J Gen Plant Pathol (2004) 70:278–283
DOI 10.1007/s10327-004-0126-7

© The Phytopathological Society of Japan
and Springer-Verlag Tokyo 2004

BACTERIAL AND PHYTOPLASMA DISEASES

Short communication

Mitsuo Horita · Kazutaka Yano · Kenichi Tsuchiya

PCR-based specific detection of *Ralstonia solanacearum* race 4 strains

Received: November 12, 2003 / Accepted: January 30, 2004

Abstract Two primer sets were designed based on the sequence of polymorphic bands that were derived from repetitive sequence-based polymerase chain reaction (rep-PCR) fingerprinting and specifically detected in *Ralstonia solanacearum* race 4 strains (ginger, mioga, and curcuma isolates). One primer set (AKIF-AKIR) amplified a single band (165 bp) from genomic DNA obtained from all mioga and curcuma and some ginger isolates; another set (21F-21R) amplified one band (125 bp) from the other ginger isolates. These primer sets did not amplify the bands from genomic DNA of other *R. solanacearum* strains or of other related bacteria. PCR detection limit for the pathogen was 2×10^2 cfu.

Key words *Ralstonia solanacearum* · Bacterial wilt · Zingiberaceae crop · PCR detection · rep-PCR

Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi et al. 1995 is one of the most important and widespread bacterial diseases in tropical, subtropical, and warm temperate regions worldwide (Hayward 1991). Based on differences in host ranges, *R. solanacearum* has been divided into five races (Buddenhagen and Kelman 1964; Hayward et al. 1967; He et al. 1983). Strains that affect Zingiberaceae crops were discriminated from other plant isolates and subsequently designated race 4 (Schaad et al. 2001). In Japan, bacterial wilt of Zingiberaceae crops such as ginger (*Zingiber officinale* Rosc.), mioga (*Z. mioga* Rosc.), and curcuma (*Curcuma alismatifolia* Hort.) has occurred since 1995 (Morita et al. 1996; Tsuchiya et al. 1999; Yano et al. 2002). Infected rhizomes are thought to have been introduced from other countries and colonized in Japanese agro-eco systems (Tsuchiya et al. 2000).

To control bacterial wilt, the ability to detect the disease at a minimal level of infection and to eliminate infection sources and spread of the pathogen is essential (Seal and Elphinstone 1994). This study describes a useful polymerase chain reaction (PCR) method for specific detection of *R. solanacearum* race 4 strains. The primer sets were generated from the nucleotide sequences of race 4-specific DNA bands obtained by repetitive sequence-based polymerase chain reaction (rep-PCR) (Louws et al. 1999).

Bacterial strains used in this study are listed in Tables 1 and 2. Most strains were from the Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF) genebank culture collection. Strains were maintained in long-term storage as suspensions in sterile distilled water or by freezing in 10% skim milk supplemented with 1% sodium glutamate at −30°C; they were revived by plating on tetrazolium chloride (TZC) medium (Kelman 1954) or nutrient agar medium (Difco, Detroit, MI, USA). Strains provided as extracted genomic DNA were stored at −30°C until use.

Bacterial cells were grown overnight at 30°C in casamino acids, peptone, and glucose (CPG) broth (Hendrick and Sequeira 1984) or nutrient broth. A 1-ml aliquot of the culture was centrifuged, and crude DNA was extracted by heating resuspended pellets to 100°C in a 1.5-ml microtube for 10 min. Boiled samples were stored at −30°C until use.

The rep-PCR method employed was adapted from previous reports (Horita and Tsuchiya 2001; Louws et al. 1994). Primer sets REP [REP1R-I (5′-IIIICGICGICATCIGGC-3′) and REP2-I (5′-ICGITTATCIGGCCTAC-3′)] and BOX [BOXA1R (5′-CTACGGCAAGGCGACGCTGAC G-3′)] were synthesized by Amersham Pharmacia Biotech (Tokyo, Japan). PCR amplifications were performed in a

M. Horita (✉)
National Agricultural Research Center for Hokkaido Region,
1 Hitsujigaoka, Toyohira-ku, Sapporo 062-8555, Japan
Tel. +81-11-857-9277; Fax +81-11-859-2178
e-mail: mhorita@affrc.go.jp

