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FLAVOBACTERIUM DEVORANS ATCC 10829: A STRAIN OF *PSEUDOMONAS PAUCIMOBILIS*

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Flavobacterium devorans ATCC 10829 (=NRRL B–54=KM 1367) was compared with *Pseudomonas paucimobilis* NCTC 11030 (type, =KM 2395), 11031 (=KM 2396), and 11032 (=KM 2397) in respect to more than 65 phenotypic tests, susceptibility to 15 antimicrobial agents, electron-microscopy, and visible absorption spectrum of yellow pigments in acetone extracts of bacterial cells. Long-chain base component of ceramide glucuronic acid in cellular lipids was quantitatively determined from thin-layer chromatogram of hexane-ether extract from the hydrolysate of acetone-dried cells of 4 strains. Characteristic profile of cellular fatty acids of strain 10829, as well as of 3 *P. paucimobilis* strains, was the presence of 2-hydroxymyristic acid, palmitic acid, and octadecenoic acid in high concentration. Because of the phenotypic and analytical similarities and high percentage of guanine plus cytosine content in deoxyribonucleic acid, *F. devorans* 10829 was reidentified as a strain of *P. paucimobilis*. Since the strain ATCC 10829 was a misidentified organism, the name *Flavobacterium devorans* can not be a senior synonym over *Pseudomonas paucimobilis*. Unless the authentic strain of the original describer of *F. devorans* or any isolate in conformity with the original description for the species were found, the name *Flavobacterium devorans* should not be retained further.

Flavobacterium was named and described as a color genus and its taxonomic heterogeneity has been frequently pointed out. Bergey *et al.* transferred *Bacillus devorans* ZIMMERMANN 1890 (*1*) to *Flavobacterium* based on a study of a strain isolated from water, and proposed a new combination *Flavobacterium devorans* (Zimmermann) BERGEY *et al.* 1923 (*2*). STEINHAUS (*3*), in his report on the bacterial flora of insects, recorded an isolation of a strain (No. 56) from alimentary

tract of *Coccinella novemnotata* (nine spotted lady beetle). He thought the strain closely resembled *F. devorans*. In spite of the statement by BREED *et al.* (*4*) that Steinhaus apparently found the same organism as *F. devorans*, Steinhaus reported nothing but the source of isolation of his organism. Furthermore, Steinhaus' isolate and Bergey's isolate are not available. Thus, it is not possible to judge at present the identity of these 2 isolates. Although *F. devorans* was included in each edition of Bergey's Manual, neither holotype nor cotype of the species is extant.

Strain 10829 (ATCC), referred to as a reference strain for *F. devorans* by WEEKS (*5*), is a sole existing strain bearing the name *F. devorans*. It is a gram-negative rod-shaped organism and produces deep yellow colonies. Broth-cultured cells of strain 10829 were chiefly atrichous and some had a single polar flagellum, contrary to the description by Weeks on flagellar morphology of *F. devorans* as peritrichous. Because of such discrepancy, bacteriological investigation of strain 10829 was performed. The guanine plus cytosine content of the strain was reported as 69.0% (*5*) or 67.7% (*6*). Cellular lipids of the strain 10829 was reported to contain a new sphingoglycolipid, ceramide glucuronic acid (N-acyl-dihydrosphingosinle-1-glucuronic acid), for the first time throughout eucaryotic and procaryotic cells (*7*).

In recent years, isolation of deep yellow-pigmented bacteria from human clinical materials and from sources in hospital environment have been reported. These bacteria were described as *Flavobacterium* sp., *Xanthomonas* sp., or pseudomonad-like organism. TATUM *et al.* (*8*) characterized pseudomonad-like organisms referred to as group II K and divided them into biotypes 1 and 2. HOLMES *et al.* (*9*) proposed a new name, *Pseudomonas paucimobilis*, for the yellow-pigmented organisms including strains of group II K biotype 1.

