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Physiology, Ecology, Phylogeny, and Genomics of Microorganisms Capable of Syntrophic Metabolism

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Syntrophic metabolism is diverse in two respects: phylogenetically with microorganisms capable of syntrophic metabolism found in the Deltaproteobacteria and in the low G+C gram-positive bacteria, and metabolically given the wide variety of compounds that can be syntrophically metabolized. The latter includes saturated fatty acids, unsaturated fatty acids, alcohols, and hydrocarbons. Besides residing in freshwater and marine anoxic sediments and soils, microbes capable of syntrophic metabolism also have been observed in more extreme habitats, including acidic soils, alkaline soils, thermal springs, and permanently cold soils, demonstrating that syntrophy is a widely distributed metabolic process in nature. Recent ecological and physiological studies show that syntrophy plays a far larger role in carbon cycling than was previously thought. The availability of the first complete genome sequences for four model microorganisms capable of syntrophic metabolism provides the genetic framework to begin dissecting the biochemistry of the marginal energy economies and interspecies interactions that are characteristic of the syntrophic lifestyle.

Key words: **syntrophy; methanogenesis; hydrogen; formate; acetogenesis; *Syntrophus*; *Syntrophomonas***

Introduction

The complete mineralization of complex organic matter to CO_2 and CH_4 occurs in anoxic environments where electron acceptors, other than CO_2, are limiting.[1–5] Examples of such environments include freshwater sediments, flooded soils, wet wood of trees, tundra, landfills, and sewage digestors. Syntrophic metabolism plays an essential role in the recycling of organic matter to methane and carbon dioxide in these environments. The degradation of natural polymers, such as polysaccharides, proteins, nucleic acids, and lipids, to CO_2 and CH_4 involves a complex microbial community. Fermentative bacteria hydrolyze the polymeric substrates, such as polysaccharides, proteins, and lipids, and ferment the hydrolysis products to acetate and longer-chain fatty acids, CO_2, formate, H_2. Acetogenic bacteria are likely involved in the fermentation of methanol derived from the demethylation of pectin and in the *O*-demethylation of low molecular-weight ligneous materials, and ferment hydroxylated and methoxylated aromatic compounds with the production of acetate.[6] Propionate and longer-chain fatty acids, alcohols, and some amino acids and aromatic compounds are syntrophically degraded to the methanogenic substrates, H_2, formate, and acetate.[2,4] The syntrophic degradation of fatty

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acids is often the rate-limiting step, so it is essential that waste treatment reactors be operated under conditions that favor the retention of bacteria capable of syntrophic metabolism. Last, two different groups of methanogens, the hydrogenotrophic methanogens and the acetotrophic methanogens, complete the process by converting the acetate, formate, and hydrogen made by other microorganisms to methane and carbon dioxide. The syntrophic metabolism and methanogenesis must be tightly coupled to accomplish anaerobic degradation.

Large amounts of organic matter are microbially degraded, making methanogenesis an integral part of the global carbon cycle. Methanogenesis also occurs in the gastrointestinal tract of animals; however, organic matter is incompletely degraded to acetate and longer-chain fatty acids, which accumulate and are absorbed and used by the host animal as energy sources.[7] Syntrophic bacteria and acetoclastic methanogens grow too slowly to be maintained in the gastrointestinal tract. The amount of energy released and harvested per unit of biomass degraded during methanogenesis is very low. For this reason, methanogenesis is a treatment of choice for complex waste digestion, because sludge yields are low and most of the energy in the original substrates is retained in the energy-rich product, methane.

The methanogenic fermentation of complex polymeric materials involves a number of diverse, interacting microbial species. The mutual dependence between interacting species can be so extreme that neither species can function without the activity of its partner, and together the partners perform functions that neither species can do alone. Syntrophy is a specialized case of tightly coupled mutualistic interactions. The term syntrophy was first used to describe the interaction between phototrophic green sulfur bacteria and chemolithotrophic, sulfur-reducing bacteria[8] and fatty acid–oxidizing microorganisms and hydrogen/formate-using microorganisms.[3] In both cases, the pool size of intermediates that are exchanged between the partners (sulfur during anaerobic photosynthesis or hydrogen/formate during fatty-acid metabolism) must be kept very low for efficient cooperation among the partners to occur. We will focus on syntrophic interactions active in methanogenic environments where hydrogen and formate are exchanged between the two partners. In these syntrophic interactions, the degradation of the parent compound, for example, the fatty acid, is thermodynamically unfavorable unless the hydrogen and formate produced by the fatty-acid degrader is kept at low levels by a second microorganism, in this case, a hydrogen/formate-consuming methanogen.[4] The thermodynamic basis for these interactions is discussed later in the chapter.

Under optimal conditions, the free energy changes involved in syntrophic metabolism are close to equilibrium[9–12] and the little free energy that is released in these reactions must be shared among partners.[4] Growth rates and growth yields are low, 7-month doubling time and 0.6 g (dry weight) per mole of methane for anaerobic methane–oxidizing consortium,[13] making biochemical investigations very difficult. Thus, it is appropriate to describe syntrophy as an extreme existence, a lifestyle that involves a marginal energy economy.

In this review, we discuss syntrophic interactions operative in natural and man-made environments from the perspective of the organisms involved, their phylogenetic relationships, the range of syntrophic substrates metabolized, and the new perspectives offered by the emerging genome sequencing information for several model syntrophic microorganisms.

