Characterization of *Pseudomonas syringae* pv. *maculicola* and Comparison with *P. s. tomato*

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ABSTRACT

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Thirty strains of *Pseudomonas syringae* pv. *maculicola* and four of *P. s. tomato* were collected from diverse localities and compared for symptom expression, host range, temperature response, and plasmid profile. The *P. s. maculicola* strains induced one of three distinct lesion types on crucifer hosts: chlorotic, water-soaked, or necrotic. Each of five *P. s. maculicola* strains that caused chlorotic lesions synthesized coronatine as confirmed by bioassay and by gas chromatographic and mass spectrometric analyses of culture filtrates. Four other strains that caused either water-soaked or necrotic lesions did not synthesize coronatine. Plasmid profiles of 25 *P. s. maculicola* strains contained from zero to seven plasmids ranging from 11 to 105 kb. The strains were less variable in host range and temperature response, but the diversity in symptom expression, coronatine synthesis, and plasmid profile denoted a high degree of heterogeneity within the pathovar. The *P. s. maculicola* and *P. s. tomato* strains were similar in many of these traits, but *P. s. maculicola* infected both crucifers and tomatoes, whereas *P. s. tomato* did not infect crucifers.

Pepper spot of crucifers, caused by *Pseudomonas syringae* pv. *maculicola* (McCulloch) Young, Dye, and Wilke, has been a minor disease in California (6) and in other parts of the world (10,33,38,44,45). However, under some conditions, such as the periods of high rainfall that occurred during the winter of 1982–1983 in California, pepper spot causes significant economic losses to some crucifers, principally cauliflower (*Brassica oleracea* L. var. *botrytis*) (6) and broccoli (*B. o. italica*) (41). The disease is becoming more important in commercial cauliflower production in the coastal valleys of California with the increasing use of greenhouse-grown transplants (A. Greathead, *personal communication*). Seedlings typically receive overhead irrigation four to six times a day, which provides ideal conditions for development and dissemination of the disease within the greenhouse and thence introduction of the pathogen to fields with transplants.

A variety of symptoms has been reported for *P. s. maculicola* on cauliflower and other *Brassica* spp. In 1911, the original report (25) described *Bacterium maculicolum* McCulloch as causing leaf spotting on cauliflower, but it was not isolated from curds of infected plants. However, a later report (14) attributed black spotting on the surface of cauliflower curds to *B. maculicolum*. Extensive necrotic lesions on stems within or below the florets, as well as along the vascular bundles of leaves, are additional features of the symptom pattern in severe outbreaks (6,40,41). Strains isolated from curds or leaves were inoculated to leaves and caused small necrotic lesions with or without a yellow halo (6,38). Some strains of *P. s. maculicola* biosynthesized the antimetabolite coronatine, which could account for the chlorotic halos (11,32). In contrast, nine strains did not synthesize coronatine (27). Thus, there is apparent variability for these traits among strains.

Tomato (*Lycopersicon esculentum* Mill.) is the only common host of bacterial speck, caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye, and Wilke (5). Symptoms include necrotic leaf lesions surrounded by a yellow chlorotic halo caused by coronatine (2,3,29,31). *P. s. maculicola* and *P. s. tomato* have been considered to be distinct but closely related species, and restriction fragment length polymorphism analysis of total DNA (9; T. Denny, *personal communication*) has shown that they are among the most closely related pathovars of *P. syringae*. Recently, it was proposed that these species be combined into one species based on physiological and pathological tests (43).

The objectives of the present study were to appraise the phenotypic diversity among *P. s. maculicola* strains, to assess host specificity of representative strains, and to evaluate the relatedness of *P. s. maculicola* to reference strains of *P. s. tomato*. Traits examined included pathogenicity, symptomatology, host range, effects of temperature on pathogenicity and host range, production and characterization of toxin, and plasmid profile. Phenotypic characters such as these may assist in providing a more meaningful taxonomic basis for classification of pseudomonads (37).

