Synonymy of *Pseudomonas gladioli* Severini 1913 and *Pseudomonas marginata* (McCulloch 1921) Stapp 1928

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An appraisal of the relationship of *Pseudomonas gladioli* Severini 1913 and *Pseudomonas marginata* (McCulloch 1921) Stapp 1928 has been conducted on the basis of an examination of the properties of these species as described in the literature and of the properties of the "lectotype" strain of *P. marginata*. Examination of this strain (ATCC 10248), which was deposited in the ATCC by McCulloch under the name *Pseudomonas marginata*, indicates that the previous descriptions of *P. marginata* were incorrect in several instances, such as in capsule formation and fluorescence of the yellow pigment it produces. Because the corrected description of *P. marginata* is identical to the original description of *P. gladioli*, the two names are regarded as synonyms, with *P. gladioli* having priority. Strain ATCC 10248 (NCPB 1891) is designated as the neotype strain of *P. gladioli*.

Two species of phytopathogenic pseudomonads which cause disease of *Gladiolus* sp. have been carried in the literature under the names *Pseudomonas gladioli* and *Pseudomonas marginata*. The first of these was described in 1913 by Severini (15) as causing a rot of gladiolus corms. The second, described by McCulloch in 1921 (11) as *Bacterium marginatum*, caused a spot and basal rot of leaves of *Gladiolus* sp.

Studies of *P. marginata* have indicated that there is confusion regarding the production of a fluorescent pigment by this organism. Both pigment production (5) and lack of pigment production (1, 19) have been noted. This confusion in the description of *P. marginata* and its apparent similarity to *P. gladioli* with regard to hosts attacked and symptoms elicited suggested that a reappraisal should be made of the relationship of these two organisms.

MATERIALS AND METHODS

Bacterial strain. The "lectotype" strain of *P. marginata*, McCulloch's strain Col. 3, was obtained from M. P. Starr (PM 107). It was deposited by McCulloch in the American Type Culture Collection, Rockville, Md., as ATCC 10248 and is held by the National Collection of Plant Pathogenic Bacteria, Harpenden, England, as NCPB 1891. To our knowledge, none of Severini's original strains of *P. gladioli*

are extant, and Severini's original description (15) of *P. gladioli* thus serves as the nomenclatural type until a neotype is designated (see below).

Methods. The methods used to characterize strain Col. 3 have been described previously (17).

RESULTS

Description comparison. It is evident that few differences distinguish these two phytopathogens (Table 1). They are alike in size, flagellation, nitrate reduction, and gelatin liquefaction. From the literature, the principal differences appear to be in the possession of capsules by *P. marginata* and pigment production by *P. gladioli*. Severini (15) described *P. gladioli* as producing "a diffusible pale yellow pigment which now and then tended to become orange, and depending upon the substrate becoming reddish." Pigment production was not reported by McCulloch (11) in the original description of *P. marginata*. In 1924, however, she (12) published a more extensive study of this species in which she stated that a faint trace of green appeared in some cultures after 8 weeks of incubation in Uschinsky's solution and that Fermi's solution became pale yellowish after 3 to 4 weeks; in Cohn's solution, the sediment became yellow, and in one set of

TABLE 1. *Properties of Pseudomonas gladioli and P. marginata*

Properties	<i>P. gladioli</i>	<i>P. marginata</i>
Gram stain	— ^a	—
Spore formation	—	—
Oxygen requirement	+	+
Gelatin liquefaction	+	+
Pectolytic enzymes	+ (11)	+ (9)
Nitrate reduction	—	—
Capsules	—	+
Indole formation	—	±
Size	0.6 by 2.3 to 2.8 μ m	0.5 to 0.8 by 0.8 to 3.4 μ m; 0.5 to 0.6 by 0.8 to 1.8 μ m and occasionally longer (12).
Flagella	One or more bipolar, 3 to 8 μ m long.	One to two polar; one to four bipolar, 3 to 8 μ m long (12).
Pigments	Diffusible pale yellow, occasional orange, and with certain substrates reddish.	None reported; Fermi's medium becomes pale yellowish-green, sediment in Cohn's medium yellowish and occasionally orange yellow (12); fluorescent green (6); brown (13); nonfluorescent yellowish-green (R. W. Ballard, Ph.D. thesis).
Disease symptoms	Corm and basal leaf rot of <i>Gladiolus</i> sp., yellow-brown exudate when squeezed.	Leaf spot and rot of <i>Gladiolus</i> sp.; spot and basal rot of leaves of <i>Gladiolus</i> sp., corm scab from which a copious gummy exudate occurs (12).

