Acquiring Reference Genomes from Uncultured Microbes by Micromanipulation and Low-complexity Metagenomics

Søren M. Karst*, Mads Albertsen, Jeppe L. Nielsen, Kåre L. Nielsen, Per H. Nielsen

Department of Biotechnology, Chemistry and Environmental Engineering,
Aalborg University, Aalborg, Denmark

*skarst06@student.aau.dk

AALBORG UNIVERSITET

Environmental Biotechnology
Aalborg University

Introduction

Access to high quality reference genomes is a pre-requisite for many of the –omics based approaches applied in environmental micro-biology today. With metagenomics and single cell genomics researchers have started to obtain genomes from the uncultured majority of bacterial diversity. However both methods face fundamental obstacles making it difficult to obtain complete genomes.

Objective

The objective was to apply the methodology of single cell genomics on bacteria forming filaments and microcolonies in complex communities. This was done to obtain low-complexity meta-genomes to be used for extraction of near complete draft genomes.

Methods

: genome from target species : genome from non-target species

Sample from Complex Microbial Community

- Activated sludge from Wastewater treatment plants
- Enrichment cultures

Isolation of Microcolony /Filament

- In-solution FISH
- Micromanipulation with glass hooks (*Figure 1*)

DNA Extraction & Amplification

- Alkaline lysis
- Multiple displacement amplification

Determine Species Content

- 16S PCR
- Sanger sequencing

Sequencing

- Illumina Sequencing
- 4 Gbp/metagenome

***De Novo* Assembly**

- CLC Genomics workbench

Contig Binning

- Tetranucleotide frequency of assembly contigs
- Extraction of contigs from target species with principle component analysis (PCA) binning (*Figure 2*)
- LCA taxonomic classification

Manual Scaffolding

- Paired end reads used to detect contig connections
- Validation with connection maps (*Figure 3*)

Draft Genome

- Completeness estimates (essential single copy genes)
- Annotation using RAST and IMG/ER

Summary

- 13 low complexity metagenomes were obtained from filaments and microcolonies.
- 5 partial genomes, up to 80% complete, were assembled.
- More genomes are being extracted from the 13 metagenomes.
- With planned optimizations the approach should provide >80% genome completeness.

Results

45 individual filaments/microcolonies were isolated of which 13 were selected for Illumina sequencing based on 16S analysis. Each resulting metagenome contained between 1 to 9 different species, representing a number of phylogenetic groups thought to be relevant in wastewater treatment i.e. *Chloroflexi, Thiothrix* and *Microthrix*. So far, 5 draft genomes have been extracted and partially assembled. The draft genomes were estimated to be up to 80% complete based on presence/absence of essential single copy genes.

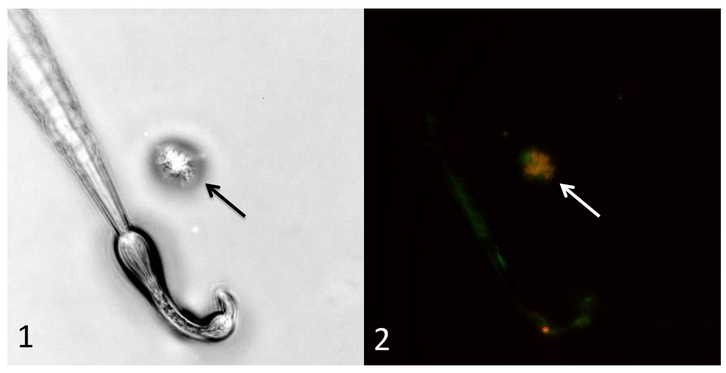


Figure 1: Pictures of a glass hook and an isolated microcolony (arrows). 400x magnification, 1: bright field, 2: fluorescence. The sample was stained using 'in solution FISH' (green = most bacteria, red = target).

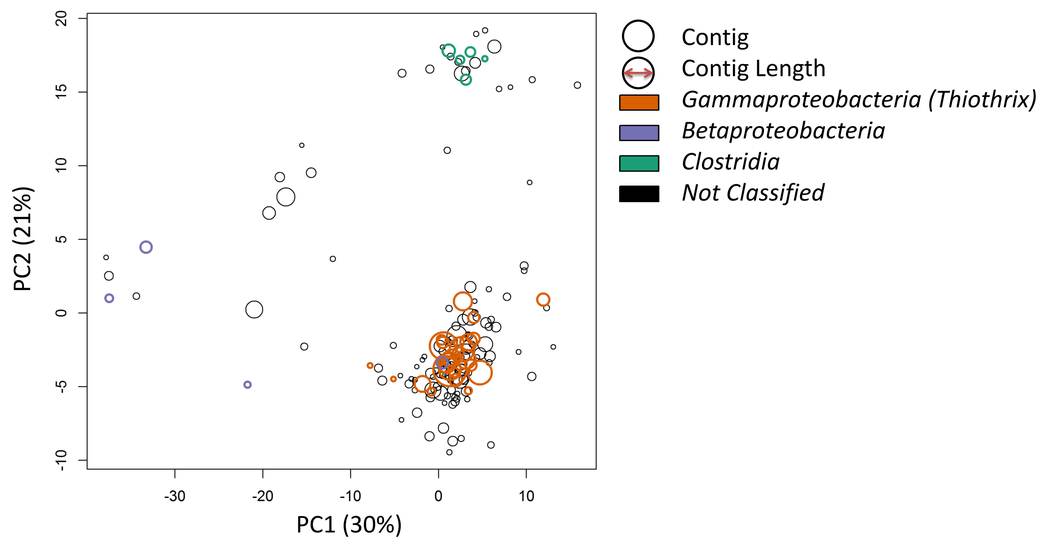
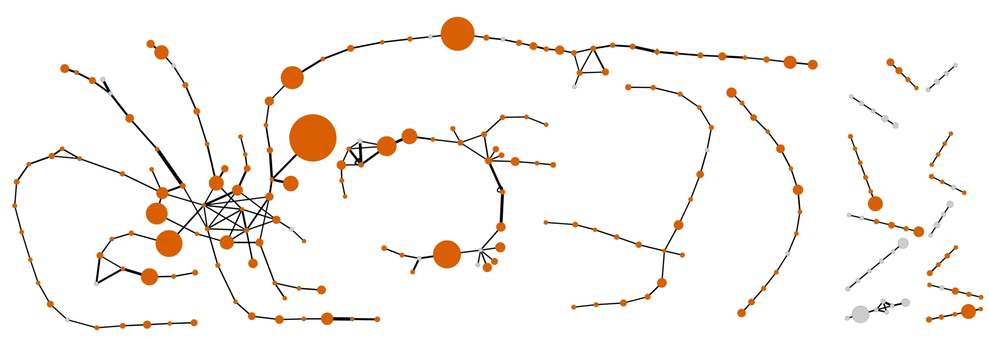


Figure 2: Scores plot from tetranucleotide frequency PCA binning of contigs from a metagenome. Distinct 'groups' are seen, which match the number of 16S genes found in the metagenome. The orange group was extracted.



: contig : contig length : contigs binned as target : connection

Figure 3: Connection map visualizing manual scaffolding of the extracted contigs from *Figure 2*. The contigs binned as belonging to the target genome (orange) connect primarily to each other.