Description of *Achromobacter xylosoxidans* Yabuuchi and Ohyama 1971

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More than 70 characters of 55 strains of *Achromobacter xylosoxidans* were compared with those of the type strain of *A. xylosoxidans*, ATCC 27061 (= KM 543). Repeated examination of these 55 strains confirmed the stability of the flagellar morphology and biochemical reaction pattern and confirmed that the species can be recognized by these characters. Two strains of *Alcaligenes faecalis*, two strains of *Alcaligenes denitrificans*, five strains of *Alcaligenes* sp., and five strains of each of King groups IIIa and IIIb were identified as strains of *A. xylosoxidans*. The base compositions of the deoxyribonucleic acids of 20 strains are given. The cellular fatty acid composition of extractable and bound lipids of five strains was determined. The minimal characters for the identification of strains of *A. xylosoxidans* are presented.

Achromobacter xylosoxidans was named and described by Yabuuchi and Ohyama (13) after a study of seven strains isolated from purulent ear discharge of patients with chronic otitis media. The type strain, KM 543, was designated and deposited in several type-culture collections. Among 183 strains of nonfermenting, gram-negative rods received from several clinical laboratories since 1969, 35 were identified as *A. xylosoxidans*. Twenty strains received from foreign investigators were subsequently identified as *A. xylosoxidans*. The uniformity of the flagellar morphology, biochemical reactions, cellular lipid and fatty acid composition, and the guanine plus cytosine (GC) mole percent of composition deoxyribonucleic acid (DNA) serve to establish the homogeneity of *A. xylosoxidans*. The purposes of this report are to offer evidence of the uniformity of the species, to amend the previous description (13), and to present the minimal characters for the identification of strains of *A. xylosoxidans*.

MATERIALS AND METHODS

The histories and corresponding accession numbers of 55 strains of *A. xylosoxidans* are listed in Table 1. Strains described in the literature (3, 6, 9) are listed in Table 2.

A. xylosoxidans ATCC 212 (= RH 137 = KM 1051) was isolated in 1923 and deposited in the American

Type Culture Collection by E. O. Jordan, University of Chicago, in 1925, and is the oldest strain of this species known to be available. The type strain, ATCC 27061 (KM 543), was isolated by H. Takeda, Kansai Medical University Hospital, from the purulent ear discharge of a 32-year-old female patient with otitis media chronica sinistra. It was isolated from a specimen collected during the patient's first visit for medical attention at Kansai Medical University Hospital on 8 July 1969, and was mixed with *Staphylococcus aureus*. The unidentified glucose nonfermenter, strain 9, was received from Takeda on 30 September 1969 and was designated KM 543 (13).

A broth culture was formalinized, washed twice with distilled water, and stained for flagella by the method of Leifson (7, 8). A distilled-water suspension, prepared from growth on an infusion agar (Difco 0045) slant, was also stained for flagella. Growth from infusion agar was suspended in formalinized saline, washed with distilled water, negatively stained with phosphotungstic acid, and observed for flagella in an electron microscope (Hitachi HU-12). Cultures for flagellar morphology were incubated at 20 °C for 18 h. Spreading, diffuse growth emanated from the line of inoculation in motility medium after 18 h of incubation at 20 °C were examined microscopically in wet-mount preparations. Nonmotile strains were inoculated in the center of motility medium in a petri dish (Gard plate) to select actively motile cultures.

Growth in the following media was determined after incubation in an anaerobic environment (BBL GasPak anaerobic system): (i) OF basal medium (Difco 0688) containing 1% glucose; (ii) nitrate broth (3) (Casitone [Difco], 10 g; yeast extract [Difco], 3 g;

TABLE 1. *Histories and corresponding numbers of 55 Achromobacter xylosoxidans strains^a*

