Cloned DNA Probes for Detection of *Pseudomonas glumae* Causing Bacterial Grain Rot of Rice

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Abstract

A 5.7-kilobase *Eco*RI fragment, PG2I, and a 3.9-kilobase *Eco*RI fragment, PG80I, were cloned randomly from the total DNA of *Pseudomonas glumae*, the causal agent of bacterial grain rot of rice. PG2I hybridized to all the 43 strains of *P. glumae* isolated from rice grain, rice seedlings, rice leaf sheaths and mung bean sprouts from different places, but PG80I did not hybridize to 5 of the 43 strains. PG2I seemed specific to this pathogen because this fragment failed to hybridize to selected strains of gram negative bacteria including *P. gladioli* pv. *gladioli*, *P. gladioli* pv. *allicola*, *P. caryophylli*, *P. plantarii*, *P. solanacearum*, *P. cepacia*, *P. avenae*, *P. cichorii*, *P. aeruginosa*, *P. syringae* pv. *phaseolicola*, *P. syringae* pv. *syringae*, *P. viridiflava*, *P. marginalis* pv. *marginalis*, *Agrobacterium radiobacter*, *A. rhizogenes*, *A. tumefaciens*, *Erwinia ananas*, *E. carotovora* subsp. *carotovora*, *E. herbicola*, *X. campestris* pv. *campestris*, and *X. campestris* pv. *oryzae*, and gram positive bacteria including *Arthrobacter illicis*, *Clavibacter michiganensis* subsp. *michiganensis*, *Cl. michiganensis* subsp. *sepedonicus*, *Cl. rathayi*, *Curtobacterium albidum*, *C. citreum*, *C. flaccumfaciens* pv. *flaccumfaciens*, *C. flaccumfaciens* pv. *oortii* and *Rhodococcus fasciens*. When PG2I was separated into two fragments, PG2Ia and PG2Ib, by digestion with *Kpn*I, PG2Ia hybridized to all the strains but PG2Ib did not hybridize to 18 of the 43 strains of *P. glumae*, indicating that PG2Ia is specific to this pathogen. Hybridization patterns of PG2Ib and PG80I to these strains showed that they were divided into 4 groups and that at least three groups were present in three localities in Oita Prefecture in 1984. These findings suggest allopatric differentiation of different strains of *P. glumae*. These probes are considered to be useful for the detection of *P. glumae* in ecological studies.

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Key words: specific probe, *Pseudomonas glumae*.

INTRODUCTION

Pseudomonas glumae Kurita and Tabei causes rice seedling rot in the nursery boxes and rice grain rot in paddy fields in Japan. Bacterial grain rot is, especially, a serious disease in the southern part of Japan because this pathogen causes serious yield loss. The bacteria exist on rice plants^{7,10,18,19} during the growing season, and the bacteria on leaf sheaths play a major role as a source of inoculum for the disease development on panicles¹⁸. Diseased panicles, which occur just after heading, are important in the focal formation in the field¹⁶. Although the bacterium can survive in rice grain stored at room temperature¹⁷, little is known about its survival in soil and other environments during winter. Tsushima *et al.*¹⁷ could not detect this pathogen from the diseased rice grain left for three months in winter on the soil surface in a field using the selective medium, S-PG²⁰. This is ascribed to the assumption that in winter the pathogen exists in grain at a lower level than the detection limit (approximately 10³ cfu/g).

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The survival of this pathogen in soil and other environments in winter still remains unknown and is important in terms of epidemiology.

DNA probes have been developed in some pathogenic bacteria for detection, identification^{3,4,12-15,21} and restriction fragment length polymorphism (RFLP) analysis^{2,6}. DNA-DNA hybridization with probe is a more rapid, sensitive, and specific method than conventional ones for identification of pathogenic bacteria^{14,21}. In these reports, DNA encoding pathogenicity gene^{2,3,12,13} or DNA selected randomly from chromosomal DNA^{4,5,14,15,21} were used as probes.

