Characterization of microbial communities in raw and homogenized bentonite samples

Rojina Shrestha[1,2], Jana Steinová[2], Lukáš Falteisek[2], Denisa Vlková[1], Alena Ševců[1,2]

(1) Faculty of Mechatronics, Informatics and Interdisciplinary Studies, Technical University of Liberec, Studentská 2, 461 17 Liberec (2) Institute for Nanomaterials, Advanced Technologies and Innovation, Technical University of Liberec, Bendlova 7, 461 17 Liberec, Czech Republic

Abstract

World-wide appreciated strategy for the management and treatment of high level and long lived radioactive waste is to dispose it in a deep and stable geological formation**.** Microbial community present in the host or buffer material may compromise the effective performance and safety of waste disposal system. In the Czech Republic, bentonite from Černý vrch locality is planned to be used as a buffer material. Therefore, microbial community was analysed in two bentonite samples (homogenized and raw bentonite) from this source.16S rRNA gene was amplified targeting the variable V4 region and sequencing was performed using Ion Torrent platform. Both bentonite samples were inhabited by relatively similar bacteria. Beta and Alpha-proteobacteria dominated both samples. Moreover, chemolithotrophs including *Thiobacillus*, *Gallionella* and *Nitrosomonas* capable of oxidising NH_3, Mn^{2+}, Fe^{2+} and S^{2-} were also present.

Introduction

The globally accepted strategy for the management and treatment of high level and long lived radioactive waste is to dispose of the waste in a deep and stable geological formation. The concept of the containment is based on a multi barrier system with different materials such as a metal container, bentonite and the host rock. The microbial community of the host rock or buffer material for the deep geological repository may compromise the effective performance and the safety of the radioactive waste disposal system [1]. Therefore, emphasis has been given to understanding the activity and diversity of microorganisms existing in repository. In the Czech Republic, bentonite from Černý vrch is planned to be used as a buffer material in the waste repository, however nothing is known about its microbial diversity. Therefore, our study is the first attempt made to investigate the structure of microbial community of the Czech bentonite.

Methods

Bentonite samples

Czech Mg-Ca bentonite originated from Černý vrch locality. Raw bentonite was unhomogenized and has of a very rough texture. Homogenized bentonite was commercially obtained from Keramost a.s.; the product is called “Bentonite a montmorillonite” BaM”.

Molecular analysis

Genomic DNA was extracted from 10 g of bentonite. SDS was used to lyse the cells. Furthermore; lysis was combined with precipitation of extracted DNA with polyethylene glycol followed by the purification step using AXG-100 cartridges [1-2].Qubit 2.0 or Agilent 2200 tape station was used for the quantification of genomic DNA. 16S rRNA gene was amplified using primers 530F [3] and 802R [4] targeting the variable V4 region. Same primers carrying Ion Torrent adaptor sequences and unique Tag barcodes were used for the amplicon preparation.We used Ion Torrent platform for amplicon sequencing. The process consists of following steps: i) emulsion PCR, ii) enrichment, iii) sequencing carried out on a 314 chip using the Ion Torrent personal genome machine system, New-generation Sequencing (NGS) technology.

Data analysis

Sequence data were analysed by the pipeline SEED v. 1.2.3 [5]. Sequences with insufficient quality or mismatches in tags were removed from the dataset. All sequences with minimal read length 275 bp were clustered into operational taxonomic units (OTUs) and chimeric sequences were removed using UPARSE implementation in USEARCH 7.0.1090 [6] with a 97% similarity threshold. The consensus from each OTU was constructed from an MAFFT alignment [7] based on the most abundant nucleotide at each position. The OTUs

were identified and their environmental requirements were assessed by megaBLAST and BLASTn algorithms against GenBank nt/nr database.

Result/Discussion

The homogenized bentonite (BaM) and the raw bentonite samples from Černý vrch were more similar than we expected in terms of the microbial community structure based on OTU. Most OTUs were shared between the two samples.Out of 126 shared OTUs with a frequency higher than 10, only 18 of them had a very asymmetric distribution (the ratio between the two samples 1:10 or 10:1).

