Pseudomonas melanogena Iizuka and Komagata 1963, a Later Subjective Synonym of *Pseudomonas maltophilia* Hugh and Ryschenkow 1960

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Strain IAM 1554 (ATCC 17806) is herein designated as the type strain of *Pseudomonas melanogena* Iizuka and Komagata 1963 and compared with ATCC 13637, the type strain of *Pseudomonas maltophilia* Hugh and Ryschenkow 1960. The three additional strains used in the original description of *P. melanogena* and five strains of *P. maltophilia* originally identified as *Pseudomonas alcaligenes* are also studied. The morphological and physiological attributes and deoxyribonucleic acid base compositions of the 10 strains are sufficiently similar so as to place these strains in a single species, the correct name of which is *P. maltophilia*. The name *P. melanogena* is reduced to being a junior synonym of *P. maltophilia*.

Pseudomonas melanogenum (sic) (the specific epithet is here corrected to *melanogena* to agree with the feminine gender of the generic name *Pseudomonas*) was named and described by Iizuka and Komagata (8) in 1963 on the basis of observations of four strains (YF-3, YF-6, YF-8, and YF-10) isolated from Japanese rice paddies. These strains produced yellow pigment and a brown coloration on nutrient agar supplemented with tyrosine and were unable to grow on glutamate agar. Although a type strain was not designated for this species when it was named and described, strain YF-3 was deposited in the American Type Culture Collection (ATCC), Rockville, Md., as the type strain; it carries the ATCC accession number 17806 (1). This strain is listed in the ATCC *Catalogue of Strains* (1) as the type strain; however, culture collection catalogues do not constitute effective publication, and so strain YF-3 is here designated as the type strain of *P. melanogena*. *P. maltophilia* was named and described by Hugh and Ryschenkow in 1960 (Bacteriol. Proc., p. 78, 1960), and in the following year these authors designated ATCC 13637 as the type strain (7). According to Buchanan et al. (2), *P. maltophilia* is a validly published and legitimate name. Komagata (12) and Iizuka and Komagata (9, 10) isolated from oil brine five strains of *Pseudomonas* that did not produce acid from glucose and identified them as strains of *Pseudomonas alcaligenes*.

Hugh and Leifson (6) reidentified these five strains as *P. maltophilia*, and Iizuka and Komagata (11) agreed with this conclusion.

A comparative study of the four original strains of *P. melanogena* and six strains of *P. maltophilia*, including the type strain, was undertaken to determine the taxonomic relationship between the two species, since both exhibit deoxyribonuclease and nucleoside phosphotransferase activities, which are only rarely observed in *Pseudomonas* species.

MATERIALS AND METHODS

Bacterial strains. The type strain of *P. maltophilia*, the five strains of *P. maltophilia* identified initially as *P. alcaligenes* (9, 10, 12), the strain designated above as the type strain of *P. melanogena*, and the three other strains on which the original description of *P. melanogena* was based were studied. The histories and corresponding numbers of these 10 strains are listed in Table 1.

Methods. The characteristics of the strains were determined by methods described previously (9, 10, 13, 18).

RESULTS AND DISCUSSION

The 10 strains studied contained gram-negative, asporogenous rods that were actively motile with polar flagella. Although the original

TABLE 1. *Strain designations, sources of isolation, and corresponding numbers of strains identified here as Pseudomonas maltophilia*

Original name	Strain designation of Iizuka and Komagata	Other strain designations ^a					Status	Source of isolation	Reference
		AJ	ATCC	IAM	KM	RH			
<i>P. maltophilia</i>		2082	13637		418	1168	Type		6
<i>P. melanogena</i>	YF-3	2220	17806	1554	318	2880	Type	Rice paddy	8
<i>P. melanogena</i>	YF-6	2221	17807	1556	798			Rice paddy	8
<i>P. melanogena</i>	YF-8	2222	17808	1577	799			Rice paddy	8
<i>P. melanogena</i>	YF-10	2223	14535	1588	800			Rice paddy	8
<i>P. alcaligenes</i>	P-1	2273		1396	412	1875		Oil brine	6, 11
<i>P. alcaligenes</i>	P-7	2277		1450	413	1876		Oil brine	6, 11
<i>P. alcaligenes</i>	P-7-2	2275		1425	414	1877		Oil brine	6, 11
<i>P. alcaligenes</i>	P-19	2274		1411	415	1878		Oil brine	6, 11
<i>P. alcaligenes</i>	P-31-2	2276		1438	416	1879		Oil brine	6, 11

