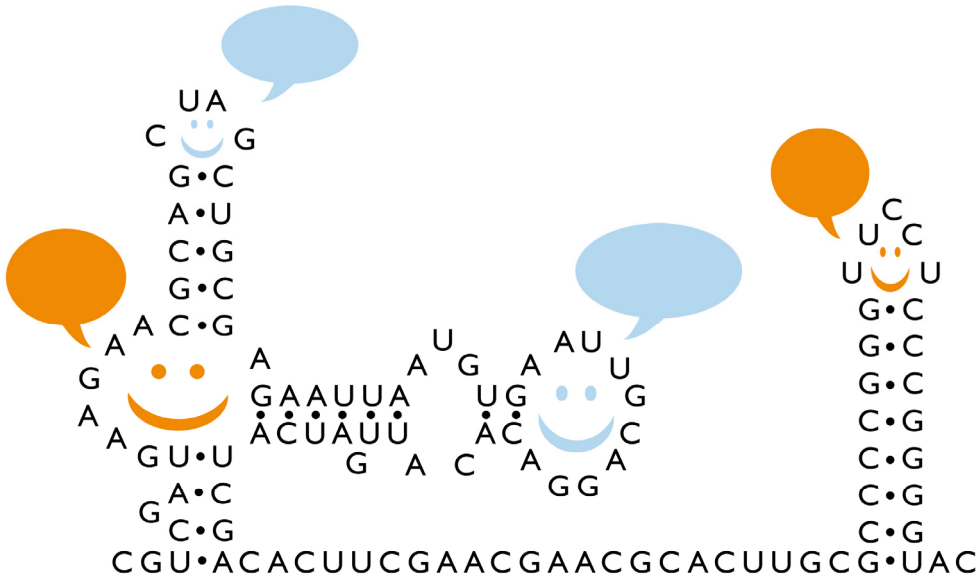
International Workshop on ribosomal RNA Technology

Program & Abstracts

April 7-9, 2008 in Bremen



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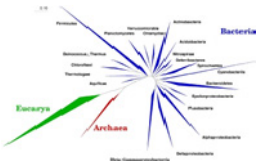

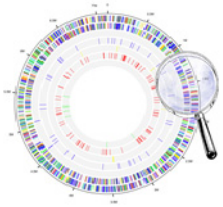

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Welcome to the International Workshop on ribosomal RNA technology in Bremen

First of all we would like to welcome you to the Max Planck Institute for Marine Microbiology in Bremen and wish you a pleasant stay and many fruitful discussions.

Thirty years have passed since Carl Woese suggested three primary kingdoms based on the phylogenetic analysis of the ribosomal RNA genes. Despite ongoing discussions about the validity of the concept, the introduction of the ribosomal RNA as a universal molecular marker has influenced the self conception of microbiology to its roots. Adopted by many researchers worldwide the ribosomal RNA has become the "gold-standard" for molecular taxonomy, diversity analysis and the identification of microorganisms. The more than 600,000 rRNA sequences in the public databases constitute an unprecedented hallmark of the richness of microbial biodiversity on earth. Nevertheless, from the ongoing genomic and metagenomic studies it is evident that we might have seen only the tip of the iceberg so far.

It is now time to sum up the current status and think about how to best proceed with this rich harvest from a technological and biological point of view. Central points that will be addressed by the conference are:

1. What kind of technology is needed to help biologists to deal with the deluge of data?
2. Which kinds and quality of data can be expected that need to be organized, stored and analysed?
3. How can the rRNA help to answer burning questions in biology?

To move on with these issues **26 experts from Europe and the US** will talk about the burning topics in the field of rRNA technology. The conference is intended to **cross boundaries** by bringing together computer scientists, bioinformaticians, geologists, statisticians and biologists to finally find new approaches that help to answer the big questions **"Who is out there?"** and **"What are they doing?".**

Again a warm welcome from the organisation committee

Frank Oliver Glöckner, MPI-Bremen, Linda Amaral-Zettler, ICoMM, Wolfgang Ludwig, TU München, Jörg Peplies, Ribocon GmbH, Bernhard Fuchs, MPI-Bremen, Alban Ramette, MPI-Bremen and our helping hands: Anke Lindström, Algrid Hillmer and Sandra Nowak

More information about the speakers and program can be found at www.arb-silva.de

Bremen, 24 March 2008
rev. 8

Welcome

Welcome to the International Workshop on ribosomal RNA technology in Bremen, April 07 to 09, 2008

We would like to welcome you to the BITZ-Bremen and the Max Planck Institute for Marine Microbiology in Bremen and wish you a pleasant and enlightening stay.

How to find us:
The BITZ-Bremen (workshop sessions) and the Max Planck Institute (welcome dinner) are located on the campus of the University Bremen which is also called the "Technologiepark". You can easily reach us by tram or taxi from the international airport Bremen or from the main train station. More information – see appendix.

The **addresses** are:

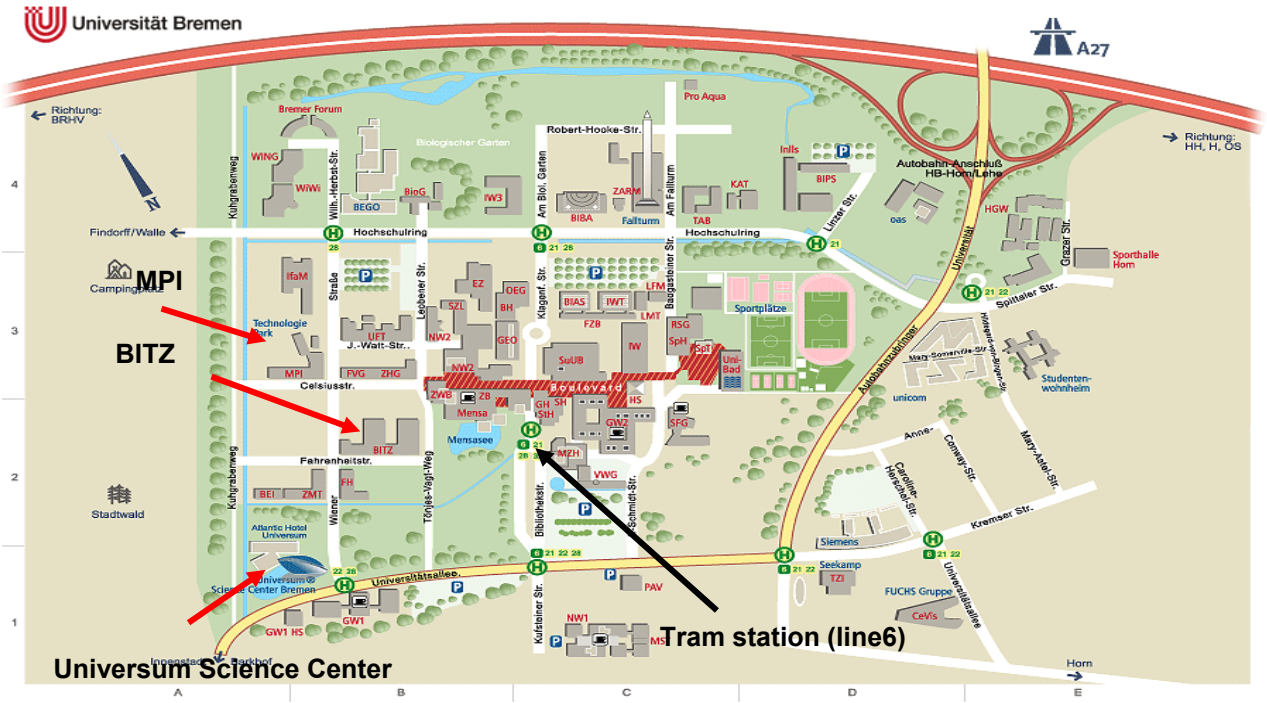
BITZ-Bremen
Fahrenheitstrasse 1
28359 Bremen

