Chapter 1
Valuable Secondary Metabolites from Fungi

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Introduction

A major contribution of microbes to the health and well-being of people began back in 1928, when Alexander Fleming discovered in a Petri dish seeded with *Staphylococcus aureus* that a compound produced by a mold killed the bacterium. The mold, *Penicillium notatum*, produced an active agent, which was named penicillin. Fleming's discovery began the microbial drug era. By using the same method, other naturally occurring substances, like chloramphenicol and streptomycin, were later isolated from bacterial fermentations. Naturally occurring antibiotics are produced by fermentation, an old technique that can be traced back almost 8,000 years, initially for beer and wine production, and recorded in the written history of ancient Egypt and Mesopotamia. During the last 4,000 years, *Penicillium roqueforti* has been utilized for cheese production and for the past 3,000 years, soy sauce in Asia and bread in Egypt represented examples of traditional fermentations [1].

Natural products (NPs) with high commercial value can be produced via primary or secondary metabolism. The present review deals with secondary metabolites. Due to technical improvements in screening programs and separation and isolation techniques, the number of natural compounds discovered exceeds one million [2]. Among them, 50–60 % are produced by plants (alkaloids, flavonoids, terpenoids, steroids, carbohydrates, etc.) and 5 % of these plant products have a microbial origin. From all the reported natural products, about 20–25 % show biological activity and of these, approximately 10 % have been obtained from microbes. Microorganisms produce many compounds with biological activity. From the 22,500 biologically active compounds so far obtained from microbes, about 40 % are produced by fungi [2, 3]. The role of fungi in the production of antibiotics and other drugs for treatment of noninfective diseases has been dramatic [4].

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Biosynthetic genes are present in clusters coding for large, multidomain, and multi-modular enzymes such as polyketide synthases, prenyltransferases, non-ribosomal peptide synthases, and terpene cyclases. Genes adjacent to the biosynthetic gene clusters encode regulatory proteins, oxidases, hydroxylases, and transporters. Aspergilli usually contain 30–40 secondary metabolite gene clusters. Strategies to activate silent genes have been reviewed by Brakhage and Schroekh [3].

Currently, with less than 1 % of the microbial world having been cultured, there have been significant advances in microbial techniques for growth of uncultured organisms as a potential source of new chemicals [5]. Furthermore, metagenomics—i.e., the extraction of DNA from soil, plants, and marine habitats and its incorporation into known organisms—is allowing access to a vast untapped reservoir of genetic and metabolic diversity [6, 7]. The potential for discovery of new secondary metabolites with beneficial use for humans is great. A method to predict secondary metabolite gene clusters in filamentous fungi has recently been devised [8].

Microbes normally produce secondary metabolites in only tiny amounts due to the evolution of regulatory mechanisms that limit production to a low level. Such a level is probably enough to allow the organism to compete with other organisms and/or coexist with other living species in nature. The industrial microbiologist, however, desires a strain that will overproduce the molecule of interest. Development of higher-producing strains involves mutagenesis and, more recently, recombinant DNA technologies [9]. Although some metabolites of interest can be made by plants or animals, or by chemical synthesis, the recombinant microbe is usually the "creature of choice." Thousandfold increases in production of small molecules have been obtained by mutagenesis and/or genetic engineering. Other important parts of industrial production include creating a proper nutritional environment for the organism to grow and produce its product, and the avoidance of negative effects such as inhibition and/or repression by carbon sources, nitrogen sources, phosphorus sources, metals, and the final product itself. Avoidance of enzyme decay is also desired [4, 10].

Applications of Microbial Natural Products

Over the years, the pharmaceutical industry extended their antibiotic screening programs to other areas [11, 12]. Since microorganisms are such a prolific source of structurally diverse bioactive metabolites, the industry extended their screening programs in order to look for microbes with activity in other disease areas. As a result of this move, some of the most important products of the pharmaceutical industry were obtained. For example, the immunosuppressants have revolutionized medicine by facilitating organ transplantation [13]. Other products include antitumor drugs, hypocholesterolemic drugs, enzyme inhibitors, gastrointestinal motor stimulator agents, ruminant growth stimulants, insecticides, herbicides, antiparasitics versus coccidia and helminths, and other pharmacological activities. Catalyzed by the use of simple enzyme assays for screening prior to testing in intact animals or in the field, further applications are emerging in various areas of pharmacology and agriculture.

