Synonymy of *Pseudomonas avenae* Manns 1905 and *Pseudomonas alboprecipitans* Rosen 1922

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The nomenspecies *Pseudomonas avenae* Manns 1909 and *Pseudomonas alboprecipitans* Rosen 1922, causal agents of leaf blight of oats and leaf blight of corn, have been compared with respect to their physiological and biological properties as described in the early literature. Except for a few minor physiological characters, most of the properties were identical to those determined herein with *P. alboprecipitans* isolates obtained in Georgia and Florida and with the "lecto" type strain (ATCC 19860). All isolates of *P. alboprecipitans* were closely related genetically as judged from deoxyribonucleic acid (DNA)-DNA reassociation experiments. On the other hand, DNA of *P. alboprecipitans* failed to reassociate with DNA of fluorescent *Pseudomonas syringae* 5D417 and *Pseudomonas andropogonis* PA146 isolated from sorghum or the nonfluorescent *Pseudomonas atrofaciens* PA13 isolated from wheat. *P. alboprecipitans* isolates also possessed DNA of relatively high guanine plus cytosine (G+C) content (72.2 mol%) as compared to the 57.8 mol% G+C of *P. atrofaciens* and *P. andropogonis*.

Pseudomonas avenae, the causal agent of leaf blight of oats, was described by Manns in 1909 (12). Several years later, Rosen (15) reported that a bacterial leaf blight of foxtail was similar to the leaf blight of oats described by Manns (12). Rosen suggested that the foxtail blighting organism was identical to *P. avenae*, but because of physiological differences a new species, *Pseudomonas alboprecipitans* (16), was generated. In 1949, Johnson et al. (8) reported that the causal agent of bacterial leaf blight of corn was the same bacterium described earlier by Rosen on foxtail.

For several seasons an unidentified bacterium has been associated with a corn leaf blight periodically during hot, humid seasons in southern Georgia. A preliminary study showed that the causal bacterium fit the description of *P. alboprecipitans* reported by Johnson et al. (8) and also fit that of *P. avenae* described earlier by Manns (12). We also found that our isolates were equally or more virulent on oats than on corn. These observations led us to investigate several *P. alboprecipitans* isolates by comparing them to the known American Type Culture Collection (ATCC) isolate 19860 and the earlier published descriptions of *P. avenae* and *P. alboprecipitans*. Attempts to obtain the original and any possible existing cultures of *P. avenae* or plant samples of the leaf blight of oats were unsuccessful.

MATERIALS AND METHODS

Bacterial strains. Five cultures of *P. alboprecipitans* were used. Isolates C-9 and C-10 were isolated from blighted corn in Georgia in 1973, isolates 072-1869 and 072-1872 were obtained from J. Miller, Florida Department of Agriculture, Gainesville, Fla., and ATCC 19860 was obtained from the ATCC. *Pseudomonas syringae* 5D417, pathogenic for sorghum and corn, was obtained from M. Schroth, Berkeley, Calif. *Pseudomonas andropogonis* PA146, isolated from sorghum, and *Pseudomonas atrofaciens* PA13, isolated from wheat, were obtained from the International Collection of Plant Pathogenic Bacteria. These cultures were maintained on nutrient agar (Difco) at 5 C.

Morphological properties. Flagella were stained according to Blenden and Goldberg (1). Cellular appearance was determined from Gram-stained smears. Colonies were examined for size, color, consistency, and elevation on yeast extract-dextrose-calcium carbonate agar (19).

Physiological and biochemical properties. For gelatin hydrolysis two different media were used: (i) the medium and plate method of Johnson et al. (8) and (ii) the medium and test tube method of Stanier et al. (17). Production of acid from various carbohydrates was observed on a medium recommended by Kado and Heskett (10). Oxidation-fermentation, indole, and hydrogen sulfide tests were studied by the methods recommended by Edwards and Ewing (2). Production of ammonia, starch hydrolysis, reaction in litmus milk, and nitrate reduction were determined by the methods of Kiraly et al. (11). Oxidase disks (Difco)

were used for the oxidase test. Rotting of potato, hydrolysis of lipid, and production of arginine dihydrolase were determined by the methods of Misaghi and Grogan (14).