Because of its polar monotrichous flagellation, deep-yellow colony formation, and high GC%, strain 10829 was compared with the type and 2 reference strains of *P. paucimobilis*.

MATERIALS AND METHODS

The reference strain of *Flavobacterium devorans* ATCC 10829 was received from the Research Institute for Microbial Diseases, Osaka University. The holotype strain, NCTC (National Collection of Type Cultures) 11030, and 2 reference strains, NCTC 11031 and 11032 of *Pseudomonas paucimobilis*, were received from the National Collection of Type Cultures, London. Histories and corresponding number of the 4 strains are shown in Table 1. Casitone (Difco, 1%)-yeast extract (Difco, 0.3%) broth containing 0.5% NaCl was routinely used for growing these organisms. Cultures were incubated at 30° unless otherwise stated.

Soma size of 10829 was determined on electron micrograph of shadow-casted cells. Twenty-hour broth culture was stained for flagella by LEIFSON's technique

Table 1. Histories and corresponding strain number of *Flavobacterium devorans* and 3 *Pseudomonas paucimobilis* strains.

Original strain designation	Received as	Corresponding strain numbers					Source of isolation	Status	Received from
		ATCC[a]	NRRL[b]	NCTC[c]	CDC[d]	KM[e]			
Acetobacter acetosum No. 16	*F. devorans*	10829	B–54			1367	Not recorded	—	RIMD[f]
CL 1/70	*P. paucimobilis*			11030		2395	Respirator	Type	NCTC
Group II K biotype 1	*P. paucimobilis*			11031	B3271	2396	Spinal fluid	—	NCTC
Group II K biotype 1	*P. paucimobilis*			11032	B4562	2397	Blood	—	NCTC

[a] American Type Culture Collection, Maryland.
[b] Northern Research and Developmental Laboratories, Illinois.
[c] National Collection of Type Cultures, London.
[d] Center for Disease Control, Georgia.
[e] Kansai Medical University, Osaka.
[f] Research Institute for Microbial Diseases, Osaka University, Osaka.

(*10*). Smears of 20-hr casitone-yeast extract broth culture were stained for fat granules by Sudan Black B staining (*11*). For electron microscopy, a suspension of twice-washed bacteria was placed on carbon-collodion coated grids. Excess amount of the suspension was drawn with a filter paper and the grids were kept for drying. Then the grids were shadow-casted with platinum-palladium at an angle of 30°. For examination and photography, Hitachi H-500 electron microscope was used.

Strain which failed to exhibit a spreading growth from the inoculation line in stabbed semisolid motility medium were tested for their motility by repeated examination of wet-mount living preparation of 20-hr broth culture and by observation of diffuse spreading growth on semisolid GARD's plates (*12*).

Assimilation of glucose, asparagine, and glutamine was determined by serial subculture in a chemically defined medium (*13*) containing each compound at a concentration of 0.2%. Bile aesculin agar (Difco 0879) was used to determine the ability of organism to grow in the presence of 40% bile and to hydrolyze aesculin. Strains unable to grow on this medium were tested for aesculin hydrolysis on a plate of blood agar base (BAB, Difco 0045) added with 0.1% aesculin and 0.05% ferric citrate. In order to determine growth-inhibitory effect of Bromothymol Blue (BTB), BAB containing 0.01, 0.02, 0.04, 0.06, or 0.08 g/*l* of BTB were used. Glucose fermentation was tested in Eiken Poa-media (Eiken, Tokyo) which contains less amount (0.003%) of BTB than in OF (oxidation and fermentation) basal medium (Difco 0688, 0.008%). Acid production from sugars and polyalcohols was tested in both OF basal medium and PYP (peptone-yeast extract-Phenol Red) basal medium (Nissui, Tokyo) (*14*). One liter of the latter medium contains peptone (Nissui), 0.5 g; yeast extract, 0.5 g; NaCl, 5.0 g; K_2HPO_4, 0.3 g; agar, 3.0 g; and Phenol Red, 0.02 g. To rehydrate the medium, 9.32 g of this medium was suspended in 900 ml of distilled water and heated to boiling for complete dissolution. After it had been autoclaved, sterile 10% solution of carbohydrates or polyols, or 30% ethanol was added to the basal medium in a volume of 1/10, and distributed aseptically in sterile cotton-plugged tubes (13×100 mm). To prepare PYP basal medium without carbohydrate as a control medium, 9.32 g of this medium was dissolved in 1 *l* of distilled water. Since dulcitol, inulin, and salicin are poorly soluble in 10% solution, these were added to the dissolved basal medium (9.32 g/*l*) at a concentration of 1%, and distributed into the tubes, and sterilized at 116° for 10 min. All the tubes were inoculated by stabbing and observed for growth of the test organism and for color change up to 2 weeks. Phosphatase activity of 4 strains was tested according to the description by HARRIGAN and MCCANCE (*15*). Anaerobic respiration by nitrate, nitrite, or fumarate was determined by testing anaerobic growth in casitone-yeast extract broth added with 0.2% nitrate or nitrite, or 0.08% sodium fumarate (*7*), under petrolatum seal. The biochemical characteristics investigated are listed in Tables 2 and 3. The methods used for these tests were described previously (*13*).

