Secondary Metabolites and their Exploitation in Medicine

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Many of the medicinal products available today are derived from secondary metabolites. Plants remain the main source of such therapeutic agents but other sources are being explored and exploited. New and old technologies are being applied in the continuing quest of finding useful secondary metabolites of plant origin or otherwise from which newer drugs can be made.

Introduction

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of organisms (Petr Karlovsky, 2008). It is in this sense they are called "secondary" but this does not imply that they are in anyway unimportant. They exert various biological effects, often at very low concentrations, and can be regarded as carriers of chemical communication among organisms.

Contrary to primary metabolites, secondary metabolites are not ubiquitous in the living organisms which produce them and they are rarely conserved over a wide taxonomical range (c.f. primary metabolism is conserved among phyla and across kingdoms). Usually, they are produced through a long chain of intermediates involving several enzymes. Generally they are created from modified primary metabolites, or from substrates of primary metabolite origin. Some are produced all the time (i.e. constitutively) while others are produced after induction.

By now, some 150,000 natural products are known, of which about 80% originate from plants.

Functions of Secondary Metabolites to the Organism

The function of secondary metabolites is usually of an ecological nature. Some are used by the organism to defend itself against its predators, parasites and diseases. Others are used for interspecies competition, or to facilitate the reproductive processes e.g the coloring agents and the attractive smells of flowers are usually secondary metabolites. They may also function in such other processes as immunity, anti-herbivory, maintaining symbiotic associations or communication between organisms (e.g. VOCs = volatile organic compounds; bacterial autoinducers).

Bacterial cells use secondary metabolites to chemically communicate with each other in a process called quorum sensing (QS). Quorum sensing in bacteria is the regulation of gene expression as a function of the cell-population density. The bacteria produce and release chemical signal molecules called autoinducers. Usually as the bacterial population density increases, the concentration of the signal molecules increases as well. When the bacterial population reaches a critical level called the quorum, the bacteria respond and modulate certain gene expression (like genes coding for virulence) almost simultaneously.

Gram-positive and Gram-negative bacteria use quorum sensing to regulate a diverse array of physiological activities such as symbiosis, competence, conjugation, antibiotic production, motility, sporulation, virulence and biofilm formation. In general, Gram-negative bacteria use acylated homoserine lactones (AHLs) as autoinducers, and Gram-positive bacteria use processed oligo-peptides to communicate. This cell-to-cell communication via autoinducers occurs both within and between bacterial species. Indeed, furanosyl borate diester has been found to be employed by certain bacteria for interspecies communication (Chen et al. 2002).

Research is showing that with quorum sensing, the bacteria are becoming 'multicellular'. And doctors should no longer think that bacteria are asocial, single cells. They are individual cells but they act in communities. Researchers in various labs all over the world are now trying to find out exactly how bacteria chemically 'talk' to each other. Hopefully, they will know how to control and manipulate

Table 1

Medical Use	Medicinal agent	Source
Anti-tumour	Vincristine, VinblastineTaxol, Ursolic acid Discodermolide	Catharanthus roseus, Taxus sp., Lichens Discodermia dissoluta
Antibiotic	Abyssomicin, Vancomycin, Streptomycin Bacitracin, Penicillin, Erythromycin, Usnic acid	Actinobacteria, Bacillus subtilis Penicillium, Saccharopolyspora erythraea, Lichens
Antiviral	Depsides, Depsidones Virsenic acid, Stictic acid Chlorophaeic acid, Podophyllin	Lichens Podophyllium
Antimalarial	Quinine	Cinchona ledgeriana
Cardiotonic	Digoxin	Digitalis lanata
Antihypertensive	Reserpine, Ajmalicine	Rauwolfia serpentina, Catharanthus roseus
Analgesic	Morphine	Papaver somniferum
Anti-pyretic Anti-inflammatory	Salicylic acid	Salix
Anti-cholinergic	Atropine, Hyoscyamine, Scopolamine	Atropa belladonna, Hyoscyamus niger Datura metel
Cholinergic	Pilocarpine	Pilocarpus sp.
Anti-gout	Cochicine	Colchicum autumnale
Antifertility	Steroids from Diosgenin	Dioscorea deltoidea
Anti-tussive, Antidiarrheal	Codeine	Papaver somniferum

