Research Paper

Isolation and characterisation of *Ralstonia solanacearum* strains from *Solanaceae* crops in Ethiopia

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Eighty one isolates of *Ralstonia solanacearum*-like bacteria on triphenyl tetrazolium chloride (TTC) medium were collected from different *Solanaceae* crops (i.e. potato, tomato and pepper plants and potato tubers) at various sites in Ethiopia. Of these, 62 strains were identified as *R. solanacearum* based on their cultural characteristics on TTC medium, tomato pathogenicity bioassay, carbon source utilisation patterns and a specific PCR-based assay. By Hayward's classification method, based on carbon source utilisation, 19 of the 62 *R. solanacearum* strains were identified as biovar I and 43 strains were identified as biovar II. The biovar I strains exhibited a high growth rate at high temperatures (37 °C). Whereas the growth rate of biovar II strains was greatest at lower temperatures (22 °C). Biovar I strains had broader host range than biovar II strains, which were limited to potato, tomato, and eggplant. To our knowledge, this is the first report of *R. solanacearum* biovar I in Ethiopia. The existence of biovar I strains in Ethiopia raises concerns because they have a broader host range than biovar II strains.

Keywords: Bacterial wilt / biovar / characteristics / Ethiopia / *Ralstonia solanacearum*

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Ralstonia solanacearum (basonym *Pseudomonas solanacearum* [Smith 1896] Smith 1914; other synonyms *Burkholderia solanacearum* (Yabucchi *et al.* 1993, "*Bacillus solanacearum*" SMITH 1896) is a widespread and economically important bacterial plant pathogen in tropical, subtropical, and temperate regions (Hayward 1991, Horita and Tsuchiya 2001). *Ralstonia solanacearum* causes "bacterial wilt", a major disease that limits production of diverse crops such as potato, tomato, eggplant, pepper, tobacco, banana and peanut (French and Sequeria 1970, Williamson *et al.* 2002). The pathogen has an extended host range including hundreds of plant species in 50 families (Hayward 1995).

R. solanacearum is one of the most important and wide spread bacterial plant pathogens in Ethiopia, mainly in the off cropping season. Yaynu (1989) indicated that bacterial wilt is an important disease threatening the production of potato and tomato in many regions of Ethiopia. Moreover, incidence of the disease has been increasing. Disease incidence as high as 45% (Yaynu and Korobko 1986) and 63% (Bekele 1996) on potato and 55% (EARO 2002) on tomato were recorded in major potato producing areas of Ethiopia.

Based upon carbon source utilisation patterns *R. solanacearum* has been divided into five biovars (Hayward 1964, Hayward *et al.* 1990, He *et al.* 1983). In a previous study, 58 strains of *R. solanacearum* from potato and tomato collected at different sites in Ethiopia were identified as *R. solanacearum* biovar II (Yaynu 1989). Biovar II was reported to infect potatoes and tomatoes, and rarely eggplant (French and Sequeira 1970). However, in Ethiopia, pepper plants are infected by the pathogen and the intensity of bacterial wilt is increasing. This may be due to introduction of new strains to Ethiopia from other parts of the world through latently infected planting materials. The aim of this study was to isolate and characterise the strains isolated from potato, tomato, and pepper plants from different localities in Ethiopia based on their cultural and physiological characteristics and to determine biovars of *R. solanacearum* in Ethiopia.

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Materials and methods

Origin and isolation: Initially 81 strains were isolated from potato, tomato, and pepper plants and potato tubers from various locations of Ethiopia during the 2003 off cropping season (February to May). Collections were from Jimma, Dedo, and Agaro in south west; Awassa and Shashemene in the south; Qarsa and Kombolcha in the east; Arjo, Kejo, and Bako in the west; and Guder, Ambo, Holeta, Gedo, Ginchi, Jeldu, Adam Tulu, and Ziway in central Ethiopia. Additional six strains were obtained from the collection of the Plant Protection Research Centre (PPRC), Ambo, Ethiopia. For comparison, five strains of *R. solanacearum* of the Göttingen Collection of Phytopathogenic Bacteria (GCPB), Göttingen University, Germany were included. The novel bacterial isolates investigated in this study were obtained from various diseased plants (potato, tomato and pepper roots and potato tubers) with symptoms of bacterial wilt. Isolations were performed at the the Plant Protection Laboratory at the Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), Jimma, Ethiopia. Rootlets and tubers were thoroughly washed in tap water, and surface sterilized by dipping into 70% (vol/vol) ethanol and flamed (OEPP/EPPO 1990). Subsequently, plant material was chopped into sterile water using a disinfected scalpel. Rootlets and tuber pieces were maintained in the water for 30 min (Wullings *et al.* 1998) to allow bacteria to migrate from plant material. After 30 min two loopfuls of the suspension was streaked onto triphenyl tetrazolium chloride (TTC) agar (Kelman 1954). After 48 h incubation, colonies with morphology similar to *R. solanacearum* were purified and individual isolates were maintained temporarily in distilled water until they were transported to the Federal Biological Research Centre for Agriculture and Forestry (BBA), Darmstadt (Germany) for further analysis.

