MARINE BIOPROSPECTING : THE PATHWAY FROM ORGANISM TO PRODUCT AND THE IMPLICATION FOR INDIGENOUS RESOURCE OWNER

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This paper covers three areas. First is to describe how marine bioprospecting takes place and advances that have been made from this kind of work. Second is to discuss how Pacific island countries can benefit from involvement in marine prospecting ventures. The third focus is what is happening in marine bioprospecting in the South Pacific region.

MARINE BIOPROSPECTING

Background

The systematic investigation of marine environments for novel biologically active agents began in earnest in the mid-1970s. From 1977-1987 about 2500 new metabolites were reported from a variety of marine sources. Marine organisms have been shown to be a rich source of bioactive compounds, many from novel chemical classes compared to those found in terrestrial sources.

Coral reefs are often crowded places with many organisms looking for space to settle and feed. Organisms that survive and multiply in such rigid space confines are likely to have chemicals that defend their territory. In addition many brightly-coloured soft sessile invertebrates are present on the reef. The fact they are not eaten is most likely due to chemicals they contain.

Methodology

Unlike plants there is almost no traditional medicinal use of marine organisms so most marine collections involve random selection of mainly soft sessile organisms. These can include:
soft corals

- sponges
- tunicates
- algae
- bryozoans
- sea whips
- molluscs (e.g. sea hares)
- nudibranchs
- ascidians

If possible up to one kilogram of wet material is collected, given an identification number and an underwater photograph taken. On the surface another photograph is taken and the sample bag containing the material in seawater is cooled. As soon as possible a small portion of the material is stored as a voucher to aid later identification and the remaining material extracted with organic solvent.

This extract is then subjected to a series of biological activity screens either by the collecting agency or a collaborator. Although pharmaceutical activity is often the main use one thinks of, a number of other activity studies may be performed:

- sun screen
- antifouling
- skin care (anti-aging)
- agricultural products (e.g. insecticides)
- food additives

In the last decade pharmaceutical screening has seen great advances in the expansion from the "whole organism" approach in which a human cell line or microorganism will be tested to the "mechanism-based approach" which uses biochemicals, enzymes and receptors to mimic the mechanism of a certain bioactivity. These latter tests can be automated to provide high throughput of samples which has been one of the reasons for the increased demand for extracts and compounds to test.

Extracts that show the most interesting activity are given priority and, using the bioactivity assay as a guide, are fractionated until the pure, active constituent(s) are identified and their structure elucidated. At this point secondary activity studies are performed and the extracted compound is transformed into a large number of derivatives to try and understand factors affecting the activity and determine the most active form to be used for clinical trials. These clinical trials take several years. A time-line for this entire drug-discovery process is given in Figure 1 which indicates that seventeen years may be taken and that one approved drug may result from 5000-10,000 extracts originally screened. It is estimated that this process may cost US$350 million dollars.

Stage/Years	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Compound success rate by stages
Discovery	----------→																		5,000-10,000 compounds screened
Pre-clinical testing (laboratory and animal testing)		----------→																	250 enter pre-clinical testing
Clinical testing Phase I						----------→													5 enter clinical testing
Phase II																			
Phase III										------→									
FDA review/approval												----------→							
Additional post-marketing testing														----------→					1 approved by the FDA

Figure 1. Compound Success Rates by Stages in Pharmaceutical Research and Development

To date no marine chemical has finished this process. Several are well advanced, including:

- Bryostatin, an anti-cancer derived from the US West Coast bryozoan *Bugula neritina*;
- Dolastatin 10, an anti-cancer agent derived from an Indian Ocean mollusk, *Dolabella auricularia*;
- Ecteinascidin 743, an anti-cancer agent derived from a sea whip, Ecteinascidia *turbinata*;
- Manoalida, a compound from *Luffrariella variabilis*, a sponge collected in the Pacific Island of Palau in 1979;
- Discodermolide, an immunosuppressant agent from the Bahamian sponge, *Discodermia dissoluta*; and
- Didemnin B, an anti-cancer agent isolated from a Caribbean tunicate of the genus *Tridemmum*.

A marine organism that has resulted in a commercial product is the sea whip *Pseudopterogorgia elisabethae* from the Bahamas. It contains potent anti-inflammatory

diterpene glycosides called pseudopterosins that are used in a skin care product marketed by Estee Lauder that prevents skin aging.

A marine microalgae called *Crypthecodinium cohnii* produces docosahexaenoic acid (DHA) which, as an essential fatty acid thought useful to aid in brain development, is being promoted for inclusion in infant formulas.

Supply Issues

The original collection of about one kilogram-wet weight of marine organism may suffice to isolate the pure active compound. Further studies require addition material. In cases of small or uncommon organisms this may raise practical difficulties or conservation concerns. Another issue is that often the same organism collected from a different environment may not have the same active chemicals. This is thought to be due to the fact that it is the symbiotic microorganisms that may be responsible for the production of the active chemicals and not the organisms themselves.

