Enrichment Metagenomes

Nikole E. Kimes
Postdoctoral Studies
Universidad Miguel Hernández

Metagenomics Course 2013

Enrichments

- Definition:
  - *Popular definition* - to make fuller, more meaningful, or more rewarding
  - *Life science definition* - to make more productive through the addition of nutrients
  - *Alternative life science definition* - to increase the proportion of a valuable entity
- Purpose: to target the growth of a specific microbial community member(s)
- Strategies:
  - adjust the nutrients available according to targeted microbe
  - remove unwanted microorganisms
  - other targeted selection (e.g., size or autofluorescence)

How does metagenomics help?

- Global effects
  - community shifts
  - functional shifts
- Targeting individual microorganimsms
  - genomes from the unculturable
  - shifting consortiums
- Targeting specific functions
  - functional diversity without culture bias

Global Effects

OPEN ACCESS Freely available online

PLOS | ONE

Soil Bacterial Community Shifts after Chitin Enrichment: An Integrative Metagenomic Approach

Samuel Jacquiod[1,2], Laure Franqueville[1], Sébastien Cécillon[1], Timothy M. Vogel[1], Pascal Simonet[1]*

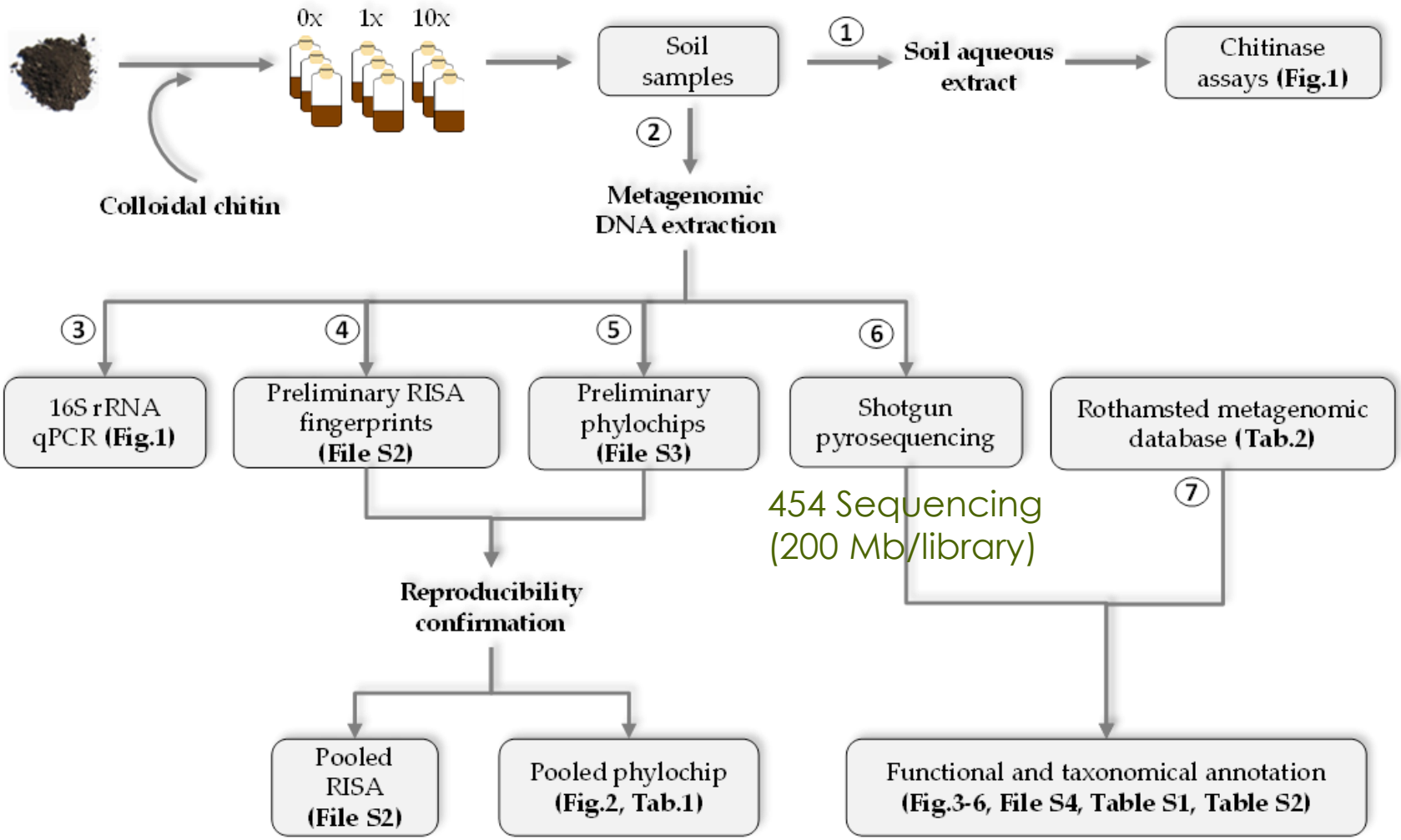
1 Environmental Microbial Genomics Group, Ecole Centrale de Lyon, Laboratoire Ampère UMR5005 CNRS, Ecully, France, **2** Microbial Molecular Ecology Group, Section of Microbiology, København Universitat, København, Denmark

Abstract

Chitin is the second most produced biopolymer on Earth after cellulose. Chitin degrading enzymes are promising but untapped sources for developing novel industrial biocatalysts. Hidden amongst uncultivated micro-organisms, new bacterial enzymes can be discovered and exploited by metagenomic approaches through extensive cloning and screening. Enrichment is also a well-known strategy, as it allows selection of organisms adapted to feed on a specific compound. In this study, we investigated how the soil bacterial community responded to chitin enrichment in a microcosm experiment. An integrative metagenomic approach coupling phylochips and high throughput shotgun pyrosequencing was established in order to assess the taxonomical and functional changes in the soil bacterial community. Results indicate that chitin enrichment leads to an increase of *Actinobacteria*, *γ-proteobacteria* and *β-proteobacteria* suggesting specific selection of chitin degrading bacteria belonging to these classes. Part of enriched bacterial genera were not yet reported to be involved in chitin degradation, like the members from the *Micrococcineae* sub-order (*Actinobacteria*). An increase of the observed bacterial diversity was noticed, with detection of specific genera only in chitin treated conditions. The relative proportion of metagenomic sequences related to chitin degradation was significantly increased, even if it represents only a tiny fraction of the sequence diversity found in a soil metagenome.

Global Effects

Figure S1. Description of the pipeline used in this study.



Jacquiod S, Franqueville L, Cécillon S, M. Vogel T, et al. (2013) Soil Bacterial Community Shifts after Chitin Enrichment: An Integrative Metagenomic Approach. PLoS ONE 8(11): e79699. doi:10.1371/journal.pone.0079699

Global Effects

Figure 4. Major taxonomical changes in the soil bacterial community after incubation and chitin enrichment.

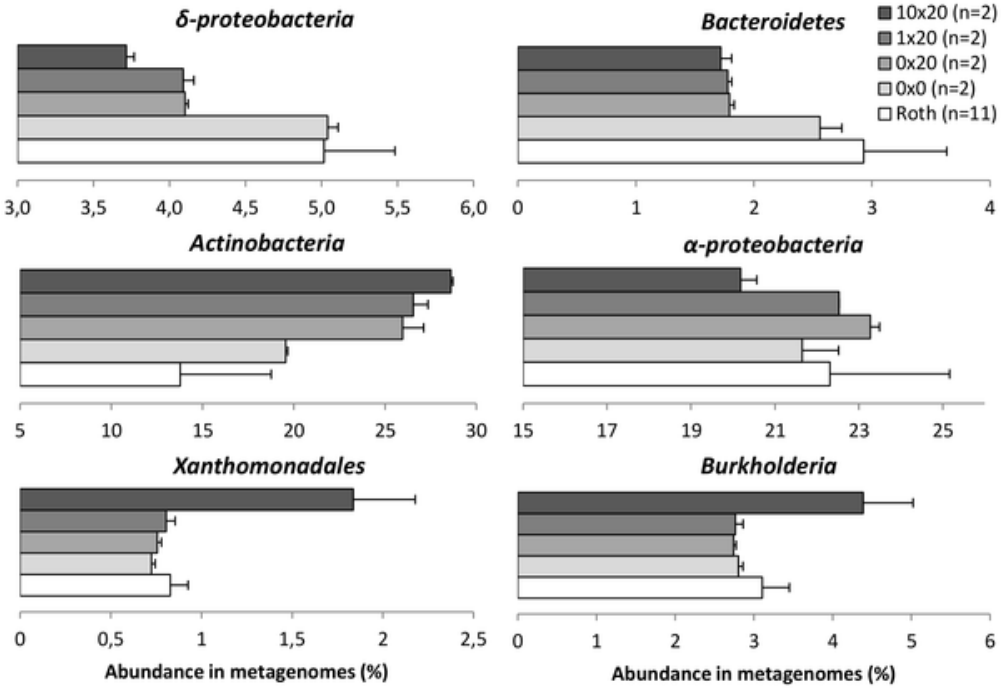
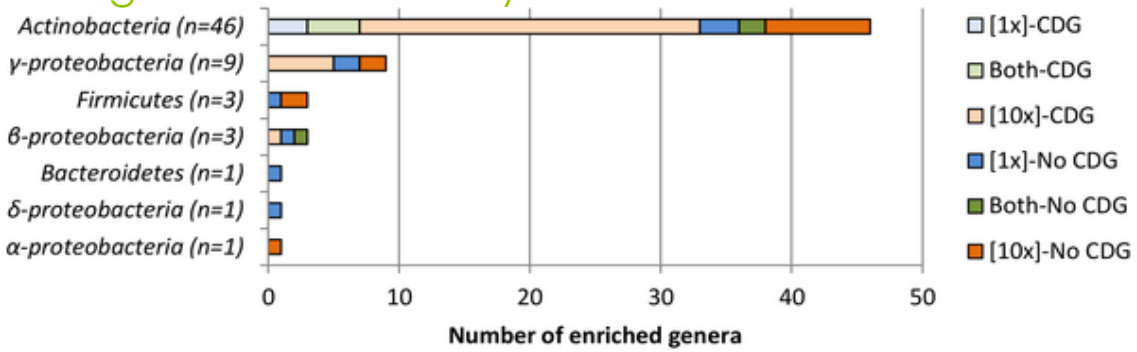