Susceptibility of 4 strains against 15 antimicrobials was tested by agar-disc diffusion technique. Each of 2 Mueller Hinton medium (Difco, 0252) plates in 70×220×10 mm square dish was inoculated with 0.2 ml of 18-hr broth culture of 4 strains by a spreader. Eight or seven 8-mm antimicrobial discs (Showa Yakuhin Kako, Tokyo) were applied on each plate and the diameter of inhibition zones

Table 2. Similar characteristics of *Flavobacterium devorans* ATCC 10829 and 3 *Pseudomonas paucimobilis* strains.

All 4 strains positive	All 4 strains negative
Gram-negative, rod-shaped	Growth at 4° and 41°
Single polar flagellum	Growth on MacConkey agar, NAC agar
Sudan Black B granule	Growth on bile-aesculin agar
Motility	Growth in 1% tryptone broth with 3% NaCl
Catalase and indophenol oxidase	Growth in KCN broth
Deep-yellow pigment, water insoluble	Glucose fermentation and fumarate respiration
Growth at 37°	Hydrogen sulfide in Kligler butt
Growth in 1% tryptone broth without NaCl	Indole production
Growth in glucose-salt broth	Acetylmethylcarbinol production
Glucose in asparagine-salt broth	Citrate-Simmons, alkalization
Citrate-Christensen, alkalization	Gas from nitrate and nitrite
Hydrolysis of aesculin and starch	Hemolysis, rabbit blood agar
Hydrolysis of Tween 20 and Tween 80	L-Lysine decarboxylase, Møller
Phenylalanine deaminase	L-Arginine dihydrolase, Møller
Phosphatase and deoxyribonuclease	L-Ornithine decarboxylase, Møller
Oxidative acid production from	Gelatinase and urease
D-arabinose, L-arabinose, xylose,	Acylamidase
fructose, galactose, glucose, mannose,	Utilization of acetate and malonate
melibiose, cellobiose, lactose, maltose,	2-Ketogluconate and 3-ketolactonate
sucrose, melezitose, trehalose	Oxidative acid production from
ethanol (3%)	dulcitol, inositol, mannitol, sorbitol,
PYP control medium alkalization	inulin, D-ribose

Table 3. Dissimilar characteristics of *Flavobacterium devorans* ATCC 10829 and 3 *Pseudomonas paucimobilis* strains.

Tests	*F. devorans*[a] 10829	*P. paucimobilis*[b] 11030	11031	11032
Kligler iron agar slant top	N[c]	A[d]	K[e]	K
Oxidative acid production from				
raffinose	+	+	−	+
rhamnose	−	−	−	+
salicin	+	+	+	−

[a] ATCC strain number
[b] NCTC strain number
[c] Neutral
[d] Acid
[e] Alkaline

including disc diameter was measured after 24-hr incubation at 30°. Susceptibility was expressed as −, +, ++, and +++, according to the manufacturer's instruction. Content of each drug in one disc and the degree of susceptibility are listed in Table 3.

