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Synonymy of *Flavobacterium pectinovorum* Dorey with *Cytophaga johnsonae* Stanier

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Several authors have suggested that *Flavobacterium pectinovorum* Dorey 1959 should be removed from the genus *Flavobacterium* and that it might be a *Cytophaga* species. An extensive study of the morphological, physiological, biochemical, and antimicrobial lytic properties of the holotypic strain NCIB 9059 (ATCC 19366) of *F. pectinovorum* has revealed that it is more properly classified as *Cytophaga johnsonae* Stanier 1947. The guanine-plus-cytosine ratio of 33 mol% agrees with the range reported for *C. johnsonae* (30 to 35 mol%).

The pectolytic *Flavobacterium pectinovorum* Dorey 1959 was originally isolated from soil in southeast England and studied for its ability to produce polygalacturonase (8). The name is validly published and legitimate (33), and the holotypic reference culture is NCIB 9059 (ATCC 19366), which is Dorey's isolate no. 81. This is also the only readily available culture. *F. pectinovorum* NCIB 9059 is an orange-yellow-pigmented, gram-negative, aflagellate rod which exhibits gliding motility (17, 18, 23), although spreading on agar media has not been evident in previous work (18, 21, 23). It has been suggested that this species be reassigned to Brisou's yellow, nonmotile genus *Empedobacter* (5; P. Kaiser, Ph.D. Thesis, University of Paris, Paris, France, 1971), but the consensus is that this organism more properly belongs in the genus *Cytophaga* (17, 18, 21, 32). No formal proposal to assign the species to a different genus and no study establishing synonymy with an established *Cytophaga* species have yet been made.

MATERIALS AND METHODS

Materials. The holotypic culture of *F. pectinovorum* NCIB 9059 (ATCC 19366) was compared in the tests described below with nine *Cytophaga johnsonae* cultures (Table 1).

Methods. The standard medium used for growing these organisms was skim acetate (SA) agar or broth (7), and incubation was at 25°C unless otherwise stated.

MORPHOLOGY

Cell morphology. The dimensions, motility, and arrangement of the cells were determined from living preparations examined at 1,000× magnification from 1-day-old SA broth cultures. Gram stains were performed on 1- and 8-day-old SA broth cultures and on 2-day-old SA plate cultures (Table 2).

CULTURAL CHARACTERISTICS

Colony morphology. Descriptions of colony form, elevation, Munsell colour (6, 22), and of optical, surface, and edge characteristics (26) were made from single colonies grown on 2-day-old Cook's cytophaga agar (7), plate count agar (Difco), and SA plates for 5 days. Colours were compared using light from a window with northern exposure during the morning or afternoon. The presence of any water-soluble pigment and spreading ability was noted on all plates used in this study (Table 3).

Growth in liquid media. Observation of the silkiness of a culture when gently shaken and of flexing movement by individual cells was made every day for 12 days on cultures grown in unshaken SA broth tubes.

PHYSIOLOGY

Salt tolerance. The dried surfaces of four SA plates made up with 0, 1, 2, and 3% NaCl, respectively, were inoculated with a central streak of the test organism in that order so that no carry-over of NaCl would occur. The amounts of growth, spreading, and lysis were noted at 14 days (Table 4, columns 2 and 3).

Atmospheric conditions for growth. SA plates were inoculated with a single, central streak of the test organism and incubated under three different atmospheres. The first group was in air, the second was in a candle jar (about 10% O_2), and the third was under hydrogen and carbon dioxide in a Gas-Pak jar (BBL, Becton, Dickinson, and Co., Cockeysville, Md.). At 7 days, estimates of the amount of growth and lysis were made (Table 4, columns 4 and 5).

Temperature limits for growth. Replicate SA streak plates were incubated at temperatures ranging from 0 to 50°C for 6 days, at which time the type and relative width of growth were recorded. The 0°C plates were incubated for 10 days, and those at 35°C or more were wrapped in polythene bags to prevent excess moisture loss (Table 4, columns 6 and 7).

pH range for growth. Batches of SA agar were adjusted with HCl or NaOH to different pH levels before autoclaving and were rechecked afterwards. When the surfaces had dried, the plates were inocu-

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TABLE 1. *Origin and designation of strains used in this study*

