

The geothermal gradient from mesophilic to thermophilic temperatures shapes microbial diversity and processes in natural gas bearing sedimentary aquifers

Comments:

The study by Katayama et al. explored the microbial diversity and processes in sedimentary aquifers and sheds lights on effects of geothermal gradient on it. The topic of work is actual. Authors has estimated microbial range in sedimentary aquifers and is good for rational analysis of geochemical processes in subsurface areas driven by microbial activities. The manuscript is scientifically sound, and the methanogenic taxa results are obtained using both culture-based and amplicon-based techniques. Used methods are described in sufficient details, and obtained experimental data is appropriate. However, there is no mention of replications of the geochemical analysis of the samples.

The presented results are shown from sample analysis and laboratory experiments for methanogenesis. Results of metagenomics as Sequence Read Archives (SRA) are submitted in DDBJ under the BioProject accession number PRJDB16863. Novel interesting results are obtained concerning microbial diversity in those areas that adds to a viewpoint of distribution of microorganisms in deep subsurface environments.

Experimental procedures are adequately described, and literature is properly cited. Therefore, based on the details provided in the manuscript, I believe it has the potential to meet the standards.

Here are some pointers for the authors-

- 1) In the deepest sample (1373 mbgs), no *mcrA* gene could be quantified. However, if we consider the acetoclastic methanogenesis in that sample, it is the highest. Why? Discuss.
- 2) Line 138: Is it *murA* gene or *mcrA* gene?
- 3) Line 198: Remove 'of sulfur'