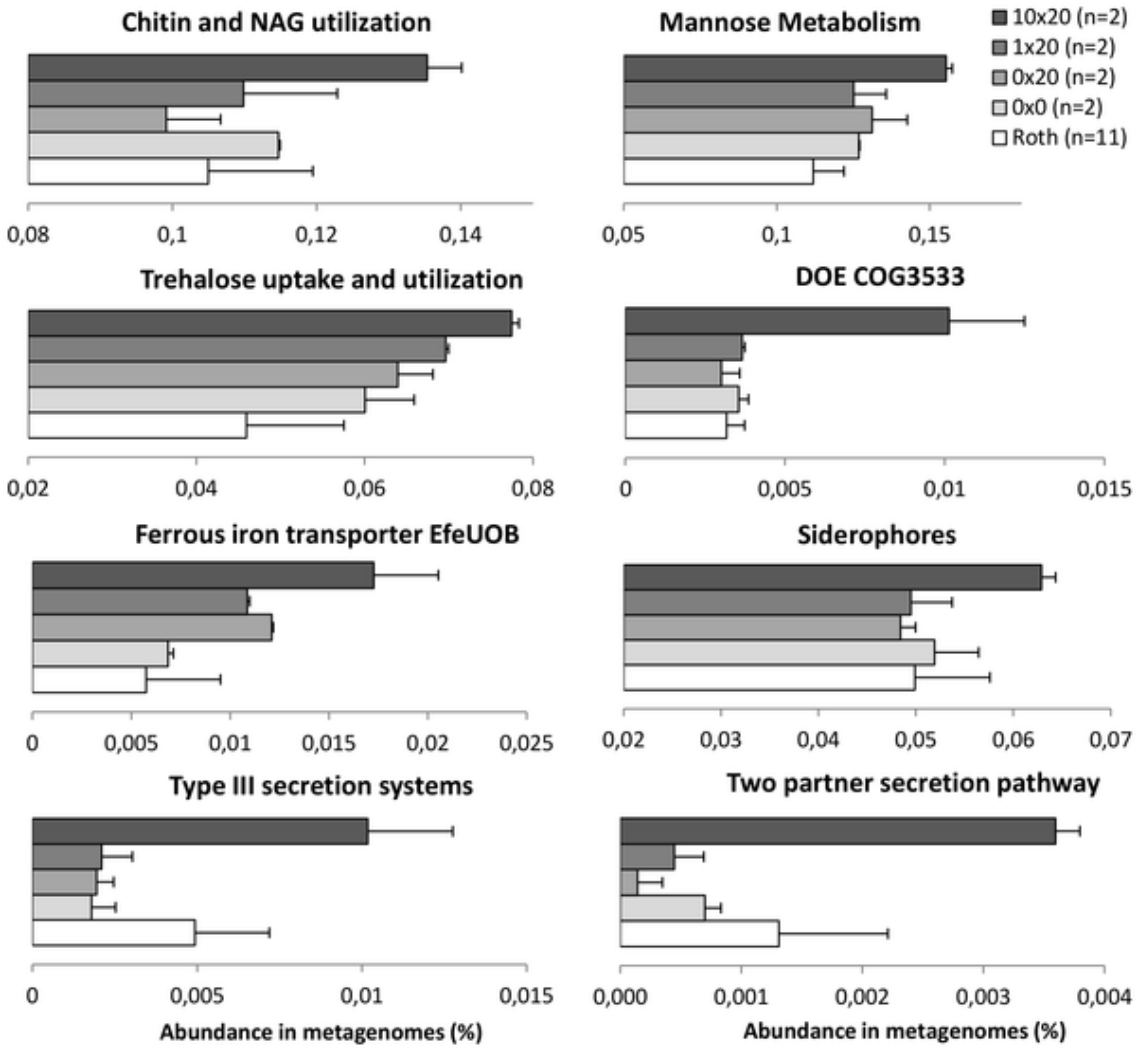
Figure 6. Bacterial genera enriched by chitin treatment.



Jacquiod S, Franqueville L, Cécillon S, M. Vogel T, et al. (2013) Soil Bacterial Community Shifts after Chitin Enrichment: An Integrative Metagenomic Approach. PLoS ONE 8(11): e79699. doi:10.1371/journal.pone.0079699

Global Effects

Figure 5. Major functional changes in the soil bacterial community after incubation and chitin



Jacquiod S, Franqueville L, Cécillon S, M. Vogel T, et al. (2013) Soil Bacterial Community Shifts after Chitin Enrichment: An Integrative Metagenomic Approach. PLoS ONE 8(11): e79699. doi:10.1371/journal.pone.0079699

Uncultured Microorganisms

OPEN ACCESS Freely available on line

PLOS | ONE

Enrichment and Genome Sequence of the Group I.1a Ammonia-Oxidizing Archaeon "*Ca.* Nitrosotenuis uzonensis" Representing a Clade Globally Distributed in Thermal Habitats

Elena V. Lebedeva[1], Roland Hatzenpichler[2], Eric Pelletier[3,4,5], Nathalie Schuster[2], Sandra Hauzmayer[2], Aleksandr Bulaev[1], Nadezhda V. Grigor'eva[1], Alexander Galushko[2], Markus Schmid[2], Marton Palatinszky[2], Denis Le Paslier[3,4,5], Holger Daims[2], Michael Wagner[2*]

1 Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia, 2 Department of Microbiology and Ecosystem Science, University of Vienna, Vienna, Austria, 3 Commissariat à l'Energie Atomique, Genoscope, Evry, France, 4 Centre National de la Recherche Scientifique, Evry, France, 5 Université d'Evry-Val-d'Essonne, Evry, France

Abstract

The discovery of ammonia-oxidizing archaea (AOA) of the phylum *Thaumarchaeota* and the high abundance of archaeal ammonia monooxygenase subunit A encoding gene sequences in many environments have extended our perception of nitrifying microbial communities. Moreover, AOA are the only aerobic ammonia oxidizers known to be active in geothermal environments. Molecular data indicate that in many globally distributed terrestrial high-temperature habits a thaumarchaeotal lineage within the *Nitrosopumilus* cluster (also called "marine" group I.1a) thrives, but these microbes have neither been isolated from these systems nor functionally characterized *in situ* yet. In this study, we report on the enrichment and genomic characterization of a representative of this lineage from a thermal spring in Kamchatka. This thaumarchaeote, provisionally classified as "*Candidatus* Nitrosotenuis uzonensis", is a moderately thermophilic, non-halophilic, chemolithoautotrophic ammonia oxidizer. The nearly complete genome sequence (assembled into a single scaffold) of this AOA confirmed the presence of the typical thaumarchaeotal pathways for ammonia oxidation and carbon fixation, and indicated its ability to produce coenzyme F_{420} and to chemotactically react to its environment. Interestingly, like members of the genus *Nitrosoarchaeum*, "*Candidatus* N. uzonensis" also possesses a putative artubulin-encoding gene. Genome comparisons to related AOA with available genome sequences confirmed that the newly cultured AOA has an average nucleotide identity far below the species threshold and revealed a substantial degree of genomic plasticity with unique genomic regions in "*Ca.* N. uzonensis", which potentially include genetic determinants of ecological niche differentiation.