and

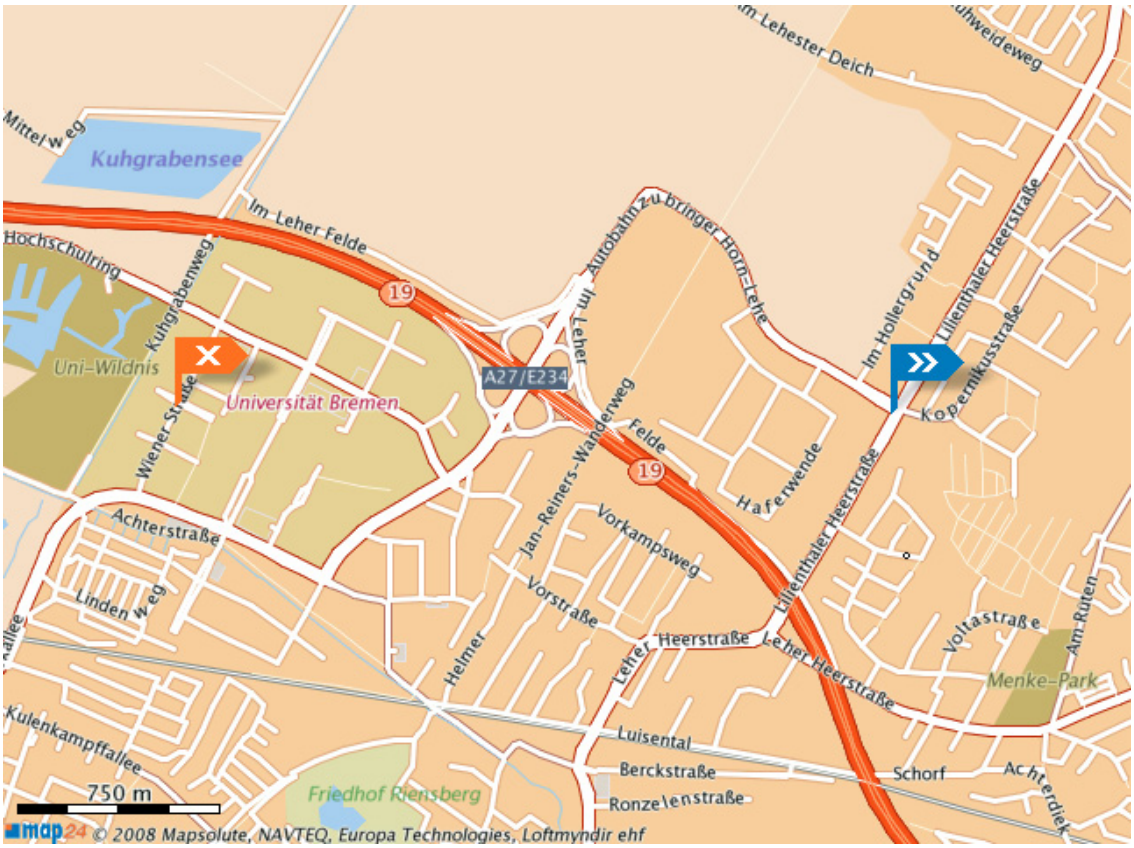
Max Planck Institute for Marine Microbiology
Celsiusstrasse 1
28359 Bremen
A description how to find the Max Planck Institute can be found here:
http://www.mpi-bremen.de/en/How_to_get_to_the_Institute.html

The BITZ-Bremen is just across the street - see map:

Map of the campus (Technologiepark): http://www.uni-bremen.de/lageplan/



The map shows the location of the **hotels Deutsche Eiche and Horner Eiche** (blue flag) and **the MPI-Bremen/BITZ** (red flag) for your orientation. Walking is possible via the Jan-Reiners-Wanderweg, but calculate at least 45 minutes and ask at the reception for a detailed map.



Accommodation:

Participants have to arrange their stay by themselves! Booking at Hotel Horner Eiche and Hotel Deutsche Eiche is recommended. More information can be found at:
http://www.arb-silva.de/rrna-workshop/hotels/

Meals:
In Germany breakfast is normally included when you stay over night in a hotel. We will provide free coffee and tea, as well as a snack for lunch during the workshop.
On Monday evening, a free dinner will be served at MPI-Bremen, all participants are welcome to join.

Program: The workshop starts on ***Monday*** the 7th at 08.45 am and ends on Wednesday 9th at 12.00 noon.
You will have time to visit the nice town of Bremen on ***Tuesday*** evening. Additional information on sightseeing in Bremen will be added to your workshop package.

More information and the complete program can be found at:
http://www.arb-silva.de/rrna-workshop/ and
http://www.arb-silva.de/rrna-workshop/program/

Welcome

Contact: If you have questions please send an e-mail to eu-workshops@mpi-bremen.de

On the next page, you will find detailed directions on how to get from the airport/main train station to the hotels Deutsche Eiche/Horner Eiche and from the hotels to the workshop location by public transport.

In **urgent cases** during the workshop (e.g. if you get lost in Bremen) please call **+49 421 2028 900 (MPI-Bremen)**.

rRNA Workshop Welcome Letter – Appendix

How to get from the Bremen airport/main train station to the hotels Horner Eiche & Deutsche Eiche by public transport:

Airport:
If you arrive at Bremen airport you first have to take tram line 6 towards Bremen central station (Bremen Hbf) and then change lines (see below)

Main train station:
A description how to find the hotel coming from the central station (Bremen Hauptbahnhof - short 'Bremen Hbf') can be found at

http://www.hotel-horner-eiche.de/HornerEiche/EN/anreise.htm#.

Actually, it is quite simple: just take the tram line 4 towards the direction 'Borgfeld' (left direction if you leave the main entrance of the central station) and leave at tram station "Kopernikusstrasse". On the left hand side is the street 'Im Hollergrund', the Hotel Horner Eiche is on the right side after about 150 m. The Hotel Deutsche Eiche is directly at the big crossroad (stop 'Koperniskusstrasse'). A single tram ticket is now EUR 2.15 and can be purchased from the driver.

More information on Bremen local traffic can be found at
http://www.bsag.de/eng/index.php.

Please consider buying a single-day-ticket for EUR 5.70 or even a 7-day-ticket for EUR 15.50 if you e.g. would like to go by tram/bus to the MPI each morning (and back of course) or to discover the city of Bremen in your free time.
The 7-day-ticket can NOT be bought in the tram but in the local traffic customer center at the central station (opening Monday-Friday 7:00-19:00, Saturday 8:00 - 18:00, Sunday 9:00-17:00).

