Contributions of marine bioscience to industrial biotechology

Industrial Biotechnology assembles international industry leaders to address current topics, in Roundtable Discussions. This issue's Roundtable focuses on marine industrial biotechnology. The Journal gratefully acknowledges the contributions of our panel of experts:

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GENERAL PERSPECTIVES & RESEARCH FOCI

MARCEL JASPARS: Marine biotechnology is a growing area worldwide. The current market is estimated at about $2.4 billion per annum, with a 10% growth predicted.

I would like to call each participant to give a description of his or her expertise and approach as an individual academic, or a company approach, to marine biotech. We'll then move into what makes the oceanic environment so special and talk about biodiversity and the implications of collecting samples worldwide, and then move into more general applications of marine biotechnology.

To introduce myself: I'm Marcel Jaspars. I am professor of chemistry at the University of Aberdeen with a focus on marine biotechnology, particularly the functions and applications of small-molecule marine natural products. I'm looking for new tools for biomedical research, small molecule catalysts, and drugs from nature.

CHARLIE BAVINGTON: I'm managing director of marine biotech company GlycoMar, Ltd., located in Scotland. My background is in medical biochemistry. The focus of the company is marine natural product drug discovery, as well as other applications in nutrition, cosmetics, and, hopefully, in industrial biotech. Our specific focus is marine glycobiology, that is, on products that compose or contain carbohydrate moieties. We are seeking applications in drug discovery and other markets, including the cosmetics and industrial area.

SURINDER CHAHAL: I'm technical director for Croda Enterprises, Ltd., which is a corporate research function for Croda International plc. My background is in chemistry, and I have been with Croda for over 20 years. Croda recognizes the importance of biotechnology, including marine biotechnology, as a source to new materials and products—"green" materials and new natural materials that will sustain future growth for our company.

JENNIFER LITTLECHILD: I'm director of the Exeter Biocatalysis Centre, an academic organization with industrial links. Our interest is exploitation of marine enzymes, which can be used in the area of cosmeceuticals, as biofilm inhibitors, and in other synthetic applications. We're interested in isolating enzymes from various marine environments, including the deep-sea environment, and some hot environments as well.

CHRIS BATTERSHILL: I'm leader of the Marine Biodiversity Team at the Australian Institute of Marine Science (AIMS). Our research efforts include projects in biodiscovery, chemical ecology, and aquaculture, including aquaculture of marine organisms for production of fine chemicals. The work is part of a wider program examining the biodiversity of Australasia.

My background is as a marine benthic ecologist, but throughout my career, I've worked with teams of chemists, biochemists, and microbiologists looking at the biodiversity in marine habitats from the tropics to the Antarctic, particularly focusing on the source, function, and production options of marine natural products that have useful industrial or pharmaceutical activities. We're currently looking at evolutionary aspects of secondary metabolite functionalities.

Our institute's model is one of partnership, where we provide a platform for collaboration, research, and development in looking for useful compounds from the sea and their production options, with a particular focus, in recent times, on the microbiology of marine systems, symbiotic organisms, and their applications.

"MARINE BIOTECH" LABEL

MARCEL JASPARS: I'd like to start with an assessment of the actual name of the subject, "marine biotechnology." Some of you, I know, have strong opinions as to whether this is a good rubric name or not.

CHARLIE BAVINGTON: I have a bit of a problem with the label "marine biotechnology," although I use it myself often enough. Essentially, marine biotechnology is the only biotechnology that is labeled by the source of its material and not by its market. From a commercial point of view, this is a problem.

I've started the company GlycoMar recently. Trying to communicate what the company is about, if we only focus on the source of the material and not on the market, doesn't make for a clear story development of a new business. I think this is also a problem in terms of developing the potential of marine-based biotechnology, in that we are failing to make connection with potential markets.

The "Eureka!" moment for me was as a medical biochemist working with marine biologists and suddenly seeing the opportunity to work across these two radically different fields—and seeing an opportunity to make a real connection from marine biology to market. My knowledge of the clinical market really brought it home for me, and I think that's something we're probably missing in the areas of industrial biotech, agricultural biotech, and nutrition and cosmetics. My point is that we shouldn't name a biotechnology based on its source but should focus instead on the market.

> *"Trying to communicate what [a] company is about, if we focus on only the source of the material and not on the market, doesn't make for a clear [business development] story."*

MARCEL JASPARS: This is a valid point. I would counter that the title has been a useful banner up until now to sell the subject to others who mightn't think to distinguish the field from terrestrial biotechnology. But I agree with the points Charlie's made. Perhaps we should be thinking about having different "subcolors" of blue (marine) biotech, or otherwise making sure that, for instance, marine microbial biotech becomes truly considered as another stream of white/industrial biotechnology.