Uncultured Microorganisms

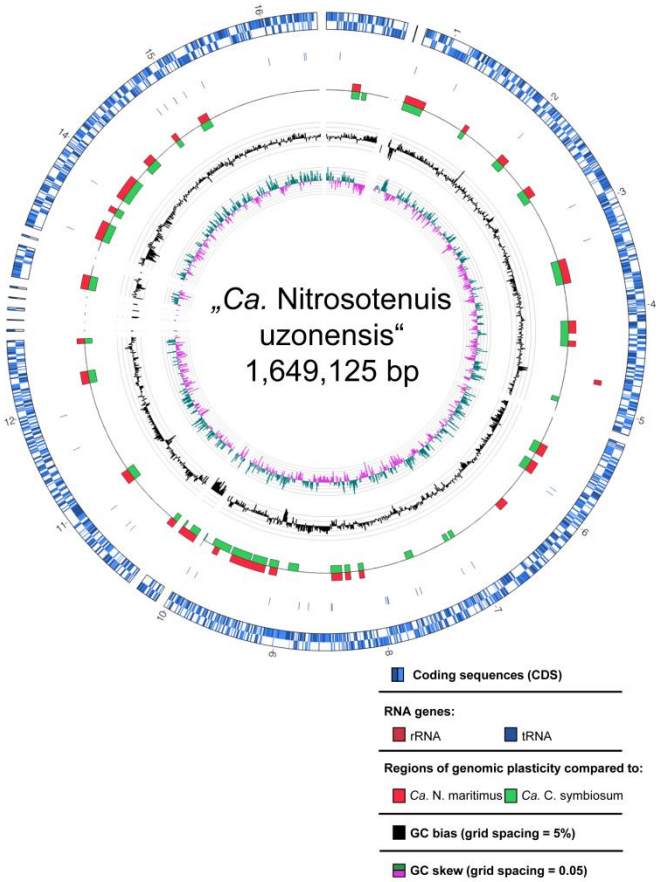


Figure S1. Circular representation of the “*Ca*. N. uzonensis” chromosome.

454 (250 Mb) coupled with Illumina (1.2Gb)

Genome size	1,649,125 bp
Average GC-content	42.25 %
Number of scaffolds	1
Number of contigs	14
Number of genomic objects (CDS, fCDS, tRNAs, rRNAs)	1,999
Number of CDS	1,958
Number of CDS with predicted function	411 (21.0%)
CDS density	1.19 CDS/kb
Average CDS length	766.3 bp
Average intergenic length	98.5 bp
Protein coding density	90.2 %
Number of 16S-23S rRNA operons	1
Number of 5S rRNAs	1
Number of tRNAs	38
Number of *amoA*, *amoB*, *amoC* genes	1 / 1 / 2
Number of *accA*/*pccB* and *hcd* genes	1 / 1

Table 1. Overview of key features of the “*Ca*. Nitrosotenuis uzonensis” genome.

Lebedeva et al. (2013) Enrichment and Genome Sequence of the Group I.1a Ammonia-Oxidizing Archaeon “*Ca*. Nitrosotenuis uzonensis” Representing a Clade Globally Distributed in Thermal Habitats. PLoS ONE 8(11): e80835. doi:10.1371/journal.pone.0080835

Consortium - Dominant

nature biotechnology

Metagenomic analysis of two enhanced biological phosphorus removal (EBPR) sludge communities

Héctor García Martín[1,5], Natalia Ivanova[1,5], Victor Kunin[1], Falk Warnecke[1], Kerrie W Barry[1], Alice C McHardy[4], Christine Yeates[2], Shaomei He[3], Asaf A Salamov[1], Ernest Szeto[1], Eileen Dalin[1], Nik H Putnam[1], Harris J Shapiro[1], Jasmyn L Pangilinan[1], Isidore Rigoutsos[4], Nikos C Kyrpides[1], Linda Louise Blackall[2], Katherine D McMahon[3], and Philip Hugenholtz[1]

Enhanced biological phosphorus removal (EBPR) is one of the best-studied microbially mediated industrial processes because of its ecological and economic relevance. Despite this, it is not well understood at the metabolic level. Here we present a metagenomic analysis of two lab-scale EBPR sludges dominated by the uncultured bacterium, "*Candidatus* Accumulibacter phosphatis." The analysis sheds light on several controversies in EBPR metabolic models and provides hypotheses explaining the dominance of *A. phosphatis* in this habitat, its lifestyle outside EBPR and probable cultivation requirements. Comparison of the same species from different EBPR sludges highlights recent evolutionary dynamics in the *A. phosphatis* genome that could be linked to mechanisms for environmental adaptation. In spite of an apparent lack of phylogenetic overlap in the flanking communities of the two sludges studied, common functional themes were found, at least one of them complementary to the inferred metabolism of the dominant organism. The present study provides a much needed blueprint for a systems-level understanding of EBPR and illustrates that metagenomics enables detailed, often novel, insights into even well-studied biological systems.

Consortium - Dominant

Plasmid (3 and 8 Kb) and
Fosmid (40 Kb) Libraries

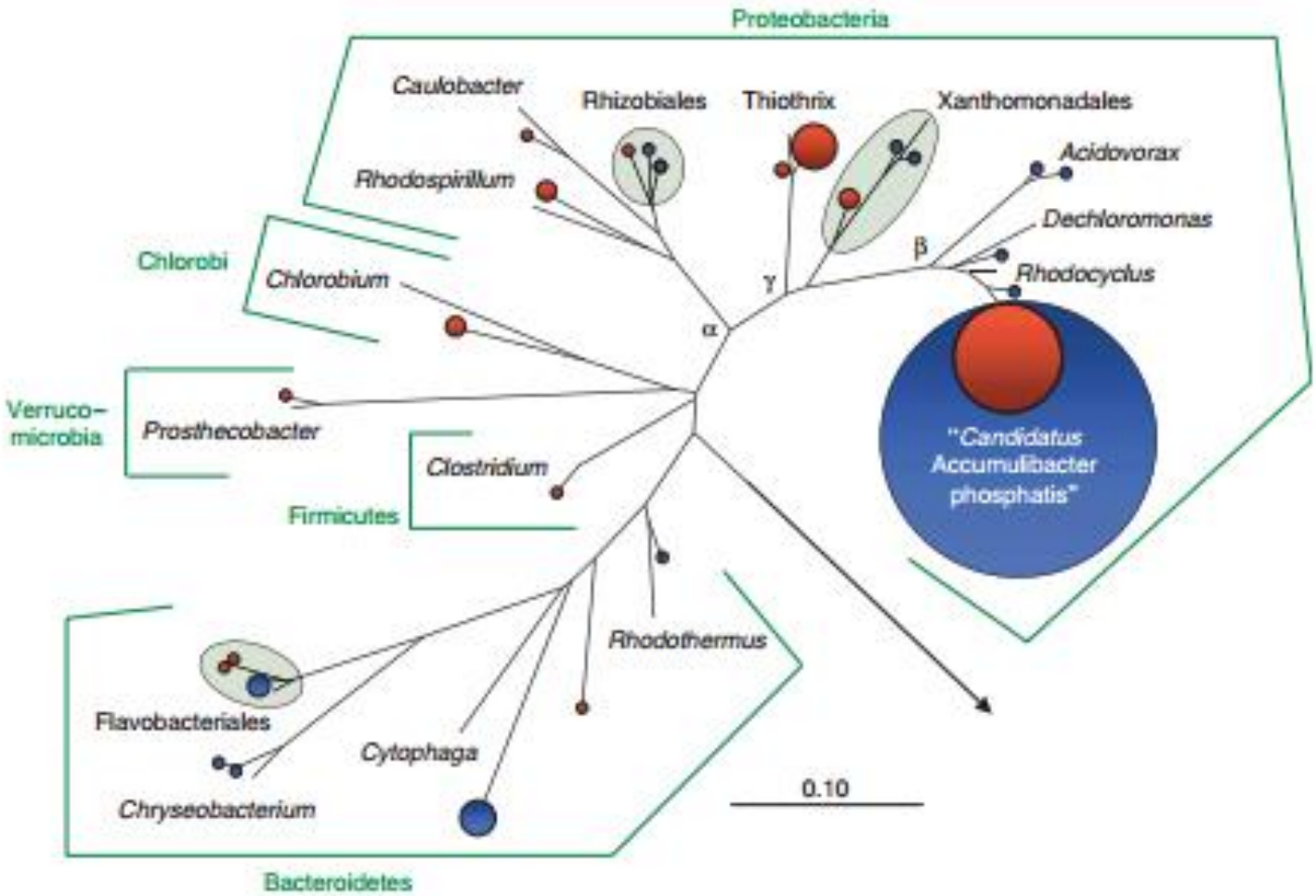


Figure 1 Maximum-likelihood tree based on partial and complete 16S rRNA genes identified on metagenomic contigs comprising at least two reads.