How to get from the hotels Horner Eiche & Deutsche Eiche to the workshop location by public transport:

Note: At http://www.uni-bremen.de/lageplan/ you can find an interactive map of the campus of the University of Bremen, where the BITZ-Bremen and the MPI-Bremen are located (grid squares B2 +A3).

* First option: Enter the bus line 21 (Bremen local traffic) at the stop 'Kopernikusstrasse' (where you first arrived) heading towards 'Main Station' and get off five stops later at 'Universität/Zentralbereich' (about 8 minutes).

From there you have to walk to the BITZ-Bremen (about 5 minutes). Please use the campus map (see link above) to find your way. You will be in the middle of the map (where the green 'H' is located just below the red 'Boulevard').
Here you can find the current time table for bus line 21 http://62.206.133.179/nds/AHF/000003e18.pdf.

* Second option: Enter the bus line 630/670 (this is not Bremen local traffic but 'VBN', see http://www.vbn.de).

You have to enter it again at stop 'Kopernikusstrasse' (towards 'Main Station') and get off at the next stop 'Universität/NW1' (about 5 minutes). Again, please use the campus map (see link above) to find your way to the BITZ. You are now located where the green 'H' is located at street 'Universitätsallee' in grid C1.
This is the link to the current time table of the bus line 630/670: http://62.206.133.179/nds/AHF/000001936.pdf.

* Third option (longest): Enter tram line 4 heading towards 'Arsten' (towards central station) at stop 'Kopernikusstrasse' and get off four stations later ('Horner Kirche', about 5 minutes).

There you have to transfer to bus line 20 towards 'Universität' and get off at 'Universität/Zentralbereich' (running time about 10 minutes). From there you have to walk to the BITZ (about 5 minutes). Please use the campus map (see link above) to find your way to the MPI. You will be located in the middle of the map (where the green 'H' is located just below the red 'Boulevard').

If you prefer to walk form the hotel to the BITZ, you have to schedule at least 45 minutes for the trip (about 4 km). In this case, please use a city map or ask the receptionist for directions. Please be aware that you may get lost on your way (it is a little complicated). If this happens, ask for the "Universum" Science Center (http://www.universum-bremen.de/index.php?lang=EN).

Everybody in Bremen should know where it is and it is close to the workshop location (http://www.uni-bremen.de/lageplan/ grid square B1).

Agenda

For your notes:

Location: **BITZ Bremen,** Fahrenheitstraße 1, 28359 Bremen

Monday 07.04.2008

08.45, Session 1: Databases

Chair: Frank Oliver Glöckner

08.45	Organizers		Welcome address
09.00	James Cole	Michigan State University, USA	The Ribosomal Database Project
09.30	Todd DeSantis	Berkeley Lab, USA	Integrating ribosomal sequence analysis with taxonomic microarray data using the Greengenes database

10.00 - 10.30 Coffee Break

10.30	Peter Dawyndt	University of Ghent, Belgium	The StrainInfo.net bioportal: reaching out to what we know about our microorganisms
11.00	Frank Oliver Glöckner	Max Planck Institute for Marine Microbiology, Bremen, Germany	The SILVA project for comprehensive, quality checked and aligned ribosomal RNA databases

11.30 - 12.00 Coffee Break

12.00, Session 2: Phylogeny

Chair: Wolfgang Ludwig

12.00	Christian von Mering	University of Zurich, Switzerland	Protein-coding phylogenetic markers: complementing rRNA in the analysis of environmental communities
12.30	Wolfgang Ludwig	Technical University Munich, Germany	rRNA based phylogeny still a backbone for taxonomy?

13.00 - 15.00 Lunch Break

15.00	Alexandros Stamatakis	Ludwig Maximilians University, Munich, Germany	Algorithms, high performance computing, and challenges for large-scale phylogenetic inference
15.30	Harald Meier	Technical University Munich, Germany	Harvesting and evaluating diagnostic fruit from a molecular phylogenetic tree – an online approach

16.00 - 16.30 Coffee Break

16.30, Session 3: Data analysis & Biogeography

Chair: Alban Ramette

16.30	Anthony Lehmann	University of Geneva, Switzerland	Generalized regression analysis and spatial predictions: from genes to communities
17.00	Angélique Gobet	Max Planck Institute for Marine Microbiology, Bremen, Germany	Contextual interpretation of 454 massive tag sequencing data. A case study applied to coastal sediments

17.30 - 18.00 Coffee Break

18.00	Thomas Pommier	Université Montpellier, France	RAMI, a new tool to characterize and visualize the biogeography of rRNA sequence clusters
18.30	Alban Ramette	Max Planck Institute for Marine Microbiology, Bremen, Germany	Analysis of high-throughput sequencing data in their environmental context

19.00, Dinner at the Max Planck Institute for Marine Microbiology, Celsiusstrasse 1

Agenda

Tuesday 08.04.2008

09.00, Session 4: Technology

Chair: Jörg Peplies

09.00	Mitch Sogin	Marine Biological Laboratory Woods Hole, USA	Massively parallel DNA sequencing and the "Rare Biosphere"
09.30	Freek Bakker	National Herbarium and University of Wageningen, The Netherlands	Global standardization & implementation of DNA barcodes: the CBOL perspective
10.00 - 10.30	**Coffee Break**		
10.30	Rudolf Amann	Max Planck Institute for Marine Microbiology, Bremen, Germany	Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology
11.00	Alexander Loy	University of Vienna, Austria	Analyzing microbes after their last meal: Raman microspectroscopy of isotope-labeled cells and nucleic acids
11.30 - 12.00	**Coffee Break**		
12.00	Barbara Pohl	biomers.net GmbH, Ulm, Germany	Oligonucleotides: Insights in synthesis, purification and modifications
12.30	Wolf Malkusch	Carl Zeiss Imaging Solutions GmbH, Munich, Germany	Traditional and future scopes of image analysis in cell biology
13.00 - 15.00	**Lunch Break**		

15.00, Session 5: Diversity & Ecology

Chair: Bernhard Fuchs

15.00	Pedro Martinez-Arbizu	DZMB Forschungsinstitut Senckenberg, Wilhelmshaven, Germany	The Census of Marine Life in Europe
15.30	Erko Stackebrandt & Sylvie Cousin	German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany	Working in the underbrush: the limitations and potential of short rRNA sequences generated in diversity studies
16.00 - 16.30	**Coffee Break**		
16.30	Dave Stahl	University of Washington, USA	Nitrification: phylotype versus ecotype revisited
17.00	Ramon Rossello-Mora	University of Palma de Mallorca, Spain	The Living Tree Project

17.30, End of Session – Free evening

Wednesday 09.04.2008

09.00, Session 5: Diversity & Ecology, cont.

Chair: Linda Amaral-Zettler

09.00	Stephen Giovannoni	Oregon State University, USA	Natural variation among ecotypes of the SAR11 clade
09.30	Anna-Louise Reysenbach	Portland State University, USA	Temporal and spatial patterns of microbial diversity at deep-sea vents

10.00 - 10.30 Coffee Break

10.30	Gerhard Herndl	Royal Netherlands Institute for Sea Research (NIOZ), Den Burg, The Netherlands	Prokaryotic community structure and activity in the North Atlantic deep-waters
11.00	Jakob Pernthaler	University of Zurich, Switzerland	rRNA as the anchor point for investigating the ecophysiology of aquatic bacteria at the single cell level

11.30, Roundup & Discussion

12.00, Official end of workshop

13.30-15.00, Preparation of workshop report
by Organizers & Representatives

Have a good trip back home!

