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A genomic view on syntrophic versus non-syntrophic lifestyle in anaerobic fatty acid degrading communities ☆

Petra Worm [a,*], Jasper J. Koehorst [b], Michael Visser [a], Vicente T. Sedano-Núñez [a], Peter J. Schaap [b], Caroline M. Plugge [a], Diana Z. Sousa [a,c], Alfons J.M. Stams [a,c]

[a] Laboratory of Microbiology, Wageningen University, Dreijenplein 10, 6703 HB Wageningen, The Netherlands
[b] Laboratory of Systems and Synthetic Biology, Wageningen University, Dreijenplein 10, 6703 HB Wageningen, The Netherlands
[c] Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

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ABSTRACT

In sulfate-reducing and methanogenic environments complex biopolymers are hydrolyzed and degraded by fermentative micro-organisms that produce hydrogen, carbon dioxide and short chain fatty acids. Degradation of short chain fatty acids can be coupled to methanogenesis or to sulfate-reduction. Here we study from a genome perspective why some of these micro-organisms are able to grow in syntrophy with methanogens and others are not. Bacterial strains were selected based on genome availability and upon their ability to grow on short chain fatty acids alone or in syntrophic association with methanogens. Systematic functional domain profiling allowed us to shed light on this fundamental and ecologically important question. Extra-cytoplasmic formate dehydrogenases (InterPro domain number; IPR006443), including their maturation protein FdhE (IPR024064 and IPR006452) is a typical difference between syntrophic and non-syntrophic butyrate and propionate degraders. Furthermore, two domains with a currently unknown function seem to be associated with the ability of syntrophic growth. One is putatively involved in capsule or biofilm production (IPR019079) and a second in cell division, shape-determination or sporulation (IPR018365). The sulfate-reducing bacteria *Desulfobacterium autotrophicum* HRM2, *Desulfomonile tiedjei* and *Desulfosporosinus meridiei* were never tested for syntrophic growth, but all crucial domains were found in their genomes, which suggests their possible ability to grow in syntrophic association with methanogens. In addition, profiling domains involved in electron transfer mechanisms revealed the important role of the Rnf-complex and the formate transporter in syntrophy, and indicate that DUF224 may have a role in electron transfer in bacteria other than *Syntrophomonas wolfei* as well. This article is a part of a Special Issue entitled: 18th European Bioenergetics Conference (Biochim. Biophys. Acta, Volume 1837, Issue 7, July 2014).

1. Introduction

Environments with a low redox potential are abundantly present on earth, especially in the deeper zones of marine and freshwater sediments. The low redox potential is created by the depletion of oxygen and the formation of hydrogen sulfide in the anaerobic degradation of organic matter. In the decomposition of sulfur-containing organic compounds such as the amino acids (cysteine and methionine) and co-factors (biotin and thiamin) hydrogen sulfide is released. Additionally, hydrogen sulfide is formed by anaerobic micro-organisms that respire with sulfate or other sulfur compounds, such as thiosulfate and elemental sulfur. This respiratory type of sulfidogenesis is quantitatively most important [1–3].

Respiratory sulfate reduction is an important process in nature, especially in marine sediments where the sulfate concentration is high (about 20 mM) [4]. In freshwater environments that are generally low in sulfate, sulfate reduction does not play an important role unless hydrogen sulfide is rapidly oxidized by sulfide-oxidizing microbes [5,6]. In sulfate-depleted anoxic environments methanogenesis is the most abundant process [7,8]. Interestingly, in marine environments methanogenesis occurs as well, especially in zones where the available sulfate is not sufficient to degrade organic matter [9]. In both marine and freshwater environments microbes involved in sulfate reduction and methanogenesis interact strongly with each other, and this interaction is strongly depending on the availability of sulfate. Generally, sulfate reduction is favored over methanogenesis when sufficient sulfate is present [4,8].

In sulfate-reducing and methanogenic environments organic material is degraded in a cascade process. Complex biopolymers are

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* Corresponding author. Tel.: +31 317 483107; fax: +31 317 483829.
E-mail address: petra.worm@wur.nl (P. Worm).

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first hydrolyzed and degraded by fermentative micro-organisms that produce hydrogen, carbon dioxide and organic compounds, typically organic acids (butyrate, propionate, acetate and formate) as products. In sulfate-reducing environments these compounds are the common substrates for sulfate-reducing micro-organisms. Phylogenetically and physiologically sulfate-reducing micro-organisms are very diverse [4]. Phylogenetically they occur in the bacterial and archaeal domain of life. Some sulfate reducers have the ability to grow autotrophically with H_2 and sulfate as energy substrates. Often these autotrophs are the sulfate reducers that are also able to degrade acetate completely to CO_2, employing the reversible Wood–Ljungdahl pathway for acetate degradation and acetate formation [10].

In methanogenic environments, methanogens use H_2/CO_2, formate and acetate as the main substrates [11]. Methanogenic archaea belong to different phylotypes. The ability to use acetate is restricted to archaea belonging to the order Methanosarcinales, with *Methanosarcina* and *Methanosaeta* as important genera. The ability to grow with H_2/CO_2 and formate occurs in most of the currently described orders of methanogens [11]. Higher organic compounds such as propionate and butyrate, that are typical intermediates in methanogenic environments, are not degraded by methanogens. Therefore, acetogenic bacteria are required to degrade such compounds to the methanogenic substrates acetate, formate and H_2/CO_2 [8,12]. For thermodynamic reasons such bacteria can only degrade propionate and butyrate when the products are efficiently taken away by methanogens. Thus, these acetogenic bacteria grow in obligate syntrophy with methanogens. The methanogenic substrates acetate and formate may be degraded by syntrophic communities as well [13,14]. Syntrophic acetate degradation especially occurs under conditions at which the activity of acetoclastic methanogens is low such as a high temperature and high levels of ammonium [13].

Though the basic concepts of sulfate reduction and methanogenesis are clear, it is not very clear how sulfate-reducing and methanogenic communities in freshwater and marine sediments are responding to changes in the sulfate availability. The metabolic flexibility of sulfate-reducing bacteria has been addressed recently [15–17]. Several sulfate reducers are able to grow acetogenically in syntrophic association with methanogens which is for instance the case for *Syntrophobacter fumaroxidans* growing with propionate. Nevertheless, not all sulfate reducers possess the ability to switch from a sulfate-dependent lifestyle to a syntrophic lifestyle. For instance, *Desulfobulbus propionicus* is a bacterium that grows with propionate and sulfate, but it is not able to grow with propionate in syntrophy with methanogens. Similarly, the thermophilic sulfate reducer *Desulfotomaculum kuznetsovii* is able to degrade propionate with sulfate, but it is not able to grow in syntrophy with methanogens, while the phylogenetically closely related non-sulfate-reducing bacterium *Pelotomaculum thermopropionicum* grows with propionate in syntrophy with methanogens [18].

This review focusses on syntrophic degradation of short chain fatty acids (SCFA) such as butyrate, propionate and acetate. In contrast to syntrophic degradation of ethanol and lactate, syntrophic SCFA degradation occurs at the limit of what is thermodynamically possible and requires at least one step with reversed electron transport [19]. Here we address a fundamental and ecologically important question: "what are the key properties that make that a SCFA-degrading bacterium is able to grow in syntrophy with methanogens and another not". The availability of genome sequences of bacteria that can and bacteria that cannot grow with SCFA in syntrophic association may allow us to identify key genes in syntrophy.

2. Microbial functions required for syntrophic growth

2.1. Functional profiling strategies

Bacterial strains were selected based on genome availability, and ability to grow on short chain fatty acids syntrophically or not. Sulfate reducers that grow on short chain fatty acids, whose genomes are available and currently have not been tested for syntrophic growth were included in our analysis (Table 1). Correct codon usage of sequences coding for selenocysteine-containing formate dehydrogenases and hydrogenases was verified (Supplementary file 1). Our strategy is to compare first bacteria that degrade propionate and butyrate, and then to identify if similarities can also be found in acetate degraders. Functional domain profiles were obtained with InterProScan 5 (version 5RC7, 27th January 2014). To get more insight into microbial functions required for syntrophic growth, domain based functional profiles of five butyrate and/or propionate-degrading syntrophs were compared with two butyrate and/or propionate-degrading non-syntrophs (Supplementary file 2). Domains only present in syntrophs are listed in Table 1. Genomes of sulfate reducers that degrade butyrate and/or propionate, but were never tested for syntrophy, were screened for these domains (Table 1).

