Syntrophomonas wolfei subsp. *saponavida* subsp. nov., a Long-Chain Fatty-Acid-Degrading, Anaerobic, Syntrophic Bacterium; *Syntrophomonas wolfei* subsp. *wolfei* subsp. nov.; and Emended Descriptions of the Genus and Species

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Most probable numbers for *Syntrophomonas*-like bacteria degrading stearate were 1.5×10^6 to 4.6×10^6 , 2.4×10^5 , and 4.6×10^2 cells per ml of municipal anaerobic digester sludge, swine waste lagoon sediment, and cow ruminal fluid, respectively. Strain SD2, which was isolated from digester sludge in coculture with the H_2 -utilizing bacterium *Desulfovibrio* sp. strain G-11, plus sulfate, was a long-chain saturated fatty-acid-using bacterium identified as a strain of the genus *Syntrophomonas*. Strain SD2 differed from *Syntrophomonas wolfei* subsp. *wolfei* subsp. nov. in its usually smaller size and in its ability to catabolize C_9 to C_{18} saturated fatty acids. It differed from *Syntrophomonas sapovorans* in not needing relatively high concentrations of Ca^{2+} and in its inability to catabolize unsaturated fatty acids, such as oleic or linoleic acid. A strain SD2 coculture was further adapted to grow on crotonate and was subsequently purified without sulfate, with cells of strain G-11 only rarely seen. A 16S ribosomal ribonucleic acid sequence analysis (H. Zhao, D. Yang, C. Woese, and M. P. Bryant, manuscript in preparation) indicated that strain SD2 is phylogenetically very closely related to *S. wolfei*. Thus, we propose the name *Syntrophomonas wolfei* subsp. *saponavida* for this organism (strain DSM 4212^T) (T = type strain) because of its ecologically important ability to use stearate and other long-chain saturated fatty acids. Emended descriptions of the genus and species are given.

Municipal anaerobic, raw sewage sludge comprises nearly equal proportions of proteins, carbohydrates, and lipids. During anaerobic digestion, the lipid fraction is degraded at a higher rate and to a greater extent than either the protein or carbohydrate fraction (6). Triglycerides, the main component of the lipid fraction (10), are rapidly hydrolyzed to free fatty acids (12). Unsaturated fatty acids are hydrogenated, and the saturated fatty acids (SFAs) are β -oxidized (12, 23). In the absence of exogenous electron acceptors other than CO_2 , SFA degradation occurs through syntrophic interactions between proton-reducing, acetogenic bacteria and hydrogen-utilizing, methanogenic bacteria (16). The thermodynamic constraints of the reactions result in growth rates of the fatty-acid-oxidizing bacteria that require solids retention times of at least 5 days at 35°C (13, 14).

Several mesophilic anaerobic bacteria with the ability to β -oxidize SFAs have been isolated in cocultures with hydrogen-utilizing bacteria from methanogenic ecosystems (16, 18, 21, 22). All of these organisms are proton-reducing acetogenic bacteria that require syntrophic interaction with H_2 -utilizing bacteria for cultivation. With one exception, *Syntrophomonas sapovorans*, a calcium-requiring, long-chain fatty-acid (LCFA)-degrading bacterium (18), only short- to medium-length (C_4 to C_{11}) SFAs serve as energy sources.

In this report we describe the isolation and characterization of a LCFA-oxidizing *Syntrophomonas* sp. strain, strain SD2, which was isolated from anaerobic sewage digester sludge in coculture with the H_2 utilizer *Desulfovibrio* sp. strain G-11. Further adaptation of the strain SD2 coculture to grow on crotonate and subsequent purification of strain SD2 are also described.

MATERIALS AND METHODS

Sources of organisms. Inocula for enrichment cultures were obtained from anaerobic sewage digester sludge (Champaign-Urbana Sanitary District), bovine ruminal fluid, and swine waste lagoon sediment (University of Illinois Agricultural Experiment Station). *Desulfovibrio* sp. strain G-11 and *Methanospirillum hungatei* JF-1 were obtained from the culture collection of M. P. Bryant. *Methanospirillum hungatei* SK (24) was supplied by F. Widdel, Philipps Universität, Marburg, Federal Republic of Germany.

