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# Pharmaceutical Bioprospecting and its Relationship to the Conservation and Utilization of Bioresources

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*Abstract:* There may be a perception amongst conservationists and the public that 'large quantities' of material are being collected from the bush or the oceans for screening for novel natural products. Whilst small amounts of material may be used for the initial stages of the drug discovery process, there is a clear desire within the pharmaceutical industry to conserve the world's biota so that more species can be examined for novel chemical molecules and, that compounds of interest are produced via routes that do not involve the destructive and costly harvesting of samples. This presentation compares and contrasts biodiversity, conservation and sampling issues involved in examining different types of biota for the production of novel natural products of pharmaceutical value. Examples of some natural products produced by microorganisms, plants and other macroorganisms are discussed to illustrate the bioresource conservation and utilization requirements of the pharmaceutical industry and how these differ for the different types of organisms.

## INTRODUCTION

### AMRAD Discovery Technologies - accessing Australian biodiversity for pharmaceutical screening

Molecules derived from natural products have an excellent record of providing novel chemical structures for development as new pharmaceuticals. For example, many of the world's most valuable and successful medicines have been derived from nature. 10 of the world's 25 top-selling pharmaceuticals were derived from natural products and accounted for global sales of almost US$14B in 1995 (ref.1).

AMRAD Discovery Technologies (ADT) was established with the aim of discovering novel lead compounds with pharmaceutical activity through the testing of extracts prepared from natural product samples.

Australia is one of the world's most biodiverse nations, and the only megadiverse, developed nation. Australian territories stretch from the tropics to the Antarctic and include

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diverse habitats harbouring an often unique range of organisms. ADT is exploring this diversity of biota from Australia, but also including SE Asia, to discover novel lead compounds for development as new pharmaceuticals.

We have in place sample collection agreements to facilitate the sourcing of the natural product samples. These agreements are secured through government authorities, research institutes, State herbaria and Aboriginal land councils and cover regions within and outside Australia.

ADT's sample extract library is prepared from these natural product samples. This sample extract library is:

- Large
- Diverse
- Continuously expanded
- Continuously refined

**Pharmaceutical bioprospecting and its relationship to the conservation and utilization of bioresources**

There is a huge interest world-wide in the conservation of biodiversity, its measurement, and the effective and non-destructive utilization of this resource. Tropical deforestation, in fact, has become the crucible of today's extinction crisis, although it should be noted that tropical species are by no means the only ones at risk - for example, temperate rain forest has shrunk as much as tropical rain forest (ref. 2). In addition, Olson (ref. 3) has noted that conservation attitudes have underestimated the potential diversity of mesic and arid regions in the tropics and Zak (ref. 4) has presented evidence that desert and semi-desert regions of the tropics may be just as important for species conservation as the more mesic regions.

There have been a number of initiatives to shape an international response to the loss of biodiversity that complement actions at local and national levels (ref. 2, ref. 5, ref. 6):

- The establishment of the Global Environment Facility by the World Bank, the United Nations Development Programme, and the United Nations Environment Programme (UNEP) in 1990.
- The release of the Global Biodiversity Strategy in 1992 by the World Resources Institute, the World Conservation Union, and UNEP.
- The Convention on Biological Diversity signed at the 1992 United Nations Conference on Environment and Development (UNCED) in Rio de Janeiro.
- The Agenda 21 plan produced at UNCED.
- The Microbial Diversity 21, BioNET-INTERNATIONAL, SPECIES 2000, Systematics Agenda 2000 International, and International Organization for Systematic and Evolutionary Biology (IOSEB) initiatives.

The natural loss due to species extinctions accompanying habitat loss is important to society for a number of reasons, including the maintenance of ecosystem function and for ethical reasons (ref. 7). But species extinction is specifically important to pharmaceutical companies in their search for novel natural products. Amongst the species being lost will be some which have the ability to produce important, but as yet undiscovered, chemical molecules.