Functional domains assigned to proteins involved in electron transport were separately analyzed. Domains that were unique for each protein were selected. Genomes of short chain fatty acid degrading syntrophs, non-syntrophs and sulfate reducers that never have been tested for syntrophy were screened for these domains (Table 2). Electron transport mechanisms in short chain fatty acid degrading syntrophs and non-syntrophs were predicted from their genomes (Supplementary files 1 and 3).

2.2. Domain based genome comparison of syntrophic and non-syntrophic propionate- and/or butyrate degraders

Six domains are present in the genomes of all analyzed butyrate and/or propionate-degrading syntrophs and not in non-syntrophs (Table 1). Domain "IPR006443" is exclusively present in the extra-cytoplasmic formate dehydrogenase (FDH) alpha subunit. Domains "IPR024064 and IPR006452" both belong to FdhE. The gene *fdh*E in *Escherichia coli* is required for maturation of the membrane bound FDH-complex [20]. The fact that extra-cytoplasmic formate dehydrogenases are only present in syntrophs and not in non-syntrophs strongly indicates that extra-cytoplasmic formate production is essential for syntrophic propionate and butyrate oxidation. It contributes to earlier indication that formate plays a major role in interspecies electron transfer [21–24]. The redox potential of the couple proton/hydrogen ($E^{0\prime} = -414$ mV) is slightly higher than the redox potential of the couple CO_2/formate (-432 mV). The relative contribution of formate and hydrogen as interspecies electron carrier in syntrophic fatty acid-degrading communities has not been clear thus far, but a syntrophic relationship in which both hydrogen and formate can be transferred would be more flexible than when only hydrogen is transferred [21]. *Syntrophobacter fumaroxidans* and *Syntrophospora bryantii* oxidize propionate and butyrate, respectively, in syntrophy with hydrogen and formate-using methanogens such as *Methanospirillum hungatei* and *Methanobacterium formicicum*, but not with the hydrogen only-using *Methanobrevibacter arboriphilus* [23]. In analogy with this, *Syntrophomonas wolfei* oxidizes butyrate faster with the formate and hydrogen-using *M. hungatei* than with the hydrogen-only using *M. arboriphilus* [24]. The importance of formate transfer in *S. wolfei* cocultures is supported further by the observed involvement of an extra-cytoplasmic formate dehydrogenase in the final reduction of CO_2 with electrons generated by the butyryl-CoA to crotonyl-CoA conversion [25]. Moreover, this extra-cytoplasmic formate dehydrogenase was more expressed during syntrophic growth compared to axenic growth [25].

Domain "IPR019079", named CapA, was found in genomes of all short chain fatty acid degrading syntrophs (including acetate oxidizers, data not shown) and was not present in the genomes of the two non-syntrophs (Table 1). CapA is part of a membrane bound complex that synthesizes poly-γ-glutamate to form a capsule or biofilm in *Bacillus subtilis*, *Bacillus anthracis*, *Staphylococcus epidermidis* and *Fusobacterium nucleatum* [41–43]. The presence of this domain in SCFA degrading

Table 1
Domain based genome comparison of syntrophic and non-syntrophic butyrate and/or propionate degraders. Domains present in genomes of all butyrate and/or propionate-degrading syntrophs and absent in those of non-syntrophs are listed and domain abundance is indicated. Syntrophs are shaded orange, non-syntrophs are shaded blue and sulfate reducers that were never tested for syntrophic growth are shaded green. The pale color green corresponds to draft genomes and the darker colors (orange, blue, green) correspond to complete genomes.

		Syntrophomonas wolfei	*Syntrophus aciditrophicus*	*Syntrophothermus lipocalidus*	*Syntrophobacter fumaroxidans*	*Pelotomaculum thermopropionicum*	*Desulfotomaculum kuznetsovii*	*Desulfobulbus propionicus*	*Desulfobulbus japonicus*	*Desulfatibacillum alkenivorans*	*Desulfatirhabdium butyrativorans*	*Desulfobacterium autotrophicum HRM2*	*Desulfospira joergensenii*	*Desulfotignum balticum*	*Desulfomonile tiedjei*	*Desulfarculus baarsii*	*Desulfosporosinus meridiei*	*Desulfotalea psychrophila*	*Desulfatibacillum aliphaticivorans*	*Desulfotomaculum gibsoniae*
Growth on butyrate [¥]																				
Growth on propionate [¥]																				
Extra-cytoplasmic FDH alpha subunit	IPR006443	1	2	1	3	1	0	0	0	2	1	5	2	0	2	0	1	0	0	1
FdhE-like protein	IPR024064	4	6	2	5	3	0	0	0	0	0	4	4	0	2	1	2	0	0	2
FDH accessory protein	IPR006452	2	3	1	2	1	0	0	0	0	0	2	2	0	1	0	1	0	0	1
Capsule synthesis protein, CapA	IPR019079	2	2	4	2	1	0	0	0	0	0	4	2	0	2	0	4	4	0	2
Cell cycle, FtsW / RodA / SpoVE,	IPR018365	1	2	2	2	1	0	0	0	2	1	3	0	1	1	2	2	0	0	0
Ribonuclease P, conserved site	IPR020539	1	1	1	1	1	0	0	0	2	1	1	0	0	1	1	1	1	0	0

[¥]The ability of substrate conversion was retrieved from literature [18,24,26–40].

bacteria may contribute to the formation of exo-polymeric substances that may facilitate syntrophic growth. Domain "IPR018365" is present in FtsW, RodA, and SpoVE, that are membrane integrated proteins involved in cell division, shape-determination and sporulation in *E. coli* and *B. subtilis* [44–46]. What the exact function of this domain is in syntrophic butyrate and propionate degraders is unclear. The domain "IPR020539" that seems exclusively present in syntrophs in our analysis belongs to the protein Ribonuclease P which removes extra residues at the 5′-side from precursor tRNA, resulting in mature tRNA. However, what its function could be in syntrophic growth is unclear. Just coincidence cannot be excluded. As can be seen from Table 1, only one copy of this domain is present in the genome of a syntrophic bacterium, whereas for the domains involved in periplasmic formate dehydrogenases, CapA-domains and cell cycle FtsW/RodA/SpoVE-domains, more copies are present. Furthermore domain co-occurrence suggests that *Desulfobacterium autotrophicum* HRM2, *Desulfomonile tiedjei* and *Desulfosporosinus meridiei* might be able to adopt a syntrophic lifestyle on SCFA.

2.3. Domain based functional profiling of electron transfer mechanisms

For syntrophic short chain fatty acid degradation, electron transfer mechanisms are required to transfer electrons to the terminal acceptor, which can be sulfate in a sulfidogenic lifestyle or protons and/or CO_2 in a syntrophic lifestyle. As the previous paragraph focussed on functional domains that are present in all syntrophic and not in non-syntrophic propionate and/or butyrate degraders, here we profile the functional domains involved in electron transfer mechanisms (Table 2). As can be seen from Table 2, cytoplasmic and extra-cytoplasmic formate dehydrogenases contain InterPro domains that are unique for each protein. "IPR006443" is only present in extra-cytoplasmic FDH's, not in cytoplasmic FDH's whereas "IPR027467", "IPR006655" and "IPR006478" of

Notes to Table 2:
Abbreviations are explained as formate dehydrogenase (FDH); NADH: ubiquinone oxidoreductase subunit 51 kDa (NUO 51 kDa); membrane-bound ferredoxin:NAD^+ oxidoreductase (Rnf) complex; butyryl-CoA dehydrogenase (Bcd); domain of unknown function 224 (DUF224).[¥]The ability of substrate conversion was retrieved from literature [18,24,26–40,50–58].
*more than 99.
[†]These IPR numbers were unique for NiFe hydrogenase alpha subunits. As the Ech complex also contains a NiFe hydrogenase alpha subunit, corresponding domains were also found in this EchE.

Table 2
Profiles of functional domains involved in electron transfer mechanisms. Profiles of functional domains involved in electron transfer mechanisms are shown. Domain abundance is indicated. Syntrophic sort chain fatty acid (SCFA) degraders are shaded orange, non-syntrophic SCFA degraders are shaded blue and sulfate reducers that were never tested for syntrophic growth are shaded green. Pale colors (orange, green) correspond to draft genomes and the darker colors (orange, blue, green) correspond to complete genomes.