Historical Origins

The first example of a thermodynamically based syntrophic interaction was ethanol metabolism performed by members of the "*Methanobacillus omelianskii*" culture.[14] Subsequently, Bryant *et al.*[15] showed that the "*Methanobacillus omelianskii*" culture was in fact a coculture of two organisms, the S organism and *Methanobacterium bryantii* strain M.O.H. (FIG. 1). The S organism fermented ethanol to acetate and hydrogen:

$$2CH_3CH_2OH + 2H_2O \rightarrow 2CH_3COOH + 4H_2$$
$$\Delta G^{\circ\prime} = 19 \text{ kJ (per 2 mol ethanol)} \quad (1)$$

The methanogen did not use ethanol, but used the H_2 made by the S organism to reduce CO_2 to CH_4:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$
$$\Delta G^{\circ\prime} = -131 \text{ kJ (per mole of } CH_4) \quad (2)$$

When the two reactions are combined, the degradation of ethanol becomes favorable:

$$2CH_3CH_2OH + CO_2 \rightarrow 2CH_3COOH + CH_4$$
$$\Delta G^{\circ\prime} = -112 \text{ kJ (per mole of } CH_4) \quad (3)$$

The importance of end-product removal on the thermodynamics of syntrophic ethanol, propionate, and butyrate degradation is illustrated in TABLE 1. Under standard conditions, the degradation of the substrates listed in TABLE 1 is endergonic. However, if the hydrogen partial pressure is low, then the degradation

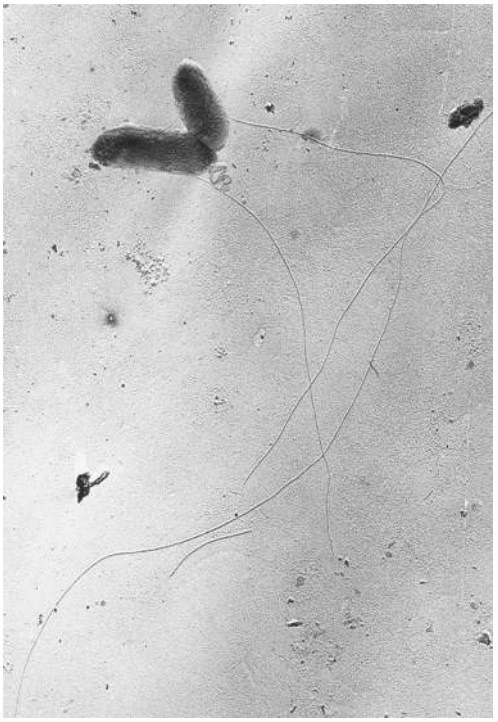

FIGURE 1. Electron micrograph of the ethanol-utilizing, rod-shaped syntrophic "S organism" isolated by Bryant and co-workers.[15] It was the first bacterium capable of syntrophic metabolism to be isolated in pure culture. The S organism possesses a single polar flagellum whose length extends approximately 10-fold the length of the cell body. (Photograph courtesy of Professor R. S. Wolfe.)

TABLE 1. Reactions involved in syntrophic metabolism

Reactions	$\Delta G^{\circ\prime}$ (footnote [a])	pH_2 for $-\Delta G'$ (footnote [b])
$Ethanol + H_2O \rightarrow Acetate^- + H^+ + 2H_2$	+9.6	$<10^{-1}$
$Propionate^- + 3H_2O \rightarrow Acetate^- + HCO_3^- + H^+ + 3H_2$	+76.1	$<10^{-4}$
$Butyrate^- + 2H_2O \rightarrow 2\ Acetate^- + H^+ + 2H_2$	+48.3	$<10^{-4}$

[a] From Reference 104.

[b] The partial pressure of hydrogen needed for the reaction to be thermodynamically favorable ($-\Delta G'$), which was calculated with concentrations of substrate and acetate of 0.1 mM and a bicarbonate concentration of 100 mM.

of these compounds is exergonic. Consistent with the thermodynamic predictions, small increases in H_2 partial pressure inhibit the degradation of butyrate and benzoate by syntrophic cocultures[4,16–20] and propionate degradation in methanogenic mixed cultures.[21]

Since this first description of syntrophic metabolism, numerous studies have led to the isolation of many novel genera and species that are capable of syntrophic metabolism. Syntrophically metabolizing bacteria have been most commonly isolated from freshwater sediments and anaerobic digesters used to treat various types of wastewater.[22–26] Several molecular studies have shown that sequences related to those of bacteria capable of syntrophic metabolism are found in a wide variety of anoxic environments.[22,24,27–30]

The theoretical basis of syntrophic metabolism was based first on the transfer of H_2 between the two partners.[2] However, we now know that interspecies transfer of formate is essential. Zindel *et al.*[31] showed that syntrophic metabolism can occur solely by interspecies formate transfer by growing an amino acid–fermenting bacterium with a sulfate reducer that used formate but not H_2. Syntrophic propionate degradation by *Syntrophobacter fumaroxidans*[32,33] and butyrate degradation by *Syntrophomonas* (*Syntrophospora*) *bryantii*[34] occurred only if the partner used both hydrogen and formate. Additionally, formate dehydrogenase levels were very high in both members of the syntrophic propionate–degrading association consistent with electron flow being coupled to interspecies formate transfer.[35]

Initially, bacteria that syntrophically oxidized fatty and aromatic acids were believed to be obligately dependent on the hydrogen/formate-using partner, since other substrates or electron donor/acceptor combinations could not be found that allowed the growth of the syntrophic metabolizer in pure culture.[3] Microbes capable of the syntrophic metabolism of fatty and aromatic acids were first called obligately proton-reducing acetogenic bacteria.[3] As discussed elsewhere in this volume, the term acetogenic is best reserved for those bacteria that synthesize acetate from CO_2 rather than from compounds with carbon–carbon bonds. Additionally, almost all of the known bacteria capable of syntrophic metabolism can be grown fermentatively in pure culture with a more oxidized derivative of their parent substrate, such as crotonate for fatty-acid degraders or fumarate for propionate degraders.[36,37] Some, like the genus *Syntrophobacter*, have diverse metabolisms and grow fermentatively with several different substrates or by anaerobic respiration using electron acceptors, such as sulfate.[18,23,38] It is noteworthy that there are a few species (*Pelotomaculum schinkii*, *Syntrophomonas zehnderi*, and *Pelotomaculum isophthalicicum*) that appear to be obligately syntrophic microorganisms.[39–41] Verification of this prediction awaits future genome sequencing studies.

