



Investigation of a putative nitrogen cycle in a subsurface radioactive thermal spring

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Background:

Previous studies on the microbial diversity [1] of the slightly radioactive thermal springs near Bad Gastein, Salzburg, Austria, suggested the occurrence of a nitrogen cycle in this subterranean environment. Microcosm experiments were performed to prove if nitrogen compounds may be used as energy sources for certain members of the microbial community of this spring

Methods:

2 x 25 l of thermal mineral water were sampled and filtered through a 0.22 μm Stericup (Millipore). Filters were excised and used as inocula for one microcosm. Stable isotope probing (SIP), was performed by using labeled nitrogen compounds to identify microorganisms, which were able to use nitrogen as the only energy source.

2 x 35 ml of natural grown biofilm were collected and used also as inocula for microcosms. Incubation was carried out as batch cultures in the dark at 30 °C or 40 °C, respectively. Two different types of media were used for incubation.

Ammonium, nitrite and nitrate were measured 3-4 times a week. PH-value was also measured and adjusted to ca. 7.5 – 7.7 if necessary.

DNA extraction was performed after 3 and 8 weeks of incubation, followed by an isopycnic centrifugation step. Clone libraries were performed only from microcosms incubated at 40 °C. To compare putative differences between the microbial communities at 30 °C with those at 40 °C, as well as the two different media, DGGE analyses were carried out.

Results:

A continuous decrease of the initial amount of ammonium was detected while the amounts of nitrite and nitrate increased simultaneously. No alterations of the initial amount of ammonium and nitrite or nitrate, could be detected with negative controls.

Mass spectrometric measurements demonstrated that the extracted DNA was highly labeled.

Phylogenetic analysis of DNA bands obtained from CsCl gradients led to differences in archaeal and bacterial communities of microcosms, which may reflect the different composition of media.

Two of the archaeal clones (FG34A3, FG34A37) belonged to the 1.1b group of the Crenarchaeota, which were known to be able for oxidizing ammonium to nitrite. The third one (FG35A23) clustered to a group of marine Euryarchaeota.

All of the bacterial clones clustered to known microorganisms, which were able to convert nitrite to nitrate, or to known denitrifiers, respectively.

DGGE-analysis of the V3 region of archaeal, as well as of bacterial 16S rDNA of the heavy DNA from microcosms inoculated with filtered microorganisms or with biofilm, showed no differences at 30 °C for Archaea between filter and biofilm but slight differences in band patterns between filter and biofilm at 40 °C. More differences

were detected for Bacteria communities between sample types and media in which they were incubated, by using DGGE analysis.

Conclusions:

Summing up, representatives of nearly all important groups of microorganisms (except ammonia oxidizing bacteria, AOBs) which were involved in a closed nitrogen-cycle have been found in our microcosm experiments. Together with the obvious oxidation of ammonium to nitrite and further to nitrate, the phylogenetic results led to the assumption that a closed nitrogen cycle could occur in the subsurface thermal spring FJQ.

References:

1. Weidler, G.W., Dornmayr-Pfaffenhuemer, M., Gerbl, F. W., Heinen, W., Stan-Lotter, H., Communities of Archaea and Bacteria in a subsurface radioactive thermal spring in the Austrian central alps, and evidence of ammonia-oxidizing Crenarchaeota. AEM, 2007. 73(1): p. 259-270.