ralstonia solanacearum* Causing Bacterial Wilt of Tomato in Nigeria

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Abstract

Bacterial wilt of tomato, caused by *Ralstonia solanacearum (Rs)*, is endemic in most tomato-growing areas of Nigeria, causing 60 to 100% loss in yield. Control measure requires definite information on race and biovar characteristics of the pathogen in those endemic areas. Soil samples were collected from seven states in Nigeria known for high incidence of tomato bacterial wilt. Isolations were performed on triphenyl tetrazolium chloride (TZC) and casamino peptone glucose (CPG) media. Morphological characterization of the pathogen was through simple staining, streaming and KOH solubility test. Molecular confirmation of pathogen's identity was through PCR amplification of genomic DNA using *Rs*-specific 759/760 primers (f:5'-GTCGCCGTCAACTCACTTTCC-3'; r:5'-GTCGCCGTAGCAATGCGGAATCG-3'). Race was determined through hypersensitive reaction on tobacco leaves and biovar characterization was through carbohydrate utilization test. Thirty-four bacterial isolates showed the characteristic creamy white colour on TZC + CPG medium. . The isolates also amplified at 280 bp, confirming the pathogen as *Rs*. Thirty isolates belonged to Race 1 Biovar III while four belonged to Race 3 Biovar II. The findings are relevant while devising a more targeted management approach to bacterial wilt of tomato in Nigeria.

Key words: Tomato, Bacterial wilt, *Ralstonia solanacearum,* pathogenicity, triphenyl tetrazolium chloride.

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Introduction

Ralstonia solanacearum (*Rs*) is a soil borne bacterial pathogen and a major limiting factor in the production of many crop plants around the world (Agrios, 1997). This organism is the causal agent of brown rot of potato, bacterial wilt or southern wilt of tomato, tobacco, eggplant and some ornamentals and moko disease of banana (Stevenson et al., 2001).

Bacterial wilt is a common bacterial disease in tropical, subtropical and some temperate regions of the world (Fegan and Prior, 2005). The species as a whole has a very broad host range, but different pathogenic races within the species occur with hosts' preferences. Generally, the pathogen infects hundreds of plant species encompassing 44 families (Hayward, 1991), including economically-important host plants in the Solanaceae or nightshade family (Stevenson, et al., 2001).

As a highly diversified pathogen, *R. solanacearum* is considered a species complex, a heterogeneous group of related strains (Fegan and Prior, 2005). There are hundreds of genetically distinct isolates of *R. solanacearum*. Historically, the species complex was subdivided into races based very loosely on host range, and into biovars based on ability to produce acid from a panel of carbohydrates (Denny and Hayward, 2001). A more phylogenetically meaningful classification scheme, based on sequence of several genes, divides the species complex into four phylotypes (Poussier et al., 2000). This approach groups strains by geographic origin, such that strains from Asia are in phylotype I, those from the Americas are in phylotype II, those from Africa are in phylotype III, and

others from Indonesia, which is the apparent center of diversity, are in phylotype IV (Fegan and Prior, 2005). Phylotypes themselves can be subgrouped into sequevars which are clusters of isolates with highly conserved DNA sequences.

Further classification of *R. Solanacearum* strains from wilt-endemic areas of the world listed strains belonging to race 1 biovar 1 (R1B1) as the most virulent. In 2002, the subgroup of *R. Solanacearum* known as race 3 biovar 2 (R3B2) was listed in the United States as a bioterrorism select agent (Lambert, 2002). Although R3B2 is best known as a pathogen of potato, these strains also can infect tomato and geranium plants, and the pathogen occasionally has been introduced into North America and Europe on geranium cuttings produced in Africa or Central America (Swanson et al., 2005). Biologically, R3B2 strains can be identified by the biovar test and by host range because, unlike many race 1 strains, they neither cause disease nor induce a hypersensitive response (HR) on universal indicator plant - tobacco.