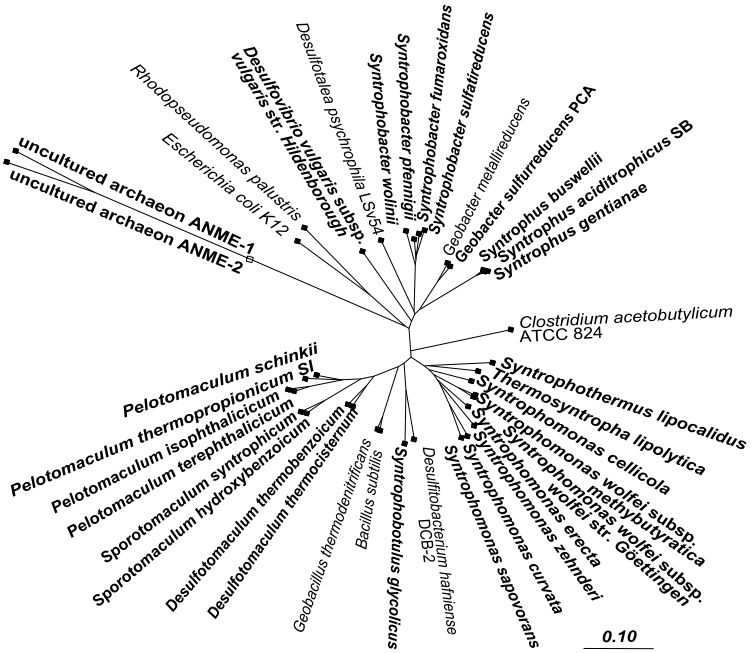

FIGURE 2. A phylogenetic tree containing representative syntrophic bacterial species. The organisms capable of syntrophy are in boldface type. The radial Neighbor-Joining tree was constructed using the ARB software package (http://www.arb-home.de/), utilizing the Greengenes 16s rRNA gene database (greengenes.lbl.gov/cgi-bin/nph-citation.cgi).[111,112] A filter was created and applied to the aligned syntrophic sequences using the maximum frequency method with a 50% minimum cutoff.

Phylogenetic Relationships of Syntrophic Metabolizers

When the 16S rRNA gene sequences from bacteria capable of syntrophic metabolism are compared, it is evident that many of these microorganisms cluster with species in *Deltaproteobacteria* and the low G+C gram-positive bacteria (FIG. 2). Genera that contain syntrophic species within the Deltaproteobacteria include *Syntrophus, Syntrophobacter, Desulfoglaeba, Geobacter, Desulfovibrio,* and *Pelobacter*. Two other groupings of microbes that perform syntrophic metabolism fall into the low G+C gram-positive bacteria (FIG. 2). One group is composed of species within the genera *Desulfotomaculum*, *Pelotomaculum*, *Sporotomaculum,* and *Syntrophobotulus*. *Syntrophomonadaceae* comprises another group of microbes that perform syntrophic metabolism in the low G+C gram-positive bacteria and includes species in the genera *Syntrophomonas*, *Syntrophothermus,* and *Thermosyntropha*. Molecular phylogenetic analyses, ^{13}C lipid isotopic determinations, and microscopic mass spectrometric analysis identified two major groups of methanogen-related archaea (ANME-1 and ANME-2) that anaerobically oxidize methane.[42,43] The close physical association between methane-oxidizing archaea and sulfate-reducing bacteria suggests a syntrophic relationship.[44] Although the ANME microbes have not yet been isolated, it does appear that syntrophic metabolism occurs in both the archaeal and bacterial lines of descent.

Diversity and Ecology of Syntrophic Metabolizers

A wide variety of compounds, including saturated fatty acids, unsaturated fatty acids, alcohols, and hydrocarbons, are syntrophically degraded in methanogenic environments.[2,38,45–47] *Syntrophomonas wolfei* was the first bacterium described that syntrophically oxidizes fatty acids in coculture with a hydrogen/formate-using microorganism.[2,3] *S. wolfei* was isolated from anaerobic digester sludge[2,3] and oxidizes saturated fatty acids, ranging from C4 to C8 in length, and isoheptanoate in coculture with hydrogen-users.[2] *S. wolfei* was shown later to grow in pure culture with crotonate.[36] *Syntrophomonas* spp. are rod-shaped, slightly motile, mesophilic, and capable of utilizing a variety of fatty acids[48] (TABLE 2). These microorganisms group

TABLE 2. Characteristics of syntrophic bacteria specializing in fatty-acid metabolism

Organism	pH range[a]	Temperature range (°C)[a]	Spore formation	Substrates used in:		Reference
				Pure culture	Coculture	
Syntrophomonas bryantii	6.5–7.5	28–34	Yes	C4:1[b]	C4–C11	49, 50, 53
Syntrophomonas wolfei subsp. *wolfei*	ND	(35–37)	No	C4:1–C6:1	C4–C8	2
Syntrophomonas wolfei subsp. *saponavida*	ND	ND	No	C4:1	C4–C18	54
Syntrophomonas sapovorans	6.3–8.1 (7.3)	25–45 (35)	No	None	C4–C18, C16:1, C18:1, C18:2	55
Syntrophomonas curvata	6.3–8.4 (7.5)	20–42 (35–37)	No	C4:1	C4–C18, C18:1	56
Syntrophomonas erecta subsp. *sporosyntropha*	5.5–8.4 (7.0)	20–48 (35–37)	Yes	C4:1	C4–C8	52
Syntrophomonas erecta subsp. *erecta*	(7.8)	*(37–40)	No	C4:1, C4 + C5:1, C4 + DMSO	C4–C8	57
Syntrophomonas zehnderi	ND	25–40 (37)	Yes	None	C4–C18, C16:1, C18:1, C18:2	41
Syntrophomonas cellicola	6.5–8.5 (7.0–7.5)	25–45 (37)	Yes	C4:1	C4–C8, C10	49
Thermosyntropha lipolytica	7.5–9.5 (8.1–8.9)	52–70 (60–66)	No	C4:1, yeast extract, tryptone, casamino acids, betaine, pyruvate, ribose, xylose	C4–C18, C18:1, C18:2; triglycerides	59
Syntrophothermus lipocalidus	6.5–7.0	45–60 (55)	No	C4:1	C4–C10; isobutyrate	58
Algorimarina butyrica	6.2–7.1	10–25 (15)	No	None	C4, isobutyrate	25

[a]Optimal condition is given in parentheses; ND = not determined.

