Characteristics of *Pseudomonas* spp. Causing Grain Discoloration and Sheath Rot of Rice, and Associated Pseudomonad Epiphytes

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ABSTRACT

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Ninety-five strains of fluorescent (presumed to be *Pseudomonas* spp.) and nonfluorescent pathogens of rice causing grain and sheath rot, dirty panicle, and *manchado de grano*, and 21 strains of nonpathogenic fluorescent pseudomonads isolated from rice grain and sheaths from 22 countries were compared with 26 reference strains (*Pseudomonas avenae*, *P. fuscovaginae*, *P. glumae*, *P. marginalis*, and *P. syringae*) with the use of morphology, serology and 77 physiological traits. A Ward's minimum variance cluster analysis grouped the strains into seven clusters corresponding to six bacterial species: *P. fuscovaginae* (two clusters), *P. avenae*, *P. fluorescens*, *P. glumae*, *P. putida*, and *P. syringae*. Every strain from Chile that caused sheath and grain rot was *P. syringae*. The rest of the pathogenic fluorescent strains were consistent with *P. fuscovaginae* but were grouped into two different clusters, suggesting that further study of this pathogen is warranted. The nonfluorescent pathogens were either *P. avenae* or *P. glumae*, and no pathogenic *Erwinia* spp. were encountered. Approximately 25% of the strains of *P. syringae* and *P. fuscovaginae* agglutinated with their respective heterologous antisera, and up to 75% of *P. avenae* and *P. glumae* agglutinated with their respective antisera. There was less than 10% agglutination between fluorescent and nonfluorescent pathogens and their respective antisera. The nonpathogenic strains did not react with any antisera. It is concluded that four bacterial species causing grain or sheath rot of rice were encountered in this study. They are *P. fuscovaginae*, *P. syringae* pv. *syringae*, *P. avenae*, and *P. glumae*, with *P. fuscovaginae* and *P. avenae* the most common. These rice pathogens, formerly believed to be of limited distribution, are shown to be distributed worldwide.

Recently, there have been numerous new reports of bacterial diseases of rice (*Oryza sativa* L.) that affect the grain, seedling, and flag leaf sheath, the occurrence and distribution of which are only now being understood (1,3,6,7,15,24,28, 33,35,36). Fluorescent and nonfluorescent pathogenic *Pseudomonas* spp. have been isolated from diseased rice in Latin America, Africa, and Asia; however, most of them have not been characterized adequately. The symptoms apparently caused by these *Pseudomonas* spp. are similar, preventing reliable diagnosis based on symptomatology only. The symptoms also resemble those caused by some fungal pathogens, such as *Sarocladium* spp. and *Dreschelera* spp. (12,33).

Typically, in seedlings a systemic discoloration of the sheath develops that may continue up the midrib or secondary veins of leaves and may lead to death of the plant. In mature plants, symptoms usually appear near flowering. They may be limited to brown necrosis of the collar of the flag leaf sheath or involve extensive necrosis of the sheath, poor panicle emergence, and grain discoloration and sterility. Alternatively, the florets become discolored, necrotic, and sterile, whereas the leaf and sheath remain healthy. The development of grain symptoms does not depend upon prior appearance of sheath symptoms. The various diseases and their causal agents and distributions, which have been described, can be grouped into three classes according to the previously mentioned symptoms (seedling necrosis, sheath rot, and grain rot; Table 1).

Various pseudomonads appear to have a causal role in sheath and grain rot of rice, whereas other bacteria, including *Erwinia* spp., are reported to cause grain discoloration or seedling stripe (7,9,26). Three *Pseudomonas* spp. and several unidentified nonpseudomonads were isolated from grain in Japan (10). Four named species of *Pseudomonas* have been associated with grain discoloration and sheath rot in rice, including *P. fuscovaginae* Miyajima, Tanii and Akita, *P. syringae* pv. *syringae* van Hall, *P. avenae* Manns, and *P. glumae* Kurita and Tabei (3,8,15,19,23,27,30,33). Also, in Chile a fluorescent *Pseudomonas* spp. similar to *P. s.* pv. *syringae* was isolated from and found to cause sheath rot of rice (36), but it has not been fully characterized. *P. s.* pv. *panici* (Elliott) Young et al causes a seedling stripe, and *P. plantarii* Azegami et al causes a seedling blight.

