Description of the Bacterium Causing Blight of Leek as *Pseudomonas syringae* pv. *porri* (pv. nov.)

R. Samson, H. Shafik, A. Benjama, and L. Gardan

First, second, and fourth authors: Institut National de la Recherche Agronomique, Centre d'Angers, Pathologie végétale et phytobactériologie, BP 57, 49071 Beaucouzé cedex, France; and third author: Institut National de la Recherche Agronomique, Station régionale de phytiairie, BP 530, Marrakech, Morocco.
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ABSTRACT

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Forty bacterial strains isolated from leek blight (*Allium porrum*) in France and other countries were studied by conventional biochemical methods, serological reactions, numerical taxonomy, DNA-DNA hybridization, and ice nucleation activity, as well as by pathogenicity on leek and other host plants. They were compared with reference strains of *Pseudomonas*, mainly pathotype strains of *P. syringae* pathovars and strains of *P. syringae* pv. *syringae* isolated from various host plants including onions. Leek strains sorted with *P. syringae* species (sensu lato) by LOPAT

tests (production of levan-sucrase, oxidase, pectinase, arginine dihydrolase, and hypersensitive reaction on tobacco). Leek strains were pathogenic to leek and produced symptoms identical to those observed in the field. They were the only strains in our study that could cause blight of leek. Thus, our results justify the creation of a new pathovar. Leek strains constituted a highly homogeneous DNA group and a discrete phenon by numerical taxonomy, and they belonged to O-serogroup POR. The name of *P. syringae* pv. *porri* is proposed for the bacterium causing leek blight. Criteria for routine identification are presented and taxonomic status is discussed.

Additional keywords: serology.

Bacterial blight of leek (*Allium porrum*) is a disease of both seedlings and seed crops and is reported in leek-production areas in Great Britain (17), France (24), New Zealand (13), the Netherlands (15), Italy (29), and very recently, the United States (16).

Leek bacterial blight appears as a foliar disease showing watery yellow lesions on young leaves leading to their typical curling and water-soaked, deep-green areas on flowering stems. The causal organism was first designated as *Pseudomonas* sp. by Lelliott (17) and then as *P. syringae* by Hale (13) after a brief description.

Preliminary studies concerned only the geographic distribution of leek blight in France, symptomatology, and the life cycle of the pathogen (24), as well as some identifying characteristics of the bacterium (23). A complete description of the pathogen was done to determine whether leek blight is due to an opportunistic and ubiquitous *P. syringae* strain or to a new pathovar. A pathovar (6) is an infrasubspecific term referring to a group of phytopathogenic bacteria principally on the basis of their host range. The purpose of this paper is to describe the leek blight pathogen using host range, numerical taxonomy, and DNA-DNA hybridization.

MATERIALS AND METHODS

Bacterial strains. From our and other laboratory collections, 40 leek (*A. porrum*) strains were selected as representative of the known geographical diversity of the leek blight agent (Table 1).

Leek strains were compared with 58 strains (including type strains and pathotype strains, designated by a superscript T after the collection number) of *P. amygdali*, *P. cichorii*, *P. ficuserectae*, *P. savastanoi*, *P. syringae*, and *P. viridiflava* obtained from the Collection Française de Bactéries Phytopathogènes (CFBP; Beau-

couzé, France). The type strain of each given species and pathovar (Fig. 1) was chosen according to published lists (32,33,34). Additional strains were added to the sets of official reference strains on the basis of pathogenicity tests and serological reactions: *P. syringae* pv. *berberidis*, CFBP 3206 (=ICMP [International Collection of Microorganisms from Plants, Auckland, New Zealand] 4116); *P. syringae* pv. *lachrymans*, CFBP 1644 (=NCPPB [National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom] 1096, *Cucumis sativus*, Hungary, 1957); *P. syringae* pv. *morsprunorum*, CFBP 2116 (*Prunus cerasus*, France, 1974); *P. syringae* pv. *persicae*, CFBP 1316 (*Prunus persica*, France, 1970); *P. syringae* pv. *phaseolicola*, CFBP 1882 (*Phaseolus vulgaris*, France, 1978); *P. syringae* pv. *striafaciens*, CFBP 1686 (=NCPPB 2480, *Avena sativa*, Zimbabwe, 1971); *P. syringae* pv. *syringae*, CFBP 1685 (=NCPPB 2264, *Zea mays*, Yugoslavia, 1965), CFBP 1764 (=ICMP 3189, *Phaseolus vulgaris*, New Zealand, 1971), CFBP 1767 (=NCPPB 2844, *Triticum aestivum*, United States, 1972), CFBP 1771 (=NCPPB 2691, *Salix* sp., New Zealand, 1968), and four strains isolated from *A. cepa*, CFBP 1787 (=NCPPB 2737), CFBP 2336, CFBP 2337, and CFBP 2338 received from Japan (M. Goto's 'a', '10', and '12', respectively); and *P. syringae* pv. *tomato*, CFBP 2545 (*Lycopersicon esculentum*, France, 1979) and CFBP 2546 (*Lycopersicon esculentum*, France, 1980).