Media and conditions of cultivation. Media were prepared and used anaerobically as described by Hungate (11) and as modified by Balch and Wolfe (1) and Bryant (5). The basal medium contained minerals, B vitamins, clarified rumen fluid (5%), sulfide or cysteine-sulfide reducing agents, and $NaHCO_3$; and the gas phase was 80% N_2 -20% CO_2 , and the pH was 7.2 (16). Liquid media were dispensed into culture tubes (18 by 150 mm) or serum tubes which were sealed with either no. 1 black rubber stoppers or black rubber serum stoppers. Solid media for roll tubes (2% agar) and shake tubes (1% agar) were dispensed into tubes (18 by 150 mm) in 7- and 9-ml portions, respectively. Slant media (1% agar) were dispensed in 3-ml portions into tubes (13 by 100 mm) which were sealed with no. 00 black rubber stoppers.

The following substrate concentrations were used in the various media (exceptions are indicated below): 2 mM stearate in all stearate liquid media, 2.5 mM tridecanoate in shake tubes, and 1.3 mM tridecanoate plus 9 mM butyrate in roll tubes. All media used with strain G-11 contained 21 mM Na_2SO_4 .

Media supplemented with insoluble fatty acids were prepared by dispensing appropriate volumes of melted suspensions from 100 mM stock solutions (pH neutral) into culture tubes prior to dispensing of prepared, nonsterile, nonreduced media. Soluble fatty acids were included in the media before dispensing or were added to individual tubes from

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filter-sterilized stock solutions. Medium supplemented with 2-bromoethanesulfonic acid was prepared by injecting portions of a filter-sterilized stock solution into individual tubes prior to inoculation (2). *Desulfovibrio* sp. strain G-11 and *Methanospirillum hungatei* were maintained in slant cultures and were transferred to liquid medium for use in cocultures (16). The liquid medium used for *Desulfovibrio* sp. strain G-11 was composed of the basal medium with double the concentration of NaHCO_3 and 21 mM Na_2SO_4 ; the gas phase was 80% H_2 -20% CO_2 (202 kPa). In the liquid medium for *Methanospirillum hungatei* Na_2SO_4 was omitted. Preparations were incubated at 37°C in the dark. Liquid cultures of *Desulfovibrio* sp. strain G-11 and *Methanospirillum hungatei* were incubated on a reciprocal shaker.

Substrate utilization. The range of utilizable fatty acids was examined by inoculating duplicate tubes of media with and without added substrate. The inocula were 0.5-ml portions of stearate-grown cocultures. Substrate concentrations are given below, but, in general, were equivalent in gram atoms of carbon to 2 mM stearate. Fatty acid esters were tested at concentrations of 0.1, 0.5, and 1% (wt/vol). Utilization was determined by measuring growth measured as absorbance at 600 nm (for soluble fatty acids only) and by measuring substrate depletion or product generation or both, as described below.

Inoculation of 0.2-ml portions of a coculture into duplicate tubes of media containing a single added energy source was used to determine the range of non-fatty acid substrates. Media were prepared by adding 0.1-ml portions from 5% sterile stock solutions to 3-ml portions of basal medium containing sulfate in tubes (13 by 100 mm). Utilization was determined by growth and, in some cases, microscopic examination.

Culture purity. Cocultures were periodically examined for purity by phase-contrast microscopy and by inoculation of aerobic and anaerobic media containing 1% Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.), 0.1% glucose, 0.1% maltose, and 0.1% cellobiose. These media did not support growth of the cocultures except small numbers of *Desulfovibrio* sp. under anaerobiosis.

