Ecord Research Grant 2016 – Intermediate Report

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Purpose
The ECORD Research Grant was used for a research stay from 29.01. – 22.03.2017 at the Environmental Microbiology Group of Prof. Dr. Mark Lever at the ETH Zürich, Zürich, Switzerland. The aim was to investigate bacterial endospores and endospore forming Firmicutes in the Nankai Trough with molecular methods.

Major changes of objectives
Compared with the initial proposal for the ECORD Research Grant 2016, two major changes did occur. Firstly, due to ship-board participation on IODP Expedition 370, the focus of the research stay changed. The current *spo0A* based quantitative polymerase chain reaction (qPCR) protocols for quantifying bacterial endospores was developed for high-biomass lake sediments (Bueche et al., 2013; Wunderlin et al., 2013 & 2014). Implementing and adapting them for the low-biomass marine sediments of site C0023 in the Nankai Trough, became the major objective. Secondly, to be able to complete this major method development work, the length of the research stay was extended from two weeks to nearly two months. By covering all the lab expenses the host, Prof. Dr. Mark Lever, made it possible for me to use the money from the ECORD Research Grant 2016 entirely on accommodation and travel and to cover the two month period.

Description of laboratory work and preliminary results
The laboratory work was in principal separated in the following parts

1) Learning a diverse set of molecular techniques and applying them on test samples and standards
2) Testing different mechanical treatments of cell disruption for DNA extraction from bacterial endospores
3) Testing multiple *spo0A* primer configurations to optimize qPCR potential
4) Producing qPCR standards of four different endospore forming strains to ensure robustness of quantification for the environmental samples

1) Molecular techniques
During the research stay the following molecular techniques were performed: DNA extraction including bead-beating, DNA purification, DNA quantification via Qubit 2.0 fluorometer and NanoDrop (Thermo Fisher Scientific), DNA amplification and quantification via PCR and qPCR, Agarose gel electrophoresis and qPCR standard production via plasmid cloning reaction.

2) Treatments for DNA extraction from bacterial endospores
Three samples from the Black Sea (M84/1) and one from the Rhône Delta (POS450) were used to test three different mechanical treatments (30 s vortexing, 30 s bead-beating and 10 min bead-beating) with three different types of beads, which resulted in 36 individual DNA extracts. The rough mechanical treatment was necessary to ensure complete DNA extraction from bacterial endospores, as their physical structure is highly resistant compared to vegetative bacterial cells.

3) spo0A primer configurations to optimize qPCR potential

Major advantages of using the *spo0A* gene for qPCR quantification instead of the traditional 16S rRNA are its specificity for the phylum Firmicutes, containing all endospore formers, and its higher sensitivity, when solely looking at this narrow group. However, one caveat of this technique is that while the *spo0A* gene is a relatively conservative gene sequence, meaning little mutation, it still has a lot more inter- and intra-species variation than the 16S rRNA gene. For example, even though forward and reverse primer pairs might work perfectly for aerobic Firmicutes of the class Bacilli, they might not work with anaerobic Firmicutes of the class Clostridia and vice versa. The task here was to find either a primer pair which would cover most of the relevant types of spore formers we expect to find in the deep biosphere of Nankai Trough, or at least, two complementing pairs.

Therefore, a total of seven primer pair modifications of the published *spo0A* primers (Bueche et al., 2013; Wunderlin et al., 2013 & 2014) were tested by PCR amplification for six different DNA extracts of spore formers. PCR conditions were optimized for the two most promising pairs via temperature gradient PCR. The final choice of the two primer pairs and PCR conditions were based on signal intensities of agarose gel electrophoresis runs.

4) qPCR standards of different endospore formers

Analysis of environmental samples via qPCR is performed by direct comparison to a parallel amplification of a qPCR standard. We were interested in producing multiple qPCR standards to cover amplification rate variations within the phylum Firmicutes and increase the chances of a parallel amplification of a standard and a sample. We attempted to obtain a total of five qPCR standards of *Desulfotomaculum carboxydivorans, Desulfosporosinus acidophilus, Thermacetogenium phaeum, Bacillus subtilis* and *Clostridium methoxy benzovorans* with the TOPO® TA Cloning® Kit (Life technologies). Standards were produced for all strains except *Clostridium methoxy benzovorans*, which leaves us with four standards and represents a major improvement to the published Bueche et al. (2013) method where only one qPCR standard was used.

To summarize, the improved tools are now in place and can be applied to environmental samples, including IODP Exp. 370 samples, during the next research stay.

Budget

Due to the covering of all lab expenses by Prof. Dr. Mark Lever, I was able to extend the research stay from two weeks to two month. Therefore, I submitted an updated budget plan to my original proposal, which is represented here by the “estimated costs”. Because I found a comparably cheap flat in Zürich and covered the costs for the return trip alternatively, I spend less money than I applied for. The money left on the account is 678 € (677.34 € precisely).

Item	**Estimated costs €**	**Actual costs €**
Travel Bremen – Zürich (by train)*	300	124
Public transport Zürich	0	163
Accommodation in Zürich for 8 weeks	2000	1280
Adapter for electronic plug	0	9
Parcel with materials	0	46
Total	**2300**	**1622**
Difference		+ 678

**Only one way trip needed, as the return trip was combined with a conference with separate funding*

I plan to return to the ETH Zürich from October to the end of December in 2017 for another research stay where I will start to analyze, amongst others, the IODP Exp. 370 samples with the newly implemented methods. I intend to spend the rest of the money for the second research stay in 2017 and will submit a final report and budget by the beginning of 2018.

References

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