16S-23Sリボゾームdnaスペーサー領域の塩基配列を利用したBurkholderia planariiおよびB.glumaeの特異的検出

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Specific Detection of *Burkholderia plantarii* and *B. glumae* by PCR Using Primers Selected from the 16S-23S rDNA Spacer Regions

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Abstract

Specific polymerase chain reaction (PCR) primers targeting genomic DNA were selected for rapid, sensitive and specific detection of *Burkholderia plantarii* and *B. glumae*, the causal agents of bacterial seedling blight of rice and bacterial seedling rot of rice, respectively. The complete sequences of the spacer region between the 16S and 23S rRNA genes of *B. plantarii, B. glumae, B. gladioli, B. cepacia, B. caryophylli, B. andropogonis, B. solanacearum* and *Pseudomonas corrugata*, were determined. Among strains of *B. plantarii* from diverse geographical regions in Japan, the degree of sequence similarity was more than 93%. All strains of *B. glumae* isolated in diverse geographical regions in Japan had the same sequence. The degree of similarity among the strains of *Burkhorderia* spp. ranged from 60% to 90%, but less than 59% between strains of *Burkholderia* spp. and strains of *P. corrugata, P. fluorescens* and *Escherichia coli*. These results suggest that the sequences are conserved within species, but are variable between species. Since strains of *B. plantarii, B. glumae* and *B. gladioli* exhibited a relatively high degree of sequence similarity (81-90%) to each other, we designed species-specific primers from the sequence of the regions that were conserved within species, but not between species. In PCR with PL-12f (5′-AGCCAGTCAGAGGATAAGTC-3′) and PL-11r (5′-CAATTGAGCCGAACATTTAAG-3′) primers, an approximately 180-bp fragment was amplified in all 45 strains of *B. plantarii*. No PCR products were obtained from other bacteria tested. Primers GL-13f (5′-ACACGGAACACCTGGGTA-3′) and GL-14r (5′-TCGCTCTCCCGAAGAGAT-3′) amplified an approximately 400-bp fragment in all 20 isolates of *B. glumae*, whereas no PCR products were obtained from other species of bacteria. Using the specific primers designed in this study, the PCR method can detect and identify *B. glumae* and *B. plantarii* in rice samples within 6 hr.

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Key words : PCR, specific detection, spacer region, *Burkholderia plantarii, Burkholderia glumae.*

INTRODUCTION

Burkholderia plantarii (Azegami, Nishiyama, Watanabe, Kadota, Ohuchi and Fukazawa 1987) Urakami, Ito-Yoshida, Araki, Kijima, Suzuki and Komagata 1994[18] and *B. glumae* (Kurita and Tabei 1967) Urakami, Ito-Yoshida, Araki, Kijima, Suzuki and Komagata 1994[18] cause bacterial seedling blight of rice and bacterial seedling rot of rice, respectively, in Japan[2,17]. They are seed-borne pathogens and cause serious damage of rice seedlings in nursery boxes, especially in the northern part of Japan. Seedlings infected with these pathogens cannot be used for transplanting.

Selective media for both *B. plantarii*[1,13] and *B. glumae*[15] have been reported, however, *B. glumae* can also be detected and identified using a serological method[9] or a specific DNA probe[16]. In contrast, no specific probe has yet been developed for *B. plantarii* which has several serological groups[7,11].

The polymerase chain reaction (PCR) has been used to detect plant pathogenic bacteria, because it is a more rapid, sensitive and specific method than conventional ones used for detecting pathogens[10,20]. The objective of this study was to select and apply PCR primers, targeting the spacer region between 16S and 23S rRNA genes of *B. plantarii* and *B. glumae*, for specific detection of these pathogens in rice samples.

