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Publication date: 2014

Document Version
Early version, also known as pre-print

Link to publication from Aalborg University

Citation for published version (APA):
Using metagenomics and metatranscriptomics to study specific bacterial species involved in biological phosphorus removal from wastewater

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**Introduction**

Enhanced Biological Phosphorous Removal (EBPR) from wastewater treatment plants is managed by Polyphosphate Accumulating Organisms (PAOs). The model PAO is “Candidatus Accumulibacter phosphatis” (Accumulibacter) and despite a large body of knowledge, their detailed physiology remains elusive.

In this study we combined metagenomics, metatranscriptomics and laboratory scale enrichment in order to examine the gene expression of the uncultured Accumulibacter and the co-enriched associated community.

**Methods**

**Enrichment**

Wastewater treatment plant → Sequencing Batch Reactor → Enrichment

**Genome recovery**

Coverage → Tetranucleotide frequency → Taxonomic classification (PhyloPythiaS+) → rRNA genes → Ecotype specific genes → Paired-end connections

**mmgenome**

A toolbox for reproducible genome extraction from metagenomes

**Assembly**

CLE

**Binning**

A GAO elished in a FDN. The putative GAO Propionibrio (magenta) is hit by the probe used to quantify the FDN Accumulibacter (white). However, it does not accumulate massive amounts of polyphosphate. Probes: PA0651 (FLUC, Accumulibacter); PA0842 (rRNA, both); PA0844 (rRNA, both); 087 (PAO, all bacteria).

**Transcriptomics**

**Results**

**Genome extraction from metagenomes.** Differential coverage binning was used as the first step in extracting individual genomes from the metagenomes. Each point represents a scaffold, colored by essential single copy genes and scaled by scaffold length. The plot was made using the metaplot function in the mmgenomics R package. Suprisingly a bacteria from the genus Propionibrio was enriched along with Accumulibacter.

**A GAO elished in a FDN.** The putative GAO Propionibrio (magenta) is hit by the probe used to quantify the FDN Accumulibacter (white). However, it does not accumulate massive amounts of polyphosphate. Probes: PA0651 (FLUC, Accumulibacter); PA0842 (rRNA, both); PA0844 (rRNA, both); 087 (PAO, all bacteria).

**Phylogenetic tree (16S-rRNA) of known PAOs and GAOs.** Until now the PAOs and GAOs have been separated into distinct phylogenetic lineages. The discovery of a GAO from the genus Propionibrio makes interpretations more difficult. The sequenced Accumulibacter genome is closely related to Accumulibacter clade IJK based on phylogenetic analysis of the ppk gene.

**Accumulibacter transcriptomics.** Relative expression of the core genes of the Accumulibacter bin under anaerobic feast (15 min) and aerobic famine (180 min) conditions.

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