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MATERIALS AND METHODS

Biological materials. Twenty-one *P. s. maculicola* strains used in various portions of this study were isolated from crucifers in California (Table 1). Strains 83-02 to 83-18 were obtained from lesions on leaves and necrotic lesions on stems within curds collected in 1983 in two cauliflower fields located in Salinas Valley. The collections made in 1984 (84-25 to 84-68) and 1988 (88-10 and 88-11) were from leaf lesions collected from cauliflower or mustard in other fields. Additional strains of *P. s. maculicola* and *P. s. tomato* were obtained from the sources shown in Table 1. The strains were identified as *P. syringae* by fluorescence on King's medium B (KB) (20) and LOPAT (23,26,34,42) tests and as *P. s. maculicola* or *P. s. tomato* by host reactions.

For short-term storage, strains were grown on 523 medium (35), stored at 5 C, and recultured at 3-mo intervals. Long-term storage was at 5 C on 6-12 mesh, nonindicating silica gel particles (Fisher Scientific Co., Fair Lawn, NJ) and at −70 C in 1 ml of 6% DMSO, nutrient broth tubes. For inoculum production, each strain was streaked on KB and incubated at 26 C for 24 hr. Bacteria were harvested in sterile 0.85% saline and adjusted spectrophotometrically to $A_{600nm} = 0.08$, equivalent to approximately 1×10^7 cfu/ml for inoculations.

Cauliflower cv. Snowball Y Improved and tomato cv. Floradade plants were used as standard hosts for leaf inoculations. Host plants were either grown directly from seed or transplanted into plastic pots (10 cm diameter) and were inoculated at the three- to four-true-leaf stage. The abaxial leaf surfaces were dusted with 500-mesh Carborundum and lightly rubbed with a cotton-tipped swab saturated with a bacterial suspension. In some tests, bacteria or culture filtrates were infiltrated into leaves with a modified Hagborg device (16). Three true leaves from each of three cauliflower plants or three terminal leaflets of the fourth true leaf from each of three tomato plants were infiltrated for each treatment. Rub-inoculated plants were covered with a clear plastic bag for 48 hr, maintained either in a greenhouse at 20–30 C or in a growth chamber with

a 14-hr photoperiod at 15, 20, 25, or 30 C, and rated for disease 12 days after inoculation. Inoculated plants were irrigated by applying water to only the soil surface to prevent secondary spread of bacteria. All inoculations were done three times.

Curds of market-stage cauliflower cv. Glacier were surface-sterilized by being dipped in 70% alcohol, then were rinsed immediately in sterile deionized water. Each curd was cut aseptically into nine to 12 sections, and a sterile florist frog, comprised of closely spaced metal pins embedded in a metal plate, was used to make multiple prick wounds. Inoculum was atomized over the wounded curd surface, and inoculated curds were incubated in the dark at 10 C for 10 days in clear plastic boxes. Each box contained a beaker of deionized water and a filter paper wick to maintain high humidity. Tomato fruits were produced on Floradade plants grown in 19-L plastic pots and fertilized weekly. Seven to 10 fruit 0.5–2.5 cm in diameter (12) on one or two plants were dusted with Carborundum and rub-inoculated with each *P. s. maculicola* or *P. s. tomato* strain. Inoculated fruit were covered with a loose-fitting plastic bag, which was removed after 2 days. Final disease observations were made 12 days later. Fruit inoculations were done twice.

Population studies. Four *P. s. maculicola* strains and *P. s. tomato* strain FLA were grown on KB plates amended with 50 μg/ml of rifampicin at 26 C to select Rif^r mutants. Mutants were inoculated onto cauliflower and tomato both by rubbing and infiltrating leaves on separate plants. Leaves were sampled immediately after inoculation (0 days) and at 2, 4, 6, and 8 days. Three leaf disks were excised with a 1.1-cm cork borer from each of three randomly selected plants, giving nine disks with a total area of 8.55 cm^2 for each replicate. Three replicates were assayed per plant species per inoculation method at each sample date. Each replicate was homogenized with a mortar and pestle in 20 ml of sterile saline, and 10 10-μl aliquots from each step of a 10-fold dilution series were spotted on rifampicin-amended KB plates (22). Plates were incubated at 26 C for 48 hr and observed with a dissecting microscope at 10× magnification. The numbers of pseudomonad colonies were averaged and calculated as log number of bacteria per square centimeter of leaf to provide a single value for each of the three replicates. The population studies were done twice.