Maintenance of cultures: All the 81 strains were stored at room temperature in test tubes containing sterile distilled water (Kelman and Person 1961, Lozano and Sequeira 1970, Wullings *et al.* 1998). Strains were routinely subcultured on TTC agar (aerobic, 30 °C) and on casamino acids-peptone-glucose (CPG) agar (Smith *et al.* 1995).

Strain identification: World-wide the most frequently used method for detection and identification of *R. solanacearum* is the isolation on TTC agar (Kelman 1954) because of the relatively low cost and simplicity. Tomato bioassay also is currently recommended by the European Plant Protection Organization (EPPO) for detection of *R. solanacearum* in soil and on potato tubers and for pathogenicity test (Elphinstone *et al.* 1996). With the development of *R. solanacearum* specific PCR methods, Ito *et al.* (1998) and Opina *et al.* (1997) were able to identify and detect viable cells of *R. solanacearum.* We combined the three methods so that the advantages of each method could be utilised and compared.

The 81 isolates, as well as the reference strains, were cultured on TTC agar on replicated plates and were incubated at 28 °C. Presumptive identification as *R. solanacearum* was made when colonies showed a characteristic red center and whitish margin on TTC medium as described by Kelman (1954).

Bioassay on tomato seedlings was performed according to Janse (1988). All isolates presumptively identified as *R. solanacearum* were inoculated on two week old tomato seedlings (cv. Matina) grown in 12 cm pots. Three replicate seedlings were inoculated with a sterile needle dipped in a suspension (~10^9 cfu/ml) of the isolates and the stem was punctured between the two cotyledons. Three replicates per bacterial suspension were used as inocula giving nine seedlings inoculated for each bacterial isolate. Seedlings inoculated with a needle dipped in sterile water was a negative control. Seedlings were held at 25 °C in greenhouse and development of typical wilt symptoms was recorded weekly. When typical symptoms were observed, the bacteria were re-isolated on TTC to confirm the presence of the bacterium (Koch's rule). In symptomless plants, stem segments around the point of inoculation were washed, surface-disinfected with ethanol, and the cut of the stem was imprinted five times on TTC medium (Prior *et al.* 1996) to check for the presence of latent infection.

Molecular identification: All presumptive *R. solanacearum* isolates that produced typical wilt symptoms in the tomato bioassay were subjected to PCR for identification. Genomic DNA was extracted using the "DNeasy Tissue" kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For PCR amplification, a thermal cycler (PeQlab Cyclone 25) was used. Reaction volumes were 50 μl and contained 1 × PCR buffer (10 mM Tris HCl [pH 8.3], 50 mM KCl), 1.5 mM $MgCl_2$, 0.05 mM of each dNTP, 25 pmol of primers 759 (5′-GTCGCCGTCAACTCACTTTCC-3′) and 760 (5′-GTCGCCGTCAGCAATGCGGAATCG-3′), 1 μl of genomic template DNA and 0.5 U of Taq DNA polymerase (Metabion, Martinsried, Germany). Samples were denaturated at 94 °C for 3 min, annealed at 53 °C for 1 min and extended at 72 °C for 1.5 min, followed by 30 cycles of 94 °C for 15 s, 60 °C for 15 s, 72 °C for 15 s, and a final extension of 72 °C for 5 min (Opina *et al.* 1997). PCR products were separated in 1.5%

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(wt/vol) agarose gels, stained with ethidium bromide at 0.5 µg/ml and visualized and photographed under UV (302 nm) irradiation.

Phenotypic characterisation: The strains identified as *R. solanacearum* by PCR and the tomato bioassay were cultured on TTC and CPG agar at 30 °C for 48 h and colony characteristics were noted. Production of fluorescent pigments was tested on KING's B agar (King *et al.* 1954). After 48 h at 30 °C plates were examined with a UV lamp (366 nm) according to Sands (1990). Tests for catalase and oxidase activity followed standard procedures (Schaad *et al.* 2001, Gerhardt *et al.* 1981). Starch hydrolysis, H_2S production, gelatineliquefication, and Tween 80 hydrolysis were tested as described by He *et al.* (1983). Nitrate reduction and NaCl tolerance tests were done as described by Hayward (1964). Temperature tolerance was tested according to Sands (1990) and Gram reaction was determined by the KOH solubility test (Fahy and Hayward 1983).