			Syntrophomonas wolfei	*Syntrophus aciditrophicus*	*Syntrophothermus lipocalidus*	*Syntrophobacter fumaroxidans*	*Pelotomaculum thermopropionicum*	*Tepidanaerobacter acetatoxydans*	*Thermoacetogenium phaeum*	*Clostridium ultunense*	*Desulfotomaculum kuznetsovii*	*Desulfobulbus propionicus*	*Desulfobulbus japonicus*	*Desulfatibacillum alkenivorans*	*Desulfatirhabdium butyrativorans*	*Desulfobacter curvatus*	*Desulfobacter postgatei*	*Desulfobacterium anilini*	*Desulfobacterium autotrophicum HRM2*	*Desulfospira joergensenii*	*Desulfotignum balticum*	*Desulfomonile tiedjei*	*Desulfohalobium retbaense*	*Desulfarculus baarsii*	*Desulfosporosinus meridiei*	*Desulfotalea psychrophila*	*Desulfatibacillum aliphaticivorans*	*Desulfobacca acetatoxidans*	*Desulfotomaculum gibsoniae*	*Desulfotomaculum acetoxidans*
Growth on SCFA [¥]		Butyrate																											
		Propionate																											
		Acetate																											
Protein (complex)	**Subunit**	**InterPro number**																											
Cytoplasmic FDH	Alpha	IPR027467	4	2	2	3	1	0	5	1	2	0	0	5	3	3	0	0	10	2	5	2	1	3	4	2	2	4	2	2
		IPR006655	2	4	0	5	2	0	11	2	2	2	1	9	5	5	1	1	9	3	4	7	3	1	7	4	1	1	6	2
		IPR006478	0	0	1	3	0	0	3	1	1	0	0	1	1	0	0	0	1	0	0	2	0	0	2	0	1	5	1	2
	NUO	IPR019575	12	12	4	8	6	4	16	4	12	8	2	12	4	0	0	2	6	0	12	9	0	8	6	8	6	12	12	6
	51kDa	IPR001949	8	6	3	4	3	2	3	1	5	4	1	6	2	0	0	1	3	0	6	4	0	4	1	2	3	6	4	3
Extra–cytopl. FDH	Alpha	IPR006443	3	4	1	5	2	0	2	0	0	0	1	3	2	0	0	2	5	4	0	2	1	0	2	0	1	0	2	0
Formate transporter	–	IPR000292	2	1	0	1	1	2	0	0	0	0	0	4	0	0	1	0	4	0	0	0	0	2	2	0	2	0	0	0
		IPR024002	2	0	0	1	0	1	0	0	0	0	0	4	0	0	1	0	2	0	0	0	0	2	2	0	2	0	0	0
FeFe–hydrogenase	Alpa	IPR004108	4	1	3	3	4	6	0	2	8	3	0	0	0	0	0	0	4	1	2	1	0	1	5	4	0	0	7	5
		IPR009016	5	1	3	3	5	4	0	2	8	3	0	0	0	0	0	0	6	1	2	1	0	1	5	6	0	0	7	5
		IPR003149	9	3	9	6	9	6	0	3	12	6	0	0	0	0	0	0	9	3	3	3	0	0	9	6	0	0	9	9
		IPR013352	3	1	3	2	3	2	0	1	4	2	0	0	0	0	0	0	3	1	1	1	0	0	3	2	0	0	3	3
NiFe–hydrogenase		IPR001501†	0	2	3	17	2	0	12	0	0	10	7	12	1	15	5	0	12	2	6	3	3	2	7	14	8	2	8	1
		IPR018194†	0	1	2	6	2	0	6	0	0	6	4	9	2	18	4	0	8	2	4	4	4	4	5	13	7	1	2	1
Rnf complex	RnfB	IPR007202	2	2	0	9	6	8	4	2	10	2	0	22	7	12	4	0	30	10	10	14	6	13	6	4	4	12	13	10
		IPR010207	0	1	0	1	0	1	0	1	0	0	0	1	0	3	1	0	5	1	0	1	2	0	0	2	1	0	0	0
	RnfC	IPR026902	0	1	0	1	0	1	2	1	0	0	1	4	0	6	2	0	2	2	1	1	1	1	3	6	1	0	0	0
		IPR010208	0	2	0	2	0	2	0	2	0	0	0	0	0	6	2	0	4	0	0	2	2	2	0	4	0	0	0	0
	RnfD	IPR004338	0	1	0	1	0	1	0	2	0	0	0	2	1	9	3	0	4	1	2	1	1	1	0	4	0	0	0	0
		IPR011303	0	1	0	1	0	1	0	2	0	0	0	2	1	3	1	0	2	1	1	1	1	1	0	2	0	0	0	0
	RnfG	IPR007329	2	2	0	2	0	2	0	16	0	0	0	4	2	18	6	0	14	2	6	2	2	2	0	8	0	0	6	0
Ech complex	EchA	IPR001750	0	0	4	6	0	0	6	0	3	14	0	4	9	7	4	0	8	6	9	6	3	0	7	11	0	6	3	5
		IPR001516	0	0	2	2	0	0	2	0	1	3	0	0	1	3	1	0	0	2	2	2	0	0	1	0	0	2	1	1
	EchB	IPR001694	0	0	3	4	0	0	3	0	2	8	0	0	2	5	1	0	2	0	1	4	0	0	4	6	0	5	2	2
	EchC	IPR006137	0	2	6	16	2	0	14	0	2	19	6	12	6	26	6	0	12	4	8	8	4	4	12	22	6	6	9	4
	EchD	IPR001268	0	0	2	2	0	0	4	0	1	3	0	0	2	5	1	0	1	0	1	2	0	0	3	4	0	2	1	2
		IPR012179	0	0	0	0	0	0	2	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	EchE	IPR001135	0	0	4	3	0	0	8	0	2	5	0	0	3	8	1	0	1	0	1	2	0	0	4	6	0	3	2	3
Etf alpha	–	IPR014731	3	1	4	4	6	1	0	1	4	2	0	10	4	5	1	2	7	4	7	3	0	3	3	3	0	1	1	3
Etf beta	–	IPR012255	3	1	4	4	7	1	0	1	4	2	0	10	4	5	1	2	5	4	7	3	0	3	3	2	0	1	1	3
Bcd	–	IPR006089	7	6	17	7	8	0	0	2	12	6	0	54	9	10	2	5	18	4	10	21	0	22	15	0	15	2	20	2
		IPR009075	18	13	28	14	20	0	0	2	20	6	0	*	36	40	10	12	64	17	36	45	0	44	22	0	34	2	30	2
		IPR006092	7	6	10	6	9	0	0	1	8	3	0	82	15	20	5	5	31	7	16	19	0	18	8	0	15	1	15	1
		IPR006091	18	13	27	14	20	0	0	2	18	6	0	*	36	40	10	12	68	17	36	45	0	43	22	0	36	2	30	2
		IPR013786	8	6	12	6	9	0	0	1	9	3	0	90	15	20	5	5	31	7	17	19	0	19	9	0	18	1	15	1
		IPR009100	9	7	14	8	11	0	0	1	9	3	0	*	18	20	5	7	34	9	19	25	0	23	12	0	18	1	15	1
DUF224	–	IPR003816	1	1	1	1	2	0	0	0	1	2	0	5	4	9	2	0	6	2	5	8	2	3	4	2	2	1	1	1
		IPR004017	6	15	10	27	13	0	16	0	26	17	8	58	25	52	11	4	54	24	34	39	20	18	34	33	10	11	31	16
		IPR023234	1	1	1	5	3	0	0	0	3	4	0	19	6	25	11	0	27	6	14	15	7	9	8	8	6	3	3	3
Cytochrome	c	IPR023155	1	1	0	6	0	0	0	0	0	12	0	12	4	10	3	0	10	4	4	20	4	11	1	3	2	1	0	0
		IPR024673	0	0	0	3	0	0	0	0	0	0	0	10	3	15	3	0	2	0	4	0	3	0	0	6	0	0	0	0
	cIII	IPR020942	0	0	0	2	0	0	0	0	0	4	2	10	5	18	4	0	11	4	10	4	9	2	0	0	1	1	0	0
		IPR002322	0	0	0	0	0	0	0	0	0	8	4	24	16	52	12	0	40	16	24	8	24	4	0	0	4	4	0	0
	b561	IPR016174	6	6	6	3	1	0	6	0	2	1	1	4	2	6	2	0	0	4	2	6	2	1	13	11	0	2	1	0
		IPR000516	0	0	4	2	0	0	6	0	0	0	0	4	2	0	0	0	0	0	0	0	2	0	10	10	0	0	2	0
	b5	IPR001199	0	3	0	3	0	0	0	0	0	0	0	0	3	0	0	0	0	4	0	3	0	0	0	0	0	0	0	0

Table 3
Gibbs free energy changes of short chain fatty acid oxidation and methane production. Values were calculated from the Gibbs free energies of formation of the reactants a concentration of 1 M, pH 7.0, T = 298 K and a partial pressure of gas of 10^5 Pa according to Thauer et al. 1977 [71].