Antimetabolite identification. Production of antimetabolites by the pseudomonads was assayed on a lawn of *Escherichia coli* (Migula) Castellani and Chalmers. Strains LL308 and HB101 of *E. coli* (provided by C. Kado, University of California-Davis) were grown on 523 medium, harvested, washed once, resuspended in *Pseudomonas* minimal medium (PMS) (11), and added to PMS agar to a concentration of 1×10^7 cfu/ml. *Pseudomonas* cultures were grown on KB, stabbed with an inoculating loop into four locations in each *E. coli*-amended PMS agar plate, and incubated at 28 C for 24 hr. The zone of growth inhibition was measured to the nearest 0.5 mm. Culture filtrates of the *Pseudomonas* strains were bioassayed for coronatine on bean cv. Red Kidney leaves. Culture filtrates were produced from strains grown for 5–7 days at ambient temperature on a rotary shaker in 50 ml of Woolley medium (46). Bacteria were removed by centrifugation at 4,340 *g* for 10 min, followed by vacuum filtration through a 0.22-μm membrane. Culture filtrates were introduced into three leaflets of the youngest trifoliate leaf of three 14-day-old bean plants by placing a 4-μl droplet on a prick made with a 20-gauge needle on the adaxial leaf surface. The plants were kept in the greenhouse for 3 days, and the diameter of each chlorotic zone was measured. Cauliflower cultivars were similarly bioassayed for coronatine sensitivity; three leaves of each of three plants were used for each strain.

The organic acids in 250-ml batches of culture filtrates of nine *P. s. maculicola* strains and *P. s. tomato* strain PT23 were compared with authentic coronatine by gas chromatograpy (GC) and mass spectrometry (MS). The organic acids were isolated by a modification of Mitchell's method (2). Culture supernatant was concentrated 10-fold by rotary evaporation at 40 C, adjusted to pH 8 with 10 N NaOH, and shaken in a separatory funnel with an equal volume of anhydrous ethyl acetate. The aqueous yellow lower layer was collected, adjusted to pH 3 with 6N HCl, and extracted three times with ethyl acetate. The final extract was slowly dripped over anhydrous Na_2SO_4, then aspirated to dryness. Dried preparations were dissolved in 0.5 ml of methanol and stored at 5 C until analyzed. Organic acid extracts were evaporated to dryness under a stream of N_2 at room temperature and derivatized with ethereal diazomethane for 1 hr. Ether was removed under a stream of N_2 at room temperature, and 10 μl of CH_2Cl_2 was added. Samples were analyzed on a Trio-2 mass spectrometer (VG Masslab, Altrincham, UK) using 70-eV electron ionization. The GC parameters included

Table 1. Origins of strains of *Pseudomonas syringae* pv. *maculicola* and *P. s. tomato* and lesion type on cauliflower leaves

Pathovar	Strain	Original host	Origin	Date	Source[a]	Lesion type[b]
maculicola	79-101	Bok Choy	Salinas, CA	1979	IA	NL
	83-02	Cauliflower	Salinas, CA	1983	IA	NL
	83-05	Cauliflower	Salinas, CA	1983	IA	NL
	83-06	Cauliflower	Salinas, CA	1983	IA	NL
	83-07	Cauliflower	Salinas, CA	1983	IA	NL
	83-10	Cauliflower	Salinas, CA	1983	IA	NL
	83-14	Cauliflower	Salinas, CA	1983	IA	NL
	83-15	Cauliflower	Salinas, CA	1983	IA	NL
	83-16	Cauliflower	Salinas, CA	1983	IA	NL
	83-17	Cauliflower	Salinas, CA	1983	IA	NL
	83-18	Cauliflower	Salinas, CA	1983	IA	NL
	84-25	Black mustard	Davis, CA	1984	IA	WS
	84-39	Cauliflower	Santa Maria, CA	1984	IA	CL
	84-59	Cauliflower	Greenfield, CA	1984	IA	CL
	84-63	Cauliflower	Salinas, CA	1984	IA	CL
	84-65	Cauliflower	Salinas, CA	1984	IA	CL
	84-66	Cauliflower	Salinas, CA	1984	IA	CL
	84-67	Cauliflower	Salinas, CA	1984	IA	CL
	84-68	Cauliflower	Salinas, CA	1984	IA	CL
	88-10	Cauliflower	Salinas, CA	1988	RNK	CL
	88-11	Cauliflower	Salinas, CA	1988	RNK	CL
	795	Cauliflower	New Zealand	1958	PDDCC	NL
	2735	Cauliflower	United States	1937	PDDCC	WS
	2744	Mustard	United Kingdom	1968	PDDCC	WS
	3936	Cauliflower	New Zealand	1965	PDDCC	WS
	4326	Radish	United States	1965	PDDCC	CL
	4335	Kale	United Kingdom	1967	PDDCC	WS
	4427	Cauliflower	United Kingdom	1966	PDDCC	WS
	4981	Cabbage	Zimbabwe	1970	PDDCC	CL
	4984	Cauliflower	United Kingdom	1965	PDDCC	WS
tomato	B113	Tomato	Italy	1966	PS	–
	B149	Tomato	California	1986	PS	–
	PT23	Tomato	Riverside, CA	1986	UCR	–
	FLA	Tomato	Florida	1984	IA	–

