J. Gen. Plant Pathol. 66 : 238-241 (2000)

Pseudomonas syringae Strains Are Classified into Five Groups by Comparing DNA Homology at the *hrp* Neighboring Regions

Yasuhiro INOUE[1,2]* and Yuichi TAKIKAWA[1]

ABSTRACT

Previously, we classified *Pseudomonas syringae* strains into at least three groups (I, II and U) by comparing DNA homology at the *hrp* cluster and its neighboring regions (Inoue and Takikawa 1999). However, heterogeneous strains remained in the undetermined group (group U). We further classify group U, using pvs. *syringae* and *coronafaciens* as references. Comparison of restriction sites for regions of each pathovar revealed distinct differences. By using probes from the two pathovars, comparisons of DNA homology at the regions separated two additional distinct groups (III and IV) from group U. Therefore, *P. syringae* strains are classified into at least five groups.

(Received November 4, 1999 ; Accepted January 27, 2000)

Key words : *Pseudomonas syringae*, grouping, DNA homology of *hrp*-neighboring regions.

Causing disease on susceptible plants and eliciting a hypersensitive response (HR) on nonhost plants are two features shared by many plant pathogenic bacteria controlled by clustered *hrp* genes. An *hrp* gene cluster has been identified in several gram-negative phytopathogenic bacteria[2]. The *hrp* genes are highly conserved, though their relative positions are considered to have been rearranged. *Pseudomonas syringae* strains have an *ca.* 80-kb homologous region encompassing the *hrp* cluster and its neighboring regions[6]. This region contains genes participating in the manifestation of pathogenicity as well as some repeating fragments[7].

We showed that *P. syringae* strains could be grouped into at least three types by comparing DNA homology at the *hrp* cluster and its neighboring regions[6]. Through hybridization studies, group I strains produced strong signals to probes from *P. s.* pv. *phaseolicola* (Psp), and group II strains gave strong signals to probes from *P. s.* pv. *maculicola* (Psm). The third group is negatively defined as strains having a moderate or weak signal to probes of either Psp or Psm. Therefore, this group was referred to as group U (U for "undetermined"). They are clearly heterogeneous, although some of them closely resembled each other. Among them, *P. s.* pv. *syringae* (Pss) and *P. s.* pv. *coronafaciens* (Psc) represent two more distinct groups, as suggested by our preliminary studies. In this study, we classify group U into several groups using large genomic regions including the *hrp* gene cluster and its neighboring regions in Pss and Psc.

Genomic libraries from Pss strain LOB2-1 and Psc strain AVPCO8101 were constructed as described before[6]. We selected cosmid clones covering more than 80-kb of the region containing the *hrp* genes. These clones were digested with appropriate restriction endonucleases, and restriction site maps were constructed. Comparison of restriction sites of the large genomic region of Pss, Psc, Psp and Psm revealed distinct differences (Fig. 1). All tested strains had homologous regions deduced to be *avrE* genes on the right outside of *hrpR*. This result suggested that *hrp* genes and its neighboring genes are located in the same order in Psc and Pss as in Psp and Psm.

The *avrE* locus has been isolated from *P. s.* pv. *tomato* and, when introduced into *P. s.* pv. *glycinea*, caused the soybean pathogen to elicit the HR on leaves of certain soybean cultivars[8]. In addition, Bogdanove *et al.*[1] reported that *avrE* genes have homology to *dsp* genes of *Erwinia amylovora*. The function of these genes is unknown in *P. syringae* strains, but they are expected to participate in pathogenicity. Therefore, this region may form a pathogenicity island in *P. syringae*[4].

