Synonymy of *Pseudomonas coronafaciens*, *Pseudomonas coronafaciens* pathovar *zeae*, *Pseudomonas coronafaciens* subsp. *atropurpurea*, and *Pseudomonas striafaciens*

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Eleven strains of *Pseudomonas coronafaciens*, four strains of *P. coronafaciens* pathovar *zeae*, six strains of *P. coronafaciens* subsp. *atropurpurea*, two strains of *P. striafaciens*, and one *Pseudomonas* sp. were compared by physiological, biochemical, serological, and pathological tests. All strains tested utilized sucrose, glucose, fructose, glycerol, and caprate as sole carbon sources, hydrolyzed Tween 80, reduced litmus milk, and grew at 0°C. All strains were arginine dihydrolase and oxidase negative and all strains failed to hydrolyze starch, reduce nitrate, or grow at 41°C. Cells of all strains were immunofluorescent positive when stained by the indirect immunofluorescence test with antiserum to cells of *P. coronafaciens* or *P. coronafaciens* subsp. *atropurpurea*. No differences among strains were detected in test tube agglutination tests. No significant differences were detected in the susceptibility of *Avena sativa* (oats), *Secale cereale* (rye), *Triticum aestivum* (wheat), *Hordeum vulgare* (barley), *Bromus inermis* (smooth brome grass), *B. japonicus* (Japanese brome), *B. secalinus* (chess brome), *B. tectorum* (cheatgrass), *Agropyron repens* (quackgrass), *Zea mays* (maize), or *Phleum pratense* (timothy) to the different strains. All strains produced toxin in Wooley medium except *P. striafaciens* and strains of *P. coronafaciens* subsp. *atropurpurea* from Italian ryegrass. The present division of strains of *P. coronafaciens* based on minor differences in pathogenicity and symptomology is untenable.

Plant pathogenic bacteria have been classified traditionally into numerous species and subspecies on the basis of their pathogenicity to different hosts. In the 7th edition of *Bergey's Manual of Determinative Bacteriology* (3) there are 89 species of *Pseudomonas* listed attacking plants in 49 families. *Pseudomonas coronafaciens* was described by Elliott in 1920 (10) as the causal agent of halo blight of oats (*Avena sativa* L.). Three years later Reddy and Godkin (31) described a new bacterial disease of smooth brome grass (*Bromus inermis* Leyss) and named the causal bacterium *Bacterium coronafaciens* var. *atropurpureum*. Stapp later changed the name to *Pseudomonas coronafaciens* var. *atropurpurea* (36). Reddy and Godkin's reasons for naming a new subspecies were that (i) *P. coronafaciens* (from oats) did not infect *Bromus inermis* and (ii) the lesions produced on oats by the two organisms were different. They stated that the two organisms were identical morphologically and physiologically. The name *P. striafaciens* was introduced in 1927 by Elliott (11) as a hitherto undescribed species on the basis of the difference in types of lesions produced on oats and the smaller size of *P. striafaciens* cells in comparison to *P. coronafaciens*. Both organisms

attacked the same hosts, gave the same results in host cultivar tests, and were identical culturally and physiologically. Although a type strain was not designated for *P. striafaciens*, Elliott's strain 70 was deposited in the American Type Culture Collection (ATCC) as strain 10730. All three bacteria are listed in the 7th edition of *Bergey's Manual* and are included in the First Draft of Approved List of Names (1) requested by the Judicial Commission of the International Committee on Systematic Bacteriology. In the 8th edition of *Bergey's Manual*, *P. coronafaciens* is included provisionally in the *P. syringae* group, and *P. striafaciens* is listed among nomen species which have been incompletely described (5). *Pseudomonas coronafaciens* subsp. *atropurpurea* is not listed. Tessi (40) studied the pathology and toxin-producing abilities of *P. coronafaciens* and *P. striafaciens* on oats in Argentina. He concluded that *P. striafaciens* strains were *P. coronafaciens* strains that had lost the ability to produce toxin. However, he refrained from combining the two species (40). Recently, Ribeiro et al. (32) designated *P. coronafaciens* infecting maize and timothy in Wisconsin as *P. coronafaciens* pathovar *zeae* on the basis of pathogenicity on maize, timothy, and

oats and similar physiological characters.