Beta- and Alphaproteobacteria dominated in both bentonites. Chemolithotrophic bacteria with a possible corrosion capability were present as well, though in lower abundances (Table 1).Typical soil bacteria, including *Massilia*, *Bradyrhizobium*, *Lysobacter*, *Methylocapsa*, *Microbacteriacea*, *Acidobacteria* were also present.These bacterial taxa are also known to inhabit oligotrophic environments.

Interestingly, chemolithotrophs that could utilize NH_3, Mn^{2+}, Fe^{2+}, S^{2-} as electron donors were present in relatively high abundances in both bentonite samples. Regularly it was *Thiobacillus*, *Gallionella*, *Rhodobacteraceae* and *Nitrosomonas*. This could be explained by slow and long-term adsorption of reduced compounds onto bentonite from the upper layers of soil in the Černý vrch mine and the consequent establishment of oxidative conditions during mining.

Table 1: Results of the NGS amplicon analysis showing selected OTUs. Numerical Value represents the number of respective microorganism present.

sample type / OTU	"BaM" homogenized bentonite	raw bentonite	determination
1	454	98	*Thiobacillus* sp.
7	19	68	*Gallionella* sp.
26	88	47	*Rhodobacteraceae*
28	112	57	*Arthrobacter* sp.
34	1	772	*Phreatobacter* sp.
44	5	211	*Aquabacterium* sp.
45	398	47	*Xanthomonadaceae*
63	81	227	*Nitrosomonas* sp.
67	395	1	*Beijerinckiaceae*
68	315	44	*Lysobacter* sp.
81	135	82	*Microbacteriaceae*
89	67	71	*Comamonadaceae*
95	23	72	*Acidobacteria*
98	62	13	unclassified
114	58	58	*Nocardioides sp.*
157	67	10	*Bacteroidetes*
214	75	3	*Bradyrhizobiaceae*

Conclusion

The microbial communities in the bentonite samples were relatively similar in both samples, although the first one was homogenized commercial material and the second one was raw bentonite sampled directly in the mine; in other words homogenization caused only small differences in the bacterial community structure. Beta- and Alphaproteobacteria dominated in both bentonite samples. *Thiobacillus*, *Gallionella*, *Rhodobacteraceae*, and *Nitrosomonas* capable of oxidizing NH_3, Mn^{2+}, Fe^{2+}, S^{2-} were present in both samples.

Acknowledgements

This work supported by the Euratom research and training programme 2014-2018 under grant agreement No. 661880 (Microbiology in Nuclear Waste Disposal - MIND).

References and Citations

[1] M. Lopez-Fernandez, A. Cherkouk, R. Vilchez-Vargas, R. Jauregui, D.Pieper, N. Boon, Sanchez-Castro and M.L. Merroun, "Bacterial diversity in bentonites, engineered barrier for deep geological disposal of radioactive wastes", *Environmental Microbiology*.DOI 10.1007/s00248-015-0630-7, 2015.

[2] S. Selenska-Pobell, G. Kampf, K. Flemming, G. Radeva and G. Satchanska, "Bacterial diversity in soil samples from two uranium waste piles as determined byrep-APD, RISA and 16Sr DNA retrieval", *Antonie van leeuwenhoek,* vol.*79*, pp. 149-161, 2001.

[3] S.E. Dowd, T.R. Callaway, R.D. Wolcott, Y. Sun, T. McKeehan, R.G. Hagevoort and T.S. Edrington, "Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP)", *BMC Microbiology*, *8*, 125, 2008.

[4] M.J. Claesson, Q. Wang, O. O'Sullivan, R. Greene-Diniz, J.R. Cole, R.P. Ross and P.W. O'Toole, "Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions", *Nucleic Acids Research,* vol.*38, No.*22, e200, 2010.

[5] T. Větrovský and P. Baldrian, "Analysis of soil fungal communities by amplicon pyrosequencing: current approaches to data analysis and the introduction of the pipeline SEED", *Biol Fertil Soils*, vol.49, pp.1027–1037, 2013.

[6] R.C. Edgar, " UPARSE: Highly accurate OTU sequences from microbial amplicon reads" *Nature Methods*, vol.10, pp. 996-998, 2013.

[7] K. Katoh, G. Asimenos and H. Toh, "Multiple alignment of DNA sequences with MAFFT", *Methods Mol Biol,* vol. 537, pp.39–64, 2009.