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The *hrpZ* and *hrpA* genes are variable, and useful for grouping *Pseudomonas syringae* bacteria

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Abstract The *hrpS* to *hrpB* regions from strains of *Pseudomonas syringae* were amplified by polymerase chain reaction (PCR) and the DNA sequence determined. The order of *hrpS*, *hrpA*, *hrpZ*, and *hrpB* was consistent among *P. syringae* strains. The sequence of *hrpS* was highly conserved. In a cluster analysis with the *hrpS* sequence, *P. syringae* strains were divided into four groups (I, II, III, and IV) and one undetermined strain, in agreement with previous studies. In contrast, the *hrpZ* sequences contained insertions, deletions, and base substitutions followed by changes in amino acids. Based on cluster analysis of *hrpA*, *hrpZ*, and *hrpB*, *P. syringae* strains could be divided into five groups. One of the four groups (group I) in the cluster analysis of *hrpS* could be further divided into two subgroups (groups IA and IB). Groups II, III, and IV were the same in the two analyses. Group-specific primers were designed, based on the DNA sequences of *hrpZ*, that could differentiate the groups of *P. syringae* strains.

Key words *Pseudomonas syringae* · Phylogenetic analysis · *hrpZ* · Group-specific primers

Introduction

Pseudomonas syringae has a number of pathovars that are in their host plants and/or disease symptoms. *P. syringae* is identifiable by several characteristics, but the phenotype of each pathovar and strain varies considerably in their nutritional and physiological characteristics (Takikawa and Inoue 2003). In recent years, DNA–DNA hybridization and DNA sequence comparisons have been used to distinguish the strains or pathovars of *P. syringae* and to classify *P. syringae* into several groups (Gardan et al. 1999; Sawada et al. 1999). Because pathogenicity on plants is one of the most important distinguishing characteristics of *P. syringae*, pathogenicity-related genes might characterize and differentiate pathovars or strains of *P. syringae*.

Causing disease on host plants and eliciting a hypersensitive response on nonhost plants are fundamental features controlled by the *hrp* genes in plant pathogenic bacteria (Bonas 1996). The *hrp*/*hrc*-encoded proteins are involved in a type-III secretion pathway (Hueck 1998). In *P. syringae*, the *hrp*/*hrc* gene cluster is flanked by a unique exchangeable effector locus (EEL) and a conserved effector locus (CEL) (Alfano et al. 2000). These loci contain the pathogenicity-related genes, and with the *hrp*/*hrc* cluster, EEL and CEL comprise a pathogenicity island (Alfano et al. 2000). The *hrpZ* operon is located in the *hrp*/*hrc* core region. The HrpZ protein was thought to be an elicitor and secreted by the type-III secretion systems (He et al. 1993; Li et al. 2002; Preston et al. 1995). The *hrpA* gene, included in the *hrpZ* operon, encodes a structural subunit protein of the Hrp pilus (Roine et al. 1997; Taira et al. 1999).

In a comparison of the DNA sequence of EEL, the EEL was found to be a variable region where many rearrangements might have occurred, but it is not useful for grouping *P. syringae* (Alfano et al. 2000; Charity et al. 2003). However, we demonstrated that *P. syringae* strains had the *hrp*/*hrc* cluster and CEL in the same order and that the strains could be grouped into at least five types by comparing DNA homology at the *hrp*/*hrc* cluster and its neighboring regions (Inoue and Takikawa 1999, 2000). Based on the comparisons of DNA homology, we thought this region might be useful to compare the sequences and structure of the *hrp*/*hrc* core regions not only for grouping *P. syringae* but also for understanding the evolutionary process by which *P. syringae* developed into a plant pathogen through the ac-

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quisition of the *hrp* gene cluster. Therefore, in this study, we compared the structure and DNA sequences of the regions, with special emphasis on *hrpZ*, *hrpA*, and their neighbors for grouping *P. syringae* pathovars. A preliminary report has been made (Inoue and Takikawa 2003).

Materials and methods

Bacterial strains, culture conditions, and DNA manipulation procedures

The 36 bacterial strains used in this study were used in our previous studies (Table 1) (Inoue and Takikawa 1999, 2000). *P. cichorri* and *P. fluorescens* were used as negative controls of group-specific polymerase chain reactions (PCRs). The DNA sequences of pv. *syringae* strain 61, pv. *glycinea* strain R4, and pv. *tomato* strain DC3000 (Preston et al. 1995) were retrieved from the GenBank database and used in our analysis. *Pseudomonas* strains were grown in YP medium (yeast extract 5g, peptone 10g, water 1 liter, pH 6.8) with or without agar (1.5%) at 27°C. *Escherichia coli* strains were grown in Luria-Bertani (LB) medium (Ausubel et al. 1992) with or without agar (1.5%) at 37°C. Genomic DNA was extracted from *Pseudomonas* strains using the CTAB procedure and plasmid DNA was isolated from *E. coli* using the boiling and alkaline lysis method (Ausubel et al. 1992).