quorum sensing and tailor make anti-quorum sensing drugs. One line of research is the demonstration that certain plants and red algae are able to mimic QS signals produced by several bacteria by secreting compounds that structurally mimic the bacterial QS molecules (Newton and Fray 2004; Bauer and Mathesius 2004). Specifically, the marine red alga Delisea pulchra produces halogenated furanones which act as QS 'mimic' compounds. These halogenated furanones have a similar structure to AHLs. Interestingly, such furanones inhibit AHL-regulated behaviours in several bacteria (Teplitski et al. 2000).

Medical Uses of Exploited Secondary Metabolites

The following valid and instructive example shows the potential of exploiting secondary metabolites for medical use. Doctors are worried that unless scientists find novel antibiotics within a short time, they would find themselves back in the era of life-threatening bacterial infections. A case in point is the superbug, MRSA, which is now resistant to most antibiotics. By exploiting marine microbes (specifically, a group of bacteria called Actinobacteria), researchers have come across a new class of antibiotics. Usually, such bacteria came from soils and researchers believed that there were no marine species of Actinobacteria. But Actinobacteria are also found in seawater sediments, from continental shelves to the abyssal depths. Indeed, a marine sediment may contain more than 1000 new species. Scientists have found a bacterium from such a sediment at a depth of 300 metres in a bay in Japan. This bacterium produces a secondary metabolite that acts as an antibiotic to control the growth of other micro-organisms in its niche. This antibiotic has been rightly named **abyssomicin**. Investigations and tests on abyssomicin are showing promising results in that it kills MRSA. Also, by studying the mode of action of abyssomicin it has been found that it disrupts pABA synthesis. Therefore, this may also make abyssomicin a potential anti-malarial drug.

Some of the medical uses of some secondary metabolites are shown in Table 1 above.

Some Specific Examples of Exploitation of Secondary Metabolites

From Plants

Plants have been used in the treatment of cancer for over 3500 years (Hartwell,1967). This is evidenced in the

folklore of many countries. For example the Chinese used podophyllum leaves and many other countries used the juice of Bittersweet (Solanum dulcamara) to treat cancers, tumors and warts. But it is only since 1959 that a systematic effort has been made to screen crude plant extracts for their inhibitory activity against tumour systems.

The number of medicines used in chemotherapy is around 20. These comprise synthetic and natural drugs (Lee, 1999). Most of the active principles act upon DNA by modifying its chemical and physical nature. On the basis of mechanisms of action the antitumour agents can be broadly categorized in 4 groups: (1) alkylating agents, (2) antimetabolites, (3) mitotic inhibitors (spindle fibre toxins) and (4) intercalating agents. Caryolysine was among the first alkylating antitumoral agent in the class of nitrogen mustard used for the treatment of human cancers. Chlorambucil, melphalan and mannomustine are other alkylating products that were developed later. Fluorouracil and methorexate are examples of antimetabolites, while colchicine, taxol and vinblastine (a vinca alkaloid) are mitotic inhibitors. Quinine, quinacrine, ellipticine, ethidium bromide, nitidine and actinomycin-D are all examples of DNA intercalating agents. Of these taxol is the latest and is obtained from the bark of yew, Taxus. Taxol is used for the treatment of breast, ovarian, lung, bladder, prostrate, melanoma, esophageal, as well as other types of solid cancers.