Agenda

For your notes:

The Ribosomal Database Project

James R. Cole

The Ribosomal Database Project (RDP) offers aligned and annotated rRNA sequence data and analysis services to the research community. These services help researchers with the discovery and characterization of microbes important to many fields beyond microbiology, including human health, agronomy and food science, environmental engineering and bioremediation, alternative fuels, earth sciences, and teaching. Updated monthly, the RDP maintained 481,650 aligned and annotated quality-controlled rRNA sequences as of February 2008 (Release 9.58). All sequences are tested for sequence anomalies, including chimeras, using Pintail from the Cardiff Bioinformatics Toolkit (Ashelford et al., 2005. *Appl. Environ. Microbiol.* 71:7724-7736). Sequences can be browsed in either a taxonomic hierarchy (Hierarchy Browser) or Publication View. An additional Genome Browser presents rRNA sequences from genome projects, along with additional information, including copy number, genome size, and links to other genome resources. The RDP maintains a suite of web-based tools to allow researchers to analyze their own sequences in the context of the RDP database. These include a naïve Bayesian classifier (RDP Classifier) that places sequences in the taxonomic hierarchy, a tool for rapid detection of the closest relatives (Sequence Match) that can also be used as a k-nearest neighbor classifier, a primer-probe testing tool (Probe Match), a tool for comparing the taxonomic content of two environmental libraries (Library Compare), an interactive phylogenetic tree building tool (Tree Builder), and a new interactive heatmap tool for visualizing the relationships between thousands of sequences at one time (Taxomatic). The *myRDP* user account feature allows researchers to maintain their own private sequence collection on the RDP servers aligned in sync with the RDP public alignment. A high-throughput Sanger sequence processing Pipeline tailored to the requirements of environmental single-read sequencing projects. It provides a complete path from sequencer output to quality-controlled alignments and analysis. To help researchers take advantage of the new ultra-high-throughput sequencing methodologies, the RDP is building a Pyrosequencing Pipeline. This new set of tools, will help automate the processing of these large data-sets, and provide researchers with the most common ecological metrics, along with the ability to download the processed data in formats suitable for common ecological and statistical packages. In addition to extensive Help Files, the RDP hosts a collection of short Video Tutorials demonstrating some of the more complex analysis tasks. User support is available through e-mail or phone (rdpstaff@msu.edu; +1-517-432-4998). RDP data and analysis services are available online at http://rdp.cme.msu.edu/.

Contact Information:

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USA

Integrating ribosomal sequence analysis with taxonomic microarray data using the Greengenes database

Todd Z. DeSantis

Greengenes (greengenes.lbl.gov) is a web application assisting molecular ecologists with data analysis. Aligning 16S rRNA gene sequences, removing chimeras, and classifying the members of a microbial community against multiple Bacterial and Archaeal taxonomies will be covered. Two advanced methods will also be discussed: integration of PhyloChip community analysis with sequencing data and how to import your greengenes pre-processed data into Arb for visualization, probe design, etc. Workshop participants may view the online tutorial from the greengenes website to explore the tools in greater depth.

Contact Information:

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USA

For your notes

Reaching out to what we know about our microorganisms

Peter Dawyndt

Microbial research is inherently distributed in nature. With many researchers in different research institutes collecting and analyzing bits and pieces of data on microbial diversity, knowledge gained on the microbial world also has a tendency to become spread across the information jungle. As today's upscaling of research projects often is hampered by the burden to integrate data extracted from several autonomous and heterogeneous information systems, the famous Newton quote "*if I have seen a little further it is by standing on the shoulders of giants*" is endangered to become rephrased as "*If I can see no more, it is because giants were standing on my shoulders*". An immediate and important challenge that lies ahead of us is that of end□to□end scientific data management, from data acquisition and data integration, to data treatment, provenance and persistence. While advances in computing, and in particular scientific data management and application development environments for science will become important in the future, what is vitally more important and dramatic in its impact is the integration of new conceptual and technological tools from computer science into the life sciences. This integration is likely to accelerate key breakthroughs in science and benefits to society, from understanding biology and revolutionizing medicine and healthcare, and from understanding the universe to the origin of life, and understanding and helping to protect the lifesupport systems of Earth on which we all depend for our survival. The StrainInfo.net bioportal (www.straininfo.net) was established to stimulate this movement towards using multi□perspective integrated information in a broadened biological and clinical context. In particular, it concentrates on establishing automated ways to collect and integrate all information that is available about the microorganisms that were deposited into a global network of biological resource centers. Fully understanding the biology of an organism (or a population of organisms) requires to take a holistic approach wherein the phenotypic and genotypic traits of the organism are inspected in light of its ecological role and geographical spread. Moreover, only by having high quality information on those organisms we already know, we will be able to further explore and understand those regions of microbial diversity that we don't know yet. In this presentation we will discuss some of the impediments against seamless data integration, propose possible ways of better collaboration and open the discussion on novel ways to exploit the vast amount of legacy data that drives microbiology.

Contact Information:

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Department of Applied Mathematics and Computer Science
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9000 Ghent,
Belgium

The SILVA project for comprehensive, quality checked and aligned ribosomal RNA databases

Frank Oliver Glöckner

Sequencing ribosomal RNA (rRNA) genes is currently the method of choice for phylogenetic reconstruction, nucleic acid based detection and quantification of microbial diversity. The resulting exponential increase of publicly available rRNA sequence data has rendered the maintenance of rRNA knowledge databases difficult over the last years. To cope with the deluge of data, the SILVA system (from Latin *silva*, forest) was implemented to provide a central, comprehensive web resource for up to date, quality controlled databases of aligned rRNA sequences from the *Bacteria*, *Archaea* and *Eukarya* domains (3). All databases are fully compatible with the worldwide used ARB software package for sequence analysis and probe design (2). For rapid and accurate alignment the new dynamic incremental profile sequence aligner (SINA) was developed. The system is able to align more than 500,000 SSU sequences overnight taking our manually curated high quality Seed alignment as the reference. All sequences are checked for anomalies using the Pintail (1) system and carry a rich set of process information. An intuitive quality ranking allows the user to get a rapid overview about the sequence and alignment quality as well as the Pintail results. The databases are designed as a central comprehensive resource by integrating multiple taxonomic classifications taken from RDP II and greengenes and the latest validly described nomenclature provided by the German Collection of Microorganisms and Cell Cultures (DSMZ). Information, if a sequence was derived from a cultivated organism, a typestrain, or belongs to a genome project has been integrated from the straininfo.net bioportal, the Living Tree project and EMBL. The latest publicly available database release 93 (February 2008) hosts a total of 668,095 sequences split into 566,047 small subunit and 102,021 large subunit rRNAs. The SILVA databases can be accessed at www.arb-silva.de

Contact Information:

Frank Oliver Glöckner
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Germany

For your notes

Protein-coding phylogenetic markers: complementing rRNA in the analysis of environmental communities

Christian von Mering

In unbiased environmental sequencing experiments "shotgun", rRNA genes are not specifically enriched and thus make up less than 1% of the sequences. This is of course true for any other phylogenetic marker gene as well, and matters are further complicated by the abundance of short and often non-overlapping sequence reads. On the positive side however, such data contain little amplification or detection bias, and functionally important 'life-style' genes are abundantly sampled in parallel to phylogenetic markers.
To derive the underlying taxonomic composition of the organisms sampled in such experiments, we developed a maximum likelihood procedure that is based on more than one marker gene, and can handle fragmented open reading frames and limited assembly. We use this procedure ("MLTreeMap") to derive detailed and standardized phylogenetic assessment for diverse metagenomics experiments. This allows us to track microbial lineages through various environments, and leads us to suggest that most lineage have clear and remarkably stable habitat preferences throughout evolution.