^a AJ, Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan; ATCC, American Type Culture Collection, Rockville, Md.; IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan; KM, Kansai Medical School Culture Collection, Osaka, Japan; RH, Rudolph Hugh, George Washington University, Washington, D.C.

description of *P. melanogena* (8) indicated that all four strains studied were motile with a single, polar flagellum, reexamination revealed cells in each strain with a tuft of polar flagella. Cells of the type strains of *P. maltophilia* and *P. melanogena*, each with a tuft of polar flagella, are shown in Fig. 1 and 2, respectively. The 10 strains grew well aerobically on nutrient agar, Leifson deoxycholate agar, and Christensen citrate agar, whereas growth on Salmonella-Shigella and nalidixic acid-cetrimide agars was variable from strain to strain. Growth was not detected in a chemically defined liquid medium containing glucose as the sole source of carbon and energy and ammonium ion as the sole source of nitrogen, on glutamate agar (12), or on Simmons citrate agar; growth was observed when these media were supplemented with L-methionine (50 mg/liter). The requirement for methionine by *P. maltophilia* was first reported by Iizuka and Komagata (11) and later confirmed on 23 strains of this species by Stanier et al. (17). All of the strains hydrolyzed gelatin, Tween 80, and deoxyribonucleic acid (DNA), and a green discoloration of the erythrocytes surrounding dense growth was observed on rabbit blood agar. A brown coloration developed in nutrient agar supplemented with 0.1% tyrosine. Growth on heart infusion agar and other media appeared lemon yellow. Lysine decarboxylase and phenylalanine deaminase activities were variable. All of the strains except YF-10 produced nitrite from nitrate. None of the strains grew anaerobi-

FIG. 1. *Pseudomonas maltophilia* ATCC 13637 (type strain) showing a tuft of polar flagella. Leifson flagella stain. $\times 1,500$.

FIG. 2. *Pseudomonas melanogena* IAM 1554 (type strain) showing a tuft of polar flagella. Leifson flagella stain. $\times 1,500$.

cally by nitrate respiration. All grew in brain heart infusion (BHI) broth containing 3% sodium chloride. Only the type strain of *P. maltophilia* grew at 41 C in BHI broth. Three strains (YF-8, YF-10, and P-7) produced a feeble oxidase reaction, but the results were recorded as negative because the reaction was slow and weak. Stanier et al. (17) reported a negative oxidase reaction for 23 strains of *P. maltophilia*, whereas Snell et al. (16) observed that the oxidase reaction of *P. maltophilia* was variable. None of the strains grew in BHI broth adjusted to pH 4.5. Erythrocytes were not hemolyzed around discrete colonies on rabbit blood agar. 2-Ketogluconate was not produced from gluconate. Arginine dihydrolase, ornithine decarboxylase, and urease were not produced. A water-soluble, fluorescent pigment was not produced on Pseudomonas F agar (Difco), but the medium slowly turned brownish after several days of incubation at room temperature. Iizuka and Komagata (8, 11) reported that their strains of *P. alcaligenes*, which were later reidentified as *P. maltophilia*, and *P. melanogena* produced hydrogen sulfide from nutrient broth supplemented with 0.05% cysteine when tested with lead acetate paper. Hydrogen sulfide was also detected by lead acetate paper suspended over an inoculated Kligler iron agar slant but not by blackening of the butt. All of the strains produced acid oxidatively from glucose, fructose, mannose, maltose, and cellobiose in OF medium (Difco 0688); a weak acid reaction was produced from sucrose, lactose,