[b]The number of carbons in the fatty acid is indicated; the number following the colon is the number of unsaturated bonds for unsaturated fatty acids. When a range of fatty acids is given, this means that the organism can use fatty acids within the indicated range of carbon numbers, but not all possibilities were tested.

phylogenetically with the low G+C gram-positive bacteria in the family Syntrophomonadaceae.[48] Despite grouping phylogenetically with the gram-positive bacteria, members of the genus *Syntrophomonas* have atypical cell walls ultrastructurally similar to gram-negative cell walls,[2,48] but lacking lipopolysaccharides. *Syntrophomonas* spp. are differentiated from each other based on substrate utilization pattern and spore formation[41,48,49] (TABLE 2). *Syntrophomonas* are capable of forming spores including *S. erecta* subsp. *sporosyntropha*, *S. cellicola*, *S. zehnderi*, and *S. bryantii*, which was recently reclassified and was formerly described as both *Clostridium bryantii* and *S. bryantii*.[41,49–53] All of the described species are able to grow in pure culture with crotonate except *S. zehnderi*.[41] *S. erecta* subsp. *sporosyntropha* has been shown to sporulate only in coculture with methanogens, not in coculture with a sulfate reducer or in pure culture.[52] *S. wolfei* subsp. *saponavida*,[54] *Syntrophomonas sapovorans*,[55] *S. zehnderi*,[41] and *Syntrophomonas curvata*[56] use C4–C18 fatty acids, and *S. bryantii* uses C4–C10 fatty acids in coculture (TABLE 2). *S. wolfei* subsp. *wolfei* has not been shown to form spores and use C4–C8 fatty acids.[2] One strain of *S. erecta* can grow in pure culture on a mixture of butyrate and pentanoate.[57]

Other members of the *Syntrophomonadaceae* include two thermophilic genera, *Syntrophothermus* and *Thermosyntropha* (TABLE 2). *Syntrophothermus lipocalidus* was isolated from granular sludge in a thermophilic upflow anaerobic sludge blanket (UASB) reactor, grows optimally at a temperature of 55°C, and metabolizes saturated fatty acids ranging from C4 to C10 and isobutyrate in coculture with a thermophilic hydrogen-using methanogen.[58] *Thermosyntropha lipolytica* was isolated from alkaline hot springs in Kenya[59] and grows at pH values of 7.15 to 9.5 and temperatures of 52°C to 70°C.[59] This organism, unlike *Syntrophomonas* spp. and *S. lipocalidus*, can use yeast extract, tryptone, casamino acids, and betaine in pure culture.[59] *T. lipolytica* and *S. lipocalidus* both use crotonate.[58,59] *T. lipolytica* uses olive oil, triacylglycerols, and both saturated and unsaturated fatty acids ranging from C4 to C18 in syntrophic association with hydrogen-using microorganisms.[59] Both *S. lipocalidus* and *T.*

TABLE 3. Characteristics of propionate-degrading syntrophic bacteria

Organism	pH range[a]	Temperature range (°C)[a]	Substrates used in: Pure culture[b]	Substrates used in: Coculture[b]	Reference
Syntrophobacter wolinii	ND	ND	C3[b] + sulfate; fumarate	C3	45, 71
Syntrophobacter pfennigii	6.2–8.0 (7.0–7.3)	20–37 (37)	C3 + sulfate, sulfite, thiosulfate; lactate	C3, lactate, propanol	38
Syntrophobacter fumaroxidans	6.0–8.0 (7.0)	20–40 (37)	C3 + sulfate or fumarate; fumarate	C3	18
Syntrophobacter sulfatireducens	6.2–8.8 (7.0–7.6)	20–48 (37)	C3 + sulfate or thiosulfate; pyruvate,	C3	23
Smithella propionica	6.3–7.8 (6.5–7.5)	23–40 (33–35)	C4:1	C3, C4, malate, fumarate	70
Pelotomaculum schinkii	ND	ND	None	C3	39
Pelotomaculum thermopropionicum	6.0–8.2 (7.0)	37–70 (55)	Pyruvate, fumarate	C3, lactate, various alcohols	63
Desulfotomaculum thermobenzoicum subsp. *thermosyntrophicum*	(7.0)	42–62 (55)	Fumarate, pyruvate, C4:1	C3, C4, benzoate	60
Desulfotomaculum thermocisternum	(6.7)	41–75 (62)	Fumarate, pyruvate	C3, C4	62

[a]Optimal condition is given in parentheses. ND = not determined.

[b]The number of carbons in the fatty acid is indicated; the number following the colon is the number of unsaturated bonds for unsaturated fatty acids.

lipolytica stain gram-negative and neither form spores.[58,59]

Certain gram-positive, spore-forming, thermophilic, sulfate-reducing bacteria from the genus *Desulfotomaculum* and other closely related genera have also been shown to degrade a variety of compounds in syntrophic association with hydrogen-using microorganisms.[60] *Desulfotomaculum* spp. are found in a variety of environments, including freshwater sediments, marine sediments, and have also been observed in hydrocarbon-degrading enrichments.[60,61] *Desulfotomaculum thermocisternum* was the first described thermophile that is capable of oxidizing propionate in syntrophic association with a hydrogen-using methanogen (TABLE 3).[62] *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum* is also capable of degrading propionate syntrophically in thermal environments (TABLE 3).[60] This organism was isolated from a thermophilic anaerobic digester treating kraft-pulp wastewater, and can be distinguished from other thermophilic, syntrophic propionate oxidizers due to its ability to oxidize benzoate in the presence of sulfate.[60]

P. schinkii and *Pelotomaculum thermopropionicum* strain SI have recently been shown to degrade propionate in syntrophic association with hydrogen-using methanogens (TABLE 3).[39,63] *P. schinkii* is currently considered to be an obligate syntrophic organism.[39] *P. thermopropionicum* grows at 55°C and metabolizes fumarate and pyruvate in pure culture, and propionate, ethanol, lactate, 1-butanol, 1-pentanol, 1,3-propanediol, 1-propanol, and ethylene glycol in coculture.[63] Other species of in the genus *Pelotomaculum* include *P. terephthalicicum* and *P. isophthalicicum* (TABLE 4). *P. isophthalicicum* has not been grown in pure culture, while *P. terephthalicicum* grows in pure culture with crotonate, 2,5-dihydroxybenzoate, and hydroquinone.[40] *P. terephthalicicum* and *P. isophthalicicum* metabolize a variety of phthalate isomers and other aromatic compounds in syntrophic association with hydrogen-using methanogens.[40] *P. schinkii*, *P. thermopropionicum*, *P. terephthalicicum*, and *P. isophthalicicum* have recently been shown to group with *Desulfotomaculum* subcluster Ih.[64] However, none of these organisms utilize sulfate as an electron acceptor.[39,40,63] Expression of *dsrAB*, which encode for the alpha and beta subunits of the dissimilatory sulfite reductase, by real-time polymerase chain reaction (PCR) was observed in only one of five propionate-degrading enrichments that contained propionate degraders that group with *Desulfotomaculum* subcluster Ih.[64] Pure cultures of *P. schinkii*, *P. thermopropionicum*, *P. terephthalicicum*, and *P. isophthalicicum*