Contact Information:

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8057 Zürich,
Switzerland

For your notes

rRNA based phylogeny still a backbone for taxonomy?

Wolfgang Ludwig

During it's more than 30 years history comparative rRNA sequence analysis 'evolved' from an expensive specialist technique to a rapid routine procedure for elucidating phylogenetic relationships, supporting microbial taxonomy and identification. The development and maintenance of appropriate software tools and special databases such as the ARB package, ARB-, and recently SILVA-databases are part of this history. Starting with the second edition of Bergey's Manual of Systematic Bacteriology, the arrangement its content follows a phylogenetic framework or road map based largely on analyses of the nucleotide sequences of the ribosomal small subunit RNA rather than on phenotypic data. Since the introduction of comparative rRNA sequencing, there has been a continuous debate concerning the justification and power of a single marker molecule for elucidating and establishing the phylogeny and taxonomy of organisms, respectively. Although generally well established in taxonomy, the polyphasic approach cannot be currently applied for sequence based analyses due to the lack of adequate comprehensive data sets for alternative marker molecules. Even in the age of genomics, the datasets for non-rRNA markers are poor in comparison to more than 500,000 rRNA primary structures available in general and special databases. Nevertheless, the data provided by the full genome sequencing projects allow defining a small set of genes representing the conserved core of prokaryotic genomes. Furthermore, comparative analyses of the core gene sequences globally support the small subunit rRNA derived view of prokaryotic evolution. Although the tree topologies reconstructed from alternative markers differ in detail, the major groups (and taxa) are verified or at least not disproved. (Bergey's Manual of Systematic Bacteriology, 2nd edition, Vol. 3, in press)

Contact Information:

Wolfgang Ludwig
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85354 Freising,
Germany

For your notes

Algorithms, high performance computing, and challenges for large-scale phylogenetic inference

Alexandros Stamatakis

Despite the impressive progress that has been achieved with the new generation of Maximum Likelihood (ML) search algorithms for phylogeny reconstruction, the analysis of large, and thus memory-intensive multi-gene datasets, still represents a major computational challenge for phyloinformatics. In the first part of my talk, I will present novel rapid Bootstrap heuristics that have been implemented in RAxML. On average, these rapid BS heuristics are 15 times faster than the standard RAxML algorithm, but yield qualitatively comparable results. In addition, they are between 18 and 495 times faster than competing programs (PHYML/GARLI) while the speed gain increases with alignment size. Coupled with parallelism the rapid BS method opens up new possibilities such as fast evaluation of a large number of alternative alignments for a single dataset or development of experimentally tested bootstopping criteria. In the second part of my talk, I will outline how ML-based inference of large multi-gene datasets can efficiently be parallelized on the IBM BlueGene supercomputer architecture and on medium-sized Linux clusters with fast interconnects. On the BlueGene we obtained a speedup of 890 on 1,024 processors for the largest, in terms of memory footprint, alignment analyzed under ML to date. I will describe how the BlueGene version can be further accelerated by a factor of 50 via integration of the rapid BS procedure and appropriate nucleotide substitution model approximations. This will allow for the computation of a small tree of life with about 10,000 sequences and data from several genes in the near future. Finally, I will address some of the future challenges such as improved models for "gappy" multi-gene alignments and methods for simultaneous alignment and tree building.

Contact Information:

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For your notes

Harvesting and evaluating diagnostic fruit from a molecular phylogenetic tree – an online approach

Harald Meier

Current small subunit ribosomal rRNA gene databases cover more than 200,000 full sequences and still grow rapidly. Therefore the search for diagnostic oligonucleotide signatures becomes a more and more computing intensive task. Extremely expensive this process gets when group specific signatures have to be searched for, because the linear increase in the number of sequences causes an exponential increase in the number of potential signature groups.
Due to the development and application of fast heuristics for molecular phylogenetic inference, however, phylogenetic trees can be reconstructed and assembled from large sequence alignments. Generally, the phylogenetic tree is represented as a static graph which allows further visual interpretation such as the phylogenetic relationship of organisms (sequences data) of interest. As shown by the work of Wolfgang Ludwig the SSU rRNA tree can have additional purposes. It represents large sequence collections as a - from a biologist's point of view - meaningfully clustered interactive graph, in order to facilitate further data handling, management and analysis. And even further information, such as the current taxonomy, can be superimposed on the SSU rRNA tree by naming subtrees.
In this talk we will show other applications of the phylogenetic SSU rRNA tree, related with the search for group specific oligonucleotide signatures, their evaluation and representation. Our implementation using web communication technologies will be introduced, and advantages as well as limitations referred to.

Contact Information:

Harald Meier
Technical University Munich
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Boltzmannstr. 3,
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Germany

For your notes

Generalized regression analysis and spatial predictions: from genes to communities

Anthony Lehmann

I will present theoretical statistical and ecological backgrounds that led many scientists to choose Generalized Linear Models (GLM) and Generalized Additive Models (GAM) as their favourite tool to model and predict species distribution. I will explore the different steps involved in species distribution modelling from data exploration, model selection, validation and interpretation, and finally spatial predictions. I will present different examples of applications of these methods in terrestrial and aquatic ecosystems. Some insights will be given on how these approaches could be transferred into Oceans metagenomic through the development of OceanDB, an environmental database describing global oceans.

Contact Information:

Anthony Lehmann
University of Geneva
Climatic Change and
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Switzerland

For your notes

Contextual interpretation of 454 massive tag sequencing data. A case study applied to coastal sediments

Angélique Gobet, Alban Ramette, Simone Böer, Justus E.E. van Beusekom, Mitchell L. Sogin, Antje Boetius

Coastal areas mainly consist of sandy sediments, but despite the important role of the latter on the recycling of carbon and nitrogen, their microbial ecology is still poorly understood. In this project, multiple environmental parameters (exopolysaccharides, pigments, nutrients, extracellular enzymes, etc.) were measured in sandy sediments over different seasons and the 454 massive tag sequencing approach was applied to obtain a high-resolution description of the microbial community composition. The 454-based analyses were compared with those obtained from Automated rRNA Intergenic Spacer Analysis (ARISA), a community fingerprinting tool commonly used in microbial ecology. Multivariate analyses were then applied to test whether an ecological signal could still be obtained from this complex dataset including thousands of sequences and a high number of environmental parameters, and to which extent the ecological description of environmental samples was method-dependent. Simple and constrained analyses were used in parallel on ARISA and 454-generated data and the comparison of sample similarities with both methods showed similar depth-related patterns for the samples. No clear trend of a seasonal (cyclic) or temporal succession was, however, detected. To disentangle the effects of covarying contextual parameters, variation partitioning analyses were applied to both cases. We found that the biological variation could be explained by the covariation of multiple environmental and depth-related factors such as chlorophyll a, nitrates and extracellular enzymes. Preliminary taxonomic analyses based on 454 data showed that the depth-related pattern was resolved at taxonomic levels below the family level and that environmental selection was similar at all taxonomic levels investigated. Overall, this study shows the usefulness of the 454 massive tag sequencing approach in generating high-resolution, primary data that can be further interpreted in their ecological context.