Analytical methods. Absorbance at 600 nm was determined by using a Spectronic 70 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.). Short-chain fatty acids were analyzed as their butyl esters (19) to allow identification of fermentation products. After it was determined that nonvolatile organic acids were not produced, short-chain fatty acids were quantitated as free acids (16) by using a Superox-FA capillary column (0.53 mm by 10 m; Alltech Associates, Inc., Deerfield, Ill.). Saturated LCFA were analyzed as free acids by using a 10% SP-215-PS-packed column (392 mm by 183 μm ; Supelco, Bellefonte, Pa.). Underivatized hexadecanol or hexadecene and unsaturated fatty acids silylated with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (Pierce Chemical Co., Rockford, Ill.) were analyzed by using a type DB-5 capillary column (0.25 mm by 30 m; J&W Scientific, Folsom, Calif.). All gas chromatographic detection was performed with flame ionization. Sulfide was quantitated colorimetrically by using the method of Fogo and Popowsky (9), as modified by M. Rasmussen (personal communication).

Enumeration. Three-tube most-probable-number estimates (16) for stearate-degrading bacteria were determined by using 1-ml inocula from samples that were serially diluted into basal medium with and without stearate. *Methanospirillum hungatei* (0.25 ml) was added to each tube. Positive results were defined as visual depletion of stearate coupled

with methane production in excess of production by controls without stearate.

RESULTS

Enumeration. We measured 1.5×10^6 to 4.6×10^6 cells per ml of anaerobic sewage digester sludge, 4.6×10^2 cells per ml of ruminal fluid, and 2.4×10^5 cells per ml of swine waste lagoon sediment. All of our samples contained a morphotype similar to strain SD2, which is described below.

Isolation. Strain SD2, an LCFA-degrading bacterium, was isolated from a stearate enrichment culture from the 10^{-6} dilution of digester sludge. Weekly 50% transfers of this enrichment culture were performed for more than 1 year. Triplicate tridecanoate shake tubes inoculated with 0.3 ml of the stearate enrichment culture were serially diluted by 1-ml transfers through seven tubes to which 0.5 ml of *Desulfovibrio* sp. strain G-11 had been added. After 6 weeks of incubation, small, white, diffuse colonies began to exhibit clear zones depleted of tridecanoate. An additional 2 weeks of incubation allowed increased growth before colonies were picked to tridecanoate slants. Cultures were transferred from the slants to stearate liquid media, where growth was followed by a second purification cycle. Colonies picked from the second series of shake tubes contained strain G-11, a *Syntrophomonas*-like rod-shaped organism, and a large sugar-fermenting rod-shaped organism. Quadruplicate tridecanoate-butyrate roll tubes were inoculated with 0.25-ml portions of serially diluted tricultures and 0.25-ml portions of a *Desulfovibrio* sp. strain G-11 culture. After 8 weeks of incubation, colonies surrounded by clear zones were picked from 10^{-5} and 10^{-6} dilution cultures to butyrate-tridecanoate slants. Growth, which was demonstrated by visual depletion of the tridecanoate, was extremely slow; the preparations required 15 weeks of incubation before transfer to stearate liquid medium. The cultures contained only *Desulfovibrio* sp. strain G-11 and the *Syntrophomonas*-like rod-shaped bacterium. On the five pure cocultures obtained, one, containing strains SD2 and G-11, was maintained for additional investigation.

Morphology. Strain SD2 was a gram-negative, slightly curved, rod-shaped bacterium with tapering ends and was morphologically similar to *Syntrophomonas wolfei*, although it was smaller than most strains of *S. wolfei* (15). The cell size was 2.0 to 4.0 by 0.4 to 0.6 μm . Slight, twitching motility was observed microscopically during growth with stearate. Subsequent staining in which either the Gray method or the Leifson method (7) was used revealed two to four flagella inserted in a linear fashion on the concave side of the organism. Cellular inclusions of strain SD2 grown with butyrate were stained by using Sudan Black, suggesting that the inclusions were composed of poly- β -hydroxybutyric acid. The ratio of cells of strain SD2 to cells of strain G-11 was approximately 1:7 when the cultures were grown with stearate.