[a] IA = isolated by authors; RNK = Rogers NK Seed Company; PDDCC = Culture Collection of Plant Diseases Division, New Zealand Department of Scientific and Industrial Research, Aukland; PS = Peto Seed Company; UCR = University of California-Riverside.
[b] CL = chlorotic, WS = water-soaked, NL = necrotic, – = none.

Table 2. Infectivity[a] of *Pseudomonas syringae* pv. *maculicola* strains at 15, 20, 25, and 30 C to *Brassica*, *Raphanus*, and *Lycopersicon* spp.

		P. s. maculicola strain and temperature (C)											
		4981				84-67				83-05			
Species	Cultivar	15	20	25	30	15	20	25	30	15	20	25	30
B. campestris	Candle	−	+	−	−	−	+	+	+	−	−	−	−
B. carinata	77-1304	−	−	−	−	−	−	−	−	−	−	−	−
B. juncea	Unnamed	−	+	+	−	−	+	+	+	−	+	−	−
B. napus	Westar	+	+	+	+	−	+	+	++	−	−	+	+
B. oleracea	Glacier	+	+	+	−	++	+	+	+	+	+	+	+
var. *botrytis*	Snowball Y Improved	+	+	+	−	+	+	++	++	+	+	+	+
B. o. capitata	Tastie	+	+	+	−	+	+	+	++	−	−	+	+
B. o. italica	Emperor	+	+	+	−	++	+	++	++	+	+	+	−
B. pekinensis	Sakata 14	−	−	−	−	−	−	−	−	−	−	−	−
B. rapa	Purple Top White Globe	−	−	+	−	+	+	++	++	−	−	+	−
L. esculentum	Floradade	++	+	+	++	+	+	++	++	−	+	+	−
R. sativus	Red Prince	−	−	−	−	−	−	−	−	−	−	−	−
	White Icicle	−	−	−	−	−	−	−	−	−	−	−	−

[a] Infectivity rating: − = no lesions; + = weakly infective, 0–10 lesions per leaf; ++ = moderately to highly infective, ≥11 lesions per leaf.

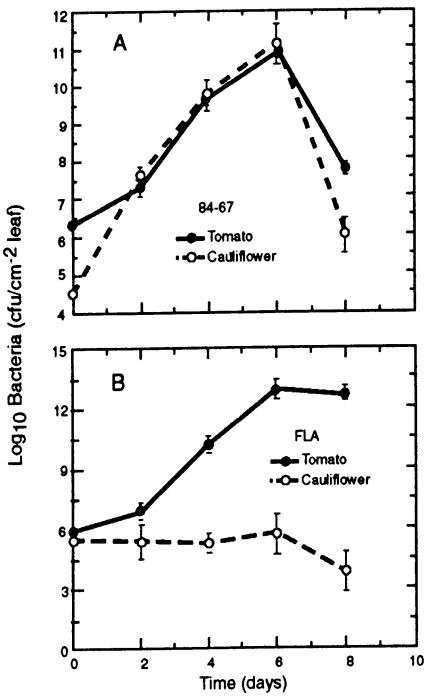

Fig. 1. Growth curves of **(A)** *Pseudomonas syringae* pv. *maculicola* strain 84-67 and **(B)** *P. s. tomato* strain FLA infiltrated into tomato and cauliflower leaves. Vertical lines represent the standard deviation on the transformed data.

a 15-m DB-5 column (0.25 mm i.d., 0.25 μm film) with helium at 35 cm/sec as the carrier gas. The temperature program was 150 (1 min) to 275 at 10/min, and a 1-μl splitless injection was used to load samples (D. Jones, *personal communication*). A purified coronatine sample (provided by C. Bender, Oklahoma State University, Stillwater) was the control for all GC-MS analyses.

