Microbial Biotechnology (2009) **2**(2), 157–163 doi:10.1111/j.1751-7915.2009.00092.x

Genomics update

Genomics of deep-sea and sub-seafloor microbes

Roland J. Siezen,[1,2,3]* and Greer Wilson[4]
[1]*Kluyver Centre for Genomics of Industrial Fermentation; TI Food and Nutrition, 6700AN Wageningen, the Netherlands.*
[2]*NIZO Food Research, 6710BA Ede, The Netherlands.*
[3]*CMBI, Radboud University Nijmegen, 6500HB Nijmegen, the Netherlands.*
[4]*Science Consultant, Bowlespark 30, 6701DS Wageningen, the Netherlands.*

Over two-thirds of the surface of the earth is covered by oceans, which have an average depth of about 3800 m. As each drop of ocean water contains $> 10^5$ cells, the $> 10^{30}$ microbial cells in the ocean represent the largest reservoir of microbes on earth (Whitman *et al*., 1998). Communities of bacteria, archaea, protists and unicellular fungi account for most of the oceanic biomass and metabolism. Marine microbes are known to play an essential role in the global cycling of nitrogen, carbon, oxygen, phosphorous, iron, sulfur and trace elements (Karl, 2007).

The largest metagenome sequencing projects undertaken to date involved surface seawater samples from the Sargasso Sea (Venter *et al*., 2004) and the Global Ocean Sampling (GOS) (Yooseph *et al*., 2007) expeditions conducted by Craig Venter and colleagues. The GOS analysis has shown that the numbers of newly discovered protein families in microbes have not yet reached a plateau, and in fact the curve is still rising. This means that there are many more functionalities to be discovered. This surface seawater metagenome sequencing did not take into account the piezophiles (= pressure-loving) and so it would be reasonable to assume that this is still a large and generally untapped source of potentially useful enzymes and products.

The microbes of the very deep differ from those isolated from shallow waters in several aspects. The GOS analysis suggests that near-surface microorganisms do not need chemotaxis, flagellae or pili to actively swim around in search of food, as they rely mostly on the plentiful O_2, CO_2 and sunlight for photosynthesis. Yet this is not the case for the deep-sea piezophilic psychrophiles. Deep-sea environments are characterized by low temperature (1–2°C), high pressure (1 MPa for every 100 m), high-salt and low-nutrient conditions. Pressure affects various aspects of metabolism in very different ways (Simonato *et al*., 2006). Little light penetrates below 500 m, the presence of food is scarce and many organisms have adapted to survive long periods without nutrition. Prokaryotic adaptation to deep sea habitats has been reviewed by Simonato and colleagues (2006) and Lauro and Bartlett (2008). It is assumed that life here will be heterotrophic and largely supported by influxes of nutrients coming down from the more 'fertile' waters above. Nutrients slowly reach the seabed in the form of dead whales, crustaceans, fish, kelp, wood and their debris. These all carry the microbiota that was associated with them as they sank to the ocean bed (Egan *et al*., 2008). These surface-associated microbes can to a certain extent also adapt to the changing conditions in which they now find themselves.

Microbial isolates or communities of some deep-sea 'oases' such as hydrothermal vents in the seafloor (Nakagawa and Takai, 2008) or whale falls (Tringe *et al*., 2005) have been sampled and studied, but these are exceptions which we do not address here.

Databases and computing tools

Marine microbe researchers are highly organized with respect to storing and sharing their data. The Community Cyberinfrastructure for Advanced Marine Microbial Ecology Research and Analysis (CAMERA) aims to develop global methods for monitoring microbial communities in the ocean and their response to environmental changes. The CAMERA's database (http://camera.calit2.net) includes environmental metagenomic and genomic sequence data, associated environmental parameters ('metadata'), pre-computed search results, and software tools to support powerful cross-analysis of environmental samples (Seshadri *et al*., 2007) (Fig. 1). The CAMERA includes the Sargasso Sea and GOS expedition data, as well as a vertical profile of marine microbial communities collected at the Hawaii Ocean Time-Series station ALOHA by Ed DeLong and his research team at MIT. In addition, the MetaLook software has been developed for visualisation, analysis and comparison of marine ecological genomic and metagenomic data with respect to habitat parameters (http://www.megx.net/metalook) (Fig. 2).

*For correspondence. E-mail r.siezen@cmbi.ru.nl; Tel. (+31) 24 3619559; Fax (+31) 24 3619395.

