Pseudomonas pomi Cole 1959 Is a Later Subjective Synonym of *Acetobacter pasteurianus* (Hansen 1879) Beijerinck 1898, and *Pseudomonas melophthora* Allen and Riker 1932 is a Nomen Dubium

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An examination of all known strains labeled *Pseudomonas melophthora* Allen and Riker 1932 and of the herein-designated type strain of *P. pomi* Cole 1959 (strain PDDCC 3878 [= NCPPB 463]) showed that they were similar to one another but atypical of members of the genus *Pseudomonas* Migula 1894. Because these organisms were found to be either motile (peritrichous cells) or nonmotile, able to grow at low pH values, and to oxidize ethanol and lactate to carbonate, it is suggested that they belong to *Acetobacter* Beijerinck 1898. *P. pomi* is regarded here as being a subjective junior synonym of *A. pasteurianus* (Hansen 1897) Beijerinck 1898, and *P. melophthora* is regarded here as being a nomen dubium. Isolates of *P. pomi* and similar organisms capable of inducing necrosis in apple fruit should be classified in *A. pasteurianus* until the relationships of pathogenic strains within the species are better understood.

Pseudomonas melophthora Allen and Riker (1) was first reported in the United States as causing a firm brown discoloration of apple (*Malus pumila* Mill.) fruit tissue after invasion by apple maggots. It and another bacterial pathogen, *Pseudomonas pomi* Cole (7), were later reported in England. The latter organism produced a similar effect but, unlike *P. melophthora*, it was encapsulated and nonmotile. Cole deposited isolates of both of these organisms with the National Collection of Plant Pathogenic Bacteria (NCPPB), Harpenden, England. One isolate of *P. pomi* and two identified as *P. melophthora*, all from the NCPPB, were the only strains of these species available for study.

In their revision of *Pseudomonas* Migula (25), Doudoroff and Palleroni (11) included *P. melophthora* in an addendum containing a list of incompletely described species and placed *P. pomi* in an addendum containing a list of species known to possess characters not in accord with the generic definition; as such, these organisms were regarded as species incertae sedis. Dye et al. (13) suggested that all validly published names of plant pathogenic species of *Pseudomonas* for which there are isolates be retained until further studies have established their relationships accurately. This paper reports a taxonomic study of *P. pomi* and of strains identified as belonging to *P. melophthora*.

MATERIALS AND METHODS

Bacterial strains. *P. melophthora* PDDCC 4167 and PDDCC 4168 (PDDCC: Plant Diseases Division Culture Collection Department of Scientific and Industrial Research, Auckland, New Zealand) (= NCPPB 461 and NCPPB 462, respectively) and *P. pomi* PDDCC 3878 (= NCPPB 463) were obtained from the PDDCC as freeze-dried cultures.

Media. For routine testing, cells were subcultured on potato-dextrose agar (Difco Laboratories) and incubated for 4 to 7 days at 25°C. Unless otherwise indicated, faintly turbid suspensions (ca. 10⁸ colony-forming units per ml) were prepared in sterile distilled water for the inoculation of broth and agar media.

Growth of cultures was compared on: nutrient agar; potato-dextrose agar; yeast-salts agar (12) containing 2% glucose; and alcohol-yeast extract-tryptone agar (3% ethanol, 0.5% yeast extract [Difco], 1% tryptone [Difco], 1.5% agar; pH 6.5).

Gram reaction, motility, and flagellation. The Hucker modification of the Gram stain was used, in which Lugol iodine replaced Burke iodine (27). The periods of staining, mordanting, counter-staining, and rinsing were carefully timed (3).

Motility was determined with 48-h-old shake cultures (yeast-salts-2% glucose broth) observed with a phase-contrast microscope. Flagella were stained by the method of Lowy and Hanson (24) and were examined with a Joel JEM 100B electron microscope.

Oxygen requirements. Each organism was inoculated into two tubes of potato-dextrose agar, one of which was placed in a Torbal jar rendered anaerobic with a GasPak (BBL Microbiology Systems, Cockeys-

ville, Md.) and held at 25°C; the other was placed in an incubator at 25°C as a check. Strains which showed visible growth after 14 days in the Torbal jar were recorded as being anaerobic. Cultures that failed to grow in anaerobic conditions were considered to be aerobes.