In this paper, we describe the prerequisite step for detecting *Pseudomonas glumae* from environment, e.g., isolation of *P. glumae* specific DNA probe from total DNA, and concomitant results on genetic differentiation in this bacterium.

MATERIALS AND METHODS

Bacterial isolates and DNA extraction. *Pseudomonas glumae* strain Kyu 82-34-2²⁰) was used for constructing DNA library. *P. glumae* was cultured at 28°C in PPG broth or on PPGA plates⁹. *Escherichia coli* strain was grown at 37°C in LB medium⁸). The bacteria used for hybridization test are listed in Table 1. Other bacteria were cultured at 25°C in PPG broth or on PPGA plates.

Total DNA was extracted from bacteria using the method of MINIPREP using CTAB/NaCl (10% hexadecyltrimethyl ammonium bromide in 0.7 M NaCl) solution¹¹. For lysis of the gram positive bacteria, 5 µl of lysozyme (100 µg/ml) were added to proteinase K (100 µg/ml) in 0.5% SDS.

DNA cloning and probe preparation. Total DNA of *P. glumae* Kyu 82-34-2 was purified as above, and digested with *Eco*RI. Digested DNA fragments were ligated into *Eco*RI digested pUC18 by Ligation Kit (Takara Ltd.) and were used to transform competent cells of *E. coli* JM109 (Takara Ltd.). Transformed *E. coli* JM109 cells were selected on LB plates containing 50 µg/ml ampicillin and cultured in LB at 37°C for 24 hr. Harvested cells were resuspended in 350ml of STET (8% sucrose, 5% TritonX-100, 50 mM EDTA, 50 mM Tris-HCl pH 8.0), treated with lysozyme (10 mg/ml), and incubated in boiling water for 40 sec. They were then precipitated in isopropanol to obtain plasmid DNA containing *P. glumae* chromosomal DNA fragments.

For the selection of plasmid DNAs containing *P. glumae* specific DNA fragment, 81 of about 300 transformed cells were screened against *P. glumae* Kyu 82-34-2 and 3 strains of *P. gladioli* species; pv. *gladioli* (MAFF 301064, ICMP 3950) and pv. *allicola* (ICMP 2804) which have similar bacteriological characteristics to *P. glumae*. One µl of plasmid DNA solution (approximately 20 ng) was blotted on Optiblot nylon membrane (International Biotechnologies, Inc.) and hybridized with total DNAs (1 µg each) from *P. glumae* and 3 strains of *P. gladioli* species which were labeled with [α -³²P]dCTP (specific activity, >3,000 Ci/mmol) using Multiprime DNA labeling system (Amersham International plc). Hybridization was carried out overnight at 68°C in hybridization buffer (7% SDS, 1% bovine serum albumin and 1 mM EDTA in 1 M sodium phosphate buffer, pH 7.0). The membranes were washed three times for 30 min in washing buffer (1% SDS, 0.1% bovine serum albumin and 1 mM EDTA in 50 mM sodium phosphate buffer, pH 7.0) at 65°C. Autoradiography was performed on Fuji X-ray films.

Dot blot hybridization with total DNA of other bacteria. Total DNA solutions (1 µg/µl) of 1 µl each from bacteria listed in Table 1 were blotted on nylon membranes and hybridized with DNA fragments specific to *P. glumae*, PG2I and PG80I, which were separated on 0.7% agarose gel after digesting PG2 and PG80 with *Eco*RI, respectively, and purified by using Bandprep (Amersham International plc). PG2I was further digested with *Kpn*I to obtain fragments, PG2Ia and PG2Ib, and separated on agarose gel. These two fragments were tested for specificity to 43 strains of *P. glumae* by dot blot hybridization.