SURINDER CHAHAL: The market is very important to consider when we're trying to develop products for different areas. But something to remember is that, certainly in fields such as cosmetics, the source of a product can be a strong marketing tool. And the marine source is very topical at the moment; it's considered natural. Companies recognize that marine biodiversity is huge, and many materials are now being developed from the marine environment that are proving popular and very successful in the cosmetics market, for example. We do need to consider the markets. In many cases, titles such as "marine" do hold quite a bit of value when it comes to actually marketing products.

JENNIFER LITTLECHILD: Yes, I would reinforce that. I think we do need to use the term "marine" because it really is an area that is underexploited. Marine biotech is sometimes called "blue" biotechnology, but I think we should subsume it under the general industrial or "white" biotechnology area and just emphasize that we're looking at marine environments. And, obviously, the market does come in here, but there's such potential that I believe we do need to emphasize the marine aspect.

CHRIS BATTERSHILL: I'd add just one thing related to marine biotechnology and the distance to market, and that's in reference to the funding models. This may not be quite the case in the northern hemisphere, but in the southern hemisphere, there is a gap in how research, leading to development and based on discovery, is funded. The very early phases (the parts where one would, say, actually get wet and dive to look at the biodiversity for leads) tend to be funded from academic sources—the university-type granting systems for appropriation funds. To then take those discoveries to market, there's quite a considerable gap.

Ron Quinn, who's known to the marine biotechnology community and likely to many of you, has described this gap as a "chasm of doom," in that, when one then applies for commercial-ready funds or biotechnology-for-business growth-type money to commercialize a product, the funding tends to be based on low-risk models: The funders are looking for "low-hanging fruit," that is, products which are close to market.

There's a considerable gap for, often, very good discoveries, which struggle for funding in early phases and often perish through starvation, for want of a better word, before they get anywhere near a proper proof of concept. This is exacerbated, I think, for the marine situation, because of perceptions that it's risky taking compounds from the sea and developing them. The perception is based, I think, on misinformation. There are enough examples around now that many marine compounds and products can be produced and progressed economically.

ANDREW MEARNS SPRAGG: Access to suitable sources of finance remains the biggest challenge for early stage marine biotechnology; marine biotechnology in particular still remains a relatively unknown quantity with many life science venture capitalists and early-stage funders, and as a consequence, finding the required funding for commercial marine biotechnology projects in the UK, at least, remains difficult.

To enable the successful commercialization of marine biotechnology and to attract the levels of VC funding required for product development and commercial exploitation, governments need to continue to support applied research and also offer financial incentives to investors to provide match-funding, to "de-risk" early-stage projects to a level where VCs become interested and where the risk in taking products to market can be greatly lessened.

MARINE BIODIVERSITY

MARCEL JASPARS: One of the topics I want to take on now is what makes the oceanic environment so special and to discuss the biodiversity we see there. Often quoted is the fact that, in terms of marine animals, we have 33 phyla, out of which 20 are unique to the oceans and only one of which is unique to land. So there have been some definite, special "selectionary" pressures operating in the marine environment that have rendered an incredible biodiversity and adaptation to different niches. Beyond that, there are extreme environments of pressure and temperature that have given rise to, for instance, bacteria that provide us some unusual, interesting enzymes.

CHRIS BATTERSHILL: Well, yes, as Marcel summarized, the seas hold over 90% of the macroorganism phyla on the planet – and probably more, actually, as new discoveries may confirm. In any one location, on any one dive, for instance, one can usually come up with brand-new species – new to science or a new record for the region. In addition, the microbial diversity in our seas is enormous, as has been recently shown by Sogin and Venter. (Sogin, for instance, identified over 20,000 microbes in a liter of seawater, and estimates emerging of 36×10^{30} cells in the oceans). It also appears that speciation may be occurring at higher rates than originally thought, especially in microbial systems.

New techniques now permit capture of this microbial diversity in particular, as the total genetic material in environmental samples can be retained, rather than just relying on the organisms that can be culture. Our ability to examine and utilize this biological resource is unprecedented.

The important thing is that the marine estate and other habitats on the planet are hosting a biodiversity that is constantly evolving; there is massive change that we're observing right now (sadly, as it happens, through rapid climate change), and the chemistry that this elicits is also changing quickly. We have a situation before us of a growing and living library of opportunity, in terms of biodiscovery.

