Pectinatus, a New Genus of the Family *Bacteroidaceae*

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The properties of an anaerobic, rod-shaped bacterium having an unusual flagellar arrangement are described. The flagella emanate from only one side of the cell body and are not limited to the center portion as they are with selenomonads. The organism is a gram-negative, nonsporeforming mesophile producing acetic, propionic, and succinic acids and traces of lactic acid from glucose fermentation. The guanine-plus-cytosine content of the deoxyribonucleic acid is 39.8 mol%. The characteristics of this organism indicate that it belongs in none of the presently described genera. For this organism, we propose a new genus, *Pectinatus*, and a new species, *Pectinatus cerevisiiphilus*, in the family *Bacteroidaceae*. The type strain, CCC B-1022, has been deposited in the American Type Culture Collection under the number 29359.

Few organisms are capable of growing in normal packaged beers because of the inherent selectivity brought about by pH, alcohol content, hop residuals, and the lack of oxygen. The organisms generally referred to as "beer spoilage bacteria" are classified in the following genera: *Acetobacter*, *Acetomonas*, *Lactobacillus*, *Pediococcus*, and *Zymomonas*. Other bacteria, such as enterobacters and flavobacteria, are considered to be problems in the early brewing process but never in the final packaged product (1). During the course of routine identification work on bacteria in our brewery, we isolated an unusual bacterium capable of growing in beer. Unlike other common organisms, this organism was a strict anaerobe, producing no growth aerobically on solid media. Under anaerobic conditions it fermented several sugars. Scanning electron microscopy (SEM) revealed that flagella were attached on only one side of the cell body, giving the cells the appearance of hair combs. The unique features of this organism clearly indicated that it was not a "typical" beer bacterium, and to our knowledge an organism of this type has not, to date, been described in the literature. The purpose of this study was to present some of the morphological and biochemical characteristics of this new isolate and to determine its taxonomic position.

MATERIALS AND METHODS

Bacterial strain. Only one strain (CCC B-1022) was studied. It was isolated from beer which had been stored at 30°C for 30 days. Stock cultures were maintained by biweekly transfers in freshly prepared MRS lactobacilli broth (Difco) with minimal head space in the tube and with incubation at 30°C. Between transfers, cultures were stored at 2°C.

Media. For sugar utilization tests and for gas chromatographic analyses of volatile and nonvolatile fatty

acids, prereduced media were used as described in the VPI *Anaerobe Laboratory Manual* (4). Cultures were grown in MRS lactobacilli broth (Difco) for determination of deoxyribonucleic acid (DNA) base composition, for preparation of cells for SEM photography, and for thin sections for electron microscopy. Thioglycolate agar with glucose (Difco) was used to obtain growth on solid media in Hungate roll tubes (5).

Biochemical tests. Peptone yeast extract, the basal medium, fortified with various carbon sources, was inoculated under oxygen-free CO₂ atmosphere with 4 drops of an actively growing culture. After 48 h of incubation, the pH of the culture was determined with a combination pH electrode (4). Volatile and nonvolatile acids were determined by the procedures described in the VPI manual, using a Hewlett-Packard 5830-A chromatograph.

Determination of DNA base composition. The guanine-plus-cytosine content of the DNA of the test organism was determined by use of the method described by Schildkraut et al. (10).

Preparation of cells for SEM photography. An actively growing broth culture (18 to 24 h old) was harvested by centrifugation at $11,400 \times g$ for 15 min. The supernatant was removed, and the cells were resuspended in sterile distilled water to yield a three-to-fourfold dilution of the original culture. The resulting suspension was slightly turbid. One drop of the dilute suspension was then placed on a grease-free glass cover slip, which was tilted so that the liquid ran slowly to the other end. To avoid breaking the flagella, a sterile glass pipette was used for transfer instead of a platinum loop. The cover slip was air dried in an empty petri dish with the lid slightly open. The air-dried cover slip was then mounted on a standard SEM aluminum stub by using 3M double-coated tape. The underside (stub side) as well as the edges of the cover slip were painted with silver conducting paint (no. 21-1 GE Electronics, Rockford, Ill.) so that a continuous conductive path from cover-slip edge to stub was established. Mounted samples were coated with a gold-palladium amalgam (60% gold-40% palladium) in a Denton DU 502 vacuum evaporator at 6×10^{-6} torr pressure. A current of 16 A was applied to the filament