Having said this, there are a variety of biodiversity, conservation and sampling issues associated with examining different types of biota for the production of novel natural products

of pharmaceutical value. In this presentation I aim to illustrate the bioresource conservation and utilization requirements of the pharmaceutical industry as I perceive them.

## MAJOR NATURAL PRODUCT SOURCES

Three of the major natural product sources examined by the pharmaceutical industry are microorganisms, plants and marine macroorganisms. Within these types of samples there are some obvious differences which are of relevance to this discussion and which might apply also to other natural product sources not discussed in this paper.

### Microorganisms

In general, microorganisms have demonstrated a greater diversity of producer genera and species per lead compound in comparison to other sample types. Table 1 shows some well-researched natural products and their producer organisms.

For example, it is rare to find a fungal natural product that is known from only one taxon or a single strain. Several taxa can produce the same metabolites, even though the producing fungi might not be taxonomically closely related. The horizontal transmission of genetic information between fungi may play a role in this phenomenon. Both Metzenberg (ref. 8) and Rodriguez-Valera (ref. 9) have speculated on the degree of horizontal transmission of genetic information in microbes in nature, noting that it may be significant. However, there also may be some phylogenetic relationship among the producers of certain compounds (e.g., ref. 10).

Again, with regard to fungi, it has also been noted that despite the diversity of habitats within the tropics there are few fungi that are strictly tropical as compared to those that extend into subtropical and temperate zones (ref. 11). Most genera of fungi are cosmopolitan as compared to plants, which have limited distributions, and most fungal species are only known from single collections, thus making it difficult to assess their distributions. Where anamorph and teleomorph connections can be made, some teleomorphs are known from one or a few tropical sites while their anamorphs are known to be cosmopolitan. This might suggest that habitat conservation for fungi is less of an issue than for plants, but this is something I'll come back to.

In addition, studies of actinomycetes from a variety of diverse Australian environments have shown that at least one-third of actinomycete types isolated appear to be area-specific (ref. 12). This suggests that habitat conservation for microbes is an important consideration if the pharmaceutical industry is going to be able to access the remaining 90-99% of the microorganisms that are always reputed to have not been cultivated in most ecosystems.

Approaches are also being developed for the utilization of uncultivable and slow-growing microorganisms, in order to expand the diversity of microbes examined. These include the isolation of genomic DNA libraries from uncultivable or slow-growing microorganisms or directly from the environment and the transfer of this DNA to an appropriate production host. These approaches are based on observations that genes required for the production of secondary metabolites are often found in clusters, and that these suites of genes could be removed intact and transferred between organisms, perhaps in a manner analogous to the horizontal transfer of genetic material that occurs in nature.

**TABLE 1.** Some natural products and their producer organisms.

| Compound | Producer organism type | Producer organism | Reference |
|---|---|---|---|
| Cyclosporins | Fungus | *Tolypocladium* spp. (*inflatum* + 5 other spp.)<br>*Chaunopycnis alba*<br>*Aphanocladium* sp.<br>*Beauveria* spp. (*bassiana*, *brongniarti*)<br>*Acremonium* spp.<br>*Paecilomyces* spp.<br>*Verticillium* spp.<br>*Isaria felina*<br>*Fusarium* spp.<br>*Trichoderma viride*<br>*Neocosmospora vasinfecta* | ref. 22<br>ref. 23<br>ref. 24<br>ref. 25<br>ref. 26<br>ref. 18 |
| Squalestatins / Zaragozic acids | Fungus | *Curvularia lunata*<br>*Exserohilum rostratum*<br>*Setosphaeria khartoumensis*<br>*Drechslera biseptata*<br>*Pseudodiplodia* sp.<br>*Sporormiella intermedia*<br>*Leptodontidium elatius*<br>*Amauroascus niger*<br>*Phoma* sp. | ref. 27<br>ref. 10 |
| Taxols | Plant<br><br>Fungus | *Taxus* spp. (*brevifolia* + 10 other spp.)<br>*Taxomyces andreanae*<br>*Pestalotia bicilia*<br>*Pestalotiopsis microspora*<br>*Fusarium lateritium*<br>*Alternaria* sp.<br>*Pithomyces* sp.<br>*Monochaetia* sp. | ref. 28<br>ref. 29 |
| Castanospermine | Plant | *Castanospermum australe*<br>*Alexa leiopetala* | ref. 30 |
| Jaspamide | Sponge | *Jaspis* spp.<br>*Dorypleres* sp.<br>*Hemiastrella minor*<br>+ other genera | ref. 31 |

