The Journal of Antibiotics (2017) 70, 347–360

www.nature.com/ja

REVIEW ARTICLE

Production of valuable compounds by molds and yeasts

Arnold L Demain[1] and Evan Martens[2]

We are pleased to dedicate this paper to Dr Julian E Davies. Julian is a giant among microbial biochemists. He began his professional career as an organic chemistry PhD student at Nottingham University, moved on to a postdoctoral fellowship at Columbia University, then became a lecturer at the University of Manchester, followed by a fellowship in microbial biochemistry at Harvard Medical School. In 1965, he studied genetics at the Pasteur Institute, and 2 years later joined the University of Wisconsin in the Department of Biochemistry. He later became part of Biogen as Research Director and then President. After Biogen, Julian became Chair of the Department of Microbiology at the University of British Columbia in Vancouver, Canada, where he has contributed in a major way to the reputation of this department for many years. He also served as an Adjunct Professor at the University of Geneva. Among Julian's areas of study and accomplishment are fungal toxins including α-sarcin, chemical synthesis of triterpenes, mode of action of streptomycin and other aminoglycoside antibiotics, biochemical mechanisms of antibiotic resistance in clinical isolates of bacteria harboring resistance plasmids, their origins and evolution, secondary metabolism of microorganisms, structure and function of bacterial ribosomes, antibiotic resistance mutations in yeast ribosomes, cloning of resistance genes from an antibiotic-producing microbe, gene cloning for industrial purposes, engineering of herbicide resistance in useful crops, bleomycin-resistance gene in clinical isolates of *Staphylococcus aureus* and many other topics. He has been an excellent teacher, lecturing in both English and French around the world, and has organized international courses. Julian has also served on the NIH study sections, as Editor for several international journals, and was one of the founders of the journal *Plasmid*. We expect the impact of Julian's accomplishments to continue into the future.
The Journal of Antibiotics (2017) **70,** 347–360; doi:10.1038/ja.2016.121; published online 12 October 2016

INTRODUCTION

Microbes have contributed significantly to improving the health and well-being of humans. The natural products that they have yielded have not only helped eradicate disease and alleviate suffering, but also greatly increased the average life expectancy. The first major contribution of microbes 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 that 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 8000 years, initially for beer and wine production, and recorded in the written history of ancient Egypt and Mesopotamia. During the past 4000 years, *Penicillium roqueforti* has been utilized for cheese production, and for the past 3000 years, soy sauce in Asia and bread in Egypt represented examples of traditional fermentations.[1]

Natural products from microbes have a broad range of therapeutic applications and are often produced via primary or secondary metabolism. Because of 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, ~20–25% show biological activity and, of these, ~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, ~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]

Biosynthetic genes are present in clusters coding for large, multidomain and multimodular enzymes. Some examples of these enzymes include polyketide synthases, prenyltransferases, nonribosomal 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]

Given that the vast majority of microbes in nature have yet to be cultured (~99%), there have been major advances in isolating and growing different microbial species.[5] Furthermore, metagenomics—that is, 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

[1]Research Institute for Scientists Emeriti (RISE), Drew University, Madison, NJ, USA and [2]Cempra, Chapel Hill, NC, USA
Correspondence: Professor AL Demain, Research Institute for Scientists Emeriti (RISE), Drew University, 36 Madison Avenue, Madison, NJ 07940, USA.
E-mail: ademain@drew.edu
Received 28 June 2016; revised 31 August 2016; accepted 1 September 2016; published online 12 October 2016

use for humans is great. A method to predict secondary metabolite gene clusters in filamentous fungi has recently been devised.[8]

Interestingly, microbial production of secondary metabolites is limited to a very low level by certain regulatory mechanisms. Despite this, the extent of such production is sufficient for the microbe to compete with other organisms or maintain a commensal/mutual relationship with other species. 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'. Thousand-fold increases in production of small molecules have been obtained by mutagenesis and/or genetic engineering. The use of genome mining to discover new fungal natural products has been reviewed by Wiemann and Keller.[10] 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, nitrogen and phosphorus sources, metals and the final product itself. Avoidance of enzyme decay is also desired.[4,11]

BROAD USE OF SECONDARY METABOLITES PRODUCED BY FUNGI

Given the diverse array of secondary metabolites that fungi are capable of producing, the pharmaceutical industry began to focus their efforts on the screening of compounds for indications other than anti-infectives.[12,13] As 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.[14] Other products include antitumor drugs, hypocholesterolemic drugs, enzyme inhibitors, gastrointestinal motor stimulator agents, ruminant growth stimulants, insecticides, herbicides and antiparasitics versus coccidia and helminths.

In the past, the treatment of noninfectious disease relied heavily upon synthetic compounds, yet only a select few turned out to be promising. 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 and zearelanone, were then successfully applied in medicine and agriculture. This led to the use of fungal products as immunosuppressive agents, hypocholesterolemic drugs and antitumor agents and for other applications.

Anti-rejection drugs (agents that suppress the immune system)

The immune system is our body's main defense against foreign antigens and pathogenic microorganisms. However, it is essential that the immune system recognizes 'native' antigens in order to avoid launching an immune response. 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.

Secondary metabolites produced by fungi have yielded compounds that function as immunosuppressants. Cyclosporin A was originally discovered in the 1970s as a narrow-spectrum antifungal peptide produced by the mold, *Tolypocladium nivenum* (previously *Tolypocladium inflatum*) in an aerobic fermentation.[15] 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 its use in heart, liver and kidney transplants and to the overwhelming success of the organ transplant field.[16] 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 that 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 ~$2 billion. Cyclosporin A also has activity against coronaviruses.[17]

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. Fingolimod (FTY720), another immunosuppressant, was approved by the US Food and Drug Administration (FDA) in 2010, specifically for relapsing forms of multiple sclerosis. The drug, initially discovered by Professors Fujita, Yoshitomi and Taito in collaboration, is a derivative of myriocin isolated from the fungus *Isaria sinclairii*. The annual world sales of fingolimod are ~$2.7 billion.

One of the first antibiotics to be discovered, with a broad spectrum of activity, was mycophenolic acid. Bartolomeo Gosio (1863–1944), an Italian physician, discovered the compound in 1893.[18] Gosio isolated a fungus from spoiled corn that he named *Penicillium glaucum*, and that was later reclassified as *Penicillium 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[19] 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 *et al.*[20] 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.[21] 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.[22] 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. Furthermore, the mycophenolic acid delayed-release tablet was approved in 2004 by the FDA as an antimetabolite immunosuppressant indicated for prophylaxis of organ rejection in kidney transplant. This delayed-release tablet is called Myfortic and is produced by Novartis, with annual sales of $637 million in 2013, $543 million in 2014 and $441 million in 2015. In addition, mycophenolic acid appears to have anti-angiogenic activity.[23]

Agents that block enzyme activity

Drugs that are enzyme inhibitors may provide key functions not only for treating human disease, but also in agriculture, enzyme structure elucidation and reaction mechanisms. Several enzyme inhibitors with various industrial uses have been isolated from microbes.[24] Among the most important are the statins and hypocholesterolemic drugs discussed below. 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.[25]

Cholesterol-lowering agents

In humans, it is estimated that ~30% of cholesterol originates from the diet, whereas the remaining 70% is synthesized primarily in the liver. Many people cannot control their level of cholesterol at a healthy level by diet alone and require hypocholesterolemic agents. High blood cholesterol leads to atherosclerosis, 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 past 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 are used to lower cholesterol by inhibiting the rate-limiting enzyme of the mevalonate pathway of cholesterol biosynthesis; that is, 3-hydroxymethyl glutaryl-CoA reductase. Inhibition of this enzyme in the liver stimulates low-density lipoprotein receptors, resulting in an increased clearance of low-density lipoprotein 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 the risk of cardiovascular disease, prevent stroke and reduce the development of peripheral vascular disease.[26]

