Direct Interspecies Electron Transfer in Methanogenic Environments: Genome-Scale Analysis of Mechanisms in Defined Co-Cultures and Natural Aggregates

Amelia-Elena Rotaru[1]* (arotaru@microbio.umass.edu), Pravin Mala Shresta[1], Fanghua Liu[1], Pier-Luc Tremblay[1], Jessica Smith[1], Dan Carlo Flores[1], Mollie Murnane[1], Harish Nagarajan[2], Karsten Zengler[2], and **Derek R. Lovley[1]**

[1] University of Massachusetts, Amherst and [2] University of California San Diego, La Jolla
http://www.geobacter.org

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 in the current research is to determine if the syntrophic associations that are central to the functioning of methanogenic terrestrial wetlands can be predictively modeled with genome-scale metabolic models.

Interspecies exchange of electrons is necessary for the proper functioning of the methanogenic ecosystems that are major contributors of atmospheric methane and for successful operation of methanogenic digestors, the most effective strategy yet devised for large-scale conversion of organic wastes to fuel. 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 is possible[1] and may be the predominant mechanism for electron exchange in some methanogenic environments[2].

In order to elucidate the mechanisms of direct electron exchange under methanogenic conditions, and the factors controlling the rate of this process, studies were conducted with natural methanogenic aggregates as well as defined co-cultures. Co-cultures were established with *Geobacter metallireducens* as the electron-donating partner and either *Methanosarcina bakeria* or *Methanosaeta harudinacea* as the methanogenic partner. A *Geobacter* species was chosen because *Geobacter* species are often abundant constituents of methanogenic aggregates in anaerobic wastewater digestors as well as in methanogenic soils, such as rice paddies. *Methanosaeta* are the most abundant methanogens in similar methanogenic environments, except when they are replaced by *Methanosarcina* species.

Co-cultures of *G. metallireducens* and *Msr. barkeri* formed aggregates that effectively converted ethanol to methane. Formate could be ruled out as a potential electron shuttle between the two organisms because *Msr. barkeri* is unable to use formate as an electron donor. Although *Msr. barkeri* has the potential to use hydrogen, hydrogen did not appear to be an important intermediate for electron exchange because the cells within the aggregates were not adapted for hydrogen utilization. These results are consistent with direct interspecies electron transfer between *G. metallireducens* and *Msr. barkeri*.

The possibility of direct interspecies electron transfer is being further evaluated with studies in which on or more of the co-culture partners are strains that are deficient in components that are considered to be important for this process. For example, the PilA-pili of *Geobacter sulfurreducens* were recently shown to possess metallic-like conductivity that permits long-range electron transfer along their length and play a role in interspecies electron transfer[3]. Therefore, a

strain of *G. metallireducens* that cannot produce PilA-pili was constructed and it is being determined whether this mutant can form syntrophic associations with *Msr. barkeri*. Furthermore, gene expression patterns in the two microorganisms are being compared with gene expression patterns determined in studies with previously described[1] co-cultures of *Geobacter* species conducting interspecies electron transfer.

Co-cultures of *G. metallireducens* and *Mst. harudinacea* that effectively converted ethanol to methane were also established. The ability to these two organisms to form syntrophic aggregates is significant because *Mst. harudinacea* is unable to use either hydrogen or formate as electron donors. Therefore, direct interspecies electron transfer is likely in this co-culture system as well. This is consistent with our previous finding that *Geobacter* and *Methanosaeta* species were the predominant microorganisms in natural methanogenic aggregates that were exchanging electrons via direct interspecies electron transfer[2]. Gene expression analysis of these co-cultures is being initiated and expected to be complete by the time of the meeting.

Analysis of gene expression of natural methanogenic aggregates provided additional evidence for direct interspecies electron transfer to the *Methanosaeta* species, which accounted for over 90 % of the methanogens in aggregates[2]. Although *Methanosaeta* are unable to use hydrogen or formate as electron donors, a full pathway for carbon dioxide reduction could be detected in *Methanosaeta* genomes. Genes in the carbon dioxide pathway of the *Methanosaeta* in the natural aggregates were expressed at high levels. These results indicate that *Methanosaeta* receive low-potential electrons that can drive carbon dioxide reduction, consistent with the concept that *Methanosaeta* species can directly accept electrons from other members of the consortia.

As summarized in a companion poster, a genome-scale metabolic model that can describe the growth of a *G. metallireducens*/*G. sulfurreducens* co-culture that functions via direct interspecies electron transfer has been constructed. The gene expression and other physiological data needed for similar genome-scale metabolic models of methanogenic communities functioning via direct interspecies electron transfer is now being collected from the defined co-cultures and natural aggregates.

The discovery of direct interspecies electron transfer is a paradigm shift in anaerobic microbial ecology. The studies summarized here are expected to provide models that will be able to predict rates of electron exchange in methanogenic communities under a diversity of environmental conditions and the impact of environmental perturbations, such a climate change, on the rates of methanogenesis. Furthermore, an understanding of the mechanisms for direct interspecies electron transfer for methane production is leading to new concepts to either promote this process for bioenergy applications or to inhibit undesirable release of methane from terrestrial environments.

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1. Summers ZM, Fogarty HE, Leang C, Franks AE, Malvankar NS, Lovley DR. 2010. *Science* 330:1413-5.
2. Morita M, Malvankar NS, Franks AE, Summers ZM, Giloteaux L, Rotaru AE, Rotaru C, and Lovley DR. 2011. *mBio* 2:e00159-11.
3. Malvankar NS, Vargas M, Nevin KP, Franks AE, Leang C, Kim BC, Inoue K, Mester T, Covalla SF, Johnson JP, Rotello VM, Tuominen MT and Lovley DR. 2011. *Nat Nanotechnol* 6:573-9.