K. Yano
Kochi Agricultural Research Center, Kochi, Japan

K. Tsuchiya
National Institute of Agro-Environmental Sciences, Ibaraki, Japan

The nucleotide sequence data reported are available in the DDBJ/EMBL/GenBank databases under accession numbers AB118756 and AB118757

Table 1. *Ralstonia solanacearum* strains used in this study

Strain	Host	Race	Geographic origin	PCR amplification[a]		
				AKI	21	AKI + 21
MAFF 211272	Curcuma (*Curcuma alismatifolia*)	4	Kochi, Japan	+	−	+
MAFF 211274	Curcuma	4	Kochi, Japan	+	−	+
MAFF 211276	Curcuma	4	Kochi, Japan	+	−	+
MAFF 211278	Curcuma	4	Kochi, Japan	+	−	+
MAFF 211490	Mioga (*Zingiber mioga*)	4	Kochi, Japan	+	−	+
MAFF 211492	Mioga	4	Kochi, Japan	+	−	+
MAFF 211493	Mioga	4	Kochi, Japan	+	−	+
MAFF 211497	Mioga	4	Kochi, Japan	+	−	+
MAFF 211479	Ginger (*Zingiber officinale*)	4	Kochi, Japan	+	−	+
MAFF 211483	Ginger	4	Kochi, Japan	+	−	+
MAFF 211484	Ginger	4	Kochi, Japan	+	−	+
419-b-1-I	Ginger	4	Thailand	+	−	+
419-b-1-III	Ginger	4	Thailand	+	−	+
412-c-1-I	Ginger	4	Thailand	+	−	+
MAFF 211471	Ginger	4	Kochi, Japan	−	+	+
MAFF 211474	Ginger	4	Kochi, Japan	−	+	+
MAFF 211476	Ginger	4	Kochi, Japan	−	+	+
MAFF 211286	Ginger	4	Australia	−	+	+
Z8a	Ginger	4	China	−	+	+
Z8b	Ginger	4	China	−	+	+
MAFF 211280	Eggplant	1	Kochi, Japan	−	−	−
MAFF 211281	Eggplant	1	Kochi, Japan	−	−	−
MAFF 211282	Eggplant	1	Kochi, Japan	−	−	−
MAFF 211514	Tomato	1	Kochi, Japan	−	−	−
MAFF 301070	Tomato	1	Kochi, Japan	−	−	−
U154	Tobacco	1	Kochi, Japan	−	−	−
MAFF 302549	Statice (*Limonium* sp.)	1	Kochi, Japan	−	−	−
MAFF 211499	Hot pepper	1	Kochi, Japan	−	−	−
MAFF 211516	Sweet pepper	1	Kochi, Japan	−	−	−
MAFF 301860	Sweet pepper	1	Ibaraki, Japan	−	−	−
MAFF 211557	Kalanchoe (*Kalanchoe* sp.)	1	Kagawa, Japan	−	−	−
MAFF 301560	Bird-of-paradise (*Strelitzia reginae*)	1	Nagasaki, Japan	−	−	−
89C-9	Pumpkin	1	Okayama, Japan	−	−	−
MAFF 211271	Potato	3	Shizuoka, Japan	−	−	−
MAFF 301559	Potato	3	Nagasaki, Japan	−	−	−
MAFF 302154[T]	Tomato	1	USA	−	−	−
MAFF 211540	Geranium (*Pelargonium capitatum*)	1	Reunion	−	−	−
13	Potato	1	Brazil	−	−	−
MAFF 211287	Potato	1	Nepal	−	−	−
MAFF 302510	Tomato	1	Indonesia	−	−	−
MAFF 302487	Cassava (*Manihot esculenta*)	1	Indonesia	−	−	−
MAFF 211290	Banana	2	Panama	−	−	−
MoD3	Banana	2	Philippine	−	−	−
MAFF 211292	Potato	3	Belgium	−	−	−
Garut1	Potato	3	Indonesia	−	−	−
MAFF 211454	Mulberry	5	China	−	−	−

AKI, AKIF-AKIR primer set; 21, 21F-21R primer set; AKI + 21, AKIF-AKIR and 21F-21R primer sets; [T], type strain
[a] +, positive; −, negative

thermocycler (PC-800; Astec, Tokyo, Japan) in 25-μl reaction volumes of 10 mM Tris-HCl at pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTP, 50 pmol each primer, 2.5 units of DNA polymerase Takara *Taq* (Takara, Otsu, Japan), and 5 μl of template DNA. The PCR thermocycler was programmed as follows: an initial denaturation step at 95°C for 7 min followed by 30 cycles of 94°C for 1 min, 44°C (REP) or 52°C (BOX) for 1 min, and 65°C for 8 min with a final extension of 65°C for 15 min.