Several of the pseudomonads described here have been associated with a poorly defined grain syndrome called *manchado de grano* (= dirty panicle and grain discoloration; 5,20,33,36). This problem has increased in significance and has received increased attention from rice researchers (17). The *Pseudomonas* spp. causing these symptoms have been shown to be seed-transmitted (13,28,33) and are recovered from discolored as well as apparently healthy seed. Although we have successfully eradicated pathogenic pseudomonads from seed (33), we have not observed a reduction in disease levels in the field when conditions are favorable. The pathogens have a wide host range and large populations may reside on weeds (37). The ubiquitous presence of nonpathogenic epiphytic pseudomonads, such as *P. putida* and *P. fluorescens*, recovered from healthy and diseased seed and sheaths complicates diagnosis and epidemiological studies and raises questions as to the ecological relationships among the pseudomonad components of epiphytic flora of rice.

Clarification of the epidemiology and etiology is needed to develop control and management strategies for this problem. This study was conducted to determine the principal bacterial pathogens involved in rice grain and sheath rot, clarify the relationship of the different causal agents and distinguish them clearly from associated epiphytic pseudomonad flora of rice, and resolve conflicting reports on their physiological characteristics.

MATERIALS AND METHODS

Strains. Thirty reference strains included were: *Pseudomonas avenae* (NCPPB 1011, 1392, 3354, and 3356–58); *P. fluorescens* Migula (from cassava, provided by J. C. Lozano, CIAT); *P. fluorescens* biovar II (pathogenic on *Centrosema* spp., provided by J. Lenne, CIAT); *P. fuscovaginae* (NCPPB 3085 [two subcultures], PDDCC 5939–5941); *P. glumae* (NCPPB 2981, PDDCC 3727–29); *P. marginalis* pv. *alfalfae* (Shinde and Lukezic) Young et al (NCPPB 2644); *P. m.* pv. *pastinacae* (Burkholder) Young et al (NCPPB 806); *P. syringae* pv. *japonica* Dye et al (NCPPB 3093, PDDCC 6305); *P. s.* pv. *panici* (NCPPB 1498, PDDCC 3955); *P. s.* pv. *striafaciens* (Elliott) Young et al (NCPPB 1898, 2394; PDDCC 4483); and *P. s.* pv. *syringae* (NCPPB 388, 1417). The 143 strains of the incompletely characterized bacteria used in the test included 113 pathogenic and nonpathogenic strains isolated from diseased rice originally collected during routine ex-

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Table 1. Symptoms, disease name, causal agents, and distribution of the principal bacterial diseases reported to affect rice seedlings, sheath, and grain

Symptom type	Disease name	Causal agent	Distribution
Seedling necrosis	Bacterial stripe	*P. syringae* pv. *panici*	Asia
	Bacterial stripe	*P. avenae*	Worldwide
	Brown stripe	*Erwinia* sp.	Brazil
	Seedling rot	*P. glumae*	Japan, South America
	Seedling blight	*P. plantarii*	Japan
Sheath rot	Bacterial sheath rot	*P. syringae* pv. *syringae*	Hungary, Australia, Asia
	Bacterial sheath brown rot	*P. fuscovaginae*	Worldwide
Grain discoloration	Black rot	*Erwinia herbicola*	
	Bacterial grain rot	*P. glumae*	Japan, Latin America
	Glume blotch	*P. syringae* pv. *syringae*	Hungary, Australia
	Manchado de grano	*P. fuscovaginae*	Worldwide
	Grain rot	*P. syringae* pv. *aptata*	Japan
	Cinnamon speck	*Xanthomonas cinnamona*	Japan
	Black-eye spot	*X. atroviridigenum*	Japan

Table 2. Geographical origin, tissue of origin, and pathogenicity on rice seedlings of test strain