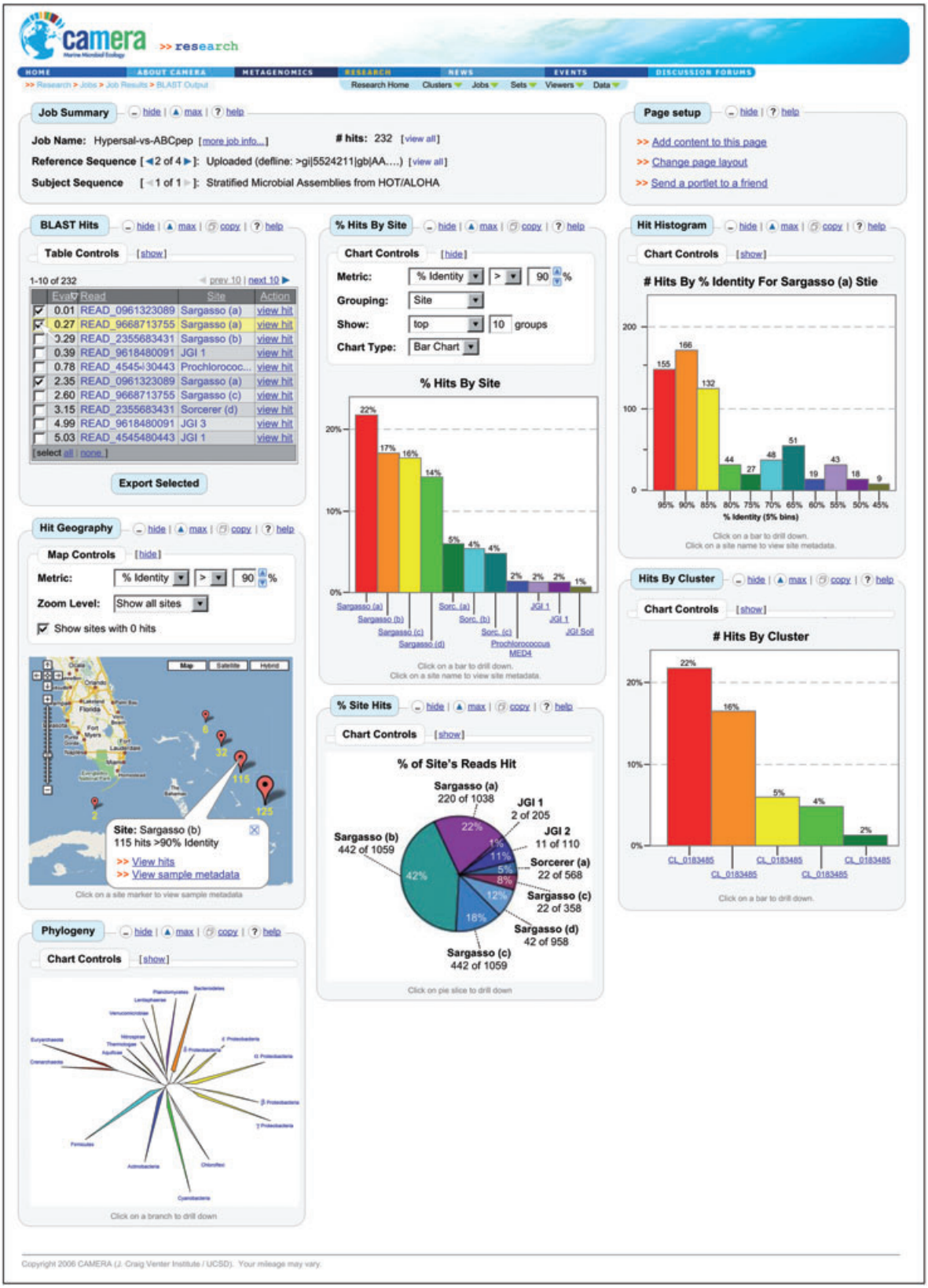

Fig. 1. Example of a BLAST search by CAMERA.