Two published polymerase chain reaction (PCR) primer sets specifically identify R3B2 strains; both primers are derived from an apparent prophage remnant present in the genomes of all tested R3B2 strains (Gabriel et al., 2006). These strains are widely distributed in Asia, Africa, South and Central America, and are found in some soils and waterways in Europe (Elphinstone, 2005). Strains of race 4 biovar 4 (R4B4) are reported in North America, while race 2 biovar 1 (R2B1) are commonly reported in some coastal parts of West Africa (Alvarez et al., 2005; Poussier et al., 2000).

Control of bacterial wilt is difficult due to high variability of the pathogen, limited possibility for chemical control, high capacity of the pathogen to survive in diverse environments and its extremely wide host range (Nguyen and Ranamukhaarachchi, 2010). The strongest foundation for an effective control measure is definitive information on the race and biovar characteristics of the pathogen in the endemic areas. The management can then be custom-tailored based on those characteristics.

This study was aimed at collecting wilt-endemic soil from seven locations across Nigeria, isolate *Ralstonia solanacearum* from the soil samples and characterize the isolated pathogen into races and biovars using a combination of cultural and molecular techniques. It was an attempt at providing baseline information for further research into solving bacterial wilt epidemics of tomato in Nigeria.

Materials and Methods

Survey of tomato farms: Soil samples were collected from major tomato bacterial wilt-endemic areas in the following States – Lagos, Ogun, Ekiti, Oyo, Plateau, Kano and Imo, representing Southwestern, Southeastern and Northern Nigeria. Samples were taken from a total of thirty-four fields in the seven states mentioned above. Soil samples were collected from 18-20 points in each location at 15-20 cm depth and were brought to laboratory. Each location samples were bulked together before analysis.

Isolation of Ralstonia solanacearum: Specific isolation medium for *Ralstonia solanacearum* was 2,3,5-triphenyltetrazolium chloride (TZC)-casein-peptone-gulcose agar. The medium contained 10.0 g peptone, 1.0 g casein hydrolysate, 5.0 g glucose, 12.0 g oxoid agar in 1 litre distilled water. The medium was adjusted to pH 7.0 and dispensed into 100 ml volumes. It was sterilized by autoclaving at 121^{o}C for 20 minutes. To the molten agar (55^{o}C) was added filter-sterilised 1% aqueous 2,3,5-triphenyl tetrazolium chloride. Dried soil samples (1 g) were mixed with 10 ml of sterile distilled water in test tubes. The samples were agitated for 5 minutes and dilutions then carried out by adding 1 ml of the sample to 9 ml of sterile distilled water to a dilution factor of 10^{-6}. Each level of dilution was plated on *Rs* isolation medium in triplicates. After 2 days, virulent strains of *Rs* appeared creamy white in the isolation plates

Characterization of the pathogen

Simple staining: Ten loopfuls of the culture was placed into a test tube containing 5 ml sterile distilled water to make a suspension of bacterial cells in the water. A loopful of this bacterial suspension was placed on a clean slide. The smear was allowed to air dry. The cells were heat-fixed by passing the slide quickly through the flame of a Bunsen burner two or three times, with the glass surface exposed to the flame (a second or two each time). The slide was flooded with crystal violet stain (0.5% aqueous) for 10 seconds. The slide was rinsed with distilled water, blotted dry, and examined under the microscope.

Streaming test: A presumptive test was carried out on infected tomato plant to diagnose the presence of *Ralstonia*

solanacearum. Stems of infected tomato plants were cut above the soil level and the cut surfaces were suspended in test tube containing clean water.

Potassium hydroxide (KOH) solubility test: This is a rapid method of distinguishing between Gram-positive and Gram-negative bacteria. A drop of KOH (3% aq., w/v) was placed on a microscope slide using a Pasteur pipette. A part of a single colony of the pathogen was removed from agar medium using a cooled sterilized wire loop. Bacteria were mixed into KOH until an even suspension is obtained. The loop was then lifted from the slide and observation of slime threads was recorded (Zubeda and Hamid, 2011).