Plasmid DNA isolation and characterization. Cultures were grown for 24 hr at 20–25 C in 50 ml of Luria-Burtani medium, and plasmid DNA was isolated by standard alkaline lysis procedures (24). Plasmid DNA was resuspended (using a Vortex mixer) in 50 μl of TE (10 mM Tris, 1 mM EDTA, pH 8.0) containing 20 μg/ml of RNase A, and the preparation was stored at −20 C. The plasmids were sized (8) by agarose gel electrophoresis (24) on minigels using the 35-, 67-, 73-, and 101-kb plasmids of *P. s. tomato* strain PT23 as standards (1,2).

RESULTS

Biological materials. All strains were fluorescent on KB except *P. s. maculicola* strains 84-59, 84-63, 84-66, and 84-67 and *P. s. tomato* strain B113, which were fluorescent when originally isolated. Likewise, *P. s. maculicola* strains 795 and 4335 were not fluorescent when received from the PDDCC (Culture Collection of Plant Diseases Division, New Zealand Department of Scientific and Industrial Research, Auckland), where they had been stored since 1968 and 1975, respectively. None of the strains induced significant soft rot of potato tissue, while all were negative to arginine dihydrolase and oxidase. All strains except 4326 produced levan and all induced a hypersensitive reaction on tobacco (*Nicotiana tabacum* L. 'Havana 425') leaves.

All *P. s. maculicola* strains infected cauliflower leaves and tomato leaves by both inoculation techniques and tomato fruit by rub-inoculation. All except 83-15 and 4326 caused dark brown to black spotting at wound sites on the upper surface of cauliflower curds. The strains were classified into three groups according to the type of lesion induced on cauliflower and tomato leaves. The chlorotic lesion (CL) type was 1–2 mm in diameter, brown, water-soaked, and surrounded by a diffuse, bright yellow zone of chlorosis extending 1–3 mm from the center. The water-soaked lesion (WS) type was identical to the CL type but without a chlorotic zone. Both CL and WS types were visible in 4 days on both leaf surfaces. The necrotic lesion (NL) type was 1–3 mm in diameter, dark gray, dry, and sunken. This type formed within 3 days on the abaxial leaf surface but occasionally penetrated to produce a lesion with a white to tan center 1–2 mm in diameter on the adaxial surface. Eleven strains representing all three lesion types caused 1- to 2-mm superficial light brown spots on tomato fruit. Strain 4335, a WS-type strain, caused lesions that were slightly larger and darker than those caused by other *P. s. maculicola* strains.

All four *P. s. tomato* strains caused dark brown spots 1–2 mm in diameter and surrounded by a chlorotic zone. These spots and accompanying chlorosis began to appear on tomato leaves in 4 days. Two strains were inoculated to tomato fruit and produced lesions that were larger and darker and penetrated more deeply into the fruit than those caused by *P. s. maculicola* strains. None of the four strains caused symptoms on either cauliflower leaves or curds.

Each of the *P. s. maculicola* and *P. s. tomato* strains were inoculated onto *Beta vulgaris* L. 'Royal Red', *Phaseolus vulgaris* L. 'Bountiful', *Cucumis melo* L. 'Top Mark', and *Lactuca sativa* L. 'Climax'. None of the strains caused lesions on any of these four species.

The pathogenicity of three *P. s. maculicola* strains was compared on the standard hosts and 11 additional hosts at 15, 20, 25, and 30 C (Table 2). *Brassica carinata* A. Braun, *B. pekinensis* (Lour.) Rupr., and *Raphanus sativus* L. were not infected by any strain at any temperature. Strain 4981 caused lesions on all other hosts at 15, 20, and 25 C except that *B. juncea* (L.) Czernj. & Coss. was not infected at 15 C, *B. campestris* L. was infected only at 20 C, and *B. rapa* L. was infected only at 25 C. Strain 84-67 caused lesions at all four temperatures, although *B. juncea*, *B. campestris*, and *B. napus* L. were not infected at 15 C. This strain was more pathogenic than either 4981 or 83-05 and more pathogenic at 25 and 30 C than at 15 or 20 C. The host range for strain 83-05 was similar

to that of strains 4981 and 84-67 except that it did not infect *B. campestris* at any temperature. *P. s. tomato* strain FLA was included in the trial but infected only tomato (*data not presented*). Typical bacterial speck lesions formed on inoculated tomato leaves at all four temperatures but were most numerous at 25 and 30 C. Because *R. sativus* was not infected by any of the three *P. s. maculicola* strains, additional inoculations were made at 25 C with strain 4326, which was originally isolated from *R. sativus*; it infected cauliflower, *B. napus*, and both *R. sativus* cultivars.

