Identification of archaea in a mixed microbial community

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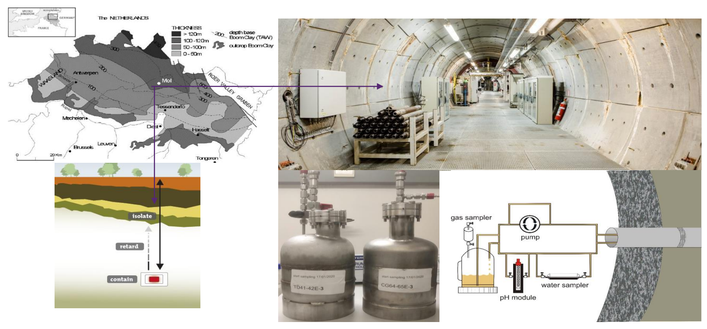
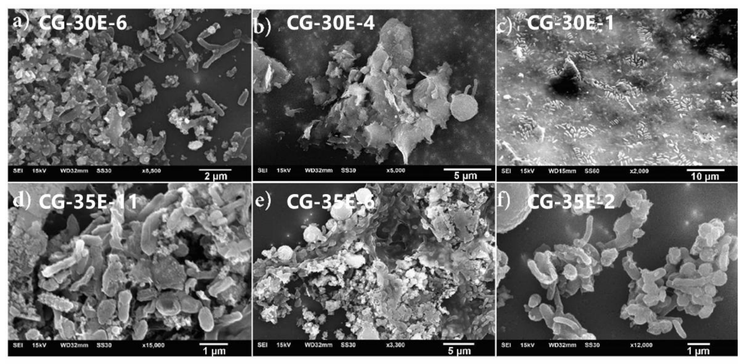
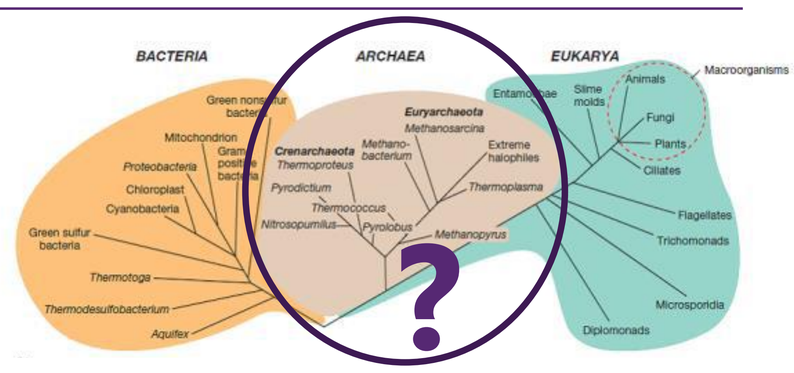
Introduction



Deep geological disposal of radioactive waste necessitates a detailed understanding of the geochemistry of the pore water.



A complex methanogenic microbial community is present in Boom Clay borehole water hampering validation of geochemical models.



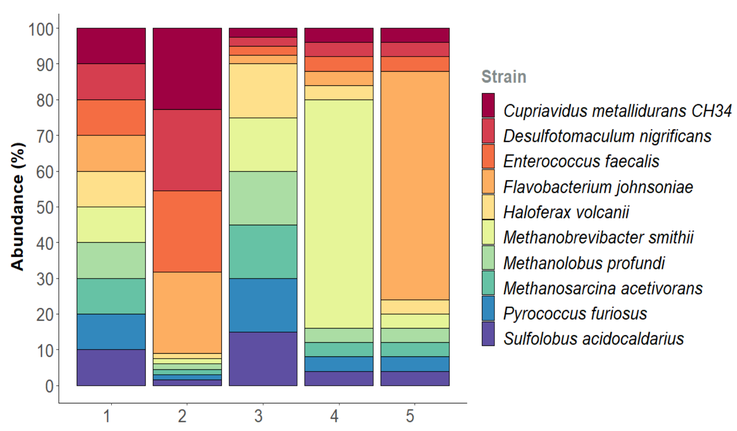
There is no validated method using a synthetic reference community (mock) to reliably identify archaea in a mixed microbial community.