RESULTS AND DISCUSSION

Plasmid DNAs, pG2 and pG80, which hybridized to *P. glumae* Kyu 82-34-2 but not two strains of *P. gladioli* pv. *gladioli* (MAFF 301064 and ICMP 3950) or *P. gladioli* pv. *allicola* (ICMP 2804), were selected

Table 1. Strains of bacteria studied^{a)}

Bacterium	Strain	Other designation
<i>Pseudomonas glumae</i>	MAFF 301094, 301095, 301096, 301098, MAFF 301099, 301169 ^T , 301170, 301171, MAFF 301172, 301386, 301389, 301441, MAFF 301682, 302382, 302383, 302384, MAFF 302394, 302395, 302396, 302397, MAFF 302421, 302422, 302423, 302437, MAFF 302438, 302439, 302440, 302441, MAFF 302442, 302443, 302444, 302445, MAFF 302446, 302447, 302448, 302449, MAFF 302450, 302453, 302463, 302553, Kyu82-34-2, Kyu82-40-1, Kyu82-41-1	
<i>P. aeruginosa</i>	MAFF 302206 ^T	ATCC 10145 ^T
<i>P. avenae</i>	MAFF 302183 ^T MAFF 301024, 301027, 301031, 301036, MAFF 301141, 301502, 301505, 301576, MAFF 301609	NCPPB 1011 ^T
<i>P. caryophylli</i>	ICMP 512 ^T MAFF 301060	
<i>P. cepacia</i>	MAFF 302528 A1, PC-10, PC-20, ALQ8281, 86130	
<i>P. cichorii</i>	MAFF 301760, 301761, 301763	
<i>P. gladioli</i> pv. <i>gladioli</i>	ICMP 3950 ^T MAFF 301064, 301580, 301584, 301586, MAFF 301588, 301728, 302385, 302537, MAFF 302543	
<i>P. gladioli</i> pv. <i>allicola</i>	ICMP 2804 ^T	
<i>P. marginalis</i> pv. <i>marginalis</i>	MAFF 302031, 302032, 302033, 302036, MAFF 302038, 302041, 302042, 302045, MAFF 302046, 302047, 302048	
<i>P. plantarii</i>	MAFF 302381, 302392, 302479	
<i>P. solanacearum</i>	MAFF 302154 ^T MAFF 301559, 301492, 301860, 301067, MAFF 301418, 301524, 301556	ATCC 11696 ^T
<i>P. syringae</i> pv. <i>phaseolicola</i>	MAFF 301766	
<i>P. syringae</i> pv. <i>syringae</i>	MAFF 302155 ^T MAFF 301861, 301862, 301863, 301864	ATCC 19310 ^T
<i>P. viridiflava</i>	MAFF 301794, 301795, 301796, 301798, MAFF 301799, 301800, 301801, 301802, MAFF 301805	
<i>Agrobacterium radiobacter</i>	MAFF 302163 ^T	ATCC 19358 ^T
<i>A. rhizogenes</i>	MAFF 302164 ^T	ATCC 11325 ^T
<i>A. tumefaciens</i>	MAFF 302165 ^T	ATCC 23308 ^T
<i>Erwinia ananas</i>	MAFF 302184 ^T	NCPPB 1846 ^T
<i>E. carotovora</i> subsp. <i>carotovora</i>	MAFF 302159 ^T	ATCC 15713 ^T
<i>E. herbicola</i>	MAFF 302186 ^T	NCPPB 2971 ^T
<i>Xanthomonas campestris</i> pv. <i>campestris</i>		
	MAFF 301443	
<i>X. campestris</i> pv. <i>oryzae</i>	MAFF 301082, 301083, 301084, 301085, MAFF 301086, 301225, 301226, 301227	
<i>Arthrobacter ilicis</i>	MAFF 302180 ^T	ATCC 14264 ^T
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>	MAFF 302187 ^T K1-1, R-1	NCPPB 2979 ^T
<i>Cl. michiganensis</i> subsp. <i>sepedonicus</i>	MAFF 302162 ^T	ATCC 33113 ^T

<i>Cl. rathayi</i>	MAFF 302197 ^T	NCPFB 2980 ^T
<i>Curtobacterium albidum</i>	MAFF 302648	IFO 15078
<i>C. citreum</i>	MAFF 302649	IFO 12677
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	NCPFB 1751, 2344	
	ATCC 33802	
<i>C. flaccumfaciens</i> pv. <i>oortii</i>	T-4	
	MAFF 301198	
<i>Rhodococcus fascians</i>	NCPFB 156	

a) ATCC ; American Type Culture Collection.