MATERIALS AND METHODS

Bacterial strains Bacterial cultures used for DNA sequencing consisted of eight strains of *Burkholderia plantarii*, six strains of *B. glumae*, five strains of *B.*

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Table 1. Bacterial strains used

Bacterium (No. of strains)	Strain	Geographic origin	Host plants
Burkholderia plantarii (45)	MAFF 311032*	Hokkaido	Rice
	Yamagata-9*	Yamagata	Rice
	Akita-1*	Akita	Rice
	MAFF 311029*	Hokkaido	Rice
	MAFF 311031*	Hokkaido	Rice
	MAFF 301723T*	Chiba	Rice
	HZ*	Oita	Rice
	MAFF 302485*	Miyagi	Rice
	IW2-11, IW3-11, IW5-11, BB1-11, BB3-12, ST1-11 ST2-31, TK2-11, TK-1-11A IK0-11, IK2-11, IK4-11, YN1-11, YN2-21, KW0-11 KM1-12, UK1-11, TG1-11 HK1-21, HT1-11, NG1-11, OT2-11, ST1-11B, IW1-11, TM1-11, MT1-11, KY92431-1, TS92421-1, NG92411-2, Tsukigata-94	Hokkaido	Rice
	MAFF 302387, MAFF 302412	Chiba	Rice
	MR8521, MR8801	Miyagi	Rice
	YR8803	Yamagata	Rice
	IR8901	Iwate	Rice
B. glumae (20)	MAFF 311028*	Hokkaido	Rice
	MAFF 301169T*	Ehime	Rice
	MAFF 301386*	Fukuoka	Rice
	MAFF 301388*	Yamagata	Rice
	MAFF 301441*	Hiroshima	Rice
	MAFF 301682*	Ibaraki	Rice
	MAFF 311026, MAFF 311027	Hokkaido	Rice
	MAFF 301171	Kagawa	Rice
	Ku8123, 8223	Fukuoka	Rice
	MAFF 301387	Kumamoto	Rice
	MAFF 301389	Akita	Rice
	MAFF 301442	Hiroshima	Rice
	Miyagi-4	Miyagi	Rice
	MR8740, MR8705, MR8781	Miyagi	Rice
	IR8912	Iwate	Rice
	7601	Okayama	Rice
B. gladioli pv. *gladioli* (3)	MAFF 311021T (ICMP 3950T)*	USA	Gladiolus
	MAFF 301066*	Chiba	Freesia
	MAFF 301588*	Tochigi	Cymbidium
B. gladioli pv. *alliicola* (1)	MAFF 311022 (ICMP 2804)*	USA	Onion
B. gladioli (3)	8726*	Japan	Rice
	8729, 8789	Japan	Rice
B. cepacia (1)	MAFF 311023T (ICMP 5796T)*	USA	Onion
B. caryophylli (1)	MAFF 311024T (ICMP512T)*	USA	Carnation
B. andropogonis (1)	MAFF 302151T (ATCC 23061T)*	USA	Sorghum
B. solanacearum (1)	MAFF 302154T (ATCC 11696T)*	USA	Tomato
Pseudomonas corrugata (1)	MAFF 302158T (ATCC 29736T)*	UK	Tomato

*Strains used for DNA sequencing.

gladioli, and type strains of *B. cepacia, B. caryophylli, B. andropogonis, B. solanacearum* and *Pseudomonas corrugata*. In addition, 37 strains of *B. plantarii*, 14 strains of *B. glumae* and two strains of *B. gladioli* were used to test the specificity of PCR amplification (Table 1).

DNA extraction Bacteria were grown on KB agar or PPGA[8]. The DNA template for PCR was extracted from cells (approximately 10^8 cfu/ml) using the InstaGene DNA purification matrix (Bio-Rad Laboratories) according to the supplier's instructions.