Strain no.[a]	Species	Source
UASM 4432	Actinomycete	F. D. Cook, from soil
UASM 4441	Actinomycete	F. D. Cook, from soil
UASM 4165	*Arthrobacter* sp.	F. D. Cook, from Prairie Regional Lab, Saskatoon, Saskatchewan, Canada
UASM 4611	*Bacillus subtilis*	F. D. Cook, from Prairie Regional Lab, Saskatoon, Saskatchewan, Canada
	Chlorella sp.	Department of Botany, Univ. of Alberta
Stanier 6	*Cytophaga hutchinsonii*	R. Y. Stanier
NCIB 10782	*C. hutchinsonii*	NCIB, from N. Palleroni
ATCC 17061	*C. johnsonae*	ATCC, from C. B. van Niel
UASM 405	*C. johnsonae*	F. D. Cook, from soil, Ottawa, Ontario, Canada
UASM ALF	*C. johnsonae*	F. D. Cook, from alfalfa roots, Edmonton, Alberta, Canada
UASM B-2-25	*C. johnsonae*	D. C. Gillespie, from diseased freshwater fish, Manitoba, Canada
UASM E-1-25	*C. johnsonae*	D. C. Gillespie, from diseased freshwater fish, Manitoba, Canada
UASM 4433	*C. johnsonae*	F. D. Cook, from moose dung, Alberta, Canada
UASM 4539	*C. johnsonae*	F. D. Cook, from moose dung, Alberta Canada
UASM 3	*C. johnsonae*	F. D. Cook, from soil, Alberta, Canada
UASM 4707	*C. johnsonae*	F. D. Cook, from soil, Alberta, Canada
UASM PC20	*Escherichia coli*	P. Christensen, from creek water, Edmonton, Alberta, Canada
NCIB 9059	*Flavobacterium pectinovorum*	NCIB, from J. J. Dorey, from soil, S. E. England
	Penicillium notatum	N. Colotelo, Edmonton, Canada
ATCC 9027	*Pseudomonas aeruginosa*	ATCC, from C. P. Hegarty, from ear infection
	Rhizopus sp.	N. Colotelo, Edmonton, Canada
	Sclerotinia sclerotiorum	N. Colotelo, Edmonton, Canada
	Serratia marcescens	Provincial Laboratory of Public Health, Edmonton, Canada
	Yeast (probably *Saccharomyces cerevisiae*)	Fleischmann's fast-rising active dry yeast, Standard Brands, Ltd., Montreal, Canada

[a] UASM, University of Alberta Soil Microbiology Laboratory, Edmonton, Alberta, Canada. UASM 405 = ATCC 29583; UASM ALF = ATCC 29584; UASM B-2-25 = ATCC 29585; UASM E-1-25 = ATCC 29586; UASM 4433 = ATCC 29587; UASM 4539 = ATCC 29588; UASM 3 = ATCC 29589; and UASM 4707 = ATCC 29590.

TABLE 2. *Dimensions of Cytophaga johnsonae strains and of Flavobacterium pectinovorum NCIB 9059 in liquid culture after 24 h of incubation*

Strain	Length (μm)	Width (μm)
C. johnsonae		
ATCC 17061	2–36	0.2
UASM 405	3–20	0.4
UASM ALF	3–25	0.2
UASM B-2-25	3–20	0.4
UASM E-1-25	3–15	0.5
UASM 4433	3–12	0.4
UASM 4539	3–20	0.5
UASM 3	3–25	0.3
UASM 4707	3–20	0.4
F. pectinovorum		
NCIB 9059	1–25	0.3

lated with spots of different test organisms, four per plate, on pH 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 agar. Growth was estimated on a comparative basis after 5 days and on the range of pH values at which growth was initially recorded (Table 4, column 8).

Nitrogen sources for growth: (i) Nitrate. Salts-glucose agar plates were made with Hutchinson and Clayton salt solution (12) (0.25% $NaNO_3$, 0.1% K_2HPO_4, 0.03% $MgSO_4 \cdot 7H_2O$, 0.01% $CaCl_2$ [anhydrous], 0.01% $FeCl_3$, pH 7.2 to 7.3) to which was added 0.1% (wt/vol) filter-sterilized glucose and 1.5% agar. A duplicate set of plates was made with the addition of 0.05% yeast extract. Streak inoculations were made and readings were taken at 9 days (Table 5, columns 2 and 3).

(ii) Ammonia. Board and Holding medium without the yeast extract was used, and lowering of the pH due to the utilization of ammonia was noted at 17 days by observing a change from blue to yellow in the colour of the medium (Table 5, column 4).

(iii) Urea. The medium was made up of two parts. Solution A consisted of 1 g of K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of $CaCl_2$ (anhydrous), 0.1 g of NaCl, 0.01 g of $FeCl_3$, and 15 g of agar in 900 ml of distilled water, adjusted to pH 6.8 to 7.0 and autoclaved. Solution B contained 20 g of urea, 5 g of glucose, and 10 ml of a 2% bromothymol blue solution in 90 ml of distilled water, and was filter-sterilized before being added to the cooled solution A prior to pouring plates. Growth and elevation of pH as a result of ammonia production upon breakdown

TABLE 3. *Colony morphology of Cytophaga johnsonae strains and Flavobacterium pectinovorum NCIB 9059 after 5 days of incubation*

Agar	Strain	Form	Surface	Edge	Elevation	Optical properties	Colour[a]
Cook's cytophaga agar	*C. johnsonae*						
	ATCC 17061	Irregular	Rough	Erose	Effuse	Transparent	1.5 YR 7/10
	UASM 405	Irregular	Rough	Undulate	Effuse	Transparent	10 YR 7/10
	7 others[b]	Irregular	Rough or smooth	Entire, lobate or erose	Effuse or raised	Transparent or translucent	7.5 YR 6/10, 10 YR 6/10-12
	F. pectinovorum						
	NCIB 9059	Irregular	Rough	Undulate	Flat	Transparent	10 YR 7/10
Skim acetate	*C. johnsonae*						
	ATCC 17061	Irregular	Rough	Erose/lobate	Effuse	Translucent	5 YR 5/10
	UASM 405	Irregular	Rough	Lobate	Effuse	Transparent	10 YR 6/8
	7 others[b]	Irregular	Rough	Erose, lobate or undulate	Effuse, umbonate, or raised	Transparent or translucent	5 YR 5.5–6/10, 7.5 YR 6/8–10 or 7/8–9
	F. pectinovorum						
	NCIB 9059	Irregular	Rough	Erose	Effuse		2.5 YR 4/12
Plate count agar	*C. johnsonae*						
	ATCC 17061	Irregular	Rough/smooth	Erose	Effuse/convex	Translucent	2.5 YR 4/10
	UASM 405	Irregular	Rough	Lobate	Effuse	Transparent	7.5 YR 5/8
	7 others[b]	Irregular or circular	Smooth or rough	Entire or undulate	Raised, convex, umbonate, or flat	Translucent or opaque	7.5 YR 5/6–10 or 6/10 or 9 YR 6/8
	F. pectinovorum						
	NCIB 9059	Roughly circular	Rough	Erose	Convex	Translucent	5 YR 5/10