Bacterial cells of 4 strains, grown on BAB plates for 20-hr at 30°, were harvested and their yellow pigment was removed by washing the cells with acetone. After centrifugation of the yellow extract in acetone, visible absorption spectrum of the supernatant was determined with Hitachi Recording Spectrophotometer Model 340. Analysis of cellular lipids and fatty acids were performed as described previously (*7*, *16*).

Qualitative determination of long-chain base component in cellular lipids was performed as follows: Acetone-dried powder (0.5 g) of 4 strains, obtained after extraction of yellow pigment, was suspended in 5 ml of a mixture of concentrated hydrochloric acid (12 N) and methanol (1: 5, v/v) and allowed to stand at 60°. After 20-hr hydrolysis, fatty acid methyl esters were extracted with hexane and removed. Residual liquid layer was then strongly alkalized with KOH grains, and long-chain base was extracted twice with hexane–ether (1: 1, v/v). Hexane-ether layer was separated and centrifuged at 2,000 rpm for 5 min. The supernatant was evaporated to dryness under flowing nitrogen gas. The dried extract was dissolved in 0.2 ml of ether and separated on a thin-layer plate with a solvent system of chloroform–methanol–water (65: 25: 4, v/v). Authentic dihydrosphingosine was simultaneously run on the same plate as control. Spots of long-chain base component were visualized by spraying ninhydrin solution.

RESULTS

Cells of strain 10829 were Gram-negative straight or slightly curved rods with rounded ends. Soma size was about 1.0×2.3–$3.0\ \mu$m. Some cells showed aggregating tendency to form rosettes (Fig. 1). Cells grown in casitone-yeast extract broth contained intracellular fat granules stained with Sudan Black B. Smears stained for flagella revealed a few cells with a single polar flagellum (Fig. 1). Spreading growth from the inoculation line in a stabbed semisolid motility agar and on a semisolid Gard plate was observed in strains 11031 and 11032 but not in 10829 and 11030. Microscopic observation of wet-mount living preparation of 20-hr broth culture of the latter 2 strains revealed a few cells actively motile. Electron micrograph of 10829 also showed a few cells with a single polar flagellum (Fig. 2). Free flagella detached from the soma were also observed in several fields.

Colonies of strain 10829 and 3 *P. paucimobilis* strains grown on BAB plates after 40-hr incubation were *ca.* 1 mm in diameter, smooth, glistening, and deep-yellow pigmented. In broth culture, the medium was uniformely turbid within 24 hr, and, after several days at room temperature, deep yellow pellicle and pre-

Fig. 1. *Flavobacterium devorans* ATCC 10829. Broth culture.
Leifson flagella stain after Formalin fixation. ×1,500. Cells are aggregating, 2 of them showing a single polar flagellum.

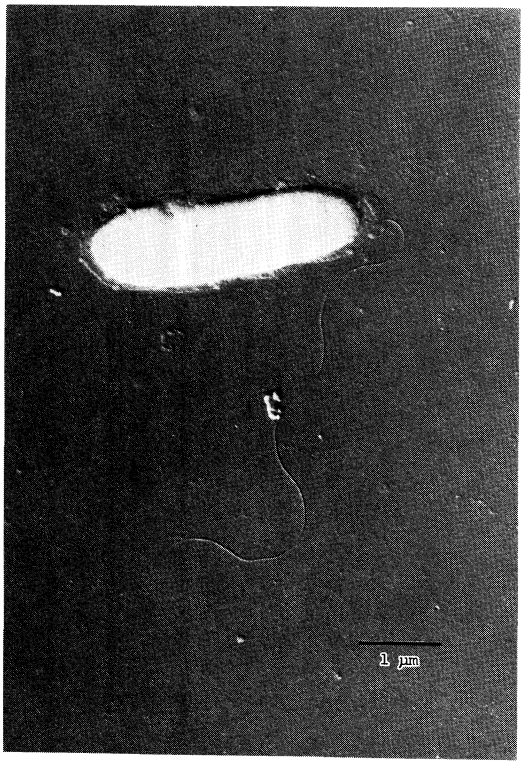

Fig. 2. Electron micrograph of *Flavobacterium devorans* ATCC 10829, showing a single polar flagellum.
Preparation was shadow-casted with platinum-palladium at an angle of 30°.