Race determination and hypersensitive reaction: The race differentiation was determined based on hypersensitive reaction (HR) on tobacco using the leaf infilteration technique of Lozano and Sequeira (1970). *Nicotiana tabacum* L. seeds were sown on plastic trays of sterilized soil and cultivated for 30 days in screen house. Each seedling was transplanted into plastic pots containing sterilized soil. Lower side of fully expanded leaves of 45 days post-transplanting tobacco were infiltrated with suspension of bacterial isolates using a syringe fitted with a fine needle (Klement et al., 1964), Sterile water served as a negative control. Leaf reactions were recorded from 24 h to 4 wk (Horita and Tsuchiya, 2001) using the template shown in Table 1.

Biovar determination through carbohydrate utilization test: Technique of Zubeda and Hamid (2011) was used. Ten ml each of 10% sugar (lactose, maltose, cellobiose, mannitol, sorbitol and dulcitol) was added in sterilized screw-capped test tubes and then heated at 100°C for 30 min to sterilize these solutions. Semi-solid TZC media in bottles were melted in water bath and cooled to70°C. Equal volume of sterilized 10% sugar solution and TZC medium were mixed to form sugar-TZC medium stock which was dispensed in aliquots of 5 ml into labeled tubes. Two drops of bacterial suspensions, prepared from 48 hrs-old cultures of individual isolates, were added to each tube of sugar-TZC medium in triplicates. The tubes were incubated at 30°C and examined after 2,7, 14 and 28 days for the presence of indicator change from olivaceous green to orange colour on the surface of medium as shown in Table 2.

Table 1: Tomato plant and tobacco leaf reaction to different races of *Ralstonia solanacearum*

Type of Race	Time (hr) after inoculation	Reaction type	
		Reaction in Tobacco	Reaction in Tomato
1	24	No visible symptoms	
	36	Dark brown lesion surrounded by a yellow zone	Wilting
	60	Vessels discolored	
	192	Leaf wilting and yellowing	
2	10-12	Hypersensitive reaction, infiltrated tissue glassy	No reaction
	60	Tissue thin, transparent, white necrosis	
3	48	Infiltrated tissue becomes yellow	Wilting

Source: Horita and Tsuchiya (2001).

Table 2: Biovar characterization of *Ralstonia solanacearum* based on sugar utilisation.

Type of Sugar	Biovar Type I	II	III	IV	V
Mannitol	-	-	+	+	+
Sorbitol	-	-	+	+	-
Dulcitol	-	-	+	+	-
Cellobiose	-	+	+	-	+
Lactose	-	+	+	-	+
Maltose	-	+	+	-	+

+ Positive reaction (colour of medium changed from green to yellow);
- Negative reaction (colour of the medium not changed).
Source: Zubeda and Hamid (2011)

Pathogenicity test of bacterial isolates on tomato: Seedlings of Beske (susceptible) and Tomachiva (resistant) tomato were raised in tray containing sterilized soil. One month-old

young tomato plants were inoculated two weeks after transplanting by soil drenching with bacterial suspensions and kept in screen-house until symptoms development according to the technique of Pradhanang et al., (2005). Sterile water served as a negative control. Prior inoculation, plants were not watered for 24 h. Watering resumed after inoculation every other day for the period of the experiment. The tested isolates were re-isolated on TZC medium.

Molecular characterization of *Ralstonia solanacearum*

Bacterial DNA extraction: Zymo® Research Fungal/ Bacterial DNA MiniPrep Kits were used. Bacterial cells (100 mg) were added to a ZR BashingBead™ Lysis Tube and 750 µl Lysis Solution added. The ZR BashingBead™ Lysis Tube was centrifuged in a microcentrifuge at 10,000 xg for 1 min. From the Lysis tube, 400 µl supernatant was transferred to Zymo-Spin™ IV Spin Filter (orange top) in a collection tube and centrifuged at 7,000 rpm (~ 7,000 x g) for 1 min. Thereafter, 1200 µl of Fungal/Bacterial DNA Binding Buffer (diluted with beta-mercaptoethanol to a final solution of 0.5% v/v i.e 500 µl per 100 ml) was added to the filtrate in the collection tube. Eight hundred microlitre of the mixture was then transferred to Zymo-Spin™ IIC Column in a Collection tube and centrifuged at 10,000 x g for 1 min. The flow through was discarded. DNA Pre-Wash Buffer (200 µl) was then added to the Zymo-Spin™ IIC Column in a Collection tube and centrifuged again at 10,000 x g for 1 min. Five hundred microlitre of Fungal/Bacterial DNA Wash Buffer was added to the Zymo-Spin™ IIC Column and centrifuged at the same 10,000 x g for 1 min. Zymo-Spin™ IIC Column was transferred to clean 1.5 ml microcentrifuge tube and 100 µl DNA Elution Buffer added and centrifuged at 10,000 x g for 30 sec to elute the DNA.