KM	ATCC	NCTC	RH	Other	Status	Source	Received from:
531						Ear discharge	Wada
543	27061	10807	2838	NRRL B4082	Type	Ear discharge	Takeda
556						Ear discharge	Takeda
563	27062	10808	2860	NRRL B4083		Ear discharge	Takeda
583	27063	10809	2861	NRRL B4084		Ear discharge	Takeda
757			2862			Ear discharge	Wada
767			2863			Ear discharge	Takeda
780						Ear discharge	Takeda
790			2864			Ear discharge	Takeda
793						Ear discharge	Takeda
949			2866	CDC B4043		Spinal fluid	Weaver
950			2867	CDC B5140		Sputum	Weaver
951			2868	CDC B5242		Brain	Weaver
952				CDC B5768		Spinal fluid	Weaver
953				CDC B6708		Urine	Weaver
954			2869	CDC B6949		Skin	Weaver
955			2870	CDC B6966		Throat	Weaver
956			2871	CDC B7076		Stool	Weaver
957				CDC B7168		Spinal fluid	Weaver
958				CDC B7176		Pus	Weaver
959							Takeda
962							Takeda
963							Takeda
964			2367			Respiratory tract	Hugh
970						Ear discharge	Tanaka
1044			2876	Lautrop AB230			De Ley
1046			2878	Lautrop AB107			Lautrop
1047			2879	Lautrop AB1098			Lautrop
1048				Lautrop AB1170			Lautrop
1049				Lautrop AB1307			Lautrop
1050	9220		136			Stool	Hugh
1051	212		137				Hugh
1052							Wada
1054				CIP 5872			Piéchaud
1055				CIP 6120			Piéchaud
1142				Sa-195		Ulcer	Saku
1143				Sa-199		Sputum	Saku
1145				Og-1		Ear discharge	Oguri
1146				Og-7		Sputum	Oguri
1147				Og-25		Sputum	Oguri
1148				SU-3		Pus	Chosa
1157				Sa-156		Ear discharge	Saku
1158				Sa-165		Ear discharge	Saku
1182						Ear discharge	Wada
1206				Sa-386		Ear discharge	Saku
1209				Sa-578		Ulcer	Saku
1210				Sa-579		Ulcer	Saku
1212				Sa-581		Burn	Saku
1213				Sa-582		Burn	Saku
1214				Sa-583		Burn	Saku
1215				Sa-584		Peritoneal dialysis	Saku
1216				Sa-585		Peritoneal dialysis	Saku
1217				Sa-586		Peritoneal dialysis	Saku
1218				Sa-587		Peritoneal dialysis	Saku
1219				Sa-588		Peritoneal dialysis	Saku

^a Abbreviations: ATCC, American Type Culture Collection, Rockville, Md; CIP, Colléction de L'Institut Pasteur, Paris; CDC, Center for Disease Control, Atlanta Ga.; KM, Kansai Medical University Culture Collection, Osaka; NCTC, National Collection of Type Cultures, London; NRRL Northern Regional Research Laboratory, Peoria, Ill.; RH Rudolph Hugh, George Washington University, Washington D.C.

TABLE 2. *Nineteen strains described in the literature and subsequently identified as strains of Achromobacter xylosoxidans*^a

KM strain no.	Received as:	Reference
949	King group IIIa	King (6)
950	King group IIIb	King (6)
951	King group IIIb	King (6)
952	King group IIIa	King (6)
953	King group IIIb	King (6)
954	King group IIIb	King (6)
955	King group IIIa	King (6)
956	King group IIIa	King (6)
957	King group IIIb	King (6)
958	King group IIIa	King (6)
1044	<i>Alcaligenes</i> sp. AB230	De Ley et al. (4)
1046	<i>Alcaligenes</i> sp. AB107	De Ley et al. (4)
1047	<i>Alcaligenes</i> sp. AB1098	De Ley et al. (4)
1048	<i>Alcaligenes</i> sp. AB1170	De Ley et al. (4)
1049	<i>Alcaligenes</i> sp. AB1307	De Ley et al. (4)
1050	<i>Alcaligenes faecalis</i> RH 136	ATCC catalogue (1)
1051	<i>A. faecalis</i> RH137	Moore and Pickett (9)
1054	<i>A. denitrificans</i> CIP 5872	De Ley et al. (4)
1055	<i>A. denitrificans</i> CIP 6120	De Ley et al. (4)

^a For abbreviations, see Table 1, footnote a.