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For your notes

RAMI, a new tool to characterize and visualize the biogeography of rRNA sequence clusters

Thomas Pommier

The most common approach to estimate microbial diversity is based on the analysis of rRNA sequences. Typically, sequences are grouped into operational taxonomic units using an arbitrary similarity threshold. This method may fail to adequately identify clusters of closely related sequences, which are important for examining the evolutionary and ecological forces shaping the structure and function of microbial communities. To ease the characterization of these clusters, we developed an integrated tool - RAMI - that discloses the phylogenetic structure of microbial communities. RAMI makes use of the patristic distances (branch lengths) retrieved from tree files produced by most tree reconstruction software to identify clusters of closely related sequences, characterize their structure, genetic variation and relationships. The program computes a number of indices to describe cluster properties that can be used for comparing the phylogenetic structure among communities. We used 16S rRNA gene sequences of several clone libraries constructed from several seawater samples to demonstrate RAMI's ability to efficiently identify and analyze closely related clusters, and to bring a geographic perspective to their occurrence in marine microbial communities. We foresee that RAMI should become an important tool for demarcating genetically coherent, and presumably ecologically distinct groups of organisms in microbial communities

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For your notes

Analysis of high-throughput sequencing data in their environmental context

Alban Ramette

Understanding the ecological and evolutionary forces that shape community composition in natural environments is a key challenge for ecologists. A critical problem that microbial ecologists currently face in their study is not only the need to analyze a large number of samples to obtain meaningful conclusions, but also the need to deal with an ever-increasing number of DNA sequences per sample. Multivariate analyses have long been used in community ecology. They can be readily and fruitfully applied to microbial studies to reduce data dimensionality and to extract the most important sources of variation. Constrained analyses may further be used to relate community diversity patterns to variation of contextual parameters. A new analytical framework will be presented in order to quantify and to test the significance of the structuring factors affecting community diversity at multiple taxonomic levels. By identifying the most significant biological or taxonomic levels that respond to specific environmental factors, the targets of ecological and evolutionary forces may thus be identified. This flexible analytical framework is illustrated on a pre-existing 16S rRNA-gene dataset obtained from microbial communities associated with obesity in mice. Using this approach, finer ecological and evolutionary insights could be obtained as compared with traditional statistical tools. Future applications of the strategy are particularly anticipated in ecology, evolution and taxonomy.

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For your notes

Massively parallel DNA sequencing and the "Rare Biosphere"

Mitchell L. Sogin

Based on surveys using rRNA gene sequences as proxies for the presence of a microbe in a sample, we now know that microbial diversity is at least 100-1000 times greater than estimates based upon cultivation-dependent surveys. Yet molecular assays that target nearly full-length rRNA sequences have only captured a fraction of microbial diversity and they rarely provide estimates of relative abundance for different kinds of microbes or operational taxonomic units (OTUs). The promise of discovering new phylotypes has fostered experimental designs where low resolution procedures e.g. restriction fragment length polymorphisms - identify putatively distinct clones for DNA sequencing. This binning procedure comes at the expense of minimizing information about the relative abundance of distinct phylotypes. Typical molecular surveys collect 1000 sequences but these data sets can only describe a small fraction of the 10^8 microbes/liter commonly found in aquatic environments (fewer than one per million cells). Dominant populations have masked the detection of low-abundance organisms and some of these may be the most interesting for predicting shifts in microbial population structures in response to ecological change. As an alternative to molecular analyses of cloned PCR amplicons, massively parallel pyrosequencing technology can generate 300,000-450,000 short "tag" sequences from rRNA hypervariable regions in a single run without requiring the construction of clones libraries or preparation of DNA templates. The collection of tags provides a first-order description of the kinds and relative abundance of distinct OTUs in a microbial community. With this technology we have targeted and compared hypervariable regions V3 and V6. The relative abundance of different OTUs in these data sets varied by more than three orders of magnitude. A relatively small number of tag sequences represent dominant bacterial populations. The vast majority (75%) of the tag sequences are very similar to each other and to entries in the reference database. Underlying the major populations is a broad distribution of distinct bacterial taxa that represent extraordinary diversity. These highly divergent, low-abundance organisms constitute a "rare biosphere" that is largely unexplored. Some of its members might serve as keystone species within complex consortia, others might simply be the products of historical ecological change with the potential to become dominant in response to shifts in environmental conditions that favor their growth. Because we know so little about the global distribution of members of the rare biosphere it is unknown if they represent specific biogeographical distributions of bacterial taxa, functional selection by particular marine environments, or cosmopolitan distribution of all microbial taxa – the "everything is everywhere" hypothesis.

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Global standardization & implementation of DNA barcodes: the CBOL perspective

Freek T. Bakker

In eukaryotes, mitochondrial encoded cox1 has been the region of choice in most DNA barcoding approaches so far (i.e. vertebrates, Lepidoptera). Although standardization (procedures, data quality) and global implementation is crucial to any DNA barcode effort, it is becoming clear that alternative and additional clade-specific barcode regions are needed for overall success. For instance, fungal and nematode DNA barcoding is largely based on rDNA, whereas for land plants multiple regions from the plastid DNA will be used for effective DNA barcoding, and for various protist clades region selection is still in its infancy. Practical/technical considerations in barcode region selection are paramount: i) ease and feasibility of PCR amplification, especially from degraded material, ii) species-level DNA sequence divergence encountered, and iii) the universality of primers used to amplify the barcodes. But structural aspects of the region proposed (length variation), reproductive biology of its target clades, as well as its possibility of matching 'unknowns' to a growing reference library are considered equally important. In order to harmonise & streamline barcode region selection as much as possible CBOL designed guidelines, and several non-CO1 DNA barcode regions are expected to be proposed to CBOL's Science Advisory Board soon. Once agreed upon, newly adopted regions can then receive the BARCODE keyword in GenBank.

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For your notes

Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology

Rudolf Amann

The ribosomal RNA (rRNA) approach to microbial evolution and ecology has become an integral part of systematic and environmental microbiology. Large database exist that encompass not only the 16S rRNA sequences of almost all validly described bacteria and archaea, but also 100 times more "environmental" 16S rRNA sequences of yet uncultured microbes. The 23S rRNA and also the internal transcribed spacer (ITS) are other high copy number targets for sequence-based identifications. With appropriate software tools rRNA-targeted oligonucleotide probes with defined specificities can be designed in a directed way. When such probes are labeled with fluorescent dyes or horseradish peroxidase they can be used to identify single microbial cells by fluorescence *in situ* hybridisation (FISH) directly in complex environmental samples. An update on methodological aspect of whole cell rRNA-probing will be given including possibilities to identify small bacterial cells in oligotrophic environments by catalyzed reporter deposition (CARD)-FISH. With optimized methods and proper controls the FISH technique can yield reliable identifications and exact cell numbers, biomasses as well as spatial distributions for phylogenetically defined populations.
FISH can also be combined with other methods thereby linking identity and function on a single cell level in complex microbial communities.