Preparation of agarose gel and confirmation of DNA: Agarose gel of 1% was prepared, microwaved at 100 ^{o}C for 3 mins. and was allowed to cool. Five microlitre of ethidium bromide (EtBr) was added to 150 ml agarose gel. It was then poured into the gel tank and allowed to solidify. Subsequently, 3 µl of loading dye was added to 5 µl each of the samples. Samples and 2 µl marker were loaded and allowed to run at 100V for 45 min before viewing on UV light source.

PCR for confirmation of Ralstonia solanacearum: The reaction mixture (25 µl) contained 30 ng of *Ralstonia solanacearum* genomic DNA, 1x PCR buffer without magnesium, 2.5 mM Mg++, 0.25 mM each dNTP, 20 pmol each of 759/760 primer (forward primer: 5'-GTCGCCGTCAACTCACTTTCC 3', reverse primer: 5'-GTCGCCGTAGCAATGCGGAATCG-3') which amplified at 280-bp fragment (Opina et al*.,* 1997), and 5 U of *Taq* polymerase. DNA was amplified by using a 3 min. hot start at 96^{o}C, followed by 30 cycles of 15 s at 94^{o}C, 58 s at 30^{o}C and 30 s at 72^{o}C. Reaction was completed within 5 min at 72^{o}C. All PCR products were electrophoresed through a 2% agarose gel stained with ethidium bromide and visualized with UV light.

Results

Surveyed states in Nigeria are shown in Table 3. Isolated strains of *Rs* on TZC- Casein Peptone Glucose medium appeared pinkish creamy colour (Plate 1, Table 3). The isolates were further subjected to KOH test to confirm the Gram-negativity of the bacterial pathogen (Table 3). Formation of slime thread is an indication of Gram-negativity. Isolates also appeared rod-shaped under the microscope following simple staining procedure (Plate 2, Table 3). Population density of the pathogen in each location was indicated in Table 3. Based on the cultural and morphological appearance, the isolated pathogens were identified as *Ralstonia solanacearum.* The identification was further supported by the molecular characterization. All isolates produced fragments at 280 bp which was characteristic of *Rs* genomic DNA amplified with universal oligonucleotides 759/760 primers (Plate 3).

Tobacco hypersensitivity test was negative 24 hrs after inoculation with no symptom of white or brown necrosis in any of the strains tested (Plate 4). Symptoms of brown necrosis with yellow halo emerged 36 hrs after inoculation of some strains, indicating that those strains belonged to Race 1. Strains from thirty locations belonged to Race 1 (Table 3). This Race is widely spread throughout the locations studied. No symptoms of Race 2 strains were observed in any of the isolates. However, in strains from four locations, infiltrated portion of tobacco leaves became yellow within 48 hrs, suggesting they belonged to Race 3 (Table 3). These two races – Race 1 and 3 also showed the symptoms of wilt when inoculated on tomato seedlings. In these two races, development of wilt on Beske susceptible

variety of tomato was rapid, resulting in complete wilting of the plant. The symptoms appeared two weeks after inoculation, and are characterized by the drooping of leaves without yellowing and death. The same pathogen was re-isolated from the diseased plants, confirming pathogenicity of the isolates.

Table 3: Cultural characterization, inoculum density, pathogenicity and race determination of *Ralstonia solanacearum*.

