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Endospore-forming bacteria as an indicator of pollution in sediments of Lake Geneva

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Abstract. Treated wastewater and runoff-water is released by the outlet of the sewage treatment plant of Vidy (Lausanne) directly into the Lake of Geneva via a pipe located 300m from the shore. Even if this water is properly treated with modern technologies, we can observe an accumulation of micro pollutants into the sediments, and particularly heavy-metals. The main objective of this project is to investigate how these elevated concentrations of heavy metals affect both abundance and diversity of prokaryotes in the sediments. A special emphasis was given to endospore-forming bacteria, which could use sporulation as a survival strategy to resist in highly contaminated areas. This study could have implications both for understanding the role of endospore-forming bacteria in the environment as well as in terms of improving the bioremediation processes.

Key words: Endospore-forming bacteria, Heavy metals, Metagenomic analysis

Introduction

Lake Geneva is the largest freshwater lake in Western Europe. Since several decades it has been the subject of close environmental monitoring. Different studies from the Forel Institute(Pote et al., 2008; J-L Loizeau et al., 2004) indicate that the sediments of the Vidy Bay contain high level of heavy-metals (HM). This is mainly due to the rejection into the bay of the runoff surface water, but also the treated wastewater from the city of Lausanne and its suburbs (412'000 equivalent inhabitant). Even if the concentration of HM in the rejected water is low, the surrounding sediments accumulate them over time. Endospore-forming bacteria (EFB) are well known for their ability to resist to harsh environmental condition over a long period of time(Vreeland et al., 2000). When conditions are not optimal, endospore formation is triggered. Spores are resistant structures intended to protect and conserve the genetic material of the organisms until the conditions become suitable for vegetative growth (Nicholson, 2002). For a long time, spores had been considered as a dormant state in which no metabolic activity takes place. Recent studies(Junier et al., 2009; Rosson and Nealson, 1982; Tebo and Obraztsova, 1998) have however demonstrated that some redox activities are active at the surface of endospores.

This study aims at evaluating an eventual link between endospore-forming species and the load of heavy-metals in the environment. A better understanding of the role of this special group of bacteria in polluted areas could lead to improved bioremediation processes in the future.

Materials and Methods

This study is a part of the "Elemo" project that aims to gain a better understanding of the biogeochemical processes taking place in the water column but also in the sediments of Lake Geneva. Thanks to the sponsors, two MIR submersibles could be used to monitor the surface of the sediments and to select the more interesting coring zones around the rejection pipe of the wastewater treatment plant of Lausanne. A first set of eight cores was retrieved from precisely selected areas by using the robotic arm of the submersible. In order to have more contrasted heavy-metal concentrations, five additional cores were retrieved from a boat during a second sampling campaign.

Cores were transported in our cold room and conserved there for no more than 4 days. During these

days, air has been bubbled at the surface of supernatant water in order to preserve the gradient of oxygenation into the sediments as well as the structure of the microbial community at the oxic/ anoxic interface.

According to the expertise we have developed in our laboratory, and in order to have a more holistic view of the microbial community, we have then opted for an indirect DNA extraction method. Sediments were split into two separated layers (0 to 3cm and 3 to 9cm deep). They were first homogenized in 1% Hexametaphosphate solution with an Ultra Turrax (IKA, Germany). Mineral particles were then separated from cells by sedimentation and gentle centrifugation. Recovered supernatants, harboring microbial cells, were filtered on 0.2µm cellulose-nitrate filters. One half of each filter was cut in pie pieces and frozen into a bead-beating tube from the FastDNA® Spin Kit for Soil (MP Biomedicals) until processing. In order to harvest DNA from very resistant structures, like spores, a modified protocol, including 4 sequential bead-beating steps, has been used. Inhibitor free DNA has then been obtained by an extra ethanol purification step at the end of the extraction procedure.