For the carbon source utilisation tests, the basal medium of Hayward (1964) was used. Lactose, maltose, cellobiose, fructose and sucrose solutions were filter-sterilized, while D-glucose, mannitol and sorbitol were autoclaved for 20 min as 10% (wt/vol) stock solutions (Hayward 1964). Dulcitol was added directly to the basal medium and was autoclaved for 20 min. Five ml of each carbon source stock solution was added to 45 ml Hayward's basal medium and 10 ml samples were distributed into test tubes (Hayward 1964); Hayward's medium without carbon source served as control.

Bacterial suspensions were prepared in 300 µl sterile water from cells grown on CPG agar for 48 h at 30 °C (Williamson *et al.* 2002). Hayward's medium amended with various carbon sources was with 30 µl of the prepared-suspension incubated at 30 °C and checked for acid production (yellow colour) for up to 5 weeks (Hayward 1964, He *et al.* 1983).

Growth experiments under static culture conditions were performed in 5 ml CPG broth in a water bath. Ten strains of biovar I, 15 of biovar II, and 3 of biovar III were investigated. Each bacterial suspension (OD_{660} = 0.05) was prepared in duplicate test tubes and incubated at 22, 27, 32, and 37 °C. Absorbance after 48 h was determined using a spectrophotometer. To determine single or interaction effects of factors (temperature by biovar), data were subjected to statistical analysis using the general linear model procedure of the SAS version 8 (SAS Institute 1999). Significant factor effects were detected by ANOVA and treatment means at different levels of the respective factors were compared using Tukey's multiple means comparison procedure.

Pathogenicity test: To assess pathogenicity on different host plants, bacterial strains were cultured on CPG agar (Smith *et al.* 1995) and inoculated (~10^9 cfu/ml) on potato (c.v. 'Secura'), tomato (c.v. 'Matina'), eggplant (c.v. 'Lange Violete'), pepper (c.v. 'Neusiedler ideal') and tobacco (c.v. 'White Burley'). Inoculation was made at the three to four true leaf stage by puncturing the stem at the axils of the third fully expanded leaves from the apex with a needle dipped in inoculum (Winstead and Kelman 1952). Nine plants of each host were inoculated with each strain. Plants inoculated with sterile water served as negative control. Inoculated plants were maintained in greenhouse at 26 to 30 °C with 12 h light and 12 h dark. Prior to inoculation, plants were not watered for 24 h (Williamsson *et al.* 2002, OEPP/EPPO 1990). The experiment was conducted two times.

Severity of wilting was recorded at weekly intervals (Horita and Tsuchiya 2001) after inoculation on the following scale: 1 = no symptom, 2 = leaf above inoculation point wilted, 3 = two or three leaves wilted, 4 = four or more leaves wilted, and 5 = plant died.

Results and discussion

Identification

All the collected 81 Ethiopian isolates showed the same colony morphology as *R. solanacearum* on TTC agar. However, in the tomato bioassay only 62 of the strains produced typical symptoms of wilting. The remaining 19 strains did not cause wilt symptoms on tomato and were presumed to be saprophytic strains. Subsequently, when the 62 strains (Table 1) that produced typical symptoms on tomato were subjected to a PCR using species specific primers 759 and 760, all produced a single 281 bp fragment (Fig. 1) which is typical of *R. solanacearum* as reported by Opina *et al.* (1997) and Ito *et al.* (1998). We concluded that cultivation on TTC agar is less accurate for identification of *R. solanacearum*, although it is less costly and simple. This is because some saprophytic bacteria have a colony morphology similar to *R. solanacearum*, which may decrease the accuracy of TTC. The tomato bioassay as recommended by the European Plant Protection Organization (EPPO) (Elphinstone *et al.* 1996) was a useful assay because all the isolates that produced typical wilt symptom on tomato were confirmed as *R. solanacearum* with the PCR assay.

Phenotypic characteristics

On the basis of cultural characteristics, all virulent *R. solanacearum* strains from Ethiopia resembled those

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Table 1. List of *Ralstonia solanacearum* strains used for this study.