ICMP ; International Collection of Micro-organisms from Plants.

IFO ; Institute for Fermentation OSAKA.

MAFF ; Ministry of Agriculture, Forestry and Fisheries of Japan.

NCPFB ; National Collection of Plant Pathogenic Bacteria.

Kyu82-34-2, 82-40-1, 82-41-1 ; S. Tsushima²⁰⁾.

A1, PC-10, PC-20, ALQ8281, 86130 ; K. Tsuchiya, National Institute of Agrobiological Resources.

K1-1, R-1, T-4 ; T. Kobayashi, Yokohama Plant Protection Station.

Table 2. Dot blot hybridization of bacterial strain probed with ³²P-labeled PG2I and PG80I

Bacterium	Total no. of strains tested	No. of strains hybridized to	
		PG2I	PG80I
<i>Pseudomonas glumae</i>	43	43	38
<i>P. aeruginosa</i>	1	0	NT ^{a)}
<i>P. avenae</i>	10	0	0
<i>P. caryophylli</i>	2	0	0
<i>P. cepacia</i>	6	0	0
<i>P. cichorii</i>	3	0	NT
<i>P. gladioli</i> pv. <i>gladioli</i>	10	0	0
<i>P. gladioli</i> pv. <i>allicola</i>	1	0	0
<i>P. marginalis</i> pv. <i>marginalis</i>	11	0	NT
<i>P. plantarii</i>	3	0	2
<i>P. solanacearum</i>	8	0	NT
<i>P. syringae</i> pv. <i>phaseolicola</i>	1	0	NT
<i>P. syringae</i> pv. <i>syringae</i>	5	0	NT
<i>P. viridiflava</i>	9	0	NT
<i>Agrobacterium radiobacter</i>	1	0	NT
<i>A. rhizogenes</i>	1	0	NT
<i>A. tumefaciens</i>	1	0	NT
<i>Erwinia ananas</i>	1	0	NT
<i>E. carotovora</i> subsp. <i>carotovora</i>	1	0	NT
<i>E. herbicola</i>	1	0	NT
<i>Xanthomonas campestris</i> pv. <i>campestris</i>	1	0	NT
<i>X. campestris</i> pv. <i>oryzae</i>	8	0	NT
<i>Arthrobacter ilicis</i>	1	0	NT
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>	3	0	NT
<i>Cl. michiganensis</i> subsp. <i>sepedonicus</i>	1	0	NT
<i>Cl. rathayi</i>	1	0	NT
<i>Curtobacterium albidum</i>	1	0	NT
<i>C. citreum</i>	1	0	NT
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	3	0	NT
<i>C. flaccumfaciens</i> pv. <i>oortii</i>	2	0	NT
<i>Rhodococcus fascians</i>	1	0	NT

a) NT; not tested.