There is need for a reexamination of the taxonomy of *P. coronafaciens* including all currently recognized variants of this species. For this purpose cultural, physiological, serological, and pathological analyses of 23 strains of the four bacteria, including lectotype strain ATCC 10730 (Elliott's strain 70) of *P. striafaciens*, were performed.

MATERIALS AND METHODS

Bacterial strains. The sources and designations of the 23 strains of *P. coronafaciens* and eight other

pseudomonads used in this study are given in Table 1. All cultures were maintained by monthly transfer on King medium B agar (KB) slants (16) at 2 to 3°C and freeze-dried for long-term storage.

Cultural and morphological properties. The media used for determining cultural characteristics were KB and Difco nutrient agar (NA). All media were sterilized for 20 min at 121°C and at a pressure of 1.1 kg/cm². Cell morphology was recorded from light microscope observations of Gram-stained smear preparations and from electron microscope observations. Flagella were observed by light microscopy following the methods of Blendon and Goldberg (2) and by electron microscopy using shadowed preparations.

Physiological and biochemical properties. For

TABLE 1. List of strains used in the study of the taxonomy of *Pseudomonas coronafaciens*

Laboratory no.	Name	Strain	Source ^a	Original host
C-19	<i>P. coronafaciens</i>	Original		Oats (<i>Avena sativa</i>)
C-20	<i>P. coronafaciens</i>	Original		Oats
C-23	<i>P. coronafaciens</i>	Original		Oats
C-28	<i>P. coronafaciens</i>	ATCC 19607	(1)	Oats
C-72	<i>P. coronafaciens</i>	NCPPB 1348	(2)	Oats
C-73	<i>P. coronafaciens</i>	NCPPB 1357	(2)	Oats
C-81	<i>P. coronafaciens</i>	NCPPB 1327	(2)	Rye (<i>Secale cereale</i>)
C-83	<i>P. coronafaciens</i>	NCPPB 600	(2)	Oats
C-86	<i>P. coronafaciens</i>	Original		Rye
C-107	<i>P. coronafaciens</i>	Original		Rye
C-117	<i>P. coronafaciens</i>	Original		Rye
C-162	<i>P. coronafaciens</i> pathovar <i>zeae</i>	ChS-1	(8)	Maize (<i>Zea mays</i>)
C-163	<i>P. coronafaciens</i> pathovar <i>zeae</i>	ChS-2	(8)	Maize
C-164	<i>P. coronafaciens</i> pathovar <i>zeae</i>	ChS-3	(8)	Maize
C-165	<i>P. coronafaciens</i> pathovar <i>zeae</i>	Ti-1	(8)	Timothy (<i>Phleum pratense</i>)
C-82	<i>P. coronafaciens</i> subsp. <i>atropurpurea</i>	NCPPB 1768	(2)	<i>Agrostis</i> sp.
C-136	<i>P. coronafaciens</i> subsp. <i>atropurpurea</i>	NCPPB 1769	(2)	Tall oatgrass (<i>Arrhenatherum elatius</i>)
C-142	<i>P. coronafaciens</i> subsp. <i>atropurpurea</i>	NCPPB 1328	(2)	<i>Bromus</i> sp.
C-159	<i>P. coronafaciens</i> subsp. <i>atropurpurea</i>	Pl-6-1	(7)	Italian ryegrass (<i>Lolium multiflorum</i>)
C-160	<i>P. coronafaciens</i> subsp. <i>atropurpurea</i>	NO55-1	(7)	Italian ryegrass
C-161	<i>P. coronafaciens</i> subsp. <i>atropurpurea</i>	NO90-1	(7)	Italian ryegrass
C-137	<i>P. striafaciens</i>	NCPPB 1898 (ATCC 10730)	(2)	Oats
C-174	<i>P. striafaciens</i>	NCPPB 2394 (Pl-19-1)	(2)	Oats
C-80	<i>Pseudomonas</i> sp.	NCPPB 2390	(2)	Timothy
C-7	<i>P. syringae</i>	B-3	(3)	Peach (<i>Prunus persica</i>)
C-18	<i>P. andropogonis</i>		(4)	Maize
C-78	<i>P. andropogonis</i>	Original		Sorghum (<i>Sorghum vulgare</i>)
C-50	<i>P. lachrymans</i>	ATCC 11965	(5)	Cucumber (<i>Cucumis sativus</i>)
C-91	<i>P. aeruginosa</i>	D-5759	(6)	
C-92	<i>P. cepacia</i>	KC-1372	(6)	
C-94	<i>P. fluorescens</i>	KC-678	(6)	
C-132	<i>P. setariae</i>	ATCC 19882	(1)	