Fifteen cauliflower cultivars were also screened for resistance to six strains of *P. s. maculicola*. The cultivars were White Rock, Late Fall FBJ, Flora Blanca, Snowpack, LABJ, Glacier, Snowball Y Improved, Serrano, Candid Charm, White Contessa, Silver Star, Vernon, White Summer, Carillon, and Montano. All cultivars were susceptible to all strains at 25 C. Some cultivars developed more lesions with individual strains but not with all strains.

Population studies. The Rif^r mutants were inoculated to cauliflower and tomato leaves by rubbing and infiltration, giving initial population levels of approximately 1×10^4 to 1×10^6 cfu/cm^2. Growth curves for Rif^r mutants of *P. s. maculicola* strains 84-67 (CL type), 4427 (WS type), and 83-05 (NL type) were very similar on both hosts and with both inoculation methods from 0 to 8 days. Figure 1A shows results of infiltration inoculation with strain 84-67. The only time the populations on the two hosts diverged was with rub-inoculated strain 83-05, which decreased after day 4 on cauliflower leaves but increased on tomato leaves through day 6. The Rif^r mutant of *P. s. tomato* strain FLA increased exponentially for 6 days on tomato with both inoculation methods (Fig. 1B) but not on cauliflower.

Antimetabolite identification. Each of 25 *P. s. maculicola* and three *P. s. tomato* strains caused a zone of growth inhibition 1–6 mm in radius in a lawn of *E. coli*, indicating the possible presence of antimetabolite(s). Culture filtrates of these strains were tested for coronatine with the droplet bean leaf bioassay. Five of the eight CL-type *P. s. maculicola* strains and all three *P. s. tomato* strains were strongly positive (Table 3), whereas *P. s. maculicola* strain 4326, a CL-type strain, was weakly positive. The other strains were indistinguishable from the culture medium control.

Culture filtrates of selected strains were tested on five cauliflower cultivars. Well-defined zones of chlorosis formed on all cultivars with CL-type *P. s. maculicola* strains 84-67 and 88-11 and *P. s. tomato* strain PT23. The chlorotic zones were generally smallest on leaves of cv. White Contessa. Culture filtrates of the NL-type strain 83-05 and WS-type strains 2735 and 4427 did not induce chlorosis.

Culture filtrates of CL-type strains 4981, 84-59, 84-66, 84-67, 88-11, and PT23 contained coronatine (Table 3), as indicated by a distinct molecular ion peak at 333 m/z and diagnostic fragment ion (m/z) peaks of 301, 191, and 163 (2,17,30). A total ion current and selected ion chromatogram peak occurred in the 700–800 m/z range for these samples. The strains had weak or no peaks at 222 and 321 m/z, indicating the nonphytotoxic intermediates coronafacic acid and coronafacoyvaline were not detected. Culture filtrates of NL-type strains 83-02 and 83-05 and WS-type strains 2735 and 4427 did not have peaks for coronatine or its intermediates.

Plasmid DNA isolation and characterization. The *P. s. maculicola* strains contained from zero to seven plasmids with sizes ranging from 11 to 105 kb. Strain 83-05 contained no detectable plasmids, 13 strains contained one, five strains contained two, five strains contained three to six, and strain 4326 contained seven plasmids (Table 4). There was no plasmid size shared by all strains, but 17 (68%) of the strains contained a 42-kb plasmid. There was no correlation between a particular plasmid size and lesion type (CL, WS, or NL). All three *P. s. tomato* strains contained the 73- and 101-kb plasmids, and some had additional plasmids. Only *P. s. maculicola* strain 2735 with a 73-kb plasmid, strain 4335 with a 67-kb plasmid, and strain 4981 with a 101-kb plasmid had plasmids similar in size to those found in any *P. s. tomato* strain.

