Biodiscovery from microbial resources: Actinomycetes leading the way

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Biodiscovery from microbial resources

Biodiscovery from microbial resources can be defined as the exploration of microbial metabolic products to detect, identify and evaluate their potential for medicinal, agricultural and biotechnological operations. Microbial resource centres house microbial compounds produced under different physical and nutritional parameters to maximise the production of metabolites and are subsequently tested in different bioassays to define targeted activity.

Microbial natural products still continue to represent an important route to the discovery of novel chemicals for the development of new therapeutic agents, and the evaluation of the potential of lesser-known and/or novel microbial genetic resources is of increasing interest to industry.

Bioactive metabolites for industrial applications

Microbial primary metabolism is composed of a series of enzyme-catalysed reactions providing living cells with energy, synthetic intermediates and key macromolecules such as protein and nucleic acids. As a result of finely balanced metabolic control systems, its intermediates, the primary metabolites, rarely accumulate. Secondary metabolites, on the other hand, to a large extent, are species-specific and in contrast to primary metabolites, often accumulate in substantial quantity, which is a key factor in their commercial importance.

Most characteristic features of the secondary metabolites are their incredible array of unique chemical structures and their very frequent occurrence and versatile bioactivities. However, in spite of the diversity of their chemical structures, of their biological activities and of the organisms which produce them, the secondary metabolites are formed by only a few basic biosynthetic pathways by using the intermediates of the primary metabolic processes.

In industrial applications, nevertheless, microbial secondary metabolites are often defined as “low molecular mass products of secondary metabolism”, which include antibiotics, pigments, toxins, effectors of ecological competition and symbiosis, pheromones, enzyme inhibitors, immunomodulating agents, antibacterial agents, and so on.

Table 1. Types of microbial products.

<table>
<thead>
<tr>
<th>Acidulants</th>
<th>Coenzymes</th>
<th>Insecticides</th>
<th>Pharmacological agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Converted sterols and steroids</td>
<td>Ionophores</td>
<td>Pigments</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Emulsifying agents</td>
<td>Iron transport factors</td>
<td>Plant growth promoters</td>
</tr>
<tr>
<td>Animal growth promoters</td>
<td>Enzymes</td>
<td>Lipids</td>
<td>Polysaccharides</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Enzyme inhibitors</td>
<td>Nucleic acids</td>
<td>Proteins</td>
</tr>
<tr>
<td>Antihelminthic agents</td>
<td>Fatty acids</td>
<td>Nucleosides</td>
<td>Solvents</td>
</tr>
<tr>
<td>Antimetabolites</td>
<td>Flavor enhancers</td>
<td>Nucleotides</td>
<td>Starter cultures</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>Herbicides</td>
<td>Natural food preservatives</td>
<td>Sugars</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>Humectants</td>
<td>Non-nutritive sweeteners</td>
<td>Stabilisers and thickeners</td>
</tr>
<tr>
<td>Antitumor agents</td>
<td>Insecticides</td>
<td>Organic acids</td>
<td>Surfactants</td>
</tr>
<tr>
<td>Coccidiostats</td>
<td>Ionophores</td>
<td>Pesticides</td>
<td>Vitamins</td>
</tr>
</tbody>
</table>

Adapted from Demain (1981).
In Focus

receptor antagonists and agonists, pesticides, antitumour agents
and growth promoters" 1-5 (Tables 1 and 2). Biodiscovery from
microbial sources then inevitably targets detection of such
compounds for their further use in health, nutrition, agricultural
and other related bioindustries.

Microbial metabolites can be employed in the following industrial
categories:

(i) As direct applications of natural/fermentation product in
medicine (Table 3), agriculture or other fields.

(ii) As starting material for subsequent chemical or
microbiological modifications.

(iii) As lead compounds, for chemical synthesis of new analogues
or as templates, in the rational drug design studies 1-3,5.

The use of microbially-derived compounds has now been
extended to other areas of industrial applications such as enzymes
in bioconversion, in production of biodegradable detergents and
in food and feed processing. In addition, paper pulp, textile
and leather industries are the fastest growing markets for the
microbially-derived industrial enzymes 6,7. Currently, to combat
mounting environmental problems, biological solutions are
sought after and applied microbiology and biotechnology is
one of the rapidly growing areas in this search 8. The design and
implementation of environmentally friendly biotechnologies now
rely on the incorporation of microbial secondary metabolites into
the application or product formation process 7,8.