Geographical origin	Tissue origin	Rice pathogenicity[a]	CIAT reference number[b]
Argentina	Seed	+	25, 26
Bolivia	Seed	+	203, 204
Brazil	Seed	+	1–8, 33, 140, 146, 173–175
Brazil	Seed	−	52, 117, 190
Brazil	Sheath	+	59
Chile	Seed	+	14–17, 48, 49
China	Seed	+	58, 84, 138, 139
China	Seed	−	187, 96–100, 118
Colombia	Seed	+	22, 28, 34–36, 55, 56, 70, 71, 78, 113, 171, 201
Colombia	Seed	−	102, 185
Colombia	Sheath	+	27, 119
Colombia	Leaf	+	31
Costa Rica	Seed	−	106, 110
Cuba	Seed	−	105
Dominican Republic	Sheath	+	30
Ecuador	Seed	+	53, 54
El Salvador	Seed	−	121
Guatemala	Seed	−	156
Guatemala	Sheath	+	32
Guatemala	Sheath	−	153
Madagascar	Seed	+	188
Mexico	Seed	+	182
Nepal	Seed	+	72, 73
Nicaragua	Seed	+	9–13
Nicaragua	Seed	−	120
Nicaragua	Sheath	+	122–132
Panama	Seed	+	172
Panama	Seed	−	166
Panama	Sheath	−	165
Peru	Seed	+	57, 137, 142–145
Peru	Sheath	+	133–136, 141
Philippines	Seed	+	20, 23, 61, 62, 64
Philippines	Seed	−	101
Philippines	Sheath	+	24, 60
Sierra Leone	Seed	+	74
Thailand	Seed	+	202
Turkey	Seed	+	116

[a] Pathogenicity determined by stem puncture of 10^7 cfu aqueous suspension of strain into 20–30 day-old rice seedlings (cv. Oryzica 1).
[b] As they appear in Fig. 1.

change of germ plasm or from an earlier distribution study (36; Table 2). Strains were maintained at 27 C on Difco nutrient agar (NA) and transfered twice weekly. At the outset of the study, subcultures of all strains were lyophilized in equal volumes of 5% peptone and 20% sucrose.

Physiological and nutritional characters. Fresh, 24-hr-old cultures on NA were tested for production of the following (22): fluorescent pigment on King's medium B, oxidase, protease (gelatin liquefaction), amylase (starch hydrolysis), levan from sucrose, arginine dihydrolase, tolerance to 5% sodium chloride, pectolytic enzymes (CVP gel pitting), soft rot of potato, hydrolysis of esculin, H_2S from cysteine, urease (modified YS broth with 0.0016% cresol red w/v), poly-β-hydroxybutyrate, hypersensitivity in tobacco (cv. Burley), and growth on sorbitol neutral red agar. The basal salt medium of Ayers et al (2) was used in carbon source (0.1% w/v) utilization tests. One ml/l of 1.6% bromthymol blue was added to detect acid production (except for 2-ketogluconate reduction and use of polygalacturonic acid). Growth at 4, 37, or 41 C was tested in yeast salts broth (4). Nitrate reduction was tested by the method of Azegami et al (3). Gram stain was determined by the KOH stringing method (25); catalase, casein hydrolysis, and lecithinase by the methods of Blair et al (4); and utilization of polygalacturonic acid and reduction of 2-ketogluconate by the methods of Misaghi and Grogan (14). A differential medium for *P. glumae* (SPG) was prepared as described by Tsushima et at (29). The clustering of the strains and the factor analysis was performed according to Ward's method of minimum variance (21). Only phenotypic characters that yielded different reactions were included in the analysis and were coded as 1 (positive) or 0 (negative). Serological reaction, cell morphology, and colony characteristics were not included in this analysis.

Pathogenicity and symptomatology. At the outset and end of the study, 20–30 day-old rice seedlings (cv. Oryzica 1) were inoculated with each strain by puncturing stems with a sterile needle that had been dipped in an aqueous suspension (10^7 cfu/ml). Strains that produced necrosis more than 5 mm beyond the wound within 7 days were considered pathogenic. If a strain lost pathogenicity, fresh colonies were retrieved from lyophilized subcultures. Where retrieval of pathogenic strains was unsuccessful, the strain was dropped from the study. However, we included two strains (CIAT 187 and 185) that lost pathogenicity before the study. Mature rice plants (cv. Oryzica 1) were inoculated by spraying the boot or panicle (5% flowering) to runoff with an aqueous suspension (10^7 cfu/ml). The plants were incubated for 48 hr at 100% RH, 24 C, transferred to the greenhouse, and observed 14 days after inoculation.

