The Relationship of Host Range, Physiology, and Genotype to Virulence on Cantaloupe in *Pseudomonas syringae* from Cantaloupe Blight Epidemics in France

Cindy E. Morris, Catherine Glaux, Xavier Latour, Louis Gardan, Régine Samson, and Michel Pitrat

First, second, and third authors: INRA, Pathologie Végétale, BP 94, 84143 Montfavet, France; fourth and fifth authors: INRA, Pathologie Végétale, BP 57, 49071 Beaucouzé, France, and sixth author: INRA, Génétique et Amélioration des Fruits et Légumes, BP 94, 84143 Montfavet, France.

Current address of X. Latour: Laboratoire de Microbiologie du Froid, IUT Evreux, 55 bd. St. Germain, 27000 Evreux, France.

Accepted for publication 11 February 2000.

ABSTRACT

Morris, C. E., Glaux, C., Latour, X., Gardan, L., Samson, R., and Pitrat, M. 2000. The relationship of host range, physiology, and genotype to virulence on cantaloupe in *Pseudomonas syringae* from cantaloupe blight epidemics in France. *Phytopathology* 90:636-646.

In 1993, a bacterial blight caused important losses of cantaloupe (*Cucumis melo* var. *cantalupensis*) in southwestern France and has now been reported in all cantaloupe-growing regions of France. The causal agent of this blight is *Pseudomonas syringae*, although on a worldwide basis this bacterium has not been a major pathogen of melon for over 50 years. To identify the pathovar of the cantaloupe pathogen, we employed biochemical tests, plasmid and chromosomal profiling, and host range studies for

23 strains from cantaloupe and 47 reference strains of 14 pathovars of *P. syringae*. Numerical analysis of 119 traits, serological typing, syringomycin production, and BOX-polymerase chain reaction profiles did not allow us to differentiate among pathovars related to *P. syringae* pv. *syringae*. Host range studies of cantaloupe and references strains on 18 plant species showed that virulence to sugar beet was a common feature of strains virulent on cantaloupe, but was not common to strains avirulent on cantaloupe. Virulence to other species of plants varied among strains, but the overall extent of the host range was proportional to aggressiveness to cantaloupe. We propose that the strains attacking cantaloupe in France be considered *P. syringae* pv. *aptata* and that adequate host range testing may reveal that this pathovar is the cause of cantaloupe blight reported in other parts of the world.

France produces about 300,000 tons of cantaloupe (*Cucumis melo* L. var. *cantalupensis* Naudin) annually on about 15,000 ha situated in the southwest, central west, and southeast regions. In 1993, a severe bacterial blight led to important losses of cantaloupe in the Tarn-et-Garonne region of southwestern France. Since 1993, bacterial blight has been reported in all major cantaloupe-growing regions of France in the southwest and central west (23) and just recently in the southeast. Disease symptoms include necrotic leaf spots that may be encircled by water-soaked halos, stem and petiole cankers, and sunken lesions on fruits that may be accompanied by large, dry-rotted cavities in the flesh of the fruit. Epidemics occurring at the time of early fruit development can lead to fruit drop. In 1996 and 1997, when the severest epidemics were observed, up to 80% of the production fields in the southwest were infected and some growers reported 100% yield losses due to bacterial blight (23). Although numerous viral and fungal diseases are common in commercial production fields of cantaloupe in France, no bacteria have been previously reported to cause serious disease problems (4).

On a worldwide basis, seven species of bacteria have been reported to be pathogenic to *C. melo*. *Acidovorax avenae* subsp. *citrulli*, a pathogen of watermelon, has caused important damage to fruit of honeydew melon (*C. melo* var. *inodorus*) in Texas (13). *Erwinia tracheiphila* causes bacterial wilt, which is one of the most devastating diseases of muskmelon (*C. melo* var. *cantalupensis*), in the midwestern and eastern United States (29). This disease also occurs in Asia (29), but is uncommon in Europe (30). *Xanthomonas melonis* causes soft rots of melon fruits in produc-

tion fields in Brazil (24). *C. melo* is listed among the host plants of *X. cucurbitae* (6), but there are few reports about the importance of this bacterium on melons. *Pseudomonas viridiflava* has recently been reported to cause leaf blight of melon (variety not specified) in Greece (9). None of these bacteria have been reported to attack cantaloupe in France. *E. carotovora* subsp. *carotovora* probably occurs in most melon-growing regions where warm and humid conditions prevail. In France, this pathogen seems to be more important when plants are cultivated under plastic tunnels than in open fields (4). Several reports have suggested that *P. syringae* is also pathogenic to melons, but it is not clear which pathovars have this capacity. Blancard et al. (4) reported that *P. syringae* occasionally causes damage in the greenhouse and plastic tunnel production of cantaloupe near Almería, Spain. However, no strains are available for the determination of pathovar identity. Smith (31) attributed sunken spots on honeydew fruit to *P. syringae* pv. *lachrymans*. In that study, the pathovar was identified on the basis of biochemical tests, but not on host range. A strain of *P. syringae* isolated from *C. melo* in New Zealand was identified as *P. syringae* pv. *syringae* based on biochemical tests, DNA-DNA homology, and pathogenicity to lilac (39). That author suggests that *C. melo* is a previously unreported host for *P. syringae* pv. *syringae*. However, he did not confirm the pathogenicity of his strain on *C. melo* or report the host range of this strain.

Although the pathovar identity of the few strains of *P. syringae* that are pathogenic on melons is debatable, over the past 50 years this species of bacterium apparently has not been an important pathogen of *C. melo*. However, in a preliminary study, we have determined that *P. syringae* is the causal agent of the severe cantaloupe blight that is widespread in France (23). The objective of this work was to characterize the population of *P. syringae* attacking cantaloupe in France and determine which pathovar(s) is(are) responsible for this new disease outbreak. To identify the patho-

Corresponding author: C. E. Morris; E-mail address: morris@avignon.inra.fr

Publication no. P-2000-0417-01R

© 2000 The American Phytopathological Society

var, we employed biochemical and physiological tests, plasmid and chromosomal profiling, and extensive host range studies.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Strains of bacteria pathogenic to cantaloupe were isolated from lesions on leaves, stems, and fruits and from seeds of fruits harvested from commercial production fields. Plant tissues were washed under running tap water for a few seconds and patted dry with clean paper towels. The zone of chlorotic or green tissues at the margin of lesions was aseptically excised and placed directly on plates of King's medium B (KB) (16) containing cycloheximide (50 mg/liter) or on plates of tryptic soy agar (TSA) (1.7 g of tryptone, 0.3 g of Bacto-Soytone, 0.25 g of glucose, 0.5 g of NaCl, 0.5 g of K₂HPO₄, 15 g of agar, and 50 mg of cycloheximide per liter). Excised tissue was also macerated in sterile distilled water, and this macerate was streaked onto KB and TSA (Difco Laboratories, Detroit). Seeds collected from diseased fruits were placed directly on plates of culture media. After incubation of the plates for 2 to 4 days at 25°C, bacterial growth was streaked for single clones on KB and TSA. Pure strains of the bacteria isolated from lesions and seeds were tested for induction of a hypersensitive response in tobacco by infiltrating fully developed leaves of *Nicotiana tabacum* L. cv. Samsun plants at the 10-leaf stage with bacterial suspensions of 48-h-old cultures at approximately 1×10^8 CFU/ml (17). Strains of bacteria that induced the hypersensitive response in plants within 48 h of incubation at 25°C were further characterized for their biochemical and physiological properties and for their pathogenicity to cantaloupe and other plant species. Origins of bacterial strains isolated from and pathogenic to cantaloupe used in this study are listed in Table 1.

Strains of bacteria obtained from culture collections are listed in Table 2. All strains were cultured once on KB and then stored in 20% glycerol at -80°C and in sterile potassium phosphate buffer (6.75 g of KH₂PO₄ and 8.75 g of K₂HPO₄ per liter, pH 7) at 4°C.

