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Treponema saccharophilum sp. nov., a Large Pectinolytic Spirochete from the Bovine Rumen

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A large, obligately anaerobic spirochete (strain PB) was isolated from bovine rumen fluid by a procedure involving rifampin as a selective agent. The helical cells measured 0.6 to 0.7 μm by 12 to 20 μm and possessed approximately 16 periplasmic flagella inserted near each end of the protoplasmic cylinder. The periplasmic flagella were arranged in a bundle wound around the cell body. Strain PB utilized as fermentable substrates various plant polysaccharides (e.g., pectin, arabinogalactan, starch, and inulin) as well as pentoses, hexoses, disaccharides, and uronic acids. Glucose was fermented to acetate, formate, and ethanol, whereas the fermentation of pectin or glucuronic acid yielded only acetate and formate as major end products. Determinations of radioactivity in end products and assays of enzymatic activities indicated that strain PB catabolized glucose via the Embden-Meyerhof pathway. Extracts of cells grown in pectin-containing media possessed relatively high levels of phospho-2-keto-3-deoxygluconate aldolase activity, an enzymatic activity typical of the Entner-Doudoroff pathway. The guanine-plus-cytosine content of the DNA of strain PB (54 mol%) was considerably higher than that of known host-associated anaerobic spirochetes. This study indicates that strain PB represents a new species of *Treponema*, for which we propose the name *Treponema saccharophilum*.

In a previous study we showed that the bovine rumen is inhabited by relatively large numbers of physiologically diverse spirochetes, and we described the characteristics of numerous strains of spirochetes isolated from rumen fluid (19). Strain PB, one of the isolates, had several properties that distinguished it from other spirochete strains. For example, the guanine-plus-cytosine content of the DNA of strain PB was considerably higher than that of other known rumen spirochetes (19) as well as of other host-associated anaerobic spirochetes (e.g., *Treponema* species) (21). Furthermore, cells of strain PB were larger than those of other rumen spirochetes and had many periplasmic flagella (periplasmic fibrils) arranged in a bundle (19).

This paper reports the results of a study on the ultrastructure, nutrition, and general physiological characteristics of strain PB. Our findings indicated that strain PB represents a new species of *Treponema*. As discussed below, the name *Treponema saccharophilum* is proposed for this spirochete and, henceforth, we will refer to it by the proposed name. The ultimate goal of our work with rumen spirochetes is to achieve a greater understanding of the physiology and ecology of these bacteria.

MATERIALS AND METHODS

Isolation of *T. saccharophilum*. *T. saccharophilum* PB was isolated from a rumen fluid sample obtained from a fistulated cow (Holstein breed) housed at the University of Massachusetts Dairy Facility, South Deerfield, Mass. The cow was fed a mixed diet of hay and corn silage. Methods used for collecting and transporting rumen fluid samples were described previously (22). Rumen fluid is defined as described earlier (22).

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T. saccharophilum was isolated with an agar medium that contained the antibiotic rifampin as a selective agent (22). A sample of rumen fluid was serially diluted (30 to 60 min after collection) into deeps of RFG agar medium (23) (see below) to which pectin (0.2 g/100 ml of medium) had been added in place of glucose as a growth substrate. Rifampin was included in the medium at a final concentration of 1 μg/ml. After incubation for 24 h at 39°C, colonies of spirochetes were readily recognizable because of their characteristic spreading appearance, which results from the ability of these bacteria to migrate through agar media (4). Isolated colonies of *T. saccharophilum* were obtained by serially diluting cells from a single colony into deeps of the agar medium described above and incubating the cultures at 39°C. *T. saccharophilum* PB was cloned by repeating this procedure three times.

Media and growth conditions. The composition of medium RFG, a rumen fluid-containing medium, was described previously by Stanton and Canale-Parola (23). Medium MVTY, used for routine growth of *T. saccharophilum*, contained a supplement of fatty acids, yeast extract, and Trypticase (BBL Microbiology Systems, Cockeysville, Md.)-peptone instead of rumen fluid. This medium was identical to medium VTY described previously (23), except that propionic and acetic acids were omitted. The pH of medium MVTY was 6.9. When glucose was omitted from medium MVTY and no other fermentable substrate was added in its place, the medium did not support detectable growth of *T. saccharophilum*. Conditions for growth of *T. saccharophilum* PB were described earlier (19).

Nutritional requirements. The ability of *T. saccharophilum* to utilize various carbon and energy sources for growth was determined by measuring the final growth yields of cultures in MVTY broth from which glucose was omitted and replaced by the substrate under test (0.2 g/100 ml of medium).