Serological reactions and morphology. Twenty-five μl of a bacterial suspension (10^5 cfu/ml of saline) was mixed with 0.5 ml of a 1:1 or 1:100 saline dilution of antisera prepared from *P. avenae* (NCPPB 1392), *P. glumae* (NCPPB 2981), *P. syringae* (CIAT 17), and *P. fuscovaginae* (PDDCC 5940; 34) in polystyrene Microelisa plates. The plates were agitated for 1 hr at 27 C, and agglutination was determined visually and compared to bacteria and saline or serum and saline checks. Cell dimensions were measured at ×1,000 with an ocular micrometer under the light microscope for 100 cells from two to six randomly chosen strains (24 hr on NA) from each cluster.

RESULTS

Physiological and nutritional characters. The test strains were grouped into seven distinct clusters (Fig. 1) based on their physiological characteristics (Table 3). Rice pathogens were found only in clusters 1, 2, 4, 5, and 7, and none clustered with the nonpathogens (clusters 3 and 6). Clusters 2 and 4–7 included reference strains from only one species (*P. fuscovaginae*, *P. syringae*, *P. avenae*, *P. marginalis, and P. glumae*, respectively), while clusters 1 and 3 included no reference strains. Cluster 1 showed extreme differences from cluster 2 only in acid production from glucose. Cluster 3 differed substantially from clusters 1 and 2 in nitrate reduction; utilization of cellobiose, maltose, valine, phenylalanine; gelatin hydrolysis; acid from mannose and dextrin; potato rotting; growth on SPG; production of lecithinase and lipase; induction of hypersensitive reaction on tobacco; and pathogenicity on rice. All pathogenic strains were able to grow on SPG, a medium reported to be specific for *P. glumae* (28).

Pathogenicity and symptomatology. Selected strains (all from Colombia, with the exception of PDDCC 3727) in clusters 1, 2, 4, 5, and 7 caused disease on rice panicles after inoculation at the booting and flowering stages. Strains in clusters 1 and 2 caused severe discoloration and rotting of the flag leaf sheath. Symptoms began as watersoaked lesions in an irregular blotched pattern that became necrotic and in severe cases covered up to 100% of the sheath. In an earlier study (36), strains from cluster 4 caused identical symptoms. Occasionally, the panicle did not emerge and rotted within the boot. Spikelet sterility exceeded 50% in some cases and grains that matured were partially to completely discolored. Frequently, the infection became systemic and extended up the midrib or veins of the flag leaf. Fluorescent pathogenic bacteria were always recovered from tissue. The symptoms caused by strains from these clusters were indistinguishable, although severity of