Statins, which had reached an annual market of nearly $30 billion before one became a generic drug, are widely used in clinical practice. The history of the statins has been described by Akira Endo, the discoverer of the first statin, compactin (mevastatin; ML-236B).[27] This first member of the group was isolated as an antibiotic product of *P. brevicompactum*.[28] At about the same time, it was found by Endo *et al.*[29] as a cholesterolemic product of *Penicillium citrinum*. 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*.[30,31] Lovastatin, developed by Merck and approved by the 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 (Figure 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.[32] Another surprising effect is its antiviral activity.[33] 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. This virus infects 400 million people and is the most common infectious disease agent in the world. The virus causes hepatocellular cancer, the leading cause of cancer death. Nucleotide analogs (lamivudine, adefovir, tenofovir, entecavir, telbuvidine) were approved for hepatitis B virus infections but they only work on 11–17% of patients. Simvastatin is synergistic with these nucleotide analogs.

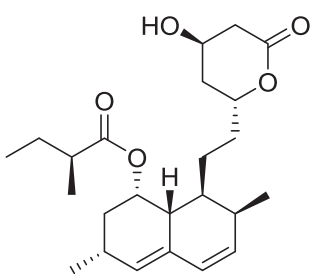

Figure 1 Chemical structure of lovastatin.

Statins also have antithrombotic, anti-inflammatory and antioxidant effects.[34] They have shown activity against multiple sclerosis, atherosclerosis, Alzheimer's Disease and ischemic stroke.[35,36] However, these applications have not yet been approved as 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.[37] The effect did not appear to be because of cholesterol lowering but rather reduction in formation of isoprenyl intermediates of the cholesterol biosynthetic process. Lovastatin has shown antitumor activity against embryonal carcinoma and neuroblastoma cells.[38]

Although simvastatin is usually made from lovastatin chemically in a multistep process, an enzymatic/bioconversion process using recombinant *Escherichia coli* has been developed.[39] Another statin, pravastatin (Pravacol) ($3.6 billion in sales per year), is made via different biotransformation processes from compactin by *Streptomyces carbophilus*[40] and *Actinomadura* sp.[41] Both simvastatin and pravastatin are synthetic variants of the naturally occurring lovastatin and compactin. Pravastatin can be produced from compactin but it involves an expensive dual-step fermentation and biotransformation process. Mclean *et al.*[42] reprogrammed *Penicillium chrysogenum* involving discovery and engineering of an enzyme involved in hydroxylation of compactin. This resulted in a single-step fermentation yielding pravastatin at $>6\ g\ l^{-1}$.

Other genera involved in production of statins are *Doratomyces*, *Eupenicillium*, *Gymnoascus*, *Hypomyces*, *Paecilomyces*, *Phoma*, *Trichoderma* and *Pleurotus*.[43] A synthetic compound, modeled from the structure of the natural statins, is Lipitor, the leading drug of the entire pharmaceutical industry in terms of market (~$14 billion per year) for many years.

Prebiotics

Prebiotics are nondigestible products stimulating growth in the colon of bacteria such as *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*.[44] They include galacto-oligosaccharides, fructo-oligosaccharides, lactulose, lactitol and its hydrolysates, malto-oligosaccharides, inulin and resistant starch. Titers are as follows: lactosucrose at $192\ g\ l^{-1}$ from lactose or sucrose by levanosucrase from *Sterigmatomyces elviae* and fructooligosaccharide at $116\ g\ l^{-1}$ from sucrose by β-fructofuranosidase from *Aspergillus japonicas*. Prebiotics are used in the nutraceutical, pharmaceutical, animal feed and aquaculture areas. They stimulate growth of beneficial intestinal bacteria and maintain health of humans by suppression of potentially harmful bacteria, improvement of defecation, elimination of ammonia, prevention of colon cancer, stimulation of mineral adsorption and lowering of cholesterol and lipids.

Food additives functioning as sugar substitutes

Aspergillus niger var. awamori, *P. roqueforti* and the plant *Thaumatococcus danielli* are all capable of producing the protein

thaumatin.[45] Thaumatin is intensely sweet (that is, 3000 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.[46] 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.[47]

Toxins

Mycotoxins, which are poisons produced by fungi, have actually been useful therapeutic agents for a variety of medical conditions and ailments. These agents (for example, ergot alkaloids) had 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.[48,49] 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 *Gibberella zeae* (syn. *Fusarium graminearum*).[50] Its reduced derivative zeranol is used as an anabolic agent in sheep and cattle that increases growth and feed efficiency. Gibberellic acid, a member of the mycotoxin group known as gibberellins, is a product of *Gibberella fujicuroi* and causes 'foolish rice seedling' disease in rice.[51] 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.[52] More than 25 are produced annually with a market of over $100 billion.

Antineoplastic drugs

In 2008, there were over 12 million new cases of cancer diagnosed throughout the world that resulted in ~7.6 million deaths. Lung (12.7%), breast (10.9%) and colorectal (9.8%) cancer had the highest incidence rates. Some of the anticancer drugs in clinical use include taxol and camptothecin, the secondary metabolites derived from plants and fungi.

Taxol (paclitaxel) is a fungal secondary metabolite first isolated from the Pacific yew tree, *Taxus brevifolia*.[53,54] It is a steroidal diterpene alkaloid that has a characteristic N-benzoylphenyl isoserine side chain and a tetracycline ring (Figure 2).

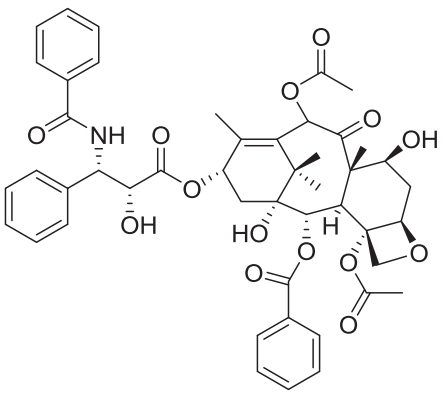

Figure 2 Chemical structure of taxol.

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* and *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.[55] 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 reached $3.7 billion annual sales in international markets.

