30. Evolution of alternative adaptive strategies sustaining two-member syntrophic communities

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Project Goals: The comparative analysis of simple two-member syntrophic communities composed of different pairings of *Desulfovibrio* and methanogenic species is used to identify evolutionarily conserved features of an environmentally relevant interspecies interaction. Initial studies have shown that although *Desulfovibrio* species have evolved alternative systems of electron and metabolite transfer for syntrophic growth in environments fluctuating in electron acceptor availability, they share similar energetic strategies involving flavin-/menaquinone-based electron confurcation and electron bifurcation (1). Alternative adaptive strategies are also associated with the syntrophic partner, revealed by the influence of different methanogenic species on the metabolism of an individual *Desulfovibrio,* and by inference the fitness of the pairing (2). Thus, continued comparative analyses of the genetic and biochemical systems sustaining this fundamental trophic interaction will contribute to a knowledgebase needed for a more predictive understanding of how complex microbial communities form and persist.

Abstract: The mineralization of organic matter in anoxic environments relies on the cooperative activities of hydrogen producers and consumers obligately linked by interspecies metabolite exchange in syntrophic consortia that may include sulphate reducing species such as *Desulfovibrio*. To evaluate the metabolic flexibility of syntrophic *Desulfovibrio* to adapt to naturally fluctuating methanogenic environments, we studied *Desulfovibrio alaskensis* str. G20 grown in chemostats under respiratory, fermentative and syntrophic conditions with alternative methanogenic partners, *Methanococcus maripaludis* and *Methanospirillum hungatei*, at different growth rates on varying energy sources. Comparative analyses of whole genome transcriptional and gene fitness (tagged transposon mutant library) data, complemented by individual G20 mutant strain growth experiments, and physiological data, revealed a significant influence of (a) energy source, (b) availability of electron donor (as controlled by dilution rate), and (c) methanogenic species on the electron transfer enzyme systems, mechanisms of energy-conservation, ratios of interspecies electron carriers, coculture population dynamics, and interspecies physical association. All data indicate that *D. alaskensis* str. G20 use both flavin- and menaquinone-based electron confurcation and bifurcation processes to drive the production of metabolites (H_2 and formate) sustaining its syntrophic association with a methanogen. During syntrophic growth on lactate, a reduced thiol/disulfide redox pair (most likely DsrC) and ferredoxin (Fd) are energetically coupled to H^+/CO_2 reduction by periplasmic formate dehydrogenase and hydrogenase via a flavin-based electron confurcation process and a menaquinone (MQ) redox loop-mediated reverse electron flow involving the membrane-bound Qmo and Qrc complexes. In contrast, *D. vulgaris* str. Hildenborough uses a larger number of cytoplasmic and periplasmic proteins linked in three intertwining pathways to couple $DsrC_{red}$ and Fd_{red} reoxidation to H^+ reduction during lactate oxidation. The faster growth of strain G20 in coculture is associated with a kinetic advantage conferred by the Qmo-MQ-Qrc loop as electron transfer system that permits higher lactate utilization rates under elevated hydrogen levels (thereby enhancing methanogenic growth), and use of formate as main electron exchange mediator (>70% electron flux), as opposed to the primarily hydrogen-based

exchange by strain Hildenborough. Although the collected data support the absence of a conserved gene core in *Desulfovibrio* that would determine the ability for syntrophic lifestyle in sulfate-reducing bacteria, systems of flavin-/menaquinone-based electron confurcation or electron bifurcation are common to both species. Remarkably, only 68 genes in *D. alaskensis* str. G20 were commonly differentially expressed under syntrophic versus respiratory lifestyle which points to its high metabolic flexibility to adjust energetically to the naturally fluctuating growth conditions in methanogenic environments. Under low energy (low growth rate) conditions, strain G20 further adapts to the metabolism of its methanogenic partners as shown by the differing gene expression of enzymes involved in the direct metabolic interactions (e.g. periplasmic hydrogenases), and the ratio shift in electron carriers used for interspecies metabolite exchange (H_2/formate). A putative monomeric [Fe-Fe] hydrogenase and Hmc complex-linked reverse MQ redox loop become increasingly important for the reoxidation of the lactate oxidation derived redox pairs, $DsrC_{red}$ and Fd_{red}, relative to the Qmo-MQ- Qrc loop. The lower growth rates also promoted close physical interspecies polar associations, presumably enabling more efficient metabolite transfer and more energy-efficient energy coupling (a similar effect was observed for strain Hildenborough cocultures). Transition from lactate to pyruvate in *D. alaskensis* str. G20 cocultures resulted in a dramatic shift in the population structure and even closer interspecies cell-to-cell interactions. Lower methane production rates in coculture than predicted from pyruvate input was attributed to redirection of electron flow to fumarate reduction. Without a methanogenic partner, accumulation of H_2 and formate resulted in greater succinate production indicating that pyruvate fermentation in strain G20 involves respiration of endogenously formed fumarate using cytoplasmic and membrane-bound energy-conserving complexes, Rnf, Hdr-Flox-1, and Hmc. At the low H_2/formate levels maintained in coculture, Rnf likely functions as proton-pumping Fd_{red}:type-I-cytochrome-c_3 oxidoreductase which transitions to a proton-pumping Fd_{red}:NADH oxidoreductase at high H_2/formate levels during fermentation in monoculture. Hdr-Flox-1 is postulated to recycle Fd_{red} via a flavin-based electron bifurcation involving NADH, Fd_{ox}, and $DsrC_{ox}$. In a menaquinone-based electron confurcation reaction, the Hmc complex is proposed to then couple $DsrC_{red}$ and periplasmic H_2/formate oxidation using the menaquinone pool to fuel a membrane-bound fumarate reductase (3).

Together these data underscore the high metabolic and energetic adaptive flexibility that likely sustains *Desulfovibrio* in naturally fluctuating methanogenic environments. These laboratory-based studies provide an important mechanistic understanding of the assembly and stability of two-member model assemblies in nature that will provide a predictive understanding of microbial processes stabilizing or destabilizing critical communities of microorganisms in the environment.

References:

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