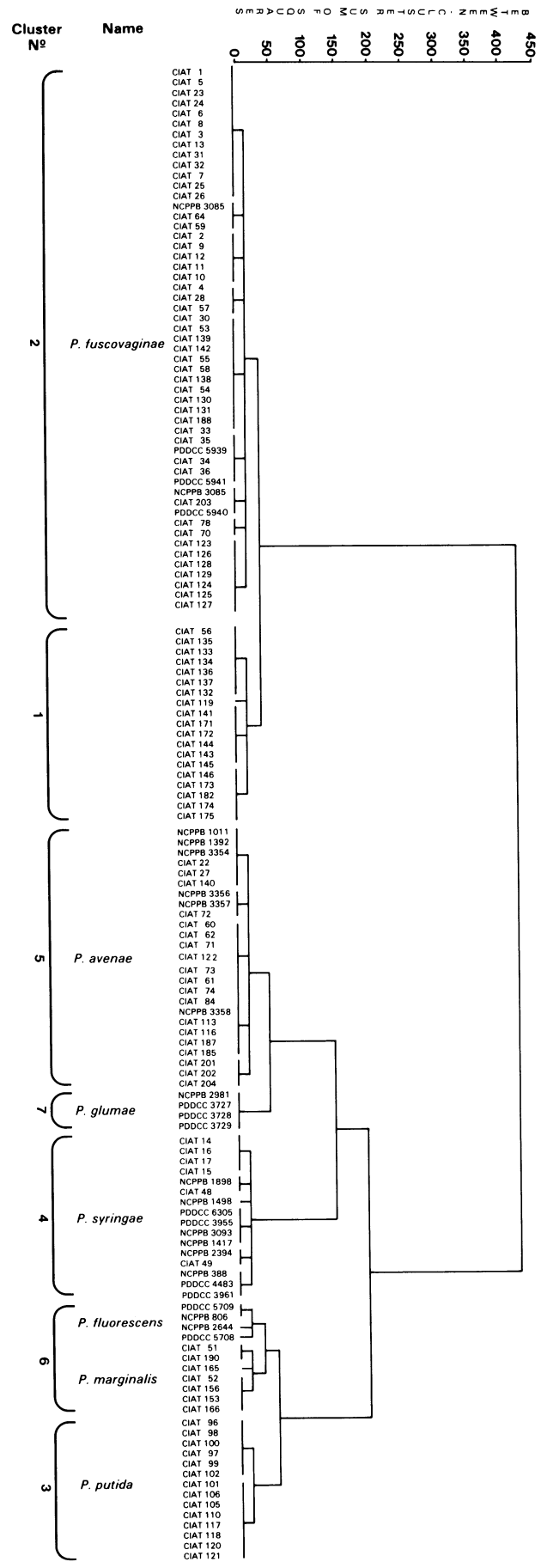

Fig. 1. Dendogram summarizing Ward's minimum variance clustering of reference strains and test strains of *Pseudomonas* spp. from rice, based on phenotypic traits.

symptom expression differed among the strains.

Strains from clusters 5 and 7 caused only slight discoloration of the flag leaf sheath, typically restricted to the collar. However, those of cluster 7 caused extreme spikelet sterility (up to 75%). Sterile spikelets were pinkish toward the distal end of the lemma and palea, with the basal portion discolored to a dark brown. Filled grains had no distinct lesions, rather a rusty to dark brown complete discoloration. Strains from cluster 5 caused considerably less spikelet sterility but identical symptoms on filled grain. Winnowed grain samples taken from plants inoculated with strains from clusters 1, 2, 4, 5, and 7 could not be distinguished reliably.

Serological reactions and morphology. On NA, all strains of clusters 1 and 2 produced convex, mucoid, cream-colored colonies with erose to entire margins and smooth to slightly rugose surfaces. After 48 hr, they reached 48 mm in diameter and, after 72 hr, crystals were produced near the colony center. Colonies produced by strains in cluster 4 were very similar, but the margin was entirely to only slightly irregular and the surface was smooth. Colonies produced by strains in cluster 5 were more mucoid with margins that were clear and extremely irregular to tenticulate. Colonies from strains in cluster 7 had entire to erose margins with slightly rugose, convex form.

All reference strains designated as *P. fuscovaginae* agglutinated with the antiserum of *P. fuscovaginae* at 1:1 dilution (Table 4); but only 75% of the test strains in clusters 1 and 2 did so. None of the remaining 25% agglutinated with any of the other antisera. Similarly, all of the strains from clusters 1 and 2 that agglutinated with antisera to strains CIAT 17 (tentatively identified as a *P. syringae*), *P. avenae*, or *P. glumae* also agglutinated with the antiserum of *P. fuscovaginae*. The strains that did not agglutinate with any antisera, or that agglutinated with antisera produced from other species, were found both in clusters 1 and 2 and showed no similarity in geographical or tissue origin.

A similar pattern of agglutination was observed between strains from cluster 4 and the antiserum to strain CIAT 17. Both reference strains from Hungary (the original *P. oryzicola*) agglutinated with antiserum from *P. fuscovaginae*, while only one agglutinated with antiserum from CIAT 17. *P. s.* pv. *phaseoli* did not agglutinate with this antiserum, while strains of *P. s.* pv. *striafaciens* and pv. *japonica* did. There was considerable reciprocal agglutination among strains of *P. glumae* and *P. avenae* and their respective antisera as well.