Morphological, staining, and physiological testings. Colony morphology was described on yeast peptone dextrose agar (YPDA) (yeast extract, 3 g/liter; Biogelytone peptone [BioMérieux, Marcy-l'Etoile, France], 5 g/liter; dextrose, 5 g/liter; and agar, 1.5 g/liter). Gram reaction was performed with 3% KOH solution. Motility was determined by direct microscopic examination in broth. Growth was determined at 4 and 41°C.

For conventional physiological characteristics (19,27), bacteria were grown at 25°C and reactions were read after 4 days unless otherwise stated. Utilization of carbon sources was tested on ARJ mineral basal medium (1) (NH₄H₂PO₄, 1 g/liter; KCl, 0.2 g/liter; MgSO₄·7H₂O, 0.2 g/liter; agar, 3 g/liter, pH 6.8; and bromothymol

Corresponding author: R. Samson; E-mail address: samson@angers.inra.fr

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blue, 0.08 g/liter). Changes in the pH indicator were recorded daily for 2 weeks. Every test was replicated at least three times, with each strain using different batches of media and inocula.

Assimilation of 147 carbon sources (49 carbohydrates, 49 organic acids, and 49 amino acids) was studied with API-50 CH, 50 AO, and 50 AA galleries (BioMérieux), which were incubated at 25°C. Growth was recorded after 6 days (8).

Numerical taxonomy. A total of 167 characteristics (147 carbon sources and 20 conventional biochemical tests: arginine dihydrolase; oxidase; gelatin; nitrate reduction; levan; fluorescence; hypersensitive reaction [HR] on tobacco; esculin; pectinolysis; Tween esterase; DNase; polypectate hydrolysis at pH 5 and 8; and utilization of sucrose, lactate, L-(+)-tartrate, D-(-)-tartrate, erythritol, mannitol, and sorbitol in ARJ medium) were included in a data bank. A distance matrix was calculated using the Jaccard coefficient (28). Cluster analysis was done using the unweighted pair group method with averages.

The amount of information contributed by each criterion was measured by calculating the diagnostic ability coefficient (DAC). DAC is based on Baye's theorem using the occurrence of various diagnoses and on the information theory. It measures the quantity of information provided by each test (5). All calculations were done with the original TAXONUM program produced by G. Hunault (Faculté des Sciences, Angers, France) and L. Gardan.

Serological methods. Three antisera (S125, S135, and S136) were prepared by immunizing rabbits with bacterial whole-cell suspensions (26). Ouchterlony double diffusion was chosen because of its qualitative property (25) using concentrated bacterial suspensions (10^{10} CFU/ml) grown in King's medium B for 2 days. For flagella serotyping, six antisera were used as described previously (12), namely antisera S223-2, S184-1, and S115-2 for the H1 serotype and S32, S183-2, and S191-2 for the H2 serotype. The position and number of flagella were also determined.

Pathogenicity tests. Leek strains, along with some other pathovar reference strains of *P. syringae*, were inoculated to a selected plant range including different *Allium* species and the pathovar differential hosts: tomato, bush bean, peach, pear, pea, cucumber, and lilac.

Leeks (*A. porrum*) cultivars Platina (Caillard, Les-Pouts-de-Cé, France) and Electra (Clause, Bretigny-sur-Orge, France) were grown in pots in greenhouse (temperatures 12 to 18°C, no supplemental light regime) and field conditions. Plants were inoculated by spraying a bacterial suspension (10^8 CFU/ml) on young growing plants (three-leaf stage) and by infiltration with a syringe of three concentrations (10^2 , 10^5 , and 10^8 CFU/ml) into the flower stem (five replicates). The following *Allium* species (vegetable or ornamental plants) were also inoculated: *A. ampeloprasum*, *A. fistulosum* (Welsh onion), and *A. viviparum* by stem infiltration; *A. cepa* (onion) and *A. cepa* var. *aggregatum* (shallot) by leaf spray and flower stem infiltration; *A. sativum* (garlic) and *A. schoenoprasum* by leaf infiltration; and *A. giganteum* and *A. moly* by leaf and stem infiltration.

Other known hosts of *P. syringae* pathovars were inoculated by the more convenient of two possible methods, plant organ infiltration and gentle spray: *Lycopersicon esculentum* (tomato cv. Montfavet 63-5) by spraying young leaves; *Phaseolus vulgaris* (French bean cv. Coco Nain Blanc) by leaf and pod infiltration; *Prunus persica* (peach tree seedlings cv. GF 305) by leaf and twig infiltration; *Pyrus communis* (pear tree seedlings cv. Fieudiere) by leaf infiltration; *Pisum sativum* (pea cv. Kelvedon Wonder) and *Cucumis sativus* (cucumber cv. Vert Long Maraicher) by spraying young plants; and *Syringa vulgaris* (Madame Lemoine clone) by leaf infiltration and leaf central vein injury. Negative controls were inoculated with sterile water. As positive controls, host plants were inoculated with homologous pathovars such as pv. *tomato* (CFBP 2546), pv. *phaseolicola* (CFBP 1882), pv. *persicae* (CFBP 1316), pv. *syringae* (strains from lilac, bean, peach, pear, and onion), pv. *pisi*^T, and pv. *lachrymans* (CFBP 1644).