melibiose, and trehalose. Acid production from D-ribose, xylose, raffinose, salicin, and ethanol varied from strain to strain. No acid was produced from L-arabinose, D-arabinose, galactose, rhamnose, melezitose, glycerol, dulcitol, inositol, adonitol, sorbitol, mannitol, or salicin. Iizuka and Komagata (8) reported that *P. melanogena* produced acid from glucose in an open tube of OF medium. Hugh and Ryschenkow (7) first reported that *P. maltophilia* did not produce acid from glucose when they used the oxidative fermentative methods of Hugh and Leifson (5) and Leifson (14), but later Hugh and Leifson (6) reported that in OF medium (Difco 0688) the type strain of *P. maltophilia* produced acid promptly from fructose, maltose, and mannose and slowly from glucose. The characteristic of the 10 strains studied are similar to those of *P. maltophilia* as given previously (3, 4, 7, 11). From the results we obtained and from those reported by others, *P. maltophilia* does produce acid oxidatively from glucose. Production of the 5'-nucleotide by the nucleoside phosphotransferase of *P. melanogena* was reported by Mitsugi et al. (15), and all of the 10 strains studied here also produced the 5'-nucleotide. The antibiotic susceptibility patterns of the 10 strains are shown in Table 2. All were susceptible to nalidixic acid and not susceptible to novobiocin. The DNA base compositions of the five strains studied, including the two type strains, are in the range of 65.1 to 66.5% guanine plus cytosine (Table 3). Sufficient differences were

TABLE 2. Antibiotic-susceptibility patterns of the strains studied

Antibiotic	Susceptibility of strain ^a									
	ATCC 13637 (Type strain of <i>P. maltophilia</i>)	YF-3 (Type strain of <i>P. melanogena</i>)	YF-6	YF-8	YF-10	P-1	P-7	P-7-2	P-19	P-31-2
Ampicillin	—	+++	—	—	—	++	—	—	—	—
Chloramphenicol	++	+++	++	++	++	+	+	+	+	+
Colistin	++	+++	++	+++	++	++	++	++	++	++
Erythromycin	—	+	—	—	—	—	—	—	—	—
Gentamicin	+	++	+	—	+	+	—	—	+	—
Kanamycin	++	—	—	—	—	—	—	—	—	—
Nalidixic acid	++	+++	+++	+	+++	++	+++	+++	++	++
Novobiocin	—	—	—	—	—	—	—	—	—	—
Penicillin	—	++	—	—	—	—	—	—	—	—
Polymyxin B	+++	+++	+	+	++	++	+	++	++	++
Streptomycin	—	—	—	—	—	—	—	—	—	—
Tetramycin	++	+++	++	—	++	+	++	+	+	+

^a +, ++, +++, Sensitive; —, insensitive. The degree of susceptibility to the antibiotic disk is expressed according to the manufacturer's statement (18).

TABLE 3. *Characteristics of the strains studied*

Characteristics ^a	Reaction of strains ^b									
	ATCC 13637 (Type strain of <i>P. mal-</i> <i>tophilia</i>)	YF-3 (Type strain of <i>P. melano-</i> <i>gena</i>)	YF-6	YF-8	YF-10	P-1	P-7	P-7-2	P-19	P-31-2
Gram-negative rods	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+
Polar tuft of flagella	+	+	+	+	+	+	+	+	+	+
Growth on LD agar	+	+	+	+	+	+	+	+	+	+
Growth on SS agar	+	—	+	+	—	—	+	—	—	+
				wk 3						
Growth on NAC agar	—	—	—	—	—	+	+	—	+	+
Growth in BHI at 41 C	+	—	—	—	—	—	—	—	—	—
Hemolysis, rabbit blood	—	—	—	—	—	—	—	—	—	—
Green discoloration, rabbit blood	+	+	+	+	+	+	+	+	+	+
H ₂ S, Kligler butt	—	—	—	—	—	—	—	—	—	—
H ₂ S, Kligler lead acetate paper	+	+	+	+	+	+	+	+	+	+
Hydrolysis of gelatin	+	+	+	+	+	+	+	+	+	+
Hydrolysis of Tween 80	+	+	+	+	+	+	+	+	+	+
Deoxyribonuclease	+	+	+	+	+	+	+	+	+	+
2-Ketogluconate	—	NG	—	NG	NG	—	—	NG	—	—
Malonate	+	+	+	+	+	+	+	+	+	+
Citrate, Simmons	—	—	—	—	—	—	—	—	—	—
Citrate, Simmons with methionine	+	+	+	+	+	+	+	+	+	+
Citrate, Christensen	+	+	+	+	+	+	+	+	+	+
Nitrate reduction to nitrite	+	+	+	+	—	+	+	+	+	+
Nitrate respiration	—	—	—	—	—	—	—	—	—	—
Acid from:										
Glucose, aerobically	+	+	+	+	+	+	+	+	+	+
Glucose, anaerobically	—	—	—	—	—	—	—	—	—	—
Ethanol	—	+	+	+	+	—	—	—	—	—
					wk 6					
Glycerol	—	—	—	—	—	—	—	—	—	—
D-Arabinose	—	—	—	—	—	—	—	—	—	—
L-Arabinose	—	—	—	—	—	—	—	—	—	—
D-Ribose	—	—	—	—	—	—	—	—	—	—
Xylose	—	+	+	+	+	—	—	—	—	—
		wk 10	wk 6	wk 4	wk 3					
Rhamnose	—	—	—	—	—	—	—	—	—	—
Mannose	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+
Galactose	—	—	—	—	—	—	—	—	—	—
Cellobiose	+	+	+	+	+	+	+	+	+	+
		wk 2	wk 4	wk	wk 4					
Lactose	+	+	+	+	+	+	+	+	+	+
		wk 4	wk 4	wk 4	wk 4	wk		wk 3	wk 3	wk 4