Antibiotics

Of the 12,000 antibiotics known in 1955, filamentous fungi produced 22 % [14, 15]. The beta-lactams are the most important class of antibiotics in terms of use. They constitute a major part of the antibiotic market. Included are the penicillins, cephalosporins, clavulanic acid, and the carbapenems. Of these, fungi are responsible for production of penicillins and cephalosporins. The natural penicillin G and the biosynthetic penicillin V had a market of $4.4 billion by the late 1990s. Major markets also included semisynthetic penicillins and cephalosporins with a market of $11 billion. In 2006, the market for cephalosporins amounted to $9.4 billion and that for penicillins was $6.7 billion. By 2003, production of all beta-lactams had reached over 60,000 t. The titer of penicillin is over 100 g L^{-1} and that for cephalosporin C is about 35 g L^{-1} [16, 17]. Recovery yields are more than 90 %. There have been more than 15,000 molecules based on penicillin that have been made by semisynthesis or by total synthesis. By the mid 1990s, 160 antibiotics and their derivatives were already on the market [15, 18]. The market in 2000 was $35 billion. Despite these impressive figures, more antibiotics are needed to combat evolving pathogens, naturally resistant microbes, and bacteria and fungi that have developed resistance to current antibiotics. A new and approved cephalosporin is ceftobiprole, which is active against methicillin-resistant *S. aureus* (MRSA) and is not hydrolyzed by a number of beta-lactamases from Gram-positive bacteria [19]. Another antibiotic of note is cerulenin, an antifungal agent produced by *Acremonium caerelens*. It was the first inhibitor of fatty acid biosynthesis discovered [20]. It alkylates and inactivates the active-site nucleophylic cysteine of the ketosynthase enzyme of fatty acid synthetase by epoxide ring opening. Other properties that are desired in new antibiotics are improved pharmacological properties, ability to combat viruses and parasites, and improved potency and safety.

Pharmacological Agents

Years ago, noninfectious diseases were mainly treated with synthetic compounds. Despite testing thousands of synthetic chemicals, only a handful of promising structures was obtained. As new synthetic lead compounds became extremely difficult to find, microbial products came into play. Poor or toxic antibiotics produced by fungi such as cyclosporin A or mycotoxins such as ergot alkaloids, gibberellins, zearelanone were then successfully applied in medicine and agriculture. This led to the use of fungal products as immunosuppressive agents, hypocholesterolemic drugs, antitumor agents, and for other applications.

Hypocholesterolemic Agents

Only about 30 % of cholesterol in humans comes from the diet. The rest is synthesized by the body, predominantly in the liver. Many people cannot control their level of cholesterol at a healthy level by diet alone and require hypocholesterolemic

Fig. 1.1 Chemical structure
of lovastatin
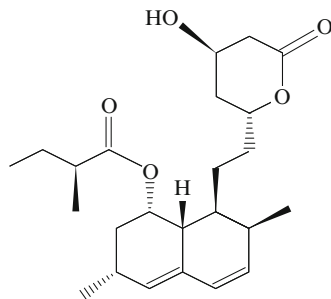
agents. High blood cholesterol leads to atherosclerosis, which is a chronic, progressive disease characterized by continuous accumulation of atheromatous plaque within the arterial wall, causing stenosis and ischemia. Atherosclerosis is a leading cause of human death. The last two decades have witnessed the introduction of a variety of anti-atherosclerotic therapies. The statins form a class of hypolipidemic drugs, formed as secondary metabolites by fungi, and used to lower cholesterol by inhibiting the rate-limiting enzyme of the mevalonate pathway of cholesterol biosynthesis; i.e., 3-hydroxymethyl glutaryl-CoA (HMG-CoA) reductase. Inhibition of this enzyme in the liver stimulates low-density lipoprotein (LDL) receptors, resulting in an increased clearance of LDL from the bloodstream and a decrease in blood cholesterol levels. They can reduce total plasma cholesterol by 20–40 %. Through their cholesterol-lowering effect, they reduce risk of cardiovascular disease, prevent stroke, and reduce development of peripheral vascular disease [21].