In addition to this, the types of compounds and materials that the marine environment is producing, have high relevance to specific industrial sectors. These products and compounds are specifically active biologically, including antifouling compounds, for instance, or enzymes that perform certain functions in extreme environments. Due to the intrinsic relevance of their mode of action, the quality of the leads in an applied sense is very high – much higher than other sources.

MARCEL JASPARS: One thing I want to interject is the difference between the topical, temperate, and cold environments that people deal with. People often say that most of the biodiversity is held in certain hot spots in tropical climates, but I find that it's now becoming apparent that, for instance, the places where cold water meets slightly warmer water, such as in the North Arctic Ocean (where the North Atlantic Drift meets the Arctic Currents), there is actually very high biodiversity there as well, which was previously unappreciated.

CHRIS BATTERSHILL: That's absolutely true and, indeed, if one did an analysis, looking at the diversity in any particular unit area (say, a 10-meter by 10-meter patch of sea floor), one would find almost the same biodiversity per unit area in the tropics as in Antarctica. What makes an area mega-biodiverse is in part due to a diversity of habitat or microhabitat within a certain geographic region, often where a variety of currents from different origins converge. The places where biodiversity increases above other parts of any coastline is, as you say, Marcel, in the tropical-temperate transitions areas. This may be as you'd expect, given that the competition amongst organisms that are at the "edges" of their niche, if you will, is intense. These are interesting locations to collect for discovery, as it's likely that there will be higher levels of chemical interaction amongst reef organisms where tropical and temperate assemblages converge, which equates to higher rates of discovery of biologically relevant compounds.

MARINE-DERIVED ENZYMES

JENNIFER LITTLECHILD: We tend to look also to quite extreme environments for novel organisms – bacteria or archaea – for discovery of different enzymes. Many of the archaea have different metabolic pathways, and so one has the opportunity actually to find new enzymes not present in bacterial species. We also tend to look at various deep sea organisms; these can be growing under very high pressures, and they can also be in thermal environments where we have the hydrothermal vents known as black smokers. In this extreme environment at the bottom of the ocean, there is a gradient from the hot area of the smoker out to the seawater, and many of the organisms we look at actually live within that gradient. We also have worked with organisms living in marine hot springs, which are aerobic, while those at the bottom of the ocean are anaerobic. So we have quite a wealth of organisms from which we can isolate DNA and clone relevant enzymes and overexpress these in *E. coli*.

In addition to the actual organisms themselves, we are very interested also in viruses from marine species. Various marine species tend to have viruses with fairly large genomes, and these code for many different proteins – many of which have open reading frames that have no similarity to other open reading frames from genomes that have been sequenced. And these can be from cold or hot environments. Some of the archaea that we've looked at from hot environments contain a whole variety of novel sorts of thermophilic virus. So the viral area is also a completely untapped area to look for novel enzyme activities. And, as alluded to earlier, there is the whole idea of metagenomics, which means one doesn't have to isolate the individual enzymes, organisms or the particular viruses, but instead examine a whole part of deep biodiversity, isolate the DNA, and look for novel enzymes. So in summary, our work involves mainly looking for novel enzymes, cloning, overexpression, and their applications in various forms of biotechnology.

SURINDER CHAHAL: From an industrial point of view (that of the chemical industry, for example), we're looking for novel enzymes that could be used for chemical transformations in a cost-effective way. There's been a lot of work done over the years identifying enzymes for chemical conversions, but this has been very much at the pharmaceutical level, where cost, relatively, tends not to be too much of an issue. The growth area is very much in standard chemical production, where we need enzymes to do chemical transformations at lower temperatures, and occasionally at higher temperatures, to provide cleaner and more specific products.

There's a great deal of interest here, with the European Union indicating that, by 2030, almost one third of all chemicals in Europe will be produced via biotechnological routes. The challenges ahead are to discover many more enzymes that are capable of meeting the requirements of higher volume and lower-cost production of chemicals.

JENNIFER LITTLECHILD: It's important to note also that using enzymes represents a much greener technology. They allow chemical transformations to be carried out with very little energy input. And we can isolate enzymes from a whole diversity of environments that have different properties, which we'll cultivate for the various chemical processes being considered.

One problem with enzymes, of course, is their stability, and, in some cases, it is good actually to isolate them from a thermophilic environment. This inherently addresses stability issues, as these enzymes can be used industrially at higher temperatures. The actual substrate or chemical upon which the enzymes acts, which is often different from that in the natural environment, may not be not liquid at room temperature.