Fig. 1. DNA dot blot of 43 strains of *Pseudomonas glumae* and 10 strains of other bacteria probed with the insert DNA fragment, PG2I and PG80I, respectively. Probes, PG2I (a) and PG80I (b), were labeled with [32 P] dCTP. Dots A (lanes 1 to 10), B (lanes 1 to 10), C (lanes 1 to 10), D (lanes 1 to 10) and E (lanes 1 to 3) represent *P. glumae* strains as following: A1, MAFF 301094; A2, MAFF 301095; A3, MAFF 301096; A4, MAFF 301098; A5, MAFF 301099; A6, MAFF 301169; A7, MAFF 301170; A8, MAFF 301171; A9, MAFF 301172; A10, MAFF 301386; B1, MAFF 301389; B2, MAFF 301441; B3, MAFF 301682; B4, MAFF 302382; B5, MAFF 302383; B6, MAFF 302384; B7, MAFF 302394; B8, MAFF 302395; B9, MAFF 302396; B10, MAFF 302397; C1, MAFF 302421; C2, MAFF 302422; C3, MAFF 302423; C4, MAFF 302437; C5, MAFF 302438; C6, MAFF 302439; C7, MAFF 302440; C8, MAFF 302441; C9, MAFF 302442; C10, MAFF 302443; D1, MAFF 302444; D2, MAFF 302445; D3, MAFF 302446; D4, MAFF 302447; D5, MAFF 302448; D6, MAFF 302449; D7, MAFF 302450; D8, MAFF 302453; D9, MAFF 302463; D10, MAFF 302553; E1, Kyu82-34-2; E2, Kyu82-40-1 and E3, Kyu82-41-1. E4 to E10; blank. Dots F (lanes 1 to 5) represent *P. gladioli* pv. *gladioli* strains as following; F1, MAFF 301064; F2, MAFF 301580; F3, MAFF 301584; F4, MAFF 301588 and F5, MAFF 302385. F (lanes 6 to 10) represent *P. marginalis* pv. *marginalis* as following: F5, MAFF 302031; F7, MAFF 302032; F8, MAFF 302033; F9, MAFF 302036 and F10, MAFF 302038.

from eighty one plasmids tested. Each insert DNA obtained from pG2 and pG80 was 5.7-kb and 3.9-kb long, respectively.

The 5.7-kb fragment, PG2I, hybridized specifically to all the 43 strains of *P. glumae* isolated from rice grain or seedlings collected from different places in Japan or mung bean sprouts but did not hybridize to *P. gladioli* pv. *gladioli* or *P. gladioli* pv. *alicolla* which was similar to *P. glumae* in bacterial characteristics (Table 2). This fragment failed to hybridize to *P. caryophylli*, *P. cepacia* and *P. solanacearum* belonging to the rRNA homology group II⁽¹¹⁾ which included *P. gladioli* pv. *gladioli* and *P. gladioli* pv. *alicolla*. Furthermore, this fragment discriminated *P. glumae* from *P. avenae* and *P. plantarii*, the pathogens of rice seedlings (Fig. 1a and Fig. 2a). PG2I showed little homology not only with other gram negative bacteria but also with gram positive bacteria (Table 2). These results show that the fragment, PG2I is useful as a probe specific to *P. glumae*.

In contrast, the 3.9-kb fragment, PG80I did not hybridize to 5 of the 43 strains of *P. glumae* (Fig. 1b) and hybridized to 2 strains of *P. plantarii*, although this DNA failed to hybridize to other bacteria tested in the rRNA homology group II (Fig. 2b). These results indicate that the PG2I is more specific to *P. glumae* and is reliable for further tests against other bacteria.

The PG2I has a *Kpn*I site which divides this DNA into 2 fragments, PG2Ia (3.1 kb) and PG2Ib (2.6 kb) (Fig. 3). Specificity of these fragments to *P. glumae* was further examined. PG2Ia hybridized to all the 43 strains of *P. glumae* but PG2Ib did not hybridize to 18 strains (Fig. 4a and 4b), indicating that PG2Ia is more specific to this pathogen than PG2Ib.

The strains of *P. glumae* were divided into 4 groups by the patterns of hybridization with PG2Ib and PG80I probes (Table 3). PG2Ib and PG80I hybridized to 13 and 14 of the 14 strains isolated from rice seedlings, respectively, and to all the 4 strains from mung bean sprouts, but failed to hybridize to 16 and 5 of the 24 strains from rice grain, respectively. In the case of strains from grains, even though they were obtained from the same prefecture in the same year, *i.e.* Oita in 1984, hybridization patterns of each strain differed from locality to locality, indicating that the strains isolated from Yamaga, Azimu and Innai in Oita Prefecture differ genetically from each other and at least three groups defined by the