[a] No water-soluble pigment observed on any plate.
[b] Strains UASM ALF, UASM B-2-25, UASM E-1-25, UASM 4433, UASM 4539, UASM 3 and UASM 4707.

TABLE 4. *Physiology of Cytophaga johnsonae strains and Flavobacterium pectinovorum NCIB 9059. I.*

Strain	NaCl causing inhibition (%)		Atmospheric conditions for:		Growth temp (°C)		pH at which growth is initiated
	Partial	Complete	Best growth	Best lysis of milk	Range	Optimum	
	14[a]		7		6 (10 for 0°C)		5
C. johnsonae							
ATCC 17061	2	3	10% O_2	10% O_2	10–35	30	7–10
UASM 405	1	2	10% O_2	10% O_2	8–35	18	5–10
UASM ALF	1–2	2	10% O_2	Air	0–30	20–25	5–10
USAM B-2-25	1	3	10% O_2	10% O_2	0–30	18	5–10
UASM E-1-25	1	2	10% O_2	10% O_2	0–30	20	5–10
UASM 4433	1	3	10% O_2	10% O_2	0–30	18	5–10
UASM 4539	1	3	10% O_2	10% O_2	0–30	18–20	5–10
UASM 3	1	2	10% O_2	10% O_2	0–35	30	5–10
UASM 4707	1	2	10% O_2	10% O_2	0–30	18–25	5–10
F. pectinovorum							
NCIB 9059	1	3	10% O_2	10% O_2	0–30	18–20	5–10
Column no.	2	3	4	5	6	7	8

[a] Days incubated.

of urea from streak inoculations were recorded at 20 days (Table 5, column 5).

(iv) **Amino acids.** Hutchinson and Clayton salts medium minus the $NaNO_3$ was supplemented with either 0.1% monosodium glutamate or 0.1% sodium asparaginate, and gelled with 1.5% agar. A filter-sterilized glucose solution was added to the cooled, autoclaved medium to a final concentration of 0.1%. Growth of the spot inocula, four per plate, was noted at 9 days (Table 5, columns 6 and 7).

Growth factors: Stimulation by yeast extract. The results from two sets of tests were compared: (i) salts + glucose ± yeast extract (see nitrate method above and Table 5, columns 2 and 3); (ii) chitin ± yeast extract (see chitin method below and Table 8, columns 14 and 15).

Antibiotic and antibacterial sensitivities: (i) SLS. Streak inoculations were made on three groups of SA agar plates containing 0, 0.01, and 0.1% sodium lauryl sulfate (SLS), respectively (7). Growth with SLS was compared with that on the control plate at 5 days (Table 6, columns 2 and 3).

(ii) Chloramphenicol, dihydrostreptomycin, penicillin, and polymyxin B. A sufficient amount of an 18-h mid-logarithmic-phase SA broth culture of a test organism was spread with a bent glass rod on the surface of an SA plate to form confluent growth. After the surface of the plate had dried, disks (BBL) of four antibiotics of the following concentrations were placed firmly on the agar: chloramphenicol, 30 μg; dihydrostreptomycin, 10 μg; penicillin G, 10 units; and polymyxin B, 300 units. The diameters of growth inhibition were noted after 2 days and scored by the Kirby-Bauer scheme (2) (Table 6, columns 4–8).

(iii) Actinomycin D. Four sets of disks were made containing 0.1, 1.0, 10, and 40 μg of actinomycin D per disk respectively. These were placed on the dried surfaces of SA plates previously inoculated with the test bacteria as in the previous method. The results were read at 2 days (Table 6, column 9).

(iv) **Nitrite.** Growth in aerobic Penassay broth tubes (Difco antibiotic medium 3) containing 0.1% KNO_2 was compared at 11 days with that in plain Penassay broth tubes to see if nitrite inhibition had taken place (Table 6, column 10).

Antimicrobial lytic action: (i) Bacteria. A selection of five bacterial species, *Escherichia coli, Pseudomonas aeruginosa, Arthrobacter* sp., *Serratia marcescens,* and *Bacillus subtilis* (Table 1), was used to screen the lytic activity of the test cultures against bacteria. Tests were made in a manner similar to antibiotic disk sensitivity tests, using 1 drop of an overnight SA shaken culture of the "predator" on a lawn of the "prey" organism. Two sets of plate count plates were used for each prey bacterium, the lawns being allowed to grow for 1 h and 2 days, respectively, before inoculation with potentially lytic test organisms. After 11 days, growth of the predators and lysis of the prey were determined (Table 7, columns 2 to 6).

