REPORT

An overview of the First Marine Biotechnology Symposium in the Southern Hemisphere-MBSH2007: "Waking up a Sleeping Beauty"

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Introduction

Marine Biotechnology targets the development of novel products and processes from marine organisms, thereby seeking to contribute to human health, the food processing and animal feed industry and many other applications such as their use to generate biofuels. It also seeks better means for protecting marine habitats and restoring those that are damaged.

In this sense, the First Marine Biotechnology Symposium in the Southern Hemisphere (MBSH2007; Viña del Mar, Chile, December 9th - 13th, 2007) promoted research and development in the field of basic and applied marine biotechnology, offering a unique opportunity for the critical examination of milestones in marine biotechnology and their significant contribution to global sustainability in the 21st century. During the intense three-and-a-half days of the conference key lectures, followed by topic-oriented roundtables, facilitated the exchange of experience among participants, with poster sessions in three main areas: general marine biotechnology, aquaculture and marine biotechnology-derived products [1].

The lecture sessions covered microalgae as green cell factories, harmful algal blooms, marine biodiscovery, fish health (immunity against diseases and diagnostics), sustainable management of marine systems and taking marine biotechnology products to the market.

Algae biotechnology

Thousands of microalgal species are spread throughout the oceans, as primary producers for the marine food chain and as the principal contributors to carbon fixation and oxygen production on Earth. Various species grow in almost every salt concentration; many are resistant to desiccation and tolerate a wide range of irradiation, pH and temperature conditions. All these characteristics make small photosynthetic protists ideal candidates for biotechnological applications, though first requiring excellent knowledge of their specific biochemistry and physiology for successful use.

In this regard, Dr. JP Cadoret (France) described the technologies developed at his laboratory to unravel the metabolism of several phytoplankters at the cellular and population levels. Dr. SP Mayfield (USA) presented results on chloroplast engineering of eukaryotic microalgae to express therapeutic proteins and produce biofuels. He and his colleagues attempted to express, in chloroplasts of the eukaryotic green alga *Chlamydomonas reinhardtii*, antibodies and antibody-toxin fusion proteins that were properly folded and assembled and able to bind and kill lymphoma tumor cells in culture. More recently, they have also developed tools to engineer algal chloroplasts for the accumulation of biofuel molecules.

Dr. M Calderon (Chile) shared his thoughts on, *inter alia*, the usefulness of microalgae to generate renewable fuels and to obtain marine oil for aquaculture. Microalgae are currently receiving much attention, due to the demand for sustainable biofuels, but still require substantial research and technological innovation for a profitable production system. He referred to the success of the Atacama BioNatural Products S.A. company (http://www.atacamabionatural.com), established in the north of Chile. At this institution, a team of scientists and engineers took advantage of the natural environmental conditions (*i.e.*, light intensity and mineral salts) to develop a profitable system for production of the pigment and Astaxanthin, a natural antioxidant used in aquaculture, from the eukaryotic microalga *Haematococcus pluvialis*. In the poster session, one of the most interesting presentations, by J Gimpel and coworkers (Chile), highlighted the use of the *H. pluvialis* for a number of biotechnological applications, including the production of Astaxanthin at high levels. In their work, they also promoted the use of microalgae for recombinant protein production. Because the genome of the *H. pluvialis* chloroplast is uncharacterized, Gimpel and colleagues used RAGE (Rapid Amplification of Genomic Ends) to identify strong promoters and regulatory sequences of highly-transcribed chloroplast genes. They proposed the potential use of chloroplast transformation vectors to construct expression cassettes for *H. pluvialis* chloroplasts.

Another poster, presented by Dr. M Llanio and co-workers (Cuba), described the anti-inflammatory, analgesic and/or antioxidant properties of extracts from *Stypopodium zonale* (collected from the Cuban shore), and its putative mechanism of action.

Other groups of scientists discussed the negative environmental impact of microalgal growth and proliferation [1]. Dr. DM Anderson (USA) described in his lecture the effects of Harmful Algal Blooms (HABs), commonly known as "red tides". HABs take many forms, affecting coastal countries through poisonous shellfish, dead fish, and changes in marine ecosystems such as erosion of submerged aquatic vegetation and increased mortality of aquatic organisms, including marine mammals. The impact of HABs on

1. Abstract Book from the First marine Biotechnology Symposium in the Southern Hemisphere-MBSH2007: "Waking up a Sleeping Beauty", December 9-13, 2007. Viña del Mar, Chile. http://www.mbsh2007.pucv.cl

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human health and in the economy is significant, with an increasing need for appropriate forecast of these events and adequate mitigation of their effects. Dr. Anderson talked about the global HABs problem and the need for developing new technologies and approaches for their monitoring, control and management. These could include methods employing molecular probes for cell detection, fast and sensitive assays for toxins, and large-scale physical/biological models to analyze previous blooms and to forecast future ones. In this direction, genomic studies have been conducted recently in organisms responsible for HABs (A.R. Place, Center for Marine Biotechnology, Baltimore, USA).