TABLE 4. Characteristics of aromatic-degrading syntrophic bacteria

Organism	pH range[a]	Temperature range (°C)[a]	Substrates used in: Pure culture	Substrates used in: Coculture	Reference
Syntrophus buswellii	6.5–7.5 (7.1–7.4)	ND	C4:1[b]; cinnamate; C4:1 + benzoate or 3-phenyl-propionate	C4:1, benzoate	67
Syntrophus gentianae	6.5–7.5 (7.1–7.4)	10–33 (28)	C4:1, hydroquinone, 2,5-diOH-benzoate	C4:1, benzoate, hydroquinone, 2,5-diOH-benzoate	69
Syntrophus aciditrophicus	6.5–7.5 (7.1–7.4)	25–42 (37)	C4:1, benzoate, cyclohex-1-ene carboxylate	Benzoate, fatty acids, unsaturated fatty acids	68
Sporotomaculum syntrophicum	6.0–7.5 (7.0–7.2)	20–45 (35–40)	C4:1; C4:1 + benzoate	Benzoate	65
Pelotomaculum terephthalicicum	6.5–7.5 (6.8–7.2)	25–45 (37)	C4:1, hydroquinone, 2,5-diOH-benzoate	Benzoate, phthalates, hydroxy-benzoates, 3-phenylpropionate	40
Pelotomaculum isophthalicicum	6.8–7.2 (7.0)	25–45 (37)	None	Benzoate, phthalates, 3-OH-benzoate	40

[a]Optimal condition is given in parentheses. ND = not determined.

[b]The number of carbons in the fatty acid is indicated; the number following the colon is the number of unsaturated bonds for unsaturated fatty acids.

also failed to yield a PCR product.[64] Therefore, the inability of the isolates and enrichments to couple propionate oxidation to sulfate reduction and the lack of *dsrAB* genes in all but one enrichment suggests that the *Desulfotomaculum* subcluster Ih consists of syntrophic metabolizers that may have lost their ability to reduce sulfate.[39,40,63,64]

Sporotomaculum syntrophicum groups with members of the *Desulfotomaculum* and metabolizes benzoate in syntrophic association with hydrogen-using methanogens (TABLE 4).[65] *S. syntrophicum* does not use sulfate as an electron acceptor.[65] *S. syntrophicum* grows in pure culture on crotonate.[65] One final example of a syntrophic metabolizer that groups with *Desulfotomaculum* is *Syntrophobotulus glycolicus*.[66] This microorganism oxidizes glycolate in syntrophic association with hydrogen-using methanogens.[66] *S. glycolicus* is most commonly observed in freshwater environments, and cannot couple the oxidation of glycolate to the reduction of sulfate.[66]

Some gram-negative bacteria affiliated with the Deltaproteobacteria are capable of syntrophic metabolism.[67–69] The first syntrophic propionate oxidizer described was *Syntrophobacter wolinii*,[45] which was isolated from primary anaerobic digestor sludge. Three other *Syntrophobacter* species have been described, *Syntrophobacter pfennigii* from an anaerobic sludge of a sewage plant,[38] *Syntrophobacter fumaroxidans* isolated from an anoxic sludge blanket reactor treating wastewater from a sugar refinery,[18] and two strains of *Syntrophobacter sulfatireducens* TB8106 and WZH410 isolated from the anoxic sludge of a reactor treating brewery wastewater or a reactor treating bean-curd wastewater, respectively.[23] *Syntrophobacter* spp. form a monophylogenetic group that is separate from other *Deltaproteobacteria*, but are most closely related to group 7 of the sulfate-reducing bacteria.[18,23,38,45,70,71] The four species are mesophilic, nonmotile, and non-spore-forming bacteria. In the absence of a methanogen, all four species are capable of axenic growth and oxidize propionate by using sulfate or fumarate as an electron acceptor (TABLE 3).[18,23,38,45,70,71] They can also grow in pure culture by fermenting fumarate, malate, or pyruvate. *Syntrophobacter* spp. have been observed in freshwater sediments, marine sediments, rice paddy sediments, acidic fens, and eutrophic bog and marsh sediments.[22,24,30,72] 13C-Propionate labeling studies showed that *Syntrophobacter* spp., *Smithella* spp., and *Pelotomaculum* spp. were active in syntrophic propionate oxidation in rice paddies.[30]

Smithella propionica is another propionate-degrading syntrophic microorganism in the *Deltaproteobacteria* (TABLE 3). It was isolated from an anaerobic filter inoculated with domestic sewage sludge enriched with propionate. Unlike *Syntrophobacter*, *S. propionica* is unable to use sulfate as an electron acceptor and needs the presence of a hydrogen user to degrade propionate.[70] *Syntrophobacter* species degrade propionate to acetate and CO_2 by using the methylmalonyl-CoA pathway.[73]

However, *S. propionica* ferments propionate to acetate with the production of traces of butyrate by a new pathway that involves the condensation of two molecules of propionate to form a six-carbon intermediate that is ultimately cleaved to form acetate and butyrate.[74] *S. propionica* grows with butyrate, malate, and fumarate in coculture with a methanogen and with crotonate in pure culture.