MARINE GLYCOBIOLOGY

CHARLIE BAVINGTON: I'd like to focus on the area of glycobiology and how diversity relates to that specifically. What we've found, looking at glycobiology in general, is that the marine environment provides us an absolutely amazing opportunity to access a whole new set of molecules. If you examine glycobiology drawn from terrestrial sources, you get a rather standard set of plant and animal molecules. But when you start exploring the marine environment, you have a whole new set of phyla to access and, very quickly, start seeing a wide range of new molecules. One can of course identify marine analogues to terrestrial products, but they have unique structural features. Digging more deeply, you start to see completely new clusters of carbohydrate-containing molecules with unique functional properties, which we're only just beginning to understand.

In glycobiology is an opportunity to combine the biological diversity of the marine environment with the chemical complexity that carbohydrate molecules offer, to render an overwhelming variety of function and structure. It's an enormous challenge, but an absolutely huge opportunity. Linked to that, also, is a unique suite of enzymes present in marine organisms. Again, we're only just beginning to scratch the surface with these; these enzymes can be synthesizing enzymes, degrading enzymes, or modifying enzymes, with applications in the synthesis and/or modification of a wide range of industrial polysaccharide products.

UN CONVENTION ON BIOLOGICAL DIVERSITY

MARCEL JASPARS: I'd like to touch on a subject important to all marine scientists, that is, the implications of the UN Convention on Biological Diversity (CBD) to collections and also, when people are collecting in deeper water, the United Nations Convention on Laws of the Sea (UNCLOS), which are both being discussed extensively at present. The CBD particularly encourages the fair and equitable sharing of anything resulting from the use of source countries' genetic resources, which includes DNA, RNA, and small molecules as well as enzymes.

CHRIS BATTERSHILL: Before the CBD was issued in 1993, for most areas around the globe, you would gain access to the sea through a permit system that controlled that the sustainability of collections, usually through local fisheries or conservation departments. There was generally no problem in getting such permits in developed nations. The issue was that, in areas that lacked those infrastructures, it could become a kind of a free-for-all; anyone wanting to sample the sea could simply talk to people they thought responsible for governing access and for providing for sustainability of any extraction, and then one could proceed. But there were no provisions with respect to downstream discoveries, or other opportunities of returning benefits back to the source, region, or country of origin.

Post-1993, the CBD came into effect, and most countries weren't prepared to handle requests for biodiscovery access, at least according to our observations in Australia. Most nations weren't prepared for the protocols now necessary for compliance with the CBD, and problems arose in terms of getting permits in some areas, because there were no processes to define benefit sharing. So the concept of a single "access and benefit-sharing" agreement arose.

There was also doubt cast regarding rights to existing collections. Difficulties arose in getting permits to re-collect leads from past discovery, and there was a great deal of legal uncertainty. It's probably fair to say that, in a lot of countries, it's taken until now to establish a legally consistent framework that facilitates biodiscovery activities through an access and benefit-sharing system. Such systems provide a framework allowing commercial interests to gain access to regional biodiversity in a sustainable manner and require up-front agreement on some sort of mechanism to share benefits—all the while recognizing the fact that those benefits may be a long time in coming and that the original source of biodiversity will be modified many, many times, with addition of IP along with way. This means that the relative value of the original biodiversity (sample) can often be relatively low, which is a surprise, I think, to some nations, who may have had unrealistic expectations of the biodiversity sample's worth.

In places like Australia, which has a complex system of jurisdictions (with states and a commonwealth, or federal, government), it can be challenging to come up with a consistent policy framework that gives investing companies, in particular, security and knowledge that they have the right to work exclusively on leads they select, all based on prior informed consent and acknowledging that sustainability of use of the biological resource is a basic premise. There are now international models (for example the 'Swiss Tool') for the types of agreements/legislation that can facilitate and promote biodiscovery in the marine estate.

JENNIFER LITTLECHILD: Very often, some of the organisms we use from the deep sea are not just found in one location in the world; you can find similar organisms in many different locations. And many of these have been collected before a lot of these guidelines were in place, and have been cultured in the UK. So obviously, in these cases, it can just be a matter of taking the DNA and amplifying up the enzymes of interest.

One area I hadn't mentioned previously is looking at enzymes not just from microorganisms, but also from algae; macroalgae is another area we work on. And these are just collected, initially, from the coastal areas. Many countries seem not have really thought about this, and I'm sure there are many enzymes exploited already that were drawn from species that had been collected long before this time. Obviously, if a commercial interest requires additional diversity, one has got to go out and collect new species and actually abide by these guidelines. But the point is, very often, you don't just find a species, for instance, *Thermococcus* (which we work with), in just in one location in the world. As I have said before, a species can be located in many different places, wherever an environment exists in which it can grow optimally.