Marine biodiscovery topics

Dr. N Blom (Senior Manager at Bioinformatics Novozymes A/S) and Dr. H. Ramlov (from the Roskilde University Section of Molecular and General Physiology) made a fascinating presentation on Galathea 3, a Danish marine expedition that circumnavigated the globe for 8 months (August 2006-April 2007). More than 200 scientists participated, conducting over 50 projects, ranging from geological surveys and atmospheric measurements to antifreeze proteins and bacterial genomics.

Dr. Blom stated that the expedition attempted to: 1) describe the microbial diversity in the coldest and deepest regions of the polar seas, 2) compare the microbial diversity from the surface down to deep waters and between northern and southern stations, 3) discover new biological processes specific for the environments of polar seas, and 4) investigate the industrial applicability of novel cold-adapted enzymes. Using electron microscopy, they obtained first glimpses of microbial diversity, further characterizing it through sequencing of a large number of 16S rRNA genes from several samples. Future studies within these projects will address the large-scale sequencing of selected samples using the 454 "next generation" sequencing technology. They will provide several hundred millions of base pairs of genomic sequence to search for known and previously undescribed genes involved in the biological processes and pathways active in these extreme environments.

Dr. Ramlov focused attention on the relevance of antifreeze proteins for marine organisms. These are a group of proteins, many only recently described present in various cold-tolerant, freeze-avoiding and cold-blooded (ectothermic) animals. These proteins are able to recognize and bind to specific ice crystal planes, thereby inhibiting their growth or possibly even their nucleation in animal body fluids, and stabilizing the supercooled state.

In another presentation, Dr. RJ Capon (Australia) gave a summary of the strategies employed at the University of Queensland to advance a range of outcomes from marine biodiscovery research in Australia. They use a collaborative, multi-disciplinary approach for investigating biodiversity with a view to discovering valuable molecular products. The breadth of biodiversity explored ranges across terrestrial and marine ecosystems, and includes plants, animals and microbes. The array of novel molecules studied is equally broad, and extends across all biosynthetic classes and includes many molecules with unprecedented structural features, and a wide spectrum of biological properties. The team includes various specialists for acquiring, purifying and characterizing bioactive molecules, using innovative bioassays and modern chromatographic and spectroscopic equipment. They work in close collaboration with external colleagues both in Australia and internationally.

Fish immunity: diseases and diagnostics

Biotechnology offers substantial opportunities to improve the health and well being of confined fish cultures and increase production, based on broodstock free of pathogens, safe and effective therapeutic agents, immune modulators, and alternative systems for administering them. There are many diseases affecting cultivated fish, causing millions of dollars in losses every year. This situation was reflected by five lectures and ten posters presented on fish immunity, diseases and diagnostics.

One of the topics addressed was salmon culture biotechnology and diagnostic methodologies. Roche Applied Science Chile (http://www.roche.cl) reported on a molecular array for the fast, easy and reliable identification of various pre-cultured pathogenic bacteria from fish, such as *Piscirickettsia salmonis*, *Renibacterium salmoninarum*, *Aeromonas salmonicida*, *Aereomonas hydrophila* and *Vibrio ordalii*. Briefly, the assay starts with PCR amplification of bacterial genomic DNA fragments, the labeled PCR fragments being hybridized to the individual array fields in one chip, where they are specifically captured by probes immobilized as spots on the bottom of each field. The data read out can be done by simple optical examination, by using a pattern matrix, or alternatively with a scanner and software. Despite it having been validated to evaluate cultures, the sensitivity of this assay is sufficient to identify bacterial specimens directly isolated from fish without previous enrichment by culture [1].

In another talk, Dr. B Dixon from the University of Waterloo (Canada), focused on the efforts carried out at his country to culture native Pacific salmon species rather than the Atlantic salmon. The studies addressed the immune behavior of Chinook salmon bred by traditional techniques and also by a novel method involving sexual selection and a semi-natural rearing environment. He reported data on the humoral and cytokine gene expression responses obtained after stimulating fish with a *Vibrio salmonicida* vaccine followed by a challenge with live pathogens. The overall objective was to determine if the offspring obtained in the semi-natural rearing environment responded better than fish bred in a conventional hatchery. Although definitive results were not yet obtained, the fish will be additionally typed for MHC allele diversity. Further study is planned to evaluate this semi-natural rearing environment as an approach for improving the genetic quality and breeding value of Pacific salmonids for aquaculture.