## Plants

It is more common to find natural products with restricted taxonomic distributions in plants. For example, taxol appears to be restricted to yews (*viz.* 11 species of the genus *Taxus*) but, interestingly, it has also been found in a number of different genera of fungal endophytes associated with yews and with endophytes from a non-yew source, *Taxodium distichum* (ref. 13). The genetic origin of fungal taxol production has been speculated to have arisen by horizontal gene transfer from *Taxus* spp. to its endophytes (ref. 14). If this is the case and the acquisition of the ability to produce certain metabolites by some microorganisms is by horizontal gene transfer from plant to microbial endophyte, the conservation of plant hosts and their indigenous microbial flora is of vital importance in the future search for new drugs.

**Marine macroorganisms**

Similarly, it would appear that many natural products have somewhat restricted taxonomic distributions in marine macroorganisms, but perhaps less than that observed in plants. Of interest also is the observation that a variety of marine macroorganisms contain large numbers of bacteria and other microorganisms, including cyanobacteria, actinomycetes and fungi. Up to 60% of the volume of sponges, for example, may be comprised of bacterial cells (Australian Institute of Marine Science, pers. comm.). There has been speculation that many bioactive compounds isolated from sponges may, in fact, be of microbial origin and this has been confirmed in a number of cases.

## COLLECTIONS AND RE-COLLECTIONS OF MATERIAL

The quantities required are not an issue with microbial samples where isolates may be stored under appropriate conditions for future use. However, there is a paucity of information available concerning the taxonomic relationships between microorganisms and information concerning their geographic distributions, as noted earlier. But, for other types of samples, there are clear collection and re-collection issues to be considered.

With the increased difficulty in discovering new medicines there has been a change of emphasis within the natural products discovery units of pharmaceutical companies from searching for novel natural products that could be delivered to market, to searching for novel lead compounds. These lead compounds would eventually find their way to market after chemical modification and/or a complete chemical synthesis being devised. This "philosophical" change to searching for new natural products has opened up opportunities for screening a variety of previously unscreened organisms for natural product production.

It may be unrealistic to collect only samples where large amounts of material are available as a hedge against requiring further material in the near future. This would severely curtail the numbers and types of samples collected. However, in the event of a successful discovery, it is imperative that raw materials be available in sufficient quantities for follow-up work and/or compound production at a reasonable scale. A balance must be reached between small initial collections requiring further follow-up re-collections with their inherent problems, and larger initial collections that may restrict the diversity of samples able to be collected.