The range of versatility of microbial compounds is enormous
and yields significant economic returns 9, yet, biodiscovery from
microbial sources depends on the following:

(i) Detection and recovery of bioactive microorganisms from
environmental sources.

(ii) Effective application of microbial metabolites in defined
targets.

(iii) Continuous improvement and/or novel design of search
and screening strategies 10-12.

Search and discovery of bioactive microorganisms

Following the identification of a biotechnological target, a search
strategy towards detection of bioactive microorganisms needs to
be implemented 10-12. Successfully applied strategies include: (i)
playing the percentage game (e.g. targeting actinomycetes, as one
of the most bioactive fraction of the microflora existing on Earth),
(ii) interrogating taxon-chemistry and taxon property databases,
(iii) focusing on novel or neglected taxa, (iv) recovering isolates
from unusual or little-explored ecosystems, and (v) screening
of known taxa for novel targets (e.g. HIV-inactivating protein

Among the bacteria, the members of the order Actinomycetales
have proved to be a particularly rich source of secondary
metabolites with extensive industrial applications (Table 4). In
particular, the capacity of the members of the genus Streptomyces
to produce commercially significant compounds, especially
antibiotics, remains unsurpassed, possibly because of the extra-
large DNA complement of these bacteria 14. Moreover, they have
interesting metabolic trends and examples include the formation
of glycosides and the uses of the shikimate route to aromatic
compounds common in the order Actinomycetales, as in the
higher plants. Whereas the polyketide route, though important,
does not play the dominant role in the same order that it plays in

Table 2. Examples of classes of organic compounds deriving from microbial secondary metabolites.

<table>
<thead>
<tr>
<th>Amino sugars</th>
<th>Glycopeptides</th>
<th>Phenazines</th>
<th>Pyrrolines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthocyanins</td>
<td>Glycosides</td>
<td>Phenoxazinones</td>
<td>Pyrrolizines</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Hydroxylamines</td>
<td>Phthaldehydes</td>
<td>Quinolines</td>
</tr>
<tr>
<td>Aziridines</td>
<td>Indole derivatives</td>
<td>Piperazines</td>
<td>Quinones</td>
</tr>
<tr>
<td>Benzoquinones</td>
<td>Lactones</td>
<td>Polycetylens</td>
<td>Salicylates</td>
</tr>
<tr>
<td>Coumarines</td>
<td>Macrolides</td>
<td>Polynes</td>
<td>Terpenoids</td>
</tr>
<tr>
<td>Diazines</td>
<td>Naphthalenes</td>
<td>Polypeptides</td>
<td>Tetracyclines</td>
</tr>
<tr>
<td>Epoxides</td>
<td>Naphthoquinones</td>
<td>Pyrazines</td>
<td>Tetronic acids</td>
</tr>
<tr>
<td>Ergot alkaloids</td>
<td>Nitriles</td>
<td>Pyridines</td>
<td>Triazines</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Nucleosides</td>
<td>Pyrones</td>
<td>Tropolones</td>
</tr>
<tr>
<td>Furans</td>
<td>Oligopeptides</td>
<td>Pyrroles</td>
<td>Vanillin</td>
</tr>
<tr>
<td>Glutarimides</td>
<td>Perylenes</td>
<td>Ppyrolidones</td>
<td>Zeaxanthin</td>
</tr>
</tbody>
</table>

Adapted from Demain (1981)2.
fungi. Formation of terpenes and steroids are common in other organisms, but rare in the order Actinomycetales. For the detection of previously unknown bioactive microorganisms, microbial systematics can be an effective tool. Information deriving from taxonomical identity can be translated into selective culturing techniques to detect rare actinomycetes. Presently, more than 50 rare actinomycete taxa are reported to be the producers of the 2500 bioactive compounds (Table 5).