Syntrophus spp. are rod-shaped bacteria capable of degrading aromatic compounds in syntrophic association with hydrogen-using microorganisms (TABLE 4).[67–69] *Syntrophus* spp. are also affiliated with the *Deltaproteobacteria* and have frequently been isolated from sewage sludge.[67–69] *Syntrophus buswellii* metabolizes benzoate in coculture with hydrogen-using microorganisms and crotonate in pure culture.[67] *Syntrophus gentianae* syntrophically metabolizes benzoate, gentisate, and 3-phenylpropionate.[69] *Syntrophus aciditrophicus* also syntrophically metabolizes benzoate, but differs from other *Syntrophus* spp. in its ability to metabolize a variety of fatty acids in syntrophic association with hydrogen users.[68] *S. gentianae* grows in pure culture with crotonate, producing butyrate and acetate,[69] whereas *S. aciditrophicus* ferments crotonate to acetate and cyclohexane carboxylate.[75]

Molecular ecological studies suggest that *Syntrophus* spp. may play an important role in a number of environments.[16,22,28,29,76] Sequences related to *Syntrophus* spp. are commonly detected in clone libraries from hydrocarbon-contaminated sites.[16,28,29,76] Several studies suggest that *Syntrophus* spp. may be involved in the degradation of benzoate, which is an important intermediate in the anaerobic degradation of aromatic hydrocarbons in hydrocarbon-contaminated sites.[16,28,29,76,77] The concentration of short-chain fatty acids have been observed to increase as a result of the degradation of hydrocarbons, which could also explain why *Syntrophus* sequences are observed at these sites.[17] Benzoate has been shown to be an important intermediate in the degradation of 3-chlorobenzoate and 2-chlorophenol, and sequences related to *Syntrophus* spp. tend to appear as benzoate degradation begins.[27] *Syntrophus* spp. sequences have also been observed in dechlorinating enrichments that contained either a mixture of trichloroethene and methanol or a mixture of vinyl chloride and methanol.[78] The degradation of alkylbenzenes[79] and halogenated aromatic compounds[80] to methane involves a consortium of microorganisms and likely involves the activity of *Syntrophus* spp.

The genus *Pelobacter* also clusters within *Deltaproteobacteria*. *Pelobacter* spp. are predominant in sediments and sludge where syntrophic alcohol oxidation occur.[81] *Pelobacter venetianus*[82] and *Pelobacter acetylenicus*[83] have been shown to syntrophically metabolize ethanol.

Syntrophococcus sucromutans is a rumen bacterium that requires an exogenous electron acceptor, either formate, methoxylated aromatic compounds, or a hydrogen/formate-using microorganism, to oxidize carbohydrates.[84] *Tepidanaerobacter syntrophicus* was also isolated from thermophilic anaerobic digesters and degrades lactate and numerous alcohols in syntrophic association with hydrogen-using methanogens.[85] This organism groups with the firmicutes, and appears to be most closely related to *Thermosediminibacter* spp. and *Thermovenabulum ferriorganovorum*.[85] Syntrophic butyrate metabolism has been described in cocultures of *Algorimarina butyrica* and hydrogen-using methanogens.[25] These cocultures were enriched from psychrophilic bay sediments, and are phylogenetically related to sulfate-reducing bacteria from the genera *Desulfonema* and *Desulfosarcina*.[25] This same study also established syntrophic propionate enrichments from these cold sediments, but the authors were unable to isolate the propionate degrader.[25]

Recent molecular characterization of enrichments for syntrophic metabolizers from a eutrophic site within the Florida Everglades revealed the presence of bacterial and archaeal sequences that were either members of novel lineages or closely related to uncultured environmental clones.[22] Subsequent cultivation-based and molecular studies revealed the presence of a novel *Methanosaeta* sp. and fatty acid–oxidizing bacteria related to *Syntrophomonas* spp. and *Syntrophobacter* spp.[22]

Also, hydrocarbons can be degraded syntrophically. A bacterium has been described that syntrophically degrades toluene in coculture with a sulfate reducer.[86] In some environments, the anaerobic oxidation of methane involves a highly organized, multicellular structure of the methane-oxidizing and sulfate-reducing bacteria.[44] More recently, *Desulfoglaeba alkanexedens* has been isolated, which can syntrophically degrade alkanes in coculture with methanogens.[87] Hexadecane-degrading enrichments contain microorganisms phylogenetically related to *Syntrophus* spp.[88] Other studies have shown that hydrocarbon loss is coupled to methane production, which would indicate that syntrophic metabolism must be involved.[46,47]

Zinder and Koch described a thermophilic acetate-degrading coculture consisting of an acetate-degrading bacterium and a H_2-consuming methanogen.[89] The syntrophic acetate oxidizer, strain AOR, appeared to be a homoacetogen in pure culture, producing acetate from H_2 and CO_2 as well as syntrophically with a

methanogen where acetate is oxidized to H_2 and CO_2, suggesting that the pathway is reversible.[90] Biochemical studies revealed that strain AOR uses the acetyl-CoA synthase pathway ("Wood–Ljungdahl" pathway), as do other homoacetogens.[90,91]

Other microbes that syntrophically oxidize acetate include *Geobacter sulfurreducens* with partners, such as *Wolinella succinogenes* or *Desulfovibrio desulfuricans*, and nitrate as the electron acceptor.[92] *Clostridium ultunense* strain BST was isolated in pure culture with substrates typically utilized by homoacetogenic bacteria, but can also syntrophically oxidize acetate.[93] Syntrophic acetate oxidation also has been observed in *Thermacetogenium phaeum*, which was isolated from thermophilic anaerobic digesters.[94] This microorganism is phylogenetically related to *Clostridium* and *Bacillus* spp.[94] A recent article described *Candidatus Contubernalis alkalaceticum*, which is capable of syntrophic acetate oxidation in coculture with *Desulfonatronum cooperativum*.[95] These microorganisms were isolated from a soda lake, and appear to group phylogenetically with uncultured low G+C gram-positive bacteria within the family *Syntrophomonadaceae*.[95] In addition to acetate, these organisms were also observed to syntrophically oxidize ethanol, propanol, isopropanol, serine, fructose, and isobutyrate.[95]

Evidence also shows that some homoacetogens are capable of syntrophic metabolism because their metabolism of methanol is affected by cocultivation with H_2-consuming anaerobes.[96,97] In pure culture, *Sporomusa acidovorans* ferments methanol and CO_2 to acetate,

$$4\,\text{Methanol} + 2\,HCO_3^- \rightarrow 3\,\text{Acetate}^- + H^+ + 4\,H_2O \quad \Delta G^{\circ\prime} = -220\ \text{kJ per mol} \tag{4}$$

