Biochimica et Biophysica Acta 1837 (2014) 2004–2016

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

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

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ARTICLE INFO

Article history:
Received 12 December 2013
Received in revised form 5 June 2014
Accepted 9 June 2014
Available online 26 June 2014

Keywords:
Syntrophy
Butyrate
Propionate
Acetate
Formate
Functional profiling

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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http://dx.doi.org/10.1016/j.bbabio.2014.06.005
0005-2728/

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