DNA sequencing The sequences of the spacer region between the 16S and 23S rRNA genes were determined by PCR by using the following method as described previously[14]. The primers used for amplification were designed on the basis of conservation of the rRNA sequences or PCR primers of *Escherichia coli*[3,4], *B. cepacia*[6] and *Pseudomonas fluorescens*[5]. The PCR primers contained the sequences of Cy-5-labeled primers for cycle sequencing reactions. The following primers were used to amplify the 16S-23S rDNA spacer regions: B-01, 5′-CGCCAGGGTTTTCCCAGTCACGACGGGTGAAGTCGTAACAAGGTA-3′, corresponding to positions 1487 to 1507 of *E. coli* 16S rDNA[3]; and B-02 5′-TTTCACACAGGAAACAGCTATGACTGATCGCCAAGGCATCCACC-3′, corresponding to positions 23 to 42 of *E. coli* 23S rDNA[4] (the underlined portions of the primers correspond to the sequences of Cy-5-labeled primers). PCR amplification of the target sequence was performed in a 50-μl (total volume) reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM $MgCl_2$, each deoxynucleoside triphosphate (dNTP) at a concentration of 200 μM, 5 pmol of each primer, 2.5 μl of template and 2.5 U of *Taq* polymerase (TOYOBO). PCR was performed with a model 480 DNA thermal cycler (Perkin-Elmer Cetus) by using the following protocol: initial denaturation at 95°C for 2.5 min, followed by 35 cycles consisting of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min and an additional extension step consisting of 72°C for 10 min. The B-02 primer was biotinylated, and the biotinylated PCR products then immobilized on streptavidin-coated paramagnetic beads (Dynabeads M-280 strepvidin; DYNAL). Single-stranded DNA templates were prepared by following the manufacturer's instructions. Both strands were then sequenced directly by cycle sequencing using ALFred autocycle sequencing kit (Pharmacia Biotech) and the following Cy-5-labeled primers: primer ALFred M13-40 (5′-CGCCAGGGTTTTCCCAGTCACGAC-3′) and reverse primer ALFred M13 (5′-TTTCACACAGGAAACAGCTATGAC-3′). The sequencing products were loaded onto a 6% polyacrylamide gel, and separation was monitored on-line with an ALFred DNA sequencer (Pharmacia Biotech).

Selection of PCR primers for specific amplification Sequence data of 16S-23S rDNA spacer regions for the strains were manually aligned. Optimal conditions for PCR were determined with a 20-μl reaction volume containing 1× *Taq* buffer (supplied with *Taq* polymerase by Gibco-BRL), 200 μM dNTPs, 2.5 mM $MgCl_2$, 0.5 U of *Taq* polymerase (Gibco-BRL), 1 μl of DNA template, 0.2 μM each primer and 0.5 μl of 10% skim milk. PCR was performed with a thermal cycler (MJ Research) by using the following protocol: initial denaturation at 94°C for 2.5 min, followed by 35 cycles consisting of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 2 min and an additional extension step at 72°C for 5 min. PCR products were resolved in a 2% agarose gel in TAE buffer and stained with ethidium bromide.

The lower limit of sensitivity for PCR amplification was determined by testing dilution series of pure cultures. Loopfuls of *B. plantarii* MAFF 311032 and *B. glumae* MAFF 311028 were suspended separately in 1 ml of sterile distilled water and diluted in a 10-fold series. The concentration of bacteria was determined by a standard plate-counting procedure on PPGA medium. One hundred μl of each dilution was held at 95°C for 8 min and centrifuged at 10,000 rpm for 3 min. The supernatant was then used for the PCR. The PCR reactions were run with the DNA extract of *B. plantarii* MAFF 311032 or *B. glumae* MAFF 311028 as positive controls and without DNA as a negative control.

PCR for detection of B. plantarii and B. glumae in rice seedlings Rice seedling samples were grown in nursery boxes from seed, which had been inoculated prior to sowing by dipping in a suspension of about 10^8 cells per ml of inoculum[12]. Severely and slightly diseased seedlings, as well as asymptomatic ones were sampled and tested for the PCR. Healthy seedlings from non-inoculated plants were used as controls.

Bacterial DNA was extracted from each seedling sample by the following method. A small piece (10-20 mg) of a seedling base was ground in a mortar with 1.0 ml of a 0.85% NaCl solution. The resulting suspension was then placed in a micro tube and centrifuged at 10,000 rpm for 5 min. The pellet was resuspended in 0.1 ml of sterile water, held at 95°C for 8 min and centrifuged at 10,000 rpm for 3 min. Five μl of this supernatant was used directly as a template for the PCR.

RESULTS

Nucleotide sequences of the 16S-23S rDNA spacer regions

The 16S-23S rDNA spacer regions of plant pathogenic *Burkholderia* species and *Pseudomonas corrugata* were determined. The sequence similarity matrix and length of the region are shown in Table 2. The spacer region of the examined strains was 496 to 660 base pairs in length and contained the genes for $tRNA^{Ile}$ and $tRNA^{Ala}$.