DNA guanine plus cytosine (G+C) analysis and reassociation experiments. Deoxyribonucleic acid (DNA) of *P. syringae* and *P. atrofaciens* was prepared as described previously (4). DNA of *P. andropogonis* and *P. alboprecipitans* was prepared similarly except for modifications of the preliminary steps. Cells of these bacteria were lysed by freezing and thawing and by digestion with lysozyme (50 µg/ml) in 0.1 M sodium phosphate buffer, pH 7.0, for 30 min at 37 C, followed by treatment with Pronase (50 µg/ml) at 37 C for 15 to 30 min. The treated cells were lysed with 1% sodium dodecyl sulfate, and the lysed material was adjusted to 0.5 M NaCl and then deproteinized with neutralized liquid phenol. All subsequent steps were the same as described earlier (4). DNA was reassociated at 51 C (about 18 C below the thermal midpoint of labeled DNA) in 50% formamide-5× SSC (0.15 M NaCl plus 0.015 M sodium citrate). All reassociation experiments were performed in a single vial containing DNA filters from all test organisms and were repeated four times (4).

Pathogenicity. For pathogenicity tests a culture growing in medium 523 (10) was adjusted to 50 Klett units (green filter) and diluted to give an inoculum containing approximately 10⁷ viable cells/ml. All test plants were restricted to the family *Graminae*, since all of the bacteria tested were pathogenic for various grass species. The following plant cultivars were used: corn, Pioneer 3369; wheat, McNair 1587; barley, Barsoy; oats, Georgia 7199; and rye, Abruzzi. Test plants at the three- to five-leaf stage were placed in a mist chamber overnight before inoculating. The inoculum was then sprayed onto plants with an atomizer.

Several additional test plants were also inoculated by placing a hypodermic needle into the sheath and inserting approximately 0.5 ml of the bacterial suspension. Control plants were sprayed or injected with sterile water.

RESULTS

The phenetic characters of *P. alboprecipitans* and *P. avenae* gathered from the literature and the five isolates of *P. alboprecipitans* studied here differed very little. Isolates of *P. avenae* tested by Manns (12) and *P. alboprecipitans* tested by Rosen (16), Johnson et al. (8), Goto and Starr (5), and by us were gram-negative, polar-flagellated rods. The bacteria in question required oxygen for growth, reduced nitrate aerobically, and were nonfluorescent. Litmus milk was peptonized but not coagulated, and indole was not produced. *P. avenae* (12) and *P. alboprecipitans* (16) were both pathogenic to corn, oats, barley, wheat, and rye.

The only properties of *P. avenae* and *P. alboprecipitans* which differed were production of ammonia, liquefaction of gelatin, and utilization of carbohydrates; there were slight variations in size and temperature optimum (Table 1).

DNA sequence homology and G+C content. *P. alboprecipitans* isolates C-10, 19860, and 072-1869 were closely related (Table 2). *P. alboprecipitans* was genetically distinct from the nonfluorescent species *P. andropogonis*, and little if any genetic relatedness existed between *P. alboprecipitans*, *P. atrofaciens*, and *P. syrin-*

TABLE 1. Comparison between differing properties of *P. avenae* and *P. alboprecipitans*^a

Properties	<i>P. avenae</i> (Manns 1905)	<i>P. alboprecipitans</i>			
		Rosen (16)	Johnson et al. (8)	Goto and Starr (5)	This paper ^b
Size (µm)	0.75 by 1.5	0.6 by 1.8	0.3-0.7 by 0.6-1.5	0.5-0.7 by 1.0-2.0	0.6 by 1.6
Capsules	ND	-	+	ND	+
Temp (growth optimum) (C)	20-30	30-35	30	ND	36
Ammonia production	-	+	+	ND	-
Hydrolysis of starch	ND	+	±	-	±
Gelatin liquefaction, plate	+	-	+	ND	+
Gelatin liquefaction, tube	+	-	-	-	V
Acid from:					
Sucrose	-	-	+	NU	-
Lactose	-	-	+	NU	-
Maltose	-	-	+	NU	-
Glycerin	(+)	-	+	+	(+)
Dextrose	+	-	+	+	+
Galactose	ND	ND	+	+	+
Mannitol	ND	-	+	+	+

^a Symbols: +, positive; -, negative; (+), delayed reaction; ±, weakly positive; V, variable + or - depending upon isolate; ND, no data available; NU, not utilized.