MARCEL JASPARS: The United Nations Convention on Laws of the Sea is being discussed at the moment with respect to what happens to those samples that are being collected currently from very deep waters, that is, in marine environments where there is no clear ownership, and whether there might be a possibility of doing damage if overcollection occurs. One idea being discussed at fairly high levels is to generate a pool of funds that can be used either for further education or to further the use of these products from the sea. It would be worth looking at this issue again in maybe four or five years' time to see how far this notion has progressed.

CHARLIE BAVINGTON: In my own experience, because the kind of diversity that we're talking about is readily available "on our doorstep" in Scotland, we don't have to go very far to sample. I wonder if there aren't many applications where, actually, we wouldn't need to butt our heads up against the CBD, so to speak, and we could just work with common, readily available species that simply are not fully characterized; we might find there the kinds of specific activity we're looking for, without going to difficult environments or having to address these issues. This could be important from a commercial point of view. We want to be environmentally sustainable but commercially sustainable as well.

MARCEL JASPARS: That's the approach that many commercial plant researchers have taken. Plants that have been transplanted around the world for hundreds of years still have an intrinsic value in terms of potential new discoveries and new downstream products. Discovery is taking place, for instance, in Kew Gardens in London, with plants being examined for particular purposes (usually medical applications). So there are examples of this approach working. Since 1993, it seems, as long as one can clearly identify the path through the samples came and one can demonstrate that collection was done under the correct permission, then problems with IP ownership don't seem to arise with regards to later trying to commercialize resulting inventions.

"There are now international models for the types of [prior informed-consent and benefit-sharing] agreements ... that can facilitate and promote biodiscovery in the marine estate."

CHRIS BATTERSHILL: The CBD has certainly provided for appropriate discussions in this arena and has effectively created a framework for establishing a "pedigree," if you will, around any particular sample in a collection—such that a company about to invest a considerable amount of money in a particular lead can be safe in the knowledge that it has the right actually to be able to do that downstream development, whether it be work with a deep-sea nematode worm or a piece of seaweed that washed up on a beach somewhere.

INDUSTRIAL APPLICATIONS

MARCEL JASPARS: Let's turn to the broad array of industrial applications of marine biotech. One major example is that of algae in food additives. More interestingly, we're now seeing polyunsaturated fatty acids used for nutraceuticals, and other products in cosmeceuticals. In agriculture and animal health, we're seeing marine-derived carotenoids in fish feeds to give natural colouring to the animals. We're also seeing, on a very large scale, the need for new antifouling materials, since the ban on the use of tributyltin for ships. (As we all know, too much fouling on ships can vastly increase transport costs for products.) So there is a need for new, clean antifouling products. Biofilm inhibitors could also prevent fouling up of pipes for water intakes, in power stations, for instance.

There is also increased interest in bioremediation using novel enzymes. Enzymes are also used in various processes such as food processing. In tissue replacement, we're finding that, for instance, sponge collagen is compatible with human collagen and that corals may have application as bone replacement materials.

I'd like each of you to describe the kinds of applications you're dealing with in your company or institution and to discuss anything novel that fits under the banner of industrial biotech.

CHARLIE BAVINGTON: GlycoMar focuses primarily on pharmaceuticals, but we are looking for opportunities for our polysaccharide products and related enzymes in nutrition and cosmetics. We are also crossing over into the reagents market, which does touch on tissue replacement area. What we're seeing is that, beyond the traditional food-additive business opportunities based on marine polysaccharides, there's an opportunity to develop new high-value products with added functional characteristics, which can be applied as nutraceuticals, either as additives or as supplements (capsules or tablets) — and also, potentially, there could be crossover into the cosmeceutical market.

In the short term, these will likely be invertebrate products, but in the longer term, we'll likely look to a marine microbial stock material, such as an exopolysaccharide that is semisynthetically modified to have the necessary functional characteristics. I'm thinking specifically of some glycosaminoglycan mimetic-type molecules that have applications both in nutrition and cosmetics. These kinds of molecules also have applications in tissue replacement, and we're specifically looking at applications in the regenerative medicine market. I realize that, strictly, industrial biotechnology may not necessarily pharmaceutical applications, but in my mind, it does include tissue replacement and applications in wound-healing and bone replacement. There's a good example of a novel application of chitin used for wound dressing; a company called HemCon Medical Technologies, in Portland, Oregon, has developed a market-leading wound dressing that has become a dressing of choice for the US Army. That dressing is made from shrimp shells – a polysaccharide product. But the company has obviously developed it to a higher level than traditional chitin.