In coculture with the H_2-consuming *Desulfovibrio desulfuricans* with nitrate, no acetate is formed, indicating that methanol is oxidized to CO_2 and H_2, a conversion that is only possible at a low hydrogen partial pressure.[97]

$$\text{Methanol} + 2\,H_2O \rightarrow HCO_3^- + H^+ + 3\,H_2 \quad \Delta G^{\circ\prime} = +24\ \text{kJ per mol} \tag{5}$$

Another syntrophic homoacetogen example may be *Methanobacillus kuznezovii*, which was described as a methanogen, but is likely to be a syntrophic coculture of a homoacetogen and a methanogen, since *M. kuznezovii* produced acetate during methanol metabolism.[98]

Syntrophic methanol-degrading enrichments were obtained from thermophilic digestors using cobalt-deficient medium to suppress methanogenesis.[99] *Moorella mulderi* and a *Desulfotomaculum* species were isolated from the enrichment.[100,101] Although the sulfate reducer used methanol in pure culture, it appeared to use the hydrogen produced by *M. mulderi* when grown in coculture. A syntrophic methanol-degrading coculture of a *Moorella* species with a H_2-utilizing *Methanothermobacter* strain was obtained when sulfate was deleted from the cobalt-deficient medium.[102] The homoacetogen grew in pure culture with methanol only when cobalt was added to the medium.[102] The effect of cobalt on the growth of the *Moorella* species is not exactly clear. It is likely that, in the presence of cobalt, a corrinoid-containing methyltransferase is used for methanol degradation, while in the absence of cobalt, a methanol dehydrogenase is used to oxidize methanol to formaldehyde, which would not be further degraded by pure cultures. *Moorella thermoautotrophica* contains a methanol dehydrogenase with pyrroloquinoline quinone as the prosthetic group.[103]

Energetics

An intriguing aspect of the metabolism of syntrophically fermenting bacteria is the fact that they must catalyze reactions that are endergonic under standard reactions conditions (i.e., positive $\Delta G^{\circ\prime}$). The activity of the partner organism is required to maintain the concentrations of hydrogen, formate, and acetate low enough to permit the metabolism of the substrate by the syntrophic metabolizer to be sufficiently exergonic to support adenosine triphosphate (ATP) synthesis, anabolism, and growth. For this reason, syntrophically fermenting bacteria are excellent model organisms to study the minimum limits of microbial energy metabolism. For example, is there a minimum amount of free energy required to conserve energy in a biologically useful form to maintain viability?

Based on the energy released by the hydrolysis of ATP and the concentrations of adenylate molecules in growing bacteria, it is estimated that a free energy change of -60 to -70 kJ per mol is required for the biochemical synthesis of ATP under physiological conditions.[104] This amount of energy does not need to be supplied in one single step, as exemplified by the formation of ATP by substrate-level phosphorylation, but rather, can be accomplished in smaller increments, for example, by membrane-bound proton-translocating redox reactions or other exergonic reactions involving the transport of sodium ions[105] that culminate in ATP synthesis through a membrane-bound ATP synthase. A ratio of three protons translocated per ATP formed has been assumed,[4] although recent research on the

structure and function of ATP synthases from different organisms indicates that this stoichiometry may vary between 3 and 5 H^+ per ATP formed or hydrolyzed.[106] Thus, the minimum increment of energy required for ATP synthesis may be as low as 12–15 kJ per mol, and values in the range of 15–20 kJ per mol reaction have been calculated for most syntrophic fermentations under *insitu* conditions.[4] In some cases, especially in syntrophic fermentation of propionate to acetate and hydrogen, the overall energetics appear to be lower, about −12 kJ per mol reaction, as calculated from *insitu* propionate, acetate, and hydrogen concentrations. This view may be incomplete, since formate exchange between the partners also appears to be required for syntrophic propionate fermentation.[35] In experiments with resting cells of the butyrate-fermenting *Syntrophus aciditrophicus* in buffer, an overall free energy change as low as −4 kJ per mol of butyrate was calculated,[10] although there was no evidence that ATP was synthesized under these conditions. The stoichiometry of ions translocated per mole substrate consumed by the syntrophic metabolizer in addition to the stoichiometry of ions consumed in support of ATP synthesis are critical issues that remain unresolved.

One specifically fascinating syntrophic system is syntrophic acetate oxidation via CO_2 and H_2 as intermediates, which was first demonstrated by Zinder and Koch[89] in a thermophilic reactor at 58°C. The overall reaction yields −35 kJ per mol for the entire two-step process under standard conditions (25°C); at 58°C, the energy yield increases to −42 kJ per mol, which is sufficient to support the growth of the two syntrophic partners. A coculture fermenting acetate syntrophically to methane and CO_2 at 37°C has been isolated.[93] The $\Delta G'$ for syntrophic acetate oxidation at 37°C is −36 kJ per mol of acetate. The hydrogen concentrations indicated that the free energy change for hydrogen production from acetate was very close to the value for hydrogen use by the methanogen, indicating that the coculture partners equally shared the available energy.[90,91] If this is the case, then the free energy available to each organism would be about −15 kJ per reaction. The growth yields also indicated that each partner equally shared the available energy. The doubling times of the coculture was in the range of 3–4 weeks, which indicates that a lower limit for efficient energy transformation was being reached under these conditions.

A new thermophilic acetate-fermenting coculture was isolated in Japan,[94] and enzyme measurements with this culture demonstrated that all enzymes of the Wood–Ljungdahl pathway were present under both acetate utilization and acetate formation conditions. Moreover, the coculture was able to immediately switch from syntrophic acetate oxidation to homoacetogenic acetate formation, indicating that the entire enzyme apparatus appears to operate in a reversible manner.[107] This is the first demonstrated case of a metabolism that is entirely reversible, thus demonstrating how close to the thermodynamic equilibrium such metabolism operates. Unresolved is how these bacteria manage to make ATP by each mode. There must be unique steps linked to ATP formation in one direction that are decoupled from ATP hydrolysis in the reverse direction, possibly some switch in electron flow in the membrane. So far, these steps have not yet been identified. The complete sequencing of the genome of the bacterium involved, *Thermacetogenium phaeum*, as well as other syntrophic acetate oxidizers, is underway and may provide insight into this exciting phenomenon soon.

