Marine Microbial Diversity and Genomics

Frank Oliver Glöckner

Seas and oceans cover over 70% of the Earth's surface and account for 97 percent of the biosphere. Marine ecosystems provide energy resources, and the basis for maritime transport, and recreation. The oceans contain the highest biological diversity on Earth; marine organisms live throughout the water column, to an extreme depth of up to 11 km, and in ocean sediments up to a further 400 m below the seafloor. Marine microorganisms in particular play a central role in the global cycling of matters and energy, for they are both a driver and indicator of global climate change. Furthermore, they are an inevitable genetic resource for new enzymes and reactions which can be used for pharmaceutical and industrial applications. Current estimates show that a millilitre of sea water hosts around 1 million cells. In sediments total cell numbers of up to 1 billion per gram can be reached. Microorganisms' total biomass represented as fixed carbon is currently estimated to be more than 300 gigatonnes (14). But apart from the huge total numbers, their diversity is also quite impressive. Although no exact numbers exist, our studies infer that around 1000 different active microorganisms persist per millilitre (1). Additionally, oceans act as a seed bank hosting a "rare biosphere" of hundreds of thousands of distinct microorganisms which become active in response to seasonal or environmental changes (9, 11).

The more than 500 microbial genome projects carried out over the last 10 years have shown that each bacterium contributes around 4000 genes (http://www.genomesonline.org/). In general around 50 percent of these genes carry out functional assignments. These are either well known genes for housekeeping pathways or variations thereof. The other half of the genes are currently only described as conserved hypothetical (which means that they have significant hits to genes in other organisms, but no specific function could be assigned so far) and hypothetical genes. The latter are said to be responsible for individual adaptations, enabling the organism to successfully survive in its ecological niche.

Traditionally, this rich genetic resource is accessed by partial or whole genome sequencing of marine isolates. Unfortunately, obtaining pure cultures of the respective organism is normally a complicated and lengthy task. This is due to the complex conditions and interactions in their natural habitat that cannot be reproduced easily in the lab. Nevertheless, many examples exist where it was possible to successfully cultivate and sequence the whole genome of environmentally relevant marine microorganisms e.g. (4, 6, 7). To support this task the Moore Foundation has launched the Marine Microbiology Initiative (http://www.moore.org/marine-micro.aspx) to sequence over 100 marine microbial isolates. Nevertheless, it is estimated that despite all ongoing efforts to improve cultivation methods 99 percent of microbial diversity has resisted isolation so far (2).

Recently, a cultivation-independent method, called metagenomics, has been introduced to gain direct and comprehensive access to our natural genetic resources. Metagenomics is defined as "the genomic analysis of microorganisms by direct extraction and cloning of DNA from an assemblage of microorganisms" (8). In practice, this means that instead of trying to cultivate single organisms, a sufficient amount of the sample (water, soil, sediment etc.) is taken, total nucleic acid (DNA) is extracted and the nucleotide composition is determined using automatic sequencers. Sequencing can be either done on cloned short (<3 kb) DNA fragments (shotgun sequencing) or on libraries with large DNA fragments which are usually between 40 and 150 kb long (Fosmid or BAC clones). The sequenced genetic inventory retrieved from the community under investigation is then subjected to bioinformatic analysis. Within this process, interesting functional genes are detected by sequence similarity searches

against databases of functionally described genes. Alternatively, the respective clone libraries are initially screened for specific enzymatic functions (activity or functional screening) and only if activity is found are the corresponding clones sequenced and further processed. The most prominent large-scale metagenomic studies carried out in the marine environment were the shotgun sequencing of the Sargasso Sea and the Global Ocean Survey by Venter et al. (10, 12). These two investigations have delivered several million new enzymes and currently represent the largest sequenced reservoir of functional genes from the marine environment. Since only a part of the samples have been analysed to date more data can be expected soon.

Coinciding with the German Presidency conference on European Maritime Policy, a side event attended by more than 40 experts on marine biotechnology research, reported that marine resources offer ample potential for natural compounds of biotechnological and medical interest (see report of the marine biotechnology meeting in Bremen). The list of interesting "blue" biotech products is growing steadily and includes a range of proteins, carbohydrates and lipids. For example, it comprises biopolymers, adhesives and colloids, exotic chemicals including powerful neurotoxins, thermostable enzymes, anti-freeze proteins, anticoagulants, immuno-stimulants, alternative biochemical pathways and hence alternative products in the form of sulfatases and cazymes (carbohydrate active enzymes) capable of converting complex carbohydrates (3). A recent example are the sulfatases within the marine planctomycete *Rhodopirellula baltica*. They are able to desulfate complex, predominantly sulfated polysaccharides which are of major interest to the chemical industry (5, 13) The adaptation of marine microorganisms to extreme environmental conditions such as low or sometimes even very high temperatures, high pressures or nutrient limitations has resulted in unique enzymatic strategies. Furthermore, 90 percent of ocean water is colder than 5°C. Thus, the majority of marine organisms are cold-adapted. The enzymes of cold-loving marine microorganisms are typically characterised by high, specific activities at low and moderate temperatures, which is still of considerable biotechnological relevance.