Table 1. Bacterial strains and summary of results of genetic analysis

Pathovar or species	Strain	Previous grouping[a]			PCR[b]	Results of cluster analysis				PCR[c]	Lane no. of Fig. 4
		A	B	C		*hrpS*	*hrpA*	*hrpZ*	*hrpB*		
Pseudomonas syringae											
phaseolicola	KZ2W	I	3	2	+	I	I-A	I-A	I-A	I-A	1
	NPS3121	I	3	2	+	I	I-A	I-A	I-A	I-A	6
glycinea	r0	I	3	2	+	I	I-A	I-A	I-A	I-A	7
tabaci	113A	I	3	2	+	I	I-A	nt	I-A	I-A	8
dendropanacis	kakuremino-1[f]	I	nt	2	+	I	I-A	I-A	I-A	I-A	9
eriobotryae	PERB8031	I	3	2	+	I	I-A	I-A	I-A	I-A	10
myricae	yamamomo801	I	3	2	+	I	I-A	I-A	I-A	I-A	11
?[d](kiwi)	KW741	I	nt	nt	+	I	I-A	I-A	I-A	I-A	12
morsprunorum	U7805	U[e]	3	2	+	I	I-A	I-A	I-A	I-A	13
mori	mori1	I	3	2	+	I	I-B	I-B	I-B	I-B	2
lachrymans	cucum-1	I	3	2	+	I	I-B	I-B	I-B	I-B	14
sesami	PSES-1	I	nt	2	+	I	I-B	I-B	I-B	I-B	15
photiniae	photinia-1	I	3	2	–	nt	nt	nt	nt	I-B	16
maculicola	R1	U	nt	nt	+	U	I-B	I-B	I-B	I-B	18
	PMC8301	II	1	3	+	II	II	II	II	II	3
tomato	ICMP2844[f]	II	1	3	+	II	II	II	II	II	19
theae	tea632	II	1	8	+	II	II	II	II	II	20
delphinii	PDDCC529[f]	II	nt	3	+	II	II	II	II	II	21
magnoliae	PMG8101	II	nt	nt	+	II	II	II	II	II	22
actinidiae	KW11[f]	II	1	nt	+	II	II	II	II	II	23
spinaciae	spin8605[f]	II	nt	nt	–	nt	nt	nt	nt	II	24
syringae	LOB2-1	III	2	1	+	III	III	III	III	III	4
japonica	BPST802	III	2	1	+	III	III	III	III	III	25
aptata	SB8601	III	2	1	+	III	III	III	III	III	26
lapsa	NCPPB2096[f]	III	2	1	+	III	III	III	III	III	27
aceris	kaede1-1	III	2	1	+	III	III	III	III	III	28
pisi	Pisum-1	III	2	1	+	III	III	III	III	III	29
coronafaciens	AVPCO8101	IV	nt	4	+	IV	IV	IV	IV	IV	5
striafaciens	avena2	IV	nt	4	+	IV	IV	IV	IV	IV	30
atropurpurea	NIAES1309	IV	nt	4	–	nt	nt	nt	nt	IV	31
oryzae	1–2[f]	IV	nt	4	+	IV	IV	IV	IV	IV	32
porri	ICMP8961[f]	IV	nt	4	–	nt	nt	nt	nt	IV	33
? (onion)	ICMP3414	IV	nt	nt	+	IV	IV	IV	IV	IV	34
Pseudomonas											
ficuserectae	L-7[g]	I	nt	2	+	I	I-B	I-B	I-B	I-B	17
cichorii	TPS ash8101				–	nt	nt	nt	nt	–	
fluorescens	IFO3507				–	nt	nt	nt	nt	–	

PCR, polymerase chain reaction; nt, not tested
[a] Column A in previous grouping is obtained from the result of Inoue and Takikawa (1999, 2000), B is from Sawada et al. (1999), and C is from Gardan et al. (1999)
[b] Result of PCR amplification using degenerated primers based on the *hrpS* and the *hrpB* sequences. +, Positive; –, negative
[c] The name of group-specific primer set that yielded positive PCR amplification
[d] Pathovar undetermined
[e] Group undetermined
[f] Pathotype strain
[g] Type strain

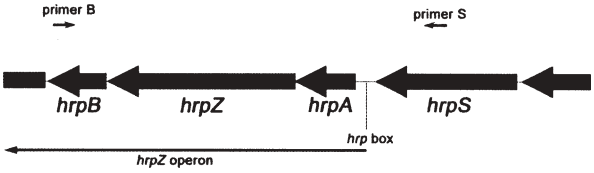


Fig. 1. Gene map from the *hrpS* to *hrpB* region of *Pseudomonas syringae* and binding position of primer B and primer S

PCR amplification

To clone the *hrpZ* and its neighboring region, DNA fragments were amplified by PCR using degenerated primers designed from the published *hrpS* and *hrpB* sequences. The primer sequences were 5′-ACGATCAASCTGGATRT CTG-3′ (primer S, designed on the *hrpS*) and 5′-TGNAGRTTNGTNAGYTTRTC-3′ (primer B, designed on the *hrpB*). The gene map from *hrpS* to *hrpB* and the binding position of primer B and primer S are shown in Fig. 1. PCR amplification was performed in a total volume of 20 µl with *ExTaq* DNA polymerase (Takara, Otsu, Japan). The reaction mixture contained 1× PCR reaction buffer supplied by the manufacturer, 200 µM of each dNTP, 0.5 U of DNA polymerase, 10 pmol of each primer, and 100 ng of purified total bacterial DNA for the template. Reactions were performed using the following cycle parameters: 2 min at 94°C; followed by 30 cycles of 30 s at 94°C, 30 s at 60°C, and 2 min at 72°C; followed by a final incubation of 10 min at 72°C. PCR products were purified and directly cloned into pCR2.1 (Invitrogen, Carlsbad, CA, USA). Group-specific PCRs were also performed under the same conditions.