^a (1) American Type Culture Collection (ATCC), Rockville, Md.; (2) National Collection of Plant Pathogenic Bacteria (NCPBP), Harpenden, Hertfordshire, England; (3) H. English, University of California, Davis; (4) A. Ullstrup, Purdue University; (5) R. W. Goth, U.S. Department of Agriculture, Beltsville, Md.; (6) R. E. Weaver, Center for Disease Control, Atlanta, Ga.; (7) K. Nishiyama, National Institute of Agricultural Science, Tokyo, Japan; (8) R. D. Durbin, University of Wisconsin, Madison.

carbon source utilization, the methods of Palleroni and Doudoroff were utilized (27). Malonate tests were performed by the methods of Edwards and Ewing (9), except that incubation was at 25°C and results were read after 3 days. Growth at 0 and 41°C was determined by streaking KB slants and incubating them in ice in a cold room and at 41°C in a Hotpack incubator (model 351910), respectively. Nitrate reduction, starch hydrolysis, and litmus milk reaction were determined by the methods of Kiraly et al. (17). The methods of Stanier et al. (35) were used to test for oxidase and gelatin liquefaction. Lipid hydrolysis was determined in Difco nutrient broth containing sodium chloride. Melanin pigment production was determined in an L-tyrosine agar medium by the method of French and Sequeira (12). Lecithinase hydrolysis was performed by the NA, egg yolk method of Colwell (7) except that the egg yolk suspension was filtered through cheese cloth, then through 8.0-, 3.0-, 0.45-, and 0.3- μ m non-sterile membrane filters (Millipore Corp.), and finally through a 0.22- μ m sterile Millipore filter. Most nutritional tests were chosen because of their previous use in characterizing fluorescent plant pathogenic pseudomonads (23, 33).

Serological tests. Inject antigens from *Pseudomonas coronafaciens* C-107 and *P. coronafaciens* subsp. *atropurpurea* C-136 were prepared by harvesting the cells from 72-h KB agar slopes in 6 ml of sterile 0.85% sodium chloride (saline). The suspension was adjusted to 100 Klett units and made 2% Formalin, and 1 ml was emulsified with 1 ml of Difco incomplete adjuvant by using a high-speed stirrer. Rabbits were given four intramuscular injections at 10-day intervals. The animals were bled 7 days after the last injection, and the sera were stored at -20°C.

Tube agglutination tests, using somatic antigens (cells treated at 100°C for 1 h), were done following the methods of Carpenter (6). Smears for the indirect fluorescent antibody stain were made from a suspension of cells in 10% Formalin-0.85% saline. The smears were made on multiwell slides (Cell-Line Associates, Inc., Minotola, N.J.) with a 0.001-ml loop. After drying, the smears were fixed with Kirkpatrick fixative (60% ethanol, 30% chloroform, 10% Formalin), stained with the antiserum, and counterstained with a fluorescent anti-rabbit globulin (GIBCO Diagnostics, Grand Island, N.Y.) as described by Goldman (13). Antiserum and anti-rabbit globulin were diluted 1:4 and 1:16, as determined by results of a block test (12). After rinsing in phosphate-buffered saline (pH 8.0) and distilled water, the slides were air-dried and mounted in 0.5 M carbonate-buffered (pH 9.0) glycerin (41). Slides were observed with a Zeiss fluorescence microscope (40 $\times$ objective) equipped with a BG 12 excitation and 50 barrier filter. The intensity of the fluorescence was classified from (-) to +3, with (-) representing no visible fluorescence and +3 indicating intense fluorescence.