(ii) Fungi, actinomycetes, and an alga. The lysis of autoclaved yeast cells was examined on yeast cell agar plates (7). The test cultures were streaked down the centre of a plate and, after 9 days, the growth of the cultures and their lytic action were noted (Table 7, column 12).

The lytic spectrum of the test cultures was estimated on the fungi *Penicillium notatum, Rhizopus* sp., and *Sclerotinia sclerotiorum,* two actinomycetes, UASM 4432 which produces a brown water-soluble pigment, and UASM 4441, which does not, and on the green alga *Chlorella* sp. (Table 1). These organisms were grown on plate count agar and were tested in the same manner as the bacteria. However, these prey organisms were allowed to grow for 1 and 4 days, respectively (2 weeks for *Chlorella*), before drops of the potential predator broth cultures were added (Table 7, columns 7 to 11 and 13).

BIOCHEMICAL REACTIONS

Oxidation-fermentation test. Poured tubes of Board and Holding medium (4) (0.5% filter-steri-

TABLE 5. *Physiology II. Nitrogen sources for Cytophaga johnsonae strains and Flavobacterium pectinovorum NCIB 9059*

Strain	Nitrogen source[a]					
	NO^-_3	NO^-_3 + yeast extract	NH_4^+	Urea	Glutamate	Asparaginate
		9[b]	17	20	9	9
C. johnsonae						
ATCC 17061	±	+	+	+	+	+
UASM 405	−	++	+	−	+	+
UASM ALF	+	+	+	+	+	+
UASM B-2-25	+	++	+	−	+	+
UASM E-1-25	+	++	+	−	+	+
UASM 4433	+	++	+	−	+	+
UASM 4539	++	++	+	−	+	+
UASM 3	++	++	+	−	+	+
UASM 4707	++	++	+	−	+	+
F. pectinovorum						
NCIB 9059	++	++	+	+	+	+
Column no.	2	3	4	5	6	7

[a] Scoring for columns 2 and 3: −, none; ±, weak; +, good; ++, excellent; for column 4: +, utilization of NH^+_4 shown by both growth and lowered pH, −, neither present; for column 5: +, both growth and high pH, due to release of NH_3; −, neither present; for columns 6 and 7: +, growth; −, no growth.

[b] Days incubated.

TABLE 6. *Physiology III. Sensitivities of Cytophaga johnsonae strains and Flavobacterium pectinovorum NCIB 9059 to antibacterial agents*[a]

Strain	% Sodium lauryl sulfate at which growth is:		Chloramphenicol (30 μg)	Streptomycin (10 μg)	Penicillin G (10 units)	Polymyxin B (300 units)		Actinomycin D	0.1% NO_2^-
	Reduced	Inhibited				Kirby-Bauer	Author's scheme		
C. johnsonae									
ATCC 17061	0.01	0.1	S	I	R	R	I	S	R
UASM 405	0.01	0.1	R	I	R	R	R	S	R
UASM ALF	0.01	0.1	S	I	R	R	R	S	R
UASM B-2-25	0.01	0.1	S	R	R	R	S	R	S
UASM E-1-25	0.01	0.1	S	R	R	R	S	S	S
UASM 4433	0.01	0.1	S	R	R	R	S	S	S
UASM 4539	0.01	0.1	S	I	R	R	I	S	S
UASM 3	0.01	0.1	I	S	I	I	S	S	R
UASM 4707	0.01	0.1	S	I	R	R	R	S	S
F. pectinovorum									
NCIB 9059	0.01	>0.1	I	I	I	R	I	S	S
Column no.	2	3	4	5	6	7	8	9	10

[a] All read at 2 days except sodium lauryl sulfate (5 days) and NO^-_2 (11 days). Abbreviations: S, sensitive; I, intermediate; R, resistant. Columns 4 to 7 are scored according to Kirby-Bauer scheme (2). Column 8 is scored on the author's scheme based on behaviour of control organisms *E. coli*, *P. aeruginosa*, *Arthrobacter* sp., *S. marcescens*, and *B. subtilis* (see Table 1). Column 9 is scored in comparison to control organisms *E. coli* and *B. subtilis*: S, more sensitive than *E. coli*, but not as sensitive as *B. subtilis*; R, same as or less sensitive than *E. coli*, i.e., resistant. Column 10: R (resistance), indicated by growth in tube; S (sensitivity), no growth.

lized glucose, 0.5% agar, 0.05% $NH_4H_2PO_4$, 0.05% K_2HPO_4, 0.05% yeast extract, 1.5 ml of 2% bromothymol blue solution per litre, pH 7.2) were inoculated by stabbing with a thin wire. One set was sealed with about 1 cm of paraffin wax, and both were incubated for 15 days, when the colours of the test and control tubes were noted (Table 8, columns 2 and 3).