Currently, there are a number of statins in clinical use. They reached an annual market of nearly $30 billion before one became a generic pharmaceutical. The history of the statins has been described by Akira Endo, the discoverer of the first statin, compactin (mevastatin; ML-236B) [22]. This first member of the group was isolated as an antibiotic product of *Penicillium brevicompactum* [23]. At about the same time, it was found by Endo and coworkers as a cholesterolemic product of *Penicillium citrinum* [24]. Although compactin was not of commercial importance, its derivatives achieved strong medical and commercial success. Lovastatin (monacolin K; mevinolin; Mevacor™), was isolated in broths of *Monascus rubra* and *Aspergillus terreus* [25, 26]. Lovastatin, developed by Merck & Co. and approved by the US Food and Drug Administration (FDA) in 1987, was the first commercially marketed statin. In its chemical structure, lovastatin has a hexahydronaphthalene skeleton substituted with a *p*-hydroxy-lactone moiety (Fig. 1.1).

A semisynthetic derivative of lovastatin is Zocor® (simvastatin), one of the main hypocholesterolemic drugs, selling for $7 billion per year before becoming generic. An unexpected effect of simvastatin is its beneficial activity on pulmonary artery hypertension [27]. Another surprising effect is its antiviral activity [28]. Simvastatin is active against RNA viruses and acts as monotherapy against chronic hepatitis C virus in humans. It has been shown to act in vitro against hepatitis B virus (HBV). This virus infects 400 million people and is the most common infectious disease agent in the world. The virus causes hepatocellular cancer, which is the leading cause of cancer

death. Nucleotide analogs (lamivudine, adefovir, tenofovir, entecavir, telbuvidine) were approved for HBV infections but they only work on 11–17 % of patients. Simvastatin is synergistic with these nucleotide analogs.

Statins also have antithrombotic, anti-inflammatory, and antioxidant effects [29]. They have shown activity against multiple sclerosis, artherosclerosis, Alzheimer's Disease, and ischemic stroke [30, 31]. However, these applications have not yet been approved since more clinical studies are required. The neuroprotective effect of statins has been demonstrated in an in vitro model of Alzheimer's disease using primary cultures of cortical neurons [32]. The effect did not appear to be due to cholesterol lowering but rather to reduction in formation of isoprenyl intermediates of the cholesterol biosynthetic process. Lovastatin has shown antitumor activity against embryonal carcinoma and neuroblastoma cells [33].

Although simvastatin is usually made from lovastatin chemically in a multistep process, an enzymatic/bioconversion process using recombinant *Escherichia coli* has been developed [34]. Another statin, pravastatin ($3.6 billion in sales per year), is made via different biotransformation processes from compactin by *Streptomyces carbophilus* [35] and *Actinomadura* sp. [36]. Other genera involved in production of statins are *Doratomyces, Eupenicillium, Gymnoascus, Hypomyces, Paecilomyces, Phoma, Trichoderma,* and *Pleurotus* [37]. A synthetic compound, modeled from the structure of the natural statins, is Lipitor®, which was the leading drug of the entire pharmaceutical industry in terms of market (about $14 billion per year) for many years.

Anticancer Drugs

More than 12 million new cases of cancer were diagnosed in the world in 2008; 6.6 million cases were in men and 6.0 million in women, resulting in 7.6 million cancer-related deaths. The tumor types with the highest incidence were lung (12.7 %), breast (10.9 %), and colorectal (9.8 %). Some of the anticancer drugs in clinical use are secondary metabolites derived from plants and fungi. Among the approved products are taxol and camptothecin.

