

Master Thesis in biology, microbiology

Working title:

Ultra-small bacteria in marine environments

Tutors:

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Research Group:

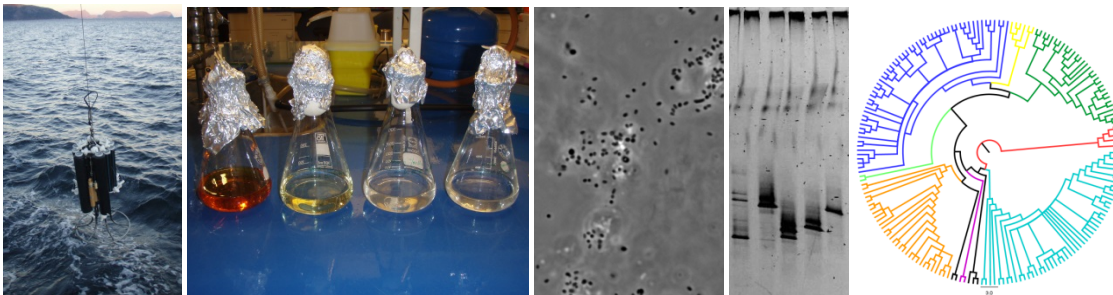
Marine microbiology

Background:

Ultra-small bacteria were recently discovered in groundwater through metagenome analyses (Luef et al., 2015). These ultra-small bacteria have a size of approximately $0.009 \pm 0.002 \mu\text{m}^3$ and genome sizes that are so small that they are on the very limit for what is needed to sustain life which makes them very interesting in an evolutionary aspect (Giovannoni et al., 2005; Morris et al., 2012). They belong to the WWE3, OP11 and OD1 candidate phyla, which are common in various environmental samples (see for instance Borrel et al (2010)), but in general little is known about them. The small size of these bacteria mean they easily go through filter sizes of $0.22 \mu\text{m}$ which is the filter size normally used to concentrate bacteria for phylogenetic diversity studies. It is therefore possible that the true diversity and abundance of these groups have been grossly underestimated in previous studies.

Aim:

The aim of this thesis is to examine the diversity of ultra-small bacteria in different marine environments and compare between these environments. Further, enrichment cultures will be prepared to attempt cultivation and isolation of ultra-small bacteria.



The two parts of the project briefly includes:

1: Screen existing samples – We have available a large number of water samples that have been prefiltered (0.45 µm), concentrated and frozen, from many different locations and environments (Fjords, the Arctic, Deep-water, etc). From these DNA will be extracted, 16S rRNA genes amplified using PCR, and clone libraries constructed to look at bacterial diversity.

2: Enrichments – To attempt cultivation of ultra-small bacteria, enrichment cultures from one or more marine locations will be prepared. Briefly, water samples will be pre-filtered to remove larger cells, and used as inoculums for enrichment cultures. Cultures will be monitored over time using microscopy and flow cytometry to determine cell abundances and cell sizes, and with PCR-based methods to determine the phylogenetic affiliations. In addition, to estimate the potential loss of ultra-small bacteria by using different filter sizes, samples from the same location(s) will be filtered through different filter sizes (0.45 and 0.22), and any remaining bacteria in the filtrate will be pelleted using ultra-centrifugation. DNA will be extracted from these filters and analysed for bacterial diversity using PCR and clone-libraries.

Methods that will be used:

Enrichments and cultivation – sampling techniques, sample preparation, preparation of growth media
Genomic-based methods - DNA extraction, PCR, Cloning, and possibly DGGE.

Microscopy - light microscopy, electron microscopy (TEM and/or SEM)

Flow cytometry

Bioinformatics

Statistics

Financing and working place:

The thesis will be linked to the research group Marine microbiology research group, department of biology, Marineholmen

References:

- Borrel, G, Lehours, A-C, Bardot, C, Bailly, X, and Fonty, G (2010): Members of candidate divisions OP11, OD1 and SR1 are widespread along the water column of the meromictic Lake Pavin (France). *Archives of Microbiology* **192**, 559-567.
- Giovannoni, SJ, Tripp, HJ, Givan, S, Podar, M, Vergin, KL, Baptista, D, Bibbs, L, Eads, J, Richardson, TH, Noordewier, M, Rappé, MS, Short, JM, Carrington, JC, and Mathur, EJ (2005): Genome Streamlining in a Cosmopolitan Oceanic Bacterium. *Science* **309**, 1242-1245.
- Luef, B, Frischkorn, KR, Wrighton, KC, Holman, H-YN, Birarda, G, Thomas, BC, Singh, A, Williams, KH, Siegerist, CE, Tringe, SG, Downing, KH, Comolli, LR, and Banfield, JF (2015): Diverse uncultivated ultra-small bacterial cells in groundwater. *Nat Commun* **6**.
- Morris, JJ, Lenski, RE, and Zinser, ER (2012): The Black Queen Hypothesis: Evolution of Dependencies through Adaptive Gene Loss. *mBio* **3**.