There are a number of strategies to counteract the supply problem. A classical alternative is chemical synthesis in which the compound of interest is built up in the laboratory from small, readily available precursors. A terrestrial example is aspirin, in which the commercial product is synthesised rather than obtained from the willow bank, its natural product source. Unfortunately most marine natural products have a quite complicated structure and are difficult and expensive, if not impossible, to synthesise in the laboratory.

An alternative is to use aquaculture techniques either in the sea or laboratory to grow the organisms of interest. Work under controlled conditions can also result in high, standard yields of the chemical of interest.

Given that microorganisms may be producing the active chemicals another approach is to culture the marine organism, isolate and purify the microorganisms obtained and then ferment the pure colonies. This may result in the chemical of interest or new active chemicals. This is already being used in the production of DHA (vide supra) and this approach using non-marine organisms has resulted in many advances ranging from pencillin antibiotics to the enzymes from *Thermus aquaticus* which led to the polymerase chain reaction (PCR).

Another approach to the use of marine microorganisms would be the use of genetic engineering techniques to produce the compounds of interest.

BENEFITS FROM MARINE BIOPROSPECTING

The previous example of *Thermus aquaticus* was collected in Yellowstone National Park in the United States. As there was no provision in the initial "scientific" collection for sharing of possible commercial benefits from the collection, this national park receives no share of the roughly $200 million

annual marketing of PCR products. This, and other examples in developing countries, led to a major shift in thinking on access and benefit from biodiversity embodied in the 1992 Convention of Biological Diversity (CBD). The objectives of the CBD are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of benefits arising from the use of genetic resources. In terms of bioprospecting, including marine, the CBD is an instrument to promote the equitable exchange, on mutually agreed terms, of access to genetic resources and associated knowledge in return for finance, technology and the opportunity to participate in research.

Countries are required to develop policies that comply with the CBD. Fiji and Samoa among Pacific island states have developed draft policies but these are not yet in practice. The CBD provisions require that access to genetic resources shall be subject to the "prior informed consent of the contracting party". A general summary of what this procedure is likely to entail is:

- submission by the applicant to the competent national authority of a proposal
- (in some procedures) public notification through publication of elements of the proposal in national and local newspapers
- negotiation of contracts between the applicant and the competent national authority and also, if appropriate, between the applicant and other parties such as private landowners, indigenous groups providing traditional knowledge or ex situ collections providing access to specimens
- approval by government of the contracts and the proposal; and
- implementation, for example periodic reporting by the applicant on the use it is making of the resources accessed

The contracts, variously called Material Transfer Agreements, Memoranda of Understanding or Letters of Collection, usually include:

- requirement of legal acquisition
- permitted use of genetic resources
- restrictions on supply of material
- benefit sharing
- other terms

Among the benefits that are commonly included in such agreements are:

MONETARY BENEFITS

- up-front fees, either for access to genetic resources, or to cover the costs of any preparation of samples, research conducted on them, and handling and shipping costs;
- milestone payments when various stages in discovery and developmnt are reached (either independent payments, or set-off against any royalties that may be incurred in the future); and

- royalties. It is important to clarify the basis of royalty payments, for example whether they are calculated on gross or net sales.

NON-MONETARY BENEFITS

- participation of source-country scientists (who may be third parties) in research;
- transfer of equipment, software and know-how;
- exchange of staff and training;
- in-kind support for conservation;
- acknowledgement of provider in research publications, patents and other forms of IPR;
- sharing of research results, including notification of discoveries and ensuring that copies of publications concerning research on the genetic resources provided are sent to the source country;
- voucher specimens to be left in national institutions; and
- terms for the licensing of technologies developed from research on the genetic resources transferred.

OTHER TERMS

In addition to terms on legal acquisition, authorised uses of materials, transfer to third parties and benefit-sharing, it is common to include a number of other clauses in material transfer agreements, which may deal with the following:

- definitions;
- duty to minimise the environmental impact of collecting activities;
- duty to maintain samples in good condition;
- ownership of materials;
- representations and warranties concerning the quality, identity of the materials transferred and any liabilities arising from their use;
- indemnity against potential liability, such as that arising from civil or criminal actions connected to access and benefit-sharing brought by source countries and other providers or recipients of genetic resources;
- length of agreement;
- notice;
- the fact that the obligations in certain clauses (e.g. benefit-sharing) survive the termination of the agreement;
- independent enforceability of individual clauses in the agreement;
- events limiting liability of either party (such as Act of God, war, fire, flood, explosion, civil commotion, industrial disputes, impossibility of obtaining gas or electricity or materials), and requirement to notify the other party in the case of any such event;
- arbitration;

- assignment or transfer of rights; and
- choice of law.

It should be realised that direct monetary benefits are not likely to be significant. Average fees for supply of a sample range from US$50-200 which also usually covers the cost of collection and postage. Any payments beyond this stage are unlikely as the chance of reaching a milestone payment stage is perhaps 1% and the royalty stage 0.01%. However slight the chance, however, the royalty policy needs to be well thought out as, even at the 2% average rate, a major drug find could result in an annual royalty in the millions of dollars. As most organisms in the Pacific occur in more than one country, a regional policy on sharing of royalties is desirable.