MARCEL JASPARS: That's an interesting application – the use of shrimp shells to make tissue replacements and other products. It raises the whole issue of the use of what would traditionally be perceived as waste product in marine biotechnology. That trend seems to be growing.

CHARLIE BAVINGTON: This represents both an opportunity and a problem for me. When people talk about the obvious potential of utilization of marine wastes, they're usually referring to waste from the fish-processing/shellfish-processing industry, and potentially from the alginate industries also. Underpinning this is the environmental pressure to make use of wastes and avoid waste-management costs, like for landfill and factors. So there is a lot of buzz about the opportunity; but to actually make a product from those wastes is quite challenging. I think the challenge for scientists is to understand the scale issues involved in making a product. Undoubtedly, we can make some very active and attractive products from these waste materials. But whether a particularly opportunity is commercially viable and competitive against existing products will remain a huge question. .Some real creativity will be needed to identify new applications where there is insufficient value to make products viable.

SURINDER CHAHAL: There are a number of products that can be derived from the waste of the fish processing industry, and Croda has in the past been involved in producing products from such wastes, for example, fish gelatin from fish skins. However, as a result of increased market demand, and consequently, additional manufacturers, it became uneconomical to produce in Europe. The economics need careful consideration, unless the products are new and novel.

CHRIS BATTERSHILL: Our experience with nonpharmaceutical products includes agrichemicals, which immediately requires a search for bioactivities relevant to a specific need. In our case, we were looking for herbicidal activities. We adopted a hypothesis-driven approach, with the argument that in those organisms (benthic invertebrates) that we found to be clean of algal biofouling, and which were clearly then defending themselves in some manner, we were likely to come up with appropriately active compounds. We rapidly came up with a number of herbicidal compounds from the surfaces of a range of marine organisms.

It's interesting here that you can go to different habitats for different targeted biological activities; a coral reef, for instance, is a microcosm where there's a lot of light and where algal fouling is a potentially a big problem. So organisms there naturally need to defend themselves. Likewise, organisms living in very polluted environments often have mechanisms to maintain themselves from a variety of cellular challenges and pathogenic attack, specific to the source pollution. A microhabitat approach in discovery, rather than a latitudinal one, is often useful.

Other opportunities include biosensor creation: coming up with tools to detect marine toxins and incorporating that detection into a handheld dipstick biosensor apparatus.

We're also exploring collaborations looking at nanomaterials. Here we are interested in utilizing the nanosized skeleton components of marine organisms. Resulting products would be siliceous and calcareous materials. New areas suggesting themselves to us are in the areas of glues, such as glues that can be used in wet environments, even "tissue glues," if you will. These are in abundance in the marine environment. The idea is to harness the ability of marine organisms to stick onto other things. It's almost an opposite of an antifouling situation.

There are also the functional foods, and, finally, we're looking at the biochemical systems of marine organisms, which are very flexible in terms of what they can do in biosynthetic terms: particularly host-symbiont relationships and what they can offer towards the creation of novel products, through directed manipulation.

MARCEL JASPARS: Surinder, you're from the biggest industrial concern among the Roundtable participants, so it would be nice to hear your viewpoint and the kind of things that Croda is achieving in the industrial biotechnology market.

SURINDER CHAHAL: We talked about algal use. One of our largest markets is in marine oils (omega-3s), which typically are obtained from fish sources. We recognize that, going forward, this may not be sustainable, particularly when considering the tremendous growth in the omega-3 market in recent years; and this growth is continuing. We need to look at alternatives to generating these lipids. There are manufacturers currently using heterotrophic growth of algae to produce DHA, for example, and that area is progressing. There is also work ongoing in developing transgenic plants to produce omega-3s, in particular, DHA and EPA. We also see that the marine environment has potentially a lot to offer and that there must be organisms in this environment capable of generating either omega-3s.

From a nutritional point of view, we see the use of microorganisms to produce fatty acids as an area of development, and many other companies are also pursuing this interest.

In the area of cosmetics, we at Croda dislike the word "cosmeceuticals." One particular reason is that it is important to differentiate what is a cosmetic is, versus what constitutes a pharmaceutical, from a regulatory point of view. To apply pharmaceutical regulations to cosmetics would be extremely damaging for the cosmetics industry.

In the cosmetics area, there is a lot of biodiversity to be exploited, not only in the marine environment, but the terrestrial environment as well. However, the marine area is of great interest and is generating a lot of market appeal at the moment. Something to take into account when considering cosmetics, which may not be as relevant in the nutritional area, is that animal products are generally not accepted by consumers. If a product manufacturer is extracting materials from marine animal-based organisms, then that manufacturer's products may struggle in the cosmetics market. The move away from using animal-derived products in cosmetics began about ten years ago and continues to this day. I still see many small biotech companies (many from Australasia, in fact) developing novel materials for cosmetics that are fish-derived or animal-derived. These products may not see great commercial success precisely for the reason that they are animal-derived.