Garcia Martin et al. (2006) Metagenomic analysis of two enhanced biological phosphorus removal (EBPR) sludge communities. Nature Biotechnology 24(10): 1263-1269. doi:10.1038/nbt1247

Consortium - Dominant

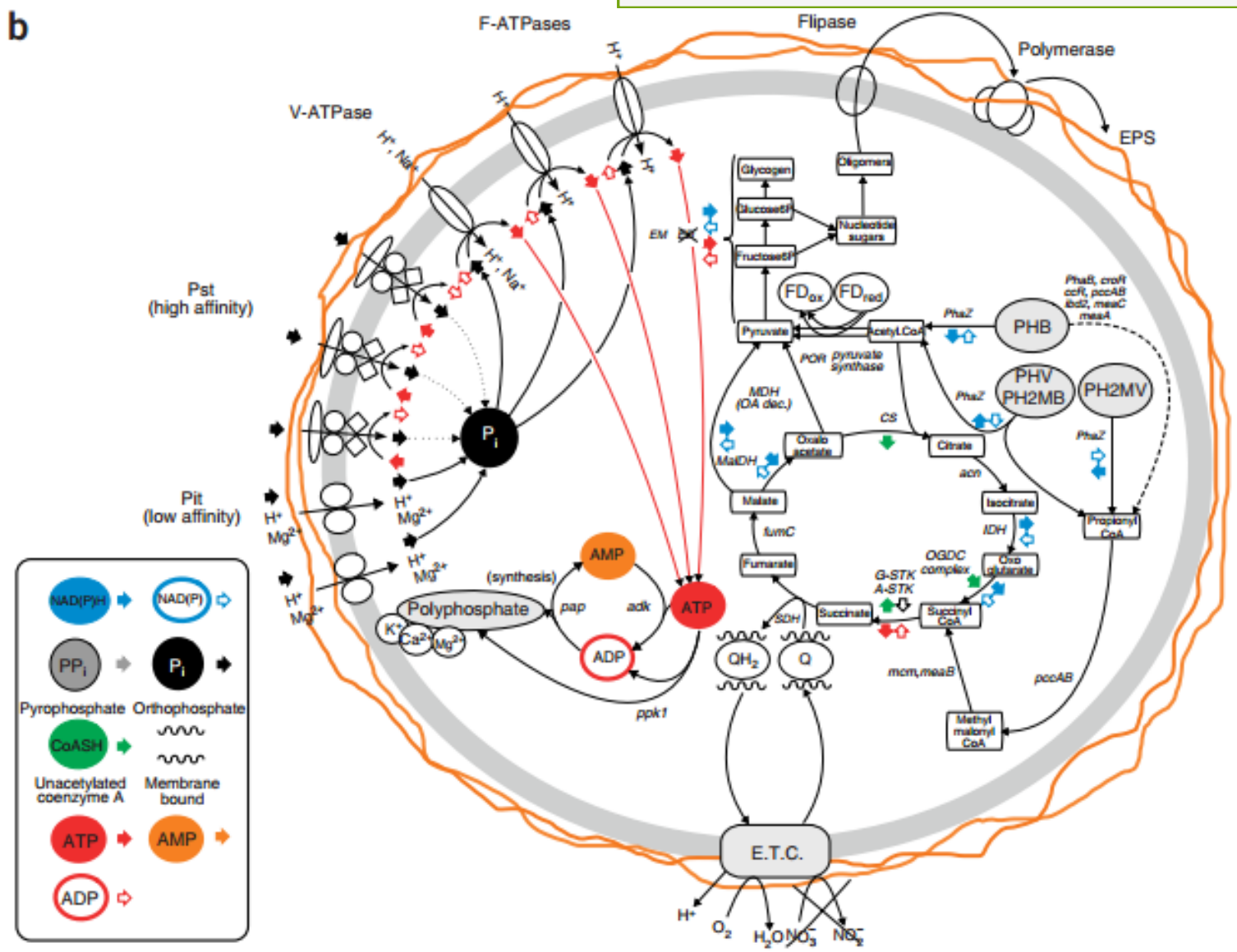


Figure 2 EBPR-relevant metabolism inferred from the A. phosphatis composite.

Garcia Martin et al. (2006) Metagenomic analysis of two enhanced biological phosphorus removal (EBPR) sludge communities. Nature Biotechnology 24(10): 1263-1269. doi:10.1038/nbt1247

Consortium - Dominant

The ISME Journal (2012) 6, 1094–1106
© 2012 International Society for Microbial Ecology All rights reserved 1751-7362/12
www.nature.com/ismej

ORIGINAL ARTICLE

A metagenome of a full-scale microbial community carrying out enhanced biological phosphorus removal

Mads Albertsen, Lea Benedicte Skov Hansen, Aaron Marc Saunders, Per Halkjær Nielsen and Kåre Lehmann Nielsen
Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Aalborg, Denmark

Enhanced biological phosphorus removal (EBPR) is widely used for removal of phosphorus from wastewater. In this study, a metagenome (18.2 Gb) was generated using Illumina sequencing from a full-scale EBPR plant to study the community structure and genetic potential. Quantitative fluorescence *in situ* hybridization (qFISH) was applied as an independent method to evaluate the community structure. The results were in qualitative agreement, but a DNA extraction bias against gram positive bacteria using standard extraction protocols was identified, which would not have been identified without the use of qFISH. The genetic potential for community function showed enrichment of genes involved in phosphate metabolism and biofilm formation, reflecting the selective pressure of the EBPR process. Most contigs in the assembled metagenome had low similarity to genes from currently sequenced genomes, underlining the need for more reference genomes of key EBPR species. Only the genome of '*Candidatus* Accumulibacter', a genus of phosphorus-removing organisms, was closely enough related to the species present in the metagenome to allow for detailed investigations. Accumulibacter accounted for only 4.8% of all bacteria by qFISH, but the depth of sequencing enabled detailed insight into their microdiversity in the full-scale plant. Only 15% of the reads matching Accumulibacter had a high similarity (>95%) to the sequenced Accumulibacter clade IIA strain UW-1 genome, indicating the presence of some microdiversity. The differences in gene complement between the Accumulibacter clades were limited to genes for extracellular polymeric substances and phage-related genes, suggesting a selective pressure from phages on the Accumulibacter diversity.

Consortium - Dominant

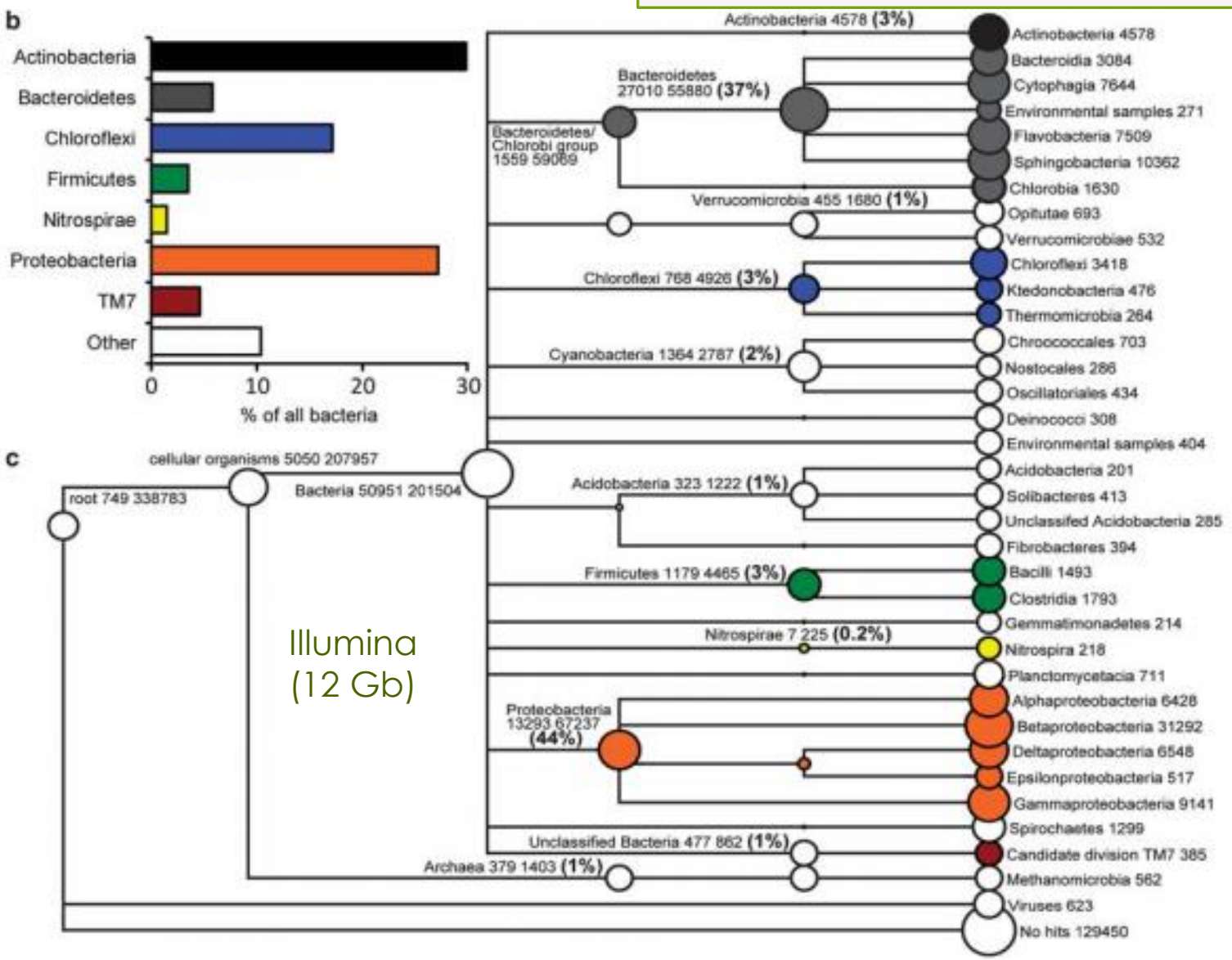


Figure 1 Comparison of the metagenome and qFISH results for Aalborg East EBPR.

Albertsen et al. (2011) A metagenome of a full-scale microbial community carrying out enhanced biological phosphorus removal. ISME J. 2012 Jun;6(6):1094-106. doi: 10.1038/ismej.2011.176.