Ice nucleation activity (INA). After 24 h of growth on King's medium B at 16°C, bacterial cells were suspended in 5 ml of distilled water and adjusted to approximately 10^9 CFU/ml. Suspensions were then diluted 10-fold in distilled water with five replicates. Dilutions were kept 1 h at 5°C before being placed in a refrigerated bath. Ice nucleating activity was tested by immersing the suspension tubes for 5 min at temperatures from -3 to -9°C (14,21).

DNA-DNA hybridizations. Bacterial DNA was extracted and purified according to Brenner et al. (2). Native DNA was labeled in vitro by nicktranslation with ³H-labeled nucleotides (Amersham International, Amersham, United Kingdom). The hybridization experiment used the S1 nuclease procedure described by Crosa et al. (3). Sheared, labeled, single-stranded test DNA (from the leek pathogen CFBP 1908) and sheared, unlabeled, single-stranded DNA in a 1:1,500 ratio were mixed in 0.42 M NaCl and incubated at 70°C for 16 h. Single-stranded DNA fragments could be specifically hydrolyzed by S1 endonuclease. The reassociated DNA could be separated from nucleotides (hydrolysis products) by precipitation with cold trichloroacetic acid and collection of precipitate by cellulose nitrate membranes (0.45 µm). The binding ratio was obtained by dividing the S1-treated sample by the untreated sample. The experiments were repeated twice.

TABLE 1. Origin of the bacteria isolated from leek (*Allium porrum*)

Strain CFBP ^a number	Country (area)	Isolator	Year of isolation
752*	France (Lot et Garonne)	J. P. Prunier	1964
1687* =NCPBP ^b 268	United Kingdom	W. J. Dowson	1949
1770* =ICMP ^c 3644	New Zealand	C. N. Hale	1973
1907*	France (Loire atlantique)	R. Samson	1978
1908*	France (Loire atlantique)	R. Samson	1978
1909*	France (Loire atlantique)	R. Samson	1978
1910	France (Indre et Loire)	R. Samson	1978
1911*	France (Haute Garonne)	R. Samson	1975
1912*	France (Loire et Cher)	R. Samson	1979
1913*	France (Maine et Loire)	R. Samson	1979
1956	France (Vienne)	B. Rat	1975
2360*	France (Gers)	R. Samson	1980
2361*	France (Aude)	R. Samson	1980
2362*	France (Pyrénées atlantiques)	R. Samson	1980
2363*	France (Manche)	R. Samson	1980
2364*	France (Ille et Vilaine)	R. Samson	1981
2365*	France (Dordogne)	R. Samson	1981
2366*	France (Nord)	R. Samson	1981
2367*	France (Lot et Garonne)	R. Samson	1982
2368*	France (Tarn)	J. F. Chauveau	1984
2369*	France (Cher)	R. Samson	1984
2395* =P1	Italy	L. Varvaro	1983
2396* =P6	Italy	L. Varvaro	1983
2397	France	J. F. Chauveau	1984
2451	France	J. F. Chauveau	1984
2474* =PD ^d 341	The Netherlands	J. Janse	1981
2475* =PD 347	The Netherlands	J. Janse	1981
2476* =PD 354	The Netherlands	J. Janse	1981
2505* =PD 415	The Netherlands	J. Janse	1982
2506* =PD 371	The Netherlands	J. Janse	1982
4235*	France (Pyrénées atlantiques)	R. Samson	1980
SE29-1	France (Lot et Garonne)	R. Samson	1985
SE80-2	France (Deux Sèvres)	R. Samson	1985
4236* =82-12-15	United States (California)	R. Campbell	1988
4237	France	M. Guenard	1989
4238	France	M. Guenard	1989
4239 =B1	Spain	C. Noval	1993
4240 =B2	Spain	C. Noval	1993
4241	Canada (Quebec)	R. Samson	1993
4242	France (Ain)	R. Samson	1994

^a Collection Francaise de Bacteries Phytopathogenes, Angers, France. * = Strains included in the numerical taxonomic study.

^b National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom.

^c International Collection of Microorganisms from Plants, Auckland, New Zealand.

^d Plantenziektenkundige Dienst, Wageningen, the Netherlands.

RESULTS

Genus and species identification of the leek strains. Leek strains were Gram-negative rods, motile by two flagella of the serotype H1. On YPDA plates, the colonies appeared creamy and semi-translucent and had undulate margins and a diameter of 1.2 to 1.5 mm within 3 days. Cultures produced a brown diffusible pigment on YPDA agar. Their growth was slower on King's medium B, where

they formed small star-shaped colonies after 3 days. All but one strain produced a green fluorescent pigment on King's medium B. They were obligate aerobes and oxidized glucose. Growth factors were not required, since they were able to grow on minimal medium with glucose. Growth occurred at 4°C, but not at 41°C. They were oxidase negative, arginine dihydrolase negative, and gave a typical HR on tobacco leaves. These characteristics lead to the assignment of this bacterium to the species *P. syringae* sensu lato (20).