KNO₃, 2 g; and distilled water, 1,000 ml); (iii) casitone-yeast extract broth (Casitone [Difco]), 10 g; yeast extract [Difco], 3 g; and distilled water, 1,000 ml; (iv) Casamino Acids broth (vitamin-free Casamino Acids [Difco 0288], 12 g; K₂HPO₄, 1 g; KH₂PO₄, 1 g; MgSO₄ · 7H₂O, 0.4 g; NaCl, 0.2 g; and distilled water, 1,000 ml, sterilized by membrane filtration).

Anaerobic growth was compared with aerobic growth response in these four media. Indophenol oxidase activity was visualized by transferring growth from infusion agar to wet filter paper containing tetramethyl paraphenyldiamine hydrochloride (Nissui cytochrome-oxidase test paper). Sodium chloride tolerance was determined in brain heart infusion (BHI) broth containing 3, 5, or 6.5% NaCl. Sensitivity to sodium azide was determined in *Streptococcus faecalis* broth (Eiken). Ability to grow in the presence of 40% bile and hydrolyze esculin was determined in bile-esculin agar (Difco 0879).

Amidase activity was determined in the following medium (2): KH₂PO₄, 2 g; MgSO₄ · 7H₂O, 0.1 g; NaCl, 5 g; acetamide or propionamide, 1 g; and distilled water, 1,000 ml, pH 6.8. This medium was distributed in tubes in 1-ml portions and autoclaved at 121 °C for 15 min. A 24-h agar slant culture of each strain was used to inoculate amidase medium. After incubation at 37 °C for 18 to 20 h, 1 drop of Nessler reagent was added to each tube. Production of ammonium due to hydrolysis of amide was indicated by a red-brown precipitate.

Assimilation of glucose was determined in a chemically defined basal medium [Na₂HPO₄, 7 g; KH₂PO₄, 3 g; (NH₄)₂SO₄, 1 g; MgSO₄ · 7H₂O, 0.2 g; and distilled water, 1,000 ml] containing a membrane-filtered 10% glucose solution to make the final concentration 0.2%. Growth from an 18-h agar slant culture was harvested and suspended in sterile distilled water, and growth was diluted to a final concentration of 10⁶ cells/ml. One drop of this suspension was aseptically added to the medium with a sterile Pasteur pipette; the culture was incubated at 30 °C and observed daily. Cultures that produced a definite turbidity within 24 to 48 h and that could be maintained by serial subculture in the same medium were regarded as positive. Culture media that remained clear or became weakly turbid after 3 or more days were regarded as negative. Other physiological and biochemical tests listed in Table 3 were performed as reported previously (13, 14).

Sensitivities of the 55 strains to 17 antibiotics were determined by the twofold serial agar dilution method. One loopful of a bacterial suspension, a 10⁻² dilution of an 18-h heart infusion broth culture, was streaked on the surface of the antibiotic-containing heart infusion agar plate. The minimal inhibitory concentration was read after 20 h of incubation at 37 °C.

The GC ratios of the DNA of 14 selected strains were determined by J. De Ley.

The cellular lipid and fatty acid compositions of five selected strains, KM 543, 954, 955, 1047, 1051, were determined according to methods described previously (15).

RESULTS

The 55 strains of *A. xylosoxidans* studied contained gram-negative, asporogenous, straight rods with rounded ends. Capsules were not seen. Most cells appeared singly and some were in pairs. Soma size was 0.8 to 1.2 × 2.5 to 3.0 µm. All strains except one (KM 953) were actively motile and peritrichous. Fifty-four strains, including the type strain, had one to nine normal peritrichous flagella. Strain KM 953 was nonmotile and atrichous after serial passage in motility medium in a petri dish. Figure 1 shows peritrichous cells of the type strain grown in a broth medium stained after

FIG. 1. *Achromobacter xylosoxidans* KM 543 peritrichous rods with nine (a) and six (b) peritrichous flagella. Broth culture. Leifson flagella stain after formalin fixation. ×2,000.