^b Data from five isolates.

TABLE 2. *G+C content and DNA homologies between various Pseudomonas sp.*

Source of unlabeled DNA	G+C (mol %) ^b	% Relative binding ^a with reference to DNA of:	
		<i>P. alboprecipitans</i> ATCC 19860	<i>P. syringae</i> 5D417
<i>P. syringae</i> 5D417	59.8 ± 0.05	1.7	100.0
<i>P. atrofaciens</i> PA13	57.8 ± 0.02	2.8	43.6
<i>P. andropogonis</i> PA146	57.8 ± 0.02	2.6	2.2
<i>P. alboprecipitans</i> C-10	71.6 ± 1.20	82.0	5.1
<i>P. alboprecipitans</i> ATCC 19860	72.2 ± 0.50	100.0	8.2
<i>P. alboprecipitans</i> 072-1869	72.8 ± 2.20	91.0	4.7

^a Percent based on specific radioactivity (counts per minute/nanomolar deoxyadenosine 5'-monophosphate).

^b Determined by thermal denaturation analysis in 0.1× SSC.

gae (Table 2). Furthermore, *P. alboprecipitans* had DNA with a much higher G+C content than the other species.

Pathogenicity. *P. avenae* was originally described on oats; however, Manns (12) also reported that barley, wheat, rye, and corn were also susceptible. Although we could not confirm Manns' work, we did determine that our five isolates of *P. alboprecipitans* infected not only corn but also barley, wheat, rye, and oats. In fact, the lesions *P. alboprecipitans* produced on wheat and oats (Fig. 1B) were similar to the symptoms on oats that Manns illustrated in his paper (ref. 12, plate XIII, Fig. 1). A preliminary test also showed that strain 19860 was more virulent when atomized onto oats, wheat, rye, and barley than onto corn. Using an inoculum of 10⁷ cells/ml, numerous lesions were produced on oats, wheat, rye, and barley, but only a few lesions were produced on corn.

Designation and description of the neo-type strain of *P. avenae*. Gram-negative, polar-flagellated rods approximately 0.6 by 1.6 μm. Colonies on yeast extract-dextrose-calcium carbonate agar are cream with tan-to-brown centers, convex, and smooth. Colonies reach a diameter of 2 to 3 mm after 3 days of incubation

FIG. 1. (A, C) Leaves of wheat, cultivar McNair, and (B) oats, cultivar Georgia 7199, 5 days after being sprayed (A, B) with approximately 10⁷ viable cells of *P. alboprecipitans* strain ATCC 19860 per ml and (C) water.

at 30 C. Old colonies are sticky and adherent to agar. Optimum growth temperature is 36 C. No fluorescent pigment is produced on King's B agar (6). The strain requires oxygen for growth, reduces nitrate aerobically but not anaerobically, and peptonizes litmus milk, and lipid is hydrolyzed. L-Leucine, D-xylose, and D-sorbitol are utilized as sole sources of carbon; L-threonine, inositol, D-arabinose, and D-trehalose are not utilized. Acid is produced from dextrose, galactose, and mannitol after 7 days and from glycerine after 10 days; no acid is produced from sucrose, lactose, or maltose. Gelatin liquefaction is variable, depending upon the isolate and method of testing. The oxidase reaction is negative, and starch is hydrolyzed only slightly. Tests for hydrogen sulfide, potato soft rot, and arginine dihydrolase are negative. The DNA contains 70.2 mol% G+C.

ATCC 19860 is here designated as the neotype strain of *Pseudomonas avenae*. A description of this strain follows.

