137. A New DIET for Methanosarcina barkeri: Direct Interspecies Electron Transfer in a Genetically Tractable Methanogen

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Project Goals: The long-term goal of our project, which is entitled "Systems Level Analysis of the Function and Adaptive Responses of Methanogenic Consortia", is to develop genome-scale metabolic models of microbial communities that play an important role in the global carbon cycle that can be coupled with the appropriate physical-chemical models to predict how the microbial communities will respond to environmental perturbations, such as climate change. The short-term objective of the current research is to elucidate the mechanisms for direct interspecies electron transfer (DIET), the diversity of methanogens that are capable of DIET, and the prevalence of DIET in soils and sediments that make important contributions to atmospheric methane.

Effective interspecies electron transfer is key to the smooth functioning of methanogenic communities. Promoting interspecies electron transfer to methanogens enhances the anaerobic digestion of wastes and appropriate models of the pathways for interspecies electron transfer are necessary in order to predictively model the response of methanogenic communities to environmental change. For over 40 years interspecies hydrogen transfer has served as the paradigm for anaerobic interspecies electron transfer. However, our recent studies demonstrated that direct interspecies electron transfer (DIET) is possible[1-5] and may be the predominant mechanism for electron exchange in some methanogenic environments[5,6].

We recently reported[5] the surprising finding that methanogens in the genus *Mehthanosaeta* can accept electrons for the reduction of carbon dioxide via DIET. *Methanosaeta* species are considered to produce more methane on earth than any other group of methanogens. They are important contributors to atmospheric methane resulting from methane production in soils and sediments and are often the most active methanogens in digesters converting wastes to methane. *Methanosaeta* were previously considered to be limited to acetate as a substrate for methane production. However, metatranscriptomic analysis of anaerobic waste digester aggregates, as well as transcriptomic, radiotracer, and genetic analysis of defined co-cultures in which *Methanosaeta harundinacea* served as the sole methane- producing partner, revealed that *Methanosaeta* species can function as the primary electron-accepting organism in syntrophic partnerships, by accepting electrons via DIET[5].

In our most recent studies the potential for other methanogens to participate in DIET was evaluated. A number of methanogens that have been reported to exclusively utilize H_2 or formate as electron donors could not participate in DIET, but *Methanosarcina barkeri* could. Like *Methanosaeta* species, *M. barkeri* is able to use acetate as a substrate for methane production, but unlike *Methanosaeta* species, *M. barkeri* can also use H_2 as an electron donor for carbon dioxide reduction. When cultured in media with ethanol as the electron donor, *M. barkeri* formed syntrophic cultures with *Pelobacter carbinolicus* in which the two species exchanged electrons via interspecies H_2 transfer, whereas in co-culture with *Geobacter metallireducens*, *M. barkeri* accepted electrons via DIET. Analysis of the transcriptome of the two syntrophic cultures revealed increased transcript abundance for genes encoding putative filaments with similarity to the electrically conductive[7] type IV pili of *Geobacter* species. Furthermore, there was a

distinctive increase in transcript abundance for genes encoding several outer surface proteins in *M. barkeri* growing via DIET. These results suggest that *M. barkeri* expresses one or more outer-surface proteins to facilitate DIET.

M. barkeri is only the second methanogen found to be capable of DIET and the first methanogen known to have the option of accepting electron either via H_2 transfer or DIET. *Methanosarcina* are often among the most abundant methanogens in methanogenic soils and sediments, landfills, and anaerobic digesters. However, other than the obvious importance of DIET in some anaerobic digesters treating brewery waste[5,6], the prevalence of DIET in other methanogenic environments is unknown. The fact that at least two major genera of methanogens have evolved the capacity for DIET suggests that there are conditions in some soils and sediments in which DIET confers a selective advantage.

Although the importance of electrically conductive pili and outer-surface cytochromes in extracellular electron exchange, including DIET, is well-known for *Geobacter* species[8], it is premature to speculate on the potential extracellular electron contacts that might permit methanogens to accept electrons via DIET. The availability of tools for genetic manipulation of *barkeri*[9] suggests that it may be the ideal candidate for functional analysis of DIET mechanisms in methanogens.

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