Substrate utilization. Strain SD2 in coculture with strain G-11 catabolized straight-chain SFA containing 4 to 18 carbon atoms (Table 1). Fatty acids which were tested but were not utilized were 2-methyl-butyric, isobutyric, isovaleric, propionic, nonadecanoic, arachidic, oleic, elaidic, and linoleic acids. The addition of equinormal concentrations of calcium to cultures with unsaturated fatty acids did not result in oxidation of the substrates. Fatty acid esters which were not utilized for growth were tripalmitin, Tween 40, and Tween 60. Hexadecanol was not catabolized.

The fermentation products suggested that degradation of butyrate, valerate, and stearate occurred through β -oxida-

TABLE 1. Utilization of saturated fatty acids by *Syntrophomonas* sp. strain SD2 and *Desulfovibrio* sp. strain G-11^a

Substrate	Concn of substrate added (mM)	Absorbance at 600 nm	Concn of sulfide produced (mM)
Butyric acid	13.7	0.19	6.5
Valeric acid	13.4	0.10	6.5
Hexanoic acid	(10) ^b	0.15	NA ^c
Heptanoic acid	(10)	0.13	NA
Octanoic acid	(7.5)	0.17	NA
Nonanoic acid	(7.5)	0.10	NA
Decanoic acid	3.3	NA	6.4
Undecanoic acid	1.1	NA	2.2
Lauric acid	1.1	NA	2.7
Tridecanoic acid	2.2	NA	5.6
Myristic acid	2.6	NA	7.8
Pentadecanoic acid	1.4	NA	4.2
Palmitic acid	2.7	NA	9.5
Heptadecanoic acid	1.3	NA	4.6
Stearic acid	2.5	NA	10.3

^a Catabolism of soluble fatty acids was determined by growth of the coculture (measured as absorbance at 600 nm). Sulfide production was quantitated to indicate utilization of insoluble fatty acids. All values are the means of triplicate cultures that were corrected for values for control cultures grown with the basal medium. Incubation was for 4.5 weeks.

^b Values in parentheses are approximations of the concentrations of substrates added.

^c NA, Not applicable.

tion (Table 2). The apparent stoichiometries for oxidation of butyrate, valerate, and stearate, respectively, were as follows:

The generation time with butyrate, calculated from change in absorbance at 600 nm, was 55 to 58 h.

Growth of the coculture containing strains SD2 and G-11 did not occur aerobically or in the absence of sulfate. The cell ratios of strains SD2 and G-11 indicated that strain SD2 did not use nitrate or fumarate as an electron acceptor during growth with stearate. Growth was not observed with non-fatty acid substrates, including adonitol, cellobiose, dextran, esculin, fructose, galactose, glucose, glycerol, glycogen, hexadecene, inositol, lactose, maltose, mannitol, melezi-

TABLE 2. Fermentation balances of *Syntrophomonas* sp. strain SD2 and *Desulfovibrio* sp. strain G-11 grown with butyrate, valerate, or stearate^a

Substrate	Concn of substrate added (mM)	Concn of products (mM)			% Recovery ^b	
		Acetate	Propionate	Sulfide	C	H
Butyrate	13.7	27.4	ND ^c	6.5	100	99
Valerate	13.4	13.4	11.3	6.5	92	99
Stearate	15.0	129.0	ND	11.3	97	98

^a The values are the means of triplicate cultures that were corrected for values for controls grown with basal medium. Incubation was for 4.5 weeks.

^b See reference 3.

^c ND, Not detected.

tose, melibiose, methanol, rhamnose, ribitol, starch, sucrose, and Trypticase soy broth.