Amplified PCR products were separated by 1.5% agarose gel electrophoresis in 0.5 × Tris-acetate-EDTA buffer (89 mM Tris-acetate pH 8.0 and 2 mM EDTA) at 4 V/cm for 5 h, stained with ethidium bromide and photographed under ultraviolet (UV) light. DNA bands were recovered from the gel by the glass beads method using an Easytrap kit (Takara, Otsu, Japan) and were cloned within the plasmid vector pPCR-Script Amp by PCR-Script Cloning Kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions.

The clones (pPS4I and pPS4II) were sequenced using the ABI PRISM 3100 DNA sequencer (PE Applied Biosystems, Foster City, CA, USA) and DNA sequencing kit with BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems) according to the instruction. Sequencing reactions were performed in a 9600 thermocycler (PE Applied Biosystems). M13 primers M3

Table 2. Bacterial strains used in this study

Species	Strain	PCR amplification		
		AKI	21	AKI + 21
Ralstonia eutropha	MAFF 311269	–	–	–
Burkholderia glumae	MAFF 301169[T]	–	–	–
Burkholderia plantarii	MAFF 301723[T]	–	–	–
Burkholderia gladioli pv. *gladioli*	MAFF 302385	–	–	–
Burkholderia cepacia	MAFF 302528	–	–	–
Burkholderia andropogonis	MAFF 301005	–	–	–
Acidovorax avenae	MAFF 301141	–	–	–
Acidovorax konjaci	MAFF 301465	–	–	–
Pseudomonas syzygii	T327	–	–	–
Pseudomonas syringae pv. *maculicola*	MAFF 302539	–	–	–
Pseudomonas marginalis pv. *marginalis*	MAFF 301512	–	–	–
Pseudomonas viridiflava	MAFF 301500	–	–	–
Pseudomonas fluorescens	MAFF 302847	–	–	–
Agrobacterium rhizogenes	MAFF 210265	–	–	–
Erwinia carotovora subsp. *carotovora*	MAFF 211376	–	–	–
Enterobacter cloacae	MAFF 811103	–	–	–
Pantoea ananas	MAFF 811106	–	–	–

and RV (Takara) were used to determine the sequences of the clones.

A homology search of the sequence was performed using the FastA program (http://www.dna.affrc.go.jp/htdocs/Blast/FastA.html). The sequences of pPS4I and pPS4II were deposited in the DDBJ/EMBL/GenBank database under accession nos. AB118757 and AB118756. Oligonucleotide primers with AKIF (5′-AACCGCACGTAAATCG TCGACA-3′) and AKIR (5′-ACGACTGCCCATTCGAC GATG-3′) to amplify a 165-bp region in pPS4I, and 21F (5′-CGACGCTGACGAAGGGACTC-3′) and 21R (5′-CTG ACACGGCAAGCGCTCA-3′) to amplify a 125-bp region in pPS4II, were designed using DNA analysis software Genetyx ver. 6 (Genetyx, Tokyo, Japan).

Amplifications by PCR with the two primer sets were performed in PC-800 in 25-μl reaction volumes of 10 mM Tris-HCl at pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTP, 50 pmol each primer, 0.5 unit of Takara *Taq*, and 5 μl of template DNA. The PCR thermocycler was programmed as follows: an initial denaturation step at 95°C for 5 min followed by 30 cycles of 94°C for 1 min, 61°C for 1 min, and 72°C for 3 min, with a final extension of 72°C for 10 min. Amplified PCR products (3–6 μl) were separated by 1.5% agarose gel electrophoresis in 0.5 × Tris-borate-EDTA buffer (89 mM Tris-HCl pH 8.0, 89 mM boric acid, and 2 mM EDTA) at 5 V/cm for 1 h, stained with ethidium bromide, and photographed under UV light.