Fig. 1. Dendrogram of the distances among 28 leek strains and 58 strains of *Pseudomonas syringae* pathovars and related bacteria tested for 167 physiological characteristics. Phenon 1: *P. syringae* pvs. *coronafaciens* 2216^T, *garcae* 1634^T, and *striaefaciens* 1674^T and 1686; Phenon 2: *P. syringae* pvs. *delphinii* 2215^T, *hibisci* 2895^T, *morsprunorum* 2116, and *passiflorae* 2346^T; Phenon 3: *P. syringae* pvs. *aceris* 2339^T, *apii* 2103^T, *aptata* 1617^T, *atrofaciens* 2213^T, *dysoxyli* 2356^T, *japonica* 2896^T, *maculicola* 1657^T, *mellea* 2344^T, *panici* 2345^T, *papulans* 1754^T, *pisi* 2105^T, *primulae* 1660^T, *ribicola* 2348^T, *tabaci* 2106^T, and *tomato* 2212^T; Phenon 4: *P. syringae* pvs. *aesculi* 2894^T, *myricae* 2897^T, *photinae* 2899^T, *tagetis* 1694^T, and *theae* 2353^T; Phenon 5: 28 leek strains (pv. *porri*); and Phenon 6: *P. syringae* pvs. *anthirrhini* 1620^T, *berberidis* 1727^T and 3206, and *philadelphia* 2898^T.

Pathogenicity. Typical symptoms of greasy blight were reproduced by leek strains when inoculated on leeks. Greasy stripes were produced when bacteria were either injected into leaves or sprayed on young plants in a nursery. Deep-green lesions on the flowering stems were easily produced by injecting bacteria with a syringe. Flowering stem inoculation was chosen to compare virulence of the strains (Fig. 2). For the most aggressive strain, an inoculum concentration of 10^2 cells per ml was sufficient to induce the disease (Table 2).

Leek strains were only pathogenic to leek, since no symptoms appeared when they were inoculated to the five other *Allium* species or to the seven other plant species listed in Materials and Methods. In comparison, typical symptoms were obtained with reference pathovars on their homologous hosts: *P. syringae* pv. *phaseolicola* (CFBP 1882) on bean, pv. *tomato* (CFBP 2546) on tomato, pv. *pisi*^T on pea, and pv. *lachrymans* (CFBP 1644) on cucumber. Suspensions containing 10^8 CFU of leek strains per ml induced HRs on the *Allium* species other than *A. porrum*, as well as on bush bean, peach, and pear leaves. When inoculated to leek, none of the aforementioned pathovars of *P. syringae* tested (pv. *phaseolicola*, *tomato*, *pisi*, *lachrymans*, and *syringae* isolated from bean, peach, pear, and onion, respectively) caused a typical watery blight.

Finally, out of 50 strains of other pathovars inoculated to leek, 32 strains induced a HR, 16 strains gave no reaction, and two strains induced slight hyperplasia (Table 3). The HR responses on leek quickly became sunken and white, in contrast with the compatible reaction that gave water-soaked leaf stripes or stem spots (Fig. 2), which developed slowly and enlarged with time.

Fig. 2. Symptoms on leek flower stem. Left: Hypersensitive reaction induced by each of 25 pathovars of *Pseudomonas syringae*. Right: Typical water-soaked lesions induced by *Pseudomonas syringae* pv. *porri*. Leek flower stems were inoculated by injecting a suspension of 10^8 bacteria per ml under the epidermis with a syringe.

DNA relatedness. The nine leek strains tested were 81 to 100% related to leek reference strain CFBP 1908 (Table 4). The reference strains of *P. syringae* pv. *syringae* (CFBP 1392^T), *P. syringae* pv. *tomato* (CFBP 2212^T), and *P. savastanoi* (CFBP 1670^T) were 45, 53, and 48% related to CFBP 1908, respectively. Thus, leek strains constituted a highly homogeneous DNA group, distinct from *P. syringae* and *P. savastanoi*.

Numerical analysis. The dendrogram displaying the distance relationships among the 86 strains studied is shown in Figure 1. At a distance of 0.23, the 28 strains studied (Table 1) and isolated from leek were clustered in a same phenon (phenon 5). A phenon corresponds to a group of strains exhibiting common phenotypical characteristics. Five additional phenons and 26 isolated phenotypes were observed (Table 1). Thus, leek strains constituted a discrete phenon. Based on their DAC, nine carbon sources delineated phenon 5 (Table 5).

INA. The ice nucleation ability was tested during a survey on the occurrence of the disease in Loire Valley, France, and was performed on 78 leek isolates. None of the strains was INA positive at -2°C , but 64 strains (82%) were INA positive at -5°C .