The use of nitrate as an alternative electron acceptor (nitrate respiration) was tested by the method of Iizuka and Komagata (19). Cultures that failed to grow in the anaerobic conditions of this test were considered to be obligate aerobes.

Glucose fermentation was tested by the method of Hugh and Leifson (18).

Other determinative tests. Production of fluorescent pigment was tested on King medium B (20).

The LOPAT tests (levan production, oxidase reaction, potato rot, arginine dihydrolase activity, and tobacco hypersensitivity reaction) were made by the methods of Lelliott et al. (23).

Production of poly- β -hydroxybutyrate inclusions was determined by the Hayward (17) modification of the Burdon (5) method.

Growth on alcohol in Hoyer-Frateur medium was determined by the method of De Ley and Frateur (10).

Growth at pH 4.5 was tested in apple juice (sp gr, 1.0) depectinized by ultrafiltration and fortified with 0.5% yeast extract. Seven-milliliter quantities of this medium (in 16-mm culture tubes) were sterilized by filtration and were inoculated and incubated as shake cultures at room temperature (20 to 22°C) for 7 days.

Production of acetic acid was examined in alcohol-yeast-extract broth and in ethanol-carbonate agar at pH 6.5 and 7.2. Oxidation of calcium lactate to carbonate and production of dihydroxyacetone (ketogenesis) from glycerol were tested for by the methods of Skerman (26).

Also tested were formation of gluconate and cellulose (6), production of 2-keto-D-gluconate (23), production of acetylmethylcarbinol (acetoin) and catalase (12), and tolerance to potassium cyanide (14). The methyl red test (12) was also performed.

Nutritional and other tests. For nutritional screening, the methods followed were those described by R. A. Lelliott in *Interlaboratory Study of LOPAT Group 1 Plant Pathogenic Pseudomonads* (circulated in 1974 by the author, Ministry of Agriculture, Fisheries and Food, Plant Pathology Laboratory, Hatching Green, Harpenden, Herts, U.K.). The carbon compounds were added to a basal medium (pH 7.2) containing: $\text{NH}_4\text{H}_2\text{PO}_4$, 1 g; KCl, 0.2 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g; agar (Davis, Christchurch, New Zealand), 12 g; and distilled water, 1 liter. Concentrated solutions of carbohydrates were filter-sterilized and added to the basal medium after autoclaving, to give a final concentration of 0.5% (wt/vol). Amino acids and organic acids were autoclaved with the basal medium to give a final concentration of 0.2% (wt/vol). Petri plates were poured thick using 30 ml of medium per plate. The carbohydrates and related carbon compounds tested were arabinose, rhamnose, xylose, dextrose, fructose, galactose, mannose, ribose, lactose, sucrose, maltose, trehalose, melibiose, cellobiose, raffinose, melezitose, starch, glycerol, erythritol, adonitol, mannitol, sorbitol, dulcitol, meso-inositol, arbutin, salicin, and α -

methyl-D-glucoside. The amino acids D-alanine, L-leucine, and DL-homoserine and sodium salts of the organic acids L-(+)-tartrate, D-(-)-tartrate, formate, malonate, gluconate, and DL-lactate were used. The plates were inoculated, 14 patches per plate, with a multipoint inoculator. Growth greater than that on basal medium was scored as positive at 3-, 5-, 7-, 14-, and 21-day intervals.

Tests for proteolysis of gelatin: action in purple milk; presence of urease; production of H_2S (from cysteine, peptone, and sodium thiosulfate), ammonia, indole, and nitrite from nitrate; and utilization of asparagine as a sole source of carbon and nitrogen were performed by the methods of Dye (12). Tests for arbutin hydrolysis and the presence of tyrosinase were made by the methods of Crosse and Garrett (8).

Growth at 30 and 34°C was tested by inoculating and incubating yeast-salts-2% glucose broth tubes in constant-temperature water baths.

Pathogenicity tests. Ripe apple (common name, "Granny Smith") fruits were surface-sterilized with 70% ethanol and pricked, 1 to 2 cm deep, at three sites per fruit with a hypodermic needle charged with bacterial suspension. Controls were prepared by inoculating fruit with sterile distilled water. The fruits were enclosed in polythene bags and incubated at 25°C for 4 weeks.