To estimate the detection sensitivity of race 4 strains, serial dilutions of the bacterial cells were prepared in sterilized distilled water and were heated at 100°C for 10 min; 5 μl from each dilution series was then added to the PCR mixture. The number of the cells in the serial dilution was confirmed by incubation on TZC agar plates at 30°C for 3 days. The PCR tests were repeated at least two times with independently prepared DNA stocks.

To apply the PCR method for detection of race 4 in infested soil, the protocols by Elphinestone et al. (1996) and Pradhanang et al. (2000) were modified. Serial dilutions of the bacterial cells were mixed well with horticultural soil (Kureha Chemical, Tokyo, Japan), and 200 μl of the soil suspended in distilled water was directly added in 4 ml of the semiselective medium [modified SMSA broth: 10 g peptone, 5 ml glycerol, 1 g casamino acids, 25 mg bacitracin (Wako, Tokyo, Japan), 100 mg polymyxin B sulfate (Wako), 5 mg chloramphenicol (Wako), 0.5 mg penicillin G sodium salt (Wako), 5 mg crystal violet, 50 mg 2,3,5-triphenyl tetrazolium chloride, and 1 l distilled water, pH 7.0] (Elphinstone et al. 1996) and incubated for 48–72 h at 30°C. After enrichment, samples of broth culture were boiled with 0.05 M NaOH for 10 min and then centrifuged; the supernatants before and after ethanol precipitation were used as a template for the PCR test. Simultaneously, serial dilution of the soil suspension was incubated on modified SMSA agar plates at 30°C for 4 days to count the viable cells. The tests were repeated two times with duplicate samples.

Genomic fingerprints of race 4 strains were generated by rep-PCR (Fig. 1). The primer sets defined two fingerprint types (I and II) after duplicate analyses. All mioga, curcuma, and some ginger isolates had type I fingerprints; the rest of the ginger isolates had type II fingerprints. Their genomic fingerprints were compared with those of other plant isolates (data not shown) and type I- or type II-specific DNA bands were identified (Fig. 1). Each DNA band was cloned using the plasmid vector and designated pPS4I (derived from type I fingerprints of MAFF 211490) or pPS4II (derived from type II fingerprints of MAFF 211471).

Insert DNA of plasmids pPS4I and pPS4II were sequenced to generate specific primers to detect *R.*