DNA sequence

DNA sequences were analyzed using a cycle sequencing method with nonlabeled primers and a BigDye Terminator Kit v.2 (Applied Biosystems, Foster City, CA, USA), following the manufacturer's instructions. DNA sequences were determined with an ABI Prism 377 DNA sequencer and Sequencing Analysis v.3.4 (Applied Biosystems).

Phylogenetic analysis

Multiple sequence alignment of the DNA and amino acid sequences was performed with Clustal X version 1.8 (Thompson et al. 1997). Neighbor-joining (NJ) trees were generated with 1000 bootstrap replicates for all sequences. The DNA sequences of the *hrpS*, *hrpN*, *hrpA*, and *hrpB* gene of *Erwinia amylovora* (Kim et al. 1997) were downloaded from the GenBank database (accession numbers AF083877, M92994, and U56662) and used as the outgroup for phylogenic analysis of the *hrpS*, *hrpZ*, *hrpA*, and *hrpB* genes of *P. syringae*.

Results

PCR amplification of the *hrpZ* neighboring region

Among the 34 strains tested, 30 strains yielded amplified products (Table 1), but strains of pathovars *atropurprea*, *photiniae*, *porri*, and *spinaciae* did not. In a comparison of DNA sequences of the amplified products, the position and orientation of the neighboring region of the *hrpZ* were the same from the *hrpS* gene to the *hrpB* gene in all the analyzed strains. A large deletion in the *hrpZ* gene was detected only in pv. *tabaci*, and its position and length were in accordance with a previous report (Taguchi et al. 2001) (data not shown).