Consortium - Dominant

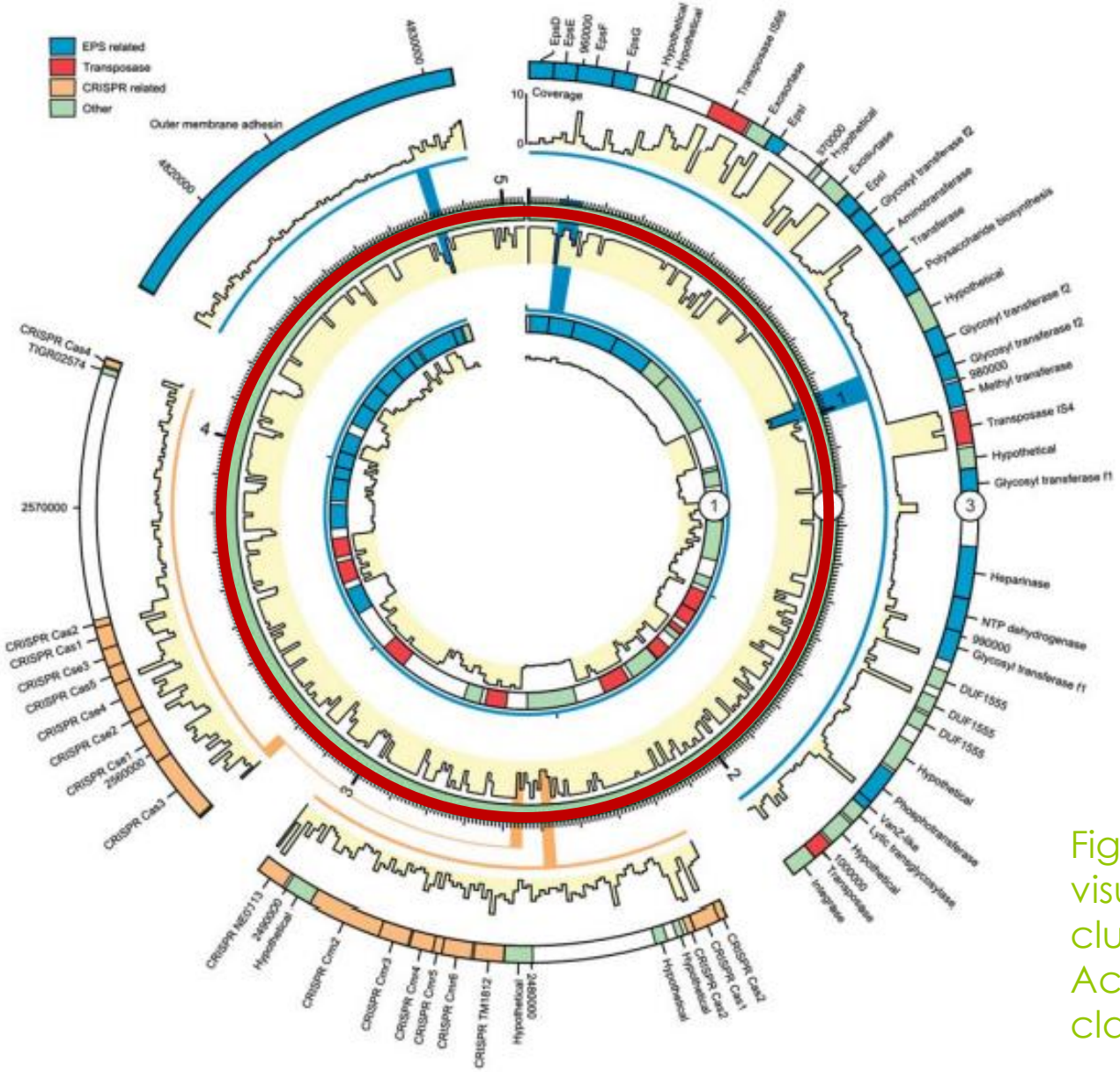


Figure 5 Circular visualization of gene clusters unique to Accumulibacter clade IIA strain UW-1.

Albertsen et al. (2011) A metagenome of a full-scale microbial community carrying out enhanced biological phosphorus removal. ISME J. 2012 Jun;6(6):1094-106. doi: 10.1038/ismej.2011.176.

Consortium - Function

Comparative metagenomics of three *Dehalococcoides*-containing enrichment cultures: the role of the non-dechlorinating community

Laura A Hug[1], Robert G Beiko[2], Annette R Rowe[3], Ruth E Richardson[3] and Elizabeth A Edwards[1,4*]

Abstract

Background: The *Dehalococcoides* are strictly anaerobic bacteria that gain metabolic energy via the oxidation of H_2 coupled to the reduction of halogenated organic compounds. *Dehalococcoides* spp. grow best in mixed microbial consortia, relying on non-dechlorinating members to provide essential nutrients and maintain anaerobic conditions. A metagenome sequence was generated for the dechlorinating mixed microbial consortium KB-1. A comparative metagenomic study utilizing two additional metagenome sequences for *Dehalococcoides*-containing dechlorinating microbial consortia was undertaken to identify common features that are provided by the non-dechlorinating community and are potentially essential to *Dehalococcoides* growth.

Results: The KB-1 metagenome contained eighteen novel homologs to reductive dehalogenase genes. The metagenomes obtained from the three consortia were automatically annotated using the MG-RAST server, from which statistically significant differences in community composition and metabolic profiles were determined. Examination of specific metabolic pathways, including corrinoid synthesis, methionine synthesis, oxygen scavenging, and electron-donor metabolism identified the Firmicutes, methanogenic Archaea, and the δ-Proteobacteria as key organisms encoding these pathways, and thus potentially producing metabolites required for *Dehalococcoides* growth.

Conclusions: Comparative metagenomics of the three *Dehalococcoides*-containing consortia identified that similarities across the three consortia are more apparent at the functional level than at the taxonomic level, indicating the non-dechlorinating organisms' identities can vary provided they fill the same niche within a consortium. Functional redundancy was identified in each metabolic pathway of interest, with key processes encoded by multiple taxonomic groups. This redundancy likely contributes to the robust growth and dechlorination rates in dechlorinating enrichment cultures.

Consortium - Function

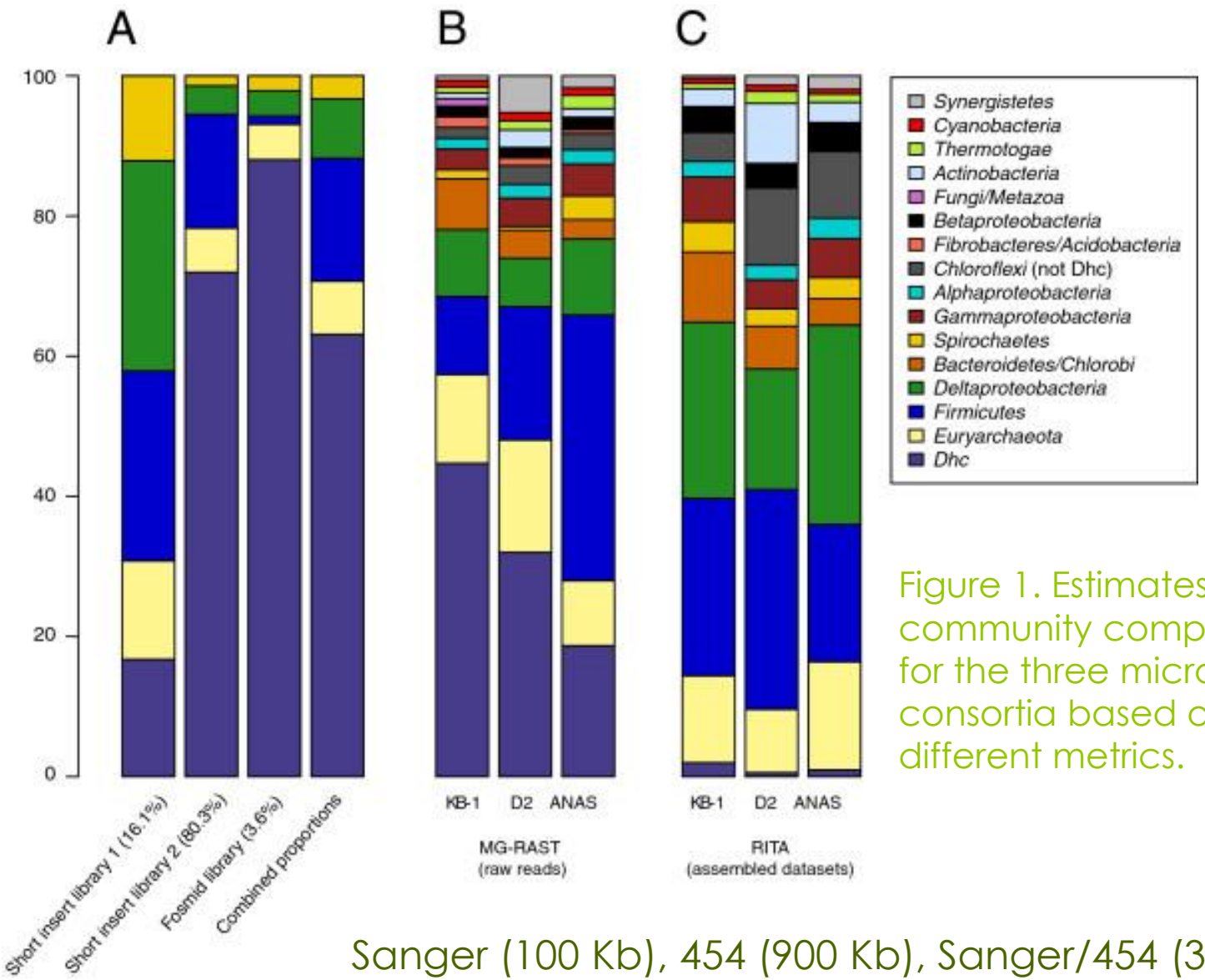


Figure 1. Estimates of community composition for the three microbial consortia based on three different metrics.