Other medically exploited secondary metabolites derived from plants include atropine, codeine, morphine, and digoxin. Atropine is extracted from deadly nightshade (Atropa belladonna), jimsonweed (Datura stramonium), mandrake (Mandragora officinarum) and other plants of the family Solanaceae. Morphine is the principal active agent in opium and other poppy saps like Papaver bracteatum. It was the first alkaloid isolated from a plant source, and was named morphium after Morpheus, the Greek god of dreams. Codeine (methylmorphine) is also found in opium and while it can be extracted from opium, most codeine is synthesized from morphine through the process of O-methylation. Digoxin (Digitalis) is a cardiac glycoside extracted from the foxglove plant, Digitalis lanata.

From Bacteria

Vancomycin is a glycopeptide antibiotic derived from the Actinobacteria species Amycolatopsis orientalis. It was first isolated from a soil sample collected from the interior jungles of Borneo by a missionary.

Streptomycin is another antibiotic, the first of a class of drugs called aminoglycosides to be discovered. It was the first antibiotic remedy for tuberculosis. It is derived from the actinobacterium Streptomyces griseus. **Neomycin** is also an aminoglycoside antibiotic that is found in many topical medications such as creams, ointments and eyedrops. It is produced naturally by the bacterium Streptomyces fradiae.

Another topical antibiotic is **Bacitracin.** It is produced by Bacillus subtilis.

From Fungi

Fungi also offer a huge source of pharmaceutically useful molecules. For the fungi the discovery of penicillin marked a new era. Penicillin is an antibiotic that is derived from the fungus Penicillium. Another useful antibiotic is erythromycin. It is produced from a strain of the actinomycete Saccharopolyspora erythraea found in soil.

The genus Claviceps is a fungus that is parasitic on certain grains and grasses. In winter the fungus forms a sclerotium usually referred to as an 'ergot'. These ergots are hard, black tuber-like bodies which consist of a compact mass of hyphae. Such ergots produce up to 40 different alkaloids. The main ergot alkaloids are ergotamine, ergocornine, ergocristine, ergocryptine, ergometrine and agroclavine. If ergot alkaloid-containing grasses and cereals are eaten, ergotism results ("St. Anthony's Fire").

Some of the ergot alkaloids have been medically exploited. Historically, controlled doses of ergot were used to induce abortions and to stop maternal bleeding after childbirth. Nowadays, one also finds nasal drops containing ergometrin (Methergin) to stop postnatal bleeding; Cafergot (containing caffeine and ergotamine or ergoline) to treat migraine headaches. Another two important ergot-derived drugs are Pergolide and Cabergoline. Pergolide (Permax) is a dopamine receptor agonist and is used for the treatment of Parkinson's disease. Pergolide products were removed from the market in 2007 because of the risk of serious damage to patients' heart valves. Nowadays, if indicated the patient should be regularly reassessed for any fibrotic reactions and valvulopathy. Cabergoline (Dostinex) is also a dopamine receptor agonist. It is indicated for the treatment of hyperprolactinemic disorders.

From Lichens

Lichens are associations between a fungus (the mycobiont) and a primitive photosynthetic organism (the photobiont). The latter is either a green alga or a

cyanobacteria. Approximately 700 secondary metabolites have been identified in lichens. Many of these compounds are the result of the symbiosis. Indeed, the majority of the organic compounds formed in lichens are secondary metabolites produced by the fungal partner (Huneck and Yoshimura, 1996).

Lichens have a long history of usage in herbal medicines. Even though their therapeutic potential has not been yet fully explored, the following biological activities of some lichen secondary metabolites have been investigated and exploited.

Usnic acid has antibiotic activity. Compositions containing usnic acid or its derivatives are used for therapeutic control of dental caries, particularly for the preventive treatment of cariogenic dental plaque. Usnic acid is bacteriostatic against the gram-positive Streptococcus mutans which is the primary pathogen in cariogenic lesions. Many tooth pastes and mouthwashes used for dental and oral cavity medications contain usnic acid.

Ursolic acid has anti-cancer activity. The use of ursolic acid for the manufacture of an anticancer agent that suppresses metastasis has been patented (Ishikawa et al, 1997). Other lichen secondary metabolites that exhibit antitumoral effects include: **nephrosternic acid**, **polyporic acid** and its derivatives and **evernic acid**.