Taxol (paclitaxel) was first isolated from the Pacific yew tree, *Taxus brevifolia* [38] and later found to be a fungal secondary metabolite [39]. It is a steroidal alkaloid diterpene alkaloid that has a characteristic *N*-benzoylphenyl isoserine side chain and a tetracycline ring (Fig. 1.2). It inhibits rapidly dividing mammalian cancer cells by promoting tubulin polymerization and interfering with normal microtubule breakdown during cell division. The benzoyl group of the molecule is particularly crucial for maintaining the strong bioactivity of taxol. The drug also inhibits several fungi (species of *Pythium, Phytophthora, Aphanomyces*) by the same mechanism. In 1992, taxol was approved for refractory ovarian cancer and today is used against breast cancer and advanced forms of Kaposi's sarcoma [40]. A formulation in which paclitaxel is bound to albumin is sold under the trademark Abraxane®. Taxol sales amounted to $1.6 billion in 2006 for Bristol Myers-Squibb, representing 10 % of the company's pharmaceutical sales and its third largest selling product. It has reached $3.7 billion annual sales in international markets.
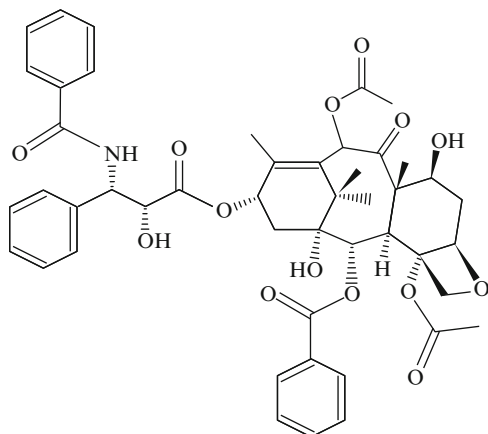
Although synthetic methods for taxol production have been tried, the chemical molecular structure is so complex that commercial synthetic production is unfeasible. Currently, Italy, the UK, the Netherlands, and other Western countries are engaged in the production of taxol by plant cell fermentation technology. Taxol production by plant cell culture of *Taxus* sp. was reported to be at 67 mg L^{-1} [41]. However, addition of methyl jasmonate, a plant signal transducer, increased production to 110 mg L^{-1}.

As stated previously, taxol has also been found to be a fungal metabolite [39, 42]. Fungi such as *Taxomyces andreanae, Pestalotiopsis microspora, Tubercularia* sp., *Phyllosticta citricarpa, Nodulisporium sylviforme, Colletotrichum gloeosporoides, Colletotrichum annutum, Fusarium maire*, and *Pestalotiopsis versicolor* produce it [39, 43–49]. The endophyte *F. maire* produces 225 µg L^{-1}. Production by *P. citricarpa* amounted to 265 µg L^{-1} [50]. Production was reported at 417 µg L^{-1} by submerged fermentation with an engineered strain of the endophytic fungus *Ozonium* sp. (EFY-21). The transformed strain overproduced the rate-limiting enzyme of taxol biosynthesis, taxadiene synthase [51]. Another endophytic fungus, *Phoma betae*, isolated from the medicinal tree *Ginkgo biloba*, produced taxol at 795 µg L^{-1} [52]. *Cladosporium cladosporoides*, an endophyte of the *Taxus media* tree, produced 800 µg L^{-1} of taxol [53]. *Metarhizium anisopiliae* H-27, isolated from the tree *Taxus chinensis*, yielded 846 µg L^{-1} [54]. Although a review of taxol production by endophytic fungi indicated that strain improvement had resulted in levels of only 0.4–1.0 mg L^{-1} [55], it was reported that another fungus, *Alternaria alternate* var. *monosporus*, from the bark of *Taxus yunanensis*, after ultraviolet and nitrosoguanidine mutagenesis, could produce taxol at 227 mg L^{-1} [56]. The endophytic fungus *P. versicolor*, from the plant *Taxus cuspidata*, produced 478 µg L^{-1} [44] and *C. annutum* from *Capsicum annuum* made 687 µg L^{-1} [45].

Another important antitumor agent is camptothecin, a modified monoterpene indole alkaloid produced by certain plants (angiosperms) and by the endophytic fungus, *Entrophospora infrequens*. The fungus was isolated from the plant

Nathapodytes foetida [38]. In view of the low concentration of camptothecin in tree roots and poor yield from chemical synthesis, the fungal fermentation is very promising for industrial production of camptothecin. It is used for recurrent colon cancer and has unusual activity against lung, ovarian, and uterine cancer [57]. Colon cancer is the second-leading cause of cancer fatalities in the USA and the third most common cancer among US citizens. Camptothecin is known commercially as Camptosar and Campto and achieved sales of $1 billion in 2003 [58]. Camptothecin's water-soluble derivatives irinotecan and topotecan have been approved and are used clinically. Metastatic colorectal cancer is treated by irinotecan whereas topotecan has use for ovarian cancer, cervical cancer, and small-cell lung cancer. A review of the activities of camptothecin and its many small and macromolecular derivatives has been published by Venditto and Simanek [59].