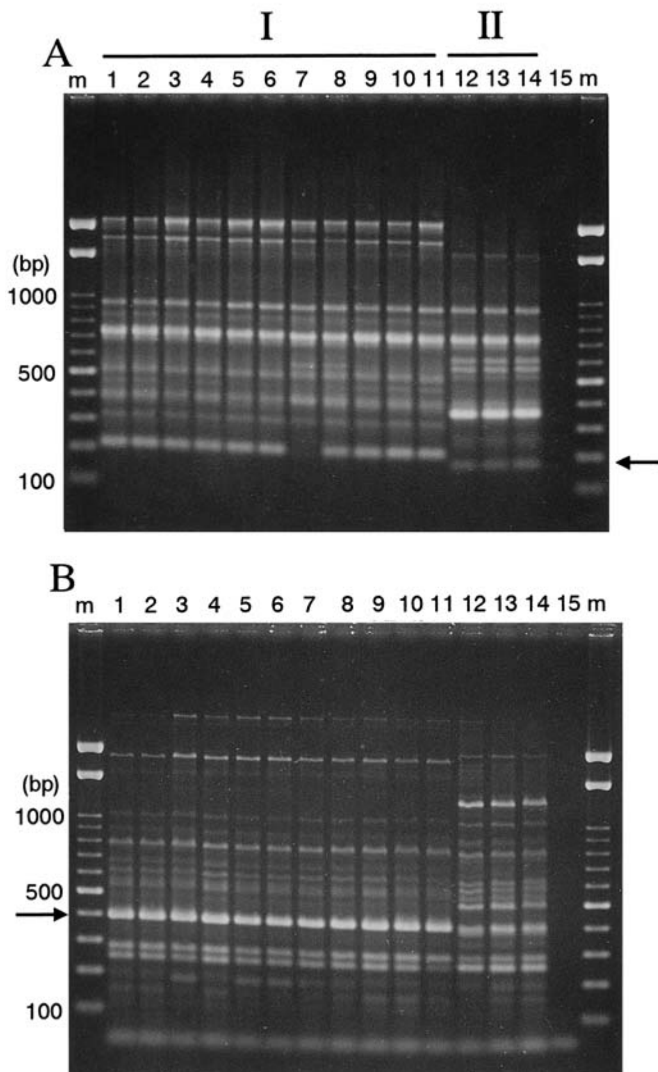

Fig. 1. Agarose gel showing representative patterns of *Ralstonia solanacearum* race 4 strains generated by repetitive sequence-based polymerase chain reaction (rep-PCR) with Box (**A**) and repetitive extragenic palindromic (REP) primers (**B**). Lanes *1–4*, curcuma isolates (MAFF 211272, MAFF 211274, MAFF 211276, MAFF 211278); lanes *5–8*, mioga isolates (MAFF 211490, MAFF 211492, MAFF 211493, MAFF 211497); lanes *9–14*, ginger isolates (MAFF 211479, MAFF 211483, MAFF 211484, MAFF 211471, MAFF 211474, MAFF 211476); lane *15*, control with no template; lane *m*, DNA molecular size marker (DNA ladder markers, 100-bp DNA ladder; Toyobo, Osaka, Japan). *Numbers* above the lane numbers indicate DNA fingerprint groups. *Arrow* indicates type I-specific (*left*) and type II-specific (*right*) DNA bands

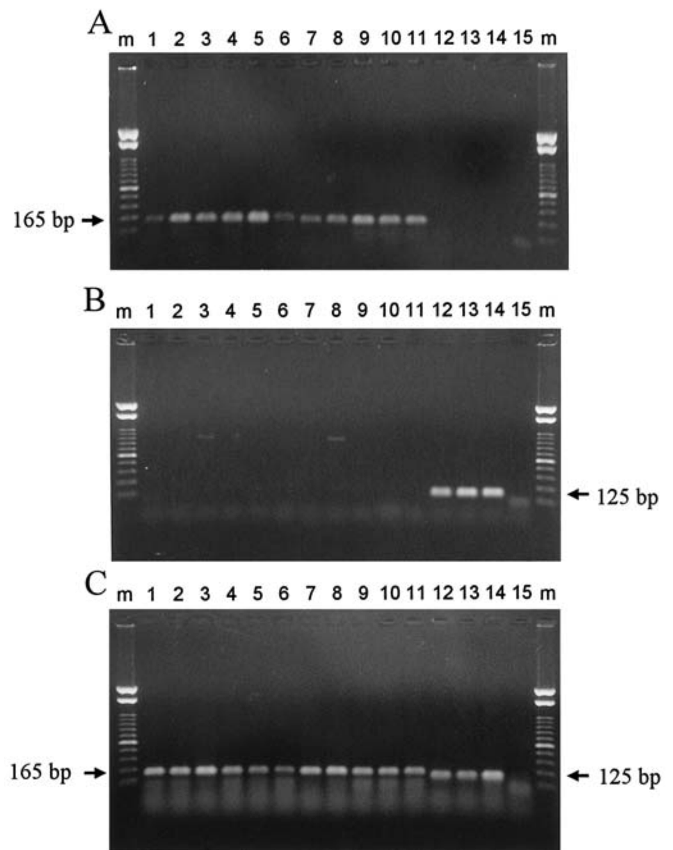

Fig. 2. PCR amplification with various primer sets: **A** AKIF-AKIR. **B** 21F-21R. **C** AKIF-AKIR and 21F-21R. PCR samples were subjected to electrophoresis on 1.5% agarose gel. Lanes *1–4*, *R. solanacearum* curcuma isolates (MAFF 211272, MAFF 211274, MAFF 211276, MAFF 211278); lanes *5–8*, mioga isolates (MAFF 211490, MAFF 211492, MAFF 211493, MAFF211497); lanes *9–14*, ginger isolates (MAFF 211479, MAFF 211483, MAFF 211484, MAFF 211471, MAFF 211474, MAFF 211476); lane *15*, control with no template; lane *m*, DNA molecular size marker (DNA ladder markers, 100-bp DNA ladder)

solanacaearum race 4. The 375-bp (pPS4I) and 173-bp (pPS4II) sequences including the primer DNA region were determined. DNA-DNA homology search showed 86.6% identity at the beginning (185 bp) of pPS4I with parts of the RS02993 and RS02991 genes (both are hypothetical) in *R. solanacearum* GMI1000 (Salanoubat et al. 2002). Other regions had no obvious homology with known bacterial genes or genome sequences.