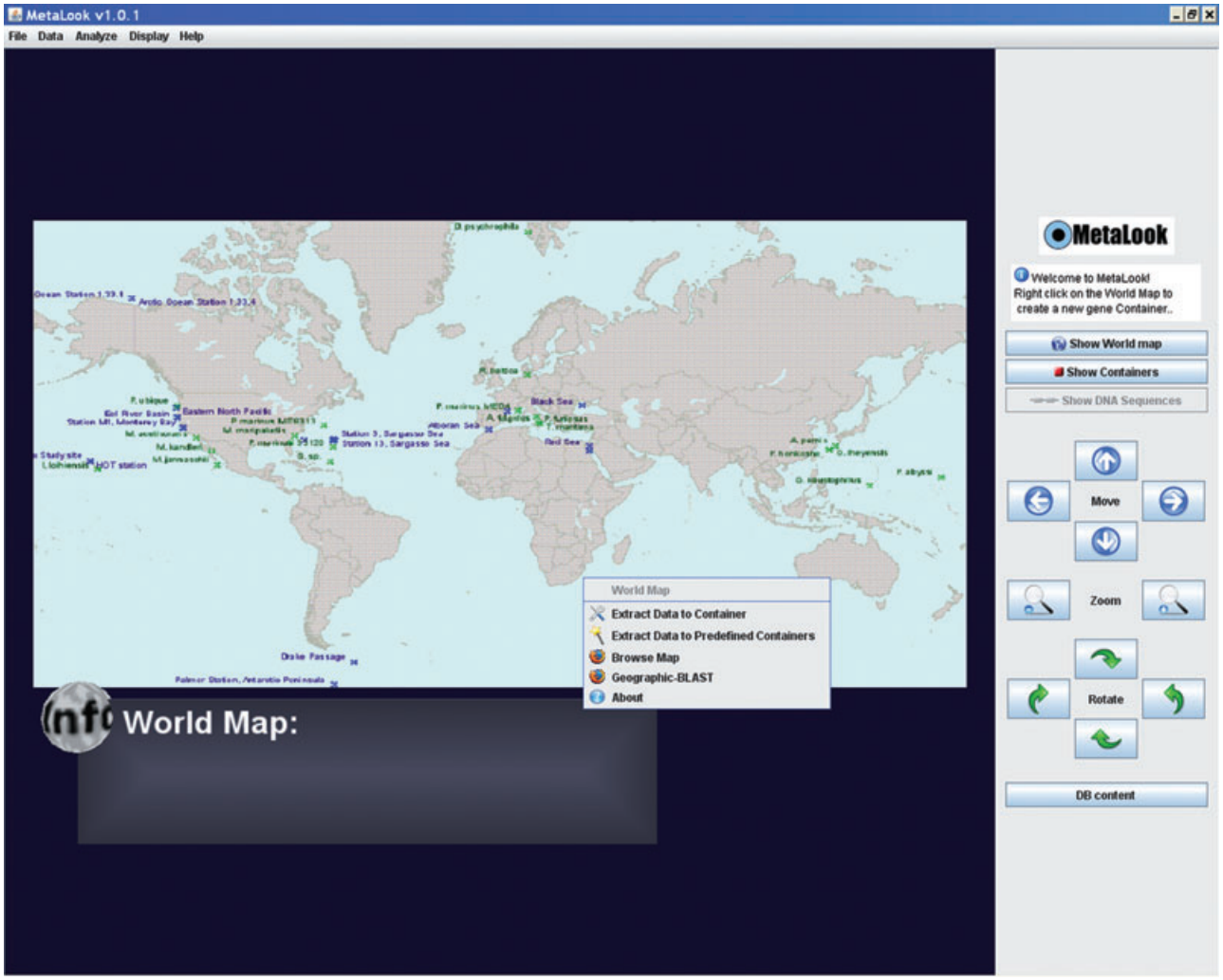

Fig. 2. The starting point of MetaLook: the world map showing genomics and metagenomics sampling sites. Clicking on a location will provide all genomics and meta data.

(Meta)genome sequencing of deep-sea microbes

Thanks to initiatives by the Gordon and Betty Moore Foundation, nearly 200 genomes of culturable marine bacteria have been sequenced since 2004 (http://www.moore.org/microgenome/). Table 1 summarizes deep-sea and sediment (meta)genome sequencing projects.