Serological reaction. Ouchterlony double diffusion gave, in 24 h, one thick and rather diffused band, typical of O-antigen recognition. All leek strains (except CFBP 4235) reacted with the three antisera and belonged to O-serogroup POR (26). The exception was strain 4235, which belonged to O-serogroup RIB, which contains rough type cultures. When strain 4235 was inoculated on leek, it gave typical watery blight. Bacteria reisolated from the lesions still typed as serogroup RIB.

The majority of the *P. syringae* pathovars gave no serological reaction at all. However, three groups of strains showed cross-reactions with leek strains (Table 6). A group including *P. syringae* pv. *coronafaciens*^T and some strains of *P. syringae* pv. *syringae* (1685, 1767, and 1771) revealed partial identity with leek strains as shown by a spur. This group belonged to O-serogroup SYR2. A second group composed of *P. syringae* pv. *atropurpurea*^T, *P. syringae* pv. *cannabina*^T, *P. syringae* pv. *garcae*^T, *P. syringae* pv. *papulans*^T, *P. syringae* pv. *syringae* strains (the type strain CFBP 1392^T and CFBP 1764), and *P. syringae* pv. *tomato* CFBP 2545, belonging to PERSAVTOM2, gave partial identity distinct from the first one. Finally, *P. syringae* pv. *viburni*^T, the four onion strains (CFBP 1787, CFBP 2336, CFBP 2337, CFBP 2338) from Japan, and *P. syringae* pv. *delphinii*^T, belonging to O-serogroup DEL, formed a third group that exhibited no strong consistent reactions except for a faint spur in some tests.

Physiological profiles. Conventional biochemical characteristics were investigated to determine tests to differentiate leek strains from serologically and taxonomically related pseudomonads (Table 6). The main differential criteria for leek strains were hydrolysis of gelatin (weak and slow); no hydrolysis of casein, esculin, or pH 5 polypectate; production of melanins from tyrosine; no utilization of inositol; and utilization of homoserine. The onion strains from Japan were clearly different from leek pathogens by their inability to utilize mannose and homoserine.

TABLE 2. Infection incidence (%) at different bacterial concentrations for 19 leek strains inoculated into leek flower stems

CFBP number	Inoculum concentration ^a (bacteria per ml)		
	10^8	10^5	10^2
1687	100 ^b	100	100
1908	100	20	20
752, 1907, 1913, 2366	100	100	0
1911, 1912, 2362, 2369	100	80	0
1770, 1956, 2360	100	60	0
1910, 2361, 2363, 2365, 2367	100	20	0
2364	100	0	0

^a Injection of 20 μl of bacterial suspension with a syringe under the flower stem epidermis.

^b Percentage of water-soaked stems 13 days after inoculation (five replicates).

DISCUSSION

The leek blight strains belong to group I of fluorescent pseudomonads (18), which constitutes the *P. syringae* species sensu lato (20). Although leek strains were collected for the current study from eight different countries, they appeared to constitute a very homogeneous group. They showed a single profile of biochemical and physiological characters. All the strains were clustered in a separate phenon when the results of phenotypical tests were analyzed by numerical taxonomy. This analysis included 58 other *Pseudomonas* strains representing six species and 49 pathovars of *P. syringae*. All but one rough strain belonged to O-serogroup POR. Furthermore, leek strains induced a typical watery blight of parenchyma cells on leek leaves and flower stems, in contrast to all of the other

TABLE 3. Symptoms produced by 38 different pathovars of *Pseudomonas syringae* on leek^a

Pathovar ^b	Symptoms			
	Hypersensitive reaction	No reaction	Light hyperplasia	Water-soaking
<i>aceris</i> ^T	X			
<i>anthrini</i> ^T	X			
<i>apii</i> ^T	X			
<i>aptata</i> ^T	X			
<i>atrofaciens</i> ^T	X			
<i>atropurpurea</i> ^T	X			
<i>berberidis</i> ^T	X			
<i>cannabina</i> ^T		X		
<i>ciccaronei</i> ^T		X		
<i>coronafaciens</i> ^T	X			
<i>delphinii</i> ^T	X			
<i>eriotryae</i> ^T	X			
<i>garcae</i> ^T	X			
<i>glycinea</i> ^T	X			
<i>helianthi</i> ^T		X		
<i>lachrymans</i> ^T		X		
1644		X		
<i>lapsa</i> ^T	X			
leek strains (40 strains)				X
<i>maculicola</i> ^T	X			
<i>mellea</i> ^T	X			
<i>mori</i> ^T		X		
<i>morsprunorum</i> ^T		X		
<i>panici</i> ^T	X			
<i>papulans</i> ^T			X	
<i>passiflorae</i> ^T	X			
<i>persicae</i> 1316			X	
<i>persicae</i> ^T		X		
<i>phaseolicola</i> ^T		X		
1888		X		
<i>pisi</i> ^T	X			
<i>primulae</i> ^T	X			
<i>ribicola</i> ^T		X		
<i>savastanoi</i> ^T		X		
<i>sesami</i> ^T		X		
<i>striaefaciens</i> ^T	X			
<i>syringae</i> ^T	X			
1685 (maize)	X			
1764 (bean)	X			
1771 (willow)	X			
1787 (onion)	X			
2336 (onion)	X			
2337 (onion)	X			
2338 (onion)	X			
<i>tabaci</i> ^T	X			
<i>tagetis</i> ^T	X			
<i>tomato</i> ^T		X		
2545		X		
2546		X		
<i>ulmi</i> ^T	X			
<i>viburni</i> ^T	X			

^a Inoculations were performed by infiltration of a 10⁸-bacteria-per-ml suspension under the flower stem epidermis.