Several DNA fragments from clones of Pss and Psc were used as probes for Southern hybridization performed as described before[6]. Briefly, 0.5 or 5 μg of digested DNA from each strain was transferred onto a nylon membrane. Hybridization was performed for 16 hr at 68°C. The membranes were washed twice in wash

[1] Plant Pathology Laboratory, Faculty of Agriculture, Shizuoka University, Ohya, Shizuoka 422-8529, Japan
[2] Present address : Orynova K. K., 700, Higashihara, Toyoda-cho, Iwata-gun, Shizuoka 438-0802, Japan
* Corresponding author (E-mail : yasuhiro.inoue@orynova.co.jp)

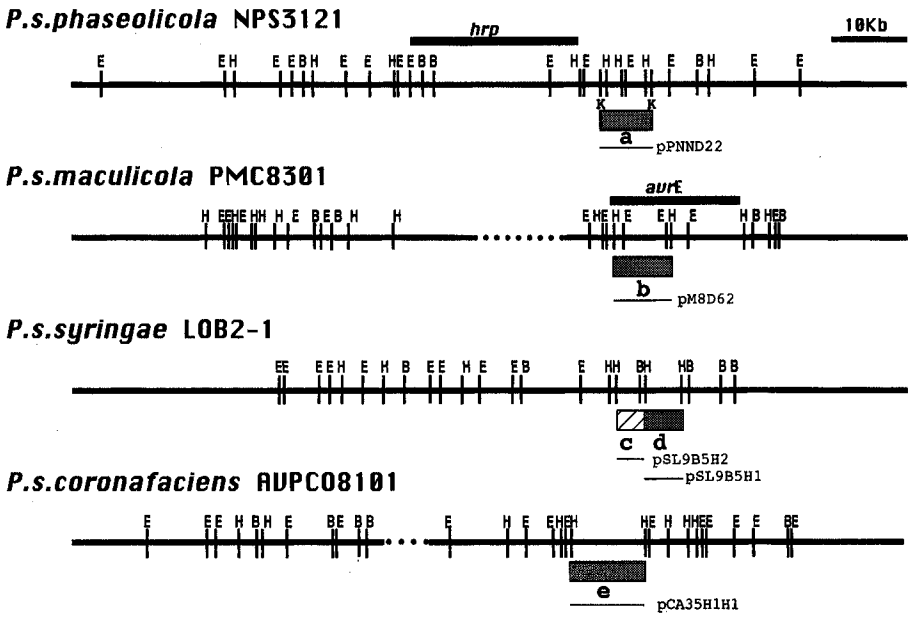

Fig. 1. Restriction map of *Pseudomonas syringae* pathovars. Filled patterns indicate hybridization probes (a-e). Lines and numbers under each probe indicate the position and the name of corresponding subclones. Dotted line is represents a gap. Vertical lines and letters on the lines indicate restriction sites : B, *Bam*HI ; E, *Eco*RI ; H, *Hin*dIII ; K, *Kpn*I.

buffer 1 (2×SSC, 0.1% SDS) at room temperature for 5 min and then in wash buffer 2 (0.5×SSC, 0.1% SDS) at 68°C for 15 min before immunological detection. Strength of the hybridization signal was ranked and scored at four levels as described before[6].

Probes corresponding to the *hrp* region hybridized to all *P. syringae* strains with relatively homogeneous strength (data not shown). However, patterns of hybridization signal strength obtained with probes of the *hrpZ* and *avrE* regions distinguished at least three types of strains in group U (Table 1). Probe c and d (corresponding to *avrE* and its neighbors) from Pss (Fig. 1) produced strong signals against strains of pvs. *syringae, aceris, aptata, japonica, lapsa* and *pisi*, but moderate or weak signals to the other pathovars. Probe e from Psc detected strong signals against the strains of pvs. *coronafaciens, atropurpurea, oryzae, porri, striafaciens* and an unnamed pathovar isolated from onion and moderate or weak signals to other pathovars. We named the two groups group III and group IV, respectively. *P. s.* pv. *morsprunorum* and *P. s.* pv. *maculicola* strain R1 were not classified into any of the four groups. Thus, they remained in group U.