Number	Strain designation	Origin	Country	Altitude (m)
1	IBC Pot 1	*Solanum tuberosum*	Mutulu, Guder, Ethiopia	2000
2	IBC Pot 5	*Solanum tuberosum*	Mutulu, Guder, Ethiopia	2000
3	IBC Pot 34	*Solanum tuberosum*	Jato, Gedo, Ethiopia	1700
4	IBC Pot 4II	*Solanum tuberosum*	Tsedey, Holeta, Ethiopia	2400
5	IBC Pot 5II	*Solanum tuberosum*	Tsedey, Holeta, Ethiopia	2400
6	IBC Pot 9II	*Solanum tuberosum*	Tsedey, Holeta, Ethiopia	2400
7	IBC Pot 10II	*Solanum tuberosum*	Tsedey, Holeta, Ethiopia	2400
8	IBC Pot 15II	*Solanum tuberosum*	Mutulu, Guder, Ethiopia	2000
9	IBC Pot 2JU	*Solanum tuberosum*	Jimma, Ethiopia	1700
10	IBC Pot 4JU	*Solanum tuberosum*	Jimma, Ethiopia	1700
11	IBC Pot 6JU	*Solanum tuberosum*	Jimma, Ethiopia	1700
12	IBC Pot 8JU	*Solanum tuberosum*	Jimma, Ethiopia	1700
13	IBC Pot 9JU	*Solanum tuberosum*	Jimma, Ethiopia	1700
14	IBC Pot 6III	*Solanum tuberosum*	Kejo, Ethiopia	1650
15	IBC Pot 10III	*Solanum tuberosum*	Kejo, Ethiopia	1650
16	IBC Pot 16III	*Solanum tuberosum*	Bako, Ethiopia	1650
17	IBC Pot 17III	*Solanum tuberosum*	Bako, Ethiopia	1650
18	IBC Pot 20III	*Solanum tuberosum*	Arjo, Ethiopia	2350
19	IBC Pot 21III	*Solanum tuberosum*	Arjo, Ethiopia	2350
20	IBC Pot 29JU	*Solanum tuberosum*	Jimma, Ethiopia	1700
21	IBC Pot 31JU	*Solanum tuberosum*	Jimma, Ethiopia	1700
22	IBC Pot 40	*Solanum tuberosum*	Ginchi, Ethiopia	2200
23	IBC Pot 42	*Solanum tuberosum*	Jeldu, Ethiopia	2600
24	IBC Pot 46	*Solanum tuberosum*	Jeldu, Ethiopia	2600
25	IBC Pot 48	*Solanum tuberosum*	Ginchi, Ethiopia	2200
26	IBC Pot 50	*Solanum tuberosum*	Ginchi, Ethiopia	2200
27	IBC Pot 55	*Solanum tuberosum*	Karafildicha, Shashemene, Ethiopia	1900
28	IBC Pot 56	*Solanum tuberosum*	Karafildicha, Shashemene, Ethiopia	1900
29	IBC Pot 57	*Solanum tuberosum*	Karafildicha, Shashemene, Ethiopia	1900
30	IBC Pot 58	*Solanum tuberosum*	Karafildicha, Shashemene, Ethiopia	1900
31	IBC Pot 59	*Solanum tuberosum*	Karafildicha, Shashemene, Ethiopia	1900
32	IBC Pot 60	*Solanum tuberosum*	Karafildicha, Shashemene, Ethiopia	1900
33	IBC Pot 61	*Solanum tuberosum*	Awassa, Ethiopia	1800
34	IBC Pot 62	*Solanum tuberosum*	Awassa, Ethiopia	1800
35	IBC Pot 65	*Solanum tuberosum*	Dedo, Jimma, Ethiopia	2200
36	IBC Pot 66	*Solanum tuberosum*	Dedo, Jimma, Ethiopia	2200
37	IBC Pot 68	*Solanum tuberosum*	Dedo, Jimma, Ethiopia	2200
38	IBC Pot 70	*Solanum tuberosum*	Dedo, Jimma, Ethiopia	2200
39	IBC Pot 71	*Solanum tuberosum*	Dedo, Jimma, Ethiopia	2200
40	IBC Pot 81	*Solanum tuberosum*	Kombolcha, east Hararge, Ethiopia	2000
41	IBC Pot 84	*Solanum tuberosum*	Ambo, Ethiopia	2600
42	IBC Pot 86	*Solanum tuberosum*	Kejo, Ethiopia	1650
43	IBC Pot 91	*Solanum tuberosum*	Karafildicha, Shashemene, Ethiopia	1900
44	IBC Pot 92	*Solanum tuberosum*	Karafildicha, Shashemene, Ethiopia	1900
45	IBC Pot 93	*Solanum tuberosum*	Karafildicha, Shashemene, Ethiopia	1925
46	IBC Pot 94	*Solanum tuberosum*	Karafildicha, Shashemene, Ethiopia	1900
47	IBC Tom 3	*Lycopersicon esculentum*	Mutulu, Guder, Ethiopia	2000
48	IBC Tom 1II	*Lycopersicon esculentum*	Tseday, Holeta, Ethiopia	2400
49	IBC Tom 6II	*Lycopersicon esculentum*	Tsedey, Holeta, Ethiopia	2400
50	IBC Tom 53	*Lycopersicon esculentum*	Karafildicha, Shashemene, Ethiopia	1900
51	IBC Tom 56	*Lycopersicon esculentum*	Adam Tulu, Ethiopia	1600
52	IBC Tom 58	*Lycopersicon esculentum*	Mutulu, Guder, Ethiopia	2000
53	IBC Tom 88	*Lycopersicon esculentum*	Ziway, Ethiopia	1850
54	IBC Pep 7	*Capsicum annum*	Mutulu, Guder, Ethiopia	2000
55	IBC Pep 58	*Capsicum annum*	Mutulu, Guder, Ethiopia	2000
56	IBC Pep 61	*Capsicum annum*	Mutulu, Guder, Ethiopia	2000
57	IBC Pot 1076PPRC*	*Solanum tuberosum*	Kombolcha, east Hararge, Ethiopia	2000
58	IBC Pot 1079PPRC*	*Solanum tuberosum*	Qarsa, east Hararge, Ethiopia	–
59	IBC Pot 262APPRC*	*Solanum tuberosum*	Goromt, Ambo, Ethiopia	2600
60	IBC Pot 1080PPRC*	*Solanum tuberosum*	Qarsa, east Hararge, Ethiopia	2000
61	IBC Pot 1091PPRC*	*Solanum tuberosum*	Agaro, Jimma, Ethiopia	1650
62	IBC Tom 768PPRC*	*Lycopersicon esculentum*	Arata Chaffa, Ziway, Ethiopia	1850