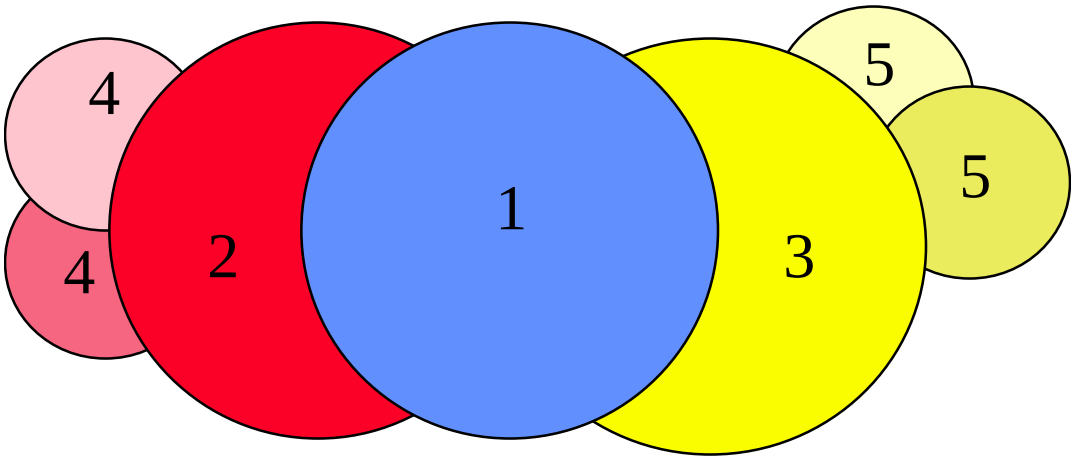
Re-collection issues certainly do arise with plants and marine macroorganisms. For example, tropical rain forest species are notoriously difficult to raise in artificial conditions. Flowering and fruiting of many species are infrequent making seed supplies unpredictable, artificial propagation is difficult and not too many species lend themselves to tissue culture on an economic basis. This means that the natural source of plant materials must be judiciously conserved and sustainably managed to ensure continuing supply. That is, not all tropical plants may be cultivable in the manner of the Pacific yew, where taxol can now be extracted from all parts of young cultivars, a renewable resource. Also, it may not be possible in every instance to use an associated organism to produce a compounds of interest, in the way that the use of the endophytic fungus, *Taxomyces andreanae*, from the Pacific yew is being investigated for the production of taxol by fermentation. It should also be borne in mind that it has taken many years of work to get to the current stage of taxol production from these renewable sources.

It has been said that drug discovery based on sourcing and direct extraction of vegetative plant material is limited by two factors: restricted access to phytochemistry due to genetic and /or metabolic regulation of secondary metabolism reflecting specific growing conditions and, the uncertainty of re-accessing interesting chemistry in subsequently sourced

samples of the same native plant. In an attempt to address these issues, plant cell culture has been used to try to provide access to a broader array of phytochemistry in each plant species through manipulated cell culture and by building a collection of plant cell cultures that can be regrown and scaled up under controlled conditions as required to provide additional material.

Our experience with both plant tissue and some microbial cultures has suggested a model of metabolite production and its relationship to biotic and abiotic stimuli (Figure 1). This model posits that a number of bioactive metabolites can be induced by a biotic interaction with another organism, some of these metabolites being induced specifically by one organism, and others by any organism. In addition, some of these biotically-inducible metabolites may also be induced by abiotic interactions such as with metal ions. That is, the circumstances - both biotic and abiotic - an organism encounters influence the metabolites produced by the organism. The organism may have a standard response to the circumstances encountered (e.g., cell death pathways triggered) and, in addition, a special response which can be non-specific or specific depending on additional biotic or abiotic factors encountered and the ecology of the organism itself.

**FIGURE 1.** A model relating metabolite production in culture to biotic and abiotic stimuli.




**Where:**

1 = metabolites produced during growth with no additional stimuli
2 = metabolites produced during growth with additional general biotic stimulation
3 = metabolites produced during growth with additional general abiotic stimulation
4 = metabolites produced during growth with additional specific biotic stimulation
5 = metabolites produced during growth with additional specific abiotic stimulation

This would suggest a requirement to apply a variety of stimuli to plant tissue or cell cultures to maximise bioactive metabolite production, in the same way as the use of a variety of growth conditions and media for microbial screening programmes. An added drawback is the lack of differentiation of plant tissue/cell culture - with its attendant lack of stimulation of developmental pathways and the metabolite production associated with these - in comparison to that observed in microorganisms. Thus, it could be suggested that plant tissue or cell culture technologies are perhaps better suited to the scale-up production of bioactive

metabolites once they have been determined to be of interest, rather than for the initial screening of plant taxa for novel metabolites.

