Syntrophic Degradation of Lactate in Methanogenic Cocultures

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VIMSS Virtual Institute for Microbial Stress and Survival — http://vimss.lbl.gov/

BACKGROUND

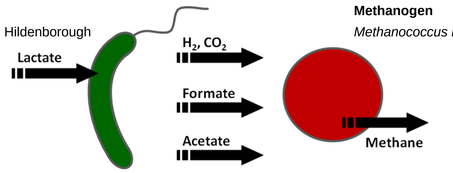
In environments where the amount of the inorganic electron acceptors (oxygen, nitrate, sulfate, sulfur or oxidized metal ions (Fe^{3+}, Mn^{4+})) is insufficient for complete breakdown of organic matter, methane is formed as the major reduced end product. In such methanogenic environments organic acids are degraded by syntrophic associations of fermenting, acetogenic bacteria (e.g. sulfate-reducing bacteria (SRB) as "secondary fermenters") and methanogenic archaea. In these consortia, the conversion of lactate to acetate, CO_2 and methane depends on the cooperating activities of both metabolically distinct microbial groups that are tightly linked by the need to maintain the exchanged metabolites (hydrogen and formate) at very low concentrations.

Energy yielding reaction for the syntrophic community on substrate lactate:

$$2\ \text{Lactate} + H_2O \rightarrow 2\ \text{Acetate} + CH_4 + H^+ + HCO_3^- \quad \Delta G'_0 = -144\ \text{kJ/mol rct.}$$

SRB
Desulfovibrio vulgaris str. Hildenborough

Methanogen
Methanococcus maripaludis



$$2\ \text{Lactate} + 4\ H_2O \rightarrow 2\ \text{Acetate} + 2\ HCO_3^- + 2\ H^+ + 4\ H_2 \quad \Delta G'_0 = -17.6\ \text{kJ/mol rct.}$$

$$HCO_3^- + H^+ + 4\ H_2 \rightarrow CH_4 + 3\ H_2O \quad \Delta G'_0 = -135.6\ \text{kJ/mol rct.}$$

The continuous hydrogen (and formate) consumption by methanogens (e.g. *Methanococcus maripaludis*) in syntrophic communities keeps the hydrogen partial pressures low enough (<10^{-3} bar) to enable *Desulfovibrio* species (e.g. *Desulfovibrio vulgaris* str. Hildenborough) to degrade lactate completely by fermentation.

TARGET OF STUDY

The current knowledge about the physiology of lactate-fermenting *Desulfovibrio* species and the metabolic interactions with their methanogenic partners in syntrophic cocultures are still limited. Therefore, the target of this study was to address the following questions:

- **syntrophic cocultures: growth characteristics of different *Desulfovibrio*-methanogen pairings**

  Question: Does a certain *Desulfovibrio* species interact identically/differently with varying methanogenic partners?

- **influence of varying cultivation conditions on growth of syntrophic cocultures**

  Question: Does e.g. the headspace-to-liquid volume ratio of the batch cultures has a general effect?

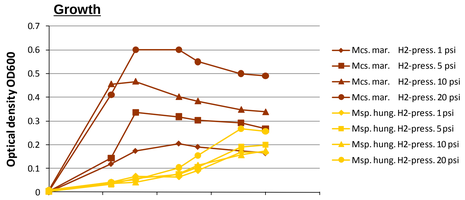
- **monocultures: growth characteristics of *Desulfovibrio* species and methanogens**

  Question: Does e.g. the H_2-production/-consumption of the *Desulfovibrio* species/methanogen correlate with the growth dynamics of the respective *Desulfovibrio*-methanogen pairing?