States	Field	Isolate	Bacterial Streaming	Plating test	KOH test	Shape	Population Density(cfu/g soil)	Pathogenicity test	Race Determination
Ekiti	Ile-ona	Rs-1	+	Pink	+	Rod	$1.4x\ 10^8$	+	1
	Awo-Ekiti	Rs-2	+	Pink	+	Rod	$2.3x10^8$	+	1
	Olorunda	Rs-3	+	Pink	+	Rod	$2.1x10^8$	+	3
	Ado-Ekiti	Rs-4	+	Pink	+	Rod	$2.1x10^8$	+	1
	Reserve Area	Rs-5	+	Pink	+	Rod	$2.0x10^8$	+	1
	Aramoko-Ekiti	Rs-6	+	Pink	+	Rod	$1.7x10^8$	+	1
Ogun	Works(FUNAAB)	Rs-7	+	Pink	+	Rod	$1.3x10^8$	+	1
	Itori	Rs-8	+	Pink	+	Rod	$2.4x10^8$	+	1
	Ogarnic	Rs-9	+	Pink	+	Rod	$1.7x10^8$	+	1
	Iwoye	Rs-10	+	Pink	+	Rod	$1.2x10^8$	+	1
	Olorunda	Rs-11	+	Pink	+	Rod	$4.7x10^7$	+	1
	Agbon	Rs-12	+	Pink	+	Rod	$2.8x10^8$	+	1
	Poro	Rs-13	+	Pink	+	Rod	$1.2x10^8$	+	1
	Imeko	Rs-14	+	Pink	+	Rod	$2.6x10^8$	+	1
	Opeji	Rs-15	+	Pink	+	Rod	$1.5x10^8$	+	3
	AMREC(FUNAAB)	Rs-16	+	Pink	+	Rod	$1.2x10^8$	+	1
Lagos	Ajara Farm	Rs-17	+	Pink	+	Rod	$1.1x10^8$	+	1
Oyo	NIHORT(Ibadan)	Rs-18	+	Pink	+	Rod	$2.3x10^8$	+	3
Platea	Barkin ladi	Rs-19	+	Pink	+	Rod	$9.1x10^7$	+	1
	Jos East	Rs-20	+	Pink	+	Rod	$2.3x10^8$	+	1
	Jos South	Rs-21	+	Pink	+	Rod	$1.5x10^8$	+	3
Kano	Kura	Rs-22	+	Pink	+	Rod	$1.9x10^8$	+	1
	Gammalla	Rs-23	+	Pink	+	Rod	$1.4x10^8$	+	1
	Yarutu	Rs-24	+	Pink	+	Rod	$1.4x10^8$	+	1
Imo	Umukabia	Rs-25	+	Pink	+	Rod	$6.5x10^7$	+	1
	Mgbidi	Rs-26	+	Pink	+	Rod	$3.2x10^7$	+	1
	Aboh	Rs-27	+	Pink	+	Rod	$6.3x10^7$	+	1
	Ata	Rs-28	+	Pink	+	Rod	$7.7x10^7$	+	1
	Ohaji	Rs-29	+	Pink	+	Rod	$7.0x10^7$	+	1
	Umuawa Ibu	Rs-30	+	Pink	+	Rod	$8.9x10^7$	+	1
	Ikereduru 2	Rs-31	+	Pink	+	Rod	$9.3x10^7$	+	1
	Ikereduru 2	Rs-32	+	Pink	+	Rod	$5.8x10^7$	+	1
	Achingali	Rs-33	+	Pink	+	Rod	$7.3x10^7$	+	1
	Umuelemi Umuichi	Rs-34	+	Pink	+	Rod	$5.9x10^7$	+	1
	LSD(0.05)						$8.7x10^7$		

The results of the biovar test showed that thirty isolates of *R. solanacearum* oxidized disaccharides (sucrose, lactose, and maltose) and sugar alcohols (mannitol, sorbitol and dulcitol) and were classified as Biovar III while isolates Rs-3, Rs-15, Rs-17 and Rs-21 failed to oxidize hexose alcohol and were classified as

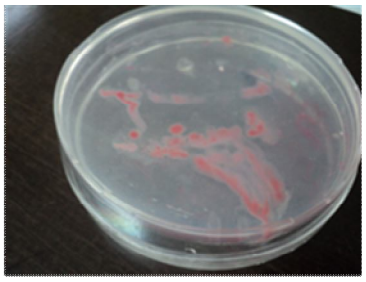

Plate 1: Cultural appearance of *Ralstonia solanacearum* strains on Triphenyl tetrazolium chloride (TZC) – casein peptone glucose aga