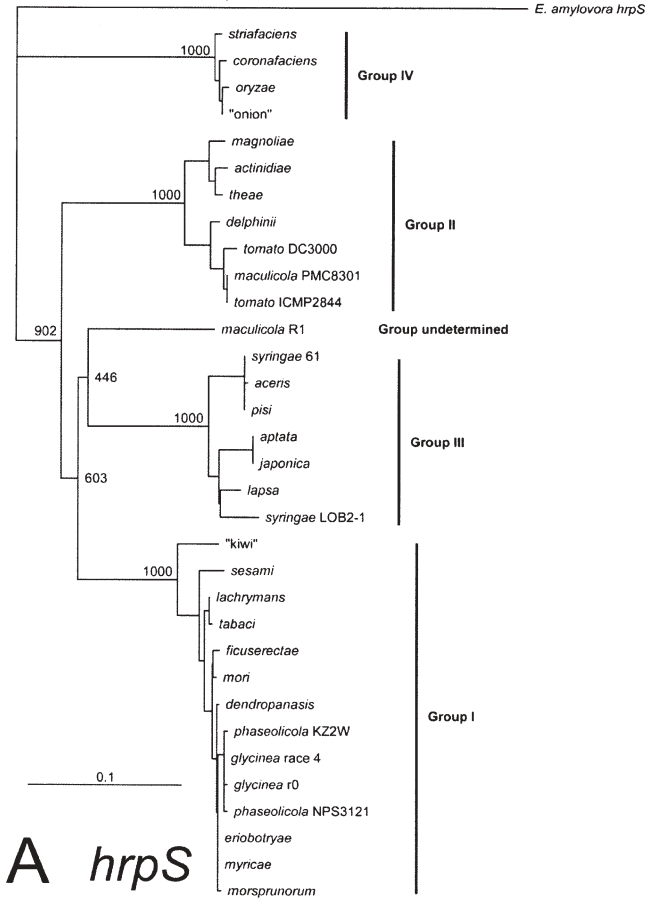
Phylogenic analysis of *hrpS*

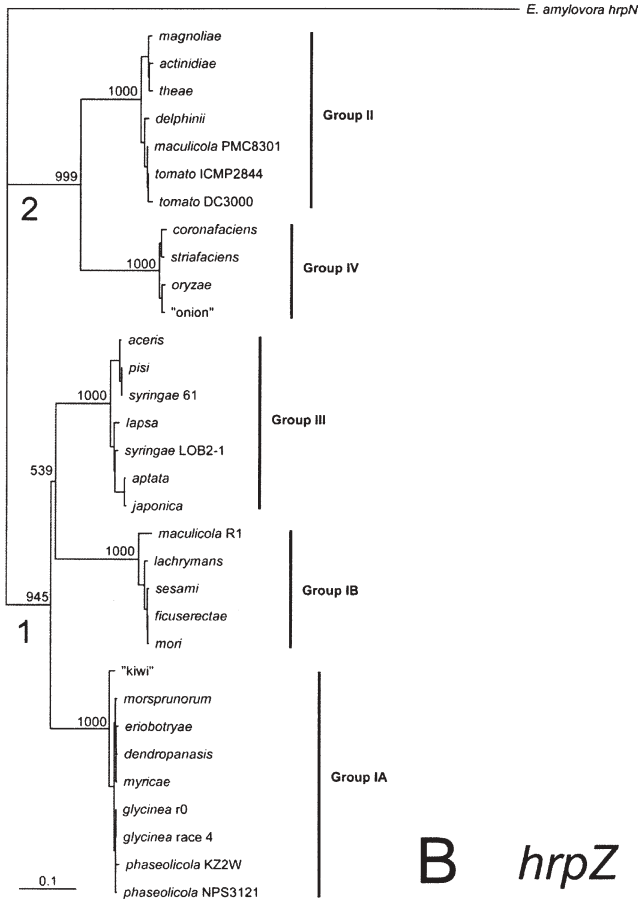
In this study, the 480-base DNA sequence near the 3′-end of *hrpS* was determined. No insertions or deletions were detected in any strain. In the cluster analysis of the *hrpS* sequence, *hrpS* of *P. syringae* strains could be divided into four groups, with one undetermined strain (pv. *maculicola* R1). DNA sequences of *hrpS* were translated into amino acid sequences. Similarities among the strains in each group were 93% or higher and those between groups ranged from 85% to 93%. Grouping by cluster analysis based on the partial HrpS sequence was consistent with that for the *hrpS* sequence.

Phylogenic analysis of *hrpA*, *hrpZ*, and *hrpB*

In the cluster analysis based on the *hrpZ* sequence, *hrpZ* genes of *P. syringae* strains could clearly be divided into two large groups (divisions 1 and 2, marked "1" and "2" in Fig. 2), and further divided into five groups with two divisions [groups IA, IB, III (division 1) and II, IV (division 2)] (Fig. 2B, Table 1). Group I strains of our previous grouping (Inoue and Takikawa 2000) were divided into two subgroups (IA and IB) based on the *hrpZ* sequence, with the undetermined strain (pv. *maculicola* R1) in group IB. DNA sequences of *hrpA* and *hrpB* were also analyzed. Cluster analysis of the sequences showed that *P. syringae* strains

Fig. 2. Cluster analysis of *Pseudomonas syringae* strains on the basis of *hrpS* (**A**), *hrpZ* (**B**), *hrpA* (**C**), and *hrpB* (**D**) DNA sequences. The neighbor-joining bootstrap tree excludes positions with gaps. Large numerals *1* and *2* indicate divisions 1 and 2. The *small numbers* show the bootstrap probability of each branch. DNA sequences of *P. syringae* pv. *glycinea* race 4, pv. *syringae* 61, and pv. *tomato* DC3000 were downloaded from DDBJ database (Acc. No. L14775, L41861-4, and U03852) for comparison with our determined sequences (Acc. No. AB112552-81)


E. amylovora hrpS
striafaciens
coronafaciens
oryzae
"onion"
Group IV
magnoliae
actinidiae
theae
delphinii
tomato DC3000
maculicola PMC8301
tomato ICMP2844
Group II
maculicola R1
Group undetermined
syringae 61
aceris
pisi
aptata
japonica
lapsa
syringae LOB2-1
Group III
"kiwi"
sesami
lachrymans
tabaci
ficuserectae
mori
dendropanasis
phaseolicola KZ2W
glycinea race 4
glycinea r0
phaseolicola NPS3121
eriobotryae
myricae
morsprunorum
Group I
1000
902
446
603
0.1
A hrpS


E. amylovora hrpN
magnoliae
actinidiae
theae
delphinii
maculicola PMC8301
tomato ICMP2844
tomato DC3000
Group II
coronafaciens
striafaciens
oryzae
"onion"
Group IV
aceris
pisi
syringae 61
lapsa
syringae LOB2-1
aptata
japonica
Group III
maculicola R1
lachrymans
sesami
ficuserectae
mori
Group IB
"kiwi"
morsprunorum
eriobotryae
dendropanasis
myricae
glycinea r0
glycinea race 4
phaseolicola KZ2W
phaseolicola NPS3121
Group IA
1000
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539
945
2
1
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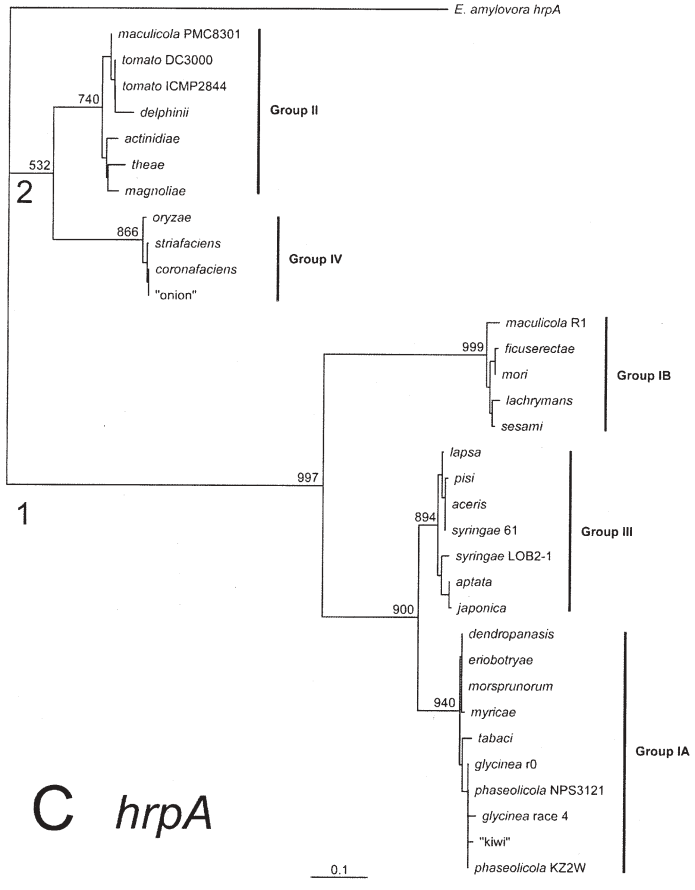
B hrpZ