Objectives

Selection of the best method to identify archaea in a mixed microbial community

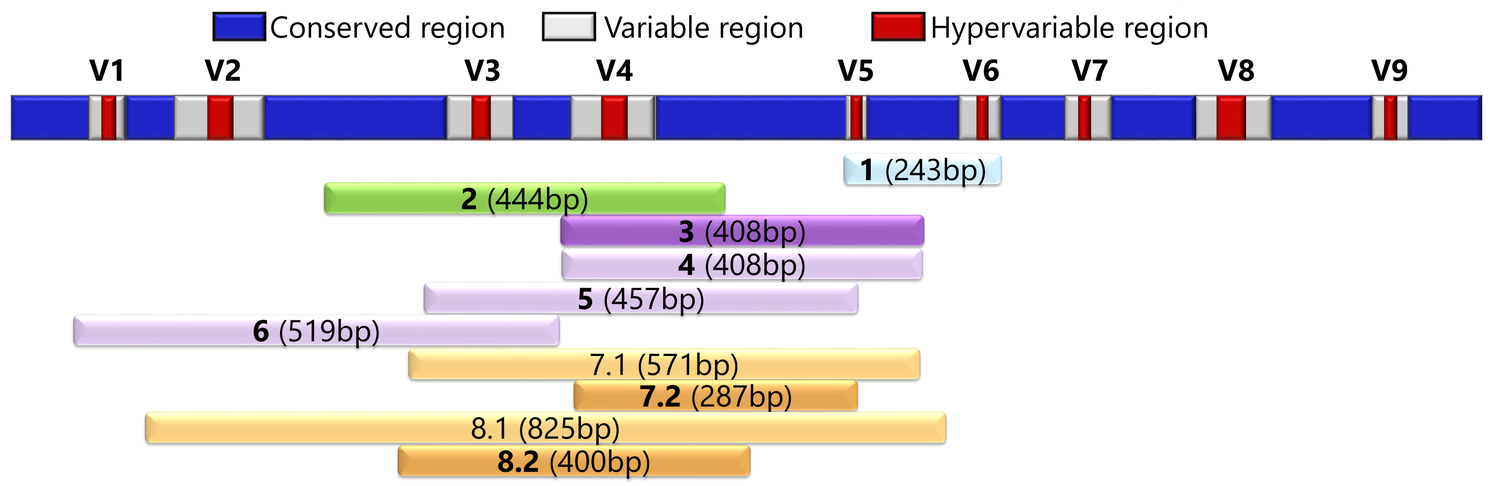
Methods

1. Development of a mock



A mock containing DNA of different archaea and bacteria was developed in different compositions to mimic different environments

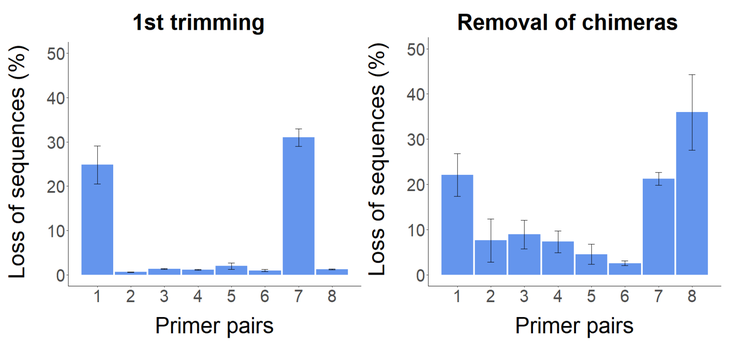
2. Selection of different methods based on the existing literature



Different regions of the 16S rRNA gene were sequenced: primers amplifying both archaea and bacteria (blue), primers specific for bacteria (green), primers specific for archaea (purple), including a commercial method (dark purple) and a 2-step PCR approach