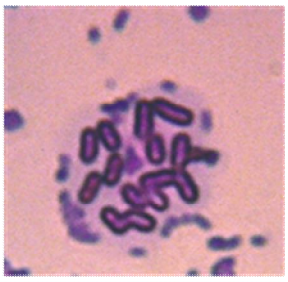

Plate 2: Rod-shaped cells of *Rastonia solanacearum* after simple staining. :x 100

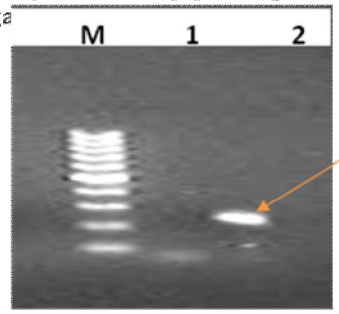

Plate 3: Polymerase chain reaction (PCR) confirmation of *Ralstonia solanacearum (Rs)* isolate. (M= 100 bp DNA ladder, 1= No DNA sample , 2= Genomic DNA of *Rs*)

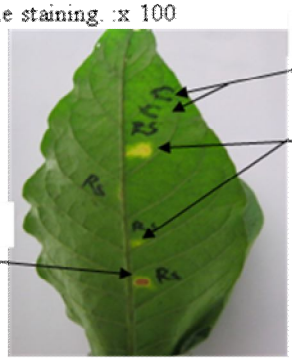

Plate 4: Tobacco hypersensitivity reaction

Biovar II (Table 4). All the Biovar III isolates were Race 1, while the Biovar II isolates were all Race 3.

Discussion

Bacterial wilt caused by *Ralstonia solanacearum* (Smith) (Yabuuchi et al., 1995) is one of the most important and widespread diseases of numerous crops in tropical, subtropical and temperate regions of the world (Hayward, 1991). According to Kelman (1954), triphenyl tetrazolium chloride (TZC) medium is used to distinguish *R. solanacearum* among other bacteria during isolation. Also when TZC medium is used with *R. solanacearum,* it shows the difference between avirulent colonies that look dark red from the fluidal virulent that are white with pink center (Klement, 1990; Rahman et al., 2010). In the present study, the colonies were fluidal whitish with a pink center, indicating virulent species of *R. solanacearum*. With the species-specific primer (759/760), the PCR produced a single band of 280 bp from the strains of *Ralstonia solanacearum* isolated. This band pattern was in agreement with the result of Opina et al. (1997) and Ito et al. (1998).

Denny and Hayward (2001) identified race of *R. solanacearum* by host specifity. The differences in reactions of tobacco leaves and susceptible tomato cultivars to infiltration/inoculation with isolates of *R. solanacearum* were used as simple and quick means of determining the race of any particular virulent isolate of this pathogen (Lozano and Sequiera, 1970; Lemessa and Zeller, 2007).

In the present study, thirty isolates belonged to Race 1 while four belonged to Race 3. *R. solanacearum* Race 1 strains attack tobacco, tomato, and many other solanaceous crops, and certain diploid bananas. Race 3 causes wilting in potato, tomato and rarely other solanaceous plants. Race 2 and 4, fortunately are not common in sub-saharan Africa, as Race 2 is known to infect triploid banana (*Musa* spp.) and *Heliconia* spp.,