Biochemical and physiological tests. Production of fluorescent pigment; presence of arginine dihydrolase, oxidase, gelatinase, levan sucrase, and Tween esterase; and reduction of nitrate and hydrolysis of esculin were tested according to Lelliott et al. (19). Pectinolytic activity was tested according to the method of Prunier and Kaiser (27) and on pectate gels at pH 5 and pH 8 (12). Utilization of DL-lactate, L-leucine, L(+)-tartrate, and D(-)-tartrate was tested in the basal medium of Stanier et al. (32) containing phenol red (0.01 g/liter). Utilization of sucrose, sorbitol, erythritol, and mannitol was tested according to Young (39). Presence of DNase was tested on DNagar (BioMérieux, La Balme-les-Grottes, France). Assimilation of an additional 99 carbon sources was tested with Biotype-100 plates (BioMérieux) that were incubated at 25°C. Growth was recorded after 4 days. Production of syringomycin was evaluated on potato dextrose agar and on syringomycin minimal medium (SRM) (10) according to the bioassay of Gross and DeVay (11), using a laboratory strain of *Geotrichum candidum* as the indicator organism. The distance between the border of the bacterial colony and the edge of the fungal lawn (zone of inhibition) was measured after 3 and 6 days of incubation.

Numerical taxonomy. Cluster analysis was performed on a database of 119 characteristics (99 carbon sources from the Biotype-100 plates, 9 additional carbon sources, 9 tests for enzyme activities, fluorescent pigment production on KB, and induction of a hypersensitive response in tobacco) as described by Gardan et al. (8). Data for reference strains not characterized in this study were taken from Gardan et al. (8). Calculations were performed with the TAXONUM program produced by G. Hunault (Faculté des Sciences, Angers, France) and L. Gardan.

Serological characterization. The serotype of cantaloupe strains was determined according to the methods used by Saunier et al. (28) to identify 23 O-serogroups of *P. syringae*. Antisera were produced in rabbits by injecting whole-cell bacteria intravenously.

Bacteria were grown in yeast peptone broth (0.3% yeast extract and 0.5% peptone) for 24 h, washed, and then resuspended at a concentration of 1×10^8 cells per ml in sterile phosphate buffer containing 0.5% formalin (28). Serological reactions were determined by Ouchterlony double-diffusion, using dense bacterial suspensions in distilled water (1×10^{10} cells per ml) and raw antisera. Lipopolysaccharide-antibody precipitate appeared as a unique band, showing total or partial identity with serological references.

Repetitive sequence-based-polymerase chain reaction (rep-PCR) fingerprinting. Characterization of genomic DNA was based on PCR using primers for the BOX element. BOX-PCR analysis was conducted for all strains isolated from cantaloupe and for reference strains from pathovars having strains that caused compatible reactions on cantaloupe. Primer sequences (obtained from Genset SA, Paris, France) were as described by Versalovic et al. (36). PCR amplifications were performed with a model 480 Thermal Cycler (Perkin-Elmer, Norwalk, CT) using the cycles described by Louws et al. (20): 1 initial cycle at 95°C for 7 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 5°C for 1 min, and extension at 65°C for 8 min; followed by a single final extension cycle at 65°C for 15 min and a final soak at 4°C. Template DNA consisted of suspensions of cells treated with 0.5 M NaOH for 15 min at 95°C. One microliter of cell suspension was added to each 24-μl aliquot of reaction mixture, which contained 50 pmol of the primer, 1.25 mM deoxynucleoside triphosphate, and 2 U of BioTaq DNA polymerase (Quantum Bioprobe, Montreuil-sous-Bois, France). Reaction mixtures were overlaid with 25 μl of sterile mineral oil before amplification. PCR products were analyzed by horizontal electrophoresis at room temperature in 11 × 14-cm gels (1.5% agarose) in Tris-acetate-EDTA buffer (TAE; 40 mM Tris, 4 mM sodium acetate, and 1 mM EDTA) at 100 V for 105 min. Gels were stained with ethidium bromide, rinsed in TAE buffer, and photographed under UV light (612 nm) with a digital camera.

Plasmid profiles. Bacterial strains were grown for 24 h at 25°C in liquid KB (without cycloheximide). The cells were pelleted by centrifugation (8,000 × g for 3 min), suspended in sterile water, and adjusted to an absorbance value at 620 nm (A₆₂₀) of 1.0. Plasmids were isolated and detected by gel electrophoresis according to the method of Kado and Liu (15) with the modifications described by Latour et al. (18).

Pathogenicity tests. The virulence of reference strains and strains isolated from cantaloupe was determined on 18 species of plants from eight families: onion (*Allium cepa* L. cv. Jaune Paille), sugar

TABLE 1. Strains of *Pseudomonas syringae* used in this study that were isolated from and pathogenic to cantaloupe

Strain	Year isolated	Organ of origin	Location collected
CC01	1995	Leaf	Tarn-et-Garonne, France
CC04	1995	Leaf	Tarn-et-Garonne, France
CC05	1995	Leaf	Tarn-et-Garonne, France
CC09	1995	Fruit	Tarn-et-Garonne, France
CC23	1996	Leaf	Agadir, Morocco
CC40	1996	Fruit	Tarn-et-Garonne, France
CC43	1996	Seed	Tarn-et-Garonne, France
CC62	1997	Leaf	Tarn-et-Garonne, France
CC66	1997	Fruit	Gers, France
CC70	1997	Leaf	Tarn-et-Garonne, France
CC73	1997	Leaf	Lot, France
CC74	1997	Leaf	Tarn-et-Garonne, France
CC84	1997	Stem	Tarn-et-Garonne, France
CC85	1997	Leaf	Gard, France
CC88	1997	Fruit	Tarn-et-Garonne, France
CC94	1997	Leaf	Tarn-et-Garonne, France
CC101	1997	Leaf	Tarn-et-Garonne, France
CC103	1997	Leaf	Gers, France
CC111	1997	Leaf	Lot, France
CC125	1997	Leaf	Lot, France
CC145	1997	Leaf	Lot-et-Garonne, France
CC159	1997	Leaf	Charente-Maritime, France
CC164	1997	Petiole	Charente-Maritime, France

beet (*Beta vulgaris* L. var. *rapa* cv. Sucrière), sunflower (*Helianthus annuus* L.), lettuce (*Lactuca sativa* L. cv. Mantila), Chinese cabbage (*Brassica pekinense* (Lour.) Rupr. cv. 106), melon (*C. melo* L. var. *cantalupensis* Naudin cv. Védraçais), cucumber (*C. sativus* L. cv. Marketer), winter wheat (*Triticum aestivum* L. cv. Soisson), oats (*Avena sativa* L.), corn (*Zea mays* L. cv. Epi d'Or), barley (*Hordeum vulgare* L.), sorghum (*Sorghum bicolor* (L.) Moench cv. Argence), soybean (*Glycine max* (L.) Merr. cv. Paoki), pinto bean (*Phaseolus vulgaris* L. cv. Pinto), pea (*Pisum sativum* L. cv. Douce Provence), tomato (*Lycopersicon esculentum* Mill. cv. Monalbo), pepper (*Capsicum annuum* L. cv. Yolo Wonder), and eggplant (*Solanum melongena* L. cv. Violette). Dicotyledonous plants were inoculated at the two-leaf stage (two trifoliate leaves for bean plants), except peas, which were inoculated at the four-leaf stage. Monocotyledonous plants were inoculated at the two- to three-leaf stage. Inoculum consisted of aqueous suspensions of 48-h-old bacterial cultures from KB plates adjusted to an A_{620} of 0.3 (approximately 1×10^8 CFU/ml). An aliquot of approximately 50 μ l of each bacterial suspension was injected into the petiole of the oldest leaf (second leaf for peas) near the junction of the petiole

and leaf blade. For each strain, three plants of each species (except cantaloupe) were inoculated in a first experiment conducted from February to June 1998 and an additional three plants were inoculated in a second experiment conducted from May to September 1998. For cantaloupe, each strain was inoculated on 11 plants planted at 11 different dates between February and September 1998 (one plant per date). Plants were incubated for 7 days in plastic chambers in the greenhouse to assure high relative humidity. The reaction of the plant was scored at 2 and 7 days after inoculation as 0 (no obvious symptoms), 1 (hypersensitive-like reaction localized at the point of inoculation), 2 (slight expansion of a necrotic zone of tissue away from the point of inoculation, necrosis and breaking of the petiole, or both), or 3 (expansion of a necrotic zone of tissue away from the point of inoculation, leading to death of the entire leaf or the entire plant). Bacteria were reisolated from selected plants to confirm that symptoms were caused by the pathogen. For all plants other than cantaloupe, the pathogenicity of the bacterial strain was defined in terms of the consistency of the response between the two inoculation dates. Strains were considered to be virulent on a given plant species if at least two of the three