Sanger (100 Kb), 454 (900 Kb), Sanger/454 (300 Kb)

Hug et al. Comparative metagenomics of three *Dehalococcoides*-containing enrichment cultures: the role of the non-dechlorinating community (2012) BMC Genomics 13:327 doi: 10.1186/1471-2164-13-327

Consortium - Function

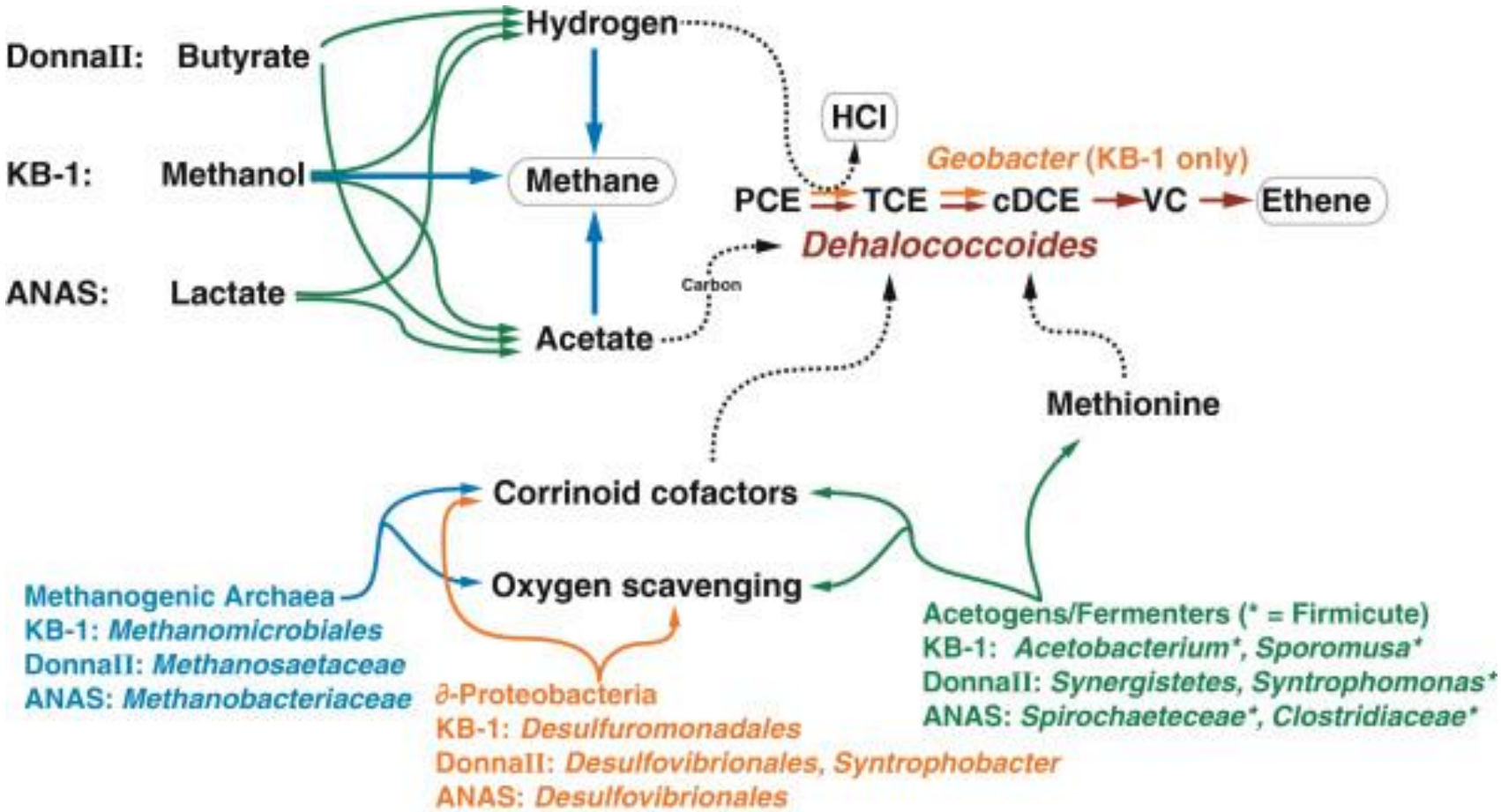


Figure 2. Schematic overview of the examined metabolic processes taking place within the dechlorinating enrichment consortia, with the organisms implicated in each process highlighted..

Hug et al. Comparative metagenomics of three *Dehalococcoides*-containing enrichment cultures: the role of the non-dechlorinating community (2012) BMC Genomics 13:327 doi: 10.1186/1471-2164-13-327

Consortium - Function

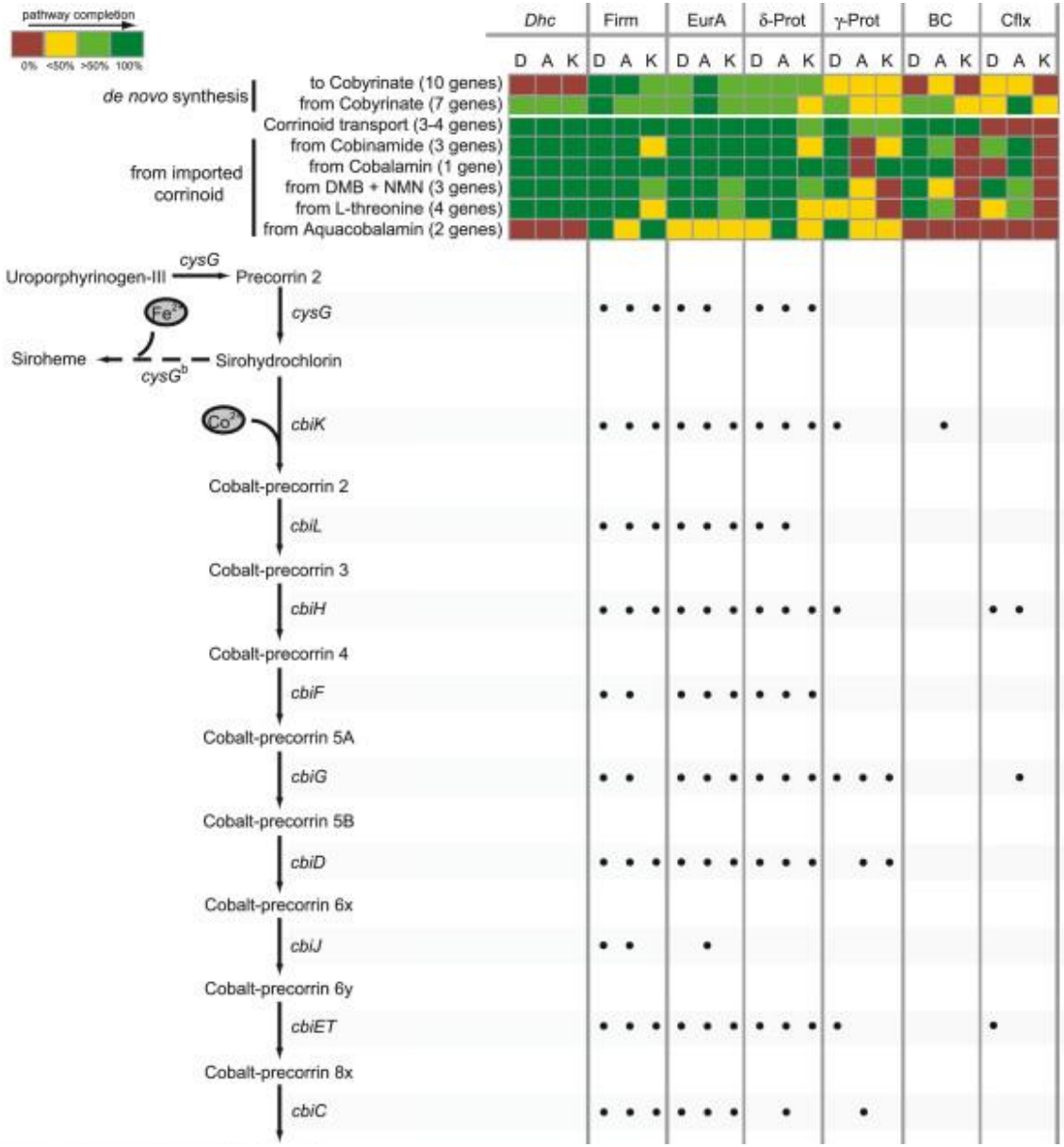


Figure 6. The anaerobic cobalamin synthesis pathway with gene distributions within the three metagenomes by taxonomic classification.