Moreover, taxonomic studies conducted on the strains shown to be producing new substances illustrated that they were close relatives of the taxa and similarly yielded novel exploitable properties. Advanced and novel screening methods and novel targets may also prove that even well-characterised members of order Actinomycetales can yield novel bioactive compounds. Examples are the discovery of the broad specificity herbicide (phosalacine), and an inhibitor of cell wall synthesis (setomimycin) from the members of the genus Kitasatospora. In addition, the antibiotic biosynthetic gene transfer in streptomycetes suggests that lateral gene transfer plays a significant role in the distribution of natural product biosynthesis across biodiversity. As a result, targeting functional diversity can be another route to discovery where, as a result of gene transfer, certain metabolic activities might be triggered in a defined ecosystem.

### Table 3. Some pharmacological activities of microbial secondary metabolites.

<table>
<thead>
<tr>
<th>ACTH-like</th>
<th>Complement inhibition</th>
<th>Haemolytic</th>
<th>Leukemogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anabolic</td>
<td>Convulsant</td>
<td>Haemostatic</td>
<td>Motility inhibition</td>
</tr>
<tr>
<td>Analeptic</td>
<td>Dermonecrotic</td>
<td>Herbicidal</td>
<td>Nephrotoxic</td>
</tr>
<tr>
<td>Anesthetic</td>
<td>Diabetogenic</td>
<td>Hormone releasing</td>
<td>Paralytic</td>
</tr>
<tr>
<td>Anorectic</td>
<td>Diuretic</td>
<td>Hypersensitising</td>
<td>Parasympathomimetic</td>
</tr>
<tr>
<td>Anticoagulant</td>
<td>Edematous</td>
<td>Hypocholesterolemic</td>
<td>Photosensitising</td>
</tr>
<tr>
<td>Antidepressive</td>
<td>Emetic</td>
<td>Hypoglycaemic</td>
<td>Relaxant (smooth muscle)</td>
</tr>
<tr>
<td>Anthelminthic</td>
<td>Enzyme inhibitory</td>
<td>Hypolipidaemic</td>
<td>Sedative</td>
</tr>
<tr>
<td>Anti-infective</td>
<td>Erythematous</td>
<td>Hypotensive</td>
<td>Serotonin antagonist</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>Estrogenic</td>
<td>Immunostimulating</td>
<td>Spasmolytic</td>
</tr>
<tr>
<td>Anti-parasitic</td>
<td>Coagulative (blood)</td>
<td>Hallucinogenic</td>
<td>Teledicial</td>
</tr>
<tr>
<td>Anti-spasmodic</td>
<td>Fertility enhancing</td>
<td>Inflammatory</td>
<td>Ulcerative</td>
</tr>
<tr>
<td>Carcinogenesis inhibition</td>
<td>Complement inhibition</td>
<td>Haemolytic</td>
<td>Vasodilatory</td>
</tr>
<tr>
<td>Coagulative (blood)</td>
<td>Hallucinogenic</td>
<td>Insecticidal</td>
<td>Anti-viral</td>
</tr>
</tbody>
</table>

### Table 4. Bioactive compounds of microbial origin.

<table>
<thead>
<tr>
<th>Source</th>
<th>Antibiotics</th>
<th>Bioactive secondary metabolites</th>
<th>Total bioactive metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>With other activity</td>
<td>No antibiotic activity</td>
</tr>
<tr>
<td>Bacteria</td>
<td>2900</td>
<td>780</td>
<td>900</td>
</tr>
<tr>
<td>Actinomycetes</td>
<td>8700</td>
<td>2400</td>
<td>1400</td>
</tr>
<tr>
<td>Fungi</td>
<td>4900</td>
<td>2300</td>
<td>3700</td>
</tr>
<tr>
<td>Total</td>
<td>16500</td>
<td>5500</td>
<td>6000</td>
</tr>
</tbody>
</table>

Adapted from Demain (1983).
Baltz critically examined the evolution of the antibiotic pathways to target isolation of novel microorganisms that might have evolved novel antibiotic pathways more recently than some of the more common antibiotic pathways. The most commonly isolated antibiotic streptomycin might be a combination of an ancient pathway originating from a divergence from the last common ancestor ~610 million years ago and horizontal gene transfer within the members of the genus *Streptomyces*. More recent evolution of the vancomycin pathway (~240 million years ago) might, however, be linked to the low frequency-occurrence of the members of the producer genus in the soil.