Most of the sequenced culturable microorganisms from the deep-sea are Alteromonadales from the Gammaproteobacteria. Unique properties of sequenced deep-sea microbes are that they all have a high ratio of rRNA operon copies per genome size, and that their intergenic regions are larger than average (Lauro and Bartlett, 2008). These properties are characteristic of bacteria with an opportunistic lifestyle and a high degree of gene regulation to respond rapidly to environmental changes when searching for food. A large number of genes are found for synthesis of mono- and poly-unsaturated fatty acids and membrane unsaturation in these deep dwelling microorganisms, as they need to maintain membrane fluidity at low temperature and high pressure (Simonato *et al.*, 2006). They also contain a larger than average repertoire of transport proteins for scavenging different types of food. Large numbers of proteins are encoded for chemotaxis, flagellar assembly and motor function to allow them to hunt for dissolved and particulate organic matter, also called 'marine snow' (Azam and Long, 2001; Kiorboe and Jackson, 2001). The latter consists mainly of diffuse gels with a variety of organisms living together in biofilms and communities highly dependent upon each other for survival. The marine snow can sink thousands of metres into the sea. This brings down to the seabed a lot of organic material, nitrogen and phosphorus, helping to sustain the communities present at depth (for a review see Azam and Malfatti, 2007). In order to make use of this food source the

Table 1. Deep-sea and sediment genome sequencing projects.

Phylum	Order	Organism	Isolation	Depth (m)	Reference/data
Euryarchaeota	Methanococci	*Methanococcus aeolicus* Nankai-3	Deep marine sediment, Nankai Trough Japan		NC_009635
Euryarchaeota	Methanomicrobia	*Methanoculleus marisnigri* JR1	Sediment, Black Sea		NC_009051
Chlorobi	Chlorobia	*Chlorobium phaeobacteroides* BS1	Chemocline, Black Sea		NC_010831
Proteobacteria	Gammaproteobacteria	*Shewanella piezotolerans* WP3	Sediment, west Pacific	1914	Wang *et al.* (2008)
Proteobacteria	Gammaproteobacteria	*Alteromonas macleodii* DSM 17117	Seawater, Urania Basin, Mediterranean	3500	NC_011138
Proteobacteria	Gammaproteobacteria	*Moritella* sp. PE36	Patton escarpment, off San Diego, Pacific		NZ_ABCQ00000000
Proteobacteria	Gammaproteobacteria	*Psychromonas* sp. CNPT3	Central north Pacific	5800	NZ_AAPG00000000
Proteobacteria	Gammaproteobacteria	*Shewanella benthica* KT99, PT99	Tonga-Kermadec Trench, Pacific	9000	NZ_ABIC00000000
Proteobacteria	Gammaproteobacteria	*Shewanella woodyi* MS32	Sediment, Strait of Gibraltar, Mediterranean	5110	NC_010506
Proteobacteria	Gammaproteobacteria	*Shewanella loihica* PV-4	Iron-rich mat, Naha Vents, Hawaii	1325	NC_009092
Proteobacteria	Gammaproteobacteria	*Shewanella* sp. W3-18-1	Sediment, Washington, Pacific	997	NC_008750
Proteobacteria	Gammaproteobacteria	*Shewanella violacea* DSS12	Sediment, Ryukyu Trench, Philippine Sea	5110	nakasone@hiro.kindai.ac.jp
Proteobacteria	Gammaproteobacteria	*Photobacterium profundum* SS9	Sulu Trough	2500	Vezzi *et al.* (2005)
Proteobacteria	Alphaproteobacteria	*Roseobacter* sp. SK209-2-6	Arabian Sea	2500	NZ_AAYC00000000
Proteobacteria	Epsilonproteobacteria	*Sulfurimonas autotrophica* OK10	Deep-sea sediment, Mid-Okinawa Trough, Japan		microbes@cuba.jgi-psf.org
Proteobacteria	Zetaproteobacteria	*Mariprofundus ferrooxydans* PV-1	Loihi Seamount, Hawaii	1100	NZ_AATS00000000
Firmicutes	Bacilli	*Oceanobacillus iheyensis* HTE831	Deep sea mud, Iheya ridge, Okinawa Japan	1050	Takami *et al.* (2002)
Firmicutes	Bacilli	*Carnobacterium* sp. AT7	Aleutian Trench	2500	NZ_ABHH00000000
Firmicutes	Clostridia	*Carboxydibrachium pacificum* JM	Okinawa Trough	1500	NZ_ABXP00000000
Metagenomes					
Unclassified	Unclassified	Marine anammox community	Deep sea		sgtringe@lbl.gov
Unclassified	Unclassified	Marine archaeal anaerobic oxidation of methane communities	Sediment, Eel River Basin, Mendocino California	520	Hallam *et al.* (2004)
Unclassified	Unclassified	Marine planktonic communities	North Pacific Ocean, ALOHA station, Hawaii	500–4000	DeLong *et al.* (2006)
Unclassified	Unclassified	Marine microbial communities	Seep water masses of the North Atlantic	500–4121	Sogin *et al.* (2006)
Unclassified	Unclassified	Marine planktonic communities	Mediterranean Sea, Ionian Km3 Station	3010	Martin-Cuadrado *et al.* (2007)
Unclassified	Unclassified	Sediment microbial communities	Sub-seafloor, Peru Margin	1229	Biddle *et al.* (2008)