Since monetary rewards are not likely to be substantial from the simple supply of raw materials, it is critical that the benefit package assists the source country to develop methods of value addition to extracts and biodiversity conservation.

Another important consideration in discussing bioprospecting agreements is that few collections are made by the companies themselves. Botanical gardens or universities usually act as intermediaries and this means that they will likely sign agreements with both the source country and the company. Obviously the terms of these agreements must be in harmony.

THE PACIFIC EXPERIENCE

Marine natural product collections have been taking place in the South Pacific for at least the last 20 years. Major collections were made by two American universities, the University of Utah and the University of California at Santa Cruz. The researchers involved satisfied the pre-CBD requirement of governments in applying for research and/or collection permits. Persistence by scientists at the University of the South Pacific in insisting that these collections should benefit the source countries have led to collaborations that are assisting the development of the Marine Natural Products Initiative at the University of the South Pacific.

Major collections were also made in Micronesia by the Marine Biotechnology Institute and, more recently in Fiji by the Coral Reef Research Foundation, based in Palau, on behalf of the United States Government National Cancer Institute (NCI).

The latter two collections, although made after the implementation of the CBD, did not involve material transfer agreements as the government departments involved did not require these. NCI does have a standard policy embodied in its "Letter of Collection" that spells out its obligations to source countries but these are more likely to be fulfilled if a local agency is aware of these obligations.

The work on these samples has resulted in isolation of a number of compounds of interesting activity, mainly from Pacific sponges. These include jaspamide from *Jaspis johnstoni*, which is undergoing clinical trials in the United States and a series of related

compounds called bengamides, isolated from *Jaspis coriacea*, which are used in cancer research and sells at US$2000 per milligram. I presume Professor Endo from MBI will discuss the positive results from their collections in the Pacific.

THE USP BIOPROSPECTING PROJECT IN VERATA PROVINCE, TAILEVU

In 1996 the University of the South Pacific applied to the Biodiversity Conservation Network (BCN), a consortium of World Wildlife Fund, the Nature Conservancy and World Resources Institute with funding by the United States Agency for International Development.

The BCN was initiated to:

(a) support site-specific biodiversity conservation efforts in the Asia/Pacific Region

(b) evaluate an enterprise-based approach to community-based conservation.

The Fiji project involved the eight villages in Verata Tikina in the central East Coast of Viti Levu. The main facets of the program were:

- creation of a bio-prospecting enterprise to provide income to the community to support conservation and development needs formerly met by the harvesting of marine resources;
- formulation of an innovative and equitable bioprospecting agreement;
- development of a community-based marine resource management plan;
- biological and socioeconomic monitoring of effects of the project (by local community members); and
- research to add value to biological extracts before being licensed for study by pharmaceutical companies overseas.

The project succeeded in setting up a bioprospecting venture with the Strathclyde Institute for Drug Research in Scotland after negotiations with SmithKline Beecham pharmaceutical firm were not fruitful (they closed down their natural products division). Licensing fees should bring about F$100,000 to Verata and much more to the stakeholders should a commercial product be developed. Verata people living in Suva have been authorised by their Tikina Council to develop a Verata Conservation Trust Fund to administer these licensing fees. During the process both the Fiji Government and the University of the South Pacific developed a policy on bioprospecting. The bioprospecting contracts have been reviewed by international experts to help ensure equitable benefits to the people of Verata and Fiji.

The University of the South Pacific has enhanced its ability to determine the activity of extracts and to identify the compounds responsible for this activity. This could increase licensing fees by factors in the hundreds.

The people of Verata, led by their chief the Ratu mai Verata, have developed a marine resource management plan which includes:

- ban of taking turtles and coral extraction
- moratorium on granting commercial fishing licenses
- size limitation of gills nets
- declaration of no-take refugia to support an enhancement of marine populations

Concurrently a workshop was held in which 20 people of Verata were trained in biological monitoring techniques. They are currently monitoring changes in "kai koso" (*Anadara sp.*) and "mana" (*Thalassina anomala*) populations, both in the no-take refugia and control areas. After eighteen months "kai koso" populations have increased by 600% in the "tabu" area and 200% in a similar area where harvesting has continued.

The administrative headmen from the eight villages have also been trained to conduct socioeconomic surveys and have designed and carried out with the assistance of SPACHEE (a regional environment group) a baseline socioeconomic survey of their villages which should help them in their future decision making.

For the communities involved, the development of the knowledge and confidence that they can conserve and wisely utilise their resources for generations to come will likely be much more important benefits than the financial ones.

Although substantial funds to undertake this pilot project have been provided by BCN, the basic paradigm of how to employ bioprospecting as an enterprise in community-based marine resource management has been developed and it is planned to replicate this experience in other communities in the Pacific. As such the conservation goals of the CBD will be especially well met. The benefits of bioprospecting should not be seen as a goal in themselves but as a tool in the sustainable development of marine resources.

The South Pacific is rich in marine resources and evidence to date suggests that these will provide chemicals of commercial value. The challenge to us all is to ensure that the benefits of such commercial development and shared equitably in the Pacific and are used to ensure the maintenance of marine biodiversity.