Two PCR primer sets (AKIF-AKIR and 21F-21R) were designed from the sequences that had no homology with the known bacterial genes and were tested. The AKIF-AKIR primer set amplified a single band (165 bp) from genomic DNA obtained from mioga, curcuma, and ginger isolates that represented type I rep-PCR fingerprints. The 21F-21R primer set amplified one band (125 bp) from ginger isolates that represented type II fingerprints (Fig. 2). Other *R. solanacearum* strains from various hosts and geographic regions were also tested. Only genomic DNA from race 4 strains yielded the expected amplified products (Fig. 3, Table 1). In addition, these bands were not amplified from related species including *R. eutropha, Pseudomonas syzygii*, or any other genera of bacteria tested (Table 2).

Samples of the serial dilutions of bacterial cells (MAFF 211471 or MAFF 211490) ranging from 2×10^4 to 2 cfu were boiled for 10 min and used as templates for the PCR reaction. The detection threshold for serially diluted cells was 2×10^2 cfu per reaction using either AKIF-AKIR or 21F-21R primer set (Fig. 4).

Samples of extracted DNA from artificially infested soils, which included serial dilutions of bacterial cells (MAFF 211471 or MAFF 211490) ranging from 3×10^7 to

Table 3. Detection of R. solanacearum race 4 strains from the soil

PCR test	Approximate no. of *R. solanacearum* in soil (cfu/g)[a]								
	3×10^7	3×10^6	3×10^5	3×10^4	3×10^3	3×10^2	3×10^1	3	Control
MAFF 211490									
After enrichment[b]	4/4[d]	4/4	0/4	0/4	0/4	0/4	0/4	0/4	0/2
Ethanol precipitation[c]	4/4	4/4	4/4	4/4	4/4	4/4	1/4	0/4	0/2
MAFF 211471									
After enrichment	4/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/2
Ethanol precipitation	4/4	4/4	4/4	4/4	4/4	4/4	1/4	0/4	0/2

[a] Cell numbers were measured by incubation on modified SMSA agar plates
[b] Soil sample was incubated in modified SMSA broth, boiled with 0.05 M NaOH, and separated from the soil by centrifugation
[c] After enrichment, the sample was precipitated with ethanol and resuspended in sterilized Milli-Q water
[d] Positive samples/tested samples

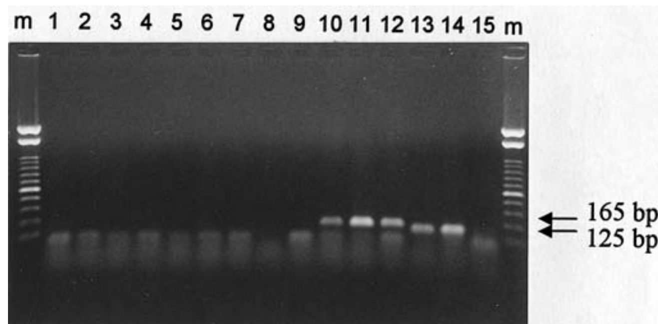

Fig. 3. PCR detection of *R. solanacearum* strains using AKIF-AKIR and 21F-21R primer sets. PCR samples were subjected to electrophoresis on 1.5% agarose gel. Lane *1*, MAFF 301070 (tomato isolate); lane *2*, MAFF 211514 (tomato); lane *3*, MAFF 211280 (eggplant); lane *4*, MAFF 211281 (eggplant); lane *5*, MAFF 211282 (eggplant); lane *6*, MAFF 211516 (sweet pepper); lane *7*, MAFF 211499 (hot pepper); lane *8*, U154 (tobacco); lane *9*, MAFF 302549 (statice); lane *10*, MAFF 211490 (mioga); lane *11*, MAFF 211272 (curcuma); lane *12*, 419-b-1-III (ginger); lane *13*, MAFF 211471 (ginger); lane *14*, MAFF 211286 (ginger); lane *15*, control with no template; lane *m*, DNA molecular size marker (DNA ladder markers, 100-bp DNA ladder; Toyobo)