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Table 1. continued.

Number	Strain designation	Origin	Country	Altitude (m)
63	GSPB 2690^T	*Capsicum annum*	Kenya	–
64	GSPB 2695^T	*Lycopersicon esculentum*	Kenya	–
65	GSPB 2709^T	*Solanum tuberosum*	Peru	2000
66	GSPB 2791^T	*Solanum tuberosum*	Peru	–
67	GSPB 2792^T	*Solanum tuberosum*	Cameroon	–

All the strains were collections of this study except those with * which were obtained from Plant Protection Research Centre (PPRC), Ambo, Ethiopia, and the reference strains with ^{T} which were procured from Göttingen Collection of Phytopathogenic Bacteria (GCPB), Göttingen, Germany.

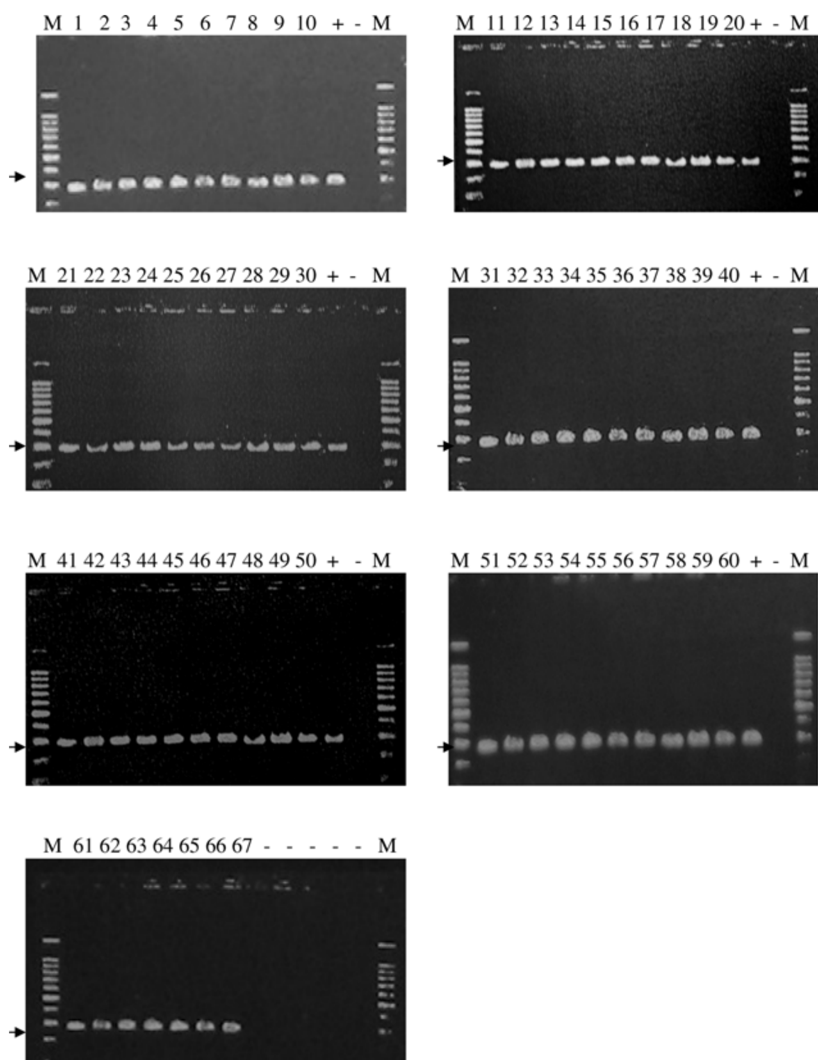

Figure 1. PCR-based confirmation of *Ralstonia solanacearum* isolates collected from Ethiopia. The 281 bp PCR product amplified is visible on the ethidium bromide stained agarose gel. M = DNA marker; + = positive control (an identified *R. solanacearum* strain from Göttingen Phytobacteriology Collection, Germany); – = negative control (reaction mixture without template DNA); Lanes 1–10, 11–20, 21–30, 31–40, 51–60 and 61–62 are strains from Ethiopia; Lanes 63–67 are strains obtained from Göttingen Phytobacteriology Collection (i.e., GSPB 2690, GSPB 2695, GSPB 2709, GSPB 2791 and GSPB 2792).

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Table 2. Comparison of carbon sources utilisation patterns of Ethiopian *Ralstonia solanacearum* strains.

Strain	Carbohydrate									Biovar
	Glucose	Fructose	Sucrose	Lactose	Maltose	Cellobiose	Dulcitol	Mannitol	Sorbitol	
IBC Pot 2JU, IBC Pot 4II, IBC Pot 8JU, IBC Pot 31JU, IBC Pot 42, IBC Pot 46, IBC Pot 48, IBC Pot 50, IBC Pot 55, IBC Pot 58, IBC Pot 59, IBC Pot 62, IBC Pot 91, IBC Pot 94, IBC Tom 6II, IBC Tom 53, IBC Pep 7, IBC Pep 58, IBC Pep 61,	+(1)	+(2)	+(1)	–	–	–	–	–	–	I