A first overview of the bacterial community has been obtained by DGGE fingerprinting on the V3 region of 16S rRNA gene. Based on this, both layers in six cores have been selected for further analyses. The quantification of the overall bacterial population has been done by qPCR on the 16S rRNA gene. Quantification of the endospore- forming subpopulation has been done by qPCR on an endosporulation specific gene (*spo0A*). Specific primers have been developed for this. 454 pyrosequencing was carried out by "Eurofins MWG GmbH" (Germany) on V1-V3 region of 16S rRNA gene. Bioinformatic identification of the approximately 20'000 sequences per sample has been done in our lab by running NCBI blastn locally.

Air dried/agate grinded sediments were used for physico-chemical analyses. A CHN analysis preceded or not by an acidic digestion step, has been used to retrieve both inorganic and organic fraction of C and N in the sediments. The total amount of HM was determined by an acidic digestion (aqua regia) followed by ICP-MS analysis.

A first set of multivariate exploratory analyses has been done with the open source R software and the library vegan. Cluster analysis and nonmetric multidimensional scaling have been used to achieve a better understanding of the main differences among the characteristic bacterial groups that shape the communities in the samples. An overview of the effect of physico-chemical factors on the prevalence of the different species has been plotted by the environmental fitting procedure described in the vegan tutorial(Oksanen, 2011). Further statistical analyses, like multiple factor analysis (MFA) and the hypothesis-driven redundancy analysis (RDA) are in progress in order to demonstrate more accurately the link between HM and specific bacterial groups.

Finally, wetlab experiments with laboratory strains are planned to achieve a better understanding of the physiological mechanisms that allow endospore-forming bacteria to cope with HM pollution. For these experiments, flow-cytometry will be used to quantify the proportion of endospore vs. vegetative cells in the different cultures amended with increasing amount of HM.

Results and Discussion

The physico-chemical analyses, as well as the composition of the bacterial community assessed by pyrosequencing, show that four out of 12 samples analyzed are clearly separated from the others among the 12 analyzed. Furthermore, the first multivariate analyses tend to demonstrate a strong link between the prevalence of endospore-forming species and the HM content of the sediments (Fig.1). As a good correlation is also observed between HM and nutrients (represented by C.org and N.total vectors) it is not clear if the first set of physico-chemical factors is sufficient to explain the prevalence of EFB in the community of these 4 particular samples. In order to answer to this uncertainty, a hypothesis-driven multivariate analysis has to be computed. RDA analysis has been chosen but results are not yet available.

Finally, quantification of EFB with the qPCR approach on *spo0A* gene gives reliable results when compared with the pyrosequencing data. Higher abundance of endospore-formers are reported in the four samples with high HM concentrations.

Conclusion

According to these first results, a clear link could be established between EFB and the load of HM in the sediments. At this point of the work we still don't know if this selection is due to the capacity of EFB to form resistance structures and therefore to escape harsh environmental conditions, or if it is due to other special features of Clostridiales. The further wetlab experiments we have planned should answer this. Additionally, we hope that we will isolate some important species that display such resistance properties. This is particularly important from an ecological point of view, because resistant species are also those that can re-colonize the empty niches after the perturbation caused by high HM loads. In other words, EFB could be involved in the resilience of ecosystems after HM pollution events. Furthermore, if we are successful with these isolation steps, we could also make a characterization of these species, and eventually discover some new depollution mechanisms that take place in these environments. This could be relevant for further bioremediation processes.