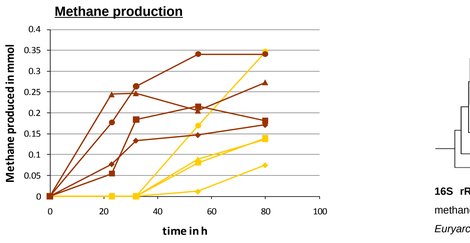
RESULTS: Monocultures of methanogens

***Methanococcus maripaludis* and *Methanospirillum hungatei*: Growth with varying hydrogen partial pressures**

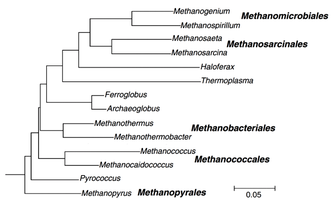
Growth



Methane production



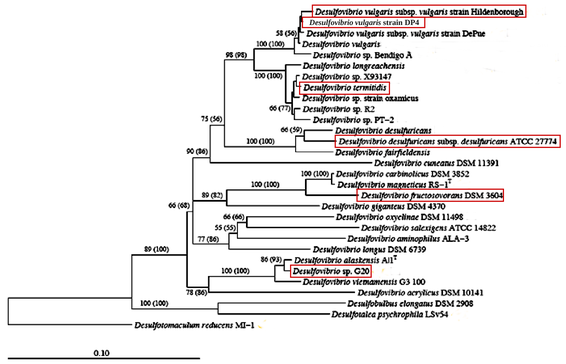
***Mcs. maripaludis* consumes H_2 faster and shows higher growth rates/biomass yields than *Msp. hungatei* under all conditions investigated**



16S rRNA phylogenetic tree: overview of the genera/families of methanogenic archaea and related archaeal lineages within the *Euryarchaeota*

RESULTS: Monocultures of *Desulfovibrio* species

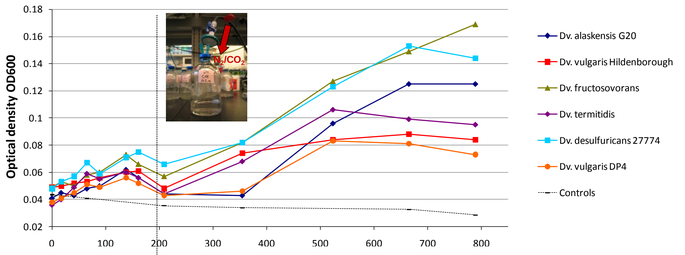
***Desulfovibrio* species: Growth on lactate in the absence of external electron acceptors or hydrogen-scavenging methanogens**



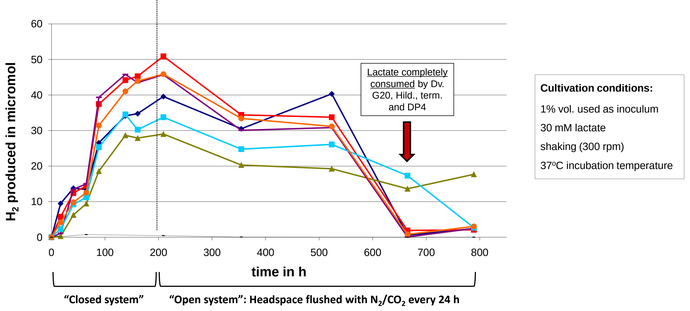
16S rRNA phylogenetic tree of the *Desulfovibrionaceae* (investigated *Desulfovibrio* species are highlighted by boxes in red color) and related lineages of sulfate-reducing bacteria within the *Deltaproteobacteria*

Lactate fermentation of *Desulfovibrio* species in batch culture

Growth



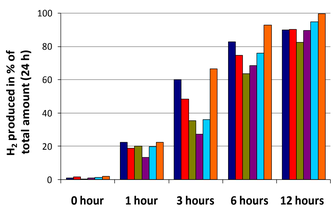
Hydrogen production

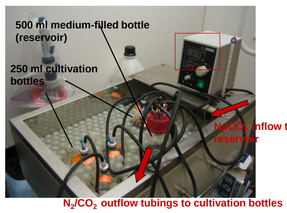


Cultivation conditions:
1% vol. used as inoculum
30 mM lactate
shaking (300 rpm)
37°C incubation temperature

all tested *Desulfovibrio* species can degrade lactate completely in the absence of H_2-consuming methanogens by fermentation to acetate and H_2, but growth rates and biomass yields are low