TABLE 2. Reference strains of pathovars of *Pseudomonas syringae* used in this study

Pathovar or group	Strain	Host of origin	Location collected	Source
pv. <i>aceris</i>	2339 type strain	<i>Acer</i> sp.	United States	CFBP ^a
pv. <i>antirrhini</i>	1620 pathotype	<i>Antirrhinum majus</i>	United Kingdom	CFBP
	1723	<i>Antirrhinum majus</i>	Australia	CFBP
	3715	<i>Antirrhinum majus</i>	United Kingdom	CFBP
pv. <i>aptata</i>	1617 type strain	<i>Beta vulgaris</i>	United States	CFBP
	1906	<i>Beta vulgaris</i>	France	CFBP
	2280	<i>Beta vulgaris</i>	France	CFBP
	2471	<i>Beta vulgaris</i>	The Netherlands	CFBP
	2507	<i>Beta vulgaris</i>	The Netherlands	CFBP
pv. <i>atrofaciens</i>	1622	<i>Triticum aestivum</i>	Canada	CFBP
	1629	Unknown	Unknown	CFBP
	2213 type strain	<i>Triticum aestivum</i>	New Zealand	CFBP
	2256	<i>Triticum aestivum</i>	Greece	CFBP
pv. <i>cannabina</i>	2341 type strain	<i>Cannabis sativus</i>	Hungary	CFBP
pv. <i>garcae</i>	1634 type strain	<i>Coffea arabica</i>	Brazil	CFBP
pv. <i>japonica</i>	2896	<i>Hordeum vulgare</i>	Japan	CFBP
pv. <i>lachrymans</i>	70	<i>Cucumis sativus</i>	France	CFBP
	2104 type strain	<i>Cucumis sativus</i>	United States	CFBP
	5823	<i>Cucumis sativus</i>	New Zealand	ICMP ^b
	7501	<i>Cucumis melo</i>	New Zealand	ICMP
pv. <i>lapsea</i>	1731 type strain	<i>Triticum</i> sp.	Unknown	CFBP
	3322	Unknown	Unknown	CFBP
pv. <i>panici</i>	2345 type strain	<i>Panicum</i> sp.	United States	CFBP
pv. <i>papulans</i>	1748	<i>Malus sylvestris</i>	United States	CFBP
	1754 type strain	<i>Malus sylvestris</i>	Canada	CFBP
	3326	<i>Malus pumila</i>	United States	CFBP
pv. <i>pisi</i>	1436	<i>Pisum sativum</i>	France	CFBP
	2258	<i>Pisum sativum</i>	France	CFBP
pv. <i>primulae</i>	1660 type strain	<i>Primula</i> sp.	United States	CFBP
pv. <i>syringae</i>	1019	<i>Prunus domestica</i>	France	CFBP
	1392 type strain	<i>Syringa vulgaris</i>	United Kingdom	CFBP
	1583	<i>Prunus persica</i>	France	CFBP
	1608	<i>Rosa</i> sp.	France	CFBP
	1679	<i>Piper nigrum</i>	Brazil	CFBP
	1693	<i>Sorghum vulgare</i>	Yugoslavia	CFBP
	1734	<i>Oryza sativa</i>	Australia	CFBP
	1778	<i>Lycopersicon esculentum</i>	New Zealand	CFBP
	1779	<i>Citrus sinensis</i>	Greece	CFBP
	1780	<i>Cucurbita</i> sp.	United States	CFBP
	1788	<i>Cucurbita maxima</i>	New Zealand	CFBP
	1957	<i>Zea mays</i>	France	CFBP
	2336	<i>Allium cepa</i>	Japan	CFBP
	5B530	<i>Phaseolus vulgaris</i>	United States	S. S. Hirano ^c
	9B1	<i>Phaseolus vulgaris</i>	United States	S. S. Hirano
	B728a	<i>Phaseolus vulgaris</i>	United States	S. S. Hirano
	3390	<i>Cucurbita maxima</i>	New Zealand	ICMP
Not specified	Grisp 322	<i>Cucumis melo</i>	France	CFBP

^a Collection Française de Bactéries Phytopathogènes (CFBP), INRA, Pathologie végétale et Phytobactériologie, BP 57, 49071 Beaucouzé, France. L. Gardan, curator.

^b International Collection of Microorganisms from Plants (ICMP), Landcare Research New Zealand Limited, Private Bag 92 170, Mt. Albert, Auckland, New Zealand. J. Young, curator.

^c University of Wisconsin, Madison, WI.

plants inoculated showed a compatible response (disease scores of 2 or 3) at both dates. Likewise, strains were considered to induce either hypersensitive-like reactions or no apparent reaction if at least two of three plants had a score of 1 or 0, respectively, in both blocks. Strains were considered virulent on cantaloupe if at least 7 of 11 plants showed a compatible reaction.

TABLE 3. Syringomycin production by reference strains of *Pseudomonas syringae* and by strains from cantaloupe

Strain	Zone of inhibition (mm) ^a	
	PDA	SRM
Strains from cantaloupe		
CC01	13	12
CC04	6	3
CC09	6	11
CC23	8	9
CC40	0	7
CC43	9	7
CC62	0	7
CC66	0	0
CC70	1	10
CC73	0	0
CC74	5	9
CC84	0	7
CC85	4	7
CC88	0	1.5
CC94	0	1
CC101	0	2
CC103	0	8
CC111	9	7
CC125	0	7
CC145	0	5
CC159	0	0
CC164	5	11
Reference strains		
<i>P. syringae</i> pv. <i>aptata</i> 1617	4	Not determined
<i>P. syringae</i> pv. <i>aptata</i> 1906	0	8
<i>P. syringae</i> pv. <i>atrofaciens</i> 2213	11	7
<i>P. syringae</i> pv. <i>atrofaciens</i> 2256	19	20
<i>P. syringae</i> pv. <i>syringae</i> 1392	5	0
<i>P. syringae</i> pv. <i>syringae</i> 1780	0	0
<i>P. syringae</i> pv. <i>syringae</i> 1788	0	0
<i>P. syringae</i> pv. <i>syringae</i> 5B530	12	9
<i>P. syringae</i> pv. <i>syringae</i> B728a	8	1.2
<i>P. syringae</i> pv. <i>syringae</i> 9B1	0	1

^a Values represent the mean width of inhibition zones for two replicates measured at 6 days after inoculation of the indicator fungus. Inhibition was determined on potato dextrose agar (PDA) and on syringomycin minimal medium (SRM) (10).

RESULTS

Biochemical and physiological characteristics. All strains produced fluorescent pigment on KB; hydrolyzed gelatin and esculin; used DL-lactate as a carbon source; gave negative reactions for the oxidase, arginine dihydrolase, and pectolytic enzyme tests; and induced distinct hypersensitive reactions in tobacco leaves within 24 to 48 h after inoculation. Production of levan was positive for 74% of the strains. Of the 23 strains tested, five, four, and none used D(–)tartrate, L(+)tartrate, and L-leucine, respectively, as single carbon sources. All strains, except CC66, CC73, and CC159, inhibited the growth of *G. candidum* on agar medium (Table 3). Zones of inhibition ranged from 1 to 13 mm wide. Strains of *P. syringae* from cantaloupe were similar to strains of *P. syringae* pvs. *aptata*, *atrofaciens*, *japonica*, and *syringae* based on their reactions in the lactate, gelatin, esculin, pectolytic enzyme, and syringomycin tests according to the results published by Young and Triggs (40) for pathovars of *P. syringae*. However, only a few of the cantaloupe strains used D(–)tartrate, whereas 80% of the *P. syringae* pv. *aptata* strains tested by Young and Triggs (40) used this carbon source. Furthermore, none of the cantaloupe strains used L-leucine, whereas about 50 to 80% of the strains of *P. syringae* pvs. *atrofaciens*, *japonica*, and *syringae* tested by Young and Triggs (40) used this substrate.