The sequence data of the strains investigated in this study have been deposited in the GenBank, EMBL, and DDBJ under following accession numbers: D87079 for MAFF 301723^T, D87080 for MAFF 301169^T, D87081 for MAFF 311021^T (ICMP 3950^T), D87082 for MAFF 311022 (ICMP 2804), D87083 for MAFF 311023^T (ICMP 5796^T), D87084 for MAFF 311024^T (ICMP 512^T), D87085 for MAFF 302151^T (ATCC 23061^T), D87086 for MAFF 302154^T (ATCC 11696^T), and D87087 for MAFF 302158^T (ATCC 29736T).

Primer design and specificity

The sequence alignment of the 16S-23S rDNA spacer regions of *B. plantarii*, *B. glumae* and *B. gladioli* is

Table 2. Sequence similarity matrix of 16S-23S rDNA spacer region of plant pathogenic *Burkholderia* spp. and other bacteria

	length (bp)	% Homology with :																	
		P1	P2	P3	P4	P5	P6	GL	GG	GF	GA	GC	GR	CE	CA	AN	SO	PC	PF
P1	569																		
P2	569	99.8																	
P3	569	99.7	99.7																
P4	557	94.2	94.1	93.9															
P5	557	94.2	94.1	93.9	99.8														
P6	560	93.6	93.4	93.2	98.8	98.9													
GL	560	89.6	89.4	88.3	88.5	88.5	89.4												
GG	548	88.3	88.1	88.3	88.5	88.5	88.2	88.5											
GF	550	88.6	88.4	88.6	88.7	88.7	88.4	88.6	99.5										
GA	557	89.4	89.2	89.5	89.6	89.6	89.1	89.1	96.1	95.5									
GC	558	89.4	89.2	89.5	90.0	90.0	89.5	89.5	96.4	96.2	99.3								
GR	596	81.8	81.7	81.9	81.9	81.9	81.5	81.4	89.8	89.6	88.8	88.3							
CE	592	76.5	76.4	76.5	78.3	78.2	77.9	77.3	80.6	80.5	79.7	79.5	75.3						
CA	660	65.7	65.6	65.8	66.8	66.8	67.4	66.0	67.2	67.1	66.0	65.9	62.8	71.4					
AN	624	66.9	67.1	66.9	66.4	66.4	67.3	67.1	67.5	68.0	67.5	67.7	64.5	69.5	66.7				
SO	497	66.5	66.5	67.0	67.2	67.2	66.5	68.1	69.1	68.8	67.8	67.5	64.1	64.0	60.5	62.0			
PC	496	57.8	56.7	56.8	58.4	57.8	57.6	58.4	57.2	58.5	57.4	58.3	56.5	53.4	49.7	52.7	57.9		
PF	516	56.8	56.6	56.6	57.2	57.1	57.0	57.5	57.2	56.9	57.5	56.8	54.2	53.0	49.5	52.3	58.0	88.7	
EC	437	51.7	51.7	51.7	53.2	53.4	53.0	53.2	53.2	52.7	52.6	52.0	49.3	51.0	47.4	48.5	55.7	58.5	58.2

P1, *Burkholderia plantarii* MAFF 311032 and Yamagata-9 ; P2, *B. plantarii* Akita-1 ; P3, *B. plantarii* MAFF 311029 and MAFF 311031 ; P4, *B. plantarii* MAFF 301723^T ; P5, *B. plantarii* HZ ; P6, *B. plantarii* MAFF 301723 ; GL, *B. glumae* MAFF 311028, MAFF 301169^T, MAFF 301386, MAFF 301388, MAFF 301441 and MAFF 301682 ; GG, *B. gladioli* pv. *gladioli* MAFF 311021^T ; GF, *B. gladioli* pv. *gladioli* MAFF 301066 ; GC, *B. gladioli* pv. *gladioli* MAFF 311588 ; GA, *B. gladioli* pv. *allicola* MAFF 311022 ; GR, *B. gladioli* 8726 ; CE, *B. cepacia* MAFF 311023^T ; CA, *B. caryophylli* MAFF 311024^T ; AN, *B. andropogonis* MAFF 302151^T ; SO, *B. solanacearum* MAFF 302154^T ; PC, *Pseudomonas corrugata* MAFF 302158^T ; PF, *P. fluorescens* Qp5[5] ; EC, *Escherichia coli* [19]