Fig. 2. DNA dot blot of 4 strains of *Pseudomonas glumae* and 42 strains of other bacteria probed with the insert DNA fragment, PG2I and PG80I, respectively. Probes, PG2I (A) and PG80I (B), were labeled with [32 P] dCTP. Dots G (lanes 1 to 4) represent *P. glumae* strains as following: G1, MAFF 301169; G2, Kyu82-34-2; G3, Kyu82-40-1; G4, Kyu82-41-1. Dots G (lanes 5 to 10) and H (lanes 1 to 4) represent *P. gladioli* pv. *gladioli* strains and H (lane 5) represent *P. gladioli* pv. *allicola* strain as following: G5, MAFF 301064; G6, MAFF 301580; G7, MAFF 301584; G8, MAFF 301586; G9, MAFF 301588; G10, MAFF 301728; H1, MAFF 302385; H2, MAFF 302537; H3, MAFF 302543; H4, MAFF 302804 and H5, MAFF 303950. Dots H (lanes 6 and 7) represent *P. caryophylli* strains as following: H6, ICMP 512 and H7, MAFF 301060. Dots H (lanes 8 to 10) represent *P. plantarii* strains as following: H8, MAFF 302381; H9, MAFF 302392 and H10, MAFF 302479. Dots I (lanes 1 to 2 and 5 to 10) represent *P. solanacearum* strains as following: I1, MAFF 302154; I2, MAFF 301559; I5 MAFF 301492; I6, MAFF 301860; I7, MAFF 301067; I8, MAFF 301418; I9, MAFF 301524 and I10, MAFF 301556. I3 and I4, blank. Dots J (lanes 1 to 6) represent *P. cepacia* strains as following: J1, MAFF 302528; J2, A1; J3, PC-10; J4, PC-20; J5, ALQ8281 and J6, 86130. Dots J (lanes 7 to 10) and K (lanes 1 to 6) represent *P. avenae* strains as follows: J7, MAFF 302183; J8, MAFF 301024; J9, MAFF 301027; J10, MAFF 301031; K1, MAFF 301036; K2, MAFF 301141; K3, MAFF 301502; K4, MAFF 301505; K5, MAFF 301576 and K6, MAFF 301609.

Fig. 3. Restriction map of a DNA fragment, PG2I. E, *EcoRI*; S, *SacI*; P, *PstI* and K, *KpnI*.

Fig. 4. DNA dot blot of 43 strains of *Pseudomonas glumae* and 10 strains of other bacteria probed with the *KpnI*-digested insert DNA fragment, PG2Ia and PG2Ib. Probes, PG2Ia (a) and PG2Ib (b), were labeled with [32 P] dCTP. Each designation, see Fig. 1.

Table 3. Hybridization of PG2Ia, PG2Ib and PG80I to isolates of *Pseudomonas glumae*^{a)}