Numerical taxonomy. The dendrogram displaying the distance relationships of 98 strains (85 reference strains and 13 cantaloupe strains) based on numerical analysis of 119 traits is shown in Figure 1. Data for 38 of the reference strains was taken from Gardan et al. (8). At a distance of 0.17 (similarity = 83%), 9 phenon and 39 unique strains were differentiated. Phenon 1 included all 13 cantaloupe strains as well as strains of *P. syringae* pvs. *aceris*, *aptata*, *atrofaciens*, *lapsa*, *panici*, *populans*, *primulae*, and *syringae*. However, strains of *P. syringae* pvs. *aptata*, *primulae*, and *syringae* were also found in other phenon. The biochemical characteristics that differentiate phenon 1 from the other main phenon identified by numerical analysis are presented in Table 4.

Serological properties. Serogroups were determined for the 23 cantaloupe strains. Eleven strains belonged to the APTPIs O-serogroup of Saunier et al. (28), nine strains were scattered into six different O-serogroups, and three strains belonged to none of the 23 O-serogroups tested (Table 5). The serogroup of a strain was not related to its geographic origin or date of isolation.

Chromosome and plasmid profiles. Plasmids were detected in only 1 of the 18 strains of *P. syringae* isolated from cantaloupe that were examined. Strain CC85 harbored one plasmid of 60 kb.

TABLE 4. Biochemical characteristics that differentiate phenon 1, containing cantaloupe strains, from phenon 2 to 9 and from strains CFBP 2280 and CFBP 2896^a

Biochemical characteristic	Phenon									Strain	
	1	2	3	4	5	6	7	8	9	CFBP 2280	CFBP 2896
Esculin	+ ^b	+	+	+	+	80	–	–	–	+	+
Betain	+	+	+	+	+	+	+	75	+	+	–
Erythritol	+	–	50	+	+	+	–	–	+	+	–
DL-lactate	+	–	50	–	50	–	–	25	–	+	–
Trigonelline	87	+	+	–	+	+	+	+	+	+	+
L(+)tartrate	–	25	50	–	–	80	–	+	+	–	–
Malonate	+	+	–	80	+	80	+	+	–	–	+
Transaconitate	+	+	+	+	+	80	–	50	+	+	+
L-histidine	87	+	+	–	+	+	+	+	+	–	+
DL-β-hydroxybutyrate	72	–	–	40	–	+	+	50	–	–	+
Caprylate	+	+	–	60	+	+	+	+	50	–	+
Caprate	84	75	–	60	+	+	+	+	50	–	+
D(–)tartrate	39	+	+	20	+	80	+	50	50	–	–
Propionate	57	75	–	60	+	60	–	–	+	–	–
Putrescine	27	–	–	–	+	+	+	–	–	–	–
L-tyrosine	–	25	–	–	+	60	+	–	–	–	–
Saccharose	+	+	+	+	–	+	+	75	50	+	+

^a The strains in each of these phenon are listed in Figure 1.

^b + = Positive for >90% of strains; – = negative for >90% of strains; and numerical values indicate % of positive strains when 11 to 89% of the strains are positive.

Fig. 1. Dendrogram of the distances among 98 strains of *Pseudomonas syringae*. The dendrogram includes 13 strains from cantaloupe described in Table 1 and 28 reference strains described in Table 2. The remaining strains are all from the Collection Française de Bactéries Phytopathogènes (cf. 8). The data for biochemical and physiological properties of these strains are presented by Gardan et al. (8).

Reference strains of *P. syringae* pvs. *cannabina*, *garcae*, *lachrymans*, and *pisi* all contained two to seven plasmids per strain having sizes of 12 to 300 kb. Reference strains of *P. syringae* pvs. *aptata*, *atrofaciens*, *japonica*, and *syringae* contained, at most, one plasmid per strain.

Genomic fingerprints, based on profiles of DNA fragments generated with BOX-PCR, were similar but not identical among cantaloupe strains. For all 23 cantaloupe strains, profiles contained common fragments of about 460, 540, 820, 1,030, 1,120, and 1,425 base pairs (bp). Profiles are shown in Figure 2A for 18 strains. The remaining five strains not shown in this figure had profiles identical to those shown in Figure 2A. The presence or absence of the 615-, 1,210-, 1,520-, and 1,620-bp fragments was the main variation observed among strains. There were no distinct fragments longer than 1,620 bp detected with any of the cantaloupe strains. For all reference strains of *P. syringae* pv. *aptata* characterized, profiles contained all of the fragments common to the cantaloupe strains and the 1,520- and 1,620-bp fragments (Fig. 2B). Furthermore, fragments longer than 1,620 bp were amplified from these strains. Reference strains of *P. syringae* pvs. *atrofaciens*, *lapsea*, and *japonica* varied in terms of the presence or absence of the fragment sizes characteristic of the *P. syringae* pv. *aptata* strains (Fig. 2B). Profiles of the reference strains of *P. syringae* pv. *syringae* were much more variable than those of the other pathovars; some contained fragment sizes not found among the other pathovars tested. Replicate BOX-PCR amplifications and gel electrophoresis were conducted at three separate dates for strains 1392, 1617, and CC94. No differences were observed in the profiles of amplified fragments among the three replicates of each of these strains.

Pathogenicity to cantaloupe and host range. Among the 23 strains of *P. syringae* isolated from cantaloupe, 10 strains consistently elicited a compatible reaction in all 11 of the cantaloupe plants that were inoculated, and 9 strains elicited a compatible reaction in 7 to 10 of the cantaloupe plants (Table 6). The remaining strains elicited reactions that were much more variable among plants, such that we could not determine if these strains were clearly virulent on cantaloupe. Among the reference strains of *P. syringae*, 3 of 5 strains of pv. *aptata*, 1 of 4 strains of pv. *atrofaciens*, and 3 of 17 strains of pv. *syringae* elicited compatible reactions in 7 to 11 of the inoculated cantaloupe plants (Table 6). Alternatively, no compatible reactions were elicited in cantaloupe by any of the strains of *P. syringae* pvs. *antirrhini*, *cannabina*, *garcae*, *lachrymans*, *papulans*, and *pisi*, or by 1 of 5 strains of pv. *aptata*, 2 of 4 strains of pv. *atrofaciens*, or 9 of 17 strains of pv. *syringae*. Among the strains that were not virulent on cantaloupe, strains of *P. syringae* pvs. *antirrhini*, *cannabina*, and *garcae* elicited mostly hypersensitive-like reactions, whereas some strains of *P. syringae* pvs. *lachrymans*, *papulans*, and *pisi* rarely elicited any apparent reaction. All other strains showed variable reactions, making it difficult to determine if they were virulent or not on cantaloupe.

The interaction of 46 strains of *P. syringae* from cantaloupe or from reference collections with 17 other plant species is presented in Table 7. The response of plants to inoculation was constant between the two inoculation dates for 498 (63%) of the total 782 host-pathogen combinations tested (Table 7). In other words, we could

clearly determine if a strain was either virulent, induced a hypersensitive-like response, or induced no apparent response for 63% of the host-pathogen combinations tested. The remaining 284 combinations resulted in reactions that varied between compatible and hypersensitive-like reactions (216 combinations) or between hypersensitive-like and no reaction (68 combinations) for the two inoculation dates. Hence, for 216 combinations, we could not determine if the bacterial strain was clearly virulent, and for 68 combinations, we could not determine if the strain was clearly avirulent on the plant tested. When compatible reactions were observed, they were similar for all host-bacterium combinations: necrotic lesions

Fig. 2. BOX-polymerase chain reaction genomic DNA profiles of *Pseudomonas syringae* strains from **A**, cantaloupe and **B**, reference strains. For cantaloupe strains, a 3.5- μ l aliquot was loaded into each lane of 1.5% agarose gels. For reference strains, a 2.5- μ l aliquot was loaded into each lane. **A**, The electrophoretic patterns show strains grouped by the region from which they were isolated. Lanes 1 to 3, Lot (group I): CC73, CC111, and CC125; lanes 4 to 13, Tarn-et-Garonne (group II): CC01, CC04, CC09, CC40, CC43, CC70, CC74, CC84, CC94, and CC101; lanes 14 and 15, Gers (group III): CC66 and CC103; lanes 16 and 17, Charente-Maritime (group IV): CC159 and CC164; and lane 18, Agadir (group V): CC23. **B**, The patterns are shown for strains grouped by *P. syringae* pathovar. Lanes 1 to 5, pv. *aptata* (group I): 1617, 1906, 2280, 2471, and 2507; lanes 6 to 9, pv. *atrofaciens* (group II): 1622, 1629, 2213, and 2256; lanes 10 to 15, pv. *syringae* (group III): 1392, 5B530, 9B1, B728a, 1957, and 3390; lane 16, pv. *japonica* (group IV): 2896; and lanes 17 and 18, pv. *lapsea* (group V): 1731 and 3322. **A and B**, The lanes labeled m show the DNA molecular size marker (1-kb ladder; Gibco BRL, Life Technologies SARL, Cergy Pointoise, France); the sizes are indicated in base pairs.