Current obstacles that need international attention if we are to fully investigate marine genetic resources are (i) access to samples from the marine system. Apart from the cost-intensive infrastructure (ships, remote operating vehicles etc.) needed to reach the sampling sites, it would be in the interest of both research and industry to have a stable framework on intellectual property rights. Furthermore, (ii) the flood of data from marine sequencing projects need to be matched by investment so that this rich harvest can be intellectually digested. Bioinformatic as well as lab infrastructure have to be enhanced if we are to translate the existing potential into biological and biotechnological knowledge.

Release: 13 June 2007

References:

1. **Alonso, C., F. Warnecke, R. Amann, and J. Pernthaler.** 2007. High local and global diversity of Flavobacteria in marine plankton. Environ Microbiol **9:**1253-1266.
2. **Amann, R. I.** 1995. *In situ* identification of microorganisms by whole cell hybridization with rRNA-targeted nucleic acid probes, p. 1-15, Molecular Microbial Ecology Manual, vol. 3.3.6. Kluwer Academic Publishers.
3. **Debashish, G., S. Malay, S. Barindra, and M. Joydeep.** 2005. Marine enzymes, p. 189-218, Marine Biotechnology I, vol. 96. SPRINGER-VERLAG BERLIN, Berlin.
4. **Dufresne, A., M. Salanoubat, F. Partensky, F. Artiguenave, I. M. Axmann, V. Barbe, S. Duprat, M. Y. Galperin, E. V. Koonin, F. Le Gall, K. S. Makarova, M. Ostrowski, S. Oztas, C. Robert, I. B. Rogozin, D. J. Scanlan, N. T. de Marsac, J. Weissenbach, P. Wincker, Y. I. Wolf, and W. R. Hess.** 2003. Genome sequence of the cyanobacterium

Prochlorococcus marinus SS120, a nearly minimal oxyphototrophic genome. Proc. Natl. Acad. Sci. USA **100:**10020-10025.

5. **Gadler, P., and K. Faber.** 2007. New enzymes for biotransformations: microbial alkyl sulfatases displaying stereo- and enantioselectivity. Trends in Biotechnology **25:**83-88.
6. **Giovannoni, S. J., H. J. Tripp, S. Givan, M. Podar, K. L. Vergin, D. Baptista, L. Bibbs, J. Eads, T. H. Richardson, M. Noordewier, M. S. Rappe, J. M. Short, J. C. Carrington, and E. J. Mathur.** 2005. Genome streamlining in a cosmopolitan oceanic bacterium. Science **309:**1242-1245.
7. **Glöckner, F. O., M. Kube, M. Bauer, H. Teeling, T. Lombardot, W. Ludwig, D. Gade, A. Beck, K. Borzym, K. Heitmann, R. Rabus, H. Schlesner, R. Amann, and R. Reinhardt.** 2003. Complete genome sequence of the marine planctomycete *Pirellula* sp. strain 1. Proc. Natl. Acad. Sci. USA **100:**8298-8303.
8. **Handelsman, J.** 2004. Metagenomics: Application of genomics to uncultured microorganisms. Microbiol. Mol. Biol. Rev. **68:**669-685.
9. **Pedros-Alio, C.** 2006. Marine microbial diversity: can it be determined? Trends Microbiol. **14:**257-263.
10. **Rusch, D. B., A. L. Halpern, G. Sutton, K. B. Heidelberg, S. Williamson, S. Yooseph, D. Wu, J. A. Eisen, J. M. Hoffman, K. Remington, K. Beeson, B. Tran, H. Smith, H. Baden-Tillson, C. Stewart, J. Thorpe, J. Freeman, C. Andrews-Pfannkoch, J. E. Venter, K. Li, S. Kravitz, J. F. Heidelberg, T. Utterback, Y.-H. Rogers, L. I. Falcón, V. Souza, G. n. Bonilla-Rosso, L. E. Eguiarte, D. M. Karl, S. Sathyendranath, T. Platt, E. Bermingham, V. Gallardo, G. Tamayo-Castillo, M. R. Ferrari, R. L. Strausberg, K. Nealson, R. Friedman, M. Frazier, and J. C. Venter.** 2007. The Sorcerer II Global Ocean Sampling Expedition: Northwest Atlantic through Eastern Tropical Pacific. PLoS. Biol. **5:**e77.
11. **Sogin, M. L., H. G. Morrison, J. A. Huber, D. M. Welch, S. M. Huse, P. R. Neal, J. M. Arrieta, and G. J. Herndl.** 2006. Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc. Natl. Acad. Sci. USA **103:**12115-12120.
12. **Venter, J. C., K. Remington, J. F. Heidelberg, A. L. Halpern, D. Rusch, J. A. Eisen, D. Y. Wu, I. Paulsen, K. E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A. H. Knap, M. W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y. H. Rogers, and H. O. Smith.** 2004. Environmental genome shotgun sequencing of the Sargasso Sea. Science **304:**66-74.
13. **Wallner, S. R., M. Bauer, C. Würdemann, P. Wecker, F. O. Glöckner, and K. Faber.** 2005. Highly enantioselective sec-Alkylsulfatase activity of the marine Planctomycete *Rhodopirellula baltica* shows retention of configuration. Angew. Chem. Int. Ed. **44:**2-4.
14. **Whitman, W. B., D. C. Coleman, and W. J. Wiebe.** 1998. Prokaryotes: The unseen majority. Proc. Natl. Acad. Sci. USA **95:**6578-6583.