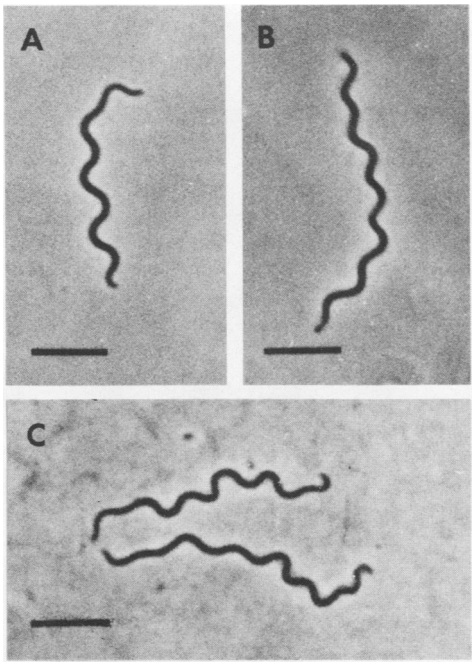

FIG. 1. Phase-contrast photomicrographs of *T. saccharophilum* cells. (A) and (B) Regularly coiled cells. (C) Irregularly coiled cells in contact with agar medium. Bar, 5 μm.

Starch, pectin, polygalacturonic acid, xylan, and arabinogalactan were washed three times in 70% ethyl alcohol or dialyzed against water to remove contaminating low-molecular-weight sugars.

Growth yields were determined after four transfers in the test medium by means of direct microscopic counts with a Petroff-Hausser counting chamber. For detection of starch hydrolysis by the spirochete, stab cultures in MVTY agar medium that initially contained 0.2% soluble starch (instead of glucose) as a growth substrate were flooded with Lugol iodine solution (6). Pectinolytic activity was determined by testing growing cells for liquefaction of RFG or MVTY medium that contained no agar but to which pectin (1.0 g/100 ml of medium) was added in place of glucose as growth substrate. Spirochete strain 606, a rumen spirochete that possesses pectinolytic enzymes (25), was used as a positive control for the determination of pectinolytic activity. Requirements for volatile fatty acids were determined by observing growth (three transfers) in medium MVTY from which one or more of the fatty acids was omitted.

Motility observations. Cells to be used for motility observations were placed in flat capillary tubes (50 by 0.2 mm, Microslides; Vitro Dynamics, Inc., Rockaway, N.J.) as previously described (7), except that deoxygenated CO_2 was used as the atmosphere instead of N_2. Procedures for the observation of cell motility and for measuring the translational velocity of cells were described previously (7).

Fermentation end products. For quantitative analyses of fermentation end products, *T. saccharophilum* was grown in MVTY broth. Growing cultures were connected to a fermentation train to determine gaseous end products (18). Formate was assayed colorimetrically as described by Lang and Lang (15). Acetoin, diacetyl, 2,3-butanediol, and glycerol were determined by means of standard procedures (18). Ethyl alcohol and glucose were assayed enzymatically with alcohol dehydrogenase and glucose oxidase, respectively (ethyl alcohol reagent kit and Statzyme glucose reagent; Worthington Diagnostics, Freehold, N.J.). All other nongaseous end products were determined by gas-liquid chromatography as described by Paster and Canale-Parola (19).

Formate production from pyruvate. Procedures for the determination of formate production from pyruvate by cell suspensions were described previously (7). Formate was assayed as described above, and pyruvate was determined enzymatically with lactic dehydrogenase (pyruvic acid diagnostic kit; Sigma Chemical Co., St. Louis, Mo.).

Determination of enzymatic activities. *T. saccharophilum* cells grown in MVTY broth (with pectin, glucuronic acid, or glucose as fermentable substrate) were used to prepare cell suspensions as described previously (7). Cell extracts were prepared by sonic treatment of cell suspensions for 90 s as reported by Lessie and Vander Wyk (16).

Fructose-bisphosphate aldolase (EC 4.1.2.13), glyceraldehyde-phosphate dehydrogenase (EC 1.2.1.12), and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activities were determined by linking the reactions to pyridine nucleotide reduction as described by Hespell and Canale-Parola (11). Glucosephosphate isomerase (EC 5.3.1.9) activity was assayed spectrophotometrically at 340 nm by measuring NADPH formed in reaction mixtures (0.4 ml) containing 0.2 M Tris buffer (pH 8.5), 5×10^{-4} M NADP, 0.01 M fructose-6-phosphate, cell-free extract (2 to 11.7 mg of protein per ml of reaction mixture), and excess of glucose-6-phosphate dehydrogenase from yeast.

Phosphogluconate dehydratase (EC 4.2.1.12) and phospho-2-keto-3-dexoy-gluconate (KdPG) aldolase (EC 4.1.2.14) activities were determined by measuring the production of pyruvate from 6-phosphogluconate. The assay mixture (0.5 ml) contained 0.2 M Tris buffer (pH 8.5), 0.01 M $MgSO_4$, *T. saccharophilum* cell-free extract (2 to 11.7 mg of protein per ml of reaction mixture), and 0.001 M 6-phosphogluconate. Excess of phosphogluconate dehydratase present in extracts of *Pseudomonas cepacia* 249-27, a mutant strain that does not possess KdPG aldolase, was added to the assay mixture for the determination of KdPG aldolase activity. Excess of KdPG aldolase present in extracts of *P. cepacia* 249-87, a mutant strain deficient in phosphogluconate dehydratase, was added to the assay mixture for the determination of phosphogluconate dehydratase activity. Production of pyruvate in either of these reaction mixtures indicated that the enzyme activity lacking in the *P. cepacia* extract was present in cell-free extracts of *T. saccharophilum*. Extracts of both mutant strains added to the assay mixture described above (i.e., without spirochete cell-free extract) provided a positive control. Reactions were run for 30 min at 30°C. Pyruvate was determined colorimetrically as the dinitrophenylhydrazone (16). The protein concentrations in cell-free extracts were determined by the method of Lowry et al. (17). The mutant strains of *P. cepacia* were grown in glycerol salts medium (16).