(20-mil-diameter tungsten) for 30 s, and then the current was raised to 22 A for an additional 30 s. The average filament-to-sample distance was 10.0 cm. During coating, the samples were on a rotating, tilting omni stage electrically rotated at 40 rpm. Coated samples were examined with an ETC Autoscan SEM at 35° tilt, 20 kV accelerating voltage, and with secondary electron imaging. Additionally, a drop of suspension air dried on a cover slip was flagella stained by the Conn and Wolfe method (3) and was then coated with gold-palladium, as described above, for a clearer view of the flagellar arrangement.

Thin sections and electron microscopy. Thin sections were prepared by the method described by Higgins in the *Handbook of Microbiology* (7). Sections were photographed with a Hitachi model HU-12 elec-

tron microscope, with an accelerating voltage of 75,000 kV.

RESULTS

Morphology. The cells of strain CCC B-1022 are slightly curved, gram-negative rods with rounded ends, 0.7 to 0.8 μm by 2.0 to 32 μm or longer in older cells (Fig. 1); they occur singly, sometimes in pairs, and only rarely in chains. Shorter, younger cells do not show a helical shape, but the elongated older cells tend to form helices. After the culture had passed the stationary and declining phases, round cell forms were often observed, and the number of these in-

FIG. 1. *Elongated cells of strain CCC B-1022 under phase-contrast microscopy. $\times 1,200$.*

creased with the time of incubation (Fig. 2). These globular forms are believed to be identical with the spheroplasts of selenomonads (6). Young cells move very actively, giving the appearance of an "X" shape as they swim. Older and longer cells appear to have a snakelike motion as they move.

The most unique morphological feature of this organism is the flagellar arrangement. SEM micrographs reveal that the flagella emanate from only one side of a cell (Fig. 3). Flagella are not limited to the center portion of the concave side of the cell as they are in the genus *Selenomonas*. The number of flagella per cell seems to depend upon the cell size and its condition, but generally they range from 10 to 23 or more. The flagellar wavelength is about 2 μm . The fact that flagella emanate from only one side of cells oriented in different directions (Fig. 4) indicates that this unique flagellar arrangement is not a result of

sample preparation. The flagella stain-gold-palladium coating preparation of this organism clearly shows the attachment of the flagella along the side of the cell body (Fig. 5 and 6).

Ultrastructure of the cell. Examination of a thin section of a cell of strain CCC B-1022 (Fig. 7) revealed a multilayered cell wall typical of gram-negative bacteria (8). The outer double layer of the cell wall appears to have a profile characteristic of lipopolysaccharide and lipoprotein as described by Murray et al. (9). Figure 8 shows a number of broken flagella still attached at only one side of the cell body.

Cultural characteristics. No growth was obtained on agar plates under aerobic conditions or under 98% CO_2 . By the Hungate roll tube technique (5) with thioglycolate agar, colonies developed after 3 to 4 days of incubation at 30°C. Colonies were circular, entire, low convex to pulvinate white, glistening, and opaque. The

FIG. 2. Globular forms (arrows) of strain CCC B-1022 under phase-contrast microscopy. $\times 1,200$.

FIG. 3. SEM micrograph illustrating flagella of strain CCC B-1022. $\times 13,680$.

organism grew fairly well in any broth medium containing fermentable sugars. With a 2% inoculum, growth was obtained in freshly prepared aerobic broth (i.e., MRS lactobacilli broth) tubed with a minimum of head space. Anaerobic conditions were not required for growth in broth cultures. The cells were actively motile in young (18- to 24-h-old) cultures, but they slowly lost motility as they grew older and eventually lost all motility.