shown in Fig. 1. On the basis of the sequence data, two pairs of PCR primers, PL-12f (5′-AGCCAGTCAGAGGATAAGTC-3′) and PL-11r (5′-CAATTGAGCCGAACATTTAAG-3′) for *B. plantarii* and GL-13f (5′-ACACGGAACACCTGGGTA-3′) and GL-14r (5′-TCGCTCTCCCGAAGAGAT-3′) for *B. glumae*, were selected for specific amplification.

In PCR with PL-12f and PL-11r primers, an approximately 180-bp fragment was amplified in all 45 strains of *B. plantarii* used in this study. No PCR products were obtained from other bacteria tested, including *B. glumae, B. gladioli, B. cepacia, B. caryophylli, B. andropogonis, B. solanacearum*, and *P. corrugata*. Primers GL-13f and GL-14r amplified an approximately 400-bp fragment in all 20 strains of *B. glumae* tested. No PCR products were obtained from other bacterial species (Table 3 and Fig. 2). Detection sensitivity of PCR for each bacterial species with the described primer pairs was 10^1 to 10^2 cfu per PCR reaction (Fig. 3).

PCR detection of B. plantarii and B. glumae in rice seedlings

PCR products were obtained using the specific primers in PCR reactions on rice seedling samples which were collected from plants infected with *B. plantarii* or *B. glumae*. Tissue samples from symptomless seedlings that had been inoculated with each of the pathogens also produced a PCR product. Healthy seedling samples from non-inoculated plants were negative in PCR detection (Fig. 4).

Fig. 1. Sequence alignment of the spacer region between 16S and 23S rRNA genes of *Burkholderia plantarii, B. glumae* and *B. gladioli*. The sequences are aligned with the sequence of *B. plantarii* MAFF 311032 and Yamagata-9. Only nucleotides that differ from those of *B. plantarii* MAFF 311032 and Yamagata-9 are shown ; identical nucleotides are indicated by dots, and deletions are indicated by dashes. The 3′ end of 16S rRNA gene and the 5′ end of the 23S rRNA gene are also shown. The location of two tRNA genes within the spacer region is also indicated. Abbreviations: P1, *B. plantarii* MAFF 311032 and Yamagata-9 ; P2, *B. plantarii* Akita-1 ; P3, *B. plantarii* MAFF 311029 and MAFF 311031 ; P4, *B. plantarii* MAFF 301723 ; P5, *B. plantarii* HZ ; P6, *B. plantarii* MAFF 301723 ; GL, *B. glumae* MAFF 311028, MAFF 301169, MAFF 301386, MAFF 301388, MAFF 301441 and MAFF 301682 ; GG, *B. gladioli* pv. *gladioli* MAFF 311021 ; GF, *B. gladioli* pv. *gladioli* MAFF 301066 ; GC, *B. gladioli* pv. *gladioli* MAFF 311588 ; GA, *B. gladioli* pv. *allicola* MAFF 311022 ; GR, *B. gladioli* 8726. The underlined regions are specific primer sites.