Hug et al. Comparative metagenomics of three *Dehalococcoides*-containing enrichment cultures: the role of the non-dechlorinating community (2012) BMC Genomics 13:327 doi: 10.1186/1471-2164-13-327

Enriched Genes

Identification and characterization of a multidomain hyperthermophilic cellulase from an archaeal enrichment

Joel E. Graham, Melinda E. Clark, Dana C. Nadler, Sarah Huffer, Harshal A. Chokhawala, Sara E. Rowland, Harvey W. Blanch, Douglas S. Clark & Frank T. Robb

Affiliations | Contributions | Corresponding authors

Nature Communications **2**, Article number: 375 | doi:10.1038/ncomms1373

Received 22 December 2010 | Accepted 02 June 2011 | Published 05 July 2011

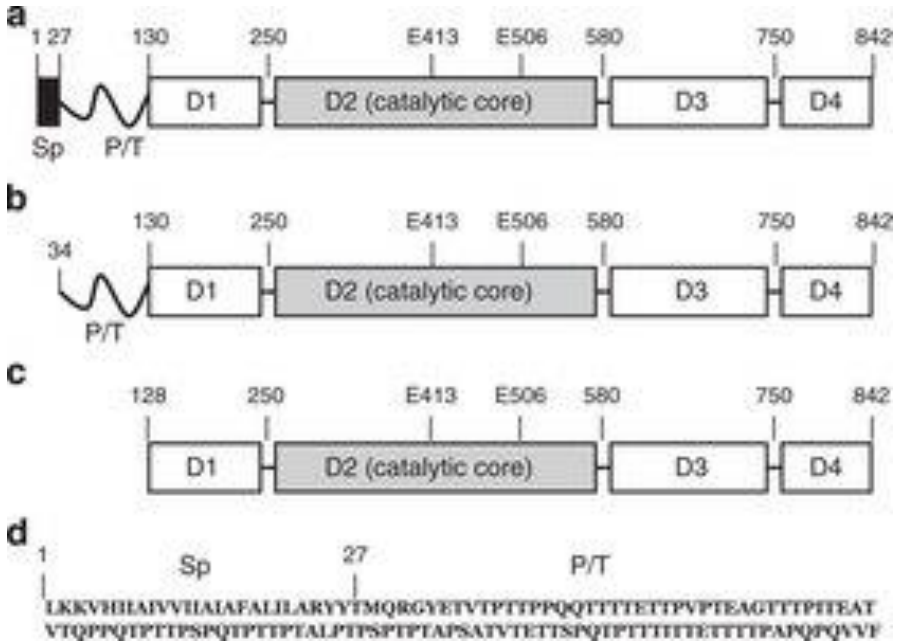


Figure 4: Sequence analysis of EBI-244 enzyme. Diagrams showing predicted signal peptide/membrane anchor (Sp), Proline threonine rich low complexity region (P/T) and structural domain architecture of EBI-244.

Enriched Genes

Applied and Environmental Microbiology, Mar. 2003, p. 1408–1416 Vol. 69, No. 3
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.3.1408–1416.2003
Copyright © 2003, American Society for Microbiology. All Rights Reserved.

Construction and Screening of Metagenomic Libraries Derived from Enrichment Cultures: Generation of a Gene Bank for Genes Conferring Alcohol Oxidoreductase Activity on *Escherichia coli*

Anja Knietsch,[1] Tanja Waschkowitz,[1] Susanne Bowien,[1] Anke Henne,[2] and Rolf Daniel[1]*

Abteilung Allgemeine Mikrobiologie[1] and Göttingen Genomics Laboratory,[2] Institut für Mikrobiologie und Genetik der Georg-August-Universität, 37077 Göttingen, Germany

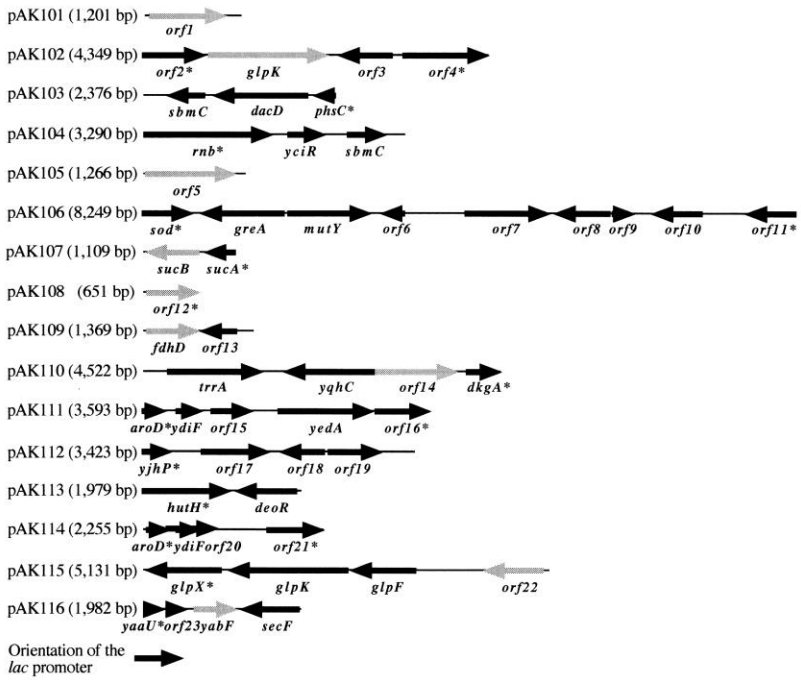


Figure 4. Genetic organization and length of the inserts of pAK101 to pAK116. The insert sizes of the plasmids are given in brackets. Arrows and arrowheads represent the lengths, locations, and orientations of the potential genes within the inserts. Incomplete open reading frames are marked with asterisks.