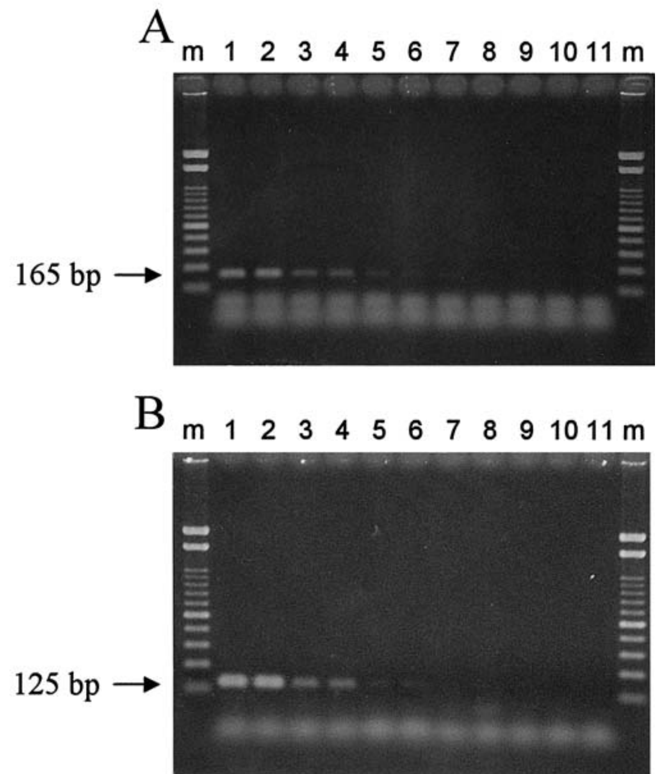

Fig. 4. PCR detection sensitivity for *R. solanacearum* race 4 strains using AKIF-AKIR and 21F-21R primer sets. **A** MAFF 211490. **B** MAFF 211471. PCR samples were subjected to electrophoresis on 1.5% agarose gel. Lanes *1* and *2*: 2×10^4 cfu; lanes *3* and *4*: 2×10^3 cfu; lanes *5* and *6*: 2×10^2 cfu; lanes *7* and *8*: 2×10^1 cfu; lanes *9* and *10*: 2 cfu; lane *11*: control with no template; lane *m*, DNA molecular size marker (DNA ladder markers, 100-bp DNA ladder; Toyobo)

3 cfu/g dry weight soil and incubated in modified SMSA broth, were used as templates for PCR. The detection thresholds were 3×10^7 cfu/g (MAFF 211471) and 3×10^6 cfu/g (MAFF 211490), respectively. Ethanol precipitation of the samples allowed detection of 3×10^2 cfu/g for each (Table 3).

Fingerprint analysis by rep-PCR has been used to differentiate species, pathovars, and strains of plant pathogenic bacteria (Horita and Tsuchiya 2001; Louws et al. 1994, 1999). In this study, we showed that these amplified polymorphic bands were also useful for designing DNA primer sets for specific detection of the pathogen. Race 4 strains have almost similar biochemical and physiological properties with race 1 strains (Horita and Tsuchiya 1999), which are commonly distributed in Japan and affect a wide range of plants (tomato, potato, eggplant, sweet pepper, tobacco, various weeds). It is difficult to differentiate these two races without a pathogenicity test. We identified race 4-specific DNA bands from the genomic fingerprints, which were not homologous to other known bacterial genes and genome sequences. For practical use of the PCR method, higher detection sensitivity of the pathogen from the infested host, soil, or natural environment is needed. We found that ethanol precipitation treatment during purification of extracted DNA efficiently enhances the detection sensitivity. Efforts to improve PCR conditions are now in progress.

Acknowledgment We thank all of those who contributed strains or genomic DNA used in this study.