Adaptation of strain SD2 coculture to grow on crotonate and purification of strain SD2. The stearate-grown coculture containing strains SD2 and G-11 was first directly transferred into liquid medium in which the stearate was replaced by 20 mM crotonate. No growth was observed after 3 months of incubation. The same experiment was repeated twice, and the same negative result was obtained. Then, a fresh coculture was transferred into liquid medium in which the stearate was replaced by 20 mM butyrate. After growth occurred, the culture was subjected to three successive 10% transfers in the butyrate medium to lower the stearate carry-over. Then, 2 ml of the coculture grown on butyrate (with Na₂SO₄) and 2 ml of *Methanospirillum hungatei* grown on H₂-CO₂ were inoculated into 7 ml of liquid medium supplemented with 20 mM butyrate but lacking Na₂SO₄. Growth occurred within 1 week of inoculation. A 1-ml portion of a fresh *Methanospirillum hungatei* culture was inoculated into each of the successive transfers in butyrate medium without Na₂SO₄. After three transfers, *Methanospirillum hungatei* became the predominant H₂ utilizer in the coculture, but strain G-11 cells were still easily observed. The same treatment was repeated seven times until strain G-11 cells were rarely observed in the culture. The final culture was transferred into medium supplemented with 10 mM butyrate plus 10 mM crotonate. After growth occurred, the culture was transferred into medium supplemented with 5 mM butyrate plus 20 mM crotonate. Growth still occurred. Finally, the culture was transferred into medium supplemented with 20 mM crotonate alone. Growth occurred within 5 weeks. This was followed by five successive 5% transfers in the crotonate medium; growth occurred within 2 weeks, and the cells of strain SD2 looked healthy. However, even in the last crotonate-grown culture, active cells of *Methanospirillum hungatei* were still present. This culture was transferred into the crotonate medium supplemented with 300 µM 2-bromoethanesulfonic acid to inhibit the methanogen (2). Growth occurred within 2 weeks, and most of the cells of *Methanospirillum hungatei* which we observed were ghostlike. In the subsequent six transfers, 2-bromoethanesulfonic acid treatment was used every other time. In the final culture *Methanospirillum hungatei* cells were no longer observed. This culture of strain SD2, in which cells of strain G-11 were only rarely seen, was used for further work on 16S ribosomal ribonucleic acid sequencing. The very small bulk of strain G-11 had no effect on sequencing.

DISCUSSION

Obligate proton-reducing, mesophilic acetogenic bacteria with the ability to β-oxidize fatty acids occur within two distinct morphotypes, which are described by the genera *Syntrophomonas* and *Clostridium* (15, 18, 21, 22). The former taxon is characterized by gram-negative, slightly curved, rod-shaped cells with tapered ends and two to eight flagella located linearly on the concave side. Strain SD2, as characterized here, was a strain of *Syntrophomonas* sp.

Strain SD2 was morphologically similar to the two previously described *Syntrophomonas* species; strain SD2 also demonstrated metabolic capabilities similar to those expressed by *S. wolfei* and *S. sapovorans*. In addition to β-oxidation of SFA as its sole catabolic activity, strain SD2 also stored a poly-β-hydroxybutyric acid-like lipid. Strain SD2 catabolized a broader range of fatty acids than *S. wolfei*; SFAs containing up to 18 rather than 8 carbon atoms per

molecule were degraded. Unlike *S. sapovorans*, strain SD2 was not inhibited by LCFA and subsequently did not require calcium supplementation. This may reflect a higher rate of β -oxidation activity in strain SD2 (8). In addition, strain SD2 lacked the ability exhibited by *S. sapovorans* to degrade oleic, elaidic, or linoleic acid. Whether *S. sapovorans* uses these unsaturated fatty acids is open to question as it contains a contaminant in addition to the H_2 user (18).

It has been suggested (20) that the hydrocarbon-degrading bacterium in a methanogenic hexadecene enrichment culture also utilizes palmitate. Because of this hypothesis, the ability of strain SD2 to utilize hexadecene was tested at several concentrations (9 to 45 mM). Growth was not observed, nor was oxidation of the hydrocarbon demonstrated. Hexadecanol, a possible intermediate of hexadecene oxidation, was not catabolized.