IBC Pot 1, IBC Pot 5, IBC Pot 34, IBC Pot 5II, IBC Pot 9II, IBC Pot 10II, IBC Pot 15II, IBC Pot 4JU, IBC Pot 6JU, IBC Pot 8JU, IBC Pot 9JU, IBC Pot 6III, IBC Pot 10III, IBC Pot 16III, IBC Pot 17III, IBC Pot 20III, IBC Pot 21III, IBC Pot 29JU, IBC Pot 40, IBC Pot 56, IBC Pot 57, IBC Pot 60, IBC Pot 61, IBC Pot 65, IBC Pot 66, IBC Pot 68, IBC Pot 70, IBC Pot 81, IBC Pot 84, IBC Pot 86, IBC Pot 93, IBC Tom 3, IBC Tom 1II, IBC Tom 56, IBC Tom 88, IBC Pot 1076PPRC, IBC Pot 1079PPRC, IBC Pot 262APPRC, IBC Pot 1080PPRC, IBC Pot 1091PPRC, IBC Tom 768PPRC, GSPB 2695^{T}, GSPB 2791^{T}	+(1)	+(2)	+(1)	+(1–2)	+(1–2)	+(1)	–	–	–	II
GSPB 2690^{T}, GSPB 2709^{T}, GSPB 2792^{T}	+(1)	+(2)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	III

+(1) = positive reaction in 2–6 days; +(2) = positive reaction in 6–14 days; – = negative reaction; ^{T} reference strains.

strains from other regions of the world (He *et al.* 1983, Kelman 1954, Williamson *et al.* 2002). They produced fluidal colonies with a pink to red centre on TTC and a fluidal creamy colony on CPG which is typical of the pathogen (Hayward 1964, He *et al.* 1983). Yaynu (1989) also reported the same result on strains from Ethiopia on TTC. On King's B medium none of the strains produced a fluorescent pigment.

All of the Ethiopian 62 strains which were confirmed to be *R. solanacearum* and the GCPB reference strains invariably were Gram negative, oxidase and catalase positive, and hydrolysed Tween 80 (data not shown). None of the strains hydrolysed starch or gelatine. All strains produced H_2S from cysteine and reduced nitrate. As characteristical for *R. solanacearum,* none of the Ethiopian strains grew on 2% (wt/vol) NaCl. All strains grew at 37 °C and but not at 41 °C. The capacity to oxidize sucrose is a stable characteristic of *R. solanacearum* (Hayward 1964). Moreover, absence of growth at 2% NaCl is another characteristic of the pathogen (Ito *et al.* 1998). In our study, all the *R. solanacearum* strains were positive for sucrose oxidation and did not grow at 2% NaCl solution. We concluded that all the 62 strains from Ethiopia were *R. solanacearum*, which agrees with the results of the tomato bioassay and the specific PCR-based assays.