^b T = type strain of the pathovar.

Pseudomonas strains tested, which induced either a HR on leek or no reaction at all. Leek strains were only pathogenic on leek. Therefore, leek strains could constitute a new pathovar of *P. syringae*. We propose to name the new pathovar as *P. syringae* pv. *porri*, pv. nov.

The symptoms caused by leek strains are distinctive. The disease affects young tissues of actively growing plants. Owing to the anatomy of monocotyledons, foliar spots spread in longitudinal stripes and the leaves typically become sickle-shaped. After an initial deep-green water-soaked phase, lesions turn yellow to orange. On flower stems, lesions enlarge to girdle the stem. Other phytopathogenic bacteria have been described on leek. *Erwinia* sp. stopped growth of the plant and reduced seed production (30). *E. carotovora* subsp. *carotovora* and *P. fluorescens* were isolated from rotted leeks (10). The former pathogen rotted leek during summer, while the latter organism caused disease under mild temperatures and high relative humidity. In our laboratory, pectinolytic *Erwinia* sp. and *P. fluorescens* were sometimes isolated as secondary invaders from lesions initially produced by *P. syringae* pv. *porri*. In contrast with the opportunistic *Erwinia* and *P. fluorescens*, *P. syringae* pv. *porri* was preferentially pathogenic on young tissues.

During several surveys of leek-production areas, saprophytic *P. syringae*-like strains could be isolated as opportunistic bacteria accompanying leek pathogens. The saprophytes were unequivocally distinguished from *P. syringae* pv. *porri* on the basis of their positive response in gelatinase, esculin, erythritol, inositol, and lactate tests; negative response in tyrosine and homoserine tests; and failure to react with POR antiserum; as well as pathogenicity to leek. The preceding tests are advised for routine identification of pv. *porri*. In our experience, no more than one of these tests varied for a given strain.

A few of the leek strains tested had been in international collections of phytopathogenic bacteria for 20 to 45 years: CFBP 1687 by Lelliott (17) in the United Kingdom, CFBP 752 in France, and CFBP 1770 in New Zealand. These three strains were listed as *P. syringae* strains isolated from leek and were included in pv. *syringae* when the pathovar classification was created (33). One may speculate that other groups of strains isolated from specific hosts may be separated from pv. *syringae* as more knowledge accumulates about their properties, especially pathogenicity. For instance, onion strains, so far only reported in Japan, might deserve a similar status change. Onion strains differ from leek strains on the basis of some physiological characters, serotype, and pathogenicity to various *Allium* spp. Onion strains also differ greatly from pv. *syringae* strains by many biochemical criteria (protease, esculin, mannose, sorbitol, and lactate tests, which are negative). Therefore, Japanese onion strains may also be classified in a specific pathovar (11).

TABLE 4. DNA relatedness of nine leek strains and the type strains of *Pseudomonas syringae*, *P. savastanoi*, and pathotype strain of *P. syringae* pv. *tomato*

Unlabelled DNA of CFBP strains ^a	Relative reassociation with labeled DNA of the leek isolate CFBP 1908 (%)	
	Leek strains ^b	
	1908	100
	2368	98
	4235	94
	1770	91
	1687	90
	2395	88
	1911	87
	2474	85
	4236	81
<i>P. syringae</i> pv. <i>syringae</i> 1392 ^T		45
<i>P. savastanoi</i> pv. <i>savastanoi</i> 1670 ^T		48
<i>P. syringae</i> pv. <i>tomato</i> 2212 ^T		53

^a T = type strain.

^b Pathovar *porri* (leek blight strains) belongs to the genomospecies 4, which includes pvs. *atropurpurea*, *coronafaciens*, *garcae*, *oryzae*, *striaefaciens*, and *zizaniae* that were 78 to 100% related to the strain CFBP 1908 of pv. *porri* (8).