Targeting of uncommon antibiotic pathways that might reside within microorganisms that are not widely dispersed in nature would be another effective route to biodiscovery. Pathways that evolved more recently (<20 million years) are more likely to be detected among common streptomycetes residing at extreme environments. Targeting such pathways can provide chemical diversity required for screening efforts.

### Future prospects and strategies

**Selective isolation of industrially important actinomycetes is no longer application of a single technique. It is design of methodologies using the information generated through eco-taxonomic disciplines**.

Metabolites to be discovered from the members of the order Actinomycetales have not been exhausted either and the predictive modelling by Wagne et al. suggested that over 150,000 bioactive metabolites are still waiting to be discovered from the members of the genus *Streptomyces* alone within this order and the current fall in discovery represents a decline in screening efforts rather than exhaustion of compounds.

Another important strategy would be targeting highly selective isolation techniques specifically designed for rare actinomycetes. The concept of general isolation medium is no longer tenable. Moreover, in an era which tends to neglect the importance of designing objective selective isolation techniques and easily classifies most organisms as “unculturable”, it is crucial that the following points are taken into consideration in the search of industrially important actinomycetes:

(i) Increased knowledge of microbial ecology and physiology with an eco-physiological perspective.

(ii) Study of neglected habitats and more intensive investigation of the better studied ones (e.g. “New microbes from old habitats”).

(iii) Improved sampling procedures designed particularly for marine and extreme habitats (e.g. targeting acidophilic, alkaliophilic, and osmophilic strains).

(iv) Development of more objective, less conservative isolation procedures, considering the functional diversity and physiological characteristics of those organisms that inhabit indigenous locations.

(v) Provision of more efficient identification systems to determine novelty (e.g. analysis of ribosomal proteins by use of MALDI-TOF Mass Spectrometry).

(vi) A holistic understanding of the organism’s biology and the natural roles of secondary metabolites.

(vii) Exploitation of new advances (e.g. use of bioinformatics: expression of antibacterial ECO-0501; use of gene disruption – Coelichelin; heterologous expression – Collinone – encoded by *Streptomyces* species).

(viii) Screening many more samples from diverse sources with objective strategies (e.g. with high-throughput whole cell antibacterial screens that process greater number of actinomycetes or while discriminating against the producers of common antibiotics by using genetically engineered

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Number of bioactive species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinosynnemataceae</td>
<td>Actinosynnema</td>
<td>51</td>
</tr>
<tr>
<td>Frankiaceae</td>
<td>Frankia</td>
<td>7</td>
</tr>
<tr>
<td>Micromonosporaceae</td>
<td>Dactylosporangium</td>
<td>58</td>
</tr>
<tr>
<td>Pseudonocardiae</td>
<td>Kibdelosporangium</td>
<td>34</td>
</tr>
<tr>
<td>Streptomycetaceae</td>
<td>Chainia</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Microellospora</td>
<td>11</td>
</tr>
<tr>
<td>Streptosporangiaceae</td>
<td>Microtetraspora</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 5. Number of rare actinomycete species producing bioactive microbial metabolites. Adapted from Bérdy (2005).
screening strains containing multiple antibiotic-resistant genes).

So long as major challenges in biotechnology and biomedicine remain (e.g. emerging diseases, established diseases, antibiotic resistance, and environmental pollution and need for renewable energy) microbial resources will be of interest to mankind providing sustainable and environmentally friendly solutions. Microorganisms continue to offer the versatility of their products providing a stimulus for interaction between different disciplines, major support from governments and agencies, as well as an understanding and supportive public and philanthropic organisations.20,21.

References

From discovery to commercialisation of vaccines

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Having spent the first 25 years of my [IG] working life involved in research on vaccine development and delivery and then a decade in industry, working for and with, companies that actually made vaccines, I am reminded of the observation, attributed to Charles Dickens:

When I was 14, I thought my Father was the stupidest person on earth. When I was 21, I was amazed at how much he had learnt in the past 7 years.

Exposure to the harsh realities of product development challenged my academic preconceptions, gave me a greater insight into the nature and complexity of the development process and a greater respect for the skills of those involved.

The public health community often laments the fact that vaccines produced by the research-based industry take so long to develop and initially cost tens, sometimes hundreds of dollars per course. They tend to assume that the high price of modern vaccines