Some differences among strains from Ethiopia were observed in carbon source utilisation tests (Table 2). Based on Hayward's classification scheme (Hayward 1964), 19 of 62 strains were classified as biovar I and 43 as biovar II (Table 2). Biovar II strains produced acid from lactose, maltose and cellobiose but failed to utilise mannitol, sorbitol and dulcitol. Biovar I strains did not utilise disaccharides or sugar alcohols even after 5 weeks of incubation. Biovar III strains from GCPB utilised all of the sugar and alcohol carbohydrates.

In 1989 Yaynu reported the existence of only biovar II in Ethiopian *R. solanacearum* population. The phenotypic tests demonstrated that the Ethiopian strains of *R. solanacearum* belong to biovar I and biovar II. Strains in biovar I were obtained from potato, tomato, and pepper and were pathogenic to potato, tomato, eggplant, pepper and/or tobacco. Biovar II strains were obtained from potato and tomato, and pathogenic to

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Table 3. Growth of *Ralstonia solanacearum* biovar I, II, and III strains at different temperatures.

Temperature (°C)	Biovar		
	I	II	III
22	0.311 B b	0.451 A a	0.280 B b
27	0.462 A a	0.471 A a	0.607 A a
32	0.564 A a	0.404 B a	0.617 A a
37	0.566 A a	0.299 B b	0.620 A a

Means within a row and column followed by the same upper case and lower case letters, respectively, are not significantly different (Tukey, $\alpha = 0.05$). Strains were grown in casamino acids-peptone-glucose (CPG) broth for 48 h under static conditions.

potato, tomato, and eggplant. In previous studies from Ethiopia, only biovar II was isolated from potato and tomato and those strains were pathogenic to potato, tomato, and eggplant (Yaynu 1989). This is the first report of biovar I of *R. solanacearum* in diseased plants in Ethiopia. Biovar I is the most widely distributed strain of *R. solanacearum* in the world (He *et al.* 1983). Hence, it is not surprising that biovar I was isolated among the strains collected from Ethiopia, as several thousands of potato genotypes have been introduced to the country (Berga *et al.* 1994) from the International Potato Centre (CIP), Peru, and other parts of the world to develop high yielding and adaptable cultivars with resistance to the major stresses. We suspect that strains of *R. solanacearum* may have been introduced through latently infected planting material. It has been demonstrated that, *R. solanacearum* can be widely disseminated through vegetative propagating material (tomato, *Heliconia,* banana, and potato) and contaminated irrigation water and latently infected planting materials play a major role in its local and international dissemination (Hayward 1991, Williamson *et al.* 2002).

The number of strains belonging to the new biovar in Ethiopia (biovar I) represented almost one third (19 out of 62) of the total number of strains collected. This may indicate that the introduction of the new strains to Ethiopia may not have occurred recently, rather occurred many years ago. One justification for this is that some of the strains in the new biovar were collected from areas distal to locations in Ethiopia (Holeta, Shashemene and Alemaya) where introduced potato genotypes are multiplied before distribution to farmers.

Growth tests under static conditions showed that temperature significantly ($F = 20.97$, $P < 0.0001$) affected growth of *R. solanacearum*. The two-way interaction between temperature and biovar was significant ($F = 20.86$, $P < 0.0001$) indicating that the effect of temperature on growth of *R. solanacearum* was associated with the biovar designation. Generally, the experiment showed that biovar II strains were able to grow preferentially at low temperature (22 °C). Biovar I and III strains (Table 3) grew at higher temperatures (32 and 37 °C). Thus, biovar II strains may be adapted to climates with relatively cooler temperatures than strains of biovars I and III, which grew well at higher temperatures. This is in agreement with previous reports that biovar II strains are adapted to cool tropical climates and biovars I and III strains are adapted to warm tropical climates (Horita *et al.* 2005, Swanson *et al.* 2005, Oepp/Eppo 2004, French *et al.* 1993, Marin and El-Nashaar 1993).

Isolates of biovar II in Ethiopia were collected from cool tropical regions with altitudes ranging from 1600 to 2600 meters above sea level. Likewise, biovar I strains from Ethiopia also were collected from areas ranging from 1630 to 2600 meters above sea level; in spite of its adaptation to warm tropical conditions. Moreover, of the three biovar III strains included as comparative controls in this study, one strain was isolated from 2000 meters above sea level in Peru (origin of the other two strains was not recorded); an environment not categorized as a warm tropical conditions. This may indicate that the strains can occur and cause disease in areas with environments to support optimal growth of the pathogen. There are reports that support that strains of *R. solanacearum* will cause disease in environments considered suboptimal for growth and survival (French *et al.* 1993). It is believed that the presence of these strains in these areas may be due to the distribution of infected potato tubers from one area to the other.