The long-disputed process of anaerobic methane oxidation appears to be a syntrophic cooperation of two microbes.[44] One organism, related to methanogens, appears to operate its methanogenic-like pathway in reverse, thus oxidizing methane. The second organism is a sulfate-reducing bacterium. The calculated free energy change of this syntrophic association is very low:

$$CH_4 + SO_4^{2-} + H^+ \rightarrow CO_2 + HS^- + 2\,H_2O$$
$$\Delta G^{\circ\prime} = -18\,\text{kJ per mol} \qquad (6)$$

The free energy available for anaerobic methane oxidation appears to be insufficient to provide both partners with an adequate amount of energy. Only under *in situ* conditions, that is, about 100 atm CH_4, would the overall process provide enough energy to "feed" both partners, and it is only under these conditions that the process can be maintained in the laboratory.[13] The preceding experimental evidence indicates that the minimum amount of energy required for synthesis of ATP must occur in small increments within the range of −15 kJ per mol reaction. It may also explain why, despite numerous efforts, nobody succeeded in the past in cultivation of anaerobic methane oxidizers in the laboratory under conditions close to standard reaction conditions.

Genomes of Syntrophs

The complete genome sequences of *Syntrophus aciditrophicus*, *S. wolfei*, *Syntrophobacter fumaroxidans*, and *Pelotomaculum thermopropionicum* have been reported (TABLE 5)[108,109] (http://www.jgi.doe.gov, http://www.integratedgenomics.com). The genome

TABLE 5. Properties of the sequenced syntrophic bacterial genomes[a]

Organism	% G+C	Genome size (Mb)	% Coding region	No. of ORF	ORF with assigned function		ORF without assigned function		ORF without similarity[b]		rRNA operons	GenBank ID
					No.	%	No.	%	No.	%		
Syntrophus aciditrophicus	52	3.18	88	3,168	2,078	65.6	1,090	34.4	422	13.3	1	NC_008346
Syntrophobacter fumaroxidans	59	4.99	82	4,098	2,809	68.5	1,289	31.5	468	11.5	2	NC_008554
Syntrophomonas wolfei subsp. *wolfei*	45	2.85	87	2,574	1,507	58.5	1,067	41.5	404	13.5	3	NC_007759
Pelotomaculum thermopropionicum	52	3.03	85	2,920	1,767[c]	60.5	1,153[c]	39.5	ND		2	NC_009454

[a]ORF (open reading frames) statistics generated by Joint Genome Institute (www.img.jgi.gov). ND = not determined.
[b]Value generated by ERGO (www.integrated genomics.com).
[c]Number determined from NC_009454 GenBank file.

sizes are in the range of about 3 MB for *S. aciditrophicus*, *S. wolfei*, and *P. thermopropionicum*, while that of *S. fumaroxidans* is higher, about 5 MB. Approximately 50–60% of the open reading frames (ORFs) were assigned tentative annotations, with the remaining 40–50% having no assigned function. It is noteworthy that a significant fraction of the ORFs in each genome lack either annotated function or similarity to proteins in any other described organism. Also noteworthy is that a best reciprocal gene comparison among these strains reveals that fewer than one-third of the genes in one strain are significantly related to those in another.[109]

While the detailed analysis of the genomic content of three of these genomes has not been reported as of the writing of this article, the genetic inventory of the *S. aciditrophicus* genome was recently published.[109] *S. aciditrophicus* appears to be self-sufficient with respect to its anabolic pathways, but in contrast is highly specialized in catabolic ability, as genes for utilization of most carbon compounds by fermentation or respiration are absent. The genome of *S. aciditrophicus* is devoid of genes for electron transport proteins common to many anaerobic fermentative or respiratory bacteria, for example, the sulfate reducers, the nitrate reducers, or other organisms able to reduce other organic or inorganic electron acceptors. Furthermore, the genetic blueprint of *S. aciditrophicus* strain SB suggests unique and apparently undescribed mechanism(s) to metabolize its substrates (i.e., crotonate, benzoate, and cyclohexane carboxylate) to acetate and other products. A distinctive feature of syntrophic metabolism is the need for reverse electron transport. The presence of a unique ion-translocating electron transfer complex, menaquinone, and membrane-bound Fe-S proteins with associated heterodisulfide reductase domains in the genome of *S. aciditrophicus* suggest mechanisms to accomplish this task. Genomic analysis indicate that *S. aciditrophicus* has multiple mechanisms to create and use ion gradients such as ion-translocating ATP synthases, pyrophosphatases, decarboxylases, and hydrogenases, which would help modulate the energy status of the cells in response to varying thermodynamic conditions. The *S. aciditrophicus* genome contains genes for 17 sigma 54–interacting transcriptional regulators and 35 transcriptional regulators with a helix-turn-helix motif.[109] Other gram-negative microbes have a larger number of transcriptional regulators with a helix-turn-helix motif, suggesting that *S. aciditrophicus* appears to have adopted a regulatory strategy reliant on sigma-54 factor coupled signal transduction pathways. Interestingly, one of the operons involved in propionate metabolism in *P. thermopropionicum* also appears to involve sigma factor regulation.[108] The complete sequencing of genomes

for other syntrophic metabolizers is under way, and the analysis of this genomic information should provide further insights into the complexity of this important microbial lifestyle.

Future Prospects

Syntrophic associations provide ideal model systems to study microbial interactions and their role in the maintenance of community structure and functional diversity within an ecosystem. Advances in cultivation techniques, molecular ecology, and genomics and functional genomics are rapidly merging and combined should allow a comprehensive approach to understand the syntrophic lifestyle at the edge of a minimal energy existence. With these techniques, it is quite likely that we will soon uncover the extent of the diversity of microorganisms capable of syntrophic metabolism with respect to the substrates that they degrade, the variety of their metabolic pathways, and their phylogenetic relatedness. We are beginning to reveal the molecular and biochemical details needed for the syntrophic lifestyle. In particular, the recent discovery of three genes, whose expression was altered during a shift from syntrophic metabolism to sulfate reduction, has provided clues about the origins of syntrophic interactions.[110] The combination of computational models with functional genomic information will allow us to interrogate the regulatory mechanisms involved in establishing and maintaining multispecies associations in order to quantify and predict the behavior of microorganisms and microbial communities in natural ecosystems.

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Conflict of Interest

The authors declare no conflicts of interest.

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