Pathogenicity tests. Cells were grown overnight at 23 to 25°C in medium 523 (15) on a rotary shaker, adjusted to 50 Klett units, and diluted to 10⁻² with sterile distilled water. Test plants were inoculated by injecting the cell suspension into the youngest leaves using a 25-gauge needle and 1.0-ml syringe. Plants were incubated for 4 to 12 days in a greenhouse at 20 to 27°C. Plant species used for pathogenicity tests

were: *Secale cereale* (rye) "Vitagraze," *Avena sativa* (oats) "Coker 66-22," *Triticum aestivum* (wheat) "Holley," *Hordeum vulgare* (barley) "Volbar," *Agropyron repens* (quackgrass), *Phleum pratense* (timothy) "Timfor," *Zea mays* (maize) "Pioneer 3030," *Bromus inermis* (smooth brome) "Sac," *Bromus japonicus* (Japanese brome) PI 239720, *Bromus secalinus* (chess brome) PI 235612, and *Bromus tectorum* (cheatgrass) PI 204433.

Toxin production. Cells were grown overnight in medium 523, and 1.5 ml of the cell suspension (approximately 100 Klett units) was added to 100 ml of Wooley medium (45) in a 250-ml Erlenmeyer flask. Cultures were grown for 5 days at 24°C on a rotary shaker. Sterile culture filtrates were prepared by Millipore filtration using a 0.22- μ m sterile filter and injected into test plant species. Incubation was identical to that used for pathogenicity tests.

RESULTS

Morphology. Cells of *P. coronafaciens* C-28, *P. coronafaciens* C-107, *P. coronafaciens* subsp. *atropurpurea* C-136, *P. coronafaciens* C-19, and *P. striafaciens* C-137 averaged 2.8 by 0.6, 2.3 by 0.8, 2.3 by 0.7, 2.8 by 1.0, and 2.3 by 0.8 μ m, respectively. Cells were rod-shaped with polar multitrichous flagella. A few cells of *P. striafaciens* C-137 were slightly curved, and filaments were present in preparations of *P. coronafaciens* C-19 and C-28.

Physiological and biochemical properties. The characteristics of 22 strains are summarized in Table 2. No significant differences among strains of *P. coronafaciens*, *P. coronafaciens* pathovar *zeae*, *P. coronafaciens* subsp. *atropurpurea*, and *P. striafaciens* were evident.

Serology. The different strains of *P. coronafaciens*, *P. coronafaciens* pathovar *zeae*, *P. coronafaciens* subsp. *atropurpurea*, *P. striafaciens*, and *Pseudomonas* sp. could not be differentiated by agglutination or fluorescent antibody tests (Table 3). All the strains reacted equally with antiserum to cells of *P. coronafaciens* or *P. coronafaciens* subsp. *atropurpurea*. Two other pseudomonads, *P. cepacia* and *P. fluorescens*, gave cross-agglutination titers similar to those of *P. coronafaciens*. However, no other pseudomonad gave a positive fluorescent antibody stain.

Host range. Individual strains differed in their pathogenicity and virulence on the 11 plant species tested. However, the only difference in host range that was detected among the strains of the supposed taxonomic groups was the failure of *P. striafaciens* strains to infect any plant species except oats (Tables 4 and 5).