Acid production from sugars and alcohols. Five sets of aerobic tubes of Board and Holding medium were made, substituting 0.5% solutions of cellobiose, sucrose, lactose, glycerol, and mannitol, respec-

TABLE 7. *Physiology IV. Antimicrobial lytic action shown by Cytophaga johnsonae strains and by Flavobacterium pectinovorum NCIB 9059*[a]

Potential predator (strain)	Potential prey organism											
	Bacteria				Actinomycetes			Fungi				Alga
	Escherichia coli	*Pseudomonas aeruginosa*	*Arthrobacter*	*Serratia marcescens*	*Bacillus subtilis*	UASM 4432	UASM 4441	*Rhizopus*	*Penicillium notatum*	*Sclerotinia sclerotiorum*	Yeast	*Chlorella*
C. johnsonae												
ATCC 17061					L		?		?	L	?	
UASM 405									?		?	
UASM ALF						?	?			?		
UASM B-2-25								?	?		?	
UASM E-1-25								?	?		?	
UASM 4433									?		L	
UASM 4539									?		?	
UASM 3									?		?	
UASM 4707							?		?			
F. pectinovorum												
NCIB 9059					L		?		?	?	?	
Column no.	2	3	4	5	6	7	8	9	10	11	12	13

[a] Results read after 11 days, except yeast at 9 days. Maximum lytic activity in each test noted as: L, good lysis; ?, possible lysis; blank, no lysis.

TABLE 8. *Biochemical reactions: Use of carbohydrates and polysaccharides by Cytophaga johnsonae strains and Flavobacterium pectinovorum NCIB 9059*[a]

Strain	O-F test on glucose		Acid production from:					Hydrolysis of:										
										Cellulose		Agar		Chitin		Starch		
	Oxidative	Fermentative	Cellobiose	Sucrose	Lactose	Glycerol	Mannitol	Alginate	Pectate	CMC	Filter paper	Tubes	Gelase test	− YE	+ YE	SYS	NBS	Potato
	15[b]		17					30				40	5	40		4	25	8
C. johnsonae																		
ATCC 17061	+	+	+	−	+	−	−	+	+	+	−	−	+	+	++	+	+	+
UASM 405	+	−	+	+	+	−	−	+	−	−	−	−	+	+	++	+	+	+
UASM ALF	+	−	+	+	+	+ (slow)	+	−	+	+	−	−	+	++	++	+	+	+
UASM B-2-25	+	+	+	+	+	+	−	−	+	+	−	−	+	+	++	+	+	+
UASM E-1-25	+	+	+	+	+	+	−	−	+	+	−	−	+	+	++	+	+	+
UASM 4433	+	−	+	−	+	−	−	−	+	+	−	−	+	++	++	+	+	+
UASM 4539	+	−	+	+	+	−	−	−	+	−	−	−	+	+	++	+	+	+
UASM 3	+	−	+	+	+	−	−	−	+	+	−	−	+	+	++	+	+	+
UASM 4707	+	−	+	+	+	+ (slow)	−	−	+	−	−	−	+	+	++	+	+	+
F. pectinovorum																		
NCIB 9059	+	−	+	+	+	−	−	+	+	+	−	−	+	+	++	+	+	+
Column no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

[a] Interpretation of symbols: Columns 1 to 7: +, positive; −, negative; columns 8, 9, 10, and 12: +, liquefaction; −, no liquefaction; column 11: +, break of paper strip; −, paper strip intact; column 13: +, unstained gelase field left around colony; −, normal yellow colour; columns 14 and 15: ++, excellent; +, moderate; −, none; columns 16, 17, and 18: +, hydrolysis; −, no hydrolysis. Abbreviations: CMC, carboxymethyl cellulose; YE, yeast extract; SYS, salts-yeast extract-starch medium; NBS, nutrient broth-starch medium.

[b] Days incubated.

tively, for the glucose. The colours of the test and control tubes were noted at 17 days (Table 8, columns 4–8).

Hydrolysis of polysaccharides: (i) Alginate, pectate, and CMC. Tubes of Hutchinson and Clayton salt solution with 0.05% yeast extract and 3% sodium alginate (15, 25), 3% sodium carboxymethyl cellulose (CMC) Fisher grade 7 HSP (9, 15), or 3% sodium polypectate (Nutritional Biochemicals Corp., Cleveland, Ohio) were inoculated with the test cultures. Estimates of liquefaction were made at intervals up to 1 month in the test and control tubes after cooling them at 10°C for 20 min, when the sloppy gel in the control tubes had attained a firm consistency. Liquefaction was taken as evidence of alginase, CMCase, and polypectase activity, respectively (Table 8, columns 9 to 11).

(ii) Cellulose. Strips of Whatman no. 1 filter paper (1 by 10 cm) (20, 28) were placed in SA broth tubes which were then inoculated. The control organism, *Cytophaga hutchinsonii* strains Stanier 6 and NCIB 10782, took about 12 days to break the paper strip at the air-water interface. Observations on the test organisms were made at intervals up to 30 days (Table 8, column 12).

(iii) Agar: (a) Agar + yeast extract tubes. Tubes of 1.5% agar (Difco) containing 0.05% yeast extract only were inoculated, and growth of the cultures and any softening of the agar were noted at intervals for 40 days (Table 8, column 13).

(b) Gelase field. The appearance of "gelase fields" around the colonies after flooding a plate with iodine is regarded by some workers as a reliable test for agar decomposition (10, 27, 31). Inoculated SA plates incubated for 5 days were used in the present study (Table 8, column 14). All of these organisms showed a gelase field, but none showed softening or liquefaction of agar in tubes. The most likely explanation for this lack of correlation is that the organisms excrete some compound which interferes with the iodine reaction.