Radioactivity measurements. The ability of growing cells of *T. saccharophilum* to fix CO_2 was tested as follows. A 10-ml culture of *T. saccharophilum* was grown in the presence of [^{14}C]sodium bicarbonate (specific activity, 10,400 cpm/μmol; Calatomic, Inc., Los Angeles, Calif.) in MVTY broth. Cells were centrifuged at 10,000 × *g* for 5 min at 5°C, and the cell pellet (washed twice in 0.2 M phosphate buffer, pH 7.0) was solubilized in 1 ml of 1 M NaOH for 24 h at 37°C. Radioactivity in the supernatant fluid, the supernatant fluid treated with acid, and the solubilized cell pellet was measured. Acid was added to part of the supernatant fluid to liberate any

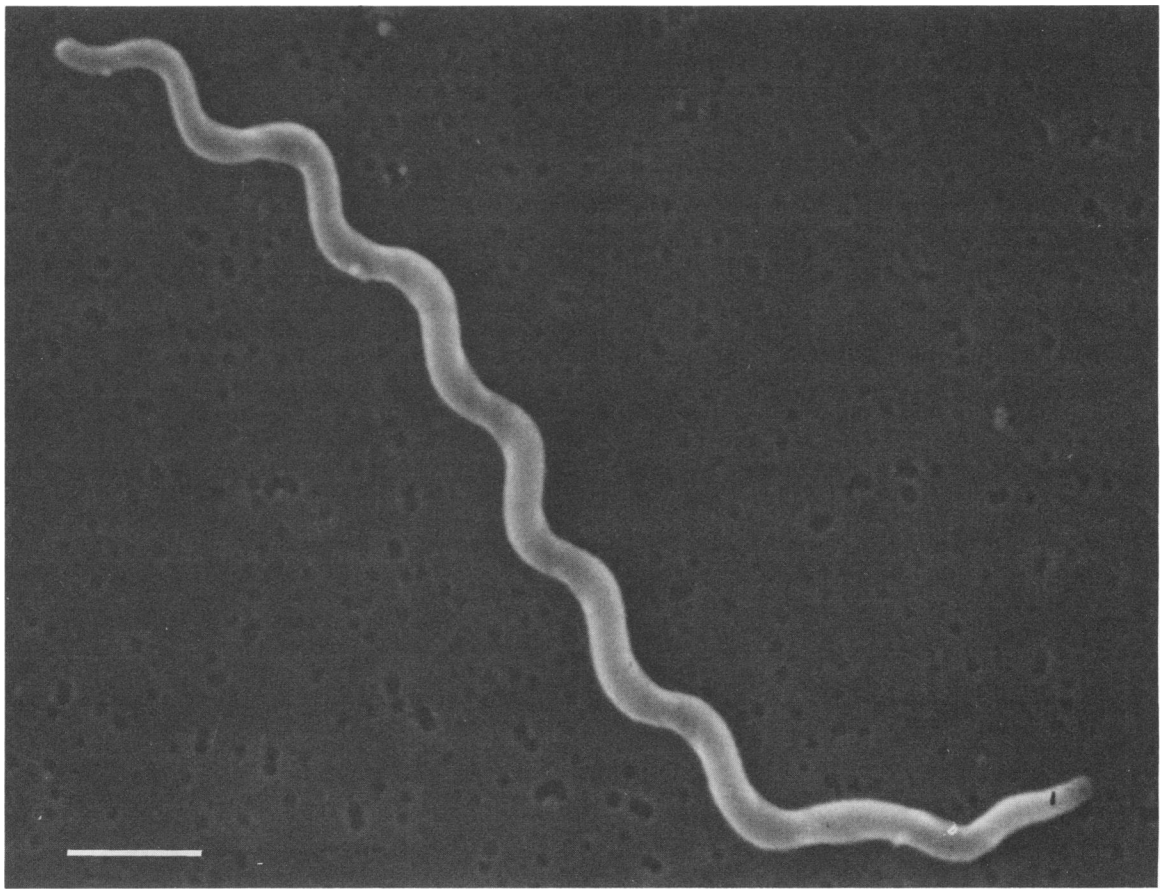

FIG. 2. Scanning electron micrograph of a regularly coiled *T. saccharophilum* cell. Bar, 1.0 μm.

remaining CO_2 in solution. Incorporation of carbon from [U-^{14}C]glucose by growing cells was measured as described previously (12). Fermentation end products of cells grown in MVTY broth in the presence of [1-^{14}C]glucose (17,400 cpm/μmol) were collected from gas-liquid chromatograph effluents in cigarette filter tips, and radioactivity was determined by methods described by Hammarstrand et al. (10).