DISCUSSION

The fluorescent *Pseudomonas* spp. that cause sheath rot and/or grain dis-

Table 3. Percent of 143 strains of *Pseudomonas* spp. that were positive for differential phenotypic characters used for assignment to seven clusters

	Percentage of strains positive						
	Pathogens (cluster no.)[a]					Nonpathogens (cluster no.)	
Character[b]	1	2	4	5	7	3	6
Fluorescent pigment*	100	100	100	0	0	100	100
Oxidase*	79	96	0	92	0	93	100
Arginine dihydrolase*	100	100	0	0	0	100	100
Nitrate reduction*	11	20	0	96	0	100	45
Utilization for growth:							
Cellobiose	89	52	0	0	100	0	0
Dextrose	15	83	87	92	50	71	81
Fructose	94	88	75	92	100	93	90
L-arabinose	84	98	100	60	100	100	100
L-rhamnose	0	0	56	84	100	0	9
Maltose	0	1	68	64	100	100	36
Sucrose*	0	0	100	0	0	0	100
Raffinose	0	1	62	0	100	0	36
Xylose	57	62	100	100	75	50	81
Acetate	100	100	75	96	100	100	100
Citrate	94	100	100	92	100	100	100
Malonate	100	98	100	100	100	100	100
Adonitol	0	3	43	4	100	0	63
Erythritol	0	0	100	0	0	0	45
Glycerol	36	30	100	100	100	14	100
Inositol*	0	7	100	0	100	0	90
Sorbitol	0	0	100	100	75	0	100
Arginine*	100	100	0	0	75	100	100
β-alanine	89	94	75	76	25	100	100
Hystidine	100	100	100	100	100	100	90
Inuline	5	0	0	92	100	0	0
Lysine	100	100	62	76	0	100	81
Ornithine	100	100	43	8	0	100	72
Phenylalanine	0	1	43	8	0	100	63
Salicin	52	7	0	0	100	0	100
Valine	100	100	0	0	0	0	0
Polygalacturonic acid	0	0	0	4	0	28	0
L-α-phosphatidylcholine	0	0	31	20	0	57	63
Growth at:							
4 C	94	92	18	100	100	100	100
37 C*	5	20	0	100	100	14	0
41 C*	0	1	0	100	100	0	0
Casein hydrolysis	0	7	68	100	100	0	36
Aesculin hydrolysis	0	0	0	8	0	0	0
Starch hydrolysis*	0	0	0	100	0	0	0
Gelatin liquefaction*	100	100	100	0	50	14	100
Levan production from sucrose*	0	0	100	0	0	0	100
H_2S from cysteine*	52	1	0	76	0	57	0
Acid from:							
Glucose*	5	90	68	76	75	100	81
Lactose	0	15	0	4	0	0	0
Mannose	100	94	31	16	25	14	63
Dulcitol	0	0	75	52	0	64	81
Mannitol	100	66	50	76	100	100	63
Dextrin	0	0	100	96	75	100	90
α-Methyl-d-glucoside	10	13	37	4	0	21	9
Urease production	5	0	0	0	0	0	0
Potato soft rot	73[c]	66[c]	43[c]	16[c]	0	0	0
Sodium chloride tolerance (5%)	5	13	100	96	100	57	36
Growth on:							
Sorbitol neutral red medium	0	1	100	100	100	0	0
SPG	100	98	100	100	100	0	0
Gas production from TSI	0	15	12	4	0	0	36
Catalase test	100	98	100	100	75	100	63
2-ketogluconate reduction	0	0	0	0	0	64	54

(*continued on next page*)

[a] From Ward's minimum variance method (Fig. 1). Includes test strains and reference strains. Reference strains: cluster 2 = P. *fuscovaginae*; cluster 4 = *P. syringae* (four pathovars); cluster 5 = *P. marginalis*; cluster 7 = *P. glumae*. No reference strains were grouped in clusters 1 and 3.

[b] All strains were negative for Gram stain (KOH), indol and acetoin production, gas production from all carbohydrates, pectate gel pitting, and acid from all carbohydrates except those shown as positive. Asterisks indicate principal characteristics of test strains in clusters.