(iv) Chitin. Chitin agar was made with 40 ml of a partially hydrolysed chitin suspension and 10 g of agar per litre of water (11, 16, 24). This produces a slightly milky gel within which digestion of the chitin can be seen as a clear zone around the organism. A duplicate set of plates with 0.05% yeast extract added was also used. Inoculated plates were kept in a damp chamber for 40 days, and the growth and clearing were noted at intervals (Table 8, columns 15 and 16).

(v) Starch: (a) Salts-yeast extract-starch plates. Two percent "soluble" starch (Fisher), 0.05% yeast extract, and 1.0% agar were added to Hutchinson and Clayton salt solution (26). Streak-inoculated plates were incubated for 4 days when growth and hydrolysis (iodine reaction) (15) were noted (Table 8, column 17).

(b) Nutrient broth-starch tubes. Two percent soluble starch (Fisher) was added to nutrient broth

TABLE 9. *Biochemical reactions: Hydrolysis of proteins by Cytophaga johnsonae strains and Flavobacterium pectinovorum NCIB 9059*

Strain	Gelatin liquefaction	Casein hydrolysis			Haemolysis of sheep red blood cells	Growth on tryptone agar	Penassay broth		Salts-Casitone broth		Salts-Casamino Acids broth	
		Milk peptonization[a]	Casein broth									
			Growth	↑ NH_3			Growth	↑ NH_3	Growth	↑ NH_3	Growth	↑ NH_3
	24[b]	7	28		12	4	11		14		7	
C. johnsonae												
ATCC 17061	+	4	+	+	−	+	+	+	+	+	+	+
UASM 405	+	3	+	+	+	+	+	+	+	+	+	+
UASM ALF	+	2	+	+	+	+	+	−	+	+	+	−
UASM B-2-25	+	7	+	+	+	+	+	+	+	+	+	+
UASM E-1-25	+	3	+	+	+	+	+	+	+	+	+	+
UASM 4433	+	3	+	+	+	+	+	+	+	+	+	+
UASM 4539	+	2	+	+	+	+	+	+	+	+	+	+
UASM 3	+	2	+	+	+	+	+	+	+	+	+	+
UASM 4707	+	2	+	+	+	+	+	+	+	+	+	+
F. pectinovorum												
NCIB 9059	+	6	+	+	+	+	+	+	+	+	+	+
Column no.	2	3	4	5	6	7	8	9	10	11	12	13

[a] Number of days for complete clearing.
[b] Days incubated.

TABLE 10. *Biochemical reactions of Cytophaga johnsonae strains and Flavobacterium pectinovorum NCIB 9059*[a]

Strain	Production of:					Reduction of NO_3^- and NO_2^-:	
	H_2S	Indole[b]	Catalase	Oxidase	Phosphatase	$NO_3^- \rightarrow NO_2^-$	$NO_2^- \rightarrow$ gas
	7[c]	7–28	5	<10 s	4	11	11
C. johnsonae							
ATCC 17061	+	−	+	+	+	−	+
UASM 405	+	−	+	+	+	+	+
UASM ALF	+	−	+	+	+	+	−
UASM B-2-25	+	−	+	+	+	+	−
UASM E-1-25	+	−	+	+	+	+	−
UASM 4433	+	−	+	+	+	+	−
UASM 4539	+	−	+	+	+	+	−
UASM 3	+	−	+	+	+	+	+
UASM 4707	+	−	+	+	+	+	−
F. pectinovorum							
NCIB 9059	+	−	+	+	+	+	−
Column no.	2	3	4	5	6	7	8

[a] Interpretation of symbols: columns 2 to 6: +, positive; −, negative; column 7: +, no NO_3^- present, no NH_3 produced, neutral pH, NO_2^- produced; −, NO_3^- still present; column 8: +, no NO_2^- present, no NO_3^- produced, no NH_3 produced, neutral pH, gas bubbles may be present; −, residual NO_2^- still present.

[b] The results for indole were negative for all four series of tests.

[c] Days incubated.

(Difco) solution (26). Growth, pH, and hydrolysis (iodine spot test) were recorded at 25 days (Table 8, column 18).

(c) Potato starch. Potato infusion agar was made up as follows: mashed potato, 20%; glucose, 0.5%; agar, 1.5%; $CaCO_3$, 0.3%; $(NH_4)_2SO_4$, 0.1%. After 8 days, the hydrolysis of starch was noted using the iodine test (Table 8, column 19).

Hydrolysis of proteins: (i) Gelatin liquefaction. Tubes of 12% gelatin in water (26) were stab-inoculated and incubated for 24 days. Liquefaction was measured after cooling the tubes at 10°C for 1 h to set unaltered gelatin (Table 9, column 2).

(ii) Casein: (a) In milk (peptonization). The number of days taken to clear SA broth and the presence or absence of a lytic zone around the culture when grown on SA agar were recorded (Table 9, column 3).

(b) In casein broth. Duplicate tubes containing only 0.5% casein (Fisher) were inoculated, and growth and ammonia production (Nessler reagent) were recorded at 28 days (Table 9, columns 4 and 5).

Haemolysis. Sheep blood agar plates (26) were inoculated with the test cultures in a manner to produce single colonies. Growth and haemolysis were noted at 12 days (Table 9, column 6).