Adapted from the GOLD database (http://www.genomesonline.org; December 2008).

Table 2. Enzymes from deep-sea microbes.

Enzyme(s)	Producing organism(s)	Isolation	Reference
α-Amylase	*Nocardiopsis* sp.	Deep sea sediment	Zhang and Zeng (2008)
Alkane hydroxylases	Metagenome	Deep-sea sediment	Xu *et al.* (2008)
β-Lactamases	*Metagenome*	Cold-seep sediment of seamount	Song *et al.* (2005)
Cellulase	*Pseudoaltermonas* sp. DY3	Deep-sea sediment	Zeng *et al.* (2006)
Esterases	Metagenome	Deep-sea hypersaline anoxic basin	Ferrer *et al.* (2005)
Esterase (alkaline)	Metagenome	Deep-sea sediment	Park *et al.* (2007)
Lipase	Metagenome	Deep-sea sediment	Hardeman and Sjoling (2007)
Lipase	Metagenome	Deep-sea sediment	Jeon *et al.* (2009)
Quinol oxidase	*Shewanella* sp. strain DB-172F	Deep-sea sediment	Qureshi *et al.* (1998)
Protease (alkaline)	*Pseudomonas strain* DY-A	Deep-sea sediment	Zeng *et al.* (2003)
Proteases (neutral, alkaline)	*Pseudoaltermonas* sp.	Deep-sea sediment	Xiong *et al.* (2007)
Protease (alkaline)	*Pseudoaltermonas* sp. SM9913	Deep-sea sediment	Chen *et al.* (2007)
Various	Metagenome	Deep sub-seafloor sediment core	Kobayashi *et al.* (2008)

Adapted from Kennedy and colleagues (2008).

microorganisms have fine-tuned hydrolytic enzymes and nutrient uptake systems.

Very few metagenomics studies of deep-sea communities have been reported so far, and include only deep water samples from the North Pacific Gyre ALOHA station (DeLong *et al.*, 2006) and the Ionian Sea in the Mediterranean (Martin-Cuadrado *et al.*, 2007), and (sub)seafloor samples from the Peru Margin (Biddle *et al.*, 2008) and the Eel Basin off the coast of California (Hallam *et al.*, 2004) (Table 1). The cold (2–5°C) North Pacific deep waters were found to contain *Deferribacteres*, *Planctomycetaceae*, *Acidobacteriales*, *Gemmatomonadaceae*, *Nitrospira*, *Alteromondaceae*, and SAR11, SAR202 and Agg47 bacterial clades (DeLong *et al.*, 2006); these microbial communities were enriched in genes encoding transposases, pilus synthesis, protein transport, polysaccharide and antibiotic synthesis, the glyoxylate cycle, and urea metabolism, relative to surface communities. The warmer (14°C) deep Mediterranean waters contained mainly Proteobacteria, but also Actinobacteria, Firmicutes, Planctomycetales, Chloroflexi, Bacteroidetes, Acidobacteria, and also Crenarchaeota (Martin-Cuadrado *et al.*, 2007). Enriched functional categories were again for pilus, polysaccharide and antibiotic synthesis, as well as peptide and amino acid transporters, while genes involved in degradation of complex biopolymers and xenobiotics were also abundant. The high percentage of genes encoding dehydrogenases, and among them *cox* genes, suggested that aerobic CO oxidation may play a role in deep seas as additional energy source.

The marine subsurface at great depths is a distinct microbial habitat. Metagenomics analysis (GS20 pyrosequencing) of ng amounts of DNA extracted from 1–50 m below the seafloor at an Ocean Drilling Program Site (seafloor depth is 1229 m) on the Peru Margin showed that only ~10% of the sequences had a detectable homology to known sequences (Biddle *et al.*, 2008). Archaea, and particularly Crenachaeota, appear to be the dominant microbes in these environments, and could be a source of as yet completely undiscovered enzymes with unique properties.