With regard to marine macroorganisms, bioactive compound production by associated endophytic/symbiotic microorganism(s) would be a desirable outcome to prevent the need for re-collection. If not, re-collection problems may be encountered, as has been reported recently in West Australia (ref. 15) where further work on a bioactive compound isolated by the Scripps Institute of the USA would require the harvesting of an 'environmentally unreasonable' 100-250 kg of the soft coral *Eleutherobia* to do thorough biotesting, thus decimating the source of the compound. The artificial cultivation of marine macroorganisms for scale-up work may well present more difficulties than those encountered with plants.

## RELATIONSHIP BETWEEN METABOLITE PRODUCTION, FUNCTIONAL DIVERSITY AND BIODIVERSITY

A compelling argument for the pharmaceutical industry's involvement in conserving biodiversity is that there are variations in secondary metabolite production between different samples of the same species from different habitats (e.g., ref. 16, ref. 17, ref. 18). It is not clear, however, whether these differences mirror the diversity of genotypes within a sampling site, or if the diversity is a result of habitat variability and epigenetic variability. Studies we have undertaken with fungi to examine this (ref. 17) have highlighted the complex relationship that exists between genotype, environment, and phenotype as expressed in secondary metabolic products. It is thought, for example, that differences in macro- and microclimatic factors influencing the fungal populations, differences in substrate patchiness, environmental disturbance, and limitations in the flow of fungal propagules within a habitat might allow more diverse populations of fungi to evolve in some habitats.

Slattery *et al.* (ref. 19) have also reported site-specific and ontogenic differences in the concentrations of bioactive compounds produced by tropical soft corals. Production of these compounds was directly correlated with predation levels, with the compounds undergoing enzymatic conversion from inactive precursors following grazing.

Such examples highlight the benefit of examining more than one population of a species and of having ecological, in addition to systematic, knowledge of the organisms one is examining in order to maximise the chances of discovering new chemicals. A greater understanding of the variation in metabolic activity of populations of species in natural ecological settings may have value in optimizing methods of searching and screening for useful natural products. Re-collections might necessitate surveys of the distribution and abundance of organisms, as well as determination of the variation of drug content in different organism parts and the fluctuation of content under different biotic and abiotic conditions.

## THE FUTURE

With regard to some of the aforementioned issues, it is likely that a keen interest will remain in the use of gene transfer technologies at both early and later stages of drug discovery programmes. These include:

- The production of hybrid compounds through modification of the genetic makeup of microorganisms such as has been done with *Streptomyces* where 'modular' gene systems occur (e.g., ref. 20)

- The screening of uncultivable and slow-growing microorganisms through gene transfer to host microorganisms in order to expand the diversity of microbes examined (*viz.* random gene transfer)
- The horizontal transfer of genetic material between a producer and another more suitable host for compound production once a compound of interest has been detected in a producer organism (*viz.* targeted gene transfer)

It should be noted that at present, however, an incomplete knowledge of many metabolic pathways limits work at the molecular level. It is also likely that the genes for making enzymes of secondary metabolism are more widely distributed than are the enzymes themselves and the metabolites - these are so-called 'silent genes' (ref. 8). Thus, it may become more difficult to predict and control chemical outcomes as larger volumes of genetic material are transferred from donor to host organisms, and as silent genes become activated, which may result in such outcomes as the instability, degeneration, and other features observed during fungal interactions by Rayner *et al.* (ref. 21).

## SUMMARY

There may be a perception amongst conservationists and the public that 'large quantities' of material are being collected from the bush or the oceans for screening for novel natural products. Whilst small amounts of material may be used for the initial stages of the drug discovery process, there is a clear desire within the pharmaceutical industry to conserve the world's biota so that more species can be examined for novel chemical molecules and, that compounds of interest are produced via routes that do not involve the destructive and costly harvesting of samples.

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