RESULTS

In pathogenicity tests, all three strains (PDDCC 3878, 4167, and 4168) inoculated into Granny Smith apples produced a similar discoloration of fruit tissue. The effect was evident as a circular brown patch around the point of inoculation within 1 week and extended on the surface as well as inside the fruit without causing collapse of tissue. The tissue inside the fruit was brown, forming a definite margin with adjacent white portions of unaffected tissue.

Nutrient agar proved to be unsuitable for culturing these bacteria, as growth was hardly evident on this medium after 7 days. Although growth was slow on potato-dextrose agar and alcohol-yeast extract-tryptone agar, these media were more suitable than nutrient agar. The colonies on these media were convex, 1 to 2 mm in diameter after 7 days, and up to 4 mm in diameter in 14 days. The colonies were pearly white on potato-dextrose agar and mauve on alcohol-yeast extract-tryptone agar; the smell of acetic acid emanated from inoculated plates of the latter medium.

Young cultures of the *P. melophthora* and *P. pomi* strains contained gram-negative, obligately aerobic cells. Cells of *P. pomi* PDDCC 3878 were oval and nonflagellated and appeared to have a capsule-like structure (Fig. 1). Cells of *P. melophthora* PDDCC 4167 and 4168 were slightly bent, peritrichous rods (Fig. 2).

The cultures did not produce fluorescent pig-

FIG. 1. Electron micrograph of cell of PDDCC 3878 (*P. pomi*). $\times 43,000$.

FIG. 2. Electron micrograph of cell of PDDCC 4167 (*P. melophthora*). $\times 23,237$.

ment in King medium B, were negative in all LOPAT tests, and did not produce poly- β -hydroxybutyrate inclusions.

All cultures grew in apple juice at pH 4.5 and oxidized ethanol to acetic acid in 2 to 4 days in alcohol-yeast extract broth and agar media at pH 6.5 but not at pH 7.2. A zone of reprecipitated calcium carbonate appeared around the bacte-

rial growth in ethanol-carbonate agar within 5 days, indicating oxidation of ethanol to acetic acid and then to CO_2 . Oxidation of lactate to CO_2 was observed after 5 days.

All strains were catalase positive, produced gluconate, and grew with ethanol. Dihydroxyacetone was not produced in glycerol medium (no ketogenesis), and the cultures did not pro-

duce 2-keto-D-gluconate or cellulose. All three strains produced gluconate and were able to grow with ethanol in Hoyer-Frateur medium within 5 to 7 days.

All of the strains of *P. melophthora* and *P. pomi* utilized glucose, ribose, glycerol, meso-inositol, salicin, D-alanine, and DL-lactate but were not shown to utilize rhamnose, lactose, maltose, raffinose, adonitol, dulcitol, arbutin, α -methyl-D-glucoside, L-leucine, DL-homoserine, L-(+)-tartrate, D-(-)-tartrate, formate, malonate, or gluconate. Strain 3878 (*P. pomi*) was the only one to show utilization of galactose, fructose, mannose, xylose, sucrose, trehalose, and sorbitol; it gave weak reactions with arabinose, cellobiose, melibiose, melezitose, erythritol, and mannitol.

The cultures of *P. melophthora* and of *P. pomi* were urease positive and grew at 30°C but not at 34°C. They did not hydrolyze arbutin, gelatin, or casein (in purple milk) and did not produce acetoin, ammonia, or indole, reduce ni-produce acetoin, ammonia, or indole, reduce nitrate to nitrite, tolerate KCN, or utilize asparagine as a sole source of carbon and nitrogen; they did not possess tyrosinase and were negative in the methyl red test.

DISCUSSION

The isolates received as *P. melophthora* and that received as *P. pomi* produced a brown discoloration of apple tissue identical with that originally described for *P. melophthora* and *P. pomi*. This ability is a major determinative character of these species. Although both Allen and Riker (1) and Cole (7) referred to the symptom of these pathogens as a "rot," neither organism exhibited pectinolytic activity, the primary symptom being a necrosis of fruit tissue.

Although the strains received as *P. melo-*

phthora were motile and peritrichous and that received as *P. pomi* was nonmotile, the cultures gave similar reactions in most tests, and it is concluded that these isolates should be classified in one species. The flagellar arrangement of strains identified as *P. melophthora* excludes them from *Pseudomonas*, and it is therefore concluded that these organisms are improperly classified in this genus (Table 1).