The inability of strain SD2 to hydrolyze lipids was consistent with results obtained with previously described SFA-oxidizing syntrophs. In natural ecosystems, lipid hydrolysis is dependent on the activity of other bacteria.

Enumeration of LCFA-oxidizing bacteria revealed significantly higher numbers in an anaerobic sewage digester and in a swine waste lagoon than in a bovine rumen. This is likely due to the growth rates of these organisms and the retention times of each system. The much longer retention times of the digester or lagoon probably allowed greater proliferation of the LCFA oxidizers than the proliferation in the rumen. *Syntrophomonas* sp. has previously been isolated from a ruminal butyrate enrichment initiated with a large inoculum (17).

In the past, efforts to isolate the "obligate" syntrophic fatty acid oxidizers in pure cultures have failed (4). Lack of pure cultures limited more detailed characterization of these organisms. Recently, *S. wolfei* was adapted to grow on crotonate (4), which is not a natural extracellular intermediate in anaerobic degradation of organic matter in methanogenic ecosystems (4, 16). Adaptation of the strain SD2 coculture to grow on crotonate and the subsequent highly purified culture made it possible to characterize strain SD2 in more aspects.

The results of a sequence analysis (H. Zhao, D. Yang, C. Woese, and M. P. Bryant, manuscript in preparation) indicated that phylogenetically strain SD2 exhibited at least 95% similarity with *S. wolfei*. Some other fatty acid-degrading syntrophs, which differed from *S. wolfei* significantly in spore formation and cell wall ultrastructure (no outer membranes), were also closely related to *S. wolfei*.

As the original description of *S. wolfei* (15) gave the same description for the genus and species, it is necessary to propose here an emended description of the genus.

Description of *Syntrophomonas* McInerney et al. 1981, 1037, emend. Lorowitz, Zhao and Bryant 1989. *Syntrophomonas* (Syn. tro. pho. mon' as. Gr. adj. *syn*, together with; Gr. n. *trophos*, one who feeds; Gr. n. *monas*, a unit, monad; M. L. fem. n. *Syntrophomonas*, monad which feeds together with [another species]). Nonsporing, gram-negative, slightly helical rods, 0.4 to 1.0 by 2.0 to 7.0 μ m, with slightly tapered rounded ends. Most cells occur singly or in pairs, with helical chains of three or more cells often observed. Multiplication by binary fission. Cells possess two to eight flagella (diameter, about 20 nm) that are laterally inserted in a linear fashion on the concave side of the cell about 130 nm or more apart. Under most conditions, cells usually exhibit only sluggish twitching motility. Cells have an unusual multilayered gram-negative wall. Muramic and *meso*-diaminopimelic

acids are present, and the organism is susceptible to penicillin. Poly- β -hydroxybutyrate is present.

Chemoorganotrophic. Cells anaerobically β -oxidize saturated fatty acids, with protons serving as the electron acceptors in cocultures with an H_2 scavenger. Growth is much faster with *Methanospirillum hungatei*, which utilizes both H_2 and formate, than with methanogens such as *Methanobacterium bryantii*, which utilizes only H_2 . (It is possible that strains contain a formate-forming CO_2 reductase.) In coculture cells produce acetate and H_2 from butyrate and longer straight-chain, even-numbered-carbon fatty acids and acetate, propionate, and H_2 from *n*-valerate and longer straight-chain, odd-numbered-carbon fatty acids. At least some strains produce acetate, isovalerate, and H_2 from iso acids, such as isoheptanoate, and some strains produce acetate, propionate, and H_2 from 2-methylbutyrate. (It is possible but has not yet been determined that anteiso acids, such as anteisooctanoate, are degraded to acetate, anteisohexanoate, and H_2 .) The strains studied so far can, with considerable difficulty, be adapted to grow slowly without coculture on crotonate, with acetate and butyrate as the products. Carbohydrates, proteinaceous materials, alcohols, or other organic compounds do not support growth. Common electron acceptors, such as fumarate, malate, nitrate, oxygen, sulfate, sulfite, sulfur, and thiosulfate, are not utilized with butyrate as the electron donor. Growth may be stimulated in cocultures by factors in rumen fluid or mixtures of vitamins or both.