Although synthetic methods for taxol production have been attempted, the chemical molecular structure is so complex that commercial synthetic production is unfeasible. Currently, Italy, United Kingdom, 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}.[56] 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.[54,57] Fungi such as *Taxomyces andreanae*, *Pestalotiopsis microspora*, *Tubercularia* sp., *Phyllosticta citricarpa*, *Nodulisporium sylviforme*, *Colletotrichum gloeosporoides*, *Colletotrichum annutum*, *Fusarium maire* and *Pestalotiopsis versicolor* produce it.[54,58–64] The endophyte *F. maire* produces 225 μg l^{-1}. Production by *P. citricarpa* amounted to 265 μg l^{-1}.[65] 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.[66] Another endophytic fungus, *Phoma betae*, isolated from the medicinal tree *Ginkgo biloba*, produced taxol at 795 μg l^{-1}.[67] *Cladosporium cladosporoides*, an endophyte of the Taxus media tree, produced 800 μg l^{-1} of taxol.[68] *Metarhizium anisopiliae* H-27, isolated from the tree *Taxus chinensis*, yielded 846 μg l^{-1}.[69] 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},[70] 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}.[71] The endophytic fungus *P. versicolor*, from the plant *Taxus cuspidata*, produced 478 μg l^{-1} [59] and *C. annutum* from *Capsicum annuum* made 687 μg l^{-1}.[60]

Camptothecin, a modified monoterpene indole alkaloid produced by certain plants (angiosperms) and by the endophytic fungus, *Entrophospora infrequens*, is another important antitumor agent. The fungus was isolated from the plant *Nathapodytes foetida*.[53] 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 cancers.[72] Colon cancer is the second leading cause of cancer fatalities in the United States 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.[73] 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.[74]

Chemically produced derivatives of camptothecin used for cancer include 10-hydroxycamptothecin, topotecan, irinotecan and SN-38. Camptothecin is a multibillion dollar anticancer drug and the fourth largest anticancer drug derived from plants after taxol, vincristine and vinblastine. Camptothecin is produced by the plants *Camptotheca acuminata* and *Nothapodyta foetida*. An endophytic fungus producing camptothecin is *E. infrequens* from the bark of *N. foetida*. Recently, it was found that *Trichoderma atroviridi* strain LY357, an endophytic fungus from *C. acuminata*, was an improved producer of camptothecin. The endophytic fungus produced 142 $\mu g\,l^{-1}$ of camptothecin in the presence of the elicitor methyljasmonate and XAD adsorbant resin.[75]

Type 1 DNA topoisomerase has been identified as the cellular target of camptothecin. 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. 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.

The process by which tumor cells recruit new blood vessels for oxygen and nutrients is known as angiogenesis. 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 that 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.

Farnesylation, which is required for the activation of Ras, a necessary step in cancer progression, has been a target for inhibitors of farnesyltransferase because of their anticancer activity. They also induce apoptosis in cancer cells. The fungus *Phoma* sp. FL-415 produces an inhibitor of farnesyltransferase known as TAN-1813.[76]

Pigments

Carotenoids, a subfamily of terpenoids, are tetra-terpenoid yellow to red pigments that are antioxidants. They are made by plants, fungi, algae and bacteria and used as nutritional supplements and food additives and in cosmetics. They can be differentiated into carotenes that are hydrocarbons, such as lycopenes and β-carotene, and their oxygenated derivatives, that is, xanthophylls, such as lutein, zeaxanthin and astaxanthin.

Production of carotenoids by microbes has been reviewed by Sanchez *et al.*[77] Over 600 carotenoids are known including lycopene, zeaxanthin, astaxanthin, β-carotene, lutein and cantaxanthin. They are used as nutrients, supplements, food ingredients, feed additives, antioxidants, anticancer agents, immune modulators and cosmetic products. Carotenoids had a 2010 market of $1.2 billion.[78] They are C_{40} isoprenoids. Carotenoids are produced on a large scale by fungi such as *Dunalliela salina*, *Xanthophyllomyces dendrorhous* (formerly *Phaffia rhodozyma*) and *Blakeslea trispora*. β-Carotene is the major carotenoid product with a 2010 market of $261 million. It is mainly produced by *Mucor*, *Phycomyces* and *B. trispora*. *B. trispora* produces β-carotene at 9 $g\,l^{-1}$.

Because of their antioxidant properties and health-related functions, xanthophylls (lutein, zeaxanthin and astaxanthin) sell for multimillion dollars each year. The astaxanthin market is $252 million for fish food and $30 million for human use. It sells for $2500 kg^{-1} for the synthetic form and $7000 kg^{-1} for the natural form. *X. dendrorhous* produces 420 $mg\,l^{-1}$ of astaxanthin. Astaxanthin, lycopene, β-carotene and cantaxanthin are used in products such as beverages, dairy foods, cereal products, cosmetics and pharmaceuticals and in aquaculture.

Adaptive laboratory evolution was used to increase microbial production of carotenoids in a genetically engineered *S. cerevisiae* strain. It was carried out by using a periodic hydrogen peroxide shocking strategy. The improved production was because of upregulation of genes related to biosynthesis of lipid and mevalonate.[79] Carotenoid production amounted to 16 $mg\,g^{-1}$ dry cell weight.

The main microbe producing carotenes is the fungus *B. trispora*. Fermentative production is stimulated by oxidative stress induced by butylated hydroxytoluene, enhanced dissolved oxygen levels, iron ions and liquid paraffin.

Lycopene and β-carotene are highly unsaturated isoprene derivatives that are pigments that stimulate the immune system and prevent degenerative diseases and cancer. Carotenes are also effective antioxidants. They are utilized as nutrient supplements, animal feeds, pharmaceuticals and as coloring agents in foods and feeds. Their market is growing at 2.3% per year.[80] They are obtained by (1) microbial production, (2) from plants and (3) synthetically. Carotenoids absorb light and in photosynthetic organisms, protect against excess light and prevent formation and reaction of reactive oxygen species. As antioxidants, they protect against oxidative damage elicited by oxidizing agents and free radicals. Astaxanthin is one of the best scavengers of reactive oxygen species, whereas β-carotene is a potent scavenger of reactive nitrogen species.

Monascus purpurea, a mold species, has played an important role in traditional Chinese food and medicine since 800 AD. Specifically, it has been used to prepare popular dishes such as koji or Angkak (red rice).[81] Monascorubramine and rubropunctatin are water-soluble red pigments formed upon reaction of the orange pigments monascorubrin and rubropunctatin with amino acids in fermentation media.[82] The fungus is used to prepare red rice, wine, soy-bean 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, antimutagenesis, antidiabetic, antiobesity, anti-inflammatory, cholesterol lowering, immunosuppressive and hypotensive.[83,84] Nutritional control of the formation of the red pigments has been described in a series of publications by Lin and Demain.[85–88]

C50 carotenoids, such as sarcinaxanthin and its glucosides, are more powerful quenchers of singlet oxygen than β-carotene. They have potential for use in nutriceuticals, pharmaceuticals and derived products such as apocarotenoids or norisoprenoids. Vitamin A is a norisoprenoid and a cleavage product of β-carotene (β-carotene is also known as provitamin A). Other norisoprenoids include safranal (providing saffron flavor to sauces and paella dishes), bixin (a pigment in annatto used to color cheeses), damascenone (a part of many perfumes) and ionones (for flavoring of soft drinks, candies and tobacco). Other norisoprenoids include the plant hormone abscisic acid and strigolactones, having functions in plants. C40 carotenoids, that is, terpenoids, can be produced by metabolically engineered

S. cerevisiae, as is β-carotene. The engineering involves introduction of three genes from the astaxanthin producer *X. dendrorhous*.

One of the most important microbial sources for preparation of the keto-carotenoid astaxanthin is *P. rhodozyma* (*X. dendrorhous*), a heterobasidiomycetous yeast.[89,90] Each year, 130 tons of astaxanthin are used for aquaculture and poultry. 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.[91] It is a very good antioxidant, 10 times more active than β-carotene and 100 times more than α-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 \$2500 kg^{-1}. 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; that is, it has a higher degree of absorption into a living system. 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, limiting its usage to trout and salmon. The yeast product is better as it is the 97% free, nonesterified (3R, 3'R) stereoisomer. The astaxanthin market was \$219 million in 2007, with 97% being synthetic. Most of the production processes with the yeast yield levels of astaxanthin $<100\ mg\ l^{-1}$. However, white light improved production to 420 $mg\ l^{-1}$,[92] and mutant strain UBv-AX2 can make 580 $mg\ l^{-1}$.[93]

Antimicrobials

The filamentous fungi produce 22% of the nearly 12 000 antibiotics that were known in 1955.[94,95] The β-lactams, which constitute a major part of the antibiotic market, and include the penicillins, cephalosporins, clavulanic acid and carbapenems, are the most important class of antibiotics in terms of use. 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 β-lactams had reached over 60 000 tons. The titer of penicillin is over $100\ g\ l^{-1}$ and that for cephalosporin C is at least $35\ g\ l^{-1}$.[96,97] Recovery yields are >90%. There have been >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 in the market.[95,98] The market in 2000 was \$35 billion.

