Title
Development of a Model, Metal-reducing Microbial Community for a System Biology Level Assessment of Desulfovibrio vulgaris as part of a Community

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Background

One of the largest experimental gaps is between the simplicity of pure cultures and the complexity of open environmental systems, particularly in metal-contaminated areas. These microbial communities form ecosystem foundations, drive biogeochemical processes, and are relevant for biotechnology and bioremediation. A model, metal-reducing microbial community was constructed as either syntrophic or competitive to study microbial cell to cell interactions, cell signaling and competition for resources. The microbial community was comprised of the metal-reducing Desulfovibrio vulgaris Hildenborough and Geobacter sulfurreducens. Additionally, Methanococcus maripaludis S2 was added to study complete carbon reduction and maintain a low hydrogen partial pressure for syntrophy to occur. Further, considerable work has been published on D. vulgaris and the D. vulgaris M. maripaludis co-culture both with and without stress. We are extending this work by conducting the same stress conditions on the model community. Additionally, this comprehensive investigation includes physiological and metabolic analyses as well as specially designed mRNA microarrays with the genes for all three organisms on one slide so as to follow gene expression changes in the various cultivation conditions as well as being comparable to the co- and individual cultures. Further, state-of-the-art comprehensive AMT tag proteomics allows for these comparisons at the protein level for a systems biology assessment of a model, metal-reducing microbial community. Preliminary data revealed that lactate oxidation by D. vulgaris was sufficient to support both G. sulfurreducens and M. maripaludis via the excretion of H₂ and acetate. Fumarate was utilized by G. sulfurreducens and reduced to succinate since neither of the other two organisms can reduce fumarate. Methane was quantified, suggesting acetate and H₂ concentrations were sufficient for M. maripaludis. Syntrophic state community cultivation will allow for a comprehensive, system biology level analysis of a metal-reducing microbial community.

Experimental Setup and Community Metabolism

Figure 1: Continuous cultivation of model communities was performed using bioreactors such as the New Brunswick Bioflo 110.

Figure 2: Cellulose and fumarate degradation along with the appearance of the metabolites acetate and succinate during growth of the Clostridium led model community.

Figure 3: (A) Scheme for the multiple trophic level, model microbial community encompassing polymerized sugar degradation which supports the overall community. (B) model community organisms and multiple carbon and electron pathways studied.

Figure 4: (A) qPCR based cell estimates showing the relative populations of the model community populations.

Figure 5: DAPI stained and fluorescently labeled species imaged under different culture conditions.

Table 1: Fermentation Balance of the Multiculture

<table>
<thead>
<tr>
<th>Compound</th>
<th>G. sulfurreducens</th>
<th>M. maripaludis</th>
<th>D. vulgaris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Fumarate</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Acetate</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Figure 6: Schema for the metal-reducing model microbial community. This community differs from the multi trophic level community by forcing the community to be dependent upon the sulfate-reducer while following complete carbon reduction. This community will be explored for the interactions between the metal-reducers during syntrophic vs. competitive growth conditions via the presence/absence of sulfite and assessing metal-reduction under these conditions.

Conclusions and Future Work

Conclusions

- Technologies such as multi-species microarrays, fluorescent antibodies and qPCR are now in place and functioning.
- Initial metabolic model of the multi-trophic level microbial community shows incomplete energy usage and preference for the primary fermenter.
- Construction and testing of the metal-reducing microbial community is nearly complete with new fluorescent antibodies for Methanococcus maripaludis S2.

Future work

- Completion of the metal-reducing microcommunity construction and analysis using all tools displayed here to determine the responses by D. vulgaris and Geobacter sulfurreducens to different community attributes.
- The metal-reducing community will be analyzed under both syntrophic and competitive conditions to assess the response of each microorganism at the gene expression level.

Acknowledgements

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