Discovery of novel enzymes from deep-sea microbes

Marine enzyme biotechnology can offer novel biocatalysts with properties like high salt tolerance, hyperthermostability, barophilicity, cold adaptivity, and ease in large-scale cultivation (reviewed by Debashish *et al.*, 2005). Metagenomics strategies are powerful tools to identify enzymes with novel biocatalytic properties from unculturable members of microbial communities (Ferrer *et al.*, 2009; Steele *et al.*, 2009). The GOS project discovered hundreds of truly novel protein and enzyme families from marine surface microbes for which no function is yet known (Yooseph *et al.*, 2007). Deep-sea and sediment microbes should provide an enormous reservoir of low-temperature and high-pressure adapted enzymes. Both sequence-based and function-based screening approaches have been used to identify enzymes with potentially interesting biocatalytic activities from cultured deep-sea microbes as well as uncultured metagenomes (Kennedy *et al.*, 2008; Kobayashi *et al.*, 2008) (Table 2).

For instance, highly salt-tolerant and pressure-tolerant esterases were identified in a metagenome expression library generated from microbes isolated from a deep-sea hypersaline anoxic basin in the Eastern Mediterranean (Ferrer *et al.*, 2005). The amino acid content of proteins in psychrophilic piezophiles is also different from that in mesophiles. There are more polar amino acids in the proteins, resulting in a loss of rigidity and increased structural flexibility for enhanced catalytic activity. The adaptive properties of psychrophilic enzymes are high specific activity, relatively low temperature optima and high thermolability (Gomes and Steiner, 2004).

A major challenge will be to develop alternative hosts and their associated vectors for heterologous expression of genes from the diverse phyla existing in the deep-sea ecosystem (Ferrer *et al*., 2009).

Applications

Cold-adapted enzymes are advantageous for waste decomposition in cold environments, for food processing, flavour enhancement (He *et al*., 2004) and preservation, and for processes that require the rapid inactivation of enzymatic reactions (Huston *et al*., 2000; O'Brien *et al*., 2004). Piezophilic enzymes could be useful for food sterilization at high pressure and low temperature, improving preservation of flavour and colour. Halophilic enzymes can be applied for non-aquatic reactions, as they have better thermostability and other unique properties in organic solvents, and could be used in anti-fouling coating and paint industries (Yebra *et al*., 2004), but also for synthesis of optically active substances. Finally, many deep-sea bacteria can synthesize interesting chemical compounds, such as omega-3 polyunsaturated fatty acids that are considered useful in reducing the risk of cardiovascular disease, and polyketides (Siezen and Khayatt, 2008) which could be used as novel antibiotics.

Acknowledgements

This project was carried out within the research programme of the Kluyver Centre for Genomics of Industrial Fermentation which is part of the Netherlands Genomics Initiative/ Netherlands Organization for Scientific Research.

References

Azam, F., and Long, R.A. (2001) Sea snow microcosms. *Nature* **414:** 497–498.

Azam, F., and Malfatti, F. (2007) Microbial structuring of marine ecosystems. *Nat Rev Microbiol* **5:** 782–791.

Biddle, J.F., Fitz-Gibbon, S., Schuster, S.C., Brenchley, J.E., and House, C.H. (2008) Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. *Proc Natl Acad Sci USA* **105:** 10583–10588.

Chen, X.L., Xie, B.B., Lu, J.T., He, H.L., and Zhang, Y. (2007) A novel type of subtilase from the psychrotolerant bacterium *Pseudoalteromonas* sp. SM9913: catalytic and structural properties of deseasin MCP-01. *Microbiology* **153:** 2116–2125.

DeLong, E.F., Preston, C.M., Mincer, T., Rich, V., Hallam, S.J., Frigaard, N.U., *et al*. (2006) Community genomics among stratified microbial assemblages in the ocean's interior. *Science* **311:** 496–503.

Debashish, G., Malay, S., Barindra, S., and Joydeep, M. (2005) Marine enzymes. *Adv Biochem Eng Biotechnol* **96:** 189–218.

Egan, S., Thomas, T., and Kjelleberg, S. (2008) Unlocking the diversity and biotechnological potential of marine surface associated microbial communities. *Curr Opin Microbiol* **11:** 219–225.