1,3-Diaminopropane (1,3-DAP) is secreted by *P. chrysogenum* and *Acremonium chrysogenum*. Both it and spermidine (that contains 1,3-DAP) increase transcription levels of the penicillin biosynthetic genes *pcbAB*, *pcbC* and *penDE*.[99] They thus stimulate production of penicillin G. The mechanism appears to involve stimulation of the expression of *laeA*, a global regulator that acts epigenetically on expression of secondary metabolism genes via heterochromatin reorganization. 1,3-DAP also stimulates production of a cephamycin in *Amycolatopsis lactamdurans*. Spermidine's activity appears to be due to 1,3-DAP. Genes coding for three enzymes involved in the conversion were found to be present in the *P. chrysogenum* genome.

Because of the emergence of resistance among fungi and bacteria to current antibiotics, naturally resistant microbes and newly evolving pathogens, more antibiotics are urgently needed. A new and approved cephalosporin is ceftobiprole that is active against methicillin-resistant *S. aureus* and is not hydrolyzed by a number of β-lactamases from Gram-positive bacteria.[100] Another antibiotic of note is cerulenin, an antifungal agent produced by *Acremonium caerelens*. It was the first inhibitor of fatty acid biosynthesis discovered.[101] 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. A new antifungal natural product is parnafungin, produced by *Fusarium lavarum*, that inhibits poly(A) polymerase in *Candida albicans* as well as a broad range of pathogenic fungi.[102]

A major antibiotic problem has been the development of resistance to carbapenem antibiotics, such as imipenem and meropenem, by Gram-negative pathogens. This is mainly because of the occurrence of extended spectrum metallo-β-lactamases such as NDM-1 (New Delhi metallo-β-lactamase). King *et al.*[103] isolated a natural product called aspergillomarasmine (AMA) from the soil fungus *Aspergillus versicolor* that inhibits NDM-1 and another metallo-β-lactamases called VIM-2. AMA is a peptide inhibitor of metalloproteinases. AMA fully restored the activity of meropenem against bacteria carrying NDM or VIM metallo-β-lactamases. The work was done by Gerard Wright and his group at McMaster University.[103–105] NDM-1 requires zinc and AMA removes zinc from the enzyme. The combination of AMA and the carbapenem has shown its effect in mice and in human cell culture.

Biofilm formation by bacteria allows pathogenic bacteria to resist dispersal and inhibition by conventional chemotherapy.[106] Biosurfactants are amphiphilic compounds containing a hydrophilic region (polar or nonpolar) and a hydrophobic region (lipid or fatty acid). They act as antibiofilm agents and include sophorolipids. Some sophorolipids are produced by *Candida* species and are active against biofilm-forming *E. coli* and *Bacillus subtilis*.

Antimalarial agent

Malaria is a major cause of illness and death, especially in tropical and subtropical areas of the world.[107] There are 500 million new cases every year, killing 1.5 million people, mainly young children and pregnant women. Quinine from the bark of the Chinchona tree and artemisinin from the Chinese herb (*Artemisia annua*) have been the two major drugs used against malaria. Quinine has been used for >1000 years but artemisinin is a newer drug. Quinine has some side effects, such as arrhythmia, thrombocythemia and cinchonism, and this is the main reason for the extensive use of artemisinin. Artemisinin is an endoperoxide sesquiterpine lactone, the most potent and effective antimalarial and is useful against multidrug-resistant *Plasmodium falciparum*. Resistance to artemisinin and its derivatives is increasing but is still mild. The level of artemisinin in *A. annua* is very low (0.01–1% of the weight of the dried leaves). Thus, genetic engineering has been pursued. A genetically engineered *S. cerevisiae* strain producing 100 $mg\ l^{-1}$ of artemisinic acid has been developed by the Keasling group in Berkeley, California. Artemisinic acid can be chemically converted to artemisinin. Keasling's company, Amyris Biotechnologies, has increased the amount of artemisinic acid produced by one million fold. The artemisinin precursor amorpha-4, 11-diene is made by the engineered *S. cerevisae* at $40\ g\ l^{-1}$.[108]

Organic acids

Carboxylic acids are made mainly by catalysis from petroleum-based precursors but interest in microbial production is increasing.[109] Annual production of these compounds is as follows (ktons): acetic

acid: 10 000; acrylic acid: 4200; 3-hydroxypropionic acid: 3600; adipic acid: 3000; citric acid: 1600; lactic acid: 450; fumaric acid: 200; gluconic acid: 87; itaconic acid: 80; malic acid: 60; glucaric acid: 42; glycolic acid: 40; and succinic acid: 37. Acetic and lactic acids are used as food preservatives. Lactic acid is also used to produce the biodegradable polymer polylactide. Citric and malic acids are food additives. Gluconic acid is used to chelate divalent and trivalent metal ions. Acrylic and adipic acids are employed to make polymers. Glycolic acid is used in the textile industry as a tanning and dyeing agent. The main acids showing promise for microbial production are succinic, lactic and itaconic acids. *S. cerevisiae* could become a leading organism for carboxylic acid production, mainly because it can grow at low pH. It was the first eukaryote to have its entire genome sequenced. Considerable genetic engineering has been done with this yeast. Also important is its naturally occurring, episomally replicating plasmid, named the 2-μ plasmid. The organism can make lactic acid at 62 g l^{-1} and malic acid at 50 g l^{-1}.

Itaconic acid is used to prepare polymers, coatings, adhesives and textiles. One such polymer is poly-itaconic acid that is used in (1) water treatment, (2) detergents, (3 as an agent for thickening, binding and sizing, (4) as an emulsifier, (5) in oral drug delivery and (6) in dental cements. Itaconic acid is made by *A. terreus* at 80 000 tons per year with a selling price of \$2 kg^{-1} in a fermentation process that is more economical than chemical synthesis. A deficiency of manganese is a critical parameter for its production.[110] Production can be completely inhibited by manganese ions. However, if the Mn concentration is kept below 5 μg l^{-1}, with an initial sugar concentration of 100 g l^{-1} or higher, the itaconic acid production by *A. terreus* is similar to that of citric acid production by *A. niger* under the same conditions (see below). A titer of 130 g l^{-1} of itaconic acid was produced.[110] Increasing pH during the production phase increased production by *A. terreus* to 146 g l^{-1}.[111] The modification was done by raising the pH from 4 to 6 or by raising pH to 3 after 2.1 days of cultivation.