TABLE 5. Distribution of the cantaloupe strains into O-serogroups

O-serogroups ^a	Cantaloupe strains
APTPIIS	CC01, CC09, CC23, CC40, CC43, CC62, CC70, CC84, CC103, CC111, CC125
PERSAVTOM1	CC73, CC88, CC94, CC145
PERSAVTOM2	CC66, CC159
PERSAVTOM3	CC101
PHA/HEL1	CC74
MOP6	CC04
None ^b	CC05, CC85, CC164

^a According to Saunier et al. (28).

^b None of the O-serogroups of Saunier et al. (28).

TABLE 6. Reaction of cantaloupe plants to inoculation with strains of *Pseudomonas syringae*

Strain	Number of plants in each reaction class		
	No reaction (0) ^a	Hypersensitive-like (1)	Compatible (2 + 3)
Strains from cantaloupe			
CC09	0	0	11
CC40	0	0	11
CC62	0	0	11
CC70	0	0	11
CC73	0	0	11
CC84	0	0	11
CC88	0	0	11
CC94	0	0	11
CC125	0	0	11
CC164	0	0	11
CC04	0	1	10
CC145	0	1	10
CC159	0	1	10
CC74	0	2	9
CC101	0	2	9
CC01	1	2	8
CC103	0	3	8
CC111	0	3	8
CC66	0	4	7
CC23	1	6	4
CC85	0	7	4
CC43	1	6	4
CC05	0	8	3
Reference strains			
pv. <i>antirrhini</i> 1620**b	0	6	0
pv. <i>antirrhini</i> 1723**	0	6	0
pv. <i>antirrhini</i> 3715**	1	5	0
pv. <i>aptata</i> 1906	0	0	11
pv. <i>aptata</i> 1617	1	0	11
pv. <i>aptata</i> 2280	0	2	9
pv. <i>aptata</i> 2507	0	7	4
pv. <i>aptata</i> 2471	0	11	0
pv. <i>atrofaciens</i> 2213	0	2	9
pv. <i>atrofaciens</i> 2256	0	9	2
pv. <i>atrofaciens</i> 1622	0	11	0
pv. <i>atrofaciens</i> 1629**	0	6	0
pv. <i>cannabina</i> 2341	0	11	0
pv. <i>garcae</i> 1634	2	9	0
pv. <i>japonica</i> 2896	0	8	3
pv. <i>lachrymans</i> 70	0	11	0
pv. <i>lachrymans</i> 2104	0	11	0
pv. <i>lachrymans</i> 5823	10	1	0
pv. <i>lachrymans</i> 7501	10	1	0
pv. <i>lapsa</i> 1731**	0	3	3
pv. <i>lapsa</i> 3322**	0	4	2
pv. <i>papulans</i> 1748**	2	4	0
pv. <i>papulans</i> 1754**	2	4	0
pv. <i>papulans</i> 3326**	3	3	0
pv. <i>pisi</i> 1436	10	1	0
pv. <i>pisi</i> 2258	1	10	0
pv. <i>syringae</i> 9b1	0	0	11
pv. <i>syringae</i> 1392	0	1	10
pv. <i>syringae</i> 3390	0	4	7
pv. <i>syringae</i> 5B530	0	6	5
pv. <i>syringae</i> B728a	0	8	3
pv. <i>syringae</i> 1957	3	0	3
pv. <i>syringae</i> 1779**	0	4	2
pv. <i>syringae</i> 1788	0	10	1
pv. <i>syringae</i> 1583**	0	6	0
pv. <i>syringae</i> 1679**	0	6	0
pv. <i>syringae</i> 1693**	0	6	0
pv. <i>syringae</i> 1019**	1	5	0
pv. <i>syringae</i> 1608**	1	5	0
pv. <i>syringae</i> 1734**	1	5	0
pv. <i>syringae</i> 1778**	4	2	0
pv. <i>syringae</i> 2236**	4	2	0
pv. <i>syringae</i> 1780	9	2	0

^a Numbers in parentheses indicate the reaction of the plant scored at 2 and 7 days after inoculation: 0 = no obvious symptoms; 1 = hypersensitive-like reaction localized at the point of inoculation; 2 = slight expansion of a necrotic zone of tissue away from the point of inoculation, necrosis and breaking of the petiole, or both; and 3 = expansion of a necrotic zone of tissue away from the point of inoculation, leading to death of the entire leaf or the entire plant.

^b Strains marked with ** were inoculated onto six cantaloupe plants. All other strains were inoculated onto 11 cantaloupe plants.

developed at the point of inoculation leading to collapse and death of the laminar and petiole tissue adjacent to the point of inoculation. In addition, some strains caused systemic symptoms leading to necrosis of the entire leaf blade or the whole plant.

Host range varied considerably among bacterial strains, comprising up to 10 susceptible plant species per strain. No strains were virulent on oats, wheat, or Chinese cabbage (Table 7). Onion and sugar beet were the species most frequently within the host range of the bacterial strains tested. Most strains of *P. syringae* virulent on cantaloupe were also virulent on onion (14 of 19 strains from cantaloupe and 6 of 7 reference strains) and on sugar beet (14 of 19 strains from cantaloupe and 5 of 7 reference strains) (Fig. 3). Virulence on onion was observed in five strains that were not virulent on cantaloupe (CC23 and reference strains 1622, 2256, 2471, and B728a) (Table 7). However, virulence to sugar beet was limited to only those strains that were virulent on cantaloupe. Furthermore, virulence to both onion and sugar beet was observed in 11 of 19 strains of *P. syringae* from cantaloupe and in 3 of 3 strains of *P. syringae* pv. *aptata* and 1 strain of *P. syringae* pv. *syringae* (9B1) virulent on cantaloupe (Fig. 3).

The host range of strains that were not virulent on cantaloupe was much more limited than that of strains virulent on cantaloupe. Among the 20 strains that were not pathogenic to cantaloupe, 10 were virulent on only one to three other plant species and 10 were not virulent on any of the plants tested (Table 7). However, all these latter 10 strains elicited a hypersensitive response in tobacco, in some of the 17 plant species tested, or in some combination of tobacco and other plant species tested.

The extent of the host range of a given bacterial strain was correlated with the aggressiveness of the strain on cantaloupe. The strains that were most aggressive on cantaloupe attacked the greatest number of plant species, whereas the strains least aggressive on cantaloupe had the most limited host range (Fig. 4). The most aggressive strains with the broadest host range (at least nine plant species) were strains CC40, CC62, CC88, CC125, CC145, 1906, and 9B1.

DISCUSSION

The identification of strains of *P. syringae* at the pathovar level poses serious practical problems. Much effort has been invested in finding biochemical or genotypic markers for pathovars so as to avoid the need for host range tests, but most of these markers have limited utility. Biochemical tests can differentiate clusters that include several, very closely related pathovars (40). For example, *P. syringae* pvs. *aptata*, *atrofaciens*, *dysoxyl*, *japonica*, and *syringae* cannot be easily separated based on biochemical tests. Fatty acid profiles of pathovars have been studied and may give inaccurate results; only 33% of *P. syringae* pv. *atrofaciens* strains and 12% of *P. syringae* pv. *syringae* strains were correctly identified with this method (33). Identification of pathovars via serotyping has been accomplished with monoclonal (25) and polyclonal (28) antibodies. However, some pathovars have identical serological profiles. Nutrient utilization profiles, such as those generated with the Biolog system, are also unreliable for certain pathovars of *P. syringae* (14). The reliability of identification based on genomic profiles from PCR amplification of repetitive elements depends on the pathovar. For example, strains of *P. syringae* pv. *morsprunorum* have identical REP-, ERIC-, and BOX-PCR profiles (20) and strains of *P. syringae* pv. *glycinea* have very similar REP-, ERIC-, and IS50-PCR profiles (37). However, in the same studies, strains within *P. syringae* pvs. *aptata*, *atrofaciens*, *syringae*, and *tomato* did not have uniform profiles (20,37). Identification of pathovars with oligonucleotide primers for PCR amplification of specific sequences is promising (2,3,26,34), but this approach is currently limited to only a few pathovars.