Table 4. Susceptibility of *Flavobacterium devorans* ATCC 10829 and 3 *Pseudomonas paucimobilis* strains to 15 antimicrobial agents, determined by single disc diffusion technique.

Antimicrobial agent	Content in one disc	*F. devorans*[a] 10829	*P. paucimobilis*[b] 11030	11031	11032
Penicillin G	20 units	+++	++	+++	++
Ampicillin	30 μg	+++	+++	+++	+++
Carbenicillin	30 μg	+++	+++	+++	+++
Cefazolin	30 μg	+++	−	+	−
Gentamicin	30 μg	+++	+++	+++	+++
Tobramycin	30 μg	+++	+++	+++	+++
Kanamycin	50 μg	+++	+++	+++	+++
Tetracyclin	200 μg	+++	+++	+++	+++
Minocyclin	200 μg	+++	+++	+++	+++
Erythromycin	50 μg	+++	+++	+++	+++
Clindamycin	30 μg	++	+	++	−
Colistin	5 μg	−	−	−	−
Polymyxin B	100 units	−	−	−	−
Nalidixic acid	50 μg	+	+	+	+

[a] ATCC strain number.
[b] NCTC strain number.

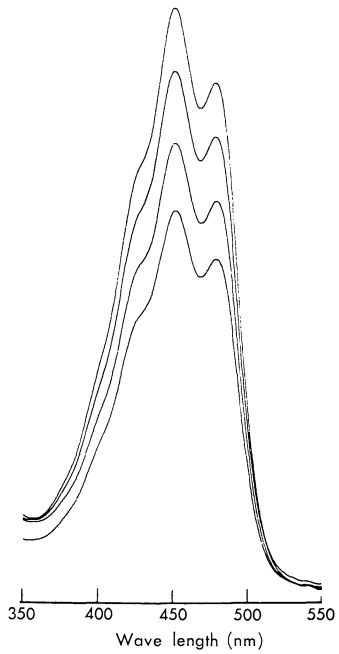

Fig. 3. Visible absorption spectrum of yellow pigments in acetone-extract.
Top, *Flavobacterium devorans* ATCC 10829; Second, *Pseudomonas paucimobilis* NCTC 11030; Third, *P. paucimobilis* NCTC 11031; Bottom, *P. paucimobilis* NCTC 11032.

cipitate formed. Broth cultured cells usually died within 2 weeks. The 4 strains grew in a chemically defined medium containing 0.2% glucose and did not require addition of any growth factor. They failed to respire nitrate, nitrite, or fumarate under anaerobic conditions.

On plates of BAB added with 0.008% BTB, strains 10829 and 11031 failed to grow and strain 11030 grew poorly, although 11032 was not affected by added BTB. The growth inhibitory effect of BTB is summarized in Table 4. Oxidative acid production from glucose and other carbon compounds was more conveniently determined in PYP basal medium than in OF basal medium, because 0.008% BTB in the latter medium inhibited the growth of some strains. Tests for which all 4 strains gave positive or negative reaction are summarized in Table 2. Tests for which different results were given by 4 strains are listed in Table 3.