Gluconic acid is made by *A. niger*.[112] It is used in construction and in production of chemicals, pharmaceuticals, foods, beverages, textiles and leather. Substrates include glucose, sucrose and golden syrup, a by-product of the process refining sugar cane juice into sugar, or by treating sugar with acid. The price varies from \$1.20 to \$8.50 per kg. Thus, 85 g l^{-1} was produced in 44 h with a productivity of 1.94 g l^{-1} h^{-1}. Previous workers had obtained 158 g l^{-1} at 0.238 g l^{-1} h^{-1} with *A. niger* immobilized on cellulose microfibers.[113]

Isocitric acid is used to make pharmaceuticals and anticoagulants. *Yarrowia lipolytica* is a yeast producing high levels of isocitric and citric acids from rapeseed oil.[114]

Yovkova *et al.*[115] engineered *Y. lipolytica* to produce a high concentration of α-ketoglutarate from raw glycerol, that is, 186 g l^{-1}. Raw glycerol is obtained as a by-product of biodiesel production and can serve as an inexpensive carbon source for many fermentations. The strain was H355A (PVCI-IDPI). The new strain overexpressed genes encoding $NADP^+$-dependent isocitrate dehydrogenase (IDP1) and pyruvate carboxylase (PYC1). Production was 19% higher than that by the parent strain (H3557). The usual by-product, pyruvic acid, was markedly decreased in the mutant fermentation. α-Ketoglutaric acid is used industrially in chemical synthesis of heterocycles or elastomers, as a dietary supplement and as an enhancer of wound healing.

Malic acid is a C4 dicarboxylic acid used in the food, feed and beverage industries as an acidulant and taste enhancer/modifier in combination with artificial sweeteners. It is also used to prepare polyester resins and coatings. Additional applications include medical uses. Metabolic engineering of *Aspergillus oryzae* NRRL 3488 has been used to overproduce malic acid at 154 g l^{-1},[116] with a selling price of \$2–3 kg^{-1}. The result was achieved by overexpressing (1) the C4-dicarboxylate transporter and (2) the cytosolic alleles of pyruvate carboxylase and malate dehydrogenase. The rate was 0.94 g l^{-1} h^{-1} and the yield on glucose was 1.38 mol mol^{-1}. *Penicillium viticola* 152 produced 168 g l^{-1} of calcium malate in a medium containing corn steep liquor.[117] The yield was 1.28 g g^{-1} glucose and productivity was 175 g l^{-1} h^{-1}.

Overproduction of pyruvic acid is carried out by *Torulopsis glabrata* (also called *Candida glabrata*), a multivitamin auxotrophic yeast.[118] The process was industrialized in 1992 by Toray Industries at 400 tons per year. Subsequently, it was found that a *S. cerevisiae* mutant could produce a higher concentration, that is, 135 g l^{-1}. However, it was not environmentally robust, had a longer lag phase, lower glucose consumption rate and lower specific growth rate. *C. glabrata* produces 94 g l^{-1}, has a high yield (0.635 g g^{-1}), high productivity (1.15 g l^{-1} h^{-1}) and high glucose tolerance. Production was increased by use of urea as a nitrogen source.[119] This organism is used for commercial production of pyruvic acid.

Approximately 95% of citric acid production is used in the food industry. Other uses include chemicals (surfactants and synthetic detergents), medicinals, textiles and metallurgy.[120] Producing microbes include *A. niger*, *A. terreus* and *Y. lipolytica*. Production by *Y. lipolytica* is favored by limitation of cell growth brought about by limiting levels of nitrogen, phosphorus or sulfur, with nitrogen limitation as the most useful. Fermentation with genetically engineered *Y. lipolytica* amounted to 154 g l^{-1} from glycerol. Citric acid production by *A. terreus* can reach 200 g l^{-1}.

Fumaric acid, a 4-carbon dicarboxylic acid, is made by species of *Rhizopus* at levels of 126–130 g l^{-1}.[121] *Rhizopus arrhizus* has been utilized by the Pfizer corporation to make 4000 tons per year.[122] DuPont patented a process using *R. arrhizus* NRRL-1526 with limited dissolved oxygen to produce 130 g l^{-1}.[123] Other producing species include *Rhizopus nigricans*, *Rhizopus formosa* and *Rhizopus oryzae*. It is used as a food acidulant, a beverage ingredient, in production of biodegradable polymers, plasticizers, polyester resins and as an animal feed supplement to reduce methane emissions.

Glycolic acid can be overproduced by *S. cerevisiae* and *Klyveromyces lactis*.[124] Engineered *S. cerevisiae* made only 1 g l^{-1}, but engineered *K. lactis* produced 15 g l^{-1} from ethanol plus D-xylose. It is polymerized to polyglycolic acid that is excellent for preparing packaging material. Glycolic acid can also be used with lactic acid to make a copolymer (poly(lactic-*co*-glycolic acid)) for medical applications in drug delivery. Glycolic acid's market in 2011 was \$93 million for the 40 million kg that were produced.

Succinic acid is made by metabolically engineered *Y. lipolytica* at 63 g l^{-1}. Lactic acid is produced by *Candida boidini* at 86 g l^{-1}.

Z. Xue *et al.* developed a new process to make eicosapentaenoic acid (EPA), a long-chain polyunsaturated fatty acid.[125] It has been produced from wild-caught ocean fish, but this source cannot keep up with the demand for polyunsaturated fatty acids that are important for human health such as for reduction of coronary disease and action against hypertriglyceridemia. The process uses a metabolically engineered strain of *Y. lipolytica* that produces EPA at 56% of its cell dry weight plus lipids at 30% of its dry weight.[126] The yeast was engineered by transformation with 21 heterologous genes encoding five different activities. The genetic manipulation included inactivation of the peroxisome biogenesis gene Pex10. The oil produced has much higher levels of EPA than natural oils. EPA is important for the anti-inflammatory activity of fish oils, thus contributing to cardiovascular

and joint health. The product has been commercialized by the DSM company.

Isoprenoids

Isoprenoids are a group of ~50 000 natural compounds used as pharmaceuticals, flavors, fragrants, dietary supplements, food ingredients, biomaterials, solvents and biofuels.[127] They are the largest and most diverse group of natural products. They include primary metabolites (sterols, carotenoids, quinines) and secondary metabolites, mainly used for medicine. They are divided according to the number of carbon atoms: hemiterpenoids (C5), monoterpenoids (C10), sesquiterpenoids (C15), diterpenoids (C20) and triterpenoids (C30).

The sesquiterpenoids are one of the largest groups of isoprenoid natural products, amounting to 7000 compounds. Acyclic sesquiterpenoids are found in essential oils and insect pheromones. They include farnesene and isomeric alcohols such as nerolidol and farnesol. They are being considered as potential diesel and jet fuel alternatives. Bisabolene, like farnesene, is also a potential diesel fuel alternative. Monocyclic sesquiterpenes are important in the pharmaceutical and perfumery industries. For example, humulene has anti-allergenic and anti-inflammatory properties. Eleniol, zingiberene and bisabolene occur in essential oils and fragrances.

The two C5 universal building blocks used to synthesize isoprenoids are isopentenyl diphosphate and its isomer dimethylallyl diphosphate. The latter is produced either from the mevalonate pathway or the methyl erythritol phosphate pathway. The mevalonate pathway is present in eukaryotes and archaea, whereas the methyl erythritol phosphate pathway is active in bacteria. Methyl erythritol phosphate pathway has been used to produce taxadiene, the isoprenoid precursor of the anticancer agent taxol. Because plants and naturally occurring microbes produce only small quantities of isoprenoids, fermentation with engineered microbes has become the way to produce carotenoids, sterols and artemisinin. Artemisinin is a potent antimalarial and also a part of antimalarial combination therapies.