Physiological and biochemical characteristics. The organism is an anaerobic mesophile. It grows between 15 and 40°C, with optimum growth occurring at about 32°C. It produced acid from adonitol, arabinose, cellobiose, dulcitol, erythritol, fructose, galactose, glucose, glycerol, lactate, maltose, mannitol, mannose, rhamnose, and ribose (Table 1). Amygdalin, milk, raffinose, salicin, sorbitol, sorbose, sucrose, trehalose, and xylose were not fermented. Nitrite was not produced from nitrate, and starch was not hydrolyzed. Acetic, propionic, succinic, and lactic acids were produced from glucose, and acetic, propionic, and succinic acids were pro-

duced from lactate. Volatile and nonvolatile fatty acid products are shown in Table 2 and Fig. 9 through 12. Catalase was not produced.

DNA base composition. The guanine-plus-cytosine content of the DNA of this organism was found to be 39.8 mol%.

DISCUSSION

The most striking structural feature of this organism is the unusual arrangement of the flagella. Members of the genus *Selenomonas* most closely resemble this organism; however, a "tuft" arrangement of flagella is described for *Selenomonas*. Due to the differences between this species and those in previously described anaerobic genera (see Tables 3 and 4), we place this species in a new genus, *Pectinatus* (*Pec.ti.na.tus*. L. part. adj. *pectinatus* combed; M.L. mas. n. *Pectinatus* combed [bacteria]). The type species is *P. cerevisiophilus* sp. nov. (*ce.re.vi.si.i.phi.lus*. L. n. *cerevisia* beer; Gr. adj. *philus* loving; M.L. adj. *cerevisiophilus* beer-loving), of which the type strain is CCC B-1022 (a culture of this strain has been deposited in the American Type Culture

FIG. 4. SEM micrograph of lateral flagellar arrangement in a 48-h-old culture of strain CCC B-1022. $\times 7,240$.

FIG. 5. SEM micrograph of a flagella stain-algarn-coated preparation of strain CCC B-1022. $\times 12,813$.

FIG. 6. SEM micrograph of a flagella stain-algarn-coated preparation of strain CCC B-1022. $\times 60,164$.

FIG. 7. Thin section of a typical cell of strain CCC B-1022 illustrating irregular, undulating cell wall layers (arrows). $\times 44,250$.

FIG. 8. Thin section of a typical cell of strain CCC B-1022 illustrating broken flagella along one side of the cell. $\times 121,675$.

TABLE 1. *Biochemical reactions of strain CCC B-1022*

Substrate or reaction	Result ^a
Utilization of:	
Adonitol	+
Amygdalin	—
Arabinose	+
Cellobiose	+
Dextrin	—
Dulcitol	+
Erythritol	+
Esculin	—
Fructose	+
Galactose	+
Glucose	+
Glycerol	+
Glycogen	—
Inositol	—
Inulin	—
Lactate ^b	+
Lactose	—
Maltose	+
Mannitol	+
Mannose	+
Melezitose	—
Melibiose	—
Raffinose	—
Rhamnose	+
Ribose	w
Salicin	—
Sorbitol	—
Sorbose	—
Starch	—
Sucrose	—
Trehalose	—
Xylose	— or w
Gelatin liquefaction	—
Indole production	—
Milk (curd or digestion)	—
Nitrate reduction	—
Peptone yeast extract	—

^a Symbols: +, pH below 5.5 in carbohydrate cultures (positive reaction); —, pH above 6.0 (negative reaction); w, pH between 5.5 and 6.0 (weak reaction).

^b Since the measurement of pH cannot be used to determine lactate utilization, gas chromatography was used to determine the by-products produced.

Collection [ATCC] under the number 29359). Because only a single strain (CCC B-1022) of this new species has been isolated to date, the description given above for this strain also serves as the species description. Obviously the description will have to be modified as more isolates are described. The same is true of the generic description, which follows.