Vaccine development and other prophylactic alternatives against relevant salmonid diseases, such as Salmonid Ricketsial Septicemia (SRS) and the Infectious Pancreatic Necrosis (IPN), were presented by Dr. S Marshall's team from the Catholic University of

Valparaiso (Chile). Prophylaxis remains as the main strategy to control these diseases. In this connection, Dr. Marshall's group identified, by substractive hybridization, salmonid genes involved in the response to infection by *Piscirickettsia salmonis*, the etiological agent of SRS. They additionally evaluated the immunogenic potential of 11 new antigens derived from ChaPs, an immunogenic protein identified as a member of the HSP60 family and regarded as an ideal candidate for the development of potential vaccines against this bacterium. They also characterized a novel, and genetically different, small infective variant of *P. salmonis*.

In this context, a research group from the Center of Marine Biotechnology, University of Maryland (USA) cloned the capsid protein genes of the IPN virus and expressed the recombinant proteins in a baculovirus expression system, and evaluated them as part of an effective, economical and safe subunit vaccine. The vaccine was injected in post-smolt fish and resulted in lower cumulative mortality rates in the injected fish after an IPN virus challenge. They also produced large quantities of recombinant IPN virus proteins in a yeast system. Fish immunized by feeding them recombinant yeast expressing the IPN virus subviral particles showed a specific immune response and reduced the viral loads after an experimental challenge with IPN virus.

Another approach was presented by Dr. MP Estrada (Cuba), Head of our Aquatic Biotechnology Department at the CIGB, who proposed the category of "metabolic modifiers" for those molecules, or their mixtures, that are able to activate or deactivate the molecular mechanisms accounting for a specific metabolic effect on a target animal (*e.g.*, growth and innate immunity). Our group characterized three of these metabolic modifiers, Acuabio 1, PACAP and neuropeptide Y, which act at early developmental stages in aquatic organisms by promoting growth and enhancing innate immunity, producing a subsequent increased resistance to pathogens. These new metabolic modifiers are emerging as new and potentially powerful biotechnological tools for aquaculture.

An interesting study on the effect of hormones in fish immune responses was presented in the poster session by M Paredes, *et al.* (from Dr. J Figueroa's group at the Austral University, Chile). They evaluated the potential of prolactin to enhance the phagocytic activity and superoxide production in Atlantic salmon macrophages *in vitro*.

In another development, antimicrobial peptides have emerged as important innate immune system effectors in fish, due to the central role of the innate immune system for survival in teleost fish, which have a less sophisticated acquired immune system [1]. Several posters touched on this topic, including studies on the role of high density lipoprotein (HDL) in the salmonids' antimicrobial defense, the expression in *Escherichia coli* of fish antimicrobial peptides by the intein system, the characterization of *Mytilus edulis chilensis* mussel histone H2A-derived antimicrobial peptides, and the bivalve *Choromytilus chorus* as a source for new antimicrobial polypeptides.

Sustainable management of marine systems

The fundamental aim of sustainable management of marine ecosystems is to preserve them and establish global responsibilities for them, while also addressing social and economic needs. The conference's first plenary speaker, Eric Mathur, (Vice President of Metagenomics at Synthetic Genomics, Inc.) gave a stimulating overview of the human benefits and economic potential of marine microorganisms as well as the incredibly important influence these organisms have on our biosphere (for example in O_2 generation, CO_2 sequestration and nutrient cycling). As an extended example, he described the metagenomics studies being undertaken in his own research, and also in collaboration with his colleague, Dr. C Venter, to identify genes from unculturable microorganisms. In particular, he summarized Venter's metagenomic sequencing of microorganisms from the nutrient-poor Sargasso Sea, near Bermuda, where more than a million ORF's were identified, the vast majority of which have no identified function. He also briefly described the current voyage of Sorcerer II, in which Venter is circumnavigating the globe, while collecting numerous marine water samples for additional metagenomic sequencing. One of the major objectives of this voyage is to collect data on marine biodiversity that might help solve some of the world's environmental problems.

Dr. V Webb explained the application of biotechnology at the National Institute of Water and Atmospheric Research at Wellington, New Zealand. Strategies such as adding value to fish factory waste and by-catch species by identifying bioactive compounds suitable for the skin care market and the use of an aquacultured instead of a naturally harvested New Zealand sponge as source for a potential anti-cancer drug were cited as successful approaches to sustainable and responsible biotechnological exploitation of marine ecosystems.

Two issues are currently influencing marine biotechnology research programs in ecosystem management: bioenergy and the global climate change. Dr. JC Hunter-Cevera, President of the University of Maryland Biotechnology Institute, discussed the possible use of marine systems to obtain bioenergy. This option is hampered by the lack of funding for research needed to elucidate and better understand the biology of marine organisms, their population dynamics and the extensive symbioses in marine environments. Her lecture included a call for funds to support research on how the oceans contribute to mitigate global climate change and how to restore the balance in threatened, fragile marine ecosystems.