No. of strain	Source	PG2I		PG80I
		PG2Ia	PG2Ib	
A) MAFF 301171	rice grain, Kagawa, 1967	+	—	—
MAFF 302446	rice grain, Oita (Yamaga), 1984	+	—	—
MAFF 302447	rice grain, Oita (Yamaga), 1984	+	—	—
MAFF 302448	rice grain, Oita (Yamaga), 1984	+	—	—
MAFF 301170	rice grain, Nagasaki, 1967	+	—	+
MAFF 301386	rice grain, Fukuoka, 1973	+	—	+
MAFF 301441	rice grain, Hiroshima, 1982	+	—	+
MAFF 302437	rice grain, Oita (Azimu), 1984	+	—	+
MAFF 302438	rice grain, Oita (Azimu), 1984	+	—	+
MAFF 302439	rice grain, Oita (Azimu), 1984	+	—	+
MAFF 302440	rice grain, Oita (Azimu), 1984	+	—	+
MAFF 302441	rice grain, Oita (Azimu), 1984	+	—	+
MAFF 302442	rice grain, Oita (Azimu), 1984	+	—	+
MAFF 302443	rice grain, Oita (Azimu), 1984	+	—	+
MAFF 302444	rice grain, Oita (Azimu), 1984	+	—	+
MAFF 302445	rice grain, Oita (Azimu), 1984	+	—	+
MAFF 301172	rice grain, Shizuoka, 1967	+	+	—
MAFF 301169	rice grain, Ehime, 1967	+	+	+
MAFF 302449	rice grain, Oita (Innai), 1984	+	+	+
MAFF 302450	rice grain, Oita (Innai), 1984	+	+	+
MAFF 302453	rice grain, Oita (Innai), 1984	+	+	+
Kyu82-34-2	rice grain, Fukuoka, 1982	+	+	+
Kyu82-40-1	rice grain, Fukuoka, 1982	+	+	+
Kyu82-41-1	rice grain, Fukuoka, 1982	+	+	+
B) MAFF 302553	rice leaf sheath, Kumamoto, 1984	+	—	+
C) MAFF 301094	rice seedling, Okayama, 1975	+	+	+
MAFF 301095	rice seedling, Okayama, 1975	+	+	+
MAFF 301096	rice seedling, Okayama, 1975	+	+	+
MAFF 301098	rice seedling, Okayama, 1975	+	+	+
MAFF 301099	rice seedling, Okayama, 1975	+	+	+
MAFF 301389	rice seedling, Akita, 1979	+	+	+
MAFF 301682	rice seedling, Ibaraki, 1982	+	+	+
MAFF 302382	rice seedling, Ibaraki, 1982	+	+	+
MAFF 302383	rice seedling, Ibaraki, 1982	+	+	+
MAFF 302384	rice seedling, Ibaraki, 1982	+	—	+
MAFF 302394	rice seedling, Miyagi, 1983	+	+	+
MAFF 302395	rice seedling, Miyagi, 1983	+	+	+
MAFF 302396	rice seedling, Miyagi, 1983	+	+	+
MAFF 302397	rice seedling, Miyagi, 1983	+	+	+
D) MAFF 302421	Mung bean sprouts, Ibaraki, 1984	+	+	+
MAFF 302422	Mung bean sprouts, Ibaraki, 1984	+	+	+
MAFF 302423	Mung bean sprouts, Ibaraki, 1984	+	+	+
MAFF 302463	Mung bean sprouts, Ibaraki, 1984	+	+	+

a) Bacterial strains were isolated from rice grain (A), rice leaf sheath (B), rice seedling (C) and mung bean sprouts (D).

patterns of hybridization were present in this prefecture. These results suggest that hybridization patterns are more diverse in the strains from rice grain than those from seedlings and, furthermore, imply the allopatric differentiation of *P. glumae* strains.

This is the first report on a DNA probe specific to *P. glumae*. The fragment PG2I obtained in this experiment is applicable as a hybridization probe to detect this pathogen in environments.

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和 文 摘 要

対馬誠也・成松千寿・水野明文・木村龍介：イネもみ枯細菌病菌検出のためのDNAプローブ

イネもみ枯細菌病菌 (*Pseudomonas glumae*) Kyu 82-34-2 から、ショットガンクローニングにより、DNA 断片 PG 2 I (5.7 kb), PG 80 I (3.9 kb) を得た。PG 2 I はドットプロットハイブリダイゼーションにより供試した本種の 43 菌株全てに高い相同性を示したが、他の 25 種 99 菌株にはほとんど反応しなかった。一方、PG 80 I は *P. glumae* 43 菌株中 5 菌株には反応せず、かつ *P. plantarii* 3 菌株中 2 菌株に高い相同性を示したことから、PG 2 I が *P. glumae* により特異的であると考えた。そこで、PG 2 I を制限酵素 *Kpn*I により 2 つの断片 (PG 2 Ia, PG 2 Ib) に分割し、*P. glumae* に対する特異性を調べた結果、PG 2 Ia (3.1 kb) が供試した全菌株に高い相同性を示し、*P. glumae* の特異的プローブとして有効であると考えた。PG 2 Ia, PG 2 Ib および PG 80 I に対する *P. glumae* 各菌株のハイブリダイゼーションのパターンから、供試菌株は籾由来の菌株で 4 群、イネ幼苗由来の菌株で 2 群、また緑豆のもやし由来の菌株で 1 群に分かれた。さらに、同一県内でも地区毎にハイブリダイゼーションのパターンが異なる菌株が分布していることが示唆された。