E. amylovora hrpA
maculicola PMC8301
tomato DC3000
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delphinii
Group II
actinidiae
theae
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oryzae
striafaciens
coronafaciens
"onion"
Group IV
maculicola R1
ficuserectae
mori
lachrymans
sesami
Group IB
lapsa
pisi
aceris
syringae 61
syringae LOB2-1
aptata
japonica
Group III
dendropanasis
eriobotryae
morsprunorum
myricae
tabaci
glycinea r0
phaseolicola NPS3121
glycinea race 4
"kiwi"
phaseolicola KZ2W
Group IA
740
532
866
999
997
894
900
940
2
1
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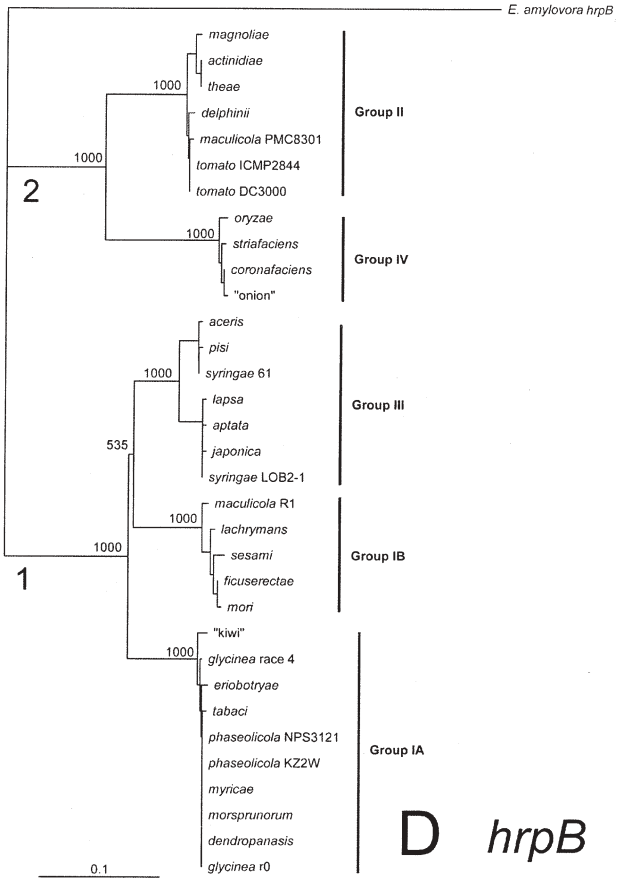
C hrpA


E. amylovora hrpB
magnoliae
actinidiae
theae
delphinii
maculicola PMC8301
tomato ICMP2844
tomato DC3000
Group II
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striafaciens
coronafaciens
"onion"
Group IV
aceris
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syringae 61
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aptata
japonica
syringae LOB2-1
Group III
maculicola R1
lachrymans
sesami
ficuserectae
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Group IB
"kiwi"
glycinea race 4
eriobotryae
tabaci
phaseolicola NPS3121
phaseolicola KZ2W
myricae
morsprunorum
dendropanasis
glycinea r0
Group IA
1000
535
2
1
0.1
D hrpB

could also be divided into five groups without contradicting the results with *hrpZ* (Fig. 2C, D; Table 1). The results of the cluster analysis based on amino acid sequences of the genes were consistent in all cases.

Comparison of the amino acid sequence of HrpZ and HrpA

The HrpZ sequences were highly diverse, having some insertions and deletions (Fig. 3A). Identities among the strains in each group were 94% or higher and those between groups ranged from 54% to 78%. Similarities among the strains between groups ranged from 64% to 86%. The length of the HrpZ sequence differed not only among groups but also among the strains in each group. Substitutions also could be detected throughout the region. Groups in division 2 were clearly distinct from groups in division 1; division 2 had more than 20 amino acids, including at least 10 glycines, inserted about 100 amino acids from the N terminal (Fig. 3A). Group-specific sequences were found also in other regions. Comparison of the HrpA sequences also revealed some insertions, deletions, and substitutions (Fig. 3B). Identities among the strains in each group were 90% or higher, and those between groups in same division and between divisions ranged from 42% to 92% and 21% to 33%, respectively. Similarities among the strains between the groups in the same division ranged from 42% to 94% and those between the divisions ranged from 44% to 53%. HrpA sequences of each group were 108 amino acids long in group IA, 104 in IB, 113 in II, 108 in III, and 115 in IV. In division 2, the similarity between HrpAs of groups II and IV was high. In division 1, the similarity between HrpAs of groups IA and III was high, but HrpA of group IB was distinct.

