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## **Title**

Metagenomics of two sludge microbial communities: population structure and biogeography

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# Metagenomics of two sludge microbial communities: population structure and biogeography

#### Abstract

Enhanced Biological Phosphate Removal (EBPR) is one of the best-studied microbially-mediated processes due to its ecological and economic relevance and tractability for study. We present a metagenomic analysis of two geographically remote EBPR sludges dominated by the recently described and as-yet uncultured bacterium Candidatus Accumulibacter phosphatis (CAP). We demonstrate that A.phosphatis represents a striking example of a highly mobile, asexual species with clonal populations that appear to lack geographical boundaries. Furthermore, the environmental distribution of this species is highly skewed, with sparse, high density point sources (i.e. EBPR sludges). This adds new data to the ongoing debate on microbial biogeography and the study illustrates that generalizations on the topic may not be appropriate.

#### Introduction

We have recently shotgun sequenced two geographically remote EBPR sludges both highly enriched in *Candidatus* Accumulibacter phosphatis (CAP). The high coverage of a single species allowed us to investigate population structure on the genomic level, while availability of genomic sequence from two remote sources provided biogeographical insights.

Genome-wide population structure has previously been observed only in microbial populations inhabiting an Acid Mine Dranage (AMD) biofilm. The Ferroplasma type II population was found to be a mosaic of at least three ancestral strains, the mosaicism arising from apparently unrestricted homologous recombination between individuals. We aimed to find whether this form of microbial sexuality is also seen in CAP, the dominant species in lab-scale Enhanced Biological Phosphorus Removal [EBPR] sludges.

The widely accepted dogma that microbial species do not form endemic species "everything is everywhere, the environment selects", has recently been challenged by studies of hyperthermophiles. In these studies it was shown using an MLST approach that *Sulfolobus* comprise endemic populations according to geographic locale, and that endemism has been overlooked due to low resolution tools for distinguishing strains. In the present study, we apply genome-scale comparisons derived from metagenomics to address the biogeography of mesophilic microbial populations.

#### Results

#### Strain abundance

Analysis of single-copy genes (see methods) demonstrated that multiple strains and several related species of CAP were observed in each sample.

A single strain dominated each sample, and was assembled in 8x and 5x coverage in the US and Australian samples respectively

### Clonality

The dominant strain was clonal: only 1 confirmed SNP was observed per 8 and 26 Kb in the US and Australian samples respectively. Even this is likely to be an overestimate with a large portion of the count resulting from erroneous co-assembly of several different strains.

#### **Biogeography**

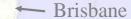
No phylogenetic separation based on geographic locale was observed. In all trees with adequate strain representation, the US and OZ CAP strains were intermingled.

The dominant strains were >98% identical on the DNA level across large parts of their genomes

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#### Celibacy

We used purpose-built software (eco-SNPvista) to detect homologous recombination (sexuality). No compelling evidence for homologous recombination was observed within or between Accumulibacter species indicating that they are asexual. However, extensive evidence for non-homologous recombination was observed.



#### Differences between dominant strains

Dominant strains were ~98% identical on DNA level, but had multiple differences in the genome. We observed multiple genomic insertions and deletions, including different exopolysaccharide operons, CRISPR as well as differences in transposable elements positioning and variety.

#### Methods

Single-copy genes were identified as members of PFAM families present in never more than a single copy and in at least half of all sequenced species. PFAM assignments were done by RP-Blast

Neighbour-joining trees of 50 single copy genes were constructed in clustal with 500 bootstrap resamplings to estimate interior node reliability. SNP calling was done using Consed viewer and EcoSNPVista.



#### Discussion

Previous metabolic reconstruction of CAP revealed genes not used in EBPR sludge, including genes for nitrogen and carbon fixation as well as flagellar and chemotaxis operons. This, combined with the universal distribution of CAP strains suggests presence of this species in the environment, which was not yet reported. This environmental stock could explain the fast distribution, strain intermingling, gene content as well as fast environmental seeding of the EBPR plants.

#### **Conclusions**

This study indicates that generalizations concerning microbial biogeography and sexuality may be ill advised and that it is more likely that a spectrum of dispersal and recombination potential exists in the microbial world. Further genome-wide studies of microbial populations will reveal the extent of this variability.

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