Patricia Berenstein described a joint project between the University Los Lagos (Chile) and Swansea University (UK), to generate systematically, scientific information for industry, farm managers and regulatory agencies that will help them to make decisions that protect genetic resources and improve their sustainable use. The project is devoted to developing a toolkit for genetic tagging and monitoring of species in the ecosystem, and also to evaluate their ecological interactions.

The resulting database will help to establish a Management Action Plan and a Code of Good Practices for the management of non-indigenous fish species and the protection of native aquatic biodiversity.

The overall message from the presentations was that the combined scientific expertise in the fields of biology, chemistry, physics, oceanography, ecology and others are required to generate products from marine organisms without damaging whole ecosystems. One general consensus is that many commercial marine species can be, and increasingly will need to be, produced through aquaculture, to ease the pressures on wild fisheries stocks and to satisfy the increasing global consumption of seafood. Dr. Y Zohar, Director of the Center of Marine Biotechnology, Baltimore (USA), in a presentation read for him at the conference, argued that for aquaculture to meet this challenge, it must become more intensive, efficient and cost-effective, while remaining compatible with the marine and coastal environments in a sustainable manner. The manipulation of molecular and hormonal aspects of reproduction, growth and genetics, using modern tools such as bioinformatics, proteomics, functional genomics, and other fields of research, along with microbial bioremediation of effluents to ensure waste containment would enhance long term commercial and environmental viability of aquaculture.

Taking marine biotechnology products to the market

The expansion of the aquaculture industry has followed a similar pattern to that of animal production in past decades, with poultry and swine industries emerging in the hands of specialized companies that can take advantage of quantitative and molecular genetic techniques to meet market demands and retain the benefits of their substantial investments.

As an example of such specialization, Dr. R. Neira (Chile) from AquaInnovo SA. (http://www.aquainnovo.com), talked about the creation AquaInnova under the framework of the 2005 National Competition of Enterprise-Technological Research Consortia in Chile. AquaInnova will face the challenge of becoming a world leader in genetics and biotechnology for salmon and ultimately for aquaculture more generally. The consortium's goal is the improvement of competitiveness and the opening of opportunities for business investments in the salmon industry. They contemplate establishing alliances with national and international research institutions and forming research and development partnerships with research leaders throughout the world.

In his talk, Dr. F Quezada from the Biotechnology Center of the Excellence Corporation (USA), discussed the new marketing program of the Woods Hole Oceanographic Institution in Massachusetts, devoted to structuring and accelerating marine biotechnological enterprises. Comparisons were made with other marine biotechnology marketing programs. Dr Quezada also commented on the future directions and challenges for expanding marine programs and taking biotechnology products to market in Chile.

Any transition of academic search results from the bench top to the market requires technology transfer, as pointed out by Dr. AJ Stevens from the Research Programs at ITEC and Director of the Office of Technology Transfer at Boston University (USA). He discussed how a robust practice of academic technology transfer has evolved over the past 25 years in the USA, and how its success has led to its implementation in Europe and Asia. Finally, he discussed application of that model to marine biotechnology.

Future directions

There are many challenges (technical, regulatory, political, and environmental) for modern marine biotechnology [2] that were discussed in this meeting:

- Technical - exploring new environments and developing new platforms, tools, and tests to discover marine organisms and applying that knowledge to develop useful products and solve environmental problems.
- Regulatory - reorganization of government regulatory requirements for drug development.
- Political - complying with regulations related to the rights of a country to its natural resources, as well as fair and equitable sharing of technologies and revenues resulting from commercialization of marine bioproducts.
- Environmental - ensuring sustainable use of marine resources with commercial potential by developing alternatives to continued harvest of marine organisms.
- Marketing - commercializing marine biotechnology discoveries, which require stronger partnerships between academic researchers, industry, and innovative small companies.

It is difficult to overstate the importance of organizing international scientific conferences such as MSBSH2007 for fostering the development of marine biotechnology and enhancing contacts among researchers. Dr. Sergio Marshall is to be commended for organizing this world-class conference in Chile, which brought scientists, government agents and businessmen from Latin America together with international experts to explore new developments and opportunities emerging from marine biotechnology. Because of the conference's success, Dr. Marshall has announced that there will be follow-up workshops this year. These workshops (DNA vaccines; Fish Immunology; microalgae and marine biotechnology) will be held on 12-19 December 2008 in beautiful Puerto Varas, Chile. Information about the workshops will soon be posted on the internet and also at The Pan-American Marine Biotechnology Association's website (http://www.pamba.ca).

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2. Pomponi AS, Baden D, Zohar Y. Marine Biotechnology: Realizing the Potential. Mar Tech Soc J (2007); 41(3):24-31.