Depsides and **depsidones** have antiviral-activity against HIV virus. They inhibit HIV-integrase and are being investigated as potential AIDS treatment. **Virsenic acid**, **stictic acid** and **chlorophaeic acid** also show important effects against HIV-1 integrase. (Neamati et al., 1996).

The screening of crude extracts of numerous species of the lichen family Graphidaceae have demonstrated inhibition of the enzyme tyrosinase. **Tyrosinase inhibitors** have become important as cosmetic and medicinal products, primarily to control melanin pigmentation.

It has been shown that some secondary metabolites from lichens (e.g. **divaricatic acid**, **atranorin**) protect against oxidative damage by inhibiting the formation of free radicals and/or trapping reactive oxygen species. (Marante et al., 2003).

Other lichen products have analgesic, anti-inflammatory, or anti-pyretic activities. For example **chrysophanol** has been shown to be a potent anti-proliferative and anti-inflammatory agent in the treatment of psoriasis (Muller, 2001).

Pannarin and **chloropannarin** have been shown to have antiprotozoal activity.

From a Sponge

Discodermolide is a recently discovered polyketide natural product found to be a potent inhibitor of tumor cell growth by hyper-stabilizing microtubules. It was first isolated in 1990 from the Caribbean **marine sponge** Discodermia dissoluta. Discodermolide also has immunosuppressive and antifungal activities. Several preclinical drug development programs are ongoing.

Current Research on Secondary Metabolites

Production of Secondary Metabolites by Bioconversion and Precursor Feeding

Plant cells are omnipotential, meaning that all the genetic information present in the plant is principally available in each cell. Hence theoretically, genes that encode enzymes of biosynthetic pathways can be brought to express themselves. It is thus feasible to produce most plant secondary metabolites using in vitro grown cultures. The successful production of antibiotics by fungi and bacteria was the stimulus for the initial plant biotechnologists to have a try with in vitro plant cell cultures. But it soon became clear that plant cell cultures do not always accumulate either qualitatively or quantitatively the same compounds found in the parent plant from which they were established. Because of this only a very limited number of secondary metabolites can be produced commercially by plant cells on a larger scale in bioreactors. High production rates are only feasible for compounds with a simple chemical structure, such as L-DOPA (an important anti-Parkinson drug).

Unfortunately, pharmacologically active compounds have a more complex chemical structure e.g. podophyllotoxin and camptothecine which exert strong cytotoxic properties. Artemisin, a very potent new antimalarial drug and also the new cytostatic agent paclitaxel, have been produced in low amounts by Taxus cell cultures, progress is slowly being made and **precursor feeding** and **bioconversion** are very promising.

If the concentration of a compound which is an intermediate in a biosynthetic pathway is increased, this may increase the yield of the final product of the pathway. This is the basis of the precursor feeding approach.

Bioconversion is the modification of added precursors (also called substrates) into more valuable products using

enzyme catalysis. The systems used for bioconversion are (i) freely suspended plant cells (ii) immobilized plant cells and (iii) enzyme preparations.

Freely suspended cells are the most simple bioconversion systems, since precursors can be supplied directly to the cultures. The cell wall and cell membrane are the only barriers a precursor meets. The best way is to use plant cells that can do a one-step bioconversion but unfortunately the precursor often undergoes more than one bioconversion, resulting in complex mixtures of products. It can also be metabolized via unknown mechanisms. Nevertheless, a number of one-step bioconversions by freely suspended cells have been done. For example papaverine has been bioconverted using free cell suspensions of Glycyrrhiza glabra.

An **immobilized plant cells system** entails either entrapment of cells or immobilisation of cells. Immobilisation of the plant cells can be done by adsorption onto a support material. Entrapment methods include gel entrapment by ionic network formation and entrapment in performed structures (Novais, 1988). The most widely used approach is entrapment by ionic network formation, especially in the form of alginate beads.