***Pectinatus* gen. nov.** Slightly curved rods, with rounded ends, occurring singly, in pairs, and only rarely in short chains. Shorter, younger cells do not show a helical shape, but the elon-

gated older cells tend to form helices. Motile by means of flagella which emanate from only one side of a cell. The attachment of the flagella is not limited to the central portion of the concave side of the cells as it is in the genus *Selenomonas*. Growth occurs between 15 and 40°C, with optimum growth occurring at about 32°C. Acetic, propionic, succinic, and lactic acids are produced from glucose, and propionic and succinic acids are produced from lactate. Nitrite is not produced from nitrate, and starch is not hydrolyzed. Strictly anaerobic. Catalase is not produced. First isolated from spoiled beer, but the habitat is not known.

The type species is *Pectinatus cerevisiiphilus*.

The general staining characteristics, cellular morphology (Fig. 1 and 2), and anaerobic requirements of the organism described here most closely resemble those of members of two families: *Bacillaceae* and *Bacteroidaceae* (2). However, the inability to demonstrate spore production by this organism was considered preliminary justification to eliminate it from membership in the family *Bacillaceae*. The most significant structural feature of this organism is its unique lateral flagellar arrangement. Among members of the family *Bacteroidaceae*, none of the three currently recognized genera (2) exhibits this structural feature. Of the gram-negative, anaerobic bacteria of uncertain affiliation (*Desulfovibrio*, *Butyrivibrio*, *Succinivibrio*, *Succinimonas*, *Lachnospira*, and *Selenomonas*) (2), members of the genus *Selenomonas* possess a flagellar arrangement which most closely resembles that of this organism. The important morphological difference between the selenomonads and this organism, however, is the "tuft" arrangement of the flagella of the selenomonads (6). The isolate described here clearly possesses a comblike flagellar arrangement along the long axis of the cell (Fig. 3 to 6, and 8). Cells were gram negative to gram variable, and the electron micrographs (Fig. 7 and 8) taken of thin sections of a cell indicate irregular, undulating cell wall layers that are usually associated with gram-negative organisms.

Strain CCC B-1022 is capable of fermenting a variety of sugars (Table 1). Gas chromatographic analysis of the products of sugar fermentation indicate that the organism produces acetic, propionic, succinic, and lactic acids from glucose (Table 2). There was no evidence of butyric acid production from any of the sugar substrates tested. The DNA base composition of the isolate is 39.8 mol% guanine plus cytosine, whereas members of the genus *Selenomonas* are reported to have a guanine-plus-cytosine value of 54 or 60 mol% (Table 4). A summary of some of the

TABLE 2. Volatile and nonvolatile fatty acids produced from various fermentable carbon sources by strain CCC B-1022

Carbon sources	Acid (meq/liter) produced ^a			
	Acetic	Propionic	Lactic	Succinic
Adonitol	70.0	19.5	—	—
Arabinose	40.0	23.8	—	1.4
Cellobiose	61.0	11.2	—	11.2
Dulcitol	—	—	—	2.7
Erythritol	12.5	39.1	—	—
Fructose	74.2	35.5	—	—
Galactose	84.2	43.0	—	—
Glucose	89.2	52.3	4.8	45.3
Glycerol	—	28.4	—	—
Lactate	186.7	63.0	—	0.5
Maltose	103.3	2.3	—	6.8
Mannitol	25.8	69.9	—	—
Mannose	65.0	33.9	—	—
Rhamnose	72.5	13.0	—	—
Ribose	20.0	3.5	—	2.0

^a —, Acid not produced.

FIG. 9. Typical chromatogram of volatile fatty acids produced by strain CCC B-1022 in peptone-yeast extract-glucose medium. Acetic acid retention time = 3.61 min. Propionic acid retention time = 4.73 min.

major characteristics of strain CCC B-1022 and closely related organisms is presented in Table 3. In most cases, these organisms possess more than one major characteristic which differs from the characteristics of the new isolate described here. Only three of the organisms listed in Table 3 demonstrate lateral flagellation of any kind:

Lachnospira, *Selenomonas*, and the new isolate. End products of glucose fermentation indicate that lachnospiras are ethanol producers. Preliminary studies on our isolate (gas chromatography and alcohol dehydrogenase tests) indicated no ethanol production from glucose.