Previous papers demonstrated the phenotypical similarity or high DNA homology values between pv. *syringae* and pv. *aptata*[5,9-12], pv. *pisi*[5,12], pv. *japonica*[15] or pv. *aceris*[14]. On the other hand, Schaad *et al.* reported the phenotypic similarity of pv. *coronafaciens*, pv. *atropurpurea* and pv. *striafaciens*, proposing that they be united into a single species as *P. coronafaciens*[13]. Our groups III and IV clearly correspond to these two previously reported groups in *P. syringae*, indicating that the distinction of *P. syringae* strains by hybridization with the probes of *hrp* genes and its neighboring regions in our studies reflects the profound diversity of the species. While preparing this paper, Gardan *et al.*[3] reported the comprehensive grouping of *P. syringae* pathovars through total DNA homology experiments. Their results almost perfectly coincide with ours (Table 1). Using a small portion of genomic DNA as probes, we demonstrated significant diversity that will lead to the easy and rapid identification of *P. syringae* pathovars.

LITERATURE CITED

1. Bogdanove, A.J., Kim, J.F., Wei, Z., Kolchinsky, P., Charkowski, A.O., Conlin, A.K., Collmer, A. and Beer, S.V. (1998). Homology and functional similarity of an *hrp*-linked pathogenicity locus, *dspEF*, of *Erwinia amylovora* and the avirulence locus *avrE* of *Pseudomonas syringae* pathovar *tomato*. Proc. Natl. Acad. Sci. USA 95 : 1325-1330.
2. Bonas, U. (1996). *hrp* genes of phytopathogenic bacteria. *In* Bacterial Pathogenesis of Plants and Animals (Dangl, J.L., ed.). pp. 79-98, Springer-Verlag, New York.
3. Gardan, L., Shafik, H., Belouin, S., Broch, R., Grimont, F. and Grimont, P.A.D. (1999). DNA relatedness among the pathovars of *Pseudomonas syringae* and description of *Pseudomonas tremae* sp. nov. and

Table 1. Summary of hybridization results

Grouping	Species or pathovar	a[c]	b	c	d	e	f[d]
Group I	*P. s. phaseolicola*[a]	+++	++	+	++	+	2
	P. s. glycinea	+++	++	+	++	+	2
	P. s. tabaci	+++	++	+	++	+	2
	P. s. lachrymans	+++	++	+	++	+	2
	P. s. mori	+++	++	+	++	+	2
	P. s. dendropanacis	+++	++	+	++	+	2
	P. s. eriobotryae	+++	++	+	++	+	2
	P. s. myricae	+++	++	+	++	+	2
	P. s. photiniae	+++	++	+	++	+	2
	P. s. sesami	+++	++	+	++	++	2
	P. s. ?(kiwi)	+++	++	+	++	++	
	P. ficuserectae	+++	++	+	++	+	2
Group II	*P. s. maculicola*[b]	++	+++	+	++	++	3
	P. s. tomato	++	+++	+	++	++	3
	P. s. theae	++	+++	+	++	++	8
	P. s. delphinii	++	+++	+	++	++	3
	P. s. magnoliae	++	+++	+	++	++	
	P. s. actinidiae	++	+++	+	++	++	
	P. s. spinaciae	++	+++	+	++	++	
Group III	*P. s. syringae*	++	++	+++	+++	+	1
	P. s. lapsa	++	++	+++	+++	++	1
	P. s. japonica	++	++	+++	+++	+	1
	P. s. aptata	++	++	+++	+++	++	1
	P. s. aceris	++	++	+++	+++	+	1
	P. s. pisi	++	++	+++	+++	+	1
Group IV	*P. s. coronafaciens*	++	++	+	++	+++	4
	P. s. striafaciens	++	++	+	++	+++	4
	P. s. ?(onion)	++	++	+	++	+++	
	P. s. atropurprea	++	++	+	++	+++	4
	P. s. oryzae	++	++	+	++	+++	4
	P. s. porri	++	++	+	++	+++	4
Group U	*P. s. morsprunorum*	++	++	+	++	+	2
	P. s. maculicola (R1)	++	++	+	++	+	
Pseudomonas spp.	*P. viridiflava*	−	−	−	−	+	6
	P. cichorii	−	−	−	−	+	
	P. corrugata	−	−	−	−	−	
	P. fluorescens	−	−	−	−	−	
	P. aeruginosa	−	−	−	−	−	
	E. coli	−	−	−	−	−	

+++, Strong hybridization signal always detected; ++, Hybridization signal detected but not always at 0.5 μg; +, Weak hybridization signal detected but not always at 5 μg and was not detected at 0.5 μg; −, No hybridization signal.

a) Containing two strains.

b) Containing three strains.

c) a to e indicate the probes shown in Fig. 1.

d) Lane f shows genomospecies assigned by Gardan *et al.*[3].