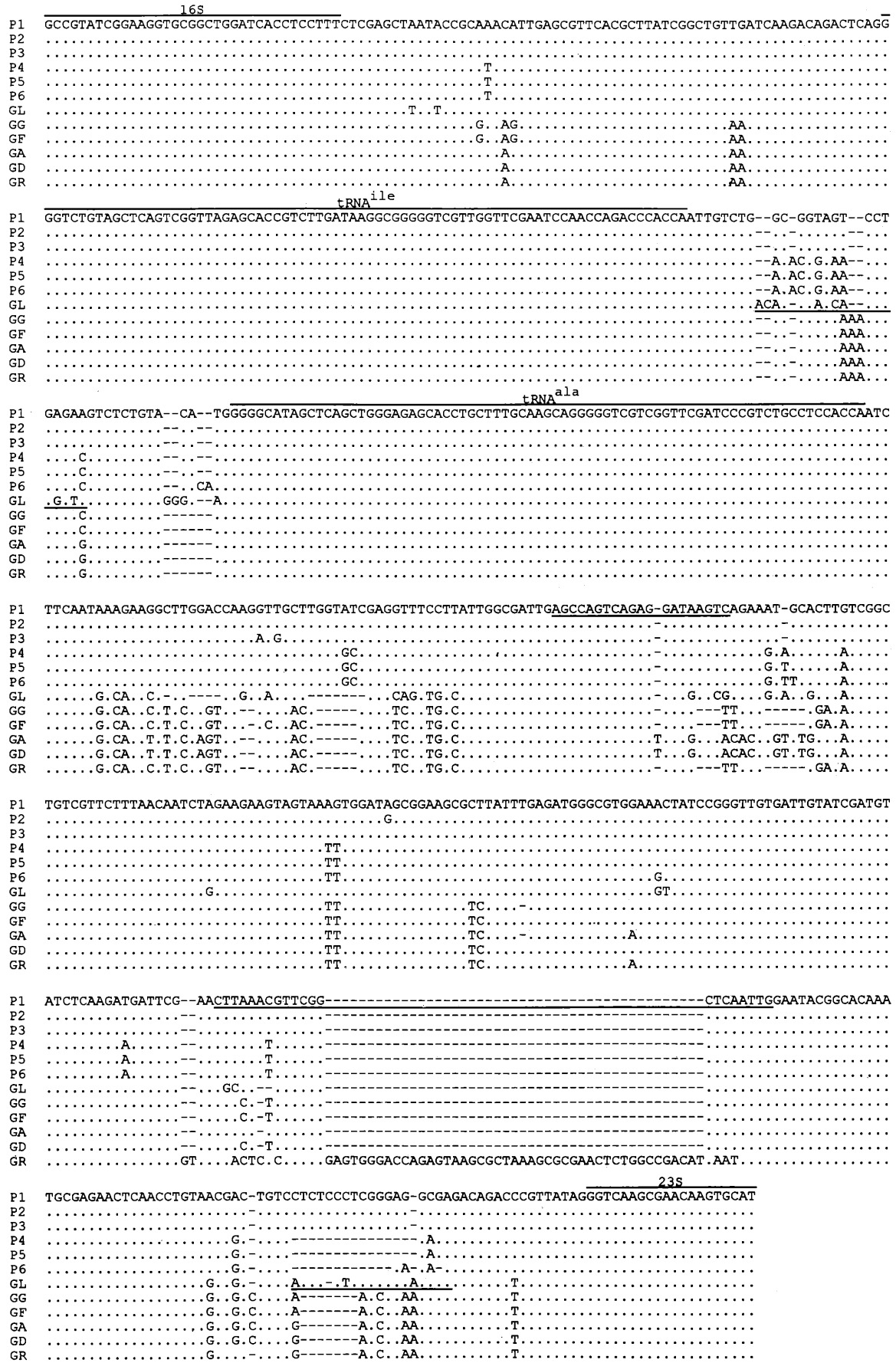

Fig. 1.

Table 3. PCR amplification of bacterial strains using two pairs of primers

Bacterium	No. of strains tested	No. of positive strains with primers:	
		PL-12f+PL-11r	GL-13f+GL-14r
Burkholderia plantarii	45	45	0
B. glumae	20	0	20
B. gladioli pv. *gladioli*	3	0	0
B. gladioli pv. *alliicola*	1	0	0
B. gladioli	3	0	0
B. cepacia	1	0	0
B. caryophylli	1	0	0
B. andropogonis	1	0	0
B. solanacearum	1	0	0
Pseudomonas corrugata	1	0	0