Some successfully applied one-step bioconversions by immobilized cells have been done. For example the bioconversion of the precursor codenione into codeine using Papaver somniferum or the bioconversion of L-tyrosine into L-DOPA using Mucuna pruriens. Immobilized cells derived from various lichens have also been employed to produce useful secondary metabolites (Vincente et al., 2003).

Enzymes from plant cells are also being used to convert foreign precursors, intermediates that are not normally biosynthesized in the plants from which the cultures are derived (Kutney, 1996: Suga and Hirata, 1990).

Bioconversions and precursor feeding are yielding new drugs but they are also being applied for the improvement of currently used drugs. Hydroxylations and gylcosylations or a combination of both are most suitable for this purpose.

Recombinant DNA technology

Plant cells often make low quantities of the desired enzyme, resulting in low bioconversion rates. A solution may be found in the transfer of plant genes, which encode the enzyme into a bacterial (e.g. E.coli) or fungal cell (yeast) and more, recently, into insect cells. The idea is to bring the gene to overexpression in a preferably rapidly growing host cell, resulting in a high production of the desired enzyme (Overbeck and Verrips, 1992). This expression of genes encoding plant enzymes in a foreign host cell is termed **heterologous expression**: a gene is transferred from one cell species, a plant cell, to another cell species, the host cell (fungal or insect cell). This heterologous expression is also being tried (with some success) for the production of certain lichen metabolites. Recombinant DNA technology has not yet reached the stage at which large numbers of foreign genes can be transferred to any host cell and then coordinately expressed. This implies that the problems regarding the expression of more complex pathways (which usually have many enzymes) under in-vitro circumstances are not likely to be solved in the near future.

With all the above problems, still, the culture of plant cells or otherwise is feasible and can be used to produce significant amounts of secondary metabolites. For the development of an industrial process, the crucial question is its economic feasibility. At the time it is only for a small number of high-value compounds that this is economically feasible. However, research and many experiments are being done to improve productivity of other secondary metabolites from stable, high-yielding cell lines. The production of secondary metabolites on an industrial scale using bioreactor system designs is important because indiscriminate removal of plants or otherwise from the wild to meet the high demand of medicine for the ever-increasing human population, is making such wild sources endangered. A case in point is the exploitation of the Taxus tree for taxol. Taxus is a small tree that grows very slowly. Taxol is derived from its bark and its extraction leads to death of the tree. It is estimated that about 50kg of taxol will be required every year for the treatment of approximately 12,500 women in the USA alone. The present world demand may touch 250kg per year. Generally 30kg of taxol are obtained from 42,000 trees. So with some calculations this means that at least three Taxus plants are needed for one patient. Such a large number of plants are not available from the wild population.

Metabolomics

The set of metabolites synthesized by an organism constitute its **metabolome**. **Metabolomics** is the quantitative and qualitative study of all the metabolites present in a cell. By comparing the **metabolic profile** of a cell producing metabolites in high quantity to a similar cell that is not producing these metabolites, differences in gene expression is being recorded. Such studies form part of **functional**

genomics and it is helping in the identification of genes which are involved in product formation. It is also elucidating key steps and bottlenecks of metabolite synthesis. Metabolic profiling of the wild type, mutant and transgenic organism, is providing useful data in **metabolic fingerprinting**. Indeed, it is becoming possible to link metabolic changes in biochemical pathways to the enzymes involved, and then to the underlying genetic alterations.

Such metabolic studies are yielding huge data sets that need to be analyzed and stored. With replications and various samples taken during experiments, the number increases considerably, generating several hundred megabytes of data per experiment (Petr Karlovsky, 2007). The raw data obtained is then processed and converted to useful biological information by a variety of data analysis techniques. Evolutionary computation-based methods such as genetic algorithms and genetic programming are being found to be ideal strategies for mining such high-dimensional data to generate useful relationships, rules and predictions (Goodcare, 2005) and thus aiding the exploitation of secondary metabolites for medical use.

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