Progress in metabolic engineering, including synthetic biology and systems biology, has been made in microbial production of isoprenoids, such as artemisinic acid, taxol, farnesene, isoprene, amorphadiene and farnesol. One of the producing microbes is *S. cerevisiae*.[128] The *S. cerevisiae* mevalonate pathway has been engineered in *E. coli* yielding the terpene farnesyl diphosphate, the precursor to amorphadiene. The amorphadiene titer was 281 mg l^{-1}. This was increased to 480 mg l^{-1} by fermentation modifications. Further genetic and fermentation modifications increased the amorphadiene titer to 27.4 g l^{-1} and then to 41 g l^{-1}. This led to production of 25 g l^{-1} of artemisinic acid. By an inexpensive chemical process, the artemisinic acid was converted into the semisynthetic artemisinin. Artemisinin has been approved as an antimalarial agent by the World Health Association and is being produced commercially by Sanofi (see Antimalarial agent section).

Farnesene has been produced by yeast at a concentration of 728 mg l^{-1} by the Amyris company. It is made from sugar cane using a laboratory-evolved strain of *S. cerevisiae*. The titer was increased to 104 g l^{-1} with a productivity of 16.9 g l^{-1} per day by use of random mutagenesis and selection. Farnesol is an acyclic sesquiterpenoid alcohol derived from farnesyl diphosphate. It is found in plant essential oils and is important in the flavor and fragrance industries. It is also an antimicrobial agent, an antitumor drug precursor and a biopesticide in aquaculture. Furthermore, it is being considered as a diesel or jet-fuel substitute. Farnesol can be produced by *C. albicans* but, more importantly, by dephosphorylation of farnesyl diphosphate in engineered *S. cerevisiae*, overproducing mevalonate pathway genes. The farnesol titer reached in such strains of *S. cerevisiae* is 5 g l^{-1}.

Proteins

Production of biopharmaceutical proteins by metabolically engineered microbes has been very successful.[129,130] Biopharmaceuticals comprise one-sixth of the pharmaceutical market and are the most rapidly growing segment. They are employed to make up for the deficiency of body proteins used for normal function. They include blood factors, monoclonal antibodies, thrombolytics, anticoagulants, vaccines, hormones, interferons, interleukins, enzymes and growth factors. These systems were responsible for almost all of the biopharmaceuticals approved to date. Of the 211 biopharmaceuticals approved by 2011, 31% were produced by yeasts. Of the yeast products, 30 were made in *S. cerevisiae* and one in *Pichia pastoris*. In 2012, 12 biopharmaceuticals were approved in the United States and Europe.[131] One was produced by *S. cerevisiae* and another by *P. pastoris*.

The work on *S. cerevisiae* has mainly dealt with increasing protein secretion. More than 40 different recombinant proteins have been made by *S. cerevisiae*. Human serum albumin, used as a plasma expander in surgery, is produced at 3 g l^{-1}, and human transferrin, used for anemia, is made at 1.8 g l^{-1}.

Enzyme use in industry has been reviewed by Hellmuth *et al.*[132] There are four major groups of industrial enzymes: (1) detergent enzymes, (2) technical enzymes, (3) food enzymes and (4) feed enzymes. The technical enzymes include those for textiles, leather, pulp and paper and fuel ethanol. The largest group are the food enzymes that include amylases, xylanases, glucose oxidase, hexose oxidase, pectinases, glucanase, invertase, glucose isomerase, protease, lipase, phosphorylase, lactase, milk-clotting enzymes, animal rennet, microbial rennet and chymosin. The main sources are molds, yeasts and bacteria. Fungal producers include *A. niger* and *K. lactis*.

Regulation of cellulolytic and hemi-cellulolytic enzyme production in filamentous fungi has been reviewed by Tani *et al.*[133] Important regulatory transcription factors include XlnR from aspergilli that is involved in D-xylose induction of xylanolytic and cellulolytic enzymes. Others include ClR-1/2 from *Neurospora*, ManR, McmA and C1bR from *Aspergillus* and Bg1R from *Trichoderma* that regulate cellulolytic and/or hemicellulolytic enzyme production.

Heterologous proteins are also made very well by the yeast *Y. lipolytica*.[134] Such recombinant proteins include lipases, proteases, amylase, mannanase, laccase, leucine aminopeptidase and insulin. Recombinant proteins are also made by other fungi such as *Hansenula polymorpha*, *K. lactis*, *Schizosaccharomycs pombe*, *C. boidini*, *A. oryzae*, *A. niger*, *Trichoderma reesei*, *T. atroviride*, *Penicillium sordida*, *Penicillium griseoroseum*, *Penicillium purpurogenum* and *R. oryzae*.[135] The products of these heterologous enzymes include citric acid, isocitric acid, α-ketoglutaric acid, succinic acid, polyunsaturated fatty acids such as γ-linoleic acid, EPA and carotenoids including lycopene and β-carotene.

The secretory pathway in yeast involves over 160 proteins that carry out different posttranslational processes such as folding and glycosylation. Of special importance is the production by *S. cerevisiae* of insulin and its analogs. The insulin market amounted to $12 billion in 2011 and has been increasing ever since.

The use of *P. pastoris*, reclassified as *Komagataella pastoris*, as a producer of heterologous proteins has been reviewed by Ahmad *et al.*[136] Among the processes developed, one of the first was the production of the plant-derived hydroxynitrile lyase at over 20 g l^{-1}.[137]

Fatty acids and lipids

Production of polyunsaturated fatty acids by fungi and other microorganisms has been reviewed by Ratledge.[138] They are used as nutriceuticals and include (1) γ-linoleic acid (18:3 omega-6) from *Mucor circinelloides*, (2) docosahexaenoic acid (DHA; 22:6 omega-3) from algae, (3) arachidonic acid (20:4 omega-6) from *Mortierella alpine* and (4) EPA from genetically modified *Y. lipolytica*. They represent a multi-billion dollar industry, mainly arachidonic acid and DHA for infant formulas. They are major components of phospholipids in cell membranes. They regulate cell fluidity, attachment of specific enzymes to cell membranes and mediate signal transduction and other metabolic processes. They are used for the biosynthesis of eicosanoids, leukotrienes, prostaglandins and resolvins that function as anti-inflammatory, antiarrhythmic and antiaggretory effectors. Many improve cardiovascular health and some improve eye function and memory in newly born infants and adults. Two of these, that is, arachidonic acid and DHA, that are added to infant formulas may also have beneficial action for Alzheimer's disease, chronic bowel disorder and cancer. Microbial fermentation can be used for their production.

Microbial oils can be produced by 30–40 species of yeast, as well as by molds and algae. They are called oleaginous microbes. Fungi can accumulate 70% of their biomass as oils. EPA plus DHA can be used to prevent cardiac problems. EPA appears to have beneficial effects in neuropsychiatric disorders such as manic depression (bipolar disorders), schizophrenia, attention deficit hyperactivity disorder in children, coronary events in heart disease patients, preventing and treating obesity, metabolic syndrome, nonalcoholic steatohepatitis, type 2 diabetes and hypertriglyceridemia.

Y. lipolytica can accumulate lipids up to 40% of its dry cell weight. It makes single-cell oil for health applications and biofuels. In one case, lipid production reached 62% of the dry cell weight.[139] In another case, a strain accumulating 90% of its dry cell weight as lipid was developed.[140] In this case, a lipid titer of $25\,g\,l^{-1}$ was achieved.

Production of intracellular lipids by yeast growing on alkali-treated corn stover was studied by Sitepu.[141] *Cryptococcus humicola* produced $15\,g\,l^{-1}$ lipids in a total biomass weight of $36\,g\,l^{-1}$. The strain (UCSEST 10–1004) came from the Phaff yeast collection at the University of California, Davis. Such lipids could become useful for biodiesel production.