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For your notes

Analyzing microbes after their last meal: Raman microspectroscopy of isotope-labelled cells and nucleic acids

Alexander Loy

Life on our planet is dominated and maintained by bacteria and archaea, most of which refuse to be cultivated in the lab. Cutting-edge technologies, which base on substrate-mediated isotopic labelling and subsequent molecular analysis, nonetheless enable us to decipher the identity and physiology of these yet uncultivated microbes. In my talk, I will introduce Raman microspectroscopy combined with fluorescence in situ hybridization as a new tool for single-cell analysis of individual community members and summarize progress on developing a Raman microspectroscopy-based stable isotope microarray method for highly parallel structure-function analysis of complex microbial communities.

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For your notes

Oligonucleotides: Insights in synthesis, purification and modifications

Barbara Pohl

Oligonucleotides are used in a tremendous number of different applications, methods, and analyses. Mostly, however, they are not the main topic of the research, but an important tool to investigate very diverse questions.
In this light it is surprising that the common knowledge about synthesis and purification of oligos is rather low. Here insights are given into the principles of oligonucleotide synthesis and its possibilities and limitations. The necessity for purification is explained and how to choose from the variety of offered purifications the most suitable for a given application.
Finally the special features of modified oligonucleotides are shortly outlined, with a focus on dye- and HRP-labelled oligos.

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For your notes

Traditional and future scopes of image analysis in cell Biology

Wolf Malkusch

In 1852 Rudolf Virchow based the Cellular Pathology and therewith the modern medicine with his statement: „each cell originates from another cell". But already in the 17th century the Protozoology started with Antoni von Leeuwenhoek, which indirectly was the headstone of the modern Cytology. Microscopes developed later on are using up to today a two step magnification. In the 19th century Ernst Abbe defined the Numerical Aperture for an objective. Since that time science is keen to reach the limits of the resulting resolution, or meanwhile even to exceed them. Using optimized condenser settings, immersion objectives, immersion fluids we managed to come quite close to the theoretical barrier.
Investigations with high resolutions needed stained specimen, which mostly could not be performed with live cells. New methods helped in the 20th century, such as the Phase Contrast (Frits Zernike) or the Differential Interference Contrast (Georges Nomarski). Discovering the GFP and its derivatives end of the 20th century we got the fluorescence labels that finally could be used in living cells. Using suitable antibodies the fluorochromes can be targeted to cell components, and visualized in a fluorescence microscope.
This immediately produced new requirements for the illumination regarding the spectra and switching speed in order to visualize interactions between proteins. Currently the development is entering a completely new area using Lasers (LSM) and LEDs (Colibri). The increasing acquisition speed of CCD cameras and the computer speed for the control and processing widely opened the doors to reach the theoretical resolution limits. For the wide field microscopy 3D deconvolution and structured illmination (ApoTome) offer new approaches to increase the resolution. Using total internal reflection fluorescence (TIRF) microscopes the theoretical resolution limit can already be reached, and new methods such as 2 photon microscopy, selective plane illuminations microscope (SPIM), stimulated emission depletion (STED), and Array Tomography let us hope for advancing in ranges below 50 nm resolution.

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For your notes

The Census of Marine Life in Europe

Pedro Martinez-Arbizu

The global Census of Marine Life project is a 10-year initiative in which it intends to assess and explain the diversity, abundance and distribution of marine organisms in the world's oceans; looking at the past, present and future. More recently, National and Regional committees (NRICs) were developed with the aim of undertaking more regional investigative science. The European Census of Marine Life (EuroCoML) is one of these twelve NRICs. In its role as an NRIC, EuroCoML supports both the International Scientific Steering Committee and Secretariat with regards to the management and promotion of international CoML. EuroCoML has been operational for almost 3 years. During this time, the project office and its two committees; the Executive and Scientific Steering committees, have had a number of key objectives: To expand partnerships and coordination with relevant European programmes and organisations, also in tandem with the general growth of the CoML; To increase participation in CoML projects where untapped potential remains; To improve marine taxonomy and species data in European waters;To improve biodiversity and ecosystem information for applied resourcemanagement in waters where European nations hold a major influence;To increase stakeholder awareness of marine biodiversity through education and outreach activities.
This workshop on Ribosomal RNA technology is just one of several workshops that EuroCoML has funded. Others include invasive species in European waters, environmental modulation of Biodiversity and Ecosystem dynamics as well as a workshop to track predators in the Atlantic. The outcomes of these workshops are, scientists committed to developing proposals to undertake large-scale investigations in European waters with the overall aim of contributing to the global CoML initiative. The international CoML is approaching the main phase of synthesis and summary as it approaches one decade of operation. Europe has much to celebrate in this remarkable programme.

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For your notes

Working in the underbrush: the limitations and potential of short rRNA sequences generated in diversity studies

Erko Stackebrandt & Sylvie Cousin

The use of complete rrn sequences for proper phylogenetic analyses and species description of novel type strains is acknowledged. Nevertheless, short 16S rRNA gene sequences are informative in a first and rapid assessment of the taxonomic position of pure cultures and composition of environmental communities, as well as in the identification of DGGE/TGGE band patterns, which do not offer an alternative. Sequences used on MLST are generally significantly smaller than the respective full length genes. For a laboratory with no access to a strong bioinformatics group, the decision to use sequences of less than ~ 1000 bp is not always due to costs, but more so to the exemption of sequence assembly and, in the absence of an automatic alignment alternative, to time. The use of the latter alternative requires absolute trust in the outcome of the resulting taxonomic affiliation as crosschecks are not part of the algorithm. Working decades with manual alignments the choice to switch to an automatic alignment system will only be done in case of the availability of a genuine automatic alternative or, when - in case of a huge number of sequences to be aligned - manual alignment is not an option.
In a recent environmental study the taxonomic affiliation of more than 5.000 partial 16S rRNA genes sequences of strains and clones needed to be evaluated. Initial BLAST analysis of about 1.800 cultures indicated moderate to high similarities with described species. The results of the phylogenetic positioning of sequences of length ~ 450 bp of the automated Greengenes/SINA/ARB systems allowed the comparison of phylogenetic position with BLAST similarities. Despite a high degree of correlation some disturbing discrepancies were noticed which may remain undetected in the absence of better knowledge, falsely mirroring phylogenetic novelty. This lecture will highlight some cases of extreme differences between <99% BLAST affiliation of sequences with type strains of species and their vastly different position (spanning phyla) in trees generated on the basis of automated alignments. This phenomenon is not novel to ARB users and informaticians who recommend the use of full length 16S rRNA sequences. At least for a pre-screening of novelty and rapid identification short sequences are still a valuable source of information. The community of users would welcome a solution which would allow them to properly place short sequences alongside full sequences for the sake of retrieving a maximum of taxonomic information.

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Nitrification: Phylotype versus ecotype revisited

David A. Stahl

Many early molecular studies of microbial distribution were based on the use of group-specific DNA probes targeting selected phylogenetic groups. The rational of this approach was primarily twofold: 1) expense – available technology could not serve for highly resolved analyses of individual species and 2) the assumption that natural coherence (phylotype) in some way reflected physiology and ecology. In turn, early studies also focused on functional assemblages showing reasonable association between phylotype and function (ecotype). Such assemblages included ammonia oxidizers, sulfate-reducers, and methanogens. One consequence of this approach to "molecular ecology" was an early focus on defined assemblages, with the possible exclusion of other functionally significant groups. This presentation will discuss some aspects of the history of probe-based studies, their relevance to the discovery of novelty (using the ammonia oxidizing *Archaea* as an example), and future applications of this general methodology.