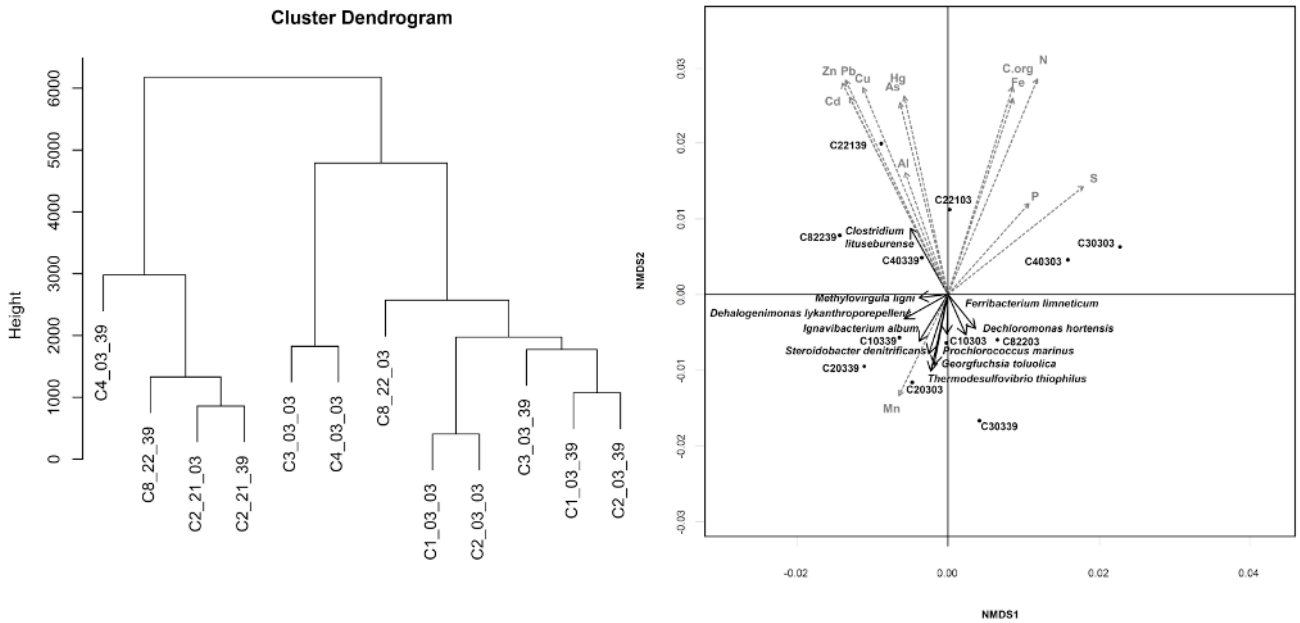

Fig. 1. Left: Cluster analyses (euclidean distance, average clustering) of the bacterial community assessed by 16S rRNA gene pyrosequencing with DNA obtained from 12 selected sediment samples. The four samples to the left form a clear separate group. Right: Nonmetric multidimensional scaling ordination (euclidean distance) of the 12 samples and environmental fitting of measured physico-chemical factors. This representation demonstrates that heavy-metals are affecting bacterial communities of these four particular samples by favoring endospore-forming species like *Clostridium lituseburense*. To refine this conclusion, more sensitive statistical methods (like redundancy analysis - RDA) are still needed.

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References

Junier P, Frutschi M, Wigginton NS, Schofield EJ, Bargar JR, Bernier-Latmani R. Metal reduction by spores of Desulfotomaculum reducens. Environ Microbiol 2009;11:3007–17.

Loizeau J-L, Pardos M, Monna F, Peytremann C, Haller L, Dominik J. The impact of a sewage treatment plant's effluent on sediment quality in a small bay in Lake Geneva (Switzerland-France). Part 2: Temporal evolution of heavy metals. Lakes & Reservoirs: Research and Management 2004; 9:53–63.

Nicholson W. Roles of Bacillus endospores in the environment. Cellular and Molecular Life Sciences 2002;59:410–6.

Oksanen J. Multivariate Analysis of Ecological Communities in R: vegan tutorial. R tutorial; October 2011. http://cc.oulu.fi/~jarioksa/opetus /metodi/vegantutor.pdf.

Pote J, Haller L, Loizeau JL, Bravo AG, Sastre V, Wildi W. Effects of a sewage treatment plant outlet pipe extension on the distribution of contaminants in the sediments of the Bay of Vidy, Lake Geneva, Switzerland. Bioresource Technology 2008; 99: 7122–31.

Rosson R, Nealson K. Manganese binding and oxidation by spores of a marine Bacillus. Journal of Bacteriology 1982;151:1027–34.

Tebo B, Obraztsova A. Sulfate-reducing bacterium grows with Cr(VI), U(VI), Mn(IV), and Fe(III) as electron acceptors. Fems Microbiology Letters 1998;162:193–8.

Vreeland R, Rosenzweig W, Powers D. Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. Nature 2000;407:897–900.