TABLE 3. Characteristics of *Achromobacter xylosoxidans*

Substrate or test ^a	Type strain ^b ATCC 27061	55 strains ^c (% positive)
Gram-negative rod-shaped cells	+	100
Motility	+	98
Peritrichous flagella	+	98
OF glucose medium open, acid	+ ₂	95 (5)
OF glucose medium sealed, acid	—	0
Assimilation of glucose	+	100
Oxidative acid production from ^d :		
Ethanol (3%)	+ ₃	24 (76)
Glycerol	+ _{1 0}	0 (73)
Adonitol, dulcitol, inositol	—	0
Mannitol, sorbitol, xylitol	—	0
Xylose	+ ₂	100
D-arabinose	+ _{1 3}	2 (36)
L-arabinose	—	2 (24)
D-ribose	—	0 (9)
Rhamnose	—	0
Fructose	—	16 (11)
Galactose	+ ₂	89 (11)
Mannose	+ ₂	58 (42)
Lactose, maltose, cellobiose	—	0
Melibiose, sucrose, trehalose	—	0
Raffinose, melezitose	—	0
Inulin, salicin	—	0
OF basal medium control, blue	+	100
Indole, methyl red	—	0
Acetylmethylcarbinol	—	0
Indophenol oxidase, catalase	+	100
Hydrogen sulfide, Kligler butt	—	0
2-Ketogluconate	+ ₂	62
3-Ketolactose	—	0
Malonate	+ ₂	100
Citrate, Simmons	+	100
Nitrate reduction to nitrite	+	100
Nitrite reduction to nitrogen gas	+ ₂	64
Water-soluble pigment	—	0
Chloroform-soluble pigment	—	0
Growth in BHI at 41 °C	+	78
Growth in BHI at pH 4.5	—	0
Growth in BHI containing 5% NaCl	+ ₂	49 (29)
Growth in BHI containing 6.5% NaCl	—	0 (9)
Growth in SF broth	—	0
Growth in the presence of 40% bile	+	96 (4)
Growth on SS agar	+	93
Growth on LD agar	+	100
Growth on Pseudose agar	—	87
Growth on NAC agar	+ ₂	84 (2)
Lysine decarboxylase, Carlquist	—	0
L-lysine decarboxylase, Møller	—	0
L-arginine dihydrolase, Møller	—	0
L-ornithine decarboxylase, Møller	—	0
Phenylalanine deaminase	—	0
Deoxyribonuclease, extracellular	—	0
Amidase for propionamide	+	100
Urease, phosphatase	—	0
Gelatin liquefaction	—	0
Hydrolysis of starch, Tween 80	—	0
Hydrolysis of esculin	—	0
Hemolysis, rabbit blood agar	—	0
Green discoloration, rabbit blood agar	—	0

^a Abbreviations: BHI, brain heart infusion; LD agar, Leifson deoxycholate agar; NAC agar, nalidixic acid-cetrimide agar; SF broth, *Streptococcus faecalis* broth; SS agar, salmonella-shigella agar.

^b Subscripts indicate days of incubation required for a positive reaction.

^c Values indicate percent positive within 1 or 2 days. Values in parentheses indicate percent positive after 3 or more days.

^d Carbohydrates except ethanol were used in 1.0% concentration.

formalin fixation. Figure 2 shows peritrichous cells of the type strain grown on agar medium and stained without formalin fixation. The electron micrograph of the type strain (Fig. 3) shows the sheathed structure of the flagella.

A. xylosoxidans strains produced a homogeneous turbidity in BHI broth within 24 h at room temperature, a pellicle and precipitate appeared and the broth became almost clear. Sixty-four percent of the strains reduced nitrate to nitrogen gas and grew luxuriantly under anaerobic conditions; the remaining 36% of the strains reduced nitrate to nitrite (not to nitrogen gas) and grew moderately well under anaerobic conditions. Casamino Acids broth, Casitone-yeast extract broth, and OF glucose medium did not support growth of the 55 strains under anaerobic conditions. They grew well in these media aerobically. Cultures of

these strains were serially maintained in a chemically defined broth medium containing glucose as a sole source of carbon and energy and ammonium ions as a sole source of nitrogen. They did not require growth factors.