The search for tests to differentiate pathovars is based on the assumption that a pathovar is a physiologically and genetically homo-

genous taxonomic unit. Given the limits of the various phenotypic and genotypic markers cited above, and the possibility of horizontal transfer of genetic factors implicated in pathogenicity (7), this assumption is probably not universally valid. For example, the work of Vauterin and Swings (35) illustrates the difficulty in reconciling the host range- and genotype-based classifications for *Xanthomonas* spp. It should also be noted that a possible complicating factor in the search for these markers is that the identification of the reference strains employed in such studies may not always be correct. The pathogenicity of strains on a standard set of host plants is rarely verified. Identifying pathovars by characterizing host range corresponds to the definition of pathovar *sensu stricto*. Furthermore, this approach yields pertinent epidemiological information. However, there are major obstacles to host range tests that may explain why they are rarely used for identification of pathovars of *P. syringae*. Probably the most important obstacle is that no standard set of host plants for differentiating pathovars of *P. syringae* has been proposed. Here, we used host plants that had the potential to differentiate among pathovars that are biochemically and

physiologically similar to *P. syringae* pv. *syringae* based on host ranges reported by Bradbury (6). A second significant obstacle is the difficulty of interpreting results. The host response may be variable among the individual plants inoculated with a given strain, which complicates the comparison of host range among strains. When cultivars are employed that are not homogenous for the genes involved in the host-pathogen interaction, this variability may be particularly important.

Based on the results of biochemical tests, serotyping, and syringomycin production assays, the strains of *P. syringae* causing cantaloupe blight in France closely resemble strains of *P. syringae* pvs. *aptata*, *atrofaciens*, *japonica*, *lapsa*, and *syringae*. In BOX-PCR profiles, the cantaloupe strains also share many bands in common with profiles of strains of these five pathovars, but they are not identical to any of them. Although these tests helped to eliminate some pathovars as possible identifications of the cantaloupe strains, none of them help us to clearly distinguish among these five pathovars.

Based on results of host range tests, we propose that the population of bacteria responsible for cantaloupe blight in France is com-

TABLE 7. The interaction of strains of *Pseudomonas syringae* with 17 plant species at two inoculation dates^a

Origin or pathovar ^b	Strain	Barley	Bean	Chinese cabbage	Corn	Cucumber	Egg-plant	Lettuce	Oats	Onion	Pea	Pepper	Sorghum	Soy-bean	Sugar beet	Sun-flower	Tomato	Wheat
Cantaloupe	CC01	HR	HR	HR	HR	V1	V1	HR	V1	V1	V1	HR	HR	HR	V1	HR	P	V1
	CC04	HR	V1	HR	V1	V1	P	P	V1	P	P	V1	V1	P	P	P	V1	V1
	CC09	P	P	HR	P	V1	P	HR	HR	V1	V1	HR	HR	P	P	V1	V1	HR
	CC23	V2	HR	HR	HR	HR	V1	HR	V2	P	V1	HR	HR	HR	V1	HR	V1	V2
	CC40	V1	P	HR	P	V1	P	P	V1	P	V1	P	P	HR	P	P	P	V2
	CC43	HR	HR	HR	HR	HR	HR	HR	V2	HR	V1	HR	HR	HR	HR	HR	V1	HR
	CC62	HR	P	V1	V1	P	V1	P	V1	P	P	P	P	V1	P	P	P	V1
	CC66	HR	HR	HR	HR	V1	V1	V1	V2	P	P	P	HR	HR	V1	V1	P	V2
	CC70	HR	P	V1	P	V1	V1	V1	V1	P	P	HR	V1	V1	P	P	V1	V1
	CC73	HR	HR	V1	V1	V1	P	P	HR	P	P	P	HR	HR	P	P	P	HR
	CC74	HR	HR	HR	P	V1	VI	HR	V1	P	V1	HR	V1	V1	P	V1	HR	V2
	CC84	HR	V1	HR	V1	V1	P	V1	V2	P	V1	P	V1	V1	P	P	V1	V2
	CC85	V2	HR	HR	HR	V2	V1	HR	V1	HR	V1	HR	HR	V1	V1	HR	HR	V1
	CC88	HR	V1	HR	P	V1	P	P	V1	V1	P	P	HR	P	P	P	P	HR
	CC94	HR	P	HR	V1	V1	V1	V1	V1	P	P	P	HR	V1	P	P	P	V2
	CC101	HR	V1	HR	HR	V1	P	V1	HR	V1	P	P	V1	V1	V1	P	V1	V2
	CC103	V1	HR	HR	V1	V1	HR	V1	V1	P	V1	V1	V1	HR	V1	V1	P	V1
	CC111	HR	HR	HR	V1	V1	V1	HR	V2	P	P	P	V1	V1	HR	P	HR	0
	CC125	P	V1	V1	P	V1	P	P	V2	P	P	P	V1	P	P	P	V1	HR
	CC145	P	P	HR	V1	HR	P	P	V1	P	P	P	V1	HR	P	P	V1	V2
	CC159	HR	HR	HR	V1	P	V1	P	V2	P	V1	P	V1	V1	P	P	P	V2
	CC164	V1	HR	HR	HR	HR	P	V1	V2	V1	HR	HR	HR	HR	P	V1	V1	V2
pv. <i>aptata</i>	1617	HR	V1	V2	HR	V1	HR	V1	V1	P	V1	HR	HR	V1	P	HR	V1	V1
	1906	HR	V1	HR	P	P	P	P	V1	P	V1	P	V1	V1	P	P	P	V1
	2280	P	HR	HR	V1	P	V1	V1	V1	P	V1	P	V1	V1	P	V1	HR	V1
	2471	HR	HR	HR	V1	HR	V1	HR	HR	P	P	HR	V1	V1	V1	HR	HR	V2
pv. <i>atrofaciens</i>	2507	HR	V1	HR	V1	HR	P	HR	V2	HR	P	HR	HR	HR	V1	HR	HR	HR
	1622	HR	HR	HR	HR	HR	V1	V2	V1	P	V1	HR	HR	HR	V1	HR	HR	V1
	2213	HR	HR	HR	V1	HR	HR	HR	V1	V1	V1	HR	V1	HR	P	V1	HR	V2
pv. <i>cannabina</i>	2256	V2	HR	V1	V1	HR	HR	HR	HR	P	P	HR	P	HR	HR	HR	V1	HR
	2341	HR	HR	HR	HR	HR	HR	HR	V2	HR	V1	HR	HR	HR	HR	HR	V1	V2
pv. <i>garcae</i>	1634	HR	HR	HR	V2	0	HR	HR	HR	V1	V1	HR	HR	HR	HR	HR	HR	V2
pv. <i>japonica</i>	2896	HR	HR	HR	HR	HR	P	V1	V1	V1	V1	V1	V1	HR	V1	HR	HR	V1
pv. <i>lachrymans</i>	70	V2	HR	HR	HR	HR	HR	HR	HR	HR	P	HR	HR	HR	HR	HR	HR	HR
	2104	HR	HR	HR	HR	HR	HR	HR	HR	HR	V1	HR	HR	HR	HR	HR	HR	0
pv. <i>pisi</i>	5823	0	V2	V2	0	0	0	V2	V2	0	0	V2	V2	V2	0	0	V2	0
	7501	0	0	V2	HR	0	0	V2	0	HR	HR	HR	V2	V2	0	V1	0	0
	1436	V2	0	0	V2	0	0	V2	0	HR	HR	HR	HR	HR	V2	V2	0	V2
	2258	V2	HR	V2	HR	HR	HR	HR	V1	V2	P	HR	HR	HR	HR	HR	HR	V2
pv. <i>syringae</i>	5B530	V2	HR	HR	V2	V1	P	HR	V2	V1	V1	P	HR	HR	V1	V1	HR	V2
	B728a	V2	HR	HR	HR	HR	V1	HR	V2	P	V1	V1	HR	HR	HR	HR	V1	V2
	9B1	HR	P	HR	HR	V1	P	P	V1	P	P	P	HR	HR	P	P	P	V1
	1392	HR	HR	HR	HR	HR	P	HR	V1	P	V1	P	HR	HR	V1	HR	V1	V2
	1780	0	0	0	0	V1	0	0	V2	0	0	0	V2	HR	V2	V2	0	0
	1788	HR	HR	HR	V1	HR	HR	HR	V2	V1	V1	HR	HR	HR	HR	HR	V1	V2
	3390	P	V1	HR	V1	V1	HR	HR	HR	P	P	HR	P	V1	V1	V1	V1	HR