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For your notes

The Living Tree Project

Ramon Rossello-Mora

Comparative sequence analysis of 16S rRNA genes provides a stable framework for the reconstruction of putative phylogenetic relationships of bacteria and archaea. The ribosome small subunit gene sequence (SSU) is generally used as a basic genetic information for any new taxon classification. This applies as well to the species category despite its lack of resolution at this level. Since molecular phylogeny was applied for the classification of prokaryotes, almost all new descriptions are accompanied with the rRNA gene sequence of the type strain of the species. We are currently in an extraordinary phase of development in the field of prokaryote taxonomy due to an enormous amount of new species descriptions. Just in year 2007, 614 new species were classified representing about 8.16% of a total of 7,521 validated at that date. In parallel, the information content of gene primary sequence databases is as well exponentially growing, with a current doubling rate of 18 months. In January 2008 out of the 109,626,755 gene entries in EMBL release 93, 1,200,423 were attributable as ribosomal RNAs gene sequences. However, just 20,754 were obtained from pure cultures grown in the laboratory, and 9,889 of them corresponded to sequences assigned to type strains (according to SILVA release 93). However, looking closer at their entries, one can easily find mistakes in strain assignation and nomenclature, as well as low sequence qualities. In addition, many species count with more than a single sequence, often with differences in their length and quality. Both facts make the achievement of a reliable tree compiling all hitherto classified species very difficult. Together with the journal "Systematic and Applied Microbiology" we are currently undergoing the "The Living Tree" project in order to provide a reliable tree, primarily gathering all classified type strains. Our intention is to reconstruct and make public an actualized tree at least two times a year, together with the complete alignment. For this, we are currently comparing and sieving all putative entries in the SILVA SSURef database for reference sequences according to the validated species listed in www.bacterio.cict.fr. For the 6,800 type strain sequences selected so far, the alignment was improved and tree reconstruction using maximum likelihood algorithms was started. The criteria of the sequence selection process and a preliminary tree will be detailed during the meeting.

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Natural variation among ecotypes of the SAR11 clade

Stephen Giovannoni

The heterotrophic alphaproteobacterial clade SAR11 accounts for approximately 25% of microbial plankton cells in the ocean surface layer, and may exceed 50% of the cells in temperate ocean gyres during the summer, making it a key to understanding the marine carbon cycle. Despite its high representation in environmental DNA from oceanic sites, early efforts to assemble SAR11 genomes from metagenomic data were confounded by very high sequence diversity. Complete genome sequences determined from three *candidatus* Pelagibacter ubique strains supported partial assemblies of metagenomic data and provided insight into natural variation in SAR11 genomes, supporting the hypothesis that genome streamlining has been an important factor in the evolution of SAR11 genomes. Extraordinarily high allelic variation and genome rearrangements appear to mask the conservation of many genome properties in native SAR11 populations, leading to overestimates of species diversity. Moreover, multilocus sequence typing indicated very high rates of recombination in one SAR11 population. Recently, evidence for the diversification of SAR11 ecotypes has emerged from FISH and tRFLP data from the Bermuda Atlantic Time series Study (BATS). This data supports the view that at least three SAR11 ecotypes, each with a different seasonal and spatial distribution pattern, occupy the surface layer at BATS.

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For your notes

Temporal and spatial patterns of microbial diversity at deep-sea vents

Anna-Louise Reysenbach

At mid-ocean ridges and back-arc basins, deep-sea hydrothermal vents are recognized as important biogeochemical environments. The actively-forming vent deposits, which span temperatures from ~2ºC to >400ºC, provide microhabitats for a diversity of Bacteria and Archaea. These microbes are supported by geochemical fluxes from Earth's interior and the chemical disequilibria that result when chemically reduced fluids vent at the seafloor and mix with oxic seawater. While numerous studies have looked for evidence of microorganisms within vent deposits, little focus has been on whether there are any patterns emerging from these diversity assessments. We have used a combination of thermocouple array deployments and direct sampling of multiple sulfides over multiple years to evaluate whether (i) diversity differs between newly formed material versus more mature deposits, (ii) community structure differs between different deposit types and between different vents sites In the same basin and between ridges, (iii) whether the community structure changes over long periods. Given the ease of sequencing 16S rRNA genes and the use of high throughput techniques such as DGGE, in many cases we can now obtain sample sizes that are statistically relevant. Our data are showing that changes in community succession occur as deposits mature, with newly formed deposits dominated by archaeal chemolithotrophs, but not always the same metabolic group. There are statistically significant differences in the community structure between different deposit types (flange versus chimney) and between different deep-sea vent sites in the same back-arc basin (Eastern Lau Spreading Center). Significant changes in the community structure were observed in sulfides collected from the East Pacific Rise 9ºN vents from 2000 to 2007. In some cases these different spatial and temporal changes may be a result of mineralogical changes, geochemical differences or microbial activity.

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For your notes

Prokaryotic community structure and activity in the North Atlantic deep-waters

Gerhard J. Herndl

Meso- and bathypelagic prokaryotic communities are apparently highly stratified as revealed by several molecular and genomic approaches. Using fluorescence in situ hybridization (FISH) and specific oligonucleotide probes, a major survey of the North Atantic deep waters revealed that there are specific latitudinal gradients in the abundance of Marine Group I Crenarchaeota from 65°N to 5°S. With the decrease in relative abundance of Marine Group I Crenarchaeota towards the equator, members of the SAR202 become increasingly abundant, comprising up to 40% of the bacterial community. Chemoautotrophic Marine Group I Crenarchaeota are more abundant in mesopelagic waters than in bathypelagic waters. In these bathypelagic waters, Crenarchaeota are utilizing unusual compounds such as D-amino acids as revealed by single cell analysis using microautoradiography in combination with CARD-FISH. Thus, overall, there are substantial differences discernable in the prokaryotic community composition and activity along a latitudinal as well as on a vertical gradient in the North Atlantic deep waters. These dynamics in the prokaryotic community in the deep waters indicate that there is more heterogeneity in these deep waters than hitherto assumed. The possible reasons of this heterogeneity will be discussed in the light of recent findings based on advances in metagenomic analyses of deep-water prokaryotes to make the point that the deep-water prokaryotes might be more depending on particulate matter than previously thought.

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For your notes

rRNA as the anchor point for investigating the ecophysiology of aquatic bacteria at the single cell level

Jakob Pernthaler

rRNA-based techniques such as fluorescence in situ hybridization (FISH) allow a distinction and quantification of microbial genotypic populations in field samples. These populations possess distinct phenotypic properties that are related to their functional role in the habitat and that moreover reflect their current growth state. One strategy of addressing specific microbial activities at close to *in situ* conditions is the combination of microscopic identification with the assessment of cell growth or substrate uptake during pulse-labeling experiments. Gradients of substrate concentrations or a combination of various substrates (as proxies for different aspects of cell metabolism) can moreover provide a better understanding of the ecophysiological properties of culturable or uncultured bacteria alike at relevant environmental conditions.

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