Isolated as cocultures from anaerobic ecosystems, such as aquatic sediments, sewage digester sludge, and rumen digesta where organic matter is degraded with CO_2 and CH_4 as major products.

The temperature range for cocultures with *Methanospirillum hungatei* is in the broad mesophilic range, with an optimum of 30 to 37°C. The pH range has not been determined.

Phylogenetically (as determined by 16S ribosomal ribonucleic acid sequencing), *Syntrophomonas* is in a so-far-unique group of gram-positive eubacteria that β -oxidize SFAs and include sporeformers without cell outer membranes and nonsporing bacteria with outer membranes.

Syntrophomonas wolfei is the type species.

Because of the proposed emendation of the genus and description of the new subspecies (see below), new descriptions of *S. wolfei* and *S. wolfei* subsp. *wolfei* (named to honor R. S. Wolfe) are needed.

Description of *Syntrophomonas wolfei* McInerney et al. 1981, 1037, emend. Lorowitz, Zhao and Bryant 1989. *Syntrophomonas wolfei* (wolf'e.i. M.L. gen.n. *wolfei*, of Wolfe). Surface colonies in roll tubes of *S. wolfei* cocultures with methanogens are smooth, convex, and circular with entire edges and may be dark to black when the organisms are cocultured with H_2 -using *Desulfovibrio* species with sulfate added.

Grows in cocultures with H_2 scavengers, using straight-chain fatty acids containing four carbon atoms (butyrate) to eight carbon atoms (octanoate) as energy sources. Strains utilize isoheptanoate, and some strains also use straight-chain fatty acids containing 9 carbon atoms (nonanoate) to 18 carbon atoms (stearate) or 2-methylbutyrate. Whether some strains use anteiso or other iso fatty acids is not known.

The type strain is strain DSM 2245A (the Gottingen strain grown in coculture with *Desulfovibrio* sp. strain G-11 with sulfate in the medium) and DSM 2245B (the same strain

grown in coculture with the type strain of *Methanospirillum hungatei*, strain DSM 864).

Description of *Syntrophomonas wolfei* subsp. *wolfei* subsp. nov. The size of the cells tends to be in the higher range of sizes for the genus. In cocultures, cells utilize as energy sources only four-carbon-atom (butyrate) to eight-carbon-atom (octanoate) straight-chain fatty acids and isoheptanoate; in axenic culture, they also use crotonate.

The type strain is strain DSM 2245A and B.

We propose that strain SD2 be placed in another new subspecies because of its ecologically very important ability to degrade LCFA, such as stearate and palmitate, and because of its large numbers in aquatic sediments and anaerobic digester sludge. McInerney et al. (16) found similar numbers of the butyrate-degrading organism *S. wolfei* (4.5×10^6 cells per g) in the same anaerobic digester but did not isolate strains from the most-probable-number tubes so that LCFA catabolism could be checked. Strain SD2 differed from *S. sapovorans* (18) as indicated above.

Description of *Syntrophomonas wolfei* subsp. *saponavida* subsp. nov. *Syntrophomonas wolfei* subsp. *saponavida* (sa.po.na.vi'da. L.n.sapo, soap; L. adj. *avida*, greedy; L. fem. adj. *saponavida*, greedy for soap). The size of the cells is in the lower range of sizes for the genus. In coculture with H_2 scavengers cells utilize as energy sources 4-carbon-atom (butyrate) to 18-carbon-atom (stearate) straight-chain fatty acids; they probably use acids such as isoheptanoate and longer-chain iso acids. Grows on crotonate without an H_2 -scavenging system.

The type strain is strain DSM 4212 (strain SD2 in coculture with *Desulfovibrio* sp. strain G-11 with sulfate in the medium).

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