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Mechanisms of Extracellular Electron Transfer in *Methanosarcina*

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Multiple observations, spanning a range of methanogenic Archaea, have suggested that methanogens are capable of extracellular electron transfer (EET). Early reports from Bond & Lovely 2002 demonstrated the capacity for iron reduction in diverse methanogens. Later reports have shown that reductive EET may be important for the acquisition of FeS for biosynthesis. Notable to this work, Rotaru and Lovely showed that methanogens from the *Methanosarcinales* could accept electrons from a *Geobacter metallireducens*, in a syntrophic fermentation of ethanol. This occurs without using the known electron shuttles in syntrophy (i.e. formate and hydrogen), and in a manner that requires the EET machinery of *Geobacter* (e.g., OmcS). **However, to date, no concrete mechanism of EET that is broadly relevant to *Methanosarcina* has been identified**. Our previous work investigated the ability of *Methanosarcina barkeri* to take-up electrons from a cathode, as a mimic for what might occur during electron transfer with *Geobacter*. In *M. barkeri,* we observed a hydrogen-independent catalytic feature, that was also present in a hydrogenase deletion mutant. An inhibitor of methanogenesis (BES) abolished catalytic activity. Catalytic activity is also maintained when the electrode is moved to an electrochemical system with fresh media supporting cell-association of electron uptake. Collectively, these results point to a low potential (~-500 mV vs SHE) electron uptake feature in *M. barkeri*. To investigate the identity of this electron uptake feature, we investigated extracellular proteins using whole cell biotin labeling coupled to proteomic identification. We identified 52 “novel proteins present in labeled vs. non-labeled controls. Only four of these proteins were predicted to be extracellular using bioinformatic methods. To identify proteins enriched under electrochemical conditions we used Tandem-Mass-Tagging (TMT), an isobaric tagging approach. We quantified 41 proteins that were significantly up regulated under electrochemical conditions compared with growth on methanol. From this list 11 proteins of interest were identified as putatively involved in *M. barkeri* EET, two of which were identified in our extracellular screen. This talk will discuss the potential role of these proteins in EET, as well as, our recent work developing gene deletion mutants in *M. barkeri* to test these hypotheses.