Design of group-specific primers

Group-specific primers were designed based on the DNA sequences of the *hrpZ* (Table 2). The expected sizes were 0.88 kbp in group IA, 0.85 kbp in IB, 1.0 kbp in II, 0.75 kbp in III, and 0.78 kbp in IV. In group IA, the expected size for pv. *tabaci* was 549 base pairs because of an internal deletion. All 34 strains of *P. syringae* produced PCR products of the expected size with their group-specific primers, although some strains produced extra bands (Fig. 4, Table 1). Strains of pathovars *atropurprea*, *photiniae*, *porri*, and *spinaciae*, which did not cluster into groups based on *hrpZ* sequence, could now be assigned to a group using PCR with our group-specific primers. Pathovar *photiniae* produced a PCR product with group IB-specific primers. Pathovar *spinaciae* produced a PCR product with group II-specific primers. Pathovars *atropurprea* and *porri* produced PCR products with group IV-specific primers. These results corresponded to our previous groupings (Inoue and Takikawa 2000). No amplification fragment was obtained from the tested strains of *P. cichorii* and *P. fluorescens* with any of the primer sets (data not shown).

Discussion

In this study, we were able to classify *hrpA*, *hrpZ*, and *hrpB*, involving the *hrpZ* operon of *P. syringae* strains, into five groups by comparing their DNA sequences. Furthermore, we showed that *hrpA*, *hrpZ*, and *hrpB* were highly diverse. Previously, we reported that *P. syringae* strains could be classified into four groups, with some strains undetermined, by comparing DNA homology for the *hrp* cluster and its neighboring regions (Inoue and Takikawa 1999, 2000). However, here we achieved a more precise grouping through analysis of the *hrpA*, *hrpZ*, and *hrpB* sequences.

The partial DNA sequence of *hrpS* was highly conserved in *P. syringae*, and neither insertion nor deletion was detected in any of the strains investigated. Result of cluster analysis of the *hrpS* sequence showed that *P. syringae* strains could be divided into four groups and one undetermined strain without contradicting previous grouping (Inoue and Takikawa 1999, 2000). Sawada et al. (1999) reported three groups in *P. syringae* through a comparison of sequences of the *hrpL*, *hrpS*, and two other housekeeping genes. They did not use strains of pathovars belonging to our group IV. Our results on the analysis of *hrpS* perfectly corresponded with theirs, and added strains of pathovars of monocotyledonous pathogens as another group.

In contrast, many insertions and deletions of the bases were observed in *hrpZ*. The comparison of the HrpZ sequences showed many substitutions of amino acids. These results suggested that the *hrpZ* is highly changeable. The comparison of the *hrpA* sequences also revealed many substitutions of amino acids. Li et al. (2002) reported that HrpA and HrpZ function as a conduit for the long-distance translocation of effector proteins. Taira et al. (1999) showed that the ability to elicit diseases did not change even if the first half of the *hrpA* gene had mutated. In the sequence alignments of HrpA, the conserved region is on the C-terminal side in each division (Fig. 3B). Therefore, *hrpA* might be an unstable gene and is highly exchangeable like *hrpZ*.

In general, the clustering analysis of *hrpA*, *hrpZ*, and *hrpB* corresponded to previous groupings, although this analysis differs in that group I strains can be divided into two categories (group IA and IB). DNA sequences of group IA and IB obviously differed from each other from the point adjacent to the end of *hrpS*. These results suggest that the evolution of *hrpA*, *hrpZ*, and *hrpB* was clearly distinct from the evolution of *hrpS* and other housekeeping genes.

In our previous study, pv. *morsprunorum* and pv. *maculicola* strain R1 were undetermined strains (Inoue and Takikawa 2000). In this study, pv. *morsprunorum* was classified into the group I. This result corresponded with the genomospecies assignment by Gardan et al. (1999). On the other hand, pv. *maculicola* strain R1 was clearly distinguished from other strains by the cluster analysis of *hrpS*. This result suggested that strain R1 evolved differently from