Visible absorption spectrum of acetone extract from 10829 and 3 *P. paucimobilis* strains has 2 peaks at 452 and 480 nm (Fig. 3). Ninhydrin-positive spot, which corresponds to that of standard dihydrosphingosine, was revealed on the chromatogram of hexane-ether extracts from the hydrolysate of acetone-powdered cells of 4 strains (Fig. 4). The ninhydrin-positive substance was confirmed as dihydrosphingosine by gas chromatography-mass spectrometry. As shown in Table 6 it is noteworthy that cellular lipids of strain 10829 contains 2-hydroxy-

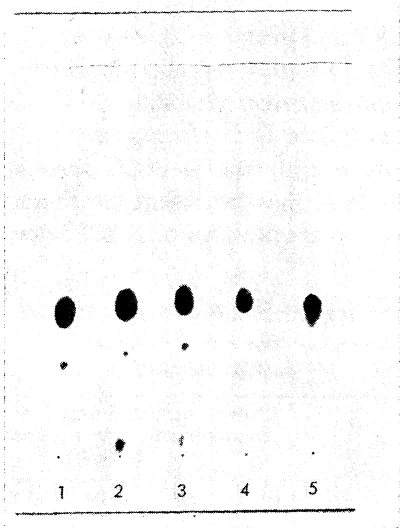

Fig. 4. Ninhydrin-positive spots of hexane-ether extracts from the hydrolysate of acetone-dried cells.

1, *Flavobacterium devorans* ATCC 10829; 2, *Pseudomonas paucimobilis* NCTC 11030; 3, *P. paucimobilis* NCTC 11031; 4, *P. paucimobilis* NCTC 11032; 5, authentic dihydrosphingosin. Silica gel 60 (Merck) plate. Chloroform–methanol–water (65: 25: 4, v/v).

myristic acid. The concentration of this sole hydroxy acid was 21% of the total extractable and 52% of the bound lipid fatty acids. Concentration of palmitic acid in extractable and octadecenoic acid in bound lipid is very high. Branched-chain fatty acids and cyclopropanoic acids were not detected. Three *P. paucimobilis* strains are quite similar in their fatty acid composition to that of strain 10829.

From the morphological, physiological, and biochemical findings, strain 10829 was identified as a strain of *P. paucimobilis*. Ninhydrin-positive spot on thin-layer chromatogram, which corresponds to the novel sphingoglycolipid reported by YAMAMOTO *et al.* (*7*) confirmed the above identification. Similarity in the cellular fatty acid composition, $C_{16:0}$, $C_{18:1}$ and 2OH-$C_{14:0}$ in high concentration, and similarity in the visible absorption spectra of yellow pigments also support this identification.

DISCUSSION

In 1940, a strain labeled as *Acetobacter acetosum* No. 16 was sent from N. Porges through F. J. de Fontenoy to the Northern Regional Research Laboratory, U.S. Department of Agriculture, Illinois, where the culture was given the NRRL accession number B–54 and reidentified there as a strain of *Flavobacterium devorans*. In 1950, HALL, *et al.* (*17*) reported that the strain NRRL B–54 produced a vitamin B_{12}-like compound. The culture was deposited in the American Type Culture Collection (ATCC) on December 22, 1950, and labeled 10829 (H. D. Hatt, personal communication). In 1974, Weeks tabulated the characteristics of 12 species of the genus *Flavobacterium* including *F. devorans* in high GC group. He designated the Porges isolate as a reference strain of the species. Since it is the only available culture bearing the name *F. devorans*, the characteristics of *F. devorans* shown in the table must represent the characteristics of strain 10829. For direct comparison, the characteristics of 10829 determined in this study were

Table 5. Inhibitory effect of Bromthymol Blue on the growth of *Flavobacterium devorans* ATCC 10829 and 3 *Pseudomonas paucimobilis* strains.

Organism	Growth on blood agar base (Difco) containing BTB at a concentration of (g/l):					Growth on control BAB without BTB
	0.01	0.02	0.04	0.06	0.08	
F. devorans 10829[a]	+	poor	−	−	−	+
P. paucimobilis 11030[b]	+	poor	poor	poor	poor	+
P. paucimobilis 11031[b]	+	poor	poor	−	−	+
P. paucimobilis 11032[b]	+	+	+	+	+	+

[a] ATCC strain number.
[b] NCTC strain number.