Toxin production. Strains of *P. coronafaciens*, *P. coronafaciens* pathovar *zeae*, *P. coronafaciens* subsp. *atropurpurea*, and *Pseudomonas* sp. from timothy produced toxin which caused the typical halo chlorosis on most test

TABLE 2. *Phenotypic characters of Pseudomonas coronafaciens, P. coronafaciens pathovar zeae, P. coronafaciens subsp. atropurpurea, and P. striafaciens*^a

Characteristic	<i>P. coronafaciens</i> (N = 11)	<i>P. coronafaciens pathovar zeae</i> (N = 4)	<i>P. coronafaciens subsp. atropurpurea</i> (N = 6)	<i>P. striafaciens</i> (N = 2)
Utilization of:				
Sucrose	+	+	+	+
Glycerol	+	+	+	+
D-Glucose	+	+	+	+
D-Fructose	+	+	+	+
Capric acid	+	+	+	+
Mannitol	V (7+)	+	+	+
D-Mannose	V (7+)	+	+	+
Citrate	+	V (2+)	+	+
L-Erythritol	V (8+)	V (3+)	V (4+)	+
Maltose	V (6+)	V (3+)	±	±
Inositol	V (6+)	+	+	+
L-Ascorbic acid	±	±	±	—
L-Rhamnose	—	—	—	—
D-Cellobiose	—	—	—	—
β-Alanine	—	—	—	—
L-Tartaric acid	—	—	—	—
L-Arabinose	—	—	—	—
Lactose	—	—	—	—
Ethanol	—	—	—	—
Ethanolamine	—	—	—	—
n-Propanol	—	—	—	—
L-Leucine	—	—	V (1+)	—
D-Serine	±	±	±	±
Growth at:				
0°C	+	+	+	+
41°C	—	—	—	—
Growth on:				
HE agar	V (7+)	+	V (4+)	+
MacConkey agar	+	+	+	+
Salmonella-shigella agar	V (7+)	ND	V (2+)	ND
Growth in:				
NB—5% NaCl	±	±	±	±
NB—7% NaCl	—	—	—	—
Lipid hydrolysis	+	+	+	+
Litmus milk	Alk	Alk	Alk	Alk
Melanin pigment production	+	+	+	+
Fluorescent pigment production	+	+	V (5+)	+
Gelatin hydrolysis	V (6+)	V (3+, 1±)	V (1±)	—
Lecithinase	V (5+)	V (3+)	V (2+)	+
Malonate	—	V (3 Alk)	V (3+)	—
Arginine dihydrolase	—	—	—	—
Nitrate reduction	—	—	—	—
Starch hydrolysis	—	—	—	—
Oxidase production	—	—	—	—

^a Symbols and abbreviations: +, test was positive; —, test was negative; ±, weak or no response after 5 days, positive after 14 days; Alk, alkaline; V, variable; numerals indicate number of strains positive (+) or weak (±); NB, Difco nutrient broth; ND, not determined.

plants (Table 4). The three strains of *P. coronafaciens* subsp. *atropurpurea* from Japan and *P. striafaciens* did not yield detectable halo-inducing toxin from culture filtrates.

DISCUSSION

The phenotypic relationship among the plant pathogenic fluorescent pseudomonads has been

studied in recent years by Lelliott et al. (18), Misaghi and Grogan (23), and Sands et al. (33). Although these workers were able to differentiate plant pathogens from saprophytic pseudomonads, they were unable to differentiate the many species of plant pathogenic pseudomonads. In the 8th edition of *Bergey's Manual* (5), all plant pathogenic pseudomonads have been

TABLE 3. Tube agglutination titers and fluorescent antibody staining with antisera to strains C-107 and C-136