[c] Weakly positive.

[d] Not included in cluster analysis. Values followed by the same letter do not differ significantly ($P = 0.05$).

Table 3. (*continued from preceding page*)

Character[b]	Percentage of strains positive: Pathogens (cluster no.)[a] 1	2	4	5	7	Nonpathogens (cluster no.) 3	6
PHB granules	0	0	0	100	100	0	0
Lecithinase*	84	96	0	4	100	0	0
Methyl red reaction	0	0	0	4	0	14	27
Lipase (Tween 80) hydrolysis*	100	100	0	0	0	28	18
Tobacco hypersensitivity	100	100	100	100	100	0	0
Pathogenicity test*	100	100	100	100	100	0	0
Cell length (μm)[d]	2.8a	2.9a	2.2b	2.0b	1.6c		
No. of strains	19	53	16	25	4	14	11

coloration of rice were grouped into three clusters based on their phenotypic characters (Fig. 1, Tables 3 and 4). Cluster 4 included the strains from Chile and all reference strains of *P. syringae*. Moreover, the antiserum developed from a strain of *Pseudomonas* from Chile was more specific in reaction to reference strains of *P. syringae* than to strains of *P. fuscovaginae*. Thus, the designation of the pathogen from Chile as *P. s.* pv. *syringae* (36) appears correct. This pathogen has been associated with diseased rice in Hungary (13), Australia (6), and several Asian countries (11). However, a second pathovar of *P. s.* pv. *aptata* (Brown and Jamieson) Young et al, has been reported to cause grain discoloration of rice in Japan as well (10). Because phenotypic characters are typically of little use in distinguishing pathovars of *P. syringae* (18,32), it is not clear why a pathogen of rice within *P. syringae* should not be pv. *syringae*.

All remaining fluorescent strains pathogenic on rice from 15 countries from Africa, Asia, and Latin America fell in clusters 1 and 2. Only differences in the percentages of strains that were positive for characters of little taxonomic importance separated these two clusters that contained the reference strains of *P. fuscovaginae*. The two subcultures of NCPPB 3085 were included in cluster 2. The characteristics of these strains were very consistent with those reported by others (15,16,19,27). However, a number of strains were weakly positive for soft rot of potato. There were no differences in geographical origin, tissue of origin, or serological reaction between the two clusters either. Therefore, the strains in the two clusters should be considered as taxonomically similar. Because the only reference strains that grouped into clusters 1 and 2 were *P. fuscovaginae*, and because most strains reacted strongly to the antiserum produced from the type strain of *P. fuscovaginae* (Table 4), they all should be considered to be this species. The incomplete description of the cinnamon speck pathogen (*Xanthomonas cinnamona* [Miyake and Tsunoda] Muko; 17) is not inconsistent with *P. fuscovaginae* as described here, while that of *X. atroviridigenum* (Miyake and Tsunoda) Tagami and Mizukami (17) differs in gelatin liquefaction. In earlier studies, *P. marginalis* appeared to be involved in sheath and grain rot and had some similarity with *P. fuscovaginae* (9,33). However, neither this study nor others (7,19) supported this similarity; therefore, *P. fuscovaginae* should be considered to be distinct from *P. marginalis* (*P. fluorescens* biovar II). The fluorescent nonpathogens, *P. putida* and *P. fluorescens*, which are commonly recovered from rice grain, have been clearly separated from the fluorescent pathogens of rice grains.

The nonfluorescent pathogens primarily caused grain discoloration and floret sterility with little or no sheath symptoms. They were morphologically distinct from the fluorescent strains as well and grouped into two different clusters. All the nonfluorescent strains in the original group (including the two strains that had lost pathogenicity) are considered to be *P. avenae* because they grouped into cluster 5 with reference strains of *P. avenae*.