Eq. no	Reaction		$\Delta G^{0\prime}$ (kJ/reaction)
1a	$Butyrate^- + 2\ H_2O$	$\rightarrow 2\ acetate^- + H^+ + 2\ H_2$	+48
1b	$Butyrate^- + 2\ H_2O + 2\ CO_2$	$\rightarrow 2\ acetate^- + 3\ H^+ + 2\ formate^-$	+55
2a	$Propionate^- + 2\ H_2O$	$\rightarrow Acetate^- + CO_2 + 3\ H_2$	+72
2b	$Propionate^- + 2\ H_2O + 2\ CO_2$	$\rightarrow Acetate^- + 3\ formate^- + 3\ H^+$	+82
3a	$Acetate^- + 2\ H_2O + H^+$	$\rightarrow 2\ CO_2 + 4\ H_2$	+95
3b	$Acetate^- + 2\ H_2O + 2\ CO_2$	$\rightarrow 4\ formate^- + 3\ H^+$	+109
4	$Formate^- + H^+$	$\rightarrow H_2 + CO_2$	−3.4
5	$4\ formate^- + 4\ H^+$	$\rightarrow CH_4 + 3\ CO_2 + 2\ H_2O$	−145
6	$4\ H_2 + CO_2$	$\rightarrow CH_4 + 2\ H_2O$	−131
7	$Acetate^- + H^+$	$\rightarrow CH_4 + CO_2$	−36

cytoplasmic FDH's, are not present in extra-cytoplasmic FDH's. Domains of cytoplasmic FDH's are present in genomes of syntrophs and non-syntrophs, whereas the domain of extra-cytoplasmic FDH's is present only in syntrophs. Formate transporter linked domains are absent in genomes of non-syntrophs whereas they are present in a number of syntrophs. These observations again point to the importance of formate as interspecies electron carrier.

The membrane bound Rnf complex that can conserve energy by the reversible translocation of protons or sodium ions from ferredoxin oxidation with NAD^+ [47] was not found in non-syntrophs but is present in several syntrophs. As syntrophs live at the limit of what is energetically possible [19,48,49] they contain mechanisms to conserve energy from ferredoxin oxidation with NAD^+. Furthermore, recently the domain with unknown function "DUF224" was shown to play a role in electron transport from an electron transfer flavoprotein (ETF) towards membrane-bound electron transfer components in *S. wolfei* [25]. DUF224 is present in 18 genomes from which 17 also contain domains linked to ETF complexes. This indicates that DUF224 may have a role in electron transfer in bacteria other than *S. wolfei* as well.

3. Energetics and metabolism of syntrophic short chain fatty acid degradation

3.1. Energy conservation mechanisms

For microbial maintenance and growth the energy that is released from catabolic reactions has to be converted into energy that can be used to perform anabolic reactions. Therefore, energy is conserved as ATP by substrate level phosphorylation or via a proton or sodium gradient over the cytoplasmic membrane, termed electron transport phosphorylation. Membrane bound enzyme complexes are required to build a proton gradient over the membrane while other membrane bound enzyme complexes are required to use the proton gradient. The membrane bound enzyme complex ATP synthase can either use the proton gradient for ATP synthesis or ATP hydrolysis to build the proton gradient.

In addition to substrate level phosphorylation and the proton gradient over the cytoplasmic membrane, an only recently discovered process called flavin-based electron bifurcation has been considered as a third mechanism for energy conservation [59]. In the last decade, several of such cytoplasmic bifurcation complexes were determined in bacteria and archaea [59–67]. Instead of coupling two redox reactions, as is performed by commonly known redox proteins, bifurcation (and the reversed reaction termed confurcation) enzyme complexes couple three redox reactions. With this concept, energy that would otherwise have been lost can be conserved or endergonic reactions can be coupled to exergonic reactions and reducing equivalents that are generated can be re-oxidized efficiently. For instance endergonic reduction of ferredoxin with NADH is coupled to the exergonic reduction of crotonyl-CoA to butyryl-CoA by the butyryl-CoA/electron transfer flavoprotein complex of *Clostridium kluyveri* [62]. Another example is the [FeFe]-hydrogenase complex of *Thermotoga maritima* that couples reversible ferredoxin reduction with hydrogen to NAD^+ reduction [63]. In addition to cytoplasmic bifurcating enzyme complexes, membrane bound complexes (Rnf-complexes) were recently shown to conserve energy by the reversible translocation of protons or sodium from ferredoxin oxidation with NAD^+ [68]. The energy conserving hydrogenase (Ech) has a similar function, but performs the proton or sodium translocation by ferredoxin oxidation with hydrogen production [69].

3.2. Syntrophic butyrate degradation

Butyrate oxidation coupled to hydrogen or formate production is endergonic under standard conditions. This is shown by the positive Gibbs free energy changes; +48 kJ and +55 kJ, respectively (Table 3). When butyrate oxidation is coupled to methane production the conversion is energetically feasible. To share this energy between the syntrophic butyrate oxidizer and the methanogen in such a manner that both micro-organisms gain enough energy to grow, the hydrogen and formate concentrations have to be kept in a certain low range (around 2 Pa) [19]. *S. wolfei*, *Syntrophus aciditrophicus* and *Syntrophothermus lipocalidus* can couple butyrate oxidation to syntrophic growth with methanogens and cannot grow in pure culture with any electron acceptor [26,27,70].

All known syntrophic butyrate degraders oxidize butyrate via the beta-oxidation pathway (Table 4, Fig. 1) [8,48]. This pathway includes two reactions that generate electron pairs and one reaction that generates ATP. This ATP partially has to be invested in the endergonic conversion of butyryl-CoA to crotonyl-CoA. The biochemical mechanism that enables investment of a fraction of ATP for the endergonic conversion of butyryl-CoA to crotonyl-CoA has recently been revealed in *S. wolfei* [25]. Electrons that are generated by the conversion of butyryl-CoA to crotonyl-CoA travel via butyryl-CoA dehydrogenase (encoded by genes with locus tags Swol_1933 and Swol_2053), an electron transfer flavoprotein (encoded by Swol_0696-7) and a membrane anchored protein that was annotated as DUF224 (encoded by Swol_0698) to the menaquinone pool in the membrane. Oxidation of reduced menaquinone is then coupled to formate generation by a membrane anchored extracytoplasmic formate dehydrogenase (encoded by Swol_0797-800) [25]. This reaction is driven by the proton motive force. The produced formate is used by the methanogen. The second reaction that generates electrons (NADH) is the conversion of hydroxybutyryl-CoA to acetoacetyl-CoA which is endergonic when coupled to hydrogen or formate production. Most likely, in *S. wolfei* this involves the [FeFe]-hydrogenase (encoded by Swol_1017-19) that forms a cytoplasmic complex with a formate dehydrogenase (Swol_0783-6) [72].

3.3. Syntrophic propionate degradation

Propionate oxidation coupled to hydrogen or formate production is endergonic under standard conditions. This is shown by the positive Gibbs free energy changes; +72 kJ and +82 kJ respectively (Table 3). However, when propionate oxidation is coupled to methane production the conversion is energetically feasible. To share energy between the

Table 4
Physiological characteristics of the short chain fatty acid degrading syntrophs and non-syntrophs.