The results of DNA-DNA hybridization presented here indicate that leek strains belong to a homogeneous DNA-DNA genomic group clearly separated from the type strain of the species *P. syringae*. The genomic heterogeneity of *P. syringae* has been mentioned by Pecknold and Grogan (22), who described three DNA-

DNA homology groups inside *P. syringae*. More recently, Denny et al. (4) showed that pv. *tomato* should be considered specifically distinct from *P. syringae*, but they did not formally propose to elevate pv. *tomato* to the species level. By contrast, Gardan et al. (7) reinstated pv. *savastanoi* as the species *P. savastanoi* including

TABLE 5. Differential assimilation of carbon sources by leek strains, pathovars of *Pseudomonas syringae*, and related bacteria

Host ^a	Differential carbon sources (diagnostic ability coefficient)									
	Trigonelline (0,90)	Betaine (0,89)	Aconitate (0,84)	L-arginin (0,82)	Esculin (0,81)	Erythritol (0,77)	dl-OH-butyrate (0,66)	d-xylose (0,59)	Sorbitol (0,57)	D-alanin (0,34)
Phenon 5 (28 leek strains)	—	—	—	—	—	—	—	+	+	+
Other phenons ^b										
1 (four pathovars)	—	+	+	d	+	+	—	+	+	+
2 (four pathovars)	d ^c	+	+	d	d	+	d	+	+	+
3 (fifteen pathovars)	+	+	+	d	d	d	+	+	+	+
4 (five pathovars)	+	+	+	+	—	—	d	d	+	d
6 (four pathovars)	+	d	+	+	d	—	d	d	+	d
Isolated phenotypes										
<i>P. amygdali</i> ^T	—	—	—	—	—	—	—	—	+	—
<i>P. cichorii</i> ^T	+	+	+	+	+	—	+	+	—	+
<i>P. ficuserectae</i> ^T	—	+	—	—	—	—	—	—	—	+
<i>P. syringae</i> pv. <i>atropurpurea</i> ^T	—	+	+	+	—	+	—	+	+	+
<i>P. syringae</i> pv. <i>avellanae</i> ^T	—	+	+	+	—	—	—	—	—	+
<i>P. syringae</i> pv. <i>cannabina</i> ^T	—	+	—	+	—	—	—	—	—	—
<i>P. syringae</i> pv. <i>ciccaronei</i> ^T	+	—	+	+	—	—	—	+	—	+
<i>P. syringae</i> pv. <i>dendropanacis</i> ^T	—	—	+	—	—	—	—	+	—	—
<i>P. syringae</i> pv. <i>erobotryae</i> ^T	+	+	+	+	+	+	—	—	+	+
<i>P. syringae</i> pv. <i>glycinae</i> ^T	+	—	+	—	—	+	—	—	—	+
<i>P. syringae</i> pv. <i>helianthi</i> ^T	+	+	+	+	—	+	—	+	+	+
<i>P. syringae</i> pv. <i>lachrymans</i> 1644	+	—	+	+	+	+	+	+	+	+
<i>P. syringae</i> pv. <i>lachrymans</i> ^T	+	—	—	—	+	—	—	+	+	+
<i>P. syringae</i> pv. <i>lapsa</i> ^T	+	+	+	—	+	—	—	+	+	—
<i>P. syringae</i> pv. <i>morii</i> ^T	—	+	+	+	—	—	—	+	—	+
<i>P. syringae</i> pv. <i>morsprunorum</i> ^T	+	—	+	+	+	+	—	+	+	+
<i>P. syringae</i> pv. <i>oryzae</i> ^T	—	+	+	+	+	+	+	+	+	+
<i>P. syringae</i> pv. <i>persicae</i> ^T	—	—	+	+	—	—	+	+	+	+
<i>P. syringae</i> pv. <i>phaseolicola</i> ^T	+	+	+	+	—	—	—	—	—	+
<i>P. syringae</i> pv. <i>savastanoi</i> ^T	+	+	—	+	—	—	—	—	—	+
<i>P. syringae</i> pv. <i>sesami</i> ^T	+	+	+	—	—	+	+	+	—	—
<i>P. syringae</i> pv. <i>syringae</i> ^T	+	+	+	—	+	+	+	—	+	+
<i>P. syringae</i> pv. <i>tremae</i> ^T	—	—	—	—	—	—	—	—	—	—
<i>P. syringae</i> pv. <i>ulmi</i> ^T	—	—	—	—	—	+	—	—	—	+
<i>P. syringae</i> pv. <i>viburni</i> ^T	—	—	+	+	—	+	—	+	+	+
<i>P. viridiflava</i> ^T	+	+	+	+	+	+	+	+	+	+

^a T = type strain of the species or the pathovar.

^b Figure 2 contains the list of pathovars of the six phenons.

^c d = at least one differing strain.