There have been different reports as to the characteristics of *P. avenae* and *P. glumae*, both of which have been included in section V of *Bergey's Manual* for those species whose relationships with the better characterized species in the genus have not been well-defined. Our results were similar to those reported by Schaad et al (23) for *P. avenae*, except for oxidase, which they reported to be negative. Azegami et al (3) compared *P. glumae* with *P. avenae* with results very similar to those reported here, including a positive oxidase reaction for *P. avenae*. However, their strains did not grow in 5% NaCl, whereas ours did. They also report negative results for tobacco hypersensitivity, lipase, and H_2S production, while our strains were positive, negative, and variable, respectively. The strains identified as *P. avenae* and *P. glumae* also accummulated poly-β-hydroxybutyrate crystals abundantly as reported by others (3,22). In the original description of *P. glumae*, the organism produced a fluorescent pigment on potato agar. However, this probably referred to the diffusible green nonfluorescent pigment that we and others (3) have observed to be produced by some strains. The "growth limit" *sic* (17) for *P. glumae* has been reported to be 40 C; however, the strains in this study all grew at 41 C and those reported by Azegami et al (3) grew at 40 C.

Although *Erwinia* spp. have been implicated in grain discoloration or sheath symptoms of rice by others (7,9,10), none of the nonfluorescent strains in this study were members of that genus. *Erwinia herbicola* (Lohnis) Dye is commonly isolated from healthy and discolored rice grain and frequently overgrows pathogenic *Pseudomonas* spp. Mixtures of strains of *E. herbicola* and pathogenic *Pseudomonas* spp. may cause symptoms on inoculated rice seedlings, but we have never found pure strains of *E. herbicola* to be pathogenic on rice.

The serological relationships among the strains are consistent with those reported elsewhere (15,19,31,36). There is some reciprocal agglutination among the rice pathogens, particularly among the fluorescent species and between the nonfluorescent species. There is little reciprocal agglutionation between the nonfluorescent and the fluorescent species and none between rice nonpathogens and any of the antisera tested. Therefore, agglutination with crude antisera offers little in the way of distinguishing pathogenic species. Similarly, agglutination could not be used to separate pathogens from nonpathogens because there may be a large number of false negative reactions. However, increased specificity offered by monoclonal antibodies should be explored.

While there are currently described at least four *Pseudomonas* spp. that can cause grain discoloration of rice, their distributions overlap and the symptoms they cause on grain may be very similar. *Pseudomonas fuscovaginae* is clearly very widespread and is probably the principal fluorescent pseudomonad causing sheath rot and grain discoloration. Because there is heterogeneity within the strains considered to be *P. fuscovaginae* in this study (clusters 1 and 2), further studies such as DNA hybridization are warranted to establish whether or not there is more than one species included in this group. The epidemiology of the bacterial component of rice grain discoloration cannot be studied easily because pathogens must always be purified and identified in order to establish which are involved. Although a differential medium has been developed to separate the pathogens (34), the development of more sophisticated tools specific to each pathogen is needed for rapid progress to be made on the epidemiology of this vexing problem.

Table 4. Percent positive reaction (serum agglutination, 1:100 dilution) of strains of *Pseudomonas* isolated from rice grains to antisera from strains of *Pseudomonas* pathogenic to rice

Strain characteristics[a]		Strains (no.)	Cluster (strain designation)	Percent strains reacting to antiserum of			
				P. fuscovaginae[b]	*P. syringae*[c]	*P. avenae*[d]	*P. glumae*[e]
Pathogen							
Fluorescent	Arg^+	73	Clusters 1 and 2 (*P. fuscovaginae*)	75	20	8	4
	Arg^-	17	Cluster 4 (*P. syringae*)	29	76	0	0
Nonfluorescent	Arg^- $Oxidase^+$	28	Cluster 5 (*P. avenae*)	7	4	93	75
	Arg^- $Oxidase^-$	4	Cluster 7 (*P. glumae*)	0	0	50	100
Nonpathogen		21	Clusters 3 and 6 (*P. fluorescens* and *P. putida*)	0	0	0	0

[a] Shown to be pathogenic in this study or reported as pathogen; Arg = Arginine dihydrolase.
[b] Produced from strain PDDCC 5940, isolated from rice from Japan.
[c] Produced from strain CIAT 17, isolated from rice from Chile.
[d] Produced from strain NCPPB 1392, isolated from rice from Japan.
[e] Produced from strain NCPPB 2981, isolated from rice from Japan.

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