	Syntrophomonas wolfei subsp. *wolfei*	*Syntrophus aciditrophicus*	*Syntrophothermus lipocalidus*	*Syntrophobacter fumaroxidans*	*Pelotomaculum thermopropionicum*	*Tepidanaerobacter acetatoxydans* Re1	*Thermoacetogeni-um phaeum*	*Clostridium ultunense* DSM 10521	*Desulfotomaculum kuznetsovii*	*Desulfobulbus propionicus*
Gram reaction	−	−	−[a]	−	−[a]	+/−[b]	+	−[a]	−[a]	−
Motility	+	−	+	−	−	+		+	+	−
Spore formation	−	−	−	−	+	+	+	+	+	−
Growth pH (range/optimum)	ND/7.2?	ND/7.0?	5.8–7.5 (6.5–7)	6.0–8.0/7	6.5–8.0/7.0	4–9.5/7?	5.9–8.4/6.8	5–10 (6.5–8)	ND	6.0–8.6 (7.1–7.5)
Growth temperature (°C) (range/optimum)	ND/35	25–42/35	45–60/55	20–40/37	45–65/55	25–55/35	40–65/58	15–50/37	50–85/60–65	10–43/39
Growth rate (d^{-1})	0.27 In coculture on butyrate with *M. hungatei*	0.22 In coculture with G11	0.93 in pure culture on crotonate 1.06 in coculture on butyrate	0.17 In coculture	0.19 coculture on propionate 2.4 coculture on ethanol	0.1	0.7	ND	ND	0.42 (propionate + sulfate)
Cytochrome b and -c menaquinone	Cyt C MK-7	MQ	Not found	Cyt b Cyt c MK-6 MK-7	MK	ND	MQ-7	Not found	ND	Cyt b Cyt c MK-4 MK-5
Metabolic pathway used	β-Oxidation	β-Oxidation	β-Oxidation	Methyl-malonyl-CoA	Methyl-malonyl-CoA	Wood Ljungdahl	Wood Ljungdahl	Wood Ljungdahl Acetyl-CoA	Wood Ljungdahl Methyl-malonyl-CoA β-Oxidation	Methyl-malonyl-CoA
Complete/incomplete oxidizer	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Complete	Complete	Incomplete?	Complete	Incomplete
Electron acceptor utilization in pure culture	None	None	None	Sulfate, thiosulfate, fumarate	Fumarate	Thiosulfate	Sulfate	None	Sulfate, sulfite, thiosulfate	Sulfate, sulfite, thiosulfate, nitrate, oxygen, Fe(III)
Substrate utilization in pure culture	Crotonate	Crotonate	Crotonate	Propionate, formate, fumarate, succinate, hydrogen, malate, aspartate, pyruvate	Propionate, fumarate, pyruvate, ethanol, lactate	Pyruvate, malate, citrate, glycerol, glucose, fructose, galactose, lactose, cellobiose, salicin, dimethylamine, histidine, cysteine, methionine, serine, cas aa, tryptone	Methanol, ethanol, n-propanol, n-butanol, 2,3-butanediol, ethanolamine, pyruvate, 3,4,5-trimethoxy-benzoate, syringate, vanillate, glycine, cysteine, formate, H_2/CO_2	Formate, betaine, glucose, pyruvate, ethylene glycol, cysteine	Formate, acetate, propionate, butyrate, valerate, lactate, malate, fumarate, succinate, methanol, ethanol, propanol, butanol, hydrogen, (up to 50%) CO	Propionate, lactate, pyruvate, ethanol, 1-propanol + 1 butanol, H_2
Substrate utilization in co-culture	Butyrate, caproate, caprylate, valerate, heptonoate, isoheptanoate	Butyrate, benzoate, hexanoate, heptanoate, octanoate, palmitate, Stearate, trans-2-pentenoate, trans-2-hexanoate, trans-3-hexanoate, 2-octenoate, methyl esters of butyrate and hexanoate,	Butyrate, isobutyrate straight-chain fatty acids from C4 to C10	Propionate	Propionate, ethanol, lactate, 1-butanol, ethylene glycol, 1-propanol, 1-pentanol, 1,3-propanediol	Acetate	Acetate	Acetate	None	None
Syntrophic partner used	*Methanospirillum hungatei* *Desulfovibrio strain G11* *Methano-bacterium bryantii* *Strain MoH* *Methano-brevibacter arboriphilus*	*Methanospirillum hungatei* *Desulfovibrio strain* G11 in the presence of sulfate	*M. thermoautotrophicum*	*Methanospirillum hungatei* *Methanobacte-rium formicicum*	*Methanothermo-bacter thermoautotro-phicus*	*Methanoculleus* sp. strain MAB2	*Methanothermo-bacter thermoautotrophicus* strain TM	*Methanoculleus* sp. strain MAB1 (reference 2)	None	None
References	[73,74] [24,70]	[26,48]	[27]	[28,75,76]	[18,77,78]	[50]	[51]	[50,52]	[66,79]	[7,30,80–84]

[a] Cells stain Gram-negative but the micro-organism has a Gram-positive cell wall ultrastructure.
[b] ND: not determined or not reported.

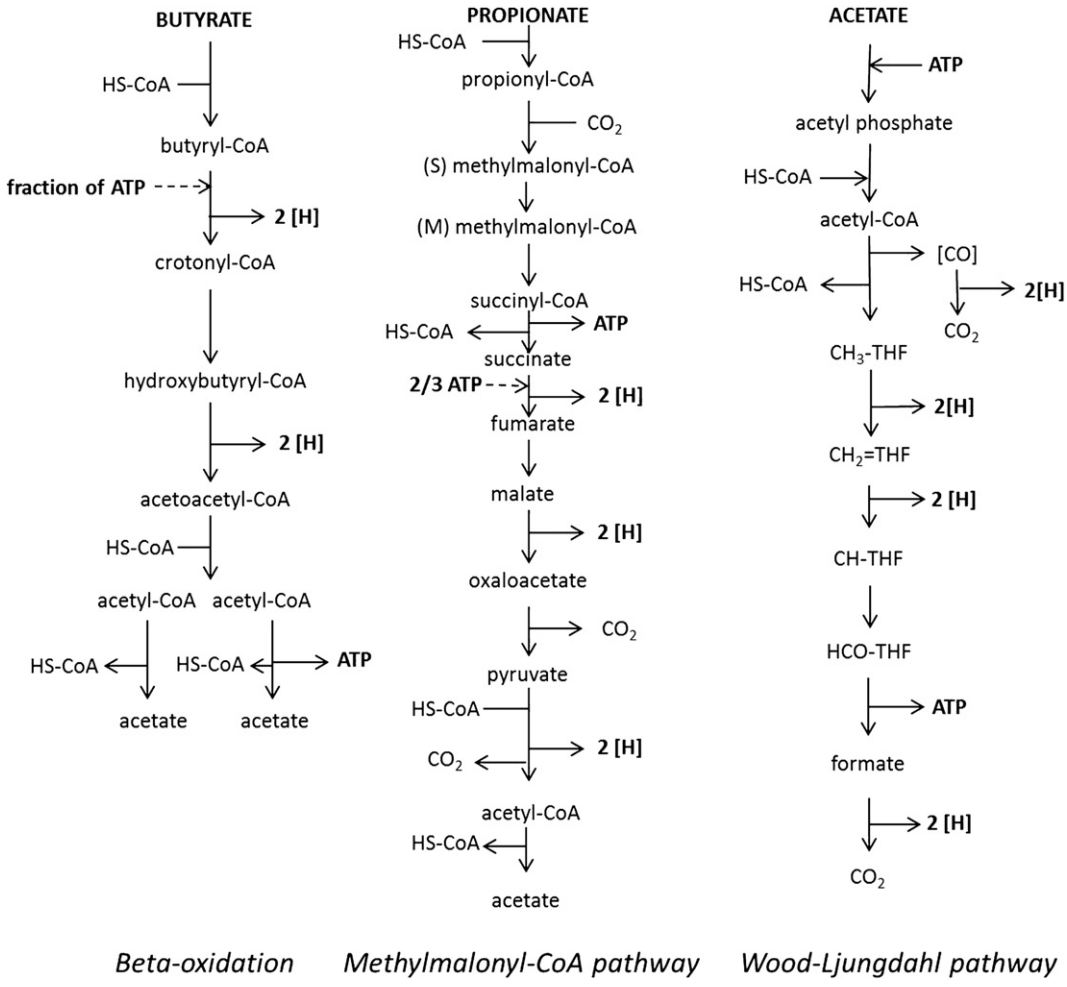


Fig. 1. Metabolic pathways that are used for acetate, propionate and butyrate conversion by bacteria that can grow in syntrophy with methanogens.

syntrophic propionate oxidizer and the methanogen in such a manner that both micro-organisms gain enough energy to grow, the hydrogen and formate concentrations have to be kept in a low range (around 40 Pa) [19]. *Smithella propionica*, *Syntrophobacter fumaroxidans* and *Pelotomaculum thermopropionicum* are able to couple propionate oxidation to syntrophic growth with methanogens [18,28,78,85]. *Smithella propionica* degrades propionate via a dismutating pathway to acetate and butyrate, which is subsequently oxidized to acetate [86]. All other known syntrophic propionate-degrading bacteria use the methylmalonyl-CoA pathway to oxidize propionate to acetate and CO_2 (Fig. 1). In this pathway one ATP is formed via substrate level phosphorylation. Part of that ATP has to be invested and three conversions in the methylmalonyl-CoA pathway generate each two electrons.