References

Buddenhagen I, Kelman A (1964) Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Annu Rev Phytopathol 2:203–230

Elphinestone JG, Hennessy J, Wilson JK, Stead DE (1996) Sensitivity of different methods for the detection of *Ralstonia solanacearum* in potato tuber extracts. EPPO Bull 26:663–678

Hayward A, Moffett M, Pegg K (1967) Bacterial wilt of ginger in Queensland. Queensland J Agric Anim Sci 24:1–5

Hayward AC (1991) Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu Rev Phytopathol 29:65–87

He L, Sequeira L, Kelman A (1983) Characteristics of strains of *Pseudomonas solanacearum* from China. Plant Dis 67:1357–1361

Hendrick CA, Sequeira L (1984) Lipopolysaccharide-defective mutants of the wilt pathogen *Pseudomonas solanacearum*. Appl Environ Microbiol 48:94–101

Horita M, Tsuchiya K (1999) Phenotypic characteristics and cluster analysis of Japanese and reference strains of *Ralstonia solanacearum*. Ann Phytopathol Soc Jpn 65:604–611

Horita M, Tsuchiya K (2001) Genetic diversity of Japanese strains of *Ralstonia solanacearum*. Phytopathology 91:399–407

Kelman A (1954) The relationship of pathogenicity of *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 44:693–695

Louws FJ, Fulbright DW, Stephens CT, de Brujin FJ (1994) Specific genomic fingerprint of phytopathogenic *Xanthomonas* and *Pseudomonas* pathovars and strains generated with repetitive sequences and PCR. Appl Environ Microbiol 60:2286–2295

Louws FJ, Rademaker JLW, de Brujin FJ (1999) The three Ds of PCR-based genomic analysis of phytobacteria: diversity, detection and disease diagnosis. Annu Rev Phytopathol 37:81–125

Morita Y, Yano K, Tsuchiya K, Kawada Y (1996) Bacterial wilt of *Curcuma alismatifolia* caused by *Pseudomonas solanacearum*. Proc Assoc Plant Protection Shikoku 31:1–6 (in Japanese with English summary)

Pradhanang PM, Elphinestone JG, Fox RTV (2000) Sensitive detection of *Ralstonia solanacearum* in soil: a comparison of different detection techniques. Plant Pathol 49:414–422

Salanoubat M, Genin S, Artiguenave F, Gouzy J, Mangenot S, Arlat M, Billault A, Brottier P, Camus JC, Cattolico L, Chandler M, Choisne N, Claudel-Renard C, Cunnac S, Demange N, Gaspin C, Lavie M, Moisan A, Robert C, Saurin W, Schiex T, Siguier P, Thebault P, Whalen M, Wincker P, Levy M, Weissenbach J, Boucher CA (2002) Genome sequence of the plant pathogen *Ralstonia solanacearum*. Nature 415:497–502

Schaad NW, Jones JB, Chun W (eds) (2001) Laboratory guide for identification of plant pathogenic bacteria. APS, St. Paul, MN

Seal SE, Elphinstone JG (1994) Advances in identification and detection of *Pseudomonas solanacearum*. In: Hayward AC, Hartman GL (eds) Bacterial wilt: the disease and its causative agent, *Pseudomonas solanacearum*. CAB International, Oxford, pp 9–24

Tsuchiya K, Yano K, Horita M, Morita Y, Kawada Y, d'Ursel CM (1999) Occurrence of bacterial wilt of ginger in Japan. Ann Phytopathol Soc Jpn 65:363 (abstract, in Japanese)

Tsuchiya K, Horita M, Hikichi Y (2000) Recent occurrence and research on bacterial wilt. Plant Protection 54:87–92 (in Japanese)

Yabuuchi E, Kosako Y, Yano I, Hotta H, Nishiuchi Y (1995) Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: proposal of *Ralstonia picketii* (Ralston, Palleroni and Doudoroff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. Microbiol Immunol 39:897–904

Yano K, Kawada Y, Tsuchiya K, Horita M (2002) First report of bacterial wilt of mioga (*Zingiber mioga*) caused by *Ralstonia solanacearum* in Japan. Jpn J Phytopathol 68:94 (abstract, in Japanese)

Copyright of Journal of General Plant Pathology is the property of Springer - Verlag New York, Inc. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.