Pseudomonas cannabina sp. nov. (ex Sutic and Dowson 1959). Int. J. Syst. Bacteriol. 49 : 469-478.

4. Hacker, J., Blum-Oehler, G., Muhldorfer, I. and Tschape, H. (1997). Pathogenicity islands of virulent bacteria : structure, function and impact on microbial evolution. Mol. Microbiol. 23 : 1089-1097.
5. Hildebrand, D.C., Schroth, M.N. and Huisman, O.C. (1982). The DNA homology matrix and non-random variation concepts as the basis for the taxonomic treatment of plant pathogenic and other bacteria. Annu. Rev. Phytopathol. 20 : 235-256.
6. Inoue, Y. and Takikawa, Y. (1999). Grouping *Pseudomonas syringae* strains by comparing DNA homology at the *hrp* gene cluster and its neighboring regions. Ann. Phytopathol. Soc. Jpn. 65 : 32-41.
7. Inoue, Y. and Takikawa, Y. (1999). Investigation of repeating sequences in *hrpL* neighboring region of *Pseudomonas syringae* strains. Ann. Phytopathol. Soc. Jpn. 65 : 100-109.
8. Mansfield, J., Jenner, C., Hockenhull, R., Bennett, M.A. and Stewart, R. (1995). Characterization of *avrPphE*, a gene for cultivar-specific avirulence from *Pseudomonas syringae* pv. *phaseolicola* which is physically linked to *hrpY*, a new *hrp* gene identified in the halo-blight bacterium. Mol. Plant-Microbe Interact. 7 : 726-739.
9. Maraite, H. and Weyns, J. (1997). *Pseudomonas syringae* pv. *aptata* and pv. *atrofaciens*, specific pathovars or members of pv. *syringae*? *In Pseudomonas syringae* Pathovars and Related Pathogens (Rudolph, K., Burr, T. J., Mansfield, J.W., Stead, D., Vivian, A. and von Kietzell, J., eds.). pp. 515-520, Kluwer Academic Publishers, Dordrecht.
10. Otta, J.D. and English, H. (1971). Serology and pathogenicity of *Pseudomonas syringae*. Phytopathology 61 : 443-452.
11. Pecknold, P.C. and Grogan, R.G. (1973). Deoxyribonucleic acid homology groups among phytopathogenic *Pseudomonas* species. Int. J. Syst. Bacteriol. 23 : 111-121.
12. Sands, D.C., Schroth, M.N. and Hildebrand, D.C. (1970). Taxonomy of phytopathogenic pseudomonads. J. Bacteriol. 101 : 9-23.
13. Schaad, N.W. and Cunfer, B.M. (1979). Synonymy of *Pseudomonas coronafaciens, Pseudomonas coronafaciens* pathovar *zeae, Pseudomonas coronafaciens* subsp. *atropurpurea*, and *Pseudomonas striafaciens*. Int. J. Syst. Bacteriol. 29 : 213-221.
14. Takikawa, Y., Tsuyumu, S. and Goto, M. (1991). Occurrence of bacterial leaf spot of maple incited by *Pseudomonas syringae* pv. *aceris* in Japan. Ann. Phytopathol. Soc. Jpn. 57 : 724-728.
15. Young, J.M. (1992). *Pseudomonas syringae* pv. *japonica* (Mukoo 1955) Dye *et al.* 1980 is a junior synonym of Ps. *syringae* pv. *syringae* van Hall 1902. Lett. Appl. Microbiol. 15 : 129-130.