Is methanogenesis related solely to methanogenic archaea?

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Introduction

Extensive methane generation is so far postulated to be feasible only with the archaeal *mcrA* gene needed in the final stage of methanogenesis (**Fig.1**). Only recently other metabolic pathways including those belonging to bacteria have been discovered[1].

Objectives

- To describe the argillite indigenous community ARGCON5 and culturable strains isolated thereof aiming at compiling new communities degrading argillite organic matter;
- Testing these communities with **native and sterilized argillite** to specify the distinct methane producing archaea / bacteria.

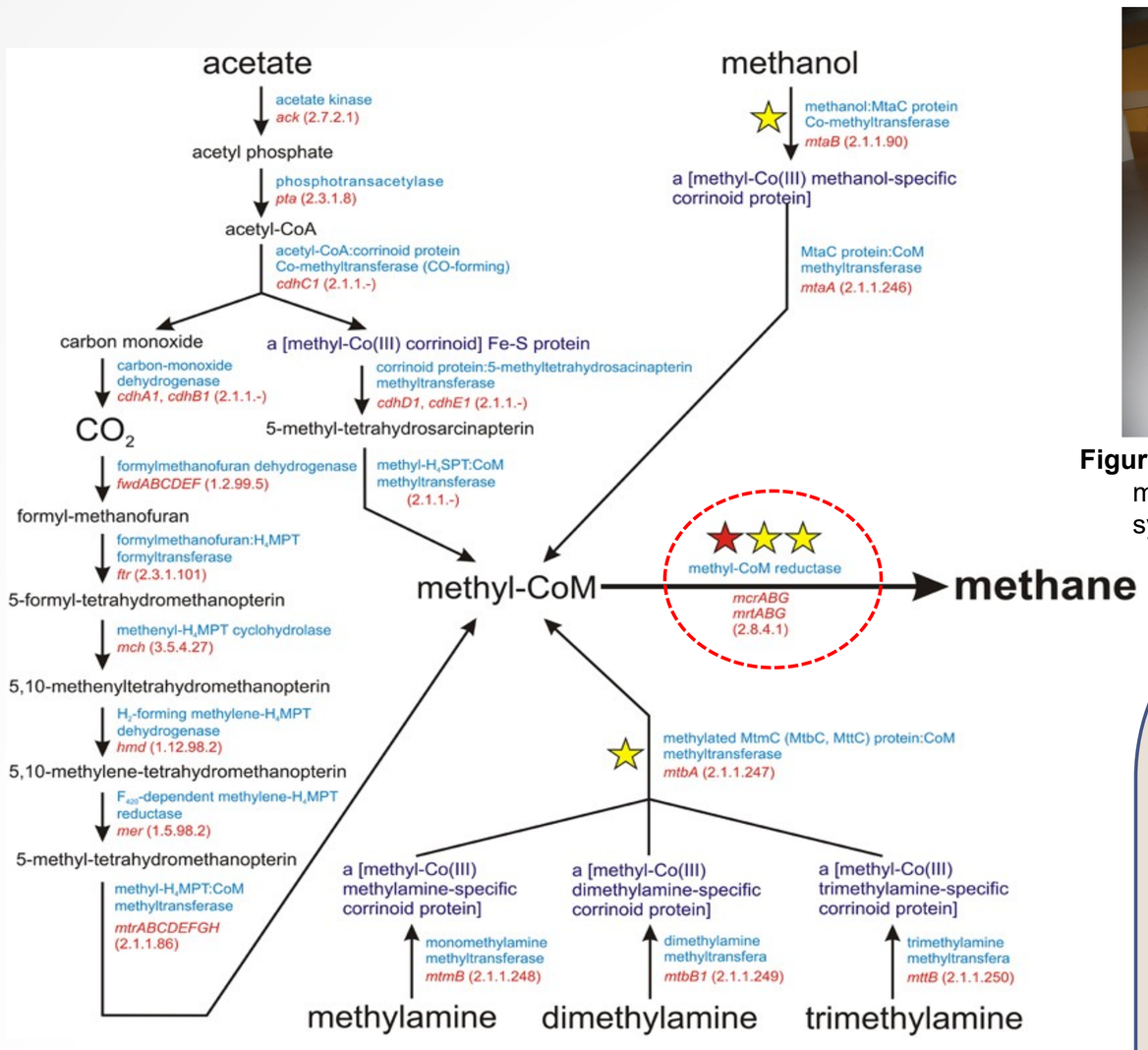

Figure 1. Schematic diagram of the pathway of methanogenesis. E.C. numbers for enzymes are shown in parentheses. The red star indicates the mcrA gene encoding subunit α of a methyl-coenzyme M reductase I, which is commonly used as a molecular marker for the detection of methanogens. The yellow stars denote molecular markers developed in study[2].