Table 6. Fatty acid composition of cellular lipids of a *Flavobacterium devorans* ATCC 10829 and 3 *Pseudomonas paucimobilis* strains

Organism	Fatty acids in extractable lipids (%)							Fatty acids in bound lipids (%)					
	14:0	16:0	16:1	18:0	18:1	2OH14:0	Other[c]	14:0	16:0	16:1	18:1	2OH14:0	Other[c]
F. devorans 10829[a]	2	13	1	—	62	21	1	3	29	6	7	52	3
P. paucimobilis 11030[b]	1	16	2	—	62	17	2	3	20	3	8	60	0
P. paucimobilis 11031[b]	1	19	2	1	58	18	1	2	14	1	11	72	0
P. paucimobilis 11032[b]	9	17	5	1	51	16	1	7	19	—	5	63	6

[a] ATCC strain number.
[b] NCTC strain number.
[c] Unidentified.

Table 7. Comparison of selected characteristics of species *Bacillus devorans*, *Flavobacterium devorans*, and of *F. devorans* ATCC 10829 by various workers.

	ZIMMERMANN (*1*) 1890	BERGEY (*2*) 1923	WEEKS (*18*) 1957	WEEKS (*5*) 1974	HAYNES[a] 1940	YABUUCHI[b] 1979
Motility	+	+	+	+	+	+
Flagellation	ND	peri	peri	peri	peri	polar mono, a few cells
Growth on agar slant	very thin spreading, gray	spreading gray	spreading gray	spreading light yell.	filiform yellow	filiform deep yellow
Relation ship to oxygen	anaerobic facultative	aerobic	anaerobic facultative	aerobic	anaerobic facultative	aerobic
Growth in media cont.						
3% NaCl	ND[c]	ND	ND	ND	ND	—
8% NaCl	ND	ND	ND	+	ND	ND
Gelatinase	+	+	+	+	+	—
Acid from lactose	ND	ND	ND	—	—	+
Acid from ethanol	ND	ND	ND	ND	+	+

[a] From the description of *F. devorans* ATCC 10829 in personal communication from L. Nakamura, NRRL.

[b] From the data in present study.

[c] No data.

compared with those of *B. devorans* described by ZIMMERMANN (*1*) and of *F. devorans* appearing in 1st (*2*), 7th (*18*), and 8th (*5*) Editions of Bergey's Manual and the cultural data in NRRL (Nakamura, personal communication) (Table 7). Discrepancies between the attributes in literature and those obtained in this study are evident, even though there must be technical differences in determining certain attributes such as acid production from carbohydrates.

Since the strain 10829 was received from the Research Institute for Microbial Diseases, Osaka University, another ampule of freeze-dried culture of NRRL B–54 supplied directly from NRRL was tested for confirmation, and entirely the same results were obtained in every respect.

The strain 10829, erroneously labeled as *F. devorans*, was reidentified as a strain of *P. paucimobilis*. It is now known that cellular fatty acid analysis is a useful tool for classification of *Pseudomonas* species (*19–23*). Cellular bound lipids of *Pseudomonas mallei, P. pseudomallei, P. caryophylli, P. cepacia, P. marginata, P. pickettii,* and *P. solanacearum* contain 3-hydroxymyristic acid in a high concentration, but not 2-hydroxymyristic acid (*21*). Extractable lipids of these species merely contain 2-hydroxymyristic acid in less than 5% of the total fatty acids. The presence of novel glycosphingolipid and 2-hydroxymyristic acid in high concentration in cellular lipids is specific and supportive evidence for this identification. Since the strain ATCC 10829 was a misidentified organism, the name *Flavobacterium devorans* can not be a senior synonym over *Pseudomonas paucimobilis*.

Unless the authentic strain of the original describer for *F. devorans* or any isolate in conformity with the original description for the species is found, the name *Flavobacterium devorans* should not be retained further.

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