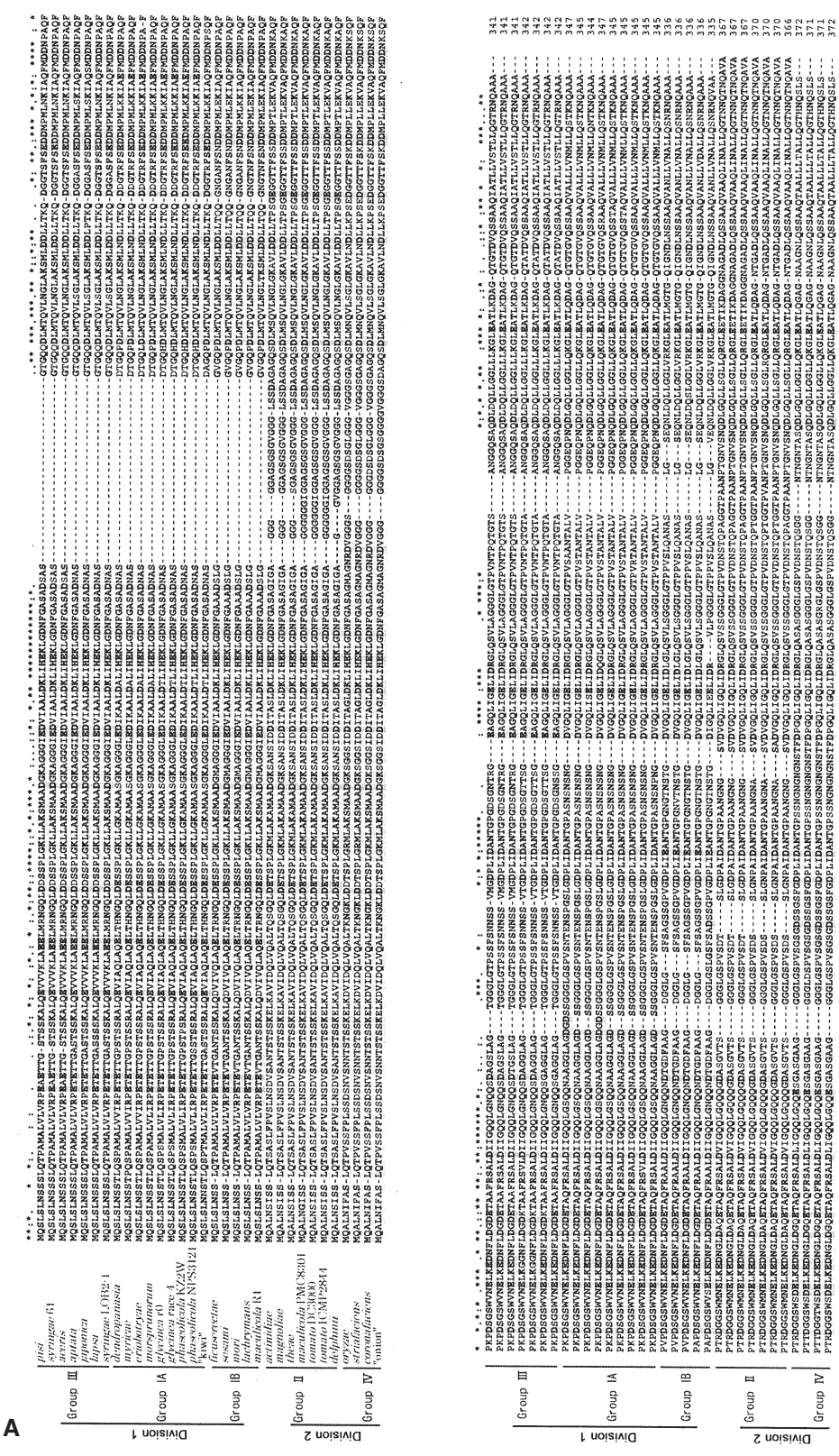

Fig. 3. Multiple sequence alignment of amino acid sequences of HrpZ (**A**) and HrpA (**B**). Divisions 1 and 2 are the two major divisions by cluster analysis. The *asterisk* indicates positions with a single, fully conserved residue. The *colon* indicates that one of the following "strong" groups is fully conserved: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, or FYW. The *period* indicates that one of the following "weaker" groups is fully conserved: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, FVLIM, or HFY

the other strains. Our unpublished result of the *rpoD* sequencing of strain R1 also supported this conclusion. However, strain R1 was included in group IB by cluster analysis of *hrpA*, *hrpZ*, and *hrpB*.

The relationship of groups IA, IB, and III is controversial. As mentioned before, group IA and IB strains are indistinguishable based on *hrpS* and other housekeeping genes. They belong to one group, i.e., group I, independent

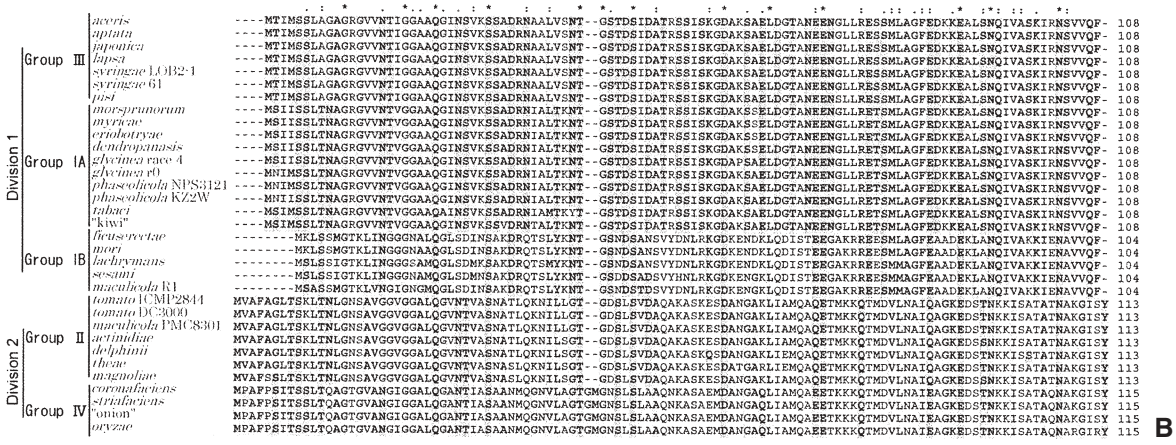

Fig. 3. (*continued*)