^a At each date, three plants were inoculated for each strain tested. Interactions were scored based on their consistency between the two inoculation dates. P, HR, and 0 indicate that compatible reactions, hypersensitive-like reactions, and no apparent reaction, respectively, were observed in at least two of three plants at both inoculation dates. V1 indicates that compatible reactions were observed in at least two of three plants at one date, but that hypersensitive-like reactions were observed in most plants at the other date. V2 indicates that most plants showed hypersensitive-like reactions at one date, but no apparent reaction at the other date.

^b *Pseudomonas syringae* pathovars.

posed principally of strains virulent on sugar beet. All strains that were virulent on sugar beet in this study were also virulent on cantaloupe. Furthermore, 25 of the 26 strains virulent on cantaloupe elicited compatible reactions in sugar beet. For 19 of these strains, the expression of virulence on sugar beet was consistent among the two inoculation dates; for six strains, compatible reactions were induced, but not consistently, between the two inoculation dates. Virulence on cantaloupe was best correlated to that on sugar beet when compared with all other hosts tested. Although most strains that were pathogenic to cantaloupe were also virulent on onion, five strains that were not pathogenic to cantaloupe were clearly virulent on onion. Hence, we propose that the strains causing cantaloupe blight should be considered *P. syringae* pv. *aptata*.

Host ranges were highly variable among the strains characterized in this study. No strains had the same host range. Furthermore, the number of plant species attacked was not characteristic of any particular group of strains or of any pathovar. Host range tended to increase with increasing aggressiveness on cantaloupe. This result suggests why there may be confusion between the concepts of virulence and aggressiveness in phytopathology. As pointed out by Andrivon (1) in the debate published in *Phytopathology* about the definition of these terms, virulence is a qualitative trait reflecting whether a pathogenic strain can or cannot cause disease on a given host. Aggressiveness, on the other hand, designates the quantity of disease induced by a pathogenic strain on a susceptible host. In the case of the pathogenicity tests conducted here, it might be possible that strains needed to be sufficiently aggressive to induce a reaction in the plant that we could detect as a

compatible interaction. Hence, some of the hypersensitive-like reactions observed may have been weak compatible interactions. This would suggest that the host range of some of the strains studied here is larger than what we report. Furthermore, the relationship we observed between aggressiveness and virulence suggests an important linkage of the genes implicated in these processes or an intimate interaction of the gene products involved.

Our work illustrates the utility of using host range tests for the identification of strains of *P. syringae*. However, it also illustrates some of the difficulties that need to be resolved before host range testing can effectively contribute to the taxonomy and identification of *P. syringae* strains on a routine basis. For example, several reference strains used here did not incite compatible reactions on their host of origin. This may have been because the cultivars that we used were resistant or tolerant to these strains or because these strains had lost the necessary virulence factors. Secondly, several of the plant species inoculated (Chinese cabbage, oats, and wheat) never showed any consistent compatible reactions. Although it is possible that none of the strains we used were virulent on these plants, it may also suggest that these plants were either difficult to inoculate or that we were not able to recognize compatible reactions for these plants. The choice of plant species and cultivars, of virulent reference strains, and of inoculation methods adapted to each of these hosts will require considerable effort but is essential for establishing of a set of differential plant species for identifying pathovars of *P. syringae*.

This is the first report of *P. syringae* pv. *aptata* as a pathogen of *C. melo* under field conditions. The only other previous report of virulence of *P. syringae* pv. *aptata* to *C. melo* of which we are aware is based on inoculation of slices of cantaloupe fruits (38). However, in light of the problems mentioned above for the identification of pathovars, it is not unlikely that *P. syringae* pv. *aptata* is the causal agent of other bacterial blights of melon reported to be caused by diverse pathovars of *P. syringae*. In previous reports of *P. syringae* as a pathogen of melons, host range studies were either omitted or were limited to cucurbit species. For strains of *P. syringae* isolated from diseased honeydew from Colorado in the 1940s (31), pathogenicity was confirmed on honeydew and cucumbers only. In the first reports of bacterial blight of field-produced cantaloupe in France (22), the bacterium was referred to as *P. syringae* pv. *lachrymans*, because it infected a cucurbit. It was assumed to be the same pathogen that causes angular leaf spot of cucumber. This same assumption has also been made for a bacterial blight of melon observed in California (information available on-line from the Statewide Integrated Pest Management Project, University of California, Davis).

Fig. 3. Host range of strains of *Pseudomonas syringae* pathogenic on cantaloupe. Solid black squares indicate a compatible reaction between the host and the strain for at least two of three inoculated plants at both inoculation dates. A total of 17 plant species was inoculated; results for three of these (Chinese cabbage, wheat, and oats) are not presented, because compatible reactions were not consistently observed at both inoculation dates for any of the strains tested.

Fig. 4. The relationship between the aggressiveness of strains on cantaloupe and the number of other plant species on which the strains were virulent. The aggressiveness of each strain is expressed as the sum of disease severity ratings for compatible reactions (disease scores 2 or 3) for the 11 cantaloupe plants inoculated. The strains and plant species represented are those presented in Figure 3.

Although bacterial blight of melons caused by *P. syringae* pv. *aptata* may occur elsewhere in the world, currently, it seems to be causing significant losses only in France. We suggest that the following factors have contributed to this apparent particularity. First, it should be noted that southwestern France was the site of the severe epidemics that first attracted our attention to the importance of this disease. This region of France is also a major site for sugar beet seed production from which strains of *P. syringae* pv. *aptata* have been recently isolated (21). These production areas may have produced inoculum for nearby cantaloupes. During the cantaloupe production seasons in southwestern France from 1993 to 1997, the climate was particularly favorable for the development of severe bacterial blights. During the production season in each of these years, there were at least one or two heavy storms (with at least 30 mm of rain per rain event) followed by several days of cool, rainy weather. Some production zones recorded the heaviest rains observed in the past 30 years. Hence, these circumstances led us to distinguish the symptoms of this disease from those caused by *Cladosporium cucumerinum* and *Colletotrichum* spp. (5), and we were able to repeatedly isolate the pathogen during several seasons of production. The identification of the causal agent of bacterial blight of cantaloupe as *P. syringae* pv. *aptata* has allowed us to hypothesize this scenario. We are currently searching for sources of inoculum and conducting detailed studies of the geographic distribution and structure of the populations of this pathogen. Furthermore, we are investigating the possibility that the cultivars of cantaloupe adapted to French production conditions may be particularly sensitive to this disease and may be a contributing factor to its importance in France.

ACKNOWLEDGMENTS

We thank S. Hirano of the University of Wisconsin and J. Young of Landcare Research New Zealand Ltd. for the generous donations of strains.