The cellular target of camptothecin is type I DNA topoisomerase. When patients become resistant to irinotecan, its use can be prolonged by combining it with the monoclonal antibody Erbitux (Cetuximab). Erbitux blocks a protein that stimulates tumor growth and the combination helps metastatic colorectal cancer patients expressing epidermal growth factor receptor (EGFR). This protein is expressed in 80 % of advanced metastatic colorectal cancers. The drug combination reduces invasion of normal tissues by tumor cells and the spread of tumors to new areas.

Angiogenesis, the recruitment of new blood vessels, is necessary for tumors to obtain oxygen and nutrients. Tumors actively secrete growth factors that trigger angiogenesis. Anti-angiogenesis therapy is now known as one of four cancer treatments; the other three are surgery, radiotherapy, and chemotherapy. By the end of 2007, 23 anti-angiogenesis drugs were in Phase III clinical trials and more than 30 were in Phase II. Fumagillin, a secondary metabolite of *Aspergillus fumigatus*, was one of the first agents found to act as an anti-angiogenesis compound. Next to come along were its oxidation product ovalacin and the fumagillin analog TNP-470 (=AGM-1470). TNP-470 binds to and inhibits type 2 methionine aminopeptidase. This interferes with amino-terminal processing of methionine, which may lead to inactivation of enzymes essential for growth of endothelial cells. In animal models, TNP-470 effectively treated many types of tumors and metastases.

Inhibitors of farnesyltransferase (FTIs) have anticancer activity because farnesylation is required for activation of Ras, a necessary step in cancer progression. They also induce apoptosis in cancer cells. The fungus *Phoma* sp. FL-415 produces an FTI known as TAN-1813 [60].

Immunosuppressant Drugs

An individual's immune system is capable of distinguishing between native and foreign antigens and to mount a response only against the latter. Suppressor cells are critical in the regulation of the normal immune response. The suppression of the immune response, either by drugs or radiation, in order to prevent the rejection of grafts or transplants or to control autoimmune diseases, is called immunosuppression.

Microbial compounds capable of suppressing the immune response have been discovered as fungal secondary metabolites. Cyclosporin A was originally discovered in the 1970s as a narrow-spectrum antifungal peptide produced by the mold, *Tolypocladium nivenum* (previously *Tolupocladium inflatum*) in an aerobic fermentation [61]. Cyclosporins are a family of neutral, highly lipophilic, cyclic undecapeptides containing some unusual amino acids, synthesized by a nonribosomal peptide synthetase, cyclosporin synthetase. Discovery of the immunosuppressive activity of this secondary metabolite led to use in heart, liver, and kidney transplants and to the overwhelming success of the organ transplant field [62]. Cyclosporin was approved for use in 1983. It is thought to bind to the cytosolic protein cyclophilin (immunophilin) of immunocompetent lymphocytes, especially *T*-lymphocytes. This complex of cyclosporin and cyclophilin inhibits calcineurin, which under normal circumstances is responsible for activating the transcription of interleukin-2. It also inhibits lymphokine production and interleukin release and therefore leads to a reduced function of effector *T*-cells. Annual world sales of cyclosporin A are approximately $2 billion. Cyclosporin A also has activity against corona viruses [63].

Studies on the mode of action of cyclosporin, and the later-developed immunosuppressants from actinomycetes, such as sirolimus (a rapamycin) and FK-506 (tacrolimus), have markedly expanded current knowledge of *T*-cell activation and proliferation. These agents act by interacting with an intracellular protein (an immunophilin), thus forming a novel complex that selectively disrupts the signal transduction events of lymphocyte activation.

Their targets are inhibitors of signal transduction cascades in microbes and humans. In humans, the signal transduction pathway is required for activation of *T* cells.

