Genomic insights into the syntrophic metabolism of propionate oxidation


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Abstract: To get insight into syntrophy revolving around propionate degradation in anaerobic digestion, we sequenced the genome and transcriptome of two propionate-degrading cultures: Pelotomaculum schinkii + Methanospirillum hungatei and Smithella propionica + Methanobacterium formicicum. Results show that formate is the preferred electron carrier for P. schinkii syntrophy, and is potentially involved in energy conservation through a newly proposed proton-pumping extrusion mechanism. We also found that P. schinkii and M. hungatei may interact through flagellar contact and complementary amino acid exchange. Further on, we successfully identified the genes and enzymes responsible for an unusual mode of propionate degradation by S. propionica. Notably, S. propionica uses a novel 2-methyl-3-hydroxy-valeryl-CoA mutase and 4-hydroxy-caproyl-CoA dehydratase that are functionality unique from any known homologs.

Keywords: propionate; syntrophy; omics

Session – Microbiology research of anaerobic digestion/genomic research

Introduction

Propionate is a common product of the anaerobic conversion of odd-chain fatty acids, amino acids, and sugars. Its subsequent conversion by propionate oxidisers is critical during the anaerobic digestion of wastes. However, knowledge on the metabolism and energy conservation in propionate oxidation is limited. Currently, propionate oxidation is known to require mutualistic symbiosis between a H₂/formate-producing propionate oxidiser (“syntroph”) and H₂/formate-oxidizing methane producer (“methanogen”). The exchange of product/substrate has been considered the basis for syntrophy, but it is possible that other molecules or mechanisms play an important role in this interaction as well.

So far, three pathways have been characterized for propionate oxidation (e.g., methylmalonyl-CoA pathway, acryl-CoA pathway, and 2-methylcitrate cycle), but work by de Bok et al. (2001) showed that Smithella propionica LYP employs a new pathway. Although stable isotope analyses revealed that two propionate molecules are condensed and oxidatively split into three acetate molecules, the detailed biochemistry of this unique pathway remains to be uncovered.

In this work we studied two metabolically distinct syntrophic propionate oxidisers: Pelotomaculum schinkii (methylmalonyl-CoA pathway) and Smithella propionica (novel pathway) to get more insights into the metabolism, energy conservation, and syntrophic behaviour of these bacteria.

Material and Methods

Syntrophic cultures of Pelotomaculum schinkii + Methanospirillum hungatei and Smithella propionica + Methanobacterium formicicum were grown in anaerobic medium containing 20 mM of propionate. Substrate consumption and product profiling were analysed over time using liquid and gas chromatography. Cultures in late exponential growth were harvested and used for genome and transcriptome sequencing. A detailed description of the procedures used for cultivation and omics analyses can be found in Hidalgo-Ahumada et al. (2018).
Results and Conclusions

**Novel energy conservation strategies of Pelotomaculum schinkii growing on propionate**

The genome of *P. schinkii* encoded the methylmalonyl-CoA pathway for propionate oxidation: propionate uptake, propionate activation to propionyl-CoA, carboxylation to methylmalonyl-CoA, subsequent isomerization to succinyl-CoA, oxidative decarboxylation to acetyl-CoA via oxaloacetate and pyruvate, acetyl-CoA dethiolation to acetate, and acetate export.

Under methanogenic conditions, syntrophic organisms like *Pelotomaculum* spp., must resort to using H⁺ or CO₂ as electron sinks to regenerate reduced electron carriers formed during substrate oxidation (e.g., NADH, reduced ferredoxin). For this, *P. schinkii* encodes confurcating hydrogenases and formate dehydrogenases. Transcriptomic examination of *P. schinkii* in co-culture with *Methanospirillum hungatei*, revealed that formate may be the preferred electron carrier for *P. schinkii* syntrophy. Unlike H₂, formate is an anion, so it cannot freely pass the cell membrane and will inevitably accumulate in the cytosol. To address this, *P. schinkii* encodes a formate transporter (FdhC). We hypothesize that FdhC can take advantage of the accumulated formate and couple exergonic formate extrusion with endergonic proton extrusion for proton motive force generation. In agreement, FdhC’s in *P. schinkii* and *M. hungatei* are coordinated in operons with their respective formate dehydrogenases. This is a novel energy conservation mechanism for syntrophic propionate degradation. *P. schinkii* did not overexpress conventional energy metabolism associated with a model syntrophic propionate degrader *Syntrophobacter fumaroxidans* (i.e., Fix and Rnf).

We further explored the genome of *P. schinkii* and the transcriptome of the syntrophic-coculture for non-catabolic behavior relevant to syntrophy. Another *Pelotomaculum* species has been demonstrated to employ flagella to physically facilitate syntrophic interactions (Shimoyama et al., 2009a). We also observed that *P. schinkii* expresses flagellum biosynthesis genes and synthetizes flagella despite being immotile. Given that all *Pelotomaculum* spp. encode complete flagellum biosynthesis machinery, “flagellum-mediated symbiosis” may be a core mechanism for *Pelotomaculum* syntrophy. The transcriptomics also revealed amino acids exchange between *P. schinkii* and *M. hungatei*.

**New insights on the Smithella propionica pathway for propionate oxidation**

By combining gene expression, in-depth protein phylogeny, and comparative protein 3D modeling, we successfully identified the genes and enzymes responsible for the unusual transformation of propionate to acetate. Notably, *S. propionica* uses a novel 2-methyl-3-hydroxy-valeryl-CoA mutase and 4-hydroxy-caproyl-CoA dehydratase that are functionally unique from any known homologs. Besides this unique propionate oxidation, inspection of the genome of *S. propionica* reveals an unusual set of enzymes for transferring reducing power from propionate oxidation to H⁺ and CO₂ respiration. For example, *S. propionica* does not depend on conventional energy conservation mechanisms (e.g., electron bifurcation) that some of the other syntrophic bacteria encode. The results suggest that the novel propionate degradation pathway is mechanistically and energetically distinct from known propionate degradation pathways, which has significant ecological implications for syntrophy in methanogenic ecosystems.

**References**