[U-^{14}C]glucose and [1-^{14}C]glucose were obtained from New England Nuclear Corp., Boston, Mass.

G+C content of DNA. The guanine-plus-cytosine (G+C) content of the DNA was determined by thermal denaturation (T_m) analysis as described by Breznak and Canale-Parola (3) and calculated by using the equation of De Ley (8). DNA from *Escherichia coli* K-12 was used in control determinations.

Microscopy. Equipment and methods used for light microscopy and photography were as described previously (19), except cell preparations (wet mounts) for photography were made by adding a drop of culture to a drop of molten agar (2% [wt/vol] Noble agar in distilled water) on a microscope slide. A glass cover slip was pressed gently on top of the mixture.

Freeze-etch replicas for transmission electron microscopy were prepared by the procedures of Holt et al. (14). Negatively stained samples, ultrathin sections, and scanning electron microscope specimens were prepared for electron microscopy as described previously (19). Equipment and materials used for electron microscopy and photography were described by Paster and Canale-Parola (19).

RESULTS

Morphology and growth characteristics. *T. saccharophilum* cells measured 0.6 to 0.7 μm by 12 to 20 μm and usually exhibited regular coiling patterns (Fig. 1A, 1B, and 2). When the spirochetes were in contact with solid surfaces, such as agar media or glass, the cell coiling often became irregular (Fig. 1C). Spherical bodies, similar in appearance to those formed by other spirochetes (3), were present in cultures in the stationary phase of growth.

Cells of *T. saccharophilum* possessed ultrastructural characteristics typical of spirochetes (4, 5, 13). A bundle of periplasmic flagella (periplasmic fibrils) was wrapped around the helical body of each cell (Fig. 2 and 3; see Fig. 5). Approximately 16 periplasmic flagella were inserted near each end of the protoplasmic cylinder (Fig. 3) and overlapped in the central region of the cell (not shown in figures). An outer sheath surrounded both the bundle of periplasmic flagella and the protoplasmic cylinder (Fig. 3, 4, and 5). The helicoid groove and crenulation present in the outer sheath of some termite hindgut spirochetes (2) were not observed in the outer sheath of *T. saccharophilum* (Fig. 2, 4, and 5).

The motility of *T. saccharophilum* was affected by temperature and by oxygen tension. At 37°C or room temperature under aerobic conditions, rapid rotation and translational motility of cells stopped permanently after 1 to 2 min. The movements of the cells then appeared sluggish and consisted only of occasional flexing and slow rotation. Under anaerobic conditions at 37°C, nearly all cells exhib-