A very old broad-spectrum antibiotic, actually the first antibiotic ever discovered, is mycophenolic acid, which has an interesting history. Bartolomeo Gosio (1863–1944), an Italian physician, discovered the compound in 1893 [64]. Gosio isolated a fungus from spoiled corn, which he named *Penicillium glaucum*, which was later reclassified as *P. brevicompactum*. He isolated crystals of the compound from culture filtrates in 1896 and found it to inhibit growth of *Bacillus anthracis*. This was the first time an antibiotic had been crystallized and the first time that a pure compound had ever been shown to have antibiotic activity. The work was forgotten but fortunately the compound was rediscovered by Alsberg and Black [65] and given the name mycophenolic acid. They used a strain originally isolated from spoiled corn in Italy called *Penicillium stoloniferum*, a synonym of *P. brevicompactum*. The chemical structure was elucidated many years later (1952) by Birkinshaw and coworkers [66] in England. Mycophenolic acid has antibacterial, antifungal, antiviral, antitumor, antipsoriasis, and immunosuppressive activities. Its antiviral activity is exerted against yellow fever, dengue virus, and Japanese encephalitis virus [67]. It was never commercialized as an antibiotic because of its toxicity, but its 2-morpholinoethylester was approved as a new immunosuppressant for kidney transplantation in 1995 and for heart transplants in 1998 [68]. The ester is called mycophenolate mofetil (CellCept) and is a prodrug that is hydrolyzed to mycophenolic acid in the body. It is sometimes used along with cyclosporin in kidney, liver, and heart transplants. Mycophenolic acid also appears to have anti-angiogenic activity [69].

Applications of Mycotoxins

Fungi produce poisons called mycotoxins, which, strangely enough, have been harnessed as medically useful agents. These agents (e.g., ergot alkaloids) caused fatal poisoning of humans and animals (ergotism) for centuries by consumption of bread made from grain contaminated with species of the fungus *Claviceps*. However, mycotoxins later were found useful for angina pectoris, hypertonia, serotonin-related disturbances, inhibition of protein release in agalactorrhea, reduction in bleeding after childbirth, and prevention of implantation in early pregnancy [70, 71]. Their physiological activities include inhibition of action of adrenalin, noradrenalin, and serotonin, as well as the contraction of smooth muscles of the uterus. Antibiotic activity is also possessed by some ergot alkaloids.

Members of the genus *Gibberella* produce zearelanone and gibberellins. Zearelanone is an estrogen made by *Gibbberella zeae* (syn. *Fusarium graminearum*) [72]. Its reduced derivative zeranol is used as an anabolic agent in sheep and cattle, which increases growth and feed efficiency. Giberellic acid, a member of the mycotoxin group known as gibberellins, is a product of *Gibberella fujikori* and causes "foolish rice seedling" disease in rice [73]. Gibberellins are employed to speed up the malting of barley, improve the quality of malt, increase the yield of vegetables, and cut the time in half for obtaining lettuce and sugar beet seed crops. They are isoprenoid growth regulators, controlling flowering, seed germination, and stem elongation [74]. More than 25 t are produced annually with a market of over $100 billion.

Inhibitors of Enzyme Activity

Enzyme inhibitors have received increased attention as useful tools, not only for the study of enzyme structures and reaction mechanisms, but also for potential utilization in medicine and agriculture. Several enzyme inhibitors with various industrial uses have been isolated from microbes [75]. Among the most important are the statins and hypocholesterolemic drugs discussed previously. Fungal products are also used as enzyme inhibitors against cancer, diabetes, poisoning, and Alzheimer's disease. The enzymes inhibited include acetylcholinesterase, protein kinase, tyrosine kinase, glycosidases, and others [76].

Pigments

Since 800 AD, *Monascus purpurea* has been grown on rice to prepare koji or Angkak (red rice), which is used as a traditional Chinese food and medicine [77]. Monascorubramine and rubropunctatin are water-soluble red pigments formed upon reaction of the orange pigments monascorubrin and rubropunctatin with amino acids in fermentation media [78]. The fungus is used to prepare red rice, wine, soybean cheese, meat, and fish. It is authorized in Japan and China for food use. There are 54 known *Monascus* pigments. They have an amazing number of activities:

antimicrobial, anticancer, anti-mutagenesis, antidiabetes, anti-obesity, anti-inflammatory, cholesterol-lowering, immunosuppressive, and hypotensive [79, 80]. Nutritional control of the formation of the red pigments has been described in a series of publications by Lin and Demain [81–84].