In Table 4, the differences between seleno-

FIG. 10. Typical chromatogram of methylated (nonvolatile) fatty acids produced by strain CCC B-1022 in peptone-yeast extract-glucose medium. Lactic acid retention time = 4.99 min. Succinic acid retention time = 16.88 min.

FIG. 11. Typical chromatogram of volatile fatty acids produced from lactate by strain CCC B-1022. Acetic acid retention time = 3.59 min. Propionic acid retention time = 4.71 min.

FIG. 12. Typical chromatogram of methylated (nonvolatile) fatty acids produced from lactate by strain CCC B-1022. Succinic acid retention time = 12.33 min. Propionic acid (background due to excess amount in medium) retention time = 6.33 min.

TABLE 3. Characteristics useful in differentiating strain CCC B-1022 from other anaerobes

Taxon	Arrangement of flagella	Spore production	Glucose by-products	G+C ratio (mol%)
<i>Clostridium</i>	Peritrichous (if flagella occur)	+		23-43
<i>Bacteroides</i>	None or peritrichous	—	Combinations of succinic, lactic, acetic, formic, and propionic acids	40-55
<i>Fusobacterium</i>	None or peritrichous	—	Butyric acid	26-34
<i>Leptotrichia</i>	None	—	Lactic acid	31-34
<i>Desulfovibrio</i>	Polar	—	Not known	46-61
<i>Butyrivibrio</i>	Polar (single flagellum)	—	Lactic and butyric acids	Not known
<i>Succinivibrio</i>	Polar (single flagellum)	—	Succinic, acetic, formic, and lactic acids	Not known
<i>Succinimonas</i>	Polar (single flagellum)	—	Succinic and acetic acids	Not known
<i>Lachnospira</i>	Subterminal to lateral	—	Ethanol, lactic, formic, and acetic acids	Not known
<i>Selenomonas</i>	Lateral (tufted)	—	Acetic, propionic, and lactic acids	53-61
Strain CCC B-1022	Lateral (comblike)	—	Acetic, propionic, succinic, and lactic acids	39.8

monads and the new isolate with respect to cell morphology, flagellation, DNA base composition, and substrate utilization are readily apparent.

The properties of the new, anaerobic, gram-negative, mesophilic rod described here are, in our opinion, sufficient to warrant placing the organism in a new genus.

TABLE 4. Characteristics useful for differentiating between *selenomonads* and *Pectinatus cerevisiiphilus* sp. nov.^a

Characteristic	<i>Selenomonas ruminantium</i>	<i>Selenomonas sputigena</i>	<i>Pectinatus cerevisiiphilus</i> sp. nov.
Cell shape	Crescent moon	Crescent moon	Slightly curved rod with round ends
Cell size (μm)	0.9–1.1 by 3.5–6.0	0.9–1.1 by 3.0–5.5	0.7–0.8 by 2.0–32 or longer
Flagella location	Tuft in the center of the concave side	Tuft in the center of the concave side	Only one side (concave), but not as a tuft
No. of flagella per cell	16	16	10–23 or more
DNA base ratio (mol% G+C)	54.0 ± 0.8	60.6 ± 0.8	39.8
Glucose fermentation products	Propionic, acetic, and lactic acids	Propionic and acetic acids	Acetic, propionic, succinic, and lactic acids
Utilization of:			
Erythritol	—	—	a
Esculin	+	+	—
Lactose	v	a	—
Melibiose	a _w	a _w	—
Raffinose	v	a	—
Sucrose	a _w	a _w	—
Xylose	a	a	—
Milk	c	c	—
Adonitol	—	—	w

^a Symbols: +, positive reaction for 90 to 100% of the strains tested; —, negative reaction for 90 to 100% of the strains tested; a, strong acid production (pH 5.5 or below); a_w, most strains produce acid, some weakly; c, curd produced in milk; v, variable acid production (strains maybe either + or —); w, weak acid production (pH 5.5–6.0).

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REPRINT REQUESTS

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