Ferrer, M., Golyshina, O.V., Chernikova, T.N., Khachane, A.N., Martins Dos Santos, V.A., Yakimov, M.M., *et al*. (2005) Microbial enzymes mined from the Urania deep-sea hypersaline anoxic basin. *Chem Biol* **12:** 895–904.

Ferrer, M., Beloqui, A., Timmis, K.N., and Golyshin, P.N. (2009) Metagenomics for mining new genetic resources of microbial communities. *J Mol Microbiol Biotechnol* **16:** 109–123.

Gomes, J., and Steiner, W. (2004) The biocatalytic potential of extremophiles and extremozymes. *Food Tech Biotechnol* **42:** 223–235.

Hallam, S.J., Putnam, N., Preston, C.M., Detter, J.C., Rokhsar, D., Richardson, P.M., and DeLong, E.F. (2004) Reverse methanogenesis: testing the hypothesis with environmental genomics. *Science* **305:** 1457–1462.

Hardeman, F., and Sjoling, S. (2007) Metagenomic approach for the isolation of a novel low-temperature-active lipase from uncultured bacteria of marine sediment. *FEMS Microbiol Ecol* **59:** 524–534.

He, H., Chen, X.L., Li, J., Zhang, Y., and Gao, P. (2004) Taste improvement of refrigerated meat treated with cold-adapted protease. *Food Chemistry* **84:** 307–311.

Huston, A.L., Krieger-Brockett, B.B., and Deming, J.W. (2000) Remarkably low temperature optima for extracellular enzyme activity from Arctic bacteria and sea ice. *Environ Microbiol* **2:** 383–388.

Jeon, J.H., Kim, J.T., Kim, Y.J., Kim, H.K., Lee, H.S., Kang, S.G., *et al*. (2009) Cloning and characterization of a new cold-active lipase from a deep-sea sediment metagenome. *Appl Microbiol Biotechnol* **81:** 865–874.

Karl, D.M. (2007) Microbial oceanography: paradigms, processes and promise. *Nat Rev Microbiol* **5:** 759–769.

Kennedy, J., Marchesi, J.R., and Dobson, A.D. (2008) Marine metagenomics: strategies for the discovery of novel enzymes with biotechnological applications from marine environments. *Microb Cell Fact* **7:** 27.

Kiorboe, T., and Jackson, G. (2001) Marine snow, organic solute plumes, and optimal chemosensory behaviour of bacteria. *Limnol Oceanogr* **46:** 1309–1318.

Kobayashi, T., Koide, O., Mori, K., Shimamura, S., Matsuura, T., Miura, T., *et al*. (2008) Phylogenetic and enzymatic diversity of deep subseafloor aerobic microorganisms in organics- and methane-rich sediments off Shimokita Peninsula. *Extremophiles* **12:** 519–527.

Lauro, F.M., and Bartlett, D.H. (2008) Prokaryotic lifestyles in deep sea habitats. *Extremophiles* **12:** 15–25.

Martin-Cuadrado, A.B., Lopez-Garcia, P., Alba, J.C., Moreira, D., Monticelli, L., Strittmatter, A., *et al*. (2007) Metagenomics of the deep Mediterranean, a warm bathypelagic habitat. *PLoS ONE* **2:** .e914.

Nakagawa, S., and Takai, K. (2008) Deep-sea vent chemoautotrophs: diversity, biochemistry and ecological significance. *FEMS Microbiol Ecol* **65:** 1–14.

O'Brien, A., Sharp, R., Russell, N., and Roller, S. (2004) Antarctic bacteria inhibit growth of food-borne microorganisms at low temperatures. *FEMS Microbiol Ecol* **48:** 157–167.

Park, H.J., Jeon, J.H., Kang, S.G., Lee, J.H., Lee, S.A., and Kim, H.K. (2007) Functional expression and refolding of new alkaline esterase, EM2L8 from deep-sea sediment metagenome. *Protein Expr Purif* **52:** 340–347.

Qureshi, M.H., Kato, C., and Horikoshi, K. (1998) Purification of a ccb-type quinol oxidase specifically induced in a deep-sea barophilic bacterium, Shewanella sp. strain DB-172F. *Extremophiles* **2:** 93–99.

Seshadri, R., Kravitz, S.A., Smarr, L., Gilna, P., and Frazier, M. (2007) CAMERA: a community resource for metagenomics. *PLoS Biol* **5:** e75.