Production of ammonia and growth in: (i) Salts-Casitone broth. A medium containing 2.0% Casitone (Difco), 0.2% $MgSO_4$, 0.1% K_2HPO_4, and 0.06% KH_2PO_4 adjusted to pH 7.6 was used. Observations on growth and ammonia production (Nessler reagent) were made at 14 days in duplicate tubes (Table 9, columns 10 and 11).

(ii) Salts-Casamino Acids broth. A solution of 1.0% vitamin-free Casamino Acids (Difco), 0.1% K_2HPO_4, 0.05% KNO_3, 0.02% $MgSO_4$, 0.01% NaCl, and 0.001% $FeCl_3$ adjusted to pH 7.0 to 7.1 was employed. Two tubes were inoculated with each organism, and growth and ammonia production (Nessler reagent) were recorded at 7 days (Table 9, columns 12 and 13).

(iii) Tryptone agar. Cultures were streaked on Cook's cytophaga agar (0.2% tryptone, 1% agar; 7) and incubated for 4 days, when the presence or absence of growth was observed (Table 9, column 7).

(iv) Penassay broth. Duplicate open tubes of Penassay broth (Difco antibiotic medium 3) were inoculated, and growth and production of ammonia (Nessler reagent) were recorded at 11 days (Table 9, columns 8 and 9).

H_2S from cysteine. Tubes containing 0.01% cysteine hydrochloride (26), sterilized by filtration, in SA broth were inoculated with the test organisms. Dried strips of filter paper impregnated with a 5% lead acetate solution were folded over the top of the tube, which was capped as usual. Blackening of the paper strip, owing to the formation of lead sulfide, indicated H_2S production (15), and this was noted at 7 days (Table 10, column 2).

Formation of indole. A small amount of Kovac reagent (26) was added to 14- and 28-day-old casein broth cultures and to 7- and 14-day-old Casitone broth cultures. After mixing and standing for up to 0.5 h, a red colour at the interface indicated the formation of indole from tryptophan (Table 10, column 3).