The observation that the strains studied were acid tolerant and oxidized ethanol to acetic acid indicates their similarity to organisms in the genera *Acetobacter* Beijerinck (4) and *Gluconobacter* Asai (2) (formerly *Acetomonas* Leifson [22]). [The generic name *Acetobacter* and the species *A. pastorianus* (sic) were first mentioned incidentally by Beijerinck (1898). No other authority seems more appropriate in light of the confused origin of this genus.] A comparison of the determinative characters of the strains with those given by De Ley and Frateur (9) for *Acetobacter* and *Gluconobacter* indicates that the cultures are members of *Acetobacter* (Table 1).

From the key given by De Ley and Frateur (10) for species of *Acetobacter* (Table 2), it is apparent that the isolates tested are representatives of *A. pasteurianus* (Hansen 1879) (16) Beijerinck 1898 (4).

Cole (7) did not designate a type strain for *P. pomi*. However, because PDDCC 3878 was the only strain described by him, this strain is the type (monotype) (Bacteriological Code, Rule 18c [21]). *P. pomi* is therefore a subjective junior synonym of *A. pasteurianus*.

Allen and Riker (1) did not designate a type strain for *P. melophthora*, and none of the strains on which they based their original description of this species is known to exist. Because the strains identified as *P. melophthora* by Cole (7) have been shown here to belong in

TABLE 1. Comparison of characters of *P. melophthora* strains and *P. pomi* with those of *Pseudomonas*, *Gluconobacter*, and *Acetobacter*^a

Character	<i>Pseudo-</i> <i>monas</i>	<i>Glucono-</i> <i>bacter</i>	<i>Acetobacter</i>	<i>P. melophthora</i>	<i>P. pomi</i>
Flagella	+ (polar)	+ (polar)/-	+ (peritrichous)/-	+ (peritrichous)	-
Growth at pH 4.5	-	+	+	+	+
Oxidation of:					
Ethanol to acetic acid	-*	+	+	+	+
Acetic acid to CO ₂	d	-	+	+	+
Lactate to CO ₂	+	-	+	+	+
Ketogenesis	-	+	d	-	-
Hydrolysis of:					
Lactose	d	-	-	-	-
Starch	d	-	-	-	-
Gelatin	d	-/w	-	-	-

^a Characters of *Pseudomonas*, *Gluconobacter*, and *Acetobacter* from De Ley and Frateur (9). Symbols: +, Positive; -, negative; w, weak; d, 11 to 89% strains positive; (), usually positive or negative; *, pH 4.5; **, pH 6.5.

TABLE 2. Comparison of characters of *P. melophthora* strains and *P. pomi* with those of species and subspecies of *Acetobacter*^a

Character	<i>P. melophthora</i> and <i>P. pomi</i>	<i>A. pasteurianus</i> subspecies <i>pasteurianus</i>	<i>A. pasteurianus</i> subspecies <i>lovanensis</i>	<i>A. pasteurianus</i> subspecies <i>estunensis</i>	<i>A. pasteurianus</i> subspecies <i>ascendens</i>	<i>A. pasteurianus</i> subspecies <i>paradoxus</i>	<i>A. peroxidans</i>	<i>A. aceti</i>
Catalase production	+	+	+	+	+	—	—	+
Ketogenesis in glycerol	—	—	—	—	—	—	—	+
Formation of: 2-Keto-D-glucosate	—	d	—	NT ^b	—	—	—	+
Cellulose	—	—	—	+	—	—	—	(—)
Gluconate	+	(+)	+	+	—	—	—	+
Growth on ethanol	+	(—)	+	+	—	—	+	d

^a *Acetobacter* sp. and subsp. characters from De Ley and Frateur (10). For an explanation of the symbols, see footnote a of Table 1.

^b NT, Not tested.

Acetobacter, there are no known strains identifiable with the species. Because Allen and Riker's (1) description is not adequate to identify the organism uniquely according to current classificatory criteria, the name *P. melophthora* is considered to be a nomen dubium according to Rule 56a(2) of the Bacteriological Code (21).

The genus *Acetobacter* is in need of taxonomic revision (10). When this revision is carried out, comparative pathogenic, biochemical, and nutritional tests will show whether all elements of *A. pasteurianus* are responsible for the necrotic reaction in apples or whether the disease is caused by a distinct pathogenic variant.

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REPRINT REQUESTS

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