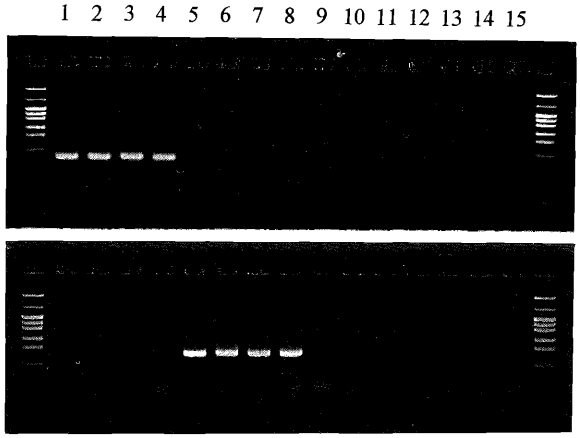

Fig. 2. Agarose gel electrophoresis of PCR products amplified using primers PL-12f and PL-11r (upper panel) and primers GL-13f and GL-14r (lower panel) from total DNA of *Burkholderia* strains. Lanes 1-4, *B. plantarii* strains MAFF 311032, MAFF 301723, MAFF 302485 and MAFF 311031, respectively; lanes 5-8, *B. glumae* strains MAFF 311028, MAFF 301369, MAFF 301386 and MAFF 301388, respectively; lane 9, *B. gladioli* pv. *gladioli* MAFF 311021; lane 10, *B. gladioli* pv. *allicola* MAFF 311022; lane 11, *B. gladioli* 8726; lane 12, *B. cepacia* MAFF 311023; lane 13, *B. caryophylli* MAFF 311024; lane 14, *B. andropogonis* MAFF 302151; lane 15, *B. solanacearum* MAFF 302154. The left and right lanes (unmarked) in each panel contain molecular weight standards (pHY marker, Takara). The seventh and eighth fragments indicate 489 and 267 bp, respectively.

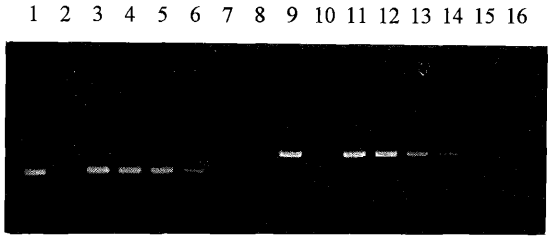

Fig. 3. Agarose gel electrophoresis of PCR products amplified using primers PL-12f and PL-11r (lanes 1-8) and primers GL-13f and GL-14r (lanes 9-16) from DNA extracts of dilution series of *Burkholderia plantarii* strain MAFF 311032 (lanes 3-8) and *B. glumae* strain MAFF 311028 (lanes 11-16). Number of bacteria added were as follows: 5×10^4 (lanes 3 and 11), 5×10^3 (lanes 4 and 12), 5×10^2 (lanes 5 and 13), 5×10^1 (lanes 6 and 14), 5 (lanes 7 and 15), 0 cfu per 20-μl PCR reaction (lanes 8 and 16). Lanes 1 and 9, positive controls (the DNA of *B. plantarii* MAFF 311032 and *B. glumae* MAFF 311028, respectively); lanes 2 and 10, negative controls (no DNA).

DISCUSSION

The complete sequences of the spacer regions between the 16S and 23S rRNA genes of plant pathogenic *Burkholderia* spp. were determined. Among strains of *B. plantarii* from diverse geographical regions in Japan, the degree of similarity was more than 93%. All strains of *B. glumae* isolated in diverse geographical regions in Japan had the same sequence. These results are consistent with the serological analysis of the pathogens reported previously[7,9,11], suggesting that *B. plantarii* contains more genetic variation than *B. glumae*.

The degree of similarity among strains of *Burkholderia* spp. ranged from 60% to 90%, but less than 59% between strains of *Burkholderia* spp. and strains of *P. corrugata, P. fluorescens* and *E. coli* (Table 2). These results suggest that the sequences are conserved within species, but are variable between species. Therefore, sequence comparison in this region may be valuable for differentiating closely related species of bacteria.