TABLE 6. Conventional biochemical tests that differentiate leek strains from serologically and taxonomically related *Pseudomonas syringae* strains

Pathovar	CFBP strain	O-serogroup ^a	Tests ^b											
			GEL	CAS	ESC	TYR	PP5	ERT	MNO	INT	SBT	LCA	DTA	HSR
<i>porri</i> (40 strains)		POR	+1 ^c	—	—	+1	—	—	+	—	(+)	—	—	(+)
<i>coronafaciens</i>	2216 ^T	SYR2	—	—	+	—	+	+	+	+	+	—	—	—
<i>atropurpurea</i>	2340 ^T	SYR2	+1	—	—	+1	—	+	+	+	+	—	—	—
<i>syringae</i> (maize)	1685	SYR2	+	+	+	—	—	+	+	+	+	+	—	—
<i>syringae</i> (wheat)	1767	SYR2	+	+	+	—	—	+	+	+	+	+	—	—
<i>syringae</i> (willow)	1771	SYR2	+	+	+	+1	—	(+)	+	(+)	+	+	—	—
<i>tomato</i>	2545	PERSAVTOM2	+1	+	+1	—	+	—	+	+	—	—	+	—
<i>cannabina</i>	2341 ^T	PERSAVTOM2	—	—	—	+	+	—	+	—	—	—	—	—
<i>garcae</i>	1634 ^T	PERSAVTOM2	+	+	+	+	—	(+)	+	+	+	—	—	—
<i>papulans</i>	1754 ^T	PERSAVTOM2	+1	—	+	—	—	(+)	+	(+)	(+)	+	—	—
<i>syringae</i> (lilac)	1392 ^T	PERSAVTOM2	+	+	+	—	—	(+)	+	+	+	+	—	—
<i>syringae</i> (bean)	1764	PERSAVTOM2	+	+	+	—	—	+	+	+	+	+	—	—
<i>viburni</i>	1702 ^T	DEL	—	—	—	—	—	—	+	(+)	(+)	—	—	—
<i>syringae</i> (onion)	1787	DEL	—	—	—	+1	—	—	—	+	—	—	—	—
<i>syringae</i> (onion)	2336	DEL	—	—	—	+1	—	(+)	—	(+)	—	—	—	—
<i>syringae</i> (onion)	2337	DEL	—	—	—	+	—	—	—	(+)	—	—	—	—
<i>syringae</i> (onion)	2338	DEL	+1	—	—	+1	—	(+)	—	(+)	—	—	—	—
<i>delphinii</i>	2215 ^T	DEL	+1	+	+	+	—	+	+	+	(+)	—	—	—

^a O-serogroups according to Saunier et al. (26).

^b Hydrolysis of gelatin (GEL), casein (CAS), esculin (ESC), tyrosine (TYR), and polypectate, pH 5 (PP5); and utilization of erythritol (ERT), mannose (MNO), inositol (INT), sorbitol (SBT), lactate (LCA), D-(–)-tartrate (DTA), and homoserine (HSR).

^c + = positive for >90% strains in 8 days, +1 = light positive, (+) = positive in 9 to 15 days, and – = negative for >90% strains in 15 days.

pv. *savastanoi*, pv. *glycinea*, and pv. *phaseolicola*. Actually, four major DNA-DNA homology groups, represented by the pathotypes *syringae*, *savastanoi*, *tomato*, and *porri*, were circumscribed within 50 of the *P. syringae* pathovars (9). Unfortunately, no distinguishing physiological and phenotypical characteristics could be deduced for the genomic groups. This lack of criteria for differentiation was not in accordance with the definition of a bacterial species (31). Accordingly, the genomic groups have not yet converted into formal species. Until they can be differentiated by biochemical tests, the status of pathovars within *P. syringae* should be kept. This is the reason why we do not propose to elevate pv. *porri* to a species rank.

Description of *P. syringae* pv. *porri*, pv. nov. *P. syringae* pv. *porri* adjective from *porrum*, the Latin name of leek (*A. porrum*).

Cells are Gram-negative, motile with several polar flagella, and obligate aerobes with oxidative metabolism of glucose. Slow growth occurs on YPDA and on King's medium B, producing colonies of 1.5 and 0.5 mm, respectively, after 3 days of incubation. Bacteria produce green fluorescent pigment on King's medium B. They are oxidase negative, levan positive, nonpectinolytic, arginine dihydrolase negative, and give a HR on tobacco.

P. syringae pv. *porri* is positive for tyrosinase activity and for utilization of mannose, mannitol, and malonate; delayed positive for hydrolysis gelatin and for utilization of sorbitol and homoserine; and negative for utilization of casein, esculin (pH 5.5), polygalacturonate, erythritol, inositol, DL-lactate, D-(−)-tartrate, and L-(+)-tartrate. Differential growth on the following carbon sources separates *P. syringae* pv. *porri* from the other *P. syringae* pathovars and related bacteria: assimilation of D-xylose, sorbitol, and D-alanine; and no assimilation of trigonelline, betaine, aconitate, L-arginine, esculin, erythritol, and *dl*-hydroxybutyrate.

Bacteria are in O-serogroup POR, and the flagella belong to the serotype H1. Bacteria display moderate INA (about 80% strains are INA positive at −5°C).

Pathogenic strains belonging to pv. *porri* specifically cause water-soaked spots on leek.

Strain 1908 is designated as the holopathotype of pv. *porri*. It was deposited, along with five other strains in three international collections: CFBP 1908^T, 752, 1911, 1912, 2360, and 2366; NCPPB 3364, 3365, 3366, 3367, 3368, and 3369; and ICMP 8961, 8960, 8962, 8963, 8964, and 8965.

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