After 2 days of incubation at 30 C, colonies on infusion agar medium were about 1 mm in diameter, smooth and glistening, and had an entire margin. Growth on infusion agar medium was grayish and semitranslucent, and after several days of incubation at room temperature it had a faint yellowish tint. Water-soluble pigment was not produced. Colonies on deoxycholate agar, salmonella-shigella agar, and nalidixic acid-cetrimide agar were discrete and gray. Rabbit erythrocytes in infusion agar medium were not hemolyzed around either discrete colonies or heavy growth. The blood agar medium around the colonies did not become greenish. The strains did not grow in BHI broth at 4 C or in BHI broth adjusted to pH 4.5. Forty-three (78%) strains grew in BHI broth containing 5% NaCl, and only five (9%) strains grew in BHI broth containing 6.5% NaCl. All 55 strains grew on bile-esculin agar without blackening the medium, and none grew in *S. faecalis* broth. Acid promptly accumulated at the surface of unsealed OF basal medium containing xylose. Repeated examination revealed weak acid production in OF ethanol, galactose, glucose, and mannose media within 2 to 4 days. Alkali accumulated in OF basal

FIG. 2. *Achromobacter xylosoxidans* KM 543 peritrichous rods with eight (a) and seven (b) flagella. Infusion agar slant culture. Leifson flagella stain without formalin fixation. $\times 2,000$.

FIG. 3. *Achromobacter xylosoxidans* KM 543 peritrichous cells with sheathed flagella. Negatively stained with phosphotungstic acid after formalin fixation. $\times 200,000$.

medium containing the following carbon compounds: rhamnose, cellobiose, lactose, maltose, sucrose, trehalose, melibiose, raffinose, melezitose, adonitol, dulcitol, inositol, mannitol, sorbitol, xylitol, inulin, and salicin. Simmons citrate and malonate media became alkaline. Indophenol oxidase, catalase, and amidase were produced. A stronger amidase reaction was observed in the propionamide medium than in the acetamide medium. Hydrogen sulfide was not detected in the butt of Kligler iron agar. Indole, acetylmethylcarbinol, and 3-ketolactose were not produced. Liquefaction of gelatin, hydrolysis of starch and Tween 80, phenylalanine deaminase, deoxyribonuclease, phosphatase, and urease reactions were negative. Carlquist ninhydrin tests for lysine decarboxylase, L-arginine dihydrolase, and L-lysine and L-ornithine decarboxylase reactions were negative in decarboxylase base Møller (Difco 0890). Of the 55 strains, 20 were inhibited by rifampin at a concentration of 12.5 $\mu\text{g/ml}$, and a few strains were inhibited by penicillin derivatives (Table 4). The GC contents of the DNA of 20 strains are recorded in Table 5; the values on six of these strains were reported by De Ley et al. (3).

Cardiolipin, phosphatidyl ethanol amine, and phosphatidyl glycerol were the major lipids extracted from five strains. The major fatty acid components of extractable lipids were C16:0, C16:1, C18:0, C18:1 straight-chain and C17 cyclopropanoic fatty acids. The major bound fatty acids in the five strains were 2-hydroxydodecanoic acid (25.6 to 33.6%),

2-hydroxytetradecanoic (16.4 to 22.4%), and 3-hydroxytetradecanoic (31.1 to 37.6%).

DISCUSSION

The morphological, physiological, and biochemical characteristics, the GC contents of DNA, and the cellular lipid and fatty acid compositions of *A. xylosoxidans* strains are very uniform. A nonfermentative, gram-negative, asporogenous rod with the attributes listed in Table 6 can be identified as a strain of *A. xylosoxidans*. These minimal characters distinguish this species from pseudomonads, *Enterobacteriaceae*, *Acinetobacter anitratus*, and oxidase-positive peritrichous rods that fail to oxidize glucose and xylose. Although the original report on this stated that most of the seven strains of *A. xylosoxidans* failed to produce distinct acidity (the media remained neutral) in open tubes of OF media containing ethanol, glucose, galactose, and mannose, a reexamination revealed that a weak acidity (greenish-yellow, pH 6.5 to 6.7) was produced from these four carbohydrates in OF media. All strains of *A. xylosoxidans* produced oxidative acidity from xylose. Some of the 55 strains slowly produced weak acidity from glycerol, D- and L-arabinose, D-ribose, and fructose. Acid was not produced from disaccharides, trisaccharides, polyalcohols inulin, or salicin.