Vitamins

Production of vitamins by microbes has been reviewed by Ledesma-Amaro *et al.*[142] Most are produced chemically but microbial production is becoming important in several cases. Vitamin D is derived chemically from cholesterol and ergosterol. However, it can be made by *S. cerevisiae*, *Saccharomyces uvarum* and *Candida utilis* at $30\,g\,g^{-1}$ of dry cells.

Riboflavin (vitamin B_2) can be made by molds (*A. gossypii*, *E. ashbyii*), yeasts (*Candida flaeri*, *Candida famata*) and bacteria. *A. gossypii* can produce it at $14\,g\,l^{-1}$. It is mainly produced by metabolically engineered microbes and is used as a feed additive (70%) and as a food additive (30%) as well as for pharmaceutical applications. The producing organisms are *A. gossypii* and the bacterium *B. subtilis* that have completely replaced chemical synthesis.[143] In the high producing mutant of *A. gossypii*, that is, strain W122032, the increased production, as compared with the wild-type ATCC 10895, is because of (1) a 9% increase in flux to pentose-5-phosphate via the pentose phosphate pathway and (2) a 16-fold increase in the flux from purine to riboflavin.[144] The result is because of increased guanosine triphosphate flux through the pentose phosphate pathway and the purine synthesis pathway.

Alcohols

Erythritol, a polyhydric alcohol, has 60–70% of the sweetness of sucrose and is used to combat obesity. It is noncarcinogenic and noncaloric as it is not digested by humans, and cannot be fermented by bacteria to cause dental caries. Repeated batch cultures of *Y. lipolytica* on crude glycerol yielded $220\,g\,l^{-1}$ with a yield of $0.43\,g\,g^{-1}$ of glycerol used and a productivity of $0.54\,g\,l^{-1}\,h^{-1}$.[145]

Xylitol, a pentahydroxy sugar alcohol originating from xylose, has applications in foods and pharmaceuticals. A review of xylitol production from lignocellulosic waste has been written by Lima de Albuquerque *et al.*[146] Xylitol is a low-calorie sweetener used by diabetics with 40% fewer calories than sucrose. It is noncariogenic and has insulin-independent metabolism properties. Xylitol has high solubility, low glycemic rate, lack of carcinogenicity and has cariostatic properties. It is used in food production. It is made by catalytic hydrogenation of xylose but this is very expensive. Its global market is over 125 000 tons per year and it sells for $4.50–5.50 per kg to pharmaceutical and food companies. It has a 12% share of the total polyol market for chewing gum and foods. It is used as a sucrose replacement for cakes, cookies, chocolate and chewing gum, and in pharmaceuticals to reduce tooth decay. It acts against oral biofilms especially against *Streptococcus mutans*. It is also active against other bacteria harmful to oral health, such as *Streptococcus pneumoniae*, *Hemophilus influenzae*, *S. aureus* and *Pseudomonas aeruginosa*. It has also been cited as a contributor to tooth calcification, and is active against diabetes, anemia, acute otitis media and osteoporosis. Of great interest is its production from xylose in lignocellulosic materials as 100% of the xylose can be converted to xylitol microbially. This is favored over chemical synthesis that uses more intense reaction conditions and yields undesirable coproducts that have to be removed. Fermentation of xylose to xylitol occurs under milder conditions of temperature and pressure, yielding lower levels of unwanted by-products. Cell-free systems have been used, but immobilized systems hold great promise because of higher levels of xylitol produced, as well as the possibility of reuse of successive cultures as a fed-batch process or prolonged fermentations during continuous processes. Systems used include stirred tank reactors, packed-bed reactors, fluidized bed reactors, bubble columns and air-lift reactors. Processes include batch culture, fed-batch or semi-continuous culture and continuous culture. Bioconversion of $300\,g\,l^{-1}$ xylose to xylitol by *Debaryomyces hansenii* amounted to $110\,g\,l^{-1}$.[147] The yield was $0.48\,g\,g^{-1}$. Repeated fed-batch fermentation (lasting 750 h) with high-cell density cultures of *Candida magnolia* TISTR 5663 in a 2-l stirred tank fermenter under oxygen limitation with feeding of xylose and nitrogen and a starting xylose concentration of $60\,g\,l^{-1}$ led to production of $284\,g\,l^{-1}$ of xylitol,[148] the highest titer ever achieved. Xylitol productivity was $1.49\,g\,l^{-1}\,h^{-1}$. *Candida tropicalis* has also been used by other groups. The overall conclusion is that immobilization of yeast cells is an excellent way to produce xylitol from xylose. Immobilization was best when entrapment in calcium alginate, followed by solid adsorption, was used.

Candida athensensis can convert vegetable waste to $100\,g\,l^{-1}$ xylitol with a yield of $0.81\,g\,g^{-1}$ and a productivity of $0.98\,g\,l^{-1}\,h^{-1}$.[149] The vegetable waste contained $200\,g\,l^{-1}$ of xylose.

Another sugar alcohol, D-arabitol, is potentially useful for oral health care and as a pharmaceutical. It has lower nutritional calories than xylitol and sucrose, making it a low-calorie natural sugar substitute for diabetics. An osmophilic strain from raw chaste honey, *Zygosaccharomyces rouxii* JM-C46, was isolated as a high D-arabitol producer.[150] Using pH control and repeated fed-batch fermentation in

a 5-l fermentor yielded 93 g l^{-1} of D-arabitol with a volumetric productivity of 1.143 g l^{-1} h^{-1}.

Mannitol is produced by genetically engineered *Y. lipolytica* at 27 g l^{-1}.

Additional compounds

Glutathione, a redox-active tripeptide thiol having the activities of antioxidation, detoxification and immune regulation can be made by an engineered strain of *S. cerevisiae* at a concentration of 317 mg l^{-1}.[151]

Coenzyme Q (ubiquinone) is an essential part of the respiratory chain producing ATP. It is an excellent antioxidant. It is composed of a quinonoid nucleus and a side chain of isoprenoids. Microbial fermentation is the best method of production as it produces no optical isomers and is the least expensive means of production. Its production has been reviewed by De Dieu Ndikubwimana and Lee.[152] It can be produced by species of the yeasts *Candida* and *Saitoella*.

Flavin adenine nucleotide is used in the pharmaceutical and food industries. It is an ophthalmic agent of which 10 tons are produced annually. It is produced at 18 g l^{-1} in a medium containing FMN and ATP by *C. famata*.[153]

Yeasts are also used to make human serum albumin, hepatitis vaccines and virus-like particles used for vaccination against human papilloma virus. *S. cerevisiae* carries out proper folding of many human proteins, secretes the proteins, and does posttranslational modifications such as proteolytic processing of signal peptides, disulfide bond formation, subunit assembly, acylation and glycosylation. A negative property of *S. cerevisiae* and other yeasts had been the high-mannose type of N-glycosylation that shortens the *in vivo* half-life and reduces efficacy. However, both *S. cerevisiae* and *P. pastoris* have been engineered to produce human-like glycosylation that includes terminal addition of sialic acid to the glycoprotein.

The yeast *Aureobasidium pullulans* strain RBF 4A3 can produce 88 g l^{-1} of pullulan.[154] Pullulan is an exopolysaccharide that has potential application in industries such as medical, food, pharmaceutical, cosmetic and agriculture.