Antigen	Strain	Agglutination titers ^a		FA staining ^b	
		C-107	C-136	C-107	C-136
<i>P. coronafaciens</i>	C-28	160	80	+3	+3
<i>P. coronafaciens</i>	C-72	160	320	+1	+2
<i>P. coronafaciens</i>	C-81	80	80	+3	+3
<i>P. coronafaciens</i>	C-83	160	320	+3	+3
<i>P. coronafaciens</i>	C-107	80	80	+3	+2
<i>P. coronafaciens</i>	C-19	40	80	+2	+3
<i>P. coronafaciens</i> pathovar <i>zeae</i>	C-162	80	320	+2	+2
<i>P. coronafaciens</i> pathovar <i>zeae</i>	C-163	80	160	+2	+2
<i>P. coronafaciens</i> pathovar <i>zeae</i>	C-164	40	160	+2	+2
<i>P. coronafaciens</i> pathovar <i>zeae</i>	C-165	40	160	+2	+2
<i>P. coronafaciens</i> subsp. <i>atropurpurea</i>	C-82	640	80	+2	+2
<i>P. coronafaciens</i> subsp. <i>atropurpurea</i>	C-136	40	80	+3	+2
<i>P. coronafaciens</i> subsp. <i>atropurpurea</i>	C-142	80	320	+3	+2
<i>P. coronafaciens</i> subsp. <i>atropurpurea</i>	C-159	40	640	+3	+3
<i>P. coronafaciens</i> subsp. <i>atropurpurea</i>	C-160	640	320	+3	+3
<i>P. coronafaciens</i> subsp. <i>atropurpurea</i>	C-161	40	160	+3	+3
<i>P. striafaciens</i>	C-137	320	80	+1	+2
<i>P. striafaciens</i>	C-174	40	80	+3	+3
<i>Pseudomonas</i> sp.	C-80	80	80	+3	+3
<i>P. syringae</i>	C-7	—	—	—	—
<i>P. andropogonis</i>	C-18	—	—	—	—
<i>P. andropogonis</i>	C-78A	—	—	—	—
<i>P. lachrymans</i>	C-50	—	—	—	—
<i>P. aeruginosa</i>	C-91	—	—	—	—
<i>P. cepacia</i>	C-92	40	10	—	—
<i>P. fluorescens</i>	C-94	160	80	—	—
<i>P. setariae</i>	C-132	10	10	—	—

^a Values given are reciprocals of highest dilution that showed agglutination; — indicates no agglutination at lowest dilution tested (1:10).

^b FA, Fluorescent antibody. The intensity of the fluorescence was classified from (—) to +3, with (—) representing no visible fluorescence and +3 indicating intense fluorescence.

placed into eight nomenclatures. Pecknold and Grogan (29), using deoxyribonucleic acid-deoxyribonucleic acid hybridization, grouped 18 species including *P. coronafaciens* into four distinct genotypic groups, "syringae," "morsprunorum," "viridiflava," and "cichorii," and two artificial groups (due to insufficient data), "tomato" and "marginalis." *Pseudomonas coronafaciens* was included in their group "tomato." *Pseudomonas coronafaciens* showed less than 60% homology to *P. syringae*, *P. aptata*, *P. morsprunorum*, *P. phaseolicola*, *P. cichorii*, and *P. viridiflava*. DNA relatedness values of 70% or higher are considered an indication that the strains belong to the same species (4).

The 24 strains of bacteria studied here conform to the original description of *P. coronafaciens* (10). Colony types varied somewhat but were within the range described for the species.

Results of our physiological tests with *P. coronafaciens* agreed with the results of Sands et al. (33), using strains of *P. coronafaciens* NCPPB 1351, 1355, and 600, *P. delphinii* NCPPB 650, *P. dysoxylis* NCPPB 225, *P. garcae*

NCPPB 1399, *P. atrofaciens* NCPPB 117, and *P. glycinea* UMPP R1 (Department of Plant Pathology, University of Minnesota, St. Paul), except for one test, utilization of arabinose. Whereas Sands et al. (33) reported that all strains in the *P. coronafaciens* group (listed above) utilized arabinose, we found that none of our strains utilized the substrate.