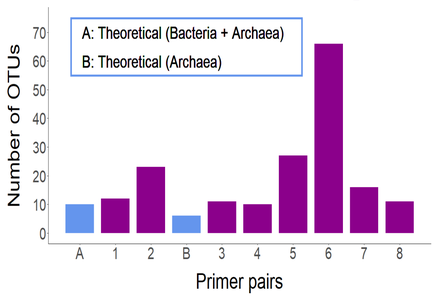
Results

1. Primer specificity & chimeras



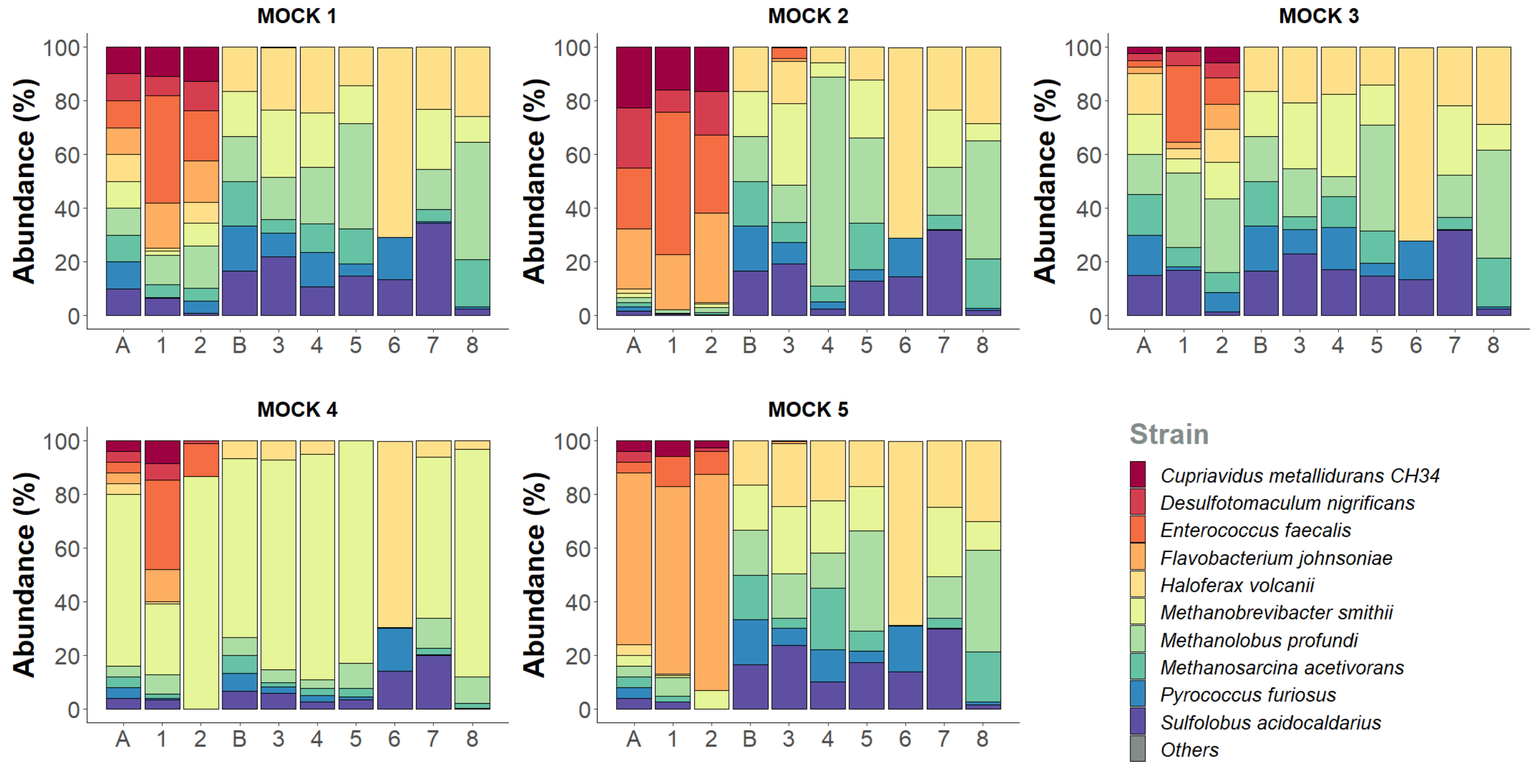
Primer pairs 1 & 7 had low specificity. A 2-step PCR approach seems more prone to chimeras.

2. Number of Operational Taxonomic units (OTUs) detected



Too many OTUs were observed with all methods.

3. Identification of the species in the different mock communities



No method could correctly asses the relative abundance of each species in the mock. Only the 2-step PCR approach was specific for archaea, with method 7 being better than method 8. However, primer bias was observed.

Conclusions

This mock constitutes a valuable tool for the optimization of archaeal identification as clear differences between the tested conditions were observed. However, none of the tested methods was optimal so further optimizations are needed.

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