Another issue with cosmetics, at the moment, relates to genetically modified organisms. Again, consumers have a preference for materials that are not genetically modified, and this preference is becoming more and more apparent, particularly in Europe. There is discussion, certainly, at the highest scientific level in the UK, suggesting that GMOs should not be considered harmful, but it will take a number of years to convince Europeans consumers of that argument. It is therefore important for the cosmetic market that new products derived from biotechnological routes are not genetically modified. In contrast, the nutritional market can accommodate some animal products, but there still remains some caution against incorporating genetically modified materials.

MARCEL JASPARS: Jenny, with your area of focus, would you speak about the applications of marine enzymes?

JENNIFER LITTLECHILD: There is an enormous potential of applications, drawing on viruses and also the macroorganisms and algae. One particular class of enzymes we're working with are enzymes able to halogenate or dehalogenate compounds. There are also a large number of brominated compounds natural to marine species, which obviously implies enzymes able to sequester bromine from seawater. Our group has been working on an algal haloperoxidase able to produce a variety of brominated antibacterial compounds that could be used for preservation of cosmetics. These compounds could also be used as biofilm inhibitors because of their antibacterial properties. And the dehalogenase enzymes are important in various bioremediation processes. This is a class of enzymes that seems to be quite pronounced in the marine environment, especially those able to brominate or otherwise potentially remove brominated compounds. It's quite interesting that, while there's a lot of chlorine, obviously, in marine environments, there is also this great abundance of brominated compounds, of which many thousands have already been identified and isolated from marine species, that have these various properties, including antibacterial properties, advantageous for preservation and antifouling.

In addition, there are various esterases and lipase enzymes with important applications, and also something that has been briefly discussed in this Roundtable, the carbohydrate-modifying enzymes. Some of these, which we've started to look at (from some of the marine viruses) are unique. We find types of esterases or lipases that are quite unrelated to enzymes that are already used commercially. These new enzymes could prove important for various food-processing applications, as well. So there's a whole wealth of marine enzymes that are new and biotechnologically useful, and these can usually be easily amplified and produced in nonmarine species, rather than having to take these enzymes directly from the marine environment (which you wouldn't want to do, from the point of view of conservation, for instance, if you were continually collecting different algal species, seaweeds, and extracting enzymes from these). So to summarize, the particular area of interest I'd like to emphasize is in the bromoperoxidases and the enzymes able to dehalogenate various compounds, which have quite a lot of potential.

CHALLENGES & OPPORTUNITIES

MARCEL JASPARS: I'd like to wind up with a discussion of the challenges and opportunities that face the field. One major issue mentioned here already is the supply issue, especially in terms of high-value chemicals. But the supply issue will also be important for the bulk production of nutraceuticals (as mentioned by Surinder) and enzymes. On the chemical side, there's the importance of having specific technologies in place to be able to do the downstream work, such as, say, fermentation. Backtracking a bit, there is also the necessity of having informed access to novel sources and benefit-sharing agreements in place. And of course, there are opportunities in looking to the markets as well.

CHRIS BATTERSHILL: I think the supply-side issues are being resolved very quickly; even ten, twenty years ago, I don't think this was a concern. You could ramp up the scale of experimentation, development, and supply to match the stage of your product in the pipeline through to commercialization. You never needed to "overcapitalize," if you like, on the supply-side options for a product. You could keep the production scale and cost sensibly in line with the progress of the target compound. This research generated some fairly useful R&D options in itself, in terms of working out how to biosynthesize/semisynthesize target molecules. Any concerns that have since arisen about the supply side, particularly with the technologies available to date, are vanishing very quickly.

I think the biggest hurdle is matching the market (and this was commented on very ably before) to the biodiversity on offer. Most sample libraries can be screened repeatedly. And if you use information derived from past screening results, even negative results (including information about how particular compounds or extracts might influence screening targets through use of phenotypic responses in cells, etc.), you can better match components of a library to certain sectors or use bioinformatics packages to prioritize samples/compounds for certain screening programs.

CHARLIE BAVINGTON: I agree. The scale of the opportunity we've got is obvious, and the challenge is to connect that opportunity to an application. And this is indeed a huge challenge, because of the nature of science. We work in discrete patterns and discrete fields, and so we don't know the breadth of applications that might be out there.