Because strains of *B. plantarii, B. glumae* and *B. gladioli* exhibited a relatively high degree of sequence similarity (81-90%) to each other, we designed the species-specific primers on the basis of the sequences of the regions that are conserved within species, but not between species (Fig. 1). The PCR method, using the specific primers designed, was found to be useful for rapid detection and identification of *B. glumae* and *B. plantarii*; the pathogens in rice samples can be identified

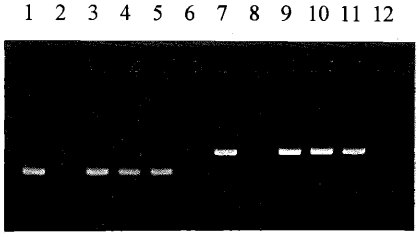

Fig. 4. Agarose gel electrophoresis of PCR products amplified using primers PL-12f and PL-11r (lanes 1-6) and primers GL-13f and GL-14r (lanes 7-12) from DNA extracts of rice seedling. Lanes 1 and 7, positive controls (the DNA of *B. plantarii* MAFF 311032 and *B. glumae* MAFF 311028, respectively); lanes 2 and 8, negative controls (no DNA); lanes 3-5 and 9-11, DNA extracts from rice seedling samples infected with *B. plantarii* or *B. glumae*, respectively; lanes 3 and 9, severely diseased seedlings; lanes 4 and 10, slightly diseased seedlings; lanes 5 and 11, asymptomatic inoculated seedlings; lanes 6 and 12, healthy seedlings from non-inoculated plants.

within 6 hr. Sensitivity of the PCR for these pathogens was almost the same as that for other bacteria reported[10,20]. In addition, the PCR detection of these two pathogens can be performed in the same set of experimental condition, including thermalcycler program. Since these pathogens are seed-borne, a method to detect them from seeds by PCR should be established in the future study.

The degree of similarity among strains of *B. gladioli*, including different pathovars and strains from diverse origins, was more than 88%, indicating that this species consists of a heterogeneous group of strains. Therefore, no species-specific primer could be designed from the sequences of this region for *B. gladioli*.

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和 文 摘 要

竹内 徹・澤田宏之・鈴木文彦・松田 泉：16S-23S リボゾームDNAスペーサー領域の塩基配列を利用した *Burkholderia plantarii* および *B. glumae* の特異的検出

イネ苗立枯細菌病菌 *Burkholderia plantarii* およびイネもみ枯細菌病菌 *B. glumae* に対して，特異的なPCRを利用した迅速で高感度な特異的検出法を開発した。*B. plantarii, B. glumae, B. gladioli, B. cepacia, B. caryophylli, B. andropogonis, B. solanacearum* および *Pseudomonas corrugata* について，16Sおよび23Sリボゾーム RNA 遺伝子間に存在するスペーサー領域の全塩基配列を比較解析した。地理的由来の異なる菌株において同種内では，*B. plantarii* では93%以上の相同性を示し，*B. glumae* では種内変異は認められなかった。*Burkholderia* 属細菌の種間では60～90%の相同性を，*Burkholderia* 属細菌と *P. corrugata, P. fluorescens* または *Escherichia coli* との間では59%以下の相同性を，それぞれ示した。以上の結果から，本領域は同種内では保存性が高く，異種間では変異が大きいことが明らかとなった。*B. plantarii, B. glumae* および *B. gladioli* は互いに比較的高い相同性（81～90%）を示したことから，これら3種の塩基配列を比較して，種内で保存性が高く，かつ種間で変異性の高い配列から種特異的なプライマーを設計した。プライマー PL-12f(5′-AGCCAGTCAGAGGATAAGTC-3′) と PL-11r (5′-CAATTGAGCCGAACATTTAAG-3′)によるPCRでは，*B. plantarii* 供試45菌株すべてから約180 bpのDNA断片の増幅が認められたが，他の細菌では認められなかった。プライマー GL-13f(5′-ACACGGAACACCTGGGTA-3′) および GL-14r (5′-TCGCTCTCCCGAAGAGAT-3′) によるPCRでは，*B. glumae* 供試20菌株すべてから約400 bpのDNA断片の増幅が認められたが，他の細菌では認められなかった。また，特異プライマーを利用したPCRによって，それぞれの病原細菌が感染したイネ苗から両菌を6時間以内で検出でき，本法がイネ組織内における両菌の迅速検出に有効であることが示された。