Phaffia rhodozyma (*Xanthophyllomyces dendrorhous*) is a heterobasidiomycetous yeast that has become the most important microbial source for preparation of the carotenoid astaxanthin [85, 86]. This oxygenated carotenoid pigment is used in the feed, food, and cosmetic industries. It is responsible for the orange to pink color of salmonid flesh and the reddish color of boiled crustacean shells. Feeding of pen-reared salmonids with a diet containing this yeast induces pigmentation of the white muscle [87]. It is a very good antioxidant, 10 times more active than beta-carotene and 100 times more than alpha-tocopherol. It is the second most important carotenoid. Astaxanthin enhances the immune system, and protects skin from radiation injury and cancer. It can be produced synthetically as hydroxyl-astaxanthin from petrochemicals with a selling price of $2,500 per kg. However, the natural product is favored because the synthetic product is a mixture of stereoisomers. Natural astaxanthin is more stable than the synthetic version and more bioavailable. The natural product is present in algae and fish as mono- and di-esters of fatty acids. However, it is difficult to hydrolyze the esters from algae, which limits its usage to trout and salmon. The yeast product is better since it is the 97 % free, non-esterified (3R, 3'R) stereoisomer. The natural product is more expensive ($7,000 per kg) than synthetic astaxanthin ($2,500 per kg). The astaxanthin market was $219 million in 2007 with 97 % being synthetic. Most of the production processes with the yeast yield levels of astaxanthin lower than 100 mg L^{-1}. However, white light improved production to 420 mg L^{-1} [88] and mutant strain UBv-AX2 can make 580 mg L^{-1} [89].

Sweeteners

Thaumatin, a protein produced by the plant *Thaumatococcus danielli*, can also be produced by *P. roqueforti and Aspergillus niger* var *awamori* [90]. Thaumatin is intensely sweet (i.e., 3,000 times sweeter than sucrose) and is approved as a food-grade ingredient. Production by *A. niger* var *awamori* was improved from 2 mg L^{-1} up to 14 mg L^{-1} by increasing gene dosage and use of a strong promoter [91]. The sweetener xylitol, normally produced by *Pichia stipitis*, can be produced by recombinant *Saccharomyces cerevisiae* in higher concentrations by transforming the *XYL1* gene of *P. stipitis* into *S. cerevisiae*. The gene encodes a xylose reductase [92].

Conclusion

Microorganisms have greatly contributed for about 85 years to the development of medicine and agriculture. However, due to different situations, pathogenic microbes have become resistant to many antibiotics creating a dangerous situation and

therefore the need for new antibiotics is imperative. Unfortunately, most of the large pharmaceutical companies have abandoned the search for new antimicrobial compounds. Due to economics, they have concluded that drugs directed against chronic diseases offer a better revenue stream than do antimicrobial agents, for which the length of treatment is short and government restriction is likely. Some small pharmaceutical and biotechnology companies are still developing antibiotics but most depend on venture capital rather than sales income, and with the present regulations, face huge barriers to enter into the market. These barriers were raised with the best intentions of ensuring public safety but they are having the opposite effect; i.e., termination of antibiotic development while resistance continues to increase [93]. However, there are some new bright possibilities. One of the more promising is the utilization of uncultivated microorganisms. Considering that 99 % of bacteria and 95 % of fungi have not yet been cultivated in the laboratory, efforts to find means to grow such uncultured microorganisms is proceeding and succeeding [5]. Furthermore, researchers are now extracting bacterial DNA from soil samples, cloning large fragments into, for example, bacterial artificial chromosomes, expressing them in a host bacterium and screening the library for new antibiotics. This metagenomic effort could open up the exciting possibility of a large untapped pool from which new natural products could be discovered [94]. Another exciting possibility is that of genome mining [95]. In addition to these relatively new techniques, chemical and biological modification of old antibiotics could still supply new and powerful drugs. These comments also apply to non-antibiotics such as antitumor agents and other microbial products. In addition, natural products must continue to be tested for desirable therapeutic activities. I believe that significant progress in identifying new antibiotics, oncology therapeutics, and other useful medicines will be made, probably not by the big pharmaceutical companies, but by biotechnology companies and small research groups from institutes and universities.

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