Table 4: Classification of isolated *R. solanacearum* into biovar

Isolate	Origin	Maltose	Lactose	Cellobiose	Dulcitol	Sorbitol	Mannitol	Biovar
Rs-1	Ile-ona	+	+	+	+	+	+	III
Rs-2	Awo-Ekiti	+	+	+	+	+	+	III
Rs-3	Olorunda	+	+	+	-	-	-	II
Rs-4	Ado-Ekiti	+	+	+	+	+	+	III
Rs-5	Reserve Area	+	+	+	+	+	+	III
Rs-6	Aramoko-Ekiti	+	+	+	+	+	+	III
Rs-7	Works(FUNAAB)	+	+	+	+	+	+	III
Rs-8	Itori	+	+	+	+	+	+	III
Rs-9	Organic farm(FUNAAB)	+	+	+	+	+	+	III
Rs-10	Iwoye	+	+	+	+	+	+	III
Rs-11	Olorunda	+	+	+	+	+	+	III
Rs-12	Agbon	+	+	+	+	+	+	III
Rs-13	Poro	+	+	+	+	+	+	III
Rs-14	Imeko	+	+	+	+	+	+	III
Rs-15	Opeji	+	+	+	-	-	-	II
Rs-16	AMREC(FUNAAB)	+	+	+	+	+	+	III
Rs-17	Ajara Farm settlement	+	+	+	-	-	-	II
Rs-18	NIHORT(Ibadan)	+	+	+	+	+	+	III
Rs-19	Barkin ladi	+	+	+	+	+	+	III
Rs-20	Jos East	+	+	+	+	+	+	III
Rs-21	Jos South	+	+	+	-	-	-	II
Rs-22	Kura	+	+	+	+	+	+	III
Rs-23	Gammalla	+	+	+	+	+	+	III
Rs-24	Yarutu	+	+	+	+	+	+	III
Rs-25	Umukabia	+	+	+	+	+	+	III
Rs-26	Mgbidi	+	+	+	+	+	+	III
Rs-27	Aboh	+	+	+	+	+	+	III
Rs-28	Ata	+	+	+	+	+	+	III
Rs-29	Ohaji	+	+	+	+	+	+	III
Rs-30	Umuawa Ibu	+	+	+	+	+	+	III
Rs-31	Ikerederu 1	+	+	+	+	+	+	III
Rs-32	Ikerederu 2	+	+	+	+	+	+	III
Rs-33	Achingali	+	+	+	+	+	+	III
Rs-34	Umuelemi Umuichi	+	+	+	+	+	+	III

+ Positive reaction, - Negative reaction.

while Race 4 attacks mulberry (He et al., 1983; OEPP/EPPO 2004). He et al. (1983) reported Race 5 from Mulberry in China. Therefore, five races have been described so far, but they differ in host range, geographical distribution and ability to survive under different environmental conditions (French, 1986).

Race 3 is highly virulent on potatoes, tomatoes and other solanaceous crops (Hudelson et al., 2002; Janse et al., 2004). Its temperature optimum is lower than that of other races and this might partly its preponderance in temperate regions of the world, and places like Jos Plateau in Nigeria.

Biovar characterization showed that most of the *R. solanacearum* isolates oxidized disaccharides (sucrose, lactose, and maltose) and sugar alcohols (manitol, sorbitol and dulcitol). The oxidation reaction was indicated by the change of colour. Thirty out of thirty-four isolates tested positive to Biovar III. The remaining four were Biovar II.

Literature revealed that the control of bacterial wilt is a very difficult one, especially for those caused by Race 1 strains. Being soil-borne, chemical control is nearly impossible to apply. Soil fumigants showed either slight or no effects (Murakoshi and Takahashi, 1984). Antibiotics such as streptomycin, ampicillin, tetracycline and penicillin also hardly showed any effect (Farag et al., 1982). Positive results were only reported with antagonistic bacteria

such as *Bacillus polymyxa* and *Pseudomonas fluorescens* as biocontrol agents, especially with potato in the Philippines (Aspiras and Cruz, 1985) and Chile (Hartman and Elphinstone, 1994). Use of resistant varieties and grafting on such resistant varieties are also possible means of control for this race and the emerging Race 3 (McAvoy et al., 2012). Intercropping and crop rotation with non-host crops such as maize could also reduce inoculum density for both Race 1 and 3 strains (Autrique and Potts, 1987).

Conclusion

In summary, strains of *R. solanacearum* from Nigeria mostly belonged to Race 1 Biovar III with few strains in the category of Race 3 Biovar I. Differences in race and biovar across wilt-endemic locations in Nigeria accounted for breakdown of resistant varieties of tomato reported in the country (Popoola et al*.,* 2012). With a definite identification of race and biovar of the pathogen in the studied area, a more targeted management approach (such as grafting with resistant rootstock) could be initiated and implemented in these areas.

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