The 55 strains failed to grow in OF glucose medium under anaerobic conditions. *A. xylosoxidans* is a strict aerobe. The 55 strains grew

TABLE 4. Antibiotic sensitivity of 55 *Achromobacter xylosoxidans* strains

Antibiotic	No. of strains sensitive at concn ($\mu\text{g/ml}$) of:										
	0.19	0.39	0.78	1.56	3.12	6.25	12.5	25.0	50.0	100	>100
Ampicillin		1			1					1	52
Carbenicillin	2						1			3	49
Sulbenicillin			1	1					3	2	48
Cephalexin										4	51
Cephazolin						1				3	51
Erythromycin							1	1	2	7	44
Kitasamycin							1		1	5	48
Rifampin						11	10	6		2	26
Chloramphenicol								9	2		44
Tetracycline								7	7	8	33
Streptomycin											55
Kanamycin									6	5	44
Dideoxy-kanamycin B									5	4	46
Gentamicin						2		4	5	10	34
Polymyxin B					1		2	4	7	5	36
Colistin						3	2	1	3	3	43
Nalidixic acid							3		1	6	45

TABLE 5. *Guanine plus cytosine mole percent of 20 Achromobacter xylosoxidans strains*^a

KM strain no.	GC mol%
563	66.9
954	66.9
956	67.4
951	67.6
955	67.9
1047	68.1
949	68.3
1046	68.4 ^b
1049	68.4 ^b
757	68.6
793	68.9
1044	68.9 ^b
1048	68.9 ^b
790	69.0
950	69.1
543	69.2
1055	69.2 ^b
583	69.5
767	69.5
1054	69.8 ^b

^a Determined by J. De Ley. KM, Kansai Medical University Culture Collection, Osaka.

^b Data from the report by De Ley et al. (4).

TABLE 6. *Minimal characters for identification of Achromobacter xylosoxidans strains*

Character	% Positive ^a	Sign
Gram-negative, asporogenous, straight rods	100	+
Peritrichous flagella	98	+
Motility	98	+
Indophenol oxidase	100	+
OF glucose medium open, acid	95 (5)	+ ^b
OF glucose medium sealed, growth	0	—
OF maltose medium, acid	0	—
OF xylose medium, acid	100	+
Citrate, Simmons	100	+
Nitrate reduction to nitrite	100	+
Urease	0	—
Hemolysis and discoloration of rabbit blood	0	—
Lysine decarboxylase, Carlquist	0	—
ninhydrin	0	—
L-arginine dihydrolase	0	—

^a Based on a study of 55 strains. Values indicate percent positive within 1 or 2 days. Values in parentheses indicate percent positive after 3 or more days.

^b Usually weaker than the acidity from xylose.

anaerobically in nitrate broth. Thirty-five (64%) strains grew luxuriantly with copious bubbling in nitrate broth. The remaining 20 (36%) strains grew moderately without gas production in nitrate broth under anaerobic conditions.

King (6) divided group III organisms into two groups: group IIIa strains reduced nitrate to nitrite and group IIIb strains reduced nitrate and nitrite to nitrogen gas. She felt that groups IIIa and IIIb belonged to the same species. Tatum (11) and Weaver et al. (12) feel that group IIIb can be regarded as a variant of group IIIa. From the results of the present study, it can be said that all known *A. xylosoxidans* strains grow anaerobically in the presence of nitrate and that the ability to reduce nitrate to nitrogen gas should be regarded as an infrasub-specific difference.

Fatty acid compositions of the extractable and bound lipids of five selected strains of *A. xylosoxidans* were very uniform. Nearly 80% or more fatty acids of the cellular bound lipids of five strains of the species were hydroxyfatty acids. The high concentration of hydroxyacids, especially 2-OH12:0, 1-OH14:0, and 3-OH14:0, in the bound lipids may prove to be characteristic of all *A. xylosoxidans* strains. A comparative investigation on the cellular lipid composition of several species of nonfermentative, gram-negative, peritrichous rods will be published elsewhere.

Although the absence of a type or neotype strain for *Achromobacter liquefaciens*, type species of the genus, has made it difficult to determine what other species should be included in the genus *Achromobacter*, *A. xylosoxidans* was included in the genus *Achromobacter* on the basis of the following definition of the genus (4): gram-negative, asporogenous, motile rods; peritrichous; OF glucose medium open, acid (+) or no change; OF glucose medium sealed, acid (—); indophenol oxidase and catalase (+); 3-ketolactose (—).