Table 2. Group-specific primers

Name of primers set	Forward primer	Reverse primer
Group IA	CAGCTTGCCCAGGAGCTGAC	ATGTTGACCAGCAGCAAGGC
Group IB	TTGGCTCAAGAGTTGACCCG	GCGCGTTGACCAGCAAGTTG
Group II	GCTGTGATCGATCAGCTGGT	TCAGGCCACAGCCTGGTTAG
Group III	AGCTGGCCGAGGAACTGATG	AACTGGTCAAGATCCTGAGC
Group IV	ATGCTCGCAAAATCGATGGC	TGACTGGCCGTATTGCCATT

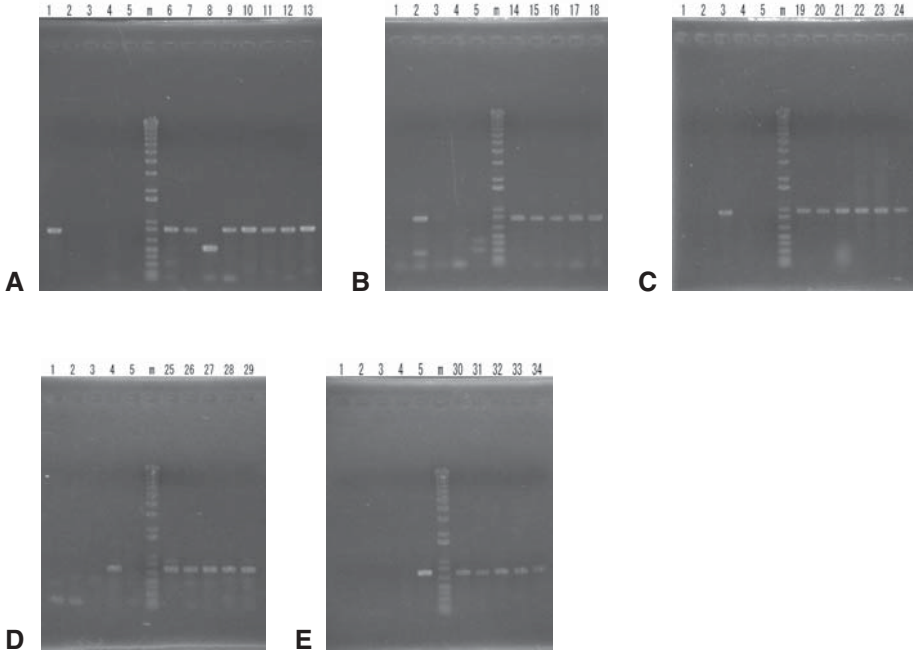

Fig. 4. Polymerase chain reaction (PCR) amplification with group-specific primers sets: **A** group IA primers sets, **B** group IB primers sets, **C** group II primers sets, **D** group III primers sets, and **E** group IV primers sets. PCR samples were subjected to electrophoresis on 0.9% agarose gel. *Lane numbers* correspond to numbers in Table 1. Lane *m*, DNA molecular size marker (1Kb Plus DNA Ladder, Invitrogen, Carlsbad, CA, USA)

of group III. In contrast, our analysis of the dendrogram obtained by clustering of *hrpA* sequences showed a potentially closer relationship between groups IA and III than between IA and IB. Although the bootstrap values are low, the analysis based on *hrpZ* and *hrpB* also suggests a similar relationship. We can think of two reasons for this. One is that the *hrpZ* operon of the common ancestor of group IA and IB might have evolved together with housekeeping genes, then diverged from a common ancestor with group III, and after the branching of I and III, the *hrpZ* operon might have been transferred horizontally from group III bacteria to group IA. Another possibility is that after

branching of group I and group III, the *hrpZ* operon might have been transferred from an unknown bacterium (such as pv. *maculicola* strain R1) to a group IB bacterium. In either case, whole *hrp* gene cluster must be analyzed before the entire process of the evolution of the *hrp* pathogenicity island can be elucidated.

Group IA contained strains causing knots or galls on trees. Our unpublished results on an analysis of all the tree gall-forming *P. syringae* pathovars in Japan showed that they all belonged to group IA. However, phenotypic analysis could not differentiate group IA from group IB. The relationship between genetic variation in this region and the type of disease caused must be investigated.

In this study, we designed group-specific primers based on the DNA sequences of the *hrpZ*. All the strains yielded PCR products of the expected size using their group-specific primers. These groups are nearly coincident with the genomospecies proposed by Gardan et al. (1999). Therefore, these primer sets may be useful for rapid genome species identification of *P. syringae*.

Although we used only one or two strains of each pathovor in this study, our results corresponded with the results of Sawada et al. (1999) and Gardan et al. (1999), who used many different strains. Therefore, our grouping of the strains should agree with the grouping of pathovors.

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