^a Unless otherwise noted, the descriptions are taken from Severini (15) and McCulloch (11) for *P. gladioli* and *P. marginata*, respectively.

cultures, the color was orange-yellow. These properties were not included in the technical description of the pathogen presented in the 1924 paper, and consequently they did not appear in later publications by other authors (3, 6, 8, 18, 19).

Bryan (5) reported in 1927 that a green fluorescent pigment was produced in certain media by *P. marginata*. This report apparently is the basis for the later descriptions (2, 4, 8, 18) of *P. marginata* as producing a green fluorescent pigment. However, it appears that this report of the production of a fluorescent pigment is in error. Starr and Burkholder (20) stated that *P. marginata* had never been known to produce a green-fluorescent diffusible pigment, although they do not mention Bryan's observation. Later, it was noted by R. W. Ballard (Ph.D. thesis, Univ. of California, Berkeley, 1969) that strains of *P. marginata*, including two strains derived from McCulloch's collection (NCPPB 1890 and 1981), formed a nonfluorescent, yellowish-green pigment in an iron-deficient medium such as King's B medium (10). This has been noted by us as well. This yellow pigment has the same appearance in sunlight as that formed by fluorescent pseudomonads and consequently could have been

confused with the fluorescent pigment by Bryan (5). We now know that the production of fluorescent diffusible pigments is easily recognized by observation under an ultraviolet source of low wave length, and this important technical detail is never mentioned by early workers in this field.

Reddish pigments have not been reported for *P. marginata*. However, Severini (15) indicated that this occurred only on certain unstated substrates. One of the media described by him was a decoction prepared from gladiolus tissue. A similar medium was prepared by us and tested as a substrate for pigment formation. This medium initially had a slight reddish cast. The color remained unchanged in control tubes but became more intense in tubes inoculated with *P. marginata* strains and was especially pronounced when viewed in direct sunlight. Several strains of *P. marginata*, including strain NCPPB 1891, formed a more intense reddish color than other strains. Reddish colors also have been noted on other occasions. The margins of *P. marginata* colonies occasionally turned a pinkish color when the organisms were grown on a polypectate gel medium (medium C) used to detect pectolytic enzymes (9). Furthermore, it should be noted that strains of

P. alliicola, an organism considered to be identical to *P. marginata* by Ballard et al. (1), produce brown pigments in culture. This pigment occasionally appears reddish during early stages of formation. A brown pigment has also been reported for *P. marginata* (13).

Symptom comparison. The question of similarity or differences in phytopathogenic symptoms should also be considered. *P. gladioli* was described as causing a rot of gladiolus corms. These symptoms were at first manifested as yellow or reddish sunken spots on the corm. The corm later became flaccid, and a yellow-brown mass could be squeezed out of it. Lesions were described as occurring on the basal portion of the leaves in addition to the corms. In severe cases, the plants break over at the base. McCulloch's first report of *P. marginata* (11) provided a somewhat similar description. Lesions were reported to occur occasionally on all parts of leaves but were most often confined to the basal portions. Under warm and moist conditions, rot spread rapidly and deeply into tissues, causing the collapse of the aerial part of the plant. Her later report (12) also described these symptoms. In addition, lesions were reported on the corm which were termed scab. These corm lesions exuded a gummy substance. Her attempts to produce rot in mature corms through wound inoculations were unsuccessful, although it was indicated that rot could be caused in immature corms under conditions of high moisture.

Properties of *P. marginata* ATCC 10248 (NCPFB 1891). The properties of *P. marginata* ATCC 10248 agree with those originally given for *P. gladioli* as follows: cells are gram-negative; endospores and capsules are not produced; cells are aerobic; gelatin is liquefied; pectolytic

enzymes are produced; nitrate is not reduced; indole is not produced after 24, 48, or 96 h; one to four polar flagella are present (Fig. 1); size ranges from 0.7 to 0.8 by 2.3 to 2.8 μ m (Fig. 2); and pigment formation is described as being diffusible, nonfluorescent pale yellow to yellowish-green with rarely a reddish pigment, depending upon the medium.