Though the natural habitat is now known, *A. xylosoxidans* has been isolated from various kinds of pathological specimens, such as blood, spinal fluid, pleural fluid, peritoneal fluid, pus, urine, stool, and swabs of eye, ear, and pharynx. The occurrence of strains of *A. xylosoxidans* in pathological specimens warrants an investigation of the clinical significance of the species. Shigeta et al. (9) reported a 9-year-old girl with purulent meningitis caused by *A. xylosoxidans*. The meningitis occurred in May and again in September after occipital trepanation and ventriculoperitoneal shunt in March. *A. xylosoxidans* was isolated from spinal fluid in pure culture. The patient's serum agglutinated the isolates up to a dilution of 1:640. During the later stage of preparation of this manuscript in 1974, Y. Kanazawa (Niigata Railway Hospital, Japan) related (personal communication to E. Y.) that a nonfermentative, gram-negative, motile rod was isolated in

pure culture from arterial blood, venous blood, and a biopsy specimen of a cervical lymph node of a 42-year-old male patient with remittent fever and cervical lymph node swelling. The three isolates were very much alike and were very sensitive to chloramphenicol among eight antibiotics tested. The patient was effectively treated with chloramphenicol. The three isolates, 1547, 1548, and 1549, were received from Kanazawa and identified as strains of *A. xylosoxidans*.

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REPRINT REQUESTS

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LITERATURE CITED

1. American Type Culture Collection. 1949. Catalogue of strains, 5th ed., p. 4. American Type Culture Collection, Rockville, Md.
2. Arai, T., M. Otake, S. Enomoto, S. Goto, and S. Kuwahara. 1970. Determination of *Pseudomonas aeruginosa* by biochemical test method. III. Acylamidase test. Jap. J. Microbiol. **14**:279-284.
3. De Ley, J., K. Kersters, J. Khan-Matsubara, and J. M. Shewan. 1970. Comparative D-gluconate metabolism and DNA base composition in *Achromobacter* and *Alcaligenes*. Antonie van Leeuwenhoek J. Microbiol. Serol. **36**:193-207.
4. Hugh, R. 1970. A practical approach to the identification of certain nonfermentative Gram-negative rods encountered in clinical specimens. Publ. Health Lab. **28**:168-187.
5. Hugh, R. 1970. *Pseudomonas* and *Aeromonas*, p. 175-190. In J. E. Blair, E. H. Lennette, and J. P. Truant (ed.), Manual of clinical microbiol. American Society for Microbiology, Bethesda, Md.
6. King, E. O. 1964. The identification of unusual pathogenic Gram-negative bacteria. CDC Publication.
7. Leifson, E. 1951. Staining, shape, and arrangement of bacterial flagella. J. Bacteriol. **62**:377-389.
8. Leifson, E. 1960. Atlas of bacterial flagellation. Academic Press Inc., London and New York.
9. Moore, H. B., and M. J. Pickett. 1960. The *Pseudomonas-Achromobacter* group. Can. J. Microbiol. **6**:35-42.
10. Shigeta, S., K. Higa, M. Ikeda, and S. Endo. 1974. A purulent meningitis caused by *Achromobacter xylosoxidans*. (In Japanese) Igaku No Ayumi **88**:336-337.
11. Tatum, H. W. 1970. Miscellaneous Gram-negative bacteria, p. 191-198. In J. E. Blair, E. H. Lennette, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Bethesda, Md.
12. Weaver, R. E., H. W. Tatum, and D. G. Hollis. 1972. The identification of unusual pathogenic Gram-negative bacteria (E. O. King). Preliminary revision. CDC Publication.
13. Yabuuchi, E., and A. Ohyama. 1971. *Achromobacter xylosoxidans* n. sp. from human ear discharge. Jap. J. Microbiol. **15**:477-480.
14. Yabuuchi, E., and A. Ohyama. 1972. Characterization of pyromelanin-producing strains of *Pseudomonas aeruginosa*. Int. J. Syst. Bacteriol. **22**:53-64.
15. Yano, I., Y. Furukawa, and M. Kusunose. 1970. 2-Hydroxy fatty acid-containing phospholipid of *Arthrobacter simplex*. Biochim. Biophys. Acta **210**:105-115.