One of the reactions that generates electrons is the endergonic oxidation of succinate to fumarate that requires investment of 2/3 ATP [19]. van Kuijk et al. (1998) [75] proposed that succinate oxidation could be coupled to extra-cytoplasmic hydrogen or formate formation via a menaquinone loop between a cytoplasmic oriented membrane-bound succinate dehydrogenase and a periplasmic oriented membrane bound hydrogenase or formate dehydrogenase. Genes coding for a periplasmic hydrogenase and three extra-cytoplasmic formate dehydrogenases were found in the genome of *S. fumaroxidans* [87]. Especially the gene Sfum_1273-74 that codes for one of the periplasmic formate dehydrogenase alpha subunits is highly transcribed during syntrophic growth [88]. Also malate oxidation to oxaloacetate generates two electrons (NADH) [89]. The third reaction that generates electrons is the conversion of pyruvate to acetyl-CoA and CO_2 by pyruvate:ferredoxin oxidoreductases [90]. Genome analysis suggests that NADH generated from malate oxidation and reduced ferredoxin generated from pyruvate oxidation could be coupled to formate or hydrogen production by confurcating formate dehydrogenases and hydrogenases [87]. Such a mechanism would use the energy that remains from ferredoxin oxidation with protons to allow the endergonic coupling of NADH oxidation to proton reduction. Formate dehydrogenases from *S. fumaroxidans* were studied for subunit-composition, enzyme activity, cofactor binding and direction of conversion. Formate dehydrogenase 1 contains W, Se, four [2Fe2S], one [4Fe4S] and is a hetero-trimer. Formate dehydrogenase 2 contains W, Se, two [4Fe4S] and is heterodimer. Both enzymes oxidize formate with benzyl viologen and reduce CO_2 with reduced methylviologen. The purified enzyme was not able to reduce NAD^+ [91]. Whether these formate dehydrogenases can confurcate electrons from NADH and reduced ferredoxin to CO_2 reduction, has never been tested.

3.4. Syntrophic acetate oxidation

Acetate oxidation coupled to hydrogen or formate production under standard conditions is endergonic, as is shown by the positive Gibbs free energy changes; +95 kJ and +109 kJ, respectively (Table 3). However, methane production from hydrogen and formate is exergonic, as is shown by the negative Gibbs free energy changes; −131 kJ and −145 kJ, respectively. When acetate oxidation is coupled to methane formation, energy (−36 kJ/reaction) is available for maintenance and growth. To share this energy between the syntrophic acetate oxidizer and the

methanogen in such a manner that both micro-organisms gain enough energy to grow, the hydrogen and formate concentrations have to be in a low range (10 to 50 Pa). *Tepidanaerobacter acetatoxydans* Re1 and *Thermacetogenium phaeum* can oxidize acetate in syntrophic relationship with methanogens [50,92] and both use the Wood–Ljungdahl pathway for acetate oxidation (Table 4). Complete acetate conversion to CO_2 involves four reactions that each generate an electron pair. One ATP is required for acetate activation (Fig. 1) and from the conversion of formyl tetrahydrofolate (HCO-THF) to formate one ATP can be generated via substrate level phosphorylation. How energy is conserved and fuelled to support growth and maintenance is unknown.

The genome of *T. phaeum*'s contains mostly single gene copies of enzymes involved in the Wood–Ljungdahl pathway, which indicates that enzymes are used bidirectional [51]. Although electron transfer mechanisms involved in acetate oxidation are unknown, they might be similar, or even the same as those used for acetate production. The acetogenic bacteria *Moorella thermoacetica* and *Acetobacterium woodii* use the Wood–Ljungdahl pathway in the direction of CO_2 reduction to acetate. *M. thermoacetica* possesses two electron-bifurcating enzymes during acetogenesis; an NADH-dependent Fd_{red}:$NADP^+$ oxidoreductase and a hydrogenase that couples NAD^+ reduction with ferredoxin [61] that probably are involved in the inter-conversion of redox couples and coupling of reducing equivalents to hydrogen production. Which reducing equivalents are involved still remains unclear. *A. woodii* might use an Rnf-complex and a hydrogenase that couples NAD^+ reduction with ferredoxin [93].

In the genome of the syntrophic acetate oxidizing bacterium *T. acetatoxydans* Re1, no genes coding for formate dehydrogenases or cytochromes were found [94], therefore it is probably not able to couple intracellular redox reactions to extracellular formate production via cytochrome and quinones. However, a gene coding for a formate transporter is present in the genome of *T. acetatoxydans* Re1.

Syntrophic acetate oxidizers use the Wood–Ljungdahl pathway in reverse, when growing on acetate, as was proposed already by Barker in 1936 and later demonstrated by Zinder and Koch (1984) with their culture of "*Reversibacter*" [95,96]. *T. acetatoxydans* cannot produce acetate by CO_2 reduction with hydrogen [50]. This is consistent with the finding that the genome of *T. acetatoxydans* lacks genes coding for a formate dehydrogenase that is essential for the first step of CO_2 reduction in the Wood Ljungdahl pathway. It does not explain why *T. acetatoxydans* cannot grow on formate [50], since the formate transporter could deliver formate directly in the cytoplasm entering the methyl-branch of the acetogenic pathway. This also implies that during syntrophic acetate oxidation by *T. acetatoxydans* Re1, formate is formed from HCO-THF by formyl tetrahydrofolate synthase (FTHFS) and is transported via the formate transporter over the membrane. Specific primers targeting FTHFS of *T. acetatoxydans* Te1 have been successfully used to quantify its abundance in anaerobic biogas reactor with increasing ammonia load [50]. As the syntrophic partner of *T. acetatoxydans* Re1 is *Methanoculleus* sp. strain MAB2 that can grow with H_2 and CO_2 and formate [97], both hydrogen and formate can be the interspecies metabolite. The absence of a formate dehydrogenase coding gene has also consequences for the acetogenic growth of the bacterium on sugars, since reducing equivalents generated during glycolysis cannot be used to reduce CO_2. The presence of a pyruvate formate lyase coding gene (TEPIRE1_470) in *T. acetatoxydans* may compensate for this and produce formate directly from pyruvate. Also in this case, formate is directly entering the methyl-branch of the acetogenic pathway.

Hydrogen formation during acetate oxidation by *T. acetatoxydans* Re1 is likely through an [FeFe]-hydrogenase that couples proton reduction to hydrogen generation.