Furthermore, our results of physiological tests with *P. striafaciens* confirms an earlier description of one of the same strains (Pl-19-1). Tomlinaga and Nishiyama (44) compared an unknown organism causing a stripe blight of oats in Japan to *P. coronafaciens* using 30 bacteriological characters and found the two organisms to be indistinguishable. The only difference between the two organisms was the failure of the stripe blight organism (strain Pl-19-1) to produce fluorescein pigment on KB medium. Because strain Pl-19-1 caused linear but not halo lesions in oats, and on the basis of results in an earlier report (11), they concluded that Pl-19-1 was *P. striafaciens* and not *P. coronafaciens*. Our results agree completely except that we did ob-

TABLE 4. *Host range of halo blight pathogens on cereals and grasses*^a

Strain	Rye (<i>Secale cereale</i>)	Oats (<i>Avena sativa</i>)	Wheat (<i>Triticum aestivum</i>)	Barley (<i>Hordeum vulgare</i>)	Quackgrass (<i>Agropyron repens</i>)	Timothy (<i>Pheleum pratense</i>)	Maize (<i>Zea mays</i>)
<i>P. coronafaciens</i>							
C-19	+,ln	ln	—	++,ln	—	+	—
C-20	sl	+,ln	—	—	—	—	—
C-23	+++	+++	+	—	ND	+	—
C-28	+++	+++	sl	+	++	+	—
C-64	+++	+++	+	++	sl	+	—
C-73	+	+	sl	+	+	+	—
C-80	++	++	+	—	sl	++	—
C-83	—	—	—	—	ND	—	—
C-86	+++	+	+++	++	ND	—	—
C-107	+++	+++	sl	++	+	+	—
<i>P. coronafaciens</i> pa- thovar <i>zeae</i>							
C-162	—	+++	—	—	+++	++	+
C-163	+++	—	—	+	+++	+++	+++
C-164	sl	+++	—	—	+++	sl	++
C-165	—	sl	—	—	+++	++	+++
<i>P. coronafaciens</i> subsp. <i>atropur- purea</i>							
C-82	+	++	—	—	sl	+	—
C-136	—	+++	—	—	+	++	—
C-142	sl	++	sl	sl	sl	sl	sl
C-159	++	++	sl	—	—	sl	+
C-160	++	+	+	—	—	sl	+
C-161	+++	+	—	—	—	sl	+
<i>P. striafaciens</i>							
C-137	—	ln	—	—	—	—	—
C-174	—	ln	—	—	—	—	—

^a Symbols and abbreviations: +, halo 1 to 3 mm in diameter; ++, 3 to 7 mm in diameter; +++, >7 mm in diameter; ln, linear necrosis; sl, slight halo; —, no symptoms; ND, not determined.

serve the presence of a fluorescent pigment when individual colonies were observed under an ultraviolet light. We do not agree that such minor differences warrant creating a new species, however.

Tominaga (42) found minor physiological differences among strains of bacteria causing bacterial blight of ryegrasses, fescues, and brome-grasses. Because the strains infected all three plant species he considered them all to be *P. coronafaciens* subsp. *atropurpurea*. In further tests, one strain from Italian ryegrass infected a wide range of small grains and other grasses, including oats and rye. Tominaga's results are consistent with the host range data presented here. Tessi (40) also reported a wide host range for a *P. coronafaciens* strain from oats. Among the susceptible hosts were rye and smooth brome-grass.

Tessi (40) presented convincing evidence that strains of *P. striafaciens* were *P. coronafaciens* strains that had lost the ability to produce toxin.

After 50 subcultures of a strain of *P. coronafaciens*, the ability to produce toxin was lost, and the symptoms produced on oats were identical to those caused by *P. striafaciens*.

Although we found that three strains of *P. coronafaciens* subsp. *atropurpurea* (C-159, C-160, and C-161) also failed to produce any detectable toxin in Wooley medium, they were similar to *P. coronafaciens* in all other characters. Furthermore, symptom expression on susceptible hosts was similar to the other strains of *P. coronafaciens* subsp. *atropurpurea* that we tested, which did produce toxin in Wooley medium. Whether or not the strains that failed to produce toxin in Wooley medium should be designated as separate biovars will require further characterization of their toxins.