Time-dependence of hydrogen production of *Desulfovibrio* species during lactate fermentation



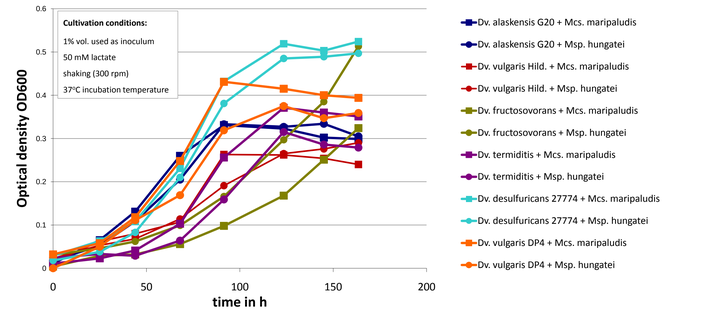


amount of H_2 produced per time unit varies among *Desulfovibrio* species

Attempts to increase growth rate by permanent flushing of headspace failed

RESULTS: Cocultures

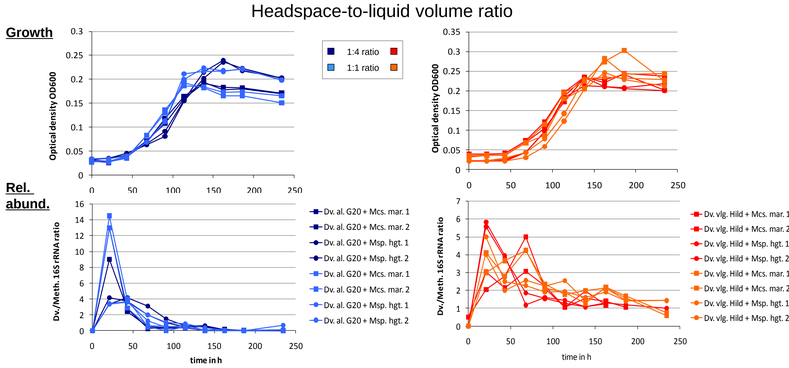
Different *Desulfovibrio*-methanogen pairings on lactate



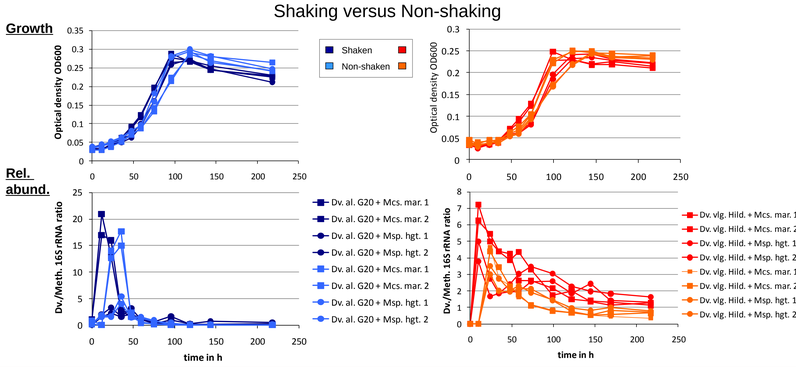
different *Desulfovibrio*-methanogen pairings show different growth dynamics during lactate degradation

Influence of varying cultivation conditions on growth of cocultures:

Headspace-to-liquid volume ratio



Shaking versus Non-shaking



CONCLUSIONS

- **all *Desulfovibrio* species form faster growing syntrophic cocultures with *Mcs. maripaludis* on lactate**
- **Exception: *Dv. fructosovorans* (lowest H_2 production) grows faster with *Msp. hungatei* (slow H_2 consumer)**
- **no clear-cut correlation between the H_2 production capability of *Desulfovibrio* species in monoculture and growth dynamics of their cocultures**
- **cultivation conditions have significant influence on growth dynamics/community structure of cocultures**

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