Table 3. Detection of coronatine in culture filtrates of *Pseudomonas syringae* pv. *maculicola*, *P. s. tomato*, and *P. s. syringae* by bean leaf bioassay and mass spectrometric analysis

Pathovar	Strain[a]	Bean leaf bioassay[b]	Mass spectrometric analysis[c]
maculicola	79-101	−	−
	83-02	−	−
	83-05	−	
	83-06	−	
	83-10	−	
	83-14	−	
	83-15	−	
	83-16	−	
	83-17	−	
	83-18	−	
	84-25	−	
	84-59*	++	+
	84-63*	−	
	84-66*	++	+
	84-67*	++	+
	88-10*	++	
	88-11*	++	+
	2735	−	−
	2744	−	
	3936	−	
	4326*	+	
	4335	−	
	4427	−	−
	4981*	−	+
	4984	−	
tomato	PT23	++	+
	FLA	++	
	B149	++	
syringae	184	−	
Control	−	−	

[a] * = Produced chlorotic lesions.
[b] − = No chlorosis or zone <5 mm in diameter, + = weak chlorosis zone 5–10 mm in diameter, ++ = strong chlorosis zone >10 mm in diameter.
[c] + = Molecular ion peak and fragment ion peaks matched those for coronatine, − = no coronatine peaks.

DISCUSSION

The strains isolated from crucifers in this study were easily assigned to *P. syringae* with standard laboratory tests such as LOPAT and fluoresence and with morphology. These tests, however, did not distinguish among *P. s. maculicola* strains for which inconsistent phenotypic characteristics have been reported, e.g., symptom expression and host range, coronatine synthesis, temperature response, and plasmid profile. The heterogeneity demonstrated herein adds validity to the conflicting reports concerning *P. s. maculicola*.

The present strains comprised three lesion types based on foliar symptoms, thus agreeing with earlier reports that a chlorotic halo occurred around leaf lesions (5,38) or was lacking (14,45). Five CL-type strains produced coronatine

Table 4. Plasmid content of *Pseudomonas syringae* pv. *maculicola* and *P. s. tomato*

Pathovar	Strain[a]	Approximate plasmid size (kb)
maculicola	79-101	40,42,59,77
	83-02	42
	83-05	None
	83-06	42
	83-10	42
	83-14	42
	83-15	42
	83-16	42
	83-17	42
	83-18	42
	84-25	42,44,48,61
	84-59*	55,70,86,105
	84-66*	55
	84-67*	55
	88-10*	89
	88-11*	89
	795	64
	2735	42,73
	2744	42,55
	3936	42,55
	4326*	11,19,21,23,40, 46,52
	4335	27,38,42,50,67
	4427	42,105
	4981*	28,42,101
tomato	4984	42,55
	B149	35,73,101
	PT23	35,67,73,101
	FLA	73,101

[a] * = Produced chlorotic lesions.

(*Cor*$^+$) as shown by GC-MS analysis of culture filtrates, which proved more sensitive than the bean leaf bioassay. Seventeen WS-type and NL-type strains were *Cor*$^-$ in the bean leaf bioassay, indicating that coronatine either was not present or was below the detectable level of 14 $\mu g\ L^{-1}$ (28). Four of these were also *Cor*$^-$ by GC-MS analysis. Four of the *Cor*$^-$ strains that induced WS-type lesions on cauliflower leaves were *Cor*$^-$ in tests by Mitchell (27). Thus, *P. s. maculicola* comprises strains that synthesize coronatine and others that do not.

The gene(s) encoding or mediating coronatine synthesis (*cor*) by pseudomonads may be located on plasmids such as the 101-kb plasmid of *P. s. tomato* strain PT23 (2), the 87-kb plasmid of *P. s. atropurpurea* (Reddy and Godkin) Young, Dye, and Wilke (36), or the 90-kb plasmid of *P. s. glycinea* (Coerper) Young, Dye, and Wilke (4). Conversely, the *cor* gene(s) may be chromosomal, as in *P. s. tomato* strain DC3000 (31). A similar variability in location of other pseudomonad genes has been noted with the IAA genes of *P. s. savastanoi* (Smith) Young, Dye, and Wilke (7). There were no common plasmids among the *Cor*$^+$ strains of *P. s. maculicola*, and only one strain had a plasmid similar to the 101-kb plasmid found in all three *P. s. tomato* strains. Thus, *Cor*$^+$ genes in *P. s. maculicola* may be on different plasmid(s) than those in *P. s. tomato* or on the chromosome, depending on the strain. This is in agreement with recent work by D. Cuppels (*personal communication*).