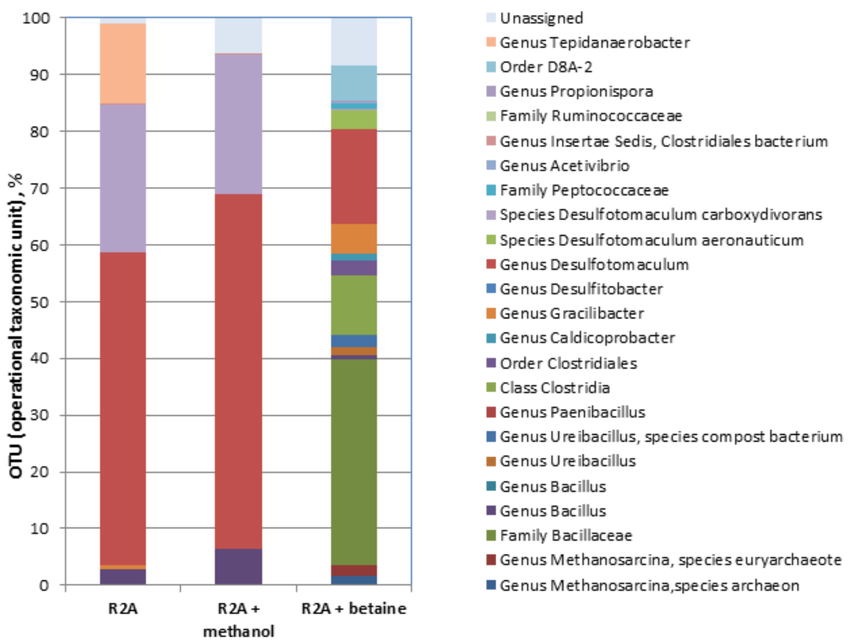

Figure 4. Taxa identified from the methane producing community with 454-tag pyrosequencing of archaeal 16S ribosomal RNA gene V2 region using primers Arch349F-A934b, in various culture media. In line with methanogens also bacterial taxa were identified.

Conclusions

- The balance between sulfate reducers and methanogens is inevitable to secure the processes towards methanogenesis.
- Is methanogenesis in these experiments initiated by methanogenic archaea in a co-culture with bacteria or by a separate bacterial pathway remains to be solved.

Figure 2. Pressure increase measurement with OxiTop system (WTW, Germany).

Materials and methods

- Methanogenesis of argillite indigenous community ARGCON5 was monitored by pressure increase with OxiTop system (**Fig. 2**);
- The species from this community were identified by 16S rRNA gene 454 sequencing;
- The isolates were tested by 16S rRNA gene Sanger sequencing, the ability to degrade argillite organic component (by methanogenesis), outplating and by biochemical properties.

Results

Heterotrophic facultative anaerobes and methanogenic archaea of argillite community ARGCON5 (CELMS No EEUT ARGCON5)[3] under anaerobic conditions are able to decompose the organic matter resulting in methane and CO_2 generation with releasing the metals (**Fig. 3**)