Additional properties of *P. marginata* strain ATCC 10248 as determined by Ballard et al. (1) reveal that poly- β -hydroxybutyric acid is accumulated but is not hydrolyzed; growth occurs at 41 C but not 4 C; it is oxidase positive and arginine dihydrolase negative; and starch is not hydrolyzed. Organic substrates utilized as sole sources of carbon and energy include: D-ribose, D-xylose, D-arabinose, L-arabinose, D-fucose, D-glucose, D-mannose, D-galactose, D-fructose, sucrose, trehalose, cellobiose, gluconate, 2-ketogluconate, saccharate, mucate, salicin, acetate, propionate, butyrate, isobutyrate, valerate, heptanoate, caprylate, caprate, caproate, pelargonate, malonate, succinate, fumarate, glutarate, adipate, azelate, sebacate, D-malate, L-malate, D-(-)-tartrate, meso-tartrate, DL- β -hydroxybutyrate, DL-lactate, glycerate, hydroxymethylglutarate, citrate, α -ketoglutarate, pyruvate, aconitate, citraconate, mesaconate, adonitol, mannitol, sorbitol, meso-inositol, glycerol, ethanol, *n*-propanol, *n*-butanol, benzoate, *p*-hydroxybenzoate, phenylacetate, quinate, glycine, D-alanine, L-alanine, β -alanine, L-serine, L-threonine, L-leucine, L-isoleucine, valine, L-aspartate, L-glutamate, L-lysine, DL-arginine, DL-ornithine, DL-citrilline, DL- α -aminobutyrate, γ -aminobutyrate, L-histidine, kynurenate, anthranilate, ethanolamine, betaine, sacrosine, hippurate, nicotinate, and trigonelline; but not maltose,

FIG. 1. Polar flagellated cells of *Pseudomonas gladioli* ATCC 10248 stained by Leifson's method. ($\times 1,200$)

Fig. 2. Cells of *Pseudomonas gladioli* ATCC 10248 grown in a mineral base plus 0.5% yeast extract and 0.2% lactate, observed under phase contrast. ($\times 1,200$)

lactose, L-rhamnose, starch, inulin, isovalerate, maleate, pimelate, suberate, glycollate, poly- β -hydroxybutyrate, levulinate, itaconate, erythritol, ethyleneglycol, propyleneglycol, 2,3-butyleneglycol, isopropanol, isobutanol, geraniol, D-mandelate, L-mandelate, benzoyl formate, *o*-hydroxybenzoate, phthalate, phenylethanediol, *m*-hydroxybenzoate, phenol, testosterone, naphthalene, DL-norleucine, DL- α -aminovalerate, δ -aminovalerate, D-tryptophan, benzylamine, putrescine, spermine, histamine, tryptamine, α -amylamine, butylamine, creatine, pantothenate, and acetamide.

DISCUSSION

It appears from the preceding that there is no justification for maintaining *P. gladioli* and *P. marginata* as separate species. The differences originally noted between these species do not appear substantiated. Our tests of *P. marginata* ATCC 10248 indicate that its properties are identical to those described for *P. gladioli*. Consequently, the few differences which were reported between these species (Table 1) were apparently due to the use of different techniques and media, such as that used for pigment formation, and to problems of interpretation, such as with indole formation and presence of capsules. Likewise, the description of the diseases caused by the two pathogens are so similar that it is evident that moderate changes in environmental conditions could account for the differences noted. This leaves the question of which species name should be recognized. The original description of both organisms are of equal quality, and consequently it can be argued that the name *P. gladioli* should be used since it was described first and has priority. On

the other hand, there are no surviving cultures of *P. gladioli*, and there has been no work conducted with it in contrast to *P. marginata*. Nevertheless, it is our recommendation that *P. gladioli* be the recognized name and that *P. marginata* be considered a later synonym of *P. gladioli*. The reasons for this recommendation are as follows: (i) priority of *P. gladioli* as previously noted; (ii) the confusion surrounding the description of *P. marginata* with regard to pigment formation. Evidence of this confusion can be seen from the fact that both fluorescent and nonfluorescent organisms are included in culture collections as *P. marginata* (14), and (iii) the similarity of the name *P. marginata* to that of *P. marginalis*, which also could lead to some confusion. We further propose that the strain previously described and designated (1) as the "lectotype" strain of *P. marginata* (ATCC 10248, NCPPB 1891) be designated as the neotype strain of *P. gladioli*.

REPRINT REQUESTS

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