Pathogenicity

With the pathogenicity test, initial symptoms of wilting in susceptible hosts appeared 3 to 4 days after stem inoculation. Initial symptoms usually consisted of wilting of the inoculated leaf and stunting of growth. In potato, tomato, and eggplants, inoculation was fol-

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Table 4. Pathogenicity of *Ralstonia solanacearum* strains from Ethiopia on five major hosts and their pathogenic groups and respective biovars.

Strains	Original host	Pathogenicity on					Pathogenic group	Biovar
		Potato	Tomato	Eggplant	Pepper	Tobacco		
IBC Pot 2JU, IBC Pot 4II, IBC Pot 8JU, IBC Pot 31JU, IBC Pot 46, IBC Pot 48, IBC Pot 50, IBC Pot 55, IBC Pot 58, IBC Pot 59, IBC Pot 94, IBC Tom 6II, IBC Tom 53, IBC Pep 58, IBC Pep 61	Potato, tomato, pepper	2.6–5[†]	2.6–5	2.6–5	1.1–5	1.1–5	1	I
GSPB 2690[T], GSPB 2709[T], GSPB 2792[T]	Potato, pepper	4.1–5	4.1–5	4.1–5	2.6–5	1.1–4	1	III
IBC Pot 42, IBC Pot 62, IBC Pot 91, IBC Pep 7	Potato, pepper	2.6–5	2.6–5	2.6–5	2.6–4	1	2	I
IBC Pot 1, IBC Pot 5, IBC Pot 34, IBC Pot 5II, IBC Pot 9II, IBC Pot 10II, IBC Pot 15II, IBC Pot 4JU, IBC Pot 6JU, IBC Pot 9JU, IBC Pot 6III, IBC Pot 10III, IBC Pot 16III, IBC Pot 17III, IBC Pot 20III, IBC Pot 21III, IBC Pot 29JU, IBC Pot 40, IBC Pot 56, IBC Pot 57, IBC Pot 60, IBC Pot 61, IBC Pot 65, IBC Pot 66, IBC Pot 68, IBC Pot 70, IBC Pot 71, IBC Pot 81, IBC Pot 84, IBC Pot 86, IBC Pot 92, IBC Pot 93, IBC Pot 1076PPRC*, IBC Pot 1079PPRC*, IBC Pot 262APPRC*, IBC Pot 1080PPRC*, IBC Pot 1091PPRC*, GSPB 2791[T], IBC Tom 3, IBC Tom 1II, IBC Tom 56, IBC Tom 58, IBC Tom 88, IBC Tom 768PPRC*, GSPB 2695[T]	Potato, tomato	2.6–5	2.6–5	2.6–5	1	1	3	II

[†] Disease severity score determined according to Horita and Tsuchiya (2001) where 1 is no symptom, and 5 is dead plant.
All the strains were collections of this study except those with * which were obtained from Plant Protection Research Centre (PPRC), Ambo, Ethiopia, and those with [T] which were procured from Göttingen Collection of Phytopathogenic Bacteria (GCPB), Göttingen, Germany.

lowed by considerable ooze and decay of the pith surrounding the point of inoculation; after which the plants wilted rapidly and died. All strains caused rapid wilting of eggplant, tomato and potato. Only 19 strains from Ethiopia and 3 strains from the Göttingen collection infected pepper and 15 strains from Ethiopia and 2 strains from Göttingen collection infected tobacco.

On the basis of host reaction, the strains studied could be categorized into three pathogenic groups (Table 4). Group 1 included strains that were virulent on all five host species; group 2 included a few strains that were virulent on all host species except tobacco; and group 3 included the majority of the strains that were virulent on potato, tomato, and eggplant but nonvirulent on pepper and tobacco. Biovar I strains fell in group 1 and 2 which showed that they have wide host range, whereas biovar II strains were placed in the narrow host range group 3; those that infect only potato, tomato, and eggplant. Strains of biovar III also infected all test plants.

Until 1989, only potato, eggplant, and tomato were infected by *R. solanacearum* in Ethiopia (Yaynu 1989). Recently, however, pepper plants with symptoms of bacterial wilt were discovered (personal observation). The disease incidence on potato (Bekele 1996) and tomato (Earo 2002) has increased in Ethiopia. This increase in the intensity of the disease and host-range of the pathogen may be due to the introduction of biovar I strains although changes in cultural practices can also result in increased disease development.

In conclusion, the study demonstrated that there are two biovars (biovar I and II) of *R. solanacearum* in Ethiopia which differ in physiological and pathogenic characteristics. This indicates that a single control measure can not effectively work for both biovars of the pathogen.

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