The fungal genus *Trichoderma* makes many secondary metabolites with useful applications.[155] Its species are commercially available as plant growth-promoting fungi and biological control agents. They have broad-spectrum antagonistic activities against a number of soil-borne phytopathogens including (1) mycoparasitism, via secretion of cell wall-degrading enzymes; (2) competition, that is, mobilizing and taking up of macro- and micro-nutrients from soil, resulting in scarcity of nutrients for other soil microbes; and (3) antibiosis via secretion of antibacterial secondary metabolites. These secondary metabolites include (1) emodin, a cathartic stimulant and tumor cell adhesion inhibitor; (2) gliotoxin, an antimalarial agent and immune system suppressor; (3) harziaolide, an antifungal agent and plant growth promoter; (4) koninginins, antifungal agents and regulators of plant growth; (5) 6-pentyl-2H-pyran-2-one, an antifungal agent as well as a promoter of plant growth and a coconut aroma used in confectionary products; (6) trichokonins, broad-spectrum antifungal agents and plant defense inducers; (7) viridofungins, potential anticancer agents and bacteriocides; (8) viridian, a broad-spectrum antifungal agent, antineoplastic and antiatherosclerosis agent; and (9) viridiol, a weedicidal agent.

Systems metabolic engineering for production of biofuels and chemicals by *Aspergillus* and *Pichia* species has been reviewed by Caspeta and Nielsen.[156] Formaldehyde is produced from methanol at 6000 tons per year by *P. pastoris*.

Many useful products are made by the basidiomycetes.[157] These fungi make carotenoids, fragrances, enzymes, astaxanthin, erythritol, lipids and oils. *Trichosporon* species produces lipids and is being considered for biodiesel production. *Pseudozyma (Candida) antartica* produces lipase for industrial use and is also a biodiesel possibility. It also produces 30 g l^{-1} of itaconic acid. *Sporobolomyces carnicolor* accumulates 82% of its biomass as intracellular lipids. *Cryptococcus* species make unique carotenoids such as plectaniaxanthin, a xanthophyll. Some cryptococci utilize glycerol and accumulate 60% of their biomass as triacylglycerols.

Biofuels

Approximately 100 billion liters of ethanol are produced per year from sugar cane and corn starch by *S. cerevisiae*. Production of ethanol and advanced biofuels at high temperature (ca 40 °C) reduces cooling costs, lowers the effects of contamination and enables more efficient hydrolysis of feedstocks. This improves productivity in the simultaneous saccharification and fermentation process. However, temperatures of 34 °C and above interfere with yeast viability and growth. Caspeta *et al.*[158] isolated *S. cerevisiae* strains with improved growth and ethanol production at 40 °C. They used adaptive laboratory evolution to obtain these mutant strains. They noted a change in sterol composition from ergosterol to fenosterol because of a mutation in the C5 sterol desaturase gene, and increased expression of sterol biosynthesis genes. The new strains grew 1.9 times faster and excreted ethanol and glycerol 1.6 times faster than the parent culture. Sterols contribute to membrane fluidity. These thermotolerant strains were improved in glucose consumption rate that was increased by 60% at 40 °C and by 300% at 42 °C.

Production of bioethanol from corn can only yield 15 billion gallons per year. It is thus desirable to produce bioethanol from lignocellulosic biomass. Bioethanol can be produced from cellulose, but lignocellulose contains not only glucose, but also C5 sugars such as xylose and arabinose that cannot be utilized by wild-type *S. cerevisiae* because it does not have a catabolic pathway for pentose utilization. However, some genetically engineered *S. cerevisiae* strains can utilize xylose. To improve the production of ethanol by a xylose-utilizing strain of *S. cerevisiae*, the HAP4 gene was knocked out.[159] This gene encodes a transcription activator that controls expression of genes involved in mitochondrial respiration and reductive pathways. By knocking out the HAP4 gene, the following were increased: maximal ethanol concentration, ethanol production rate and ethanol yield. A new strain, *S. cerevisiae* B42-DHAP4, could produce ethanol from xylose as sole carbon source under aerobic conditions. The rate of ethanol production and its yield from a detoxified hydrolysate of wood chips were markedly improved.

Alcohol tolerance in *S. cerevisiae* can be increased by adding potassium and raising the pH of the fermentation medium with KOH.[160] This increases cell growth. Ethanol titer was increased by these modifications to 127 g l^{-1}.

Product titers achieved by fungi growing on Jerusalem artichokes include 154 g l^{-1} of ethanol by a mixed culture of *S. cerevisiae* and *A. niger* and 109 g l^{-1} by *S. cerevisiae* alone.[161]

During pretreatment of biomass, a problem is the liberation of the inhibitor furfural. However, tolerance to furfural can be achieved by overexpression of *S. cerevisiae* genes encoding (1) yeast transcription activator MSN2;[162] (2) ZWF1 of the pentose phosphate pathway;[163] (3) ADH1 encoding alcohol dehydrogenase 1; and (4) TAL1, encoding transaldolase 1.[164]

P. stipitis can produce ethanol from C5 sugars and also clean up concentrated toxins liberated during lignocellulose degradation.[165]

This yeast can produce ethanol from pretreated sources of biomass such as red oaks, wheat straw, sugar cane bagasse, rice straw, corn cobs, corn stover, aspen wood, pinewood and poplar wood. From aspen wood chips, ethanol titer was $41\,g\,l^{-1}$ with a yield of $0.47\,g\,l^{-1}$.[166] In a chemically defined medium, $61\,g\,l^{-1}$ could be produced by *P. stipitis*.[167] Attributes of *P. stipitis* include consumption of acetic acid, and reduction of the furan ring toxins such as hydroxymethylfurfural and furfural present in cellulosic biomass conversions.

2,3-Butanediol can be produced at $96\,g\,l^{-1}$ by an engineered strain of *S. cerevisiae*.[168] It is a fuel with a high heating value ($27\,000\,J\,g^{-1}$) and has been used as a liquid fuel or fuel additive.

A comprehensive review of biodiesel production and application of genetic engineering is that of Lin *et al.*[169] This ideal substitute for petroleum-based diesel is made from triglycerides by transesterification with alcohols. Today, crude oil is consumed at 11.6 million tons per day that cannot last for a long time. Biodiesel use requires no engine modification. It is blended with diesel in Germany, Italy and Malaysia. It is better than diesel as it is biodegradable, nontoxic and releases less toxicant when burned as a fuel. Chemical catalysis is presently used to make biodiesel but enzymatic catalysis is looked upon as better from the aspects of mild reaction conditions and easy product separation. Current biodiesel production suffers from the lack of a stable, sufficient feedstock supply system, inconsistent performance and challenging economics. Microbial production could overcome these problems because of short producing period, little labor requirement, easy scale-up and independence from problems of venue, season, climate change, etc. So-called 'grease microorganisms' (oleaginous microbes that supply fatty acids and alcohols and convert them to biodiesel) could supply the fatty acids needed. Their composition of the fatty acids is similar to vegetable oils that are used to make biodiesel. They have $>50\%$ lipid content, can be used industrially, grow rapidly, are not polluting and oil can be easily extracted. Microalgae have been considered as an oil feedstock but their use is restricted by their low growth rate, strict breeding conditions and large up-front investment requirement. Alcohol is needed in the transesterification process to generate the fatty acid ester. Methanol or ethanol can be used to generate the fatty acid ester. Methanol makes fatty acid methyl esters, whereas ethanol yields fatty acid ethyl esters. Fatty acid methyl esters are cheaper, more reactive and volatile. However, ethanol is less toxic, and more renewable than methanol. *S. cerevisiae* has been genetically engineered for *de novo* biosynthesis of biodiesel. Of potential importance is *Y. lipolytica* for microbial biodiesel production.

Potential of being a more effective producer

Fungi harbor many more secondary metabolite gene clusters than those expressed under normal laboratory conditions