

Molecular characterization of the microbial diversity in natural and engineered intraterrestrial environments
A focus on igneous rock aquifers

Ernest Chi Fru

Department of Cell and Molecular Biology, Microbiology, Göteborg University, Medicinaregatan 9C, Box 462, SE-405-30, Göteborg, Sweden

Abstract

Microbial diversity in natural and engineered intraterrestrial environments in igneous rock aquifers used or targeted for the disposal of spent nuclear fuel (SNF) was investigated using 16S rRNA and functional gene-based techniques. Methods were optimized including DNA acquisition from bentonite to selective application of a combination of molecular techniques to enhance specificity and accuracy of community diversity estimates. Investigated systems included biofilms in an operating interim SNF disposal basin located ~30 m underground, deep granitic rock groundwater (70-1000 m) and accompanying realistic simulated SNF disposal systems. In some systems total microbial community diversity was estimated while in others specific groups of microorganisms were in focus. Results indicated that in the interim SNF storage facility a *Meiothermus* sp. was the dominant biofilm forming organism and posed corrosion-related problems for the steel linings of the storage pool. In the oxygenated and microaerophilic tunnel environments at Äspö, a *Gallionella* dominated bacteriogenic iron oxides (BIOS) community was found to sustain several metabolic life styles from sulfate, iron, and manganese reducers to nitrite, ammonia, methane, iron, hydrogen and sulfur oxidizing bacteria communities to aromatic hydrocarbon degraders. In five year old bentonite retrieved from 450 m deep simulated SNF disposal systems bacilli were singly detected at temperatures as high as 110 °C. *Desulfosporosinus*, *clostridia* and *pseudomonads* were prominent at temperatures of 19 and 35-67 °C. Generally 84.1 % of the investigated microbial diversity in the buffer was gram positives with bacilli constituting a majority. In granitic groundwater from over nine boreholes at Äspö (70-626 m) screened for sulfate-reducing prokaryotes (SRP) by targeting the dissimilatory sulfite reductase (*dsrAB*) and 16S rRNA gene sequencing, it was revealed that SRP at Äspö were diversified, ancient with yet-to culture lineages and that *Desulfovibrio aespoeensis* was dominant and widespread. Finally, by targeting the α -subunit of the particulate methane monooxygenase gene (*pmoA*) and with the use of type I and II specific 16S rRNA fluorescent in situ hybridization gene probes, thirty sampled boreholes (3 to ca. 1000 m depths) distributed over three geographical distant regions in the Fennoscandian Shield were demonstrated to contain several lineages of aerobic methanotrophs. Up to 5000 methanotrophs mL⁻¹ of groundwater was estimated. Type I methanotrophs dominated in an SNF prototype repository with estimated numbers greater than for surrounding rock-logged groundwater. Altogether the *pmoA* gene phylogeny exhibited site and geographical dependence. The implications of the studies in relation to the safe disposal of SNF in underground engineered barriers are several; many lineages of the detected microorganisms fall into categories that can reduce, mineralize or bioaccumulate radioactive metals such as U(VI) (e.g. SRP and bacilli), can induce the corrosion of SNF storage canisters and metal linings (e.g. SRP, *Meiothermus*, *clostridia*), can influence the migration of radioactive metals through the production of complexing agents (e.g. *pseudomonads*) and provides large reactive surfaces for metal adsorption (e.g. *Gallionella* produced BIOS), and can potentially eradicate oxygen trapped in closed SNF repositories (e.g. methanotrophs).

Keywords: CLAB; Äspö; Olkiluoto; Forsmark; groundwater; bentonite; high level radioactive waste.

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