Symptoms caused by CL-type and WS-type *P. s. maculicola* strains were very similar aside from the coronatine-induced chlorotic halo, and the strains behaved almost identically with respect to host range, time to symptom appearance, and population development over time. If strains that were originally *Cor*$^+$ lost coronatine gene(s) function during long-term storage, they might be classified as WS types. The loss of toxin production by pseudomonads during laboratory maintenance has been well documented (15,19,39). Unfortunately, descriptions of the symptoms caused by most of the WS-type strains on the original host are not available, so it is unknown if these strains were *Cor*$^+$ or *Cor*$^-$ at the time of isolation.

Distinguishing between symptoms induced by WS-type and NL-type strains was difficult because both were *Cor*$^-$. WS lesions typically had a pronounced water-soaked appearance, and bacterial streaming was profuse in cross sections mounted in water and viewed with a microscope. The sunken, dry, rapidly developing leaf lesions caused by NL-type strains were very similar to, and might be interpreted as, a hypersensitive reaction (10,21). However, the exponential population increase and high population density of a representative NL-type strain on and in inoculated leaves indicated infection and colonization of tissue. NL-type strains were weak pathogens, and wounding by Carborundum during inoculation may have enhanced leaf infection (13).

Aside from lesion type, there were minor differences among the *P. s. maculicola* strains in host range and the influence of temperature on infection. The strains from radish infected radish but three strains from cauliflower did not. At 30 C, strains 84-67 and 83-05 infected several crucifer hosts that strain 4981 did not. The strains were similar in that all of those tested caused symptoms on tomato fruit and leaves, and the foliar lesions were similar to those of the respective strain on cauliflower. Furthermore, one strain of each lesion type multiplied as well in tomato leaves as in cauliflower leaves. The ability to infect tomato confirmed an observation made by M. N. Schroth (*personal communication*) and leads to the question of the identity of *P. s. maculicola* and *P. s. tomato*.

P. s. maculicola and *P. s. tomato* have been regarded as closely related pathovars that have more than 75% DNA homology (T. Denny, *personal communication*). Therefore, we used four recently isolated strains of *P. s. tomato* as reference cultures while studying heterogeneity within *P. s. maculicola*, our primary objective. These strains of *P. s. tomato* caused lesions that were indistinguishable from those caused by CL-type *P. s. maculicola* strains on tomato leaves, but fruit lesions caused by *P. s. tomato* were larger and darker. Because both pathovars can infect tomato, the possible role of *P. s. maculicola* in tomato bacterial speck should be investigated. In a recent report (43), some strains of *P. s. maculicola* and *P. s. tomato* had similar characteristics, cross-infected both hosts, and were proposed as one pathovar. Because our reference strains of *P. s. tomato* failed to infect cauliflower, we have retained both pathovars as distinct entities. Crucifer-infecting strains might be found in a larger sample.

All 15 cauliflower cultivars were equally susceptible to foliar infection by *P. s. maculicola*, so the possibility of resistance (18) was not verified. Leaf lesions caused by *Cor*$^+$ strains had a smaller chlorotic halo on White Contessa than on other cultivars, apparently because it was less sensitive to the toxin.

Superficial lesions that occur on the upper surface of cauliflower curds are termed cauliflower curd discoloration (CCD) in the industry. There is a single report that *P. s. maculicola* causes CCD (14). Isolations were made from several lesions on each of 43 curds collected from Salinas to Santa Maria, California, throughout the 1985 season (R. N. Campbell and S. T. Sim, *unpublished*). Many bacteria were isolated, but not *P. s. maculicola*, even though special attention was given to detecting it. All the types of bacteria isolated, as well as *P. s. maculicola*, caused necrotic lesions when inoculated to wounds on healthy curds, as in the present study. The lesions caused by *P. s. maculicola* were darker than those caused by the other bacteria. *P. s. maculicola* can infect wounds in the upper surface of curds, but the failure to find *P. s. maculicola* in CCD lesions and the inability of the putative *P. s. maculicola* isolated by Goldsworthy (14) to cause foliar lesions on young seedlings argue that *P. s. maculicola* has a limited role, if any, in CCD.

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