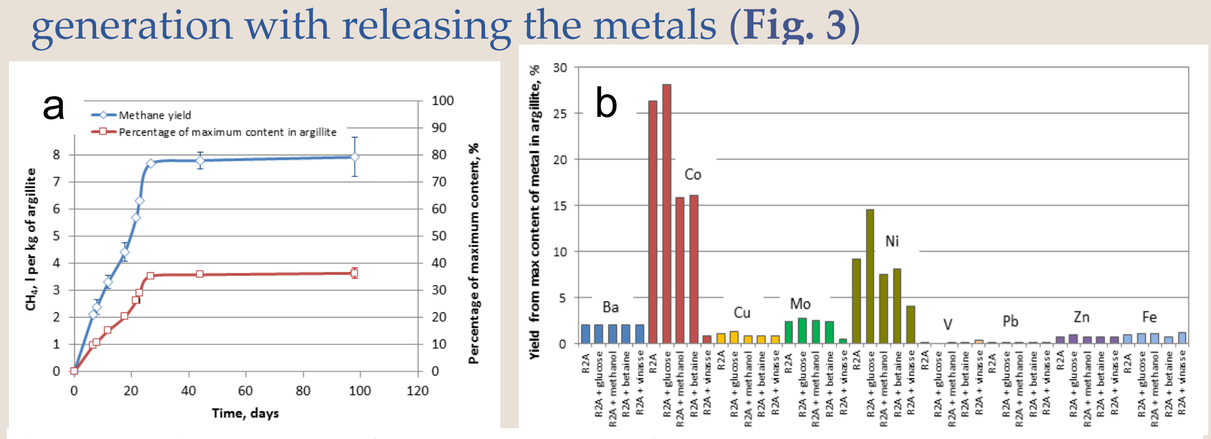

Figure 3. a. The dynamics of methane evolvement from argillite with inherent adapted culture ARGCON5. **b.** Leaching of metals from argillite with various cultivation media, yield of metals from maximum content of metal in argillite (enrichment value) on experiment day 76.

The microbial community stimulating methanogenesis is dominated by bacterial class *Bacilli* with the presence of archaeal genus *Methanosarcina*. Microbial communities lacking methanogenesis are dominated by the class *Clostridia,* mainly the genus *Desulfotomaculum* known to be involved in sulfur metabolism (**Fig. 4**).

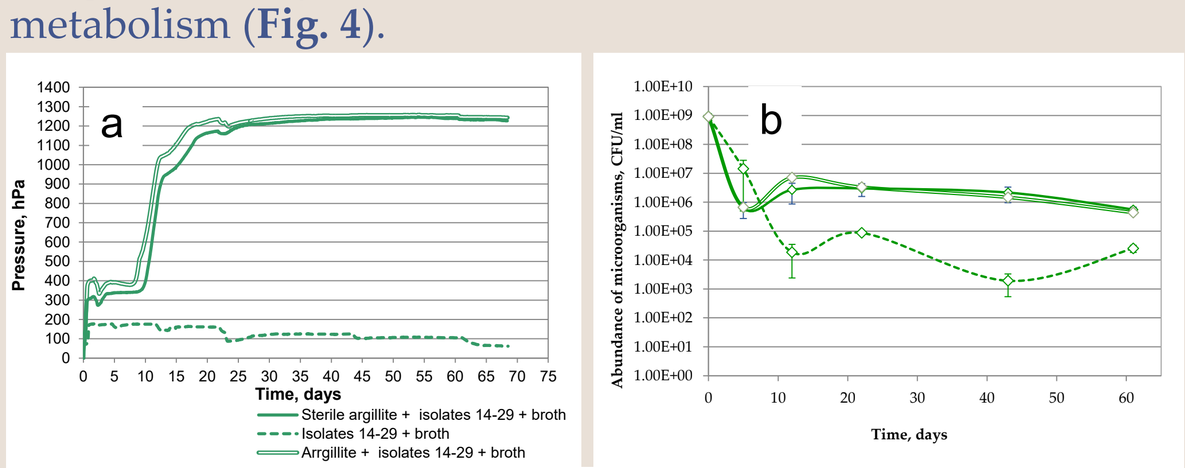

Figure 5. a. Gas production by community of 15 micro-consortia (isolates) in cultivation experiments with native and sterilized argillite**. b.** Abundance of culturable facultative anaerobes / aerobes from the same cultivation experiment.

15 bacterial micro-consortia isolated from ARGCON5 community demonstrated methanogenic activity also with sterilized substrate (**Fig. 5**). Among the culturable taxa identified were species belonging to the genera *Bacillus, Rothia, Microbacterium, Staphylococcus*, etc. (**poster Korb 61**)

Acknowledgements

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References

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