Pseudomonas coronafaciens subsp. *atropurpurea* was described on the basis that strains of *P. coronafaciens* from oats did not infect smooth brome-grass, whereas those from smooth brome-grass were able to infect oats (31). Simi-

TABLE 5. *Host range of halo blight pathogens on Bromus species*^a

Strain	Smooth brome (<i>B. inermis</i>)	Japanese brome (<i>B. japonicus</i>)	Chess brome (<i>B. secalinus</i>)	Cheatgrass (<i>B. tectorum</i>)
<i>P. coronafaciens</i>				
C-28	++	++	++	++
C-73	+	+	+	+
C-80	+	++	++	++
C-86	++	++	++	++
C-107	+++	—	+	+
<i>P. coronafaciens</i> pathovar <i>zeae</i>				
C-162	+++	+++	+++	+++
C-163	+++	++	+++	+++
C-164	+++	++	+++	++
C-165	+++	+++	+++	+++
<i>P. coronafaciens</i> subsp. <i>atropurpurea</i>				
C-82	+	+	++	—
C-136	++	++	++	+
C-142	++	++	++	++
C-159	+++	+++	+++	+++
C-160	++	++	+++	+++
C-161	+	+++	++	+++
<i>P. striafaciens</i>				
C-137	—	—	—	—
C-174	—	—	—	—

^a Symbols: —, no infection; +, halos 1 to 3 mm in diameter; ++, 3 to 7 mm in diameter; +++, >7 mm in diameter.

larly, *P. coronafaciens* pathovar *zeae* was described on the basis that strains of *P. coronafaciens* failed to infect corn and the new organism failed to infect oats. However, we found that, with the exception of corn, *P. coronafaciens* pathovar *zeae* infected the same hosts as *P. coronafaciens*. To create a pathovar or subspecies on such small differences would create chaos in bacterial taxonomy. On the other hand, there is some merit in considering *P. striafaciens* a pathovar of *P. coronafaciens*, since it infected only a single common host and produced very distinct symptoms on that host. However, until additional strains are available for study we feel that pathovar status is unwarranted.

Serological techniques have recently shown promise for identifying pseudomonads, including *P. atrofaciens* (28), *P. fuscovaginae* (38), *P. lachrymans* (21, 37; R. G. Grogan, L. T. Lucas, and K. Kimble, *Phytopathology* 55:1060, 1965), *P. phaseolicola* (14, 39; Grogan et al., *Phytopathology* 55:1060, 1965), *P. pseudoalcaligenes* subsp. *citrulli* (34), *P. solanacearum* (8, 24, 30), *P. syringae* (20, 22, 25, 26), *P. tabaci* (19), *P. avenae* (43), and *P. andropogonis* (43). The present study reveals that antiserum to cells of *P. coronafaciens* contains heteroagglutinins to *P. fluorescens* and *P. cepacia*. With these exceptions, agglutination tests with the various pseu-

domonads (Table 3) were highly specific. Fluorescent antibody staining was highly specific. No cross-reaction with another pseudomonad was observed. The failure of our antiserum to *P. coronafaciens* and *P. coronafaciens* var. *atropurpurea* to react with *P. andropogonis* agrees with a previous report that antiserum to *P. andropogonis* failed to react with *P. coronafaciens* var. *atropurpurea* (43).

Because of the variability in virulence of strains of *P. coronafaciens*, it is important to inoculate oats, corn, and a *Bromus* sp. Pathogenicity on any one of the above hosts would suggest *P. coronafaciens*; however, identification should be confirmed by serology, preferably indirect immunofluorescent staining.

As evident from the data presented in Tables 2 through 5, there are only minor differences among *P. coronafaciens*, *P. coronafaciens* pathovar *zeae*, *P. coronafaciens* subsp. *atropurpurea*, and *P. striafaciens*. Therefore, we regard all these names as subjective synonyms.

Pseudomonas coronafaciens, being the earliest name, has priority and is therefore the correct name of this species; *P. coronafaciens* subsp. *atropurpurea* and *P. striafaciens* become junior synonyms of *P. coronafaciens*. The neotype strain of *P. coronafaciens* is strain ATCC 19607 (C-28). Whether or not *P. coronafaciens*

should be included with or is a synonym of another established *Pseudomonas* species will await more detailed studies.

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REPRINT REQUESTS

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