LITERATURE CITED

- Andrivon, D. 1993. Nomenclature for pathogenicity and virulence: The need for precision. *Phytopathology* 83:889-890.
- Arnold, D. L., Athey-Pollard, A., Gibbon, M. J., Taylor, J. D., and Vivian, A. 1996. Specific oligonucleotide primers for the identification of *Pseudomonas syringae* pv. *pisi* yield one of two possible DNA fragments by PCR amplification: Evidence for phylogenetic divergence. *Physiol. Mol. Plant Pathol.* 49:233-245.
- Bereswill, S., Bugert, P., Völksch, B., Ullrich, M., Bender, C. L., and Geider, K. 1994. Identification and relatedness of coronatine-producing *Pseudomonas syringae* pathovars by PCR analysis and sequence determination of the amplified products. *Appl. Environ. Microbiol.* 60:2924-2930.
- Blancard, D., Lecoq, H., and Pitrat, M. 1991. Maladies des Cucurbitacées: Observer, identifier, lutter. PHM Revue Horticle, Limoges and INRA, Paris.
- Blancard, D., and Morris, C. E. 1998. Quel est votre diagnostic? PHM Rev. Hortic. 391:57-58.
- Bradbury, J. F. 1986. Guide to Plant Pathogenic Bacteria. CAB International Mycological Institute, Kew, Surrey, England.
- Gabriel, D. W. 1999. Why do pathogens carry avirulence genes? *Physiol. Mol. Plant Pathol.* 55:205-214.
- Gardan, L., Bollet, C., Ghorrah, M. A., Grimont, F., and Grimont, P. A. D. 1992. DNA relatedness among the pathovar strains of *Pseudomonas syringae* subsp. *savastanoi* Janse (1982) and proposal of *Pseudomonas savastanoi* sp. nov. *Int. J. Syst. Bacteriol.* 42:606-612.
- Goumas, D. E., and Chatzaki, A. K. 1998. Characterization and host range evaluation of *Pseudomonas viridiflava* from melon, blite, tomato, chrysanthemum and eggplant. *Eur. J. Plant Pathol.* 104:181-188.
- Gross, D. C. 1985. Regulation of syringomycin synthesis in *Pseudomonas syringae* pv. *syringae* and defined conditions for its production. *J. Appl. Bacteriol.* 58:167-174.
- Gross, D. C., and DeVay, J. E. 1977. Population dynamics and pathogenesis of *Pseudomonas syringae* in maize and cowpea in relation to the in vitro production of syringomycin. *Phytopathology* 67:475-483.
- Hildebrand, D. C. 1971. Pectate and pectin gels for differentiation of *Pseudomonas* sp. and other bacterial plant pathogens. *Phytopathology* 61:1430-1436.
- Isakeit, T., Black, M. C., Barnes, L. W., and Jones, J. B. 1997. First report of infection of honeydew with *Acidovorax avenae* subsp. *citrulli*. *Plant Dis.* 81:694.
- Jones, J. B., Chase, A. R., and Harris, G. K. 1993. Evaluation of the Biolog GN MicroPlate System for identification of some plant-pathogenic bacteria. *Plant Dis.* 77:553-558.
- Kado, C. I., and Liu, S. T. 1981. A rapid procedure for detection and isolation of large and small plasmids. *J. Bacteriol.* 145:1365-1373.
- King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.* 44:301-307.
- Klement, Z., Farkas, G. L., and Lovrekovich, L. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. *Phytopathology* 54:474-477.
- Latour, X., Corberand, T., Laguerre, G., Allard, F., and Lemanceau, P. 1996. The composition of fluorescent pseudomonad populations associated with roots is influenced by plant and soil type. *Appl. Environ. Microbiol.* 62:2449-2456.
- Lelliott, R. A., Billing, E., and Hayward, A. C. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. *J. Appl. Bacteriol.* 29:470-489.
- Louws, F. J., Fulbright, D. W., Stephens, C. T., and de Bruijn, F. J. 1994. Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas* pathovars and strains generated with repetitive sequences and PCR. *Appl. Environ. Microbiol.* 60:2286-2295.
- Maraitte, H., and Weyns, J. 1997. *Pseudomonas syringae* pv. *aptata* and pv. *atrofaciens*, specific pathovars or members of pv. *syringae*? Pages 515-520 in: *Pseudomonas syringae* Pathovars and Related Pathogens. K. Rudolph, T. J. Burr, J. W. Mansfield, D. Stead, A. Vivian, and J. von Kietzell, eds. Kluwer Academic Publishers, Dordrecht, the Netherlands.
- Monnet, Y. 1995. Maladies bactériennes inféodées aux cultures légumières françaises. *Phytoma Déf. Végét.* 468:36-38.
- Morris, C. E., and Pitrat, M. 1998. La bactériose du melon: Connaissances acquises et travaux en cours. *PHM Rev. Hortic.* 393:44-47.
- Neto, J. R., Sugimori, M. H., and Oliveira, A. R. 1984. A bacterial rot of muskmelon (*Cucumis melo* L.) caused by *Xanthomonas campestris* pv. *melonis* pv. nov. *Summa Phytopathol.* 10:217-233.
- Ovod, V., Rudolph, K., and Krohn, K. 1997. Serological classification of *Pseudomonas syringae* pathovars based on monoclonal antibodies towards the lipopolysaccharide O-chains. Pages 526-531 in: *Pseudomonas syringae* Pathovars and Related Pathogens. K. Rudolph, T. J. Burr, J. W. Mansfield, D. Stead, A. Vivian, and J. von Kietzell, eds. Kluwer Academic Publishers, Dordrecht, the Netherlands.
- Prosen, D., Hatziloukas, E., Schaad, N. W., and Panopoulos, N. J. 1993. Specific detection of *Pseudomonas syringae* pv. *phaseolicola* DNA in bean seed by polymerase chain reaction-based amplification of a phaseolotoxin gene region. *Phytopathology* 83:965-970.
- Prunier, J. P., and Kaiser, P. 1961. Etude de l'activité pectinolytique chez des bactéries phytopathogènes et saprophytes des plantes. I. Recherche des enzymes pectinolytiques. *Ann. Epiphyt.* 15:205-219.
- Saunier, M., Malandrin, L., and Samson, R. 1996. Distribution of *Pseudomonas syringae* pathovars into twenty-three O serogroups. *Appl. Environ. Microbiol.* 62:2360-2374.
- Sherf, A. F., and MacNab, A. A. 1986. Cucurbits. Pages 307-311 in: *Vegetable Diseases and their Control*. A. F. Sherf and A. A. MacNab, eds. Wiley, New York.
- Smith, I. M., Dunez, J., Lelliott, R. A., Philipps, D. H., and Archer, S. A. 1988. *European Handbook of Diseases*. Blackwell Scientific Publications, Oxford.
- Smith, M. A. 1946. Bacterial spot of honeydew melon. *Phytopathology* 36:943-949.
- Stanier, R. Y., Palleroni, N. J., and Douderoff, N. 1966. The aerobic pseudomonads: A taxonomic study. *J. Gen. Microbiol.* 43:159-271.
- Stead, D. E., Hennessy, J., Elphinstone, J. G., and Wilson, J. K. 1997. Modern methods for classification of plant pathogenic bacteria including *Pseudomonas syringae*. Pages 427-434 in: *Pseudomonas syringae* Pathovars and Related Pathogens. K. Rudolph, T. J. Burr, J. W. Mansfield, D. Stead, A. Vivian, and J. von Kietzell, eds. Kluwer Academic Publishers, Dordrecht, the Netherlands.
- Takahashi, Y., Omura, T., Hibino, H., and Sato, M. 1996. Detection and identification of *Pseudomonas syringae* pv. *atropurpurea* by PCR amplification of specific fragments from an indigenous plasmid. *Plant Dis.* 80:783-788.
- Vauterin, L., and Swings, J. 1997. Are classification and phytopathological diversity compatible in *Xanthomonas*. *J. Indust. Microbiol. Biotechnol.* 19:77-82.
- Versalovic, J., Schneider, M., de Bruijn, F. J., and Lupski, J. R. 1994.

- Genomic fingerprinting of bacteria using repetitive sequence based (rep-PCR). *Methods Cell. Mol. Biol.* 5:25-40.
37. Weingart, H., and Völksch, B. 1997. Genetic fingerprinting of *Pseudomonas syringae* pathovars using ERIC-, REP-, and IS50-PCR. *J. Phytopathol.* 145:339-345.
 38. Yarwood, C. E., Resconich, E. C., Ark, P. A., Schlegel, D. E., and Smith, K. M. 1961. So-called beet latent virus is a bacterium. *Plant Dis. Rep.* 45:85-89.
 39. Young, J. M. 1991. Pathogenicity and identification of the lilac pathogen, *Pseudomonas syringae* pv. *syringae* van Hall 1902. *Ann. Appl. Biol.* 118:283-298.
 40. Young, J. M., and Triggs, C. M. 1994. Evaluation of determinative tests for pathovars of *Pseudomonas syringae* van Hall 1902. *J. Appl. Bacteriol.* 77:195-207.