3.5. Syntrophic formate degradation

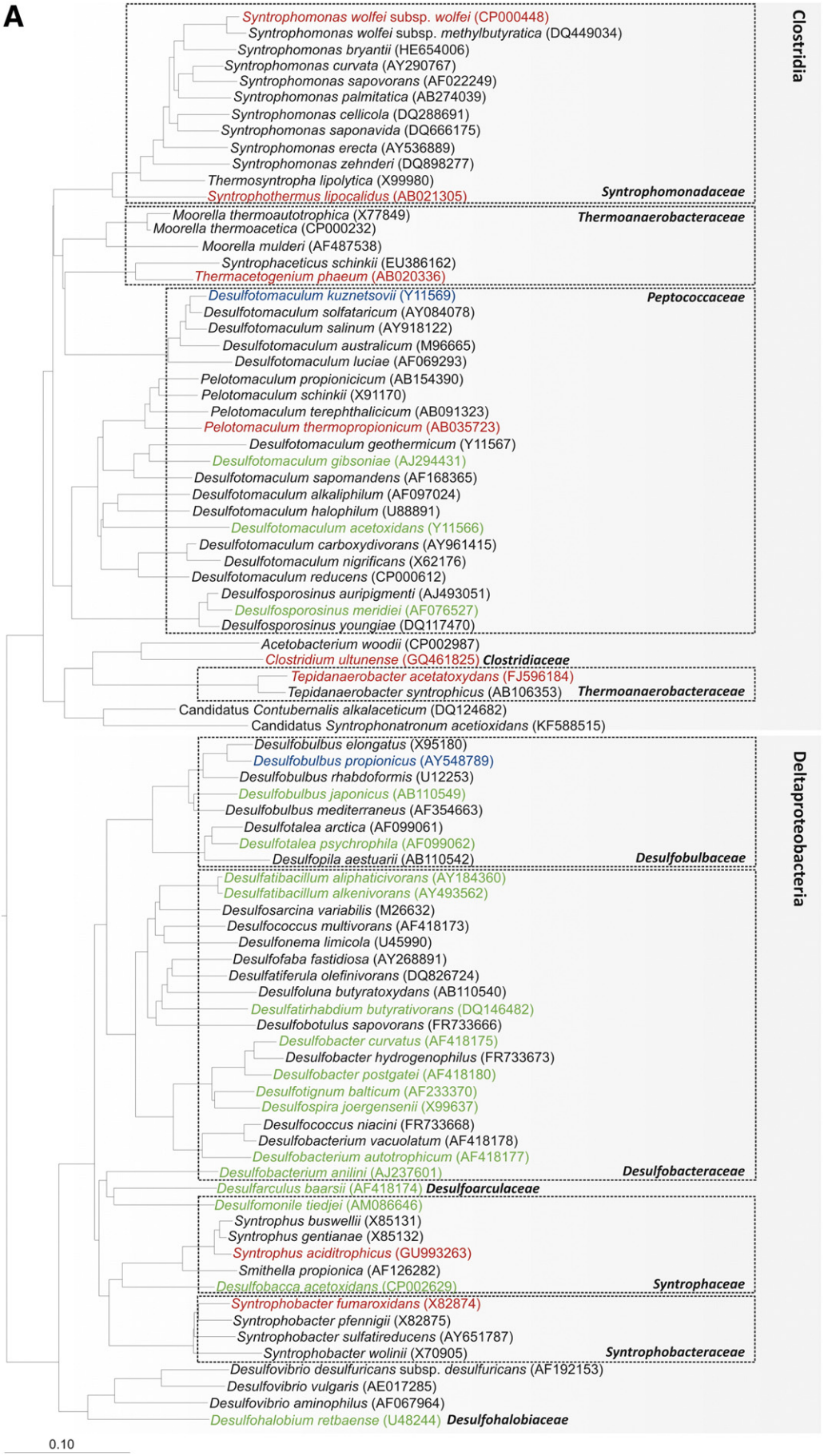
In the degradation of SCFA, formate and hydrogen play an important role as electron shuttling components. Interestingly syntrophic growth with formate occurs as well. Formate oxidation coupled to hydrogen is endergonic under standard conditions. This is shown by the Gibbs free energy change that is close to zero; −3.4 kJ (Table 3). However, when formate oxidation is coupled to methane production the conversion is energetically feasible. To share energy between the syntrophic propionate oxidizer and the methanogen in such a manner that both micro-organisms gain enough energy to grow, the hydrogen concentration has to be kept in a low range (between 40 and 100 Pa) [14]. The thermophilic *Moorella* sp. strain AMP and mesophilic *Desulfovibrio* sp. strain G11 are able to couple formate oxidation to syntrophic growth with methanogens that can only use hydrogen as electron donor [14]. The electron transfer mechanism that allows syntrophic formate degradation is not known. It was proposed that an extracytoplasmic formate dehydrogenase is coupled to a membrane integrated, cytoplasmic oriented hydrogenase which generates a proton motive force that can be used to drive ATP synthesis [14]. To what extent and in what types of anaerobic microbial environments syntrophic formate degradation can compete with formate degradation by methanogens is not known.

4. Phylogeny of short chain fatty acid degraders does not predict syntrophy

Syntrophic methanogenic growth on butyrate is performed by bacteria belonging to the Firmicutes (*Syntrophomonas*, *Syntrophothermus*, *Thermosyntropha* genera) and Deltaproteobacteria (*S. aciditrophicus*). *Syntrophomonas* is the best represented genus within syntrophic fatty-acid degraders (in terms of available isolates), with 11 species and/or subspecies described thus far [98]. Nevertheless, only the genome of *S. wolfei* subsp. *wolfei* has been sequenced [99]. Propionate can be syntrophically utilized by *Pelotomaculum*- and *Syntrophobacter* species [100]. In addition, *S. propionica* can degrade propionate in syntrophy with methanogens [85]. *Syntrophobacter* species can use propionate in syntrophy with hydrogenotrophic methanogens, or alone if sulfate is available in the environment [16]. *Pelotomaculum* species do not possess the ability to grow with propionate and sulfate. The genomes of *S. fumaroxidans* and *P. thermopropionicum* are available [77,101]. A restricted number of bacterial species is known to degrade acetate in syntrophy with hydrogenotrophic methanogens, namely *Clostridium ultunense* [52], *T. phaeum* [92], *Thermotoga lettingae* [102], *Syntrophaceticus schinkii* [103], *Tepidanaerobacter syntrophicus* [104], *T. acetatoxydans* [50] and Candidatus "*Syntrophonatronum acetioxidans*" [105]. These bacteria are all affiliated with the phylum Firmicutes, except *T. lettingae* that belongs to the phylum Thermotogae. *T. phaeum*, *T. lettingae* and *T. acetatoxydans* are the syntrophic acetate oxidizers that have their genome sequenced and available, thus far. Although *T. phaeum* and *T. acetatoxydans* both belong to the *Thermoanaerobacteraceae* family, these two species share only 83% 16S rRNA gene sequence identity.

Dissimilatory sulfate-reducing bacteria able to use fatty-acids are very diverse. Sulfate-reducing bacteria analyzed in the scope of this review are distributed among Deltaprobacteria and Firmicutes phyla (Fig. 2a, species highlighted in green color). *Desulfotomaculum* species belong to *Peptococcaceae* family, the same family of the syntrophic *Pelotomaculum* species. Recently, it was shown that the genomes of *D. kuznetsovii* and *P. thermopropionicum* have a high similarity [79]. The genes involved in propionate metabolism of these two strains are similar, but main differences were found in genes involved in the electron acceptor metabolism. Some *Desulfotomaculum* species – *D. thermobenzoicum* subsp. *thermosyntrophicum* and *D. thermocisternum* – were also shown to grow on propionate in syntrophy with a hydrogenotrophic methanogen (without sulfate) [106,107].

Two phylogenetic trees were constructed (Fig. 2). Fig. 2A shows a 16SrRNA gene based tree which shows bacterial species used in this study and their classification and phylogeny with species whose genome has not been sequenced. Fig. 2B shows a genome wide functional domain based tree of the bacterial species included in this study. Both


A
Clostridia
Deltaproteobacteria
Syntrophomonas wolfei subsp. wolfei (CP000448)
Syntrophomonas wolfei subsp. methylbutyratica (DQ449034)
Syntrophomonas bryantii (HE654006)
Syntrophomonas curvata (AY290767)
Syntrophomonas sapovorans (AF022249)
Syntrophomonas palmitatica (AB274039)
Syntrophomonas cellicola (DQ288691)
Syntrophomonas saponavida (DQ666175)
Syntrophomonas erecta (AY536889)
Syntrophomonas zehnderi (DQ898277)
Thermosyntropha lipolytica (X99980)
Syntrophothermus lipocalidus (AB021305)
Syntrophomonadaceae
Moorella thermoautotrophica (X77849)
Moorella thermoacetica (CP000232)
Moorella mulderi (AF487538)
Syntrophaceticus schinkii (EU386162)
Thermacetogenium phaeum (AB020336)
Thermoanaerobacteraceae
Desulfotomaculum kuznetsovii (Y11569)
Desulfotomaculum solfataricum (AY084078)
Desulfotomaculum salinum (AY918122)
Desulfotomaculum australicum (M96665)
Desulfotomaculum luciae (AF069293)
Pelotomaculum propionicicum (AB154390)
Pelotomaculum schinkii (X91170)
Pelotomaculum terephthalicicum (AB091323)
Pelotomaculum thermopropionicum (AB035723)
Desulfotomaculum geothermicum (Y11567)
Desulfotomaculum gibsoniae (AJ294431)
Desulfotomaculum sapomandens (AF168365)
Desulfotomaculum alkaliphilum (AF097024)
Desulfotomaculum halophilum (U88891)
Desulfotomaculum acetoxidans (Y11566)
Desulfotomaculum carboxydivorans (AY961415)
Desulfotomaculum nigrificans (X62176)
Desulfotomaculum reducens (CP000612)
Desulfosporosinus auripigmenti (AJ493051)
Desulfosporosinus meridiei (AF076527)
Desulfosporosinus youngiae (DQ117470)
Peptococcaceae
Acetobacterium woodii (CP002987)
Clostridium ultunense (GQ461825)
Clostridiaceae
Tepidanaerobacter acetatoxydans (FJ596184)
Tepidanaerobacter syntrophicus (AB106353)
Thermoanaerobacteraceae
Candidatus Contubernalis alkalaceticum (DQ124682)
Candidatus Syntrophonatronum acetioxidans (KF588515)
Desulfobulbus elongatus (X95180)
Desulfobulbus propionicus (AY548789)
Desulfobulbus rhabdoformis (U12253)
Desulfobulbus japonicus (AB110549)
Desulfobulbus mediterraneus (AF354663)
Desulfotalea arctica (AF099061)
Desulfotalea psychrophila (AF099062)
Desulfopila aestuarii (AB110542)
Desulfobulbaceae
Desulfatibacillum aliphaticivorans (AY184360)
Desulfatibacillum alkenivorans (AY493562)
Desulfosarcina variabilis (M26632)
Desulfococcus multivorans (AF418173)
Desulfonema limicola (U45990)
Desulfofaba fastidiosa (AY268891)
Desulfatiferula olefinivorans (DQ826724)
Desulfoluna butyratoxydans (AB110540)
Desulfatirhabdium butyrativorans (DQ146482)
Desulfobotulus sapovorans (FR733666)
Desulfobacter curvatus (AF418175)
Desulfobacter hydrogenophilus (FR733673)
Desulfobacter postgatei (AF418180)
Desulfotignum balticum (AF233370)
Desulfospira joergensenii (X99637)
Desulfococcus niacini (FR733668)
Desulfobacterium vacuolatum (AF418178)
Desulfobacterium autotrophicum (AF418177)
Desulfobacterium anilini (AJ237601)
Desulfobacteraceae
Desulfarculus baarsii (AF418174)
Desulfoarculaceae
Desulfomonile tiedjei (AM086646)
Syntrophus buswellii (X85131)
Syntrophus gentianae (X85132)
Syntrophus aciditrophicus (GU993263)
Smithella propionica (AF126282)
Desulfobacca acetoxidans (CP002629)
Syntrophaceae
Syntrophobacter fumaroxidans (X82874)
Syntrophobacter pfennigii (X82875)
Syntrophobacter sulfatireducens (AY651787)
Syntrophobacter wolinii (X70905)
Syntrophobacteraceae
Desulfovibrio desulfuricans subsp. desulfuricans (AF192153)
Desulfovibrio vulgaris (AE017285)
Desulfovibrio aminophilus (AF067964)
Desulfohalobium retbaense (U48244)
Desulfohalobiaceae
0.10