TABLE 3.—Continued

Characteristics ^a	ATCC 13637 (Type strain of <i>P. maltophila</i>)	YF-3 (Type strain of <i>P. melanogena</i>)	YF-6	YF-8	YF-10	P-1	P-7	P-7-2	P-19	P-31-2
Maltose	+	+ ₂	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+ ₄	+	+	+	+	+	+
	wk 4	wk 10	wk 6		wk 3	wk	wk 3	wk 3	wk 4	wk 4
Trehalose	+	+ ₄	+	+	+	+	+	+	+	+
	wk 4		wk 6	wk 4	wk 3	wk	wk 4	wk 6	wk 4	wk 4
Melibiose	+ ₄	+	+	+	+ ₄	+	+ ₃	+	+	+
		wk 5	wk 4	wk 4				wk 4	wk 6	wk 6
Raffinose	+	+	—	+	+	+ ₃	—	—	—	+
	wk 4	wk 10		wk 4	wk 4					wk 6
Melezitose	—	—	—	—	—	—	—	—	—	—
Adonitol	—	—	—	—	—	—	—	—	—	—
Dulcitol	—	—	—	—	—	—	—	—	—	—
Inositol	—	—	—	—	—	—	—	—	—	—
Mannitol	—	—	—	—	—	—	—	—	—	—
Sorbitol	—	—	—	—	—	—	—	—	—	—
Inulin	—	—	—	—	—	—	—	—	—	—
Salicin	—	—	—	—	—	—	—	—	—	—
OF basal medium control, blue	+	+	+	+	+	+	+	+	+	+
Carlquist, ninhydrin	+	+	+	+	+	+	—	—	+	+
L-Lysine decarbox- ylase, Møller	—	+ ₂	+ ₂	—	+ ₂	—	—	—	+ ₂	+ ₂
L-Arginine dihydro- lase, Møller	—	—	—	—	—	—	—	—	—	—
L-Ornithine decar- boxylase, Møller	—	—	—	—	—	—	—	—	—	—
Oxidase	—	—	—	—	—	—	—	—	—	—
Catalase	+	+	+	+	+	+	+	+	+	+
Urease	—	—	—	—	—	—	—	—	—	—
Phenylalanine deamin- ase	+	—	—	—	—	+	—	—	—	—
Growth in BHI con- taining 3% NaCl	+	+	+	+ ₂	+ ₂	+	+	+	+	+
% Guanine plus cytosine	65.1	65.2	NT	NT	66.3	66.5	66.5	NT	NT	NT

^a LD, Leifson deoxycholate agar; SS, Salmonella-Shigella agar; NAC, nalidixic acid-cetrimide agar; BHI, brain heart infusion.

^b +, Positive reaction on the first day. Subscripts indicate the day the test became positive. —, Negative reaction; wk, weak reaction; NG, No growth; NT, not tested.

not detected among the guanine plus cytosine ratios of the five strains examined.

As is evident from the data presented in Tables 2 and 3, there are only a few slight differences between the type strains of *P. maltophilia* Hugh and Ryschenkow 1960 and *P. melanogena* Iizuka and Komagata 1963. Therefore, we regard these two names as subjective synonyms. *P. maltophilia*, being the earlier of the two names, has priority and is therefore the correct name of this species; *P. melanogena* becomes a junior synonym of *P. maltophilia*. The reidentification as *P. maltophilia* of several

strains previously labeled *P. alcaligenes* (9, 10, 12) is confirmed.

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