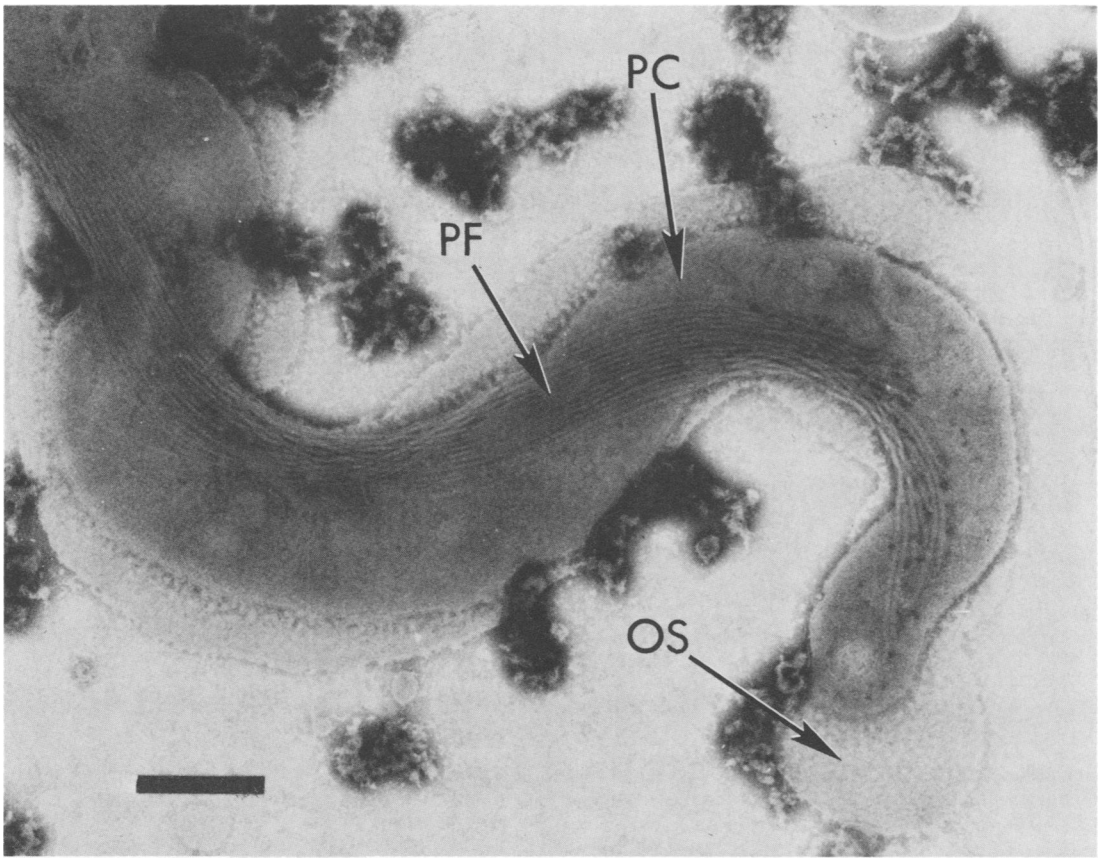

FIG. 3. Transmission electron micrograph of an end of a negatively stained cell of *T. saccharophilum*. The outer sheath (OS), the protoplasmic cylinder (PC), and the bundle of periplasmic flagella (PF) are visible. Bar, 0.3 μm.

ited translational motility by swimming through the liquid medium or by creeping or crawling on the surfaces of the glass capillary tubes. Although all translational motility ceased when the temperature of the anaerobic sample was lowered to 23°C, translational motility was restored when the temperature was raised to 37°C. Similar effects of temperature on motility were observed in studies on other spirochetes (7, 23). The velocity of cells swimming in liquid medium was in excess of 60 μm/s, whereas the creeping velocity was approximately 20 μm/s. Cells appeared to rotate about their longitudinal axes while swimming. Creeping or crawling motility on solid surfaces was similar to that described by Blakemore and Canale-Parola (1).

T. saccharophilum grew only under anaerobic conditions. Colonies in deeps of RFG or MVTY agar medium were spherical and opaque with diffuse edges (see Fig. 1c in reference 19). The colonies measured 3 to 4 mm in diameter after 24 h of incubation and, as described previously (19), their morphology varied according to the concentration of fermentable substrate in the medium.

Optimum temperature for growth was 37 to 39°C. At these temperatures, the final growth yields of *T. saccharophilum* varied from approximately 3.5×10^8 cells per ml in MVTY broth to 7×10^8 cells per ml in RFG broth, and the population doubling time in either medium averaged 90 min. The pH of MVTY broth cultures was 5.9 after 36 h of growth at 37°C. Growth yields were lower and doubling times were greater when the cells were grown at 30°C, and there was no detectable growth at 23 or 45°C.

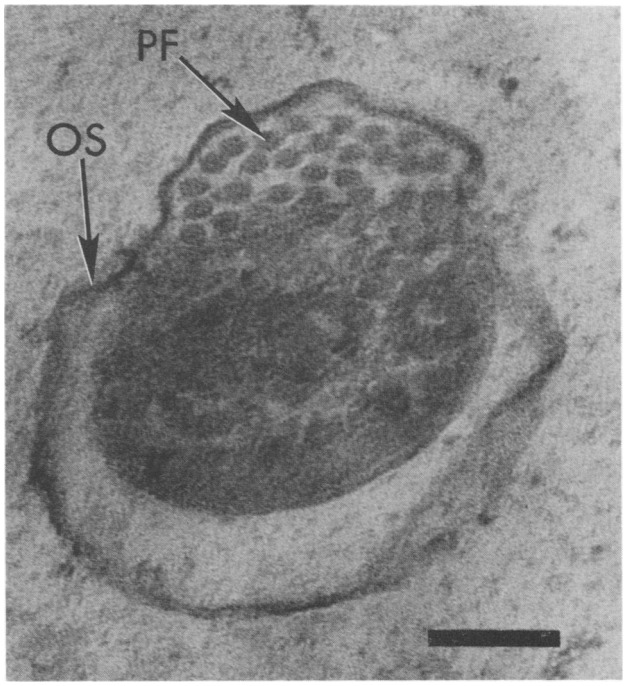

FIG. 4. Transmission electron micrograph of a thin section of a *T. saccharophilum* cell. The periplasmic flagella (PF) are present as a bundle and are surrounded by the outer sheath (OS). Bar, 0.15 μm.

