Microbial ecology in deep granitic groundwater – activity and impact of viruses

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Abstract

The deep subsurface environments in granitic rock can be studied and sampled at the Äspö hard rock laboratory (HRL) close to Oskarshamn in Sweden. Here, the groundwater is anaerobic and total counts give numbers of 10^4 to 10^6 cells mL^{-1}. However, these measurements do not reveal if the cells are active and alive. Subsequently, an ATP assay measuring biovolume, has in this thesis been shown to be reliable for estimations of the viable number of cells.

Viruses are the most abundant biological agent on earth and microbial ecology in all environments is greatly affected by viruses. The world's viral populations in marine ecosystems are known to influence the mortality of bacteria and archaea and biogeochemical cycles, and to have great genetic diversity. At the Äspö HRL, 10^5 to 10^7 mL^{-1} viral like particles were found when samples down to a depth of 450 m were studied. Using transmission electron microscopy, the viral populations were shown to be morphological diverse and likely to infect both archaea and bacteria. From the viral populations lytic bacteriophages infecting the indigenous sulphate-reducing bacterium *Desulfovibrio aespoeensis* were detected at four out of ten tested sites. Bacteriophages were isolated from the cultures and characterised as within the phage group *Podoviriade* with a genome size of 40,700 base pairs and to have a narrow host range. When the phage and host were grown in batch cultures, bacterial cells gained immunity towards the phages. In addition, bacteria belonging to the genus *Desulfovibrio* immune to the isolated lytic phages were isolated from subsurface samples. Temperate phages were found in four out of the ten bacterial isolates, and the presence of prophages in the bacterial genomes might be a protection for the cells towards lytic infection of a similar second phage.

Viruses are proposed as a factor controlling the number of micro-organisms in the environment and may allow a diversity of micro-organisms and viruses to co-exist in stable and nutrient limited groundwater systems. The found viruses are further suggested to sustain the activity of the cells in a similar way to how viral populations of marine environments are known to influence the cycling of nutrients via a viral shunt. Viruses are dependent upon the metabolism of their host to multiply. Hence, the viruses found in the deep subsurface imply active microbial communities. The activity of the microbial communities is further supported by ATP assay analysis data since the amount of ATP and the activity and cultivability of micro-organisms have been shown to correlate for subsurface samples. Sulphate-reducing bacteria producing sulphide are potential sources of corrosion on the copper surrounding the spent nuclear waste in the proposed Swedish KBS-3 storage model, and are known to live in the subsurface. Their numbers and the activity of sulphide producing populations are hence important factors for the storage.

Keywords: ATP, *Desulfovibrio*, groundwater, bacteriophage, *Podoviridae*, sulphate-reducing bacteria, viral ecology

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Papers included in this thesis:

I. **Eydal, H. S. C.** and Pedersen, K. 2007. Use of an ATP assay to determine viable microbial biomass in Fennoscandian Shield groundwater from depths of 3–1000 m. Journal of Microbial Methods, 70, 363-373.

II. Kyle, J. E., **Eydal, H. S. C.**, Ferris, F. G. and Pedersen, K. 2008. Viruses in granitic groundwater from 69 to 450m depth of the Äspö hard rock laboratory, Sweden. ISME Journal, 2, 571-574.

III. **Eydal, H. S. C.**, Jägevall, S., Hermansson, M., Pedersen, K. 2009. Bacteriophage lytic to *Desulfovibrio aespoeensis* isolated from deep groundwater. Accepted for publication in the ISME Journal in May 2009.

IV. **Eydal, H. S. C**. and Pedersen, K. 2009. Characterisation of bacteriophages of *Desulfovibrio* from deep granitic groundwater. Manuscript.