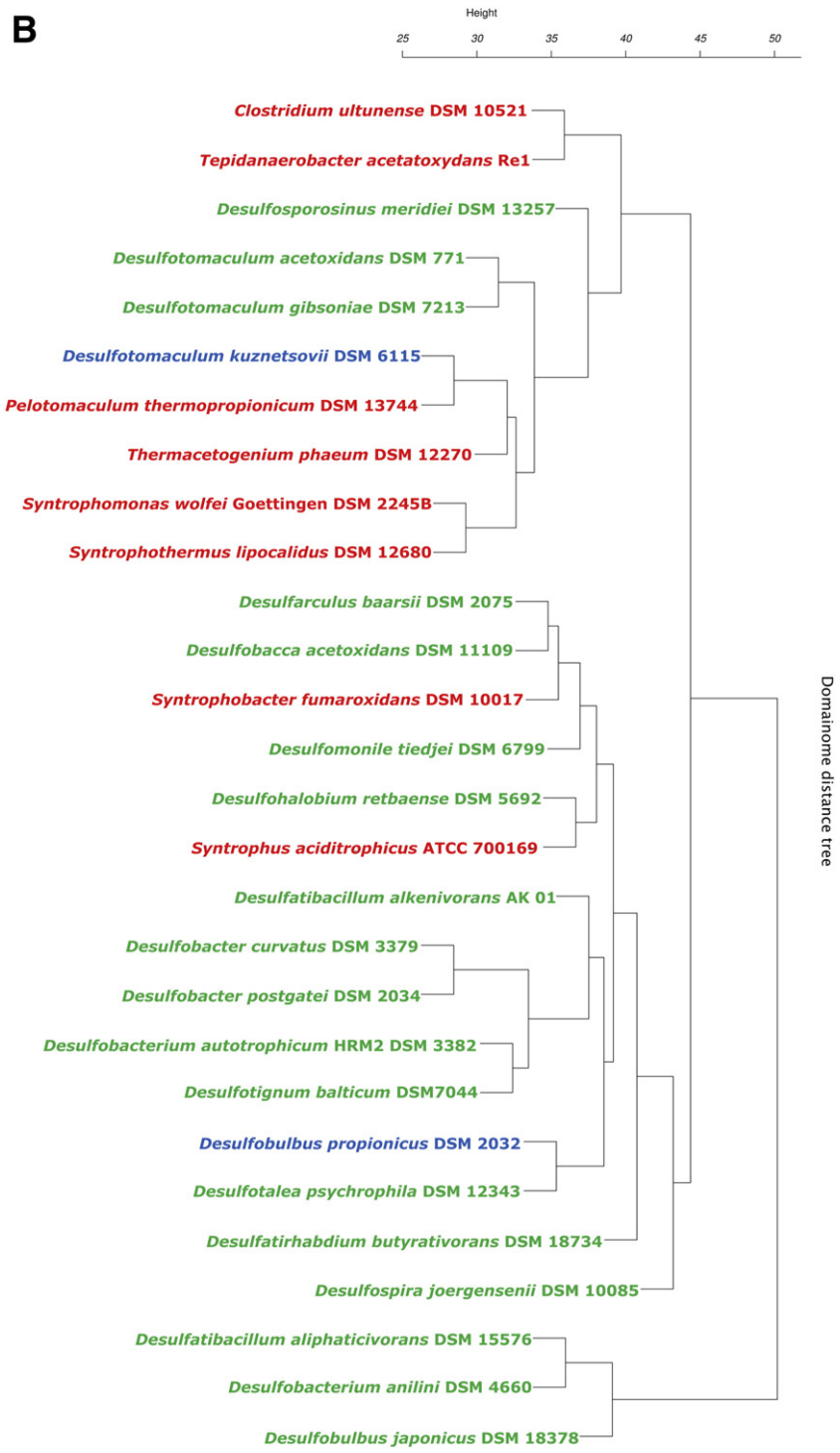


Fig. 2. Neighbor joining tree based on 16S rRNA gene sequences (A) and a tree based on the presence and absence of functional domain (B), both showing the phylogenetic affiliation of bacteria analyzed in this work. In red — bacteria that have been previously shown to grow in syntrophy with methanogens; in blue — sulfate-reducing bacteria. In the 16S rRNA gene based tree sequences of different *Thermotoga* species were used as an outgroup but were pruned from the tree. The scale bar represents 10% sequence divergence.

trees show that the ability to grow syntrophically is widely distributed, meaning that phylogeny of short chain fatty acid degraders does not predict syntrophy. The ability to grow in syntrophy is either first evolved and then lost or acquired by horizontal gene transfer from a syntroph to a non-syntroph. Multiple horizontal gene transfers of dissimilatory sulfite reductase genes (*dsrAB*) in sulfate-reducing prokaryotes have been suggested by [108]. These authors found that the topology of a tree based on a large fragment of the *dsrAB* did not match completely with the corresponding 16S based tree.

5. Conclusions

Systematic functional profiling of genomes shed light on the question: "what are the key properties that make that a SCFA degrading bacterium is able to grow in syntrophy with methanogens and another not". The presence or absence of extra-cytoplasmic formate dehydrogenases, including their maturation proteins is clearly a difference between syntrophic and non-syntrophic butyrate and/or propionate-degraders. Further biochemical examination and knock-out

experiments of genes involved in extra-cytoplasmic formate dehydrogenase activity and maturation would give more insight in the importance of this enzyme complex during syntrophy. Genetic manipulation protocols for SCFA degrading syntrophic bacteria have to be developed. Furthermore the presence or absence of two domains, both linked to membrane integrated proteins with a currently unknown function in syntrophy, appears to make a difference as well. Both are membrane integrated proteins. One is putatively involved in capsule or biofilm formation and a second in cell division, shape-determination or sporulation. Capsule formation, cell division, shape-determination and sporulation by these bacteria during syntrophic growth could be assessed with microscopic techniques.

Sulfate-reducing bacteria such as *D. autotrophicum* HRM2, *D. tiedjei* and *D. meridiei* were never tested for syntrophic growth, but all crucial domains discussed in this review were found in corresponding genomes, which suggests their possible ability to grow in syntrophic association with methanogens. In addition, profiling domains involved in electron transfer mechanisms revealed the important role of the Rnf-complex and the formate transporter in syntrophy, and indicate that DUF224 may have a role in electron transfer in bacteria other than *S. wolfei* as well.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbabio.2014.06.005.

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