The Mediterranean Sea – Alicante, Spain

Pelagic Seawater

UMH - Enrichments

Size Enrichment

20 µm

5 µm

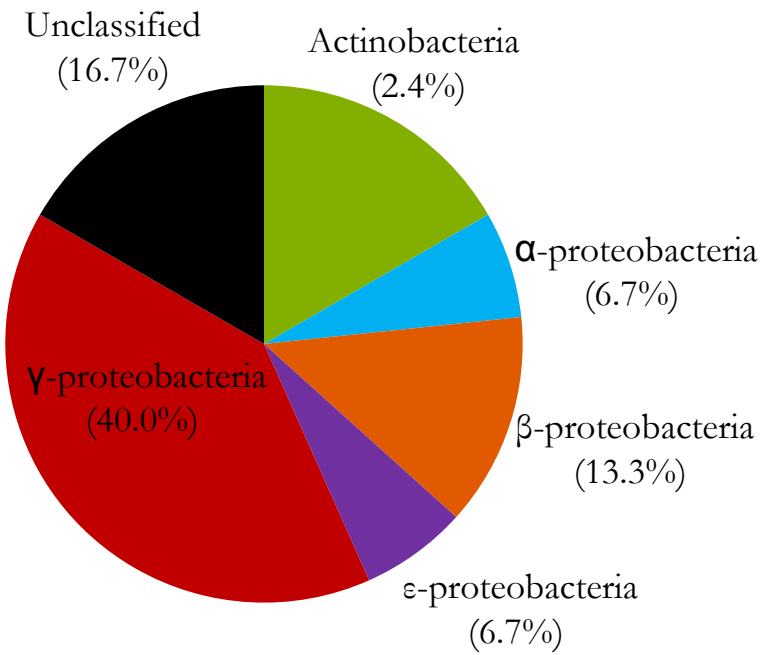
0.2 µm

0.025 µm

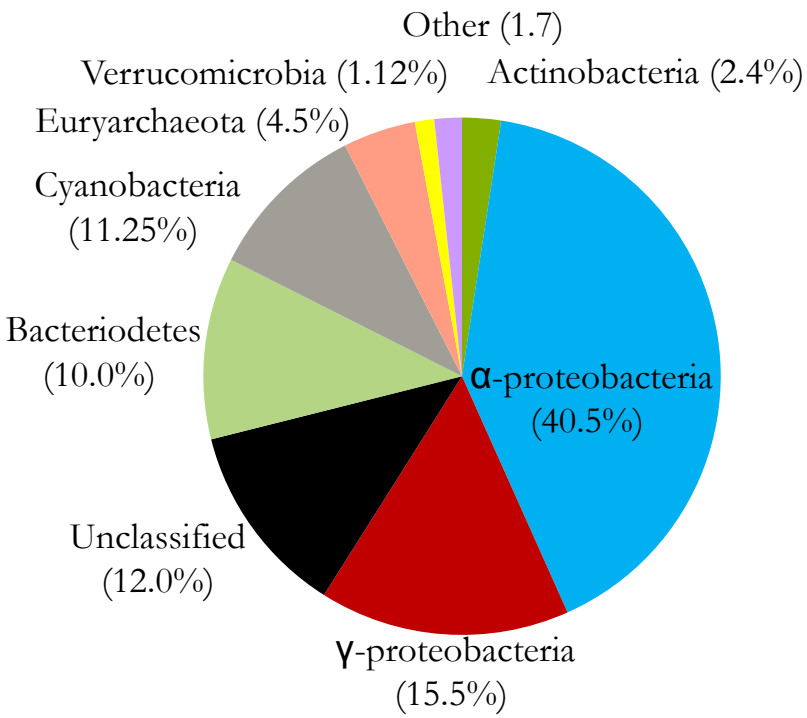
Metagenomics
Ultra DCM

Phylogenetic composition using 16S rRNA genes

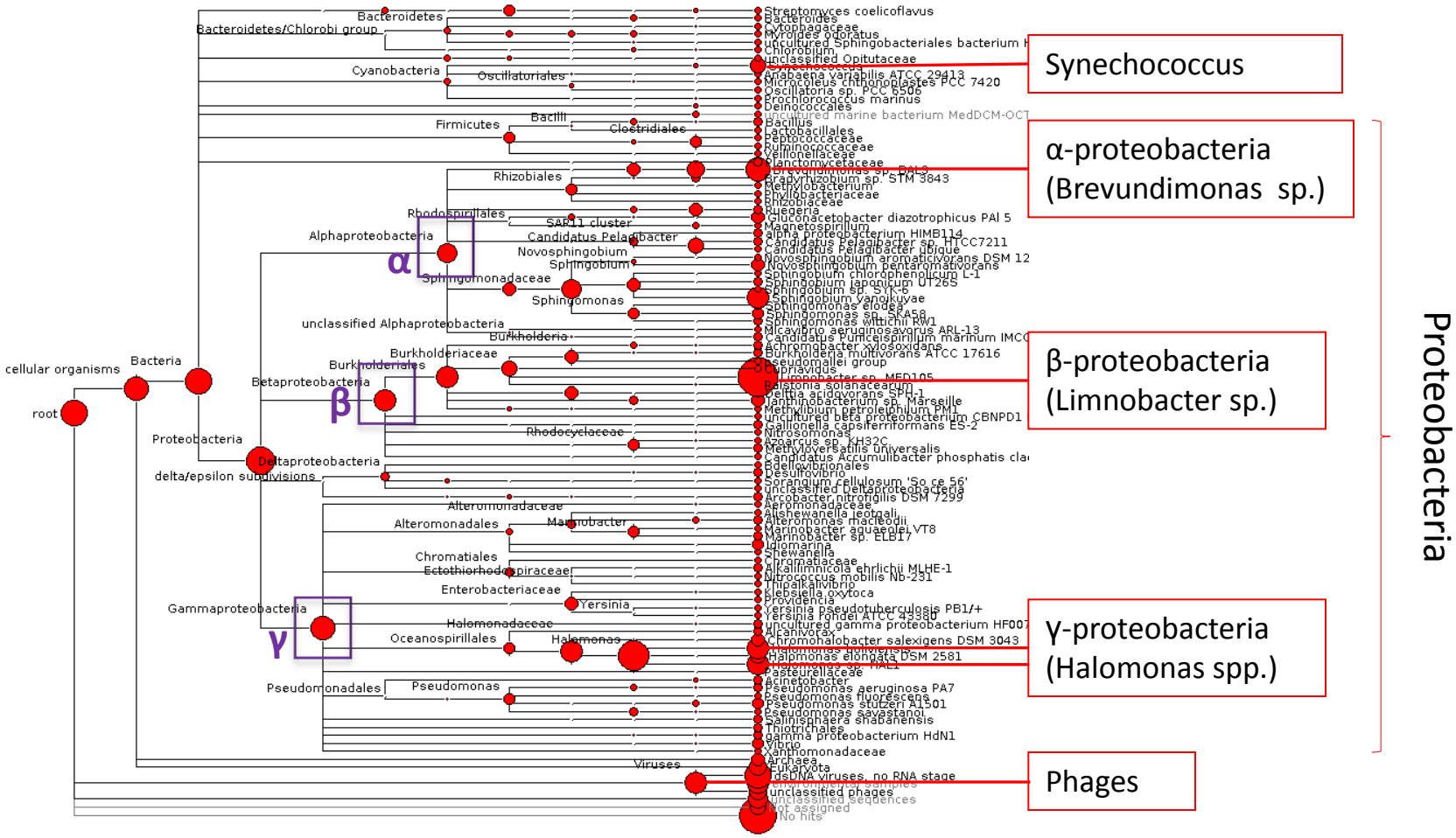
DCM Ultra Metagenome (<0.2 µm)
12 Mb (N=32)



DCM Metagenome (>0.2 µm)
312 Mb (N=1038)

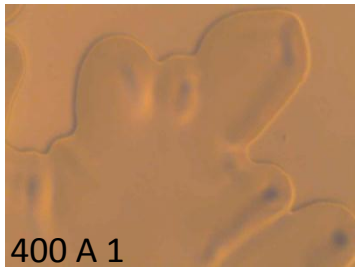


Phylogenetic composition using all 20,000 reads



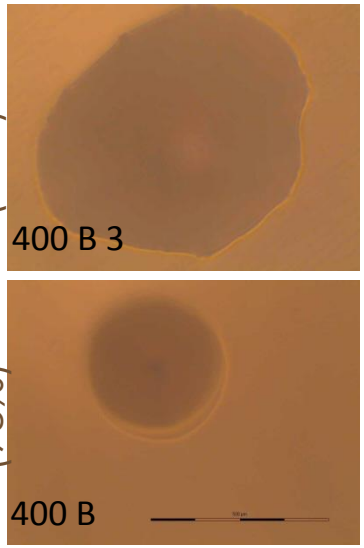
UMH - Enrichments

After 5 weeks…



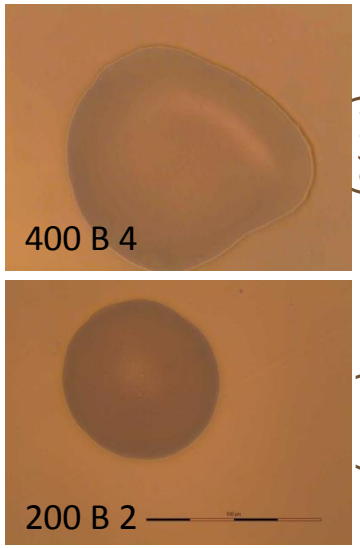
Limnobacter thiooxidans (99-98%) X3

After 8 weeks…



Brevundimonas mediterranea (99%) X4

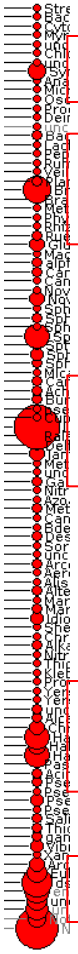
Brevundimonas aurantiaca (98%)



Rhizobium sp. (99%)

Rhizobium sp. (98%)

16S PCR Sequencing



Synechococcus

α-proteobacteria (Brevundimonas sp.)

β-proteobacteria (Limnobacter sp.)

γ-proteobacteria (Halomonas spp.)

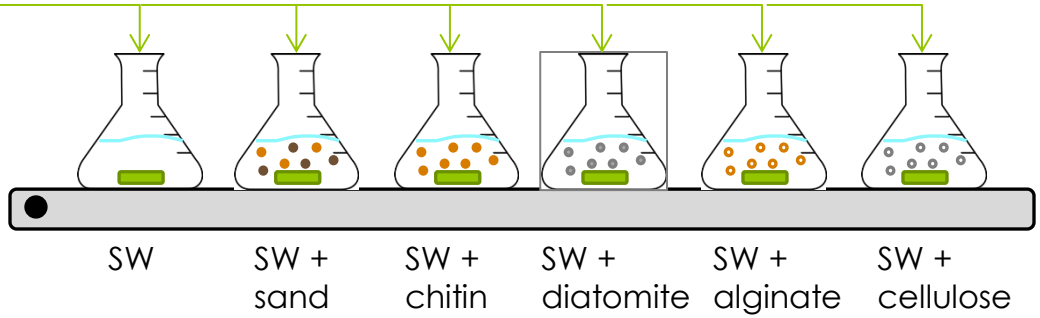
Phages

UMH - Enrichments

May 20, 2013
50m, 16C

Ammended with:
0.1% pyruvate
0.01% yeast extract

Particle-attached Enrichment



16°C and12 h light cycles, suspension via stirring
2 week incubation

stop stirring and allow particles to settle
rinse with sterilized seawater, repeat X3

vacum filter using sterile chamber
rinse with sterile seawater X 3

DNA extraction from substrate

13,500 rpm
30 min

DNA extraction from cell pellet

Particle-attached Metagenomes

UMH - Enrichments

Particle-attached Metagenomes

1/24th Illumina HiSeq lane per sample

		SW	SW + sand	SW + cellulase	SW + chitin	SW + diatomite
Post QC	Number of reads (mil)	23.3	22.2	18.6	18.5	19.8
	Mean length (bp)	91	88.3	88	90.2	89.3
	Median length (bp)	101	101	101	101	101
	Total data amount (Gb)	2	1.9	1.1	1.1	1.1
Assembly	Assembled reads(%)	74	62	63	72	67
	Number of nodes	84,372	76,127	178,334	143,370	146,810
	n50	1378	1971	612	589	580
	Maximum length (bp)	227,611	227,919	136,078	295,336	350,697
	Contigs > 10 Kb	212	400	220	220	132
	Contigs > 1 Kb	10,240	11,149	8,317	6,073	6,342

UMH - Enrichments

Use 16S rRNA for classification

Thank You

Use assemblies to show top microorganisms assembled