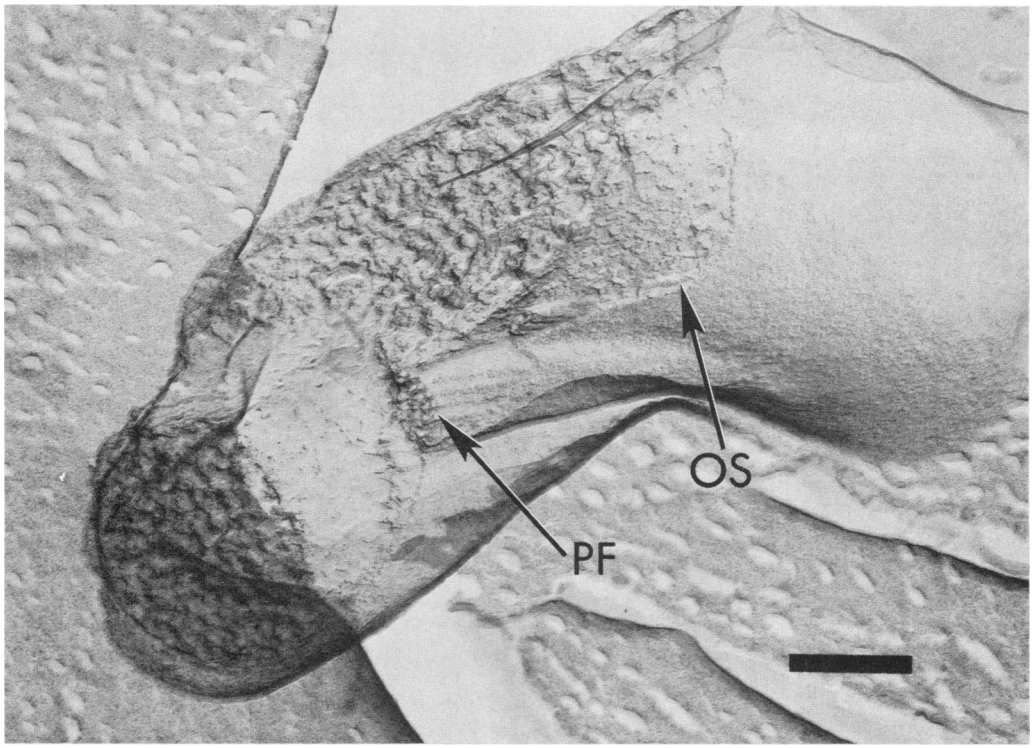

FIG. 5. Transmission electron micrograph of a freeze-etched replica of a portion of a cell of *T. saccharophilum*. A layered outer sheath (OS) covers the bundle of periplasmic flagella (PF) and the protoplasmic cylinder. Note that the outer layer of the outer sheath lacks surface structure. Bar, 0.2 μm.

TABLE 1. Fermentable substrates utilized by growing cells of *T. saccharophilum*[a]

Compound	Growth yield (10^8 cells per ml)
No addition	No growth
L-Arabinose	1.8
D-Galactose	1.8
D-Glucose	3.6
D-Mannose	2.2
D-Fructose	3.0
D-Galacturonic acid	0.2
D-Glucuronic acid[b]	3.5
Cellobiose	3.0
Lactose	0.1
Maltose	3.0
Sucrose	3.0
D-Raffinose	0.6
Dextrin	2.2
Inulin	0.8
Starch (soluble)[c]	2.8
Pectin	3.6
Polygalacturonic acid	3.4
Arabinogalactan	0.2

[a] The following compounds did not support growth: L-rhamnose, D-xylose, L-sorbose, D-ribose, cellulose (ball-milled filter paper), dextran, Casamino Acids (Difco Laboratories, Detroit, Mich.), D-arabitol, dulcitol, mannitol, ribitol, sorbitol, xylitol, glycerol, potassium galactonate, potassium gluconate, sodium acetate, sodium formate, sodium lactate, sodium succinate, potassium fumarate, Tween 80 (final concentration, 0.02% [vol/vol]), glucosamine hydrochloride, xylan.

[b] Two successive transfers of *T. saccharophilum* were necessary in medium with glucuronic acid before optimum growth was obtained.

[c] Starch hydrolysis was detected. See text.

Nutrition. *T. saccharophilum* was an obligate sugar fermenter inasmuch as it utilized various monosaccharides, disaccharides, and polysaccharides, as well as uronic acids and uronic acid polymers, but did not use amino acids or other compounds as fermentable substrates for growth (Table 1). Media containing pectin as fermentable substrate supported abundant growth of *T. saccharophilum* (Table 1). Growing cells liquefied RFG or MVTY medium that did not contain agar but to which pectin was added as fermentable substrate. Furthermore, the addition of 5 ml of culture supernatant fluid to 10 ml of a 3% pectin solution (in 0.1 M phosphate buffer, pH 7.1) caused a 30% reduction in viscosity in 2 h of incubation at 30°C (Weber and Canale-Parola, unpublished data).

$NaHCO_3$ in medium and a CO_2 atmosphere were not required for growth, but could be replaced by phosphate buffer (pH 7.0; final concentration, 0.1 M) and an N_2 atmosphere. However, under the latter growth conditions, cell yields were lower. When cells were grown in the presence of $[^{14}C]NaHCO_3$, radioactive label from CO_2 was not incorporated into cellular material or into fermentation end products. These observations indicated that growing cells of *T. saccharophilum* do not require substrate amounts of CO_2.