With my scientific background in medical research, I can also say that there is generally a basic lack of fundamental knowledge about many of the organisms we're dealing with. For companies developing marine biotechnology-based products, this lack of learning can represent a continuous and daily challenge, but also an opportunity in the areas we are working in, in that it's something of an unknown sphere. Particular challenges will arise in terms of downstream processing, but these issues are being addressed in the industry.

> *"What's needed is a lot more basic science on marine species. Collaboration among [all scientists] with an interest in marine species is vital to enable the potential contributions of marine biotechnology to be realized."*

JENNIFER LITTLECHILD: One specific challenge is getting enough of the enzymes, if they are going to be used in some kind of process for chemical transformations; however, technologies are available and are able to produce proteins at a fairly high level.

Another challenge is to know what kind of process the enzyme is going to be used for, so it's important to develop a range of enzymes, so that one then has a tool chest of biocatalysts to utilize as, and when, particular demands dictate. With these biocatalysts, one can either use the purified enzyme or a whole-cell system, where an enzyme has been cloned into *E. coli* source or even the original, or almost natural, source, if that's easier to grow.

One limitation in growing a lot of these particular marine species is the fact that they have to grow in salt water, and so one has to invest in special fermentation technology, if the proteins are taken from a natural source. But these technical challenges can be easily overcome with various glass-type fermentors and other new fermentation technology. When working with enzymes, issues of stability and immobilization are also important, but again, many of these challenges can easily be met with current technologies.

ANDREW MEARNS SPRAGG: Bioprocess challenges for scale-up and large-scale production of marine derived products is an important challenge. And though we're not focusing in this Roundtable Discussion on medical applications, Aquapharm itself focuses on antibiotic drug development, in addition to developing products for food and cosmetics. So I'll still mention that many natural products isolated from marine organisms have demonstrated great pharmaceutical potential, for example, the anticancer agent bryostatin and many others derived from marine sponges.

So a challenge faced by our industry is how to manufacture these high-value compounds at large scale and within a salty environment. Often, these compounds are structurally complex and difficult to synthesize, and therefore obtaining enough material for clinical trials (for clinical applications) without damaging the environment is a balance that needs to be addressed.

But there are good examples of how this issue can indeed be addressed. In mariculture (farming), PharmaMar's Yondelis® is a recently approved new anti-tumor drug derived from the marine organism *Ecteinascidia turbinata,* a tunicate or so-called sea squirt found in the Caribbean and Mediterranean seas. The compound is a naturally occurring compound and has been extracted from farmed *E. turbinata,* and a semisynthetic process has been developed so the drug can be manufactured chemically.

I believe the development of salt-tolerant fermentation offers the best platform for sustainable production of natural compounds of marine microorganisms including those from recombinant technologies. Fermentation offers a sustainable production platform for production of high-value compounds without the need to re-collect environmentally sensitive species.

Regarding opportunities: As Marcel mentioned earlier, it is estimated that the global market for marine biotechnology products is worth about $2.5 billion—and growing at a rate of 10% per year. This rate is set to soar over the next five years, as more new compounds and processes receive market approval.

There are the examples in pharmaceuticals, as I mentioned. The marine environment has generated thousands of novel compounds with pharmaceutical activities ranging from anticancer, central nervous system, anti-inflammatory, and anti-infective activity. Sponges in particular have been responsible for the discovery of a great many of these novel natural products, but pharmaceutically important compounds have also been derived from marine actinomycetes, algae, and fungi. And as was discussed here, other opportunities are in the sustainable supply of functional food ingredients (e.g., omega-3s from marine algae), new, natural cosmetic ingredients, and extremophile microorganisms as a powerful new source of novel enzyme technologies.

MARCEL JASPARS: I'd like to close the Roundtable Discussion by adding my take on marine biotechnology. I personally think what's needed is a lot more basic science on marine species. Collaboration among taxonomists, physiologists, biochemists, molecular biologists, pharmacologists, and chemists with an interest in marine species is vital to enable the potential contributions of marine biotechnology to be realized. Many of the marine phyla haven't really been accessed at all. So I advocate more basic science to combat our ignorance of the fundamental physiology of many of these species, what they're doing, why they're there, what the environmental challenges are, and even the basis of their taxonomy. A lot of the work that has been done on terrestrial species needs to be replicated on all the phyla in the marine environments.

Marine biotech does offer a wealth of opportunities for new discoveries in a number of fields, ranging from applications in foods, to cosmetics, biomaterials, enzymes, antifoulants, and biofilm inhibitors. I expect to see fast growth in new and unexpected applications stem from what I'd call "blue seas thinking," rather than "blue skies" thinking. A great future lies ahead.