Siezen, R.J., and Khayatt, B.I. (2008) Natural products genomics. *Microbial Biotechnol* **1:** 275–282.

Simonato, F., Campanaro, S., Lauro, F.M., Vezzi, A., D'Angelo, M., Vitulo, N., *et al*. (2006) Piezophilic adaptation: a genomic point of view. *J Biotechnol* **126:** 11–25.

Sogin, M.L., Morrison, H.G., Huber, J.A., Mark Welch, D., Huse, S.M., Neal, P.R., *et al*. (2006) Microbial diversity in the deep sea and the underexplored 'rare biosphere'. *Proc Natl Acad Sci USA* **103:** 12115–12120.

Song, J.S., Jeon, J.H., Lee, J.H., Jeong, S.H., Jeong, B.C., Kim, S.J., *et al*. (2005) Molecular characterization of TEM-type beta-lactamases identified in cold-seep sediments of Edison Seamount (south of Lihir Island, Papua New Guinea). *J Microbiol* **43:** 172–178.

Steele, H.L., Jaeger, K.E., Daniel, R., and Streit, W.R. (2009) Advances in recovery of novel biocatalysts from metagenomes. *J Mol Microbiol Biotechnol* **16:** 25–37.

Takami, H., Takaki, Y., and Uchiyama, I. (2002) Genome sequence of *Oceanobacillus iheyensis* isolated from the Iheya Ridge and its unexpected adaptive capabilities to extreme environments. *Nucleic Acids Res* **30:** 3927–3935.

Tringe, S.G., von Mering, C., Kobayashi, A., Salamov, A.A., Chen, K., Chang, H.W., *et al*. (2005) Comparative metagenomics of microbial communities. *Science* **308:** 554–557.

Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A., *et al*. (2004) Environmental genome shotgun sequencing of the Sargasso Sea. *Science* **304:** 66–74.

Vezzi, A., Campanaro, S., D'Angelo, M., Simonato, F., Vitulo, N., Lauro, F.M., *et al*. (2005) Life at depth: Photobacterium profundum genome sequence and expression analysis. *Science* **307:** 1459–1461.

Wang, F., Wang, J., Jian, H., Zhang, B., Li, S., Wang, F., *et al*. (2008) Environmental adaptation: genomic analysis of the piezotolerant and psychrotolerant deep-sea iron reducing bacterium *Shewanella piezotolerans* WP3. *PLoS ONE* **3**: .e1937.

Whitman, W.B., Coleman, D.C., and Wiebe, W.J. (1998) Prokaryotes: the unseen majority. *Proc Natl Acad Sci USA* **95:** 6578–6583.

Xiong, H., Song, L., Xu, Y., Tsoi, M.Y., Dobretsov, S., and Qian, P.Y. (2007) Characterization of proteolytic bacteria from the Aleutian deep-sea and their proteases. *J Ind Microbiol Biotechnol* **34:** 63–71.

Xu, M., Xiao, X., and Wang, F. (2008) Isolation and characterization of alkane hydroxylases from a metagenomic library of Pacific deep-sea sediment. *Extremophiles* **12:** 255–262.

Yebra, D., Kiil, S., and Dam-Johansen, K. (2004) Antifouling techniques – past, present and future steps towards efficient and environmentally friendly antifouling coatings. *Prog Org Coat* **50:** 75–104.

Yooseph, S., Sutton, G., Rusch, D.B., Halpern, A.L., Williamson, S.J., Remington, K., *et al*. (2007) The Sorcerer II Global Ocean Sampling expedition: expanding the universe of protein families. *PLoS Biol* **5**: .e16.

Zeng, R., Zhang, R., Zhao, J., and Lin, N. (2003) Cold-active serine alkaline protease from the psychrophilic bacterium *Pseudomonas* strain DY-A: enzyme purification and characterization. *Extremophiles* **7:** 335–337.

Zeng, R., Xiong, P., and Wen, J. (2006) Characterization and gene cloning of a cold-active cellulase from a deep-sea psychrotrophic bacterium *Pseudoalteromonas* sp. DY3. *Extremophiles* **10:** 79–82.

Zhang, J.W., and Zeng, R.Y. (2008) Purification and characterization of a cold-adapted alpha-amylase produced by *Nocardiopsis* sp 7326 isolated from Prydz Bay, Antarctic. *Mar Biotechnol (NY)* **10:** 75–82.