DISCUSSION

Not long after *P. avenae* was described by Manns in Ohio (12), Jones et al. (9) and Elliott (3) reported that a blight of oats in Wisconsin was caused by a strain of the same bacterium. The last known reference to *P. avenae* was that of Rosen in 1919 (15). Describing an unknown disease of foxtail in Arkansas, Rosen stated: "The effect on oats is not unlike the halo blight recently described by Miss Elliott and [it] is likely that the organism under discussion is the same as Manns' *Pseudomonas avenae*." Three years later, however, Rosen (16) considered the foxtail organism as a new species and named it *P. alboprecipitans* sp. nov.

Properties of all the *P. alboprecipitans* isolates we tested agreed with the original description (12) of *P. avenae*, which serves as a nomenclatural type in the absence of a type strain. It is clear from earlier reports (3, 8, 9, 12, 16) and our results that only minor differences occurred between the description of *P. avenae* and *P. alboprecipitans*. These minor differences were probably due not only to improved methods, as suggested by Rosen (16), but also to the lack of standardized tests. Rosen listed three main reasons for naming the bacterium he isolated from foxtail as a new species. (i) It did not liquefy gelatin; (ii) it did not produce acid from carbohydrates; and (iii) it hydrolyzed starch. Subsequent results have, however, disagreed. For example, using nutrient gelatin and petri dishes, Johnson et al. (8) reported that gelatin was liquefied by *P. alboprecipitans*. Our

results not only confirmed the results of Johnson et al. (8) but also showed that the liquefaction of gelatin in test tubes (method of Rosen) varied with the isolate. Whereas four of our isolates failed to liquefy gelatin in tubes, isolate 072-1872 was positive after 21 days. Such variation in liquefaction is not new, as other species have been shown to vary with respect to gelatin liquefaction (2). Concerning acid production from carbohydrates, again the results of Johnson et al. (8) disagreed with those of Rosen (16). On the other hand, results of Goto and Starr (5) and ours were identical to those of Manns (12); that is, acid was produced from glycerol and dextrose but not from sucrose, lactose, or maltose. Finally, it is difficult to accept hydrolysis of starch as a reason for naming a new species when hydrolysis of starch was not included in the original description of *P. avenae*.

Rosen (16) also discussed the fact that his foxtail organism, unlike *P. avenae*, did not contain endospores. Manns (12) did not, however, say that *P. avenae* definitely contained spores. He stated: "When mounts of such cultures [old] are made, . . . and stained with hot carbofuchsin for three or four minutes, these bodies retain the stain similar to spores." Describing his photograph of the above (ref. 12, plate LX, Fig. 2) he stated: "Showing what appear to be spores of the white bacterium (*Pseudomonas avenae* n. sp.) . . ."

The apparent taxonomic ambiguity between *P. avenae* and *P. alboprecipitans* would have been best resolved by DNA reassociation experiments. However, cultures of *P. avenae*, including those isolated by Rosen and Manns, no longer exist. Therefore, comparative studies on the genetic relatedness between original *P. avenae* and *P. alboprecipitans* cultures were not possible. Percent G+C values and DNA homology experiments did, however, show that *P. alboprecipitans* was genetically different from three other *Pseudomonas* spp. A G+C value of greater than 70 mol% suggests that *P. alboprecipitans* is a genetically distinct pseudomonad.

In our opinion there is no justification for maintaining two epithets for the same organism. *P. avenae* is the older of the two names and was accepted by other authors (3, 9). On the other hand, there are no surviving cultures of *P. avenae* and there has been no work conducted with it since 1918 (3). In contrast, cultures of *P. alboprecipitans* are available and work has been published recently (5, 18). Nevertheless, it is our recommendation that *P. avenae* be the recognized name and that *P. alboprecipitans* be considered a later synonym of *P. ave-*

nae. The reasons for this recommendation are as follows: (i) near identity in the phenetic characters of the pathogen reported by Manns and Rosen (7, rule 17a and 26); (ii) failure of Rosen to adopt the earliest legitimate epithet (7, rule 24b); (iii) the acceptance of Manns' epithet by others prior to Rosen; and (iv) the similarities in pathogenicity of *P. avenae* and *P. alboprecipitans*.

We propose that, since no strain was designated as the type strain of *P. alboprecipitans*, ATCC strain 19860 be designated as the neotype strain of *P. avenae*.

REPRINT REQUESTS

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