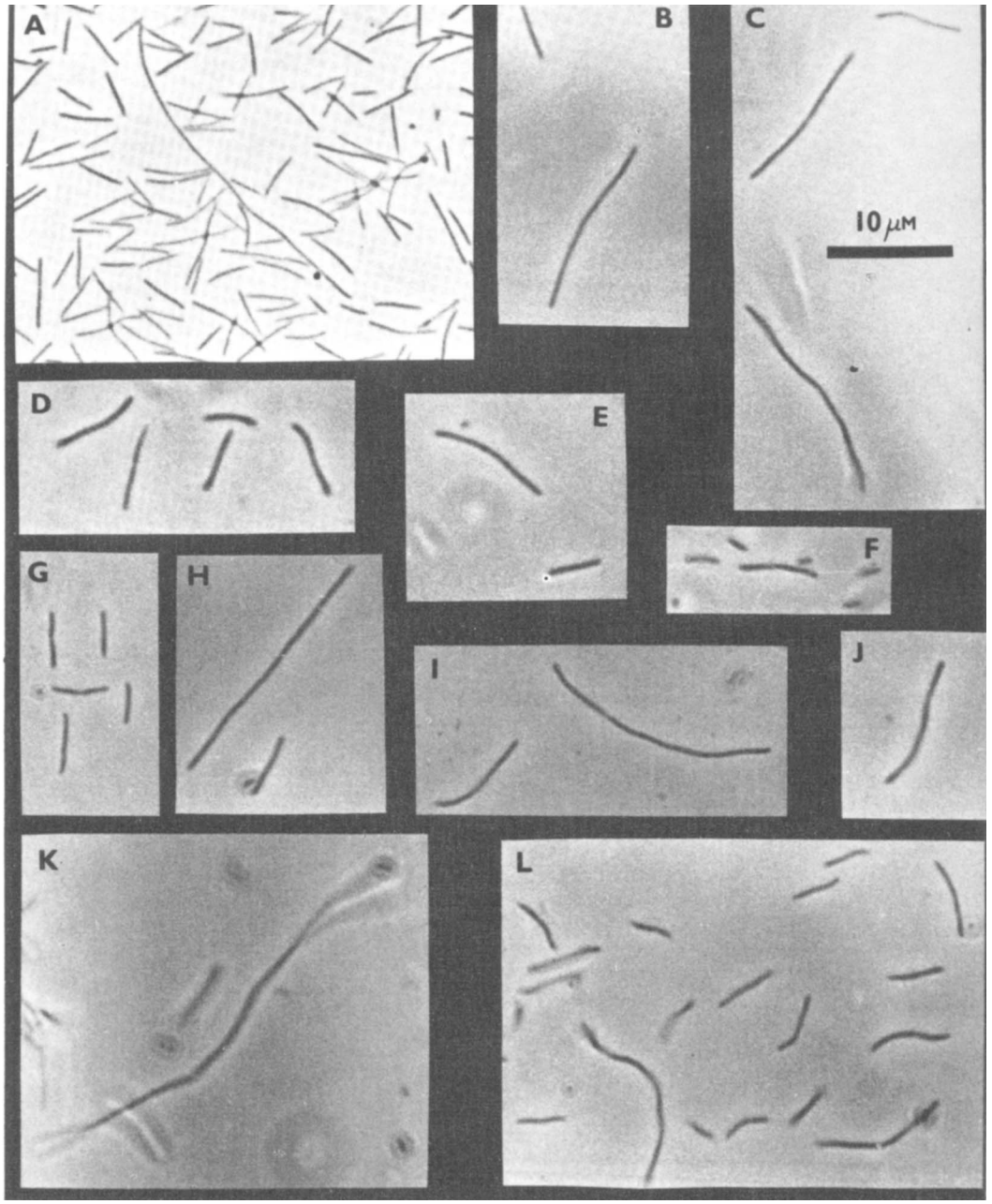

FIG. 1. *Cells of Cytophaga johnsonae strains and Flavobacterium pectinovorum NCIB 9059. (A and B) ATCC 17061; (C) UASM 3; (D) UASM 4539; (E) UASM 4707; (F) UASM E-1-25; (G) UASM 4433; (H and K) NCIB 9059; (I) UASM 405; (J) UASM B-2-25; (L) UASM ALF. Media: (A) from the spreading edge of a 5-day-old colony on yeast cell agar; (B, C, D, E, H, J, K, and L) from 60-h-old skim acetate broth; (F and G) from 24-h-old skim acetate broth. All of these 10 strains had flexible cells which exhibited gliding motility.*

Catalase production. After 5 days of incubation of streak cultures, SA plates were flooded with 10% hydrogen peroxide (26). Copious evolution of bubbles was evidence for catalase activity and hence an aerobic respiration (Table 10, column 4).

Oxidase production. A freshly prepared solution of 0.1 g of tetramethyl-*para*-phenylenediamine dihydrochloride in 10 ml of distilled water (13) was allowed to stand for 15 min and then drops were placed on a piece of Whatman no. 1 filter paper. Test

TABLE 11. *DNA base ratios for Cytophaga johnsonae strains and Flavobacterium pectinovorum NCIB 9059*

Strain	Mol% G+C	Reference
C. johnsonae		
ATCC 17061	33	19
UASM 405	34.6 (T_m)-35 (buoyant density)	19
UASM 405	30-32	M. Mandel, personal communication to F. D. Cook
F. pectinovorum		
NCIB 9059	32.9	21
NCIB 10021	32.7	21

colonies from various agar plates were smeared on the reagent-saturated paper. If oxidase was present, a dark purple colour developed within 10 s (2) (Table 10, column 5).

Phosphatase production. A 20-ml volume of a filter-sterilized 0.5% phenolphthalein diphosphoric acid solution was added to 1 litre of routine SA agar just before pouring plates, to give a final concentration of 0.1% (3). Cultures were spotted four per plate, and after 4 days of incubation the plates were exposed to ammonia vapour. Colonies producing sufficient phosphatase to liberate free phenolphthalein became bright pink; others were unchanged (Table 10, column 6).

Nitrate reduction in anaerobic system. Duplicate tubes of Penassay broth (Difco antibiotic medium 3) containing 0.1% KNO_3 (1) were inoculated with the test organisms and incubated in Gas-Pak jars under hydrogen and carbon dioxide to test for reduction of NO_3^- to NO_2^-. Spot plate tests for ammonia production (Nessler reagent), pH (bromothymol blue), nitrate and nitrite (acidified diphenylamine), and nitrite (acidified Trommsdorf reagent), and a hot wire test for nitrogen gas bubbles were carried out at 11 days (Table 10, column 7). A similar series of tests was done with tubes containing 0.1% KNO_2 to test for the denitrification of NO_2^- to a gas (Table 10, column 8).

RESULTS AND DISCUSSION

No type culture has yet been designated for *C. johnsonae*. All of these isolates conformed to the published description of *C. johnsonae* (14, 29, 30), except that ATCC 17061 and UASM 405 were not found to use NO_3^- as a nitrogen source, ATCC 17061 and UASM 4433 were sucrose negative, and UASM ALF utilized mannitol (P. J. Christensen, Ph.D. Thesis, University of Alberta, Edmonton, Alberta, Canada, 1973).

All of the 10 cultures examined were gram-negative, flexing rods which were arranged singly and showed gliding but not flagellar motility (Fig. 1). All showed thin, spreading, orange-yellow growth with no water-soluble pigment on Cook's cytophaga agar and skim acetate agar and were silky in broth culture. The morphological, physiological, and biochemical characteristics of *F. pectinovorum* NCIB 9059 were found to correspond closely to those of the nine *C. johnsonae* strains investigated (Tables 2 to 10). The deoxyribonucleic acid (DNA) base ratio of 33 mol% guanine plus cytosine for NCIB 9059 falls in the centre of the reported range for *C. johnsonae* (Table 11). Another strain of *F. pectinovorum*, NCIB 10021, which is no longer available, was also reported to have a DNA base ratio within this range (Table 11).

The strain NCIB 9059 (ATCC 19366) has been rejected as a species of the genus *Flavobacterium* on account of its gliding motility (32), and several authors have remarked upon the similarity of the properties of this organism with those of the genus *Cytophaga* (17, 18, 21; O. B. Weeks, personal communication). The data reported above on a wide range of properties of strain NCIB 9059 suggest that this organism may be more properly classified as *Cytophaga johnsonae* Stanier 1947 (29). Since this strain was the holotypic culture of *Flavobacterium pectinovorum* Dorey 1959 (8), the latter name is considered to be a junior subjective synonym.

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