Isobutyric acid was essential for growth of *T. saccharophilum*, whereas valeric acid was stimulatory (i.e., growth yields were higher when it was included in the medium). Butyric, D,L-2-methylbutyric, and isovaleric acids were not required for growth of *T. saccharophilum*.

Fermentation products. Cells of *T. saccharophilum* growing in MVTY broth fermented glucose primarily to formate, acetate, and ethanol (Table 2). Acetate and formate were major end products of pectin and glucuronic acid fermenta-

TABLE 2. Products of glucose fermentation by growing cells of *T. saccharophilum*

Product[a]	Amt (μmol/100 μmol of glucose)
Formate	150.0
Acetate	91.2
Ethyl alcohol	79.4
Carbon recovery in products	81.9%
[U-^{14}C]glucose carbon incorporated into cells[b]	14.5%
Total carbon recovery	96.4%
Oxidation-reduction balance	0.94

[a] Products not detected: H_2, CO_2, pyruvate, lactate, succinate, propionate, butyrate, acetoin, diacetyl, 2,3-butanediol, and glycerol. An unidentified end product of glucose fermentation was detected in minor amounts by gas-liquid chromatography. The compound was extracted in chloroform with nonvolatile acids.

[b] Separate experiments in which [U-^{14}C]glucose was used as substrate. Percentage given is the average of two experiments. Specific activity of [U-^{14}C]glucose, 13,800 cpm/μmol.

tion. Similar end products of pectin degradation have been reported for other pectinolytic spirochetes (24, 26, 27). When cells were grown with [1-^{14}C]glucose as fermentable substrate, radioactive label was recovered in acetate and ethanol but not in formate. This pattern of radioactive labeling is characteristic of bacteria that utilize enzymes of the Embden-Meyerhof pathway in the dissimilation of glucose. Furthermore, key enzymatic activities of the Embden-Meyerhof pathway were present in cell-free extracts of *T. saccharophilum* (Table 3). Cell suspensions of *T. saccharophilum* produced 69 μmol of formate per 100 μmol of pyruvate utilized. This finding suggests that pyruvate is metabolized via a coliform-type clastic reaction.

Enzymes of the Entner-Doudoroff pathway. Cell-free extracts of *T. saccharophilum* possessed relatively high levels of KdPG aldolase activity when the cells were grown with pectin as the fermentable substrate and considerably lower levels when the substrate was glucose or glucuronic acid (Table 3). Glucose-6-phosphate dehydrogenase and phosphogluconate dehydratase activities were not detected in any of the extracts tested (Table 3).

G+C content of the DNA. The G+C content of the DNA isolated from *T. saccharophilum* was 54 ± 1 mol% (T_m), as reported earlier (19). This value represents an average of five determinations.

DISCUSSION

Throughout this article the name *Treponema saccharophilum* was used in referring to strain PB, the large spirochete we isolated from the bovine rumen. Strain PB is appropriately classified in the genus *Treponema* because it is an obligately anaerobic spirochete indigenous to a mammalian host (5). However, strain PB differs from other species of *Treponema* in several important respects. For example, cells of recognized *Treponema* species measure 0.1 to 0.4 μm by 5 to 20 μm and have a maximum of 9 periplasmic flagella inserted near each end (21), whereas strain PB cells are 0.6 to 0.7 μm by 12 to 20 μm in size and have approximately 16 periplasmic flagella inserted near each end. In addition, all known species of *Treponema* have DNA with a G+C content (25 to 43 mol%) considerably lower than that of strain PB DNA (54 mol%), except for *T. pallidum*, whose DNA has a G+C content (53 mol%) similar to that of strain PB. However, strain PB and *T. pallidum* differ drastically from each other in physiology and morphology and, therefore, the similarity in the G+C contents of their DNAs cannot be taken as an indication of genetic relatedness.

In view of the differences summarized above, we concluded that strain PB represents a new species of *Treponema* for which we propose the specific epithet "*saccharophilum*" (see below) to indicate its ability to use a broad spectrum of carbohydrates as fermentable substrates.

A recent study (20) in which various spirochetes were characterized by rRNA oligonucleotide cataloging indicated that *T. saccharophilum* PB is part of a phylogenetic subcluster that includes *T. succinifaciens* (G+C content, 36 mol%), *T. bryantii* (G+C content, 36 mol%), a pectinolytic oral spirochete (strain P5, G+C content, 30 mol%), and rumen spirochetes CA (G+C content, 42 mol%) and 6A (G+C content, 43 mol%). The spirochetes in this subcluster can be readily differentiated from *T. saccharophilum* on the basis of the G+C content of their DNAs and of various phenotypic characteristics (7, 19, 23, 24).

The pathways for aerobic degradation of pectin, galacturonic acid, or glucuronic acid by bacteria lead to the common intermediate 2-keto-3-deoxygluconic acid (9). This compound is then phosphorylated to KdPG, which is split into pyruvate and 3-phosphoglyceraldehyde through the mediation of KdPG aldolase, a key enzyme of the Entner-Doudoroff pathway. Only limited information is available on the pathways of anaerobic degradation of pectin or uronic acids. Our data suggest that the anaerobic degradation of pectin by *T. saccharophilum* proceeds, at least in part, via the Entner-Doudoroff pathway, since a relatively high level of KdPG aldolase activity was present in cell-free extracts when the spirochete was grown with pectin as the fermentable substrate (Table 3). Apparently, KdPG aldolase was induced when the spirochetes were grown in media containing pectin as an energy source.

Many of the spirochetes present in bovine rumen grow at the expense of plant polymers such as pectin, polygalacturonate, xylan, arabinogalactan, and starch (19).

TABLE 3. Enzyme activities in cell extracts of *T. saccharophilum*

Enzymatic activity	Sp act (nmol per min/mg of protein) with following fermentable substrate in growth medium:		
	Glucose	Glucuronic acid	Pectin
Glucosephosphate isomerase[a] (EC 5.3.1.9)	4,154	NA[b]	2,068
Fructose-bisphosphate aldolase[c] (EC 4.1.2.13)	3.1	NA	1.7
Glyceraldehyde-phosphate dehydrogenase[c] (EC 1.2.1.12)	34.6	NA	12.9
Glucose 6-phosphate dehydrogenase (EC 1.1.1.49)	ND[d]	ND	ND
KdPG aldolase[e] (EC 4.1.2.14)	55.2	27.2	405.0
Phosphogluconate dehydratase (EC 4.2.1.12)	ND	ND	ND

[a] Expressed as nanomoles of NADP reduced per minute per milligram of protein.

[b] NA, Not assayed.

[c] Expressed as nanomoles of NAD reduced per minute per milligram of protein.

[d] ND, Not detected.

[e] Expressed as nanomoles of pyruvate formed per minute per milligram of protein.

These plant polymers abound in the rumen ecosystem. Some rumen spirochetes appear to be quite limited nutritionally inasmuch as they utilize pectin as fermentable substrate for growth but do not ferment any other substrate tested (27). In comparison, *T. saccharophilum* is very versatile in its ability to utilize not only various plant polymers but also pentoses, hexoses, disaccharides, and uronic acids that are products of the hydrolysis of such polymers. Thus, *T. saccharophilum* seems to be well suited for survival in the rumen.

Description of *T. saccharophilum*. *Treponema saccharophilum* sp. nov. (sac.cha.ro.phi'lum. Gr.n. *sacchar* sugar; Gr.adj. *philus* loving; M.L. adj. *saccharophilum* sugar-loving).

Helical cells, 0.6 to 0.7 μm to 12 to 20 μm. Cell coiling is regular except when cells are in contact with solid surfaces. A bundle of periplasmic flagella is wrapped around the cell body. Approximately 16 periplasmic flagella are inserted near each end of the protoplasmic cylinder and overlap in the central region of the cell. An outer sheath surrounds both the flagellar bundle and the protoplasmic cylinder.

In liquids, at 37°C, cells swim at velocities in excess of 60 μm/s, but no translational motility is observed at 23°C. Translational motility ceases 1 to 2 min after the cells are exposed to air. Cells in contact with solid surfaces exhibit creeping motility.

Obligate anaerobe. Optimum growth is at 37 to 39°C. At these temperatures, the final growth yield in rumen fluid-glucose-sodium bicarbonate-salts broth is 7×10^8 cells per ml and the population doubling time is 90 min. No growth at 23°C or 45°C. Subsurface colonies in agar media are spherical and opaque with diffuse edges.

Utilizes as fermentable substrates for growth: L-arabinose, D-galactose, D-glucose, D-mannose, D-fructose, D-galacturonic acid, D-glucuronic acid, cellobiose, lactose, maltose, sucrose, D-raffinose, dextrin, inulin, starch, pectin, polygalacturonic acid, and arabinogalactan. Do not serve as fermentable growth substrates: L-rhamnose, D-xylose, L-sorbose, D-ribose, cellulose, dextran, amino acids, D-arabitol, dulcitol, mannitol, ribitol, sorbitol, xylitol, glycerol, potassium galactonate, potassium gluconate, sodium acetate, sodium formate, sodium lactate, sodium succinate, potassium fumarate, Tween 80, glucosamine, and xylan. Exogenous isobutyric acid is required for growth, valeric acid is stimulatory. Neither $NaHCO_3$ in media nor a CO_2 atmosphere is required for growth.

Fermentation end products of growing cells (micromoles per 100 μmol of glucose utilized): formate, 150; acetate, 91.2; ethanol, 79.4. Approximately 15% of the glucose carbon consumed by growing cells is assimilated in cell material. Acetate and formate are major end products of pectin or glucuronic acid fermentation. Pyruvate is metabolized via a coliform-type clastic reaction.

Isolated from bovine rumen fluid by using an agar medium that contained rifampin as a selective agent and pectin as a fermentable substrate.

The G+C content of the DNA is 54 mol% (T_m).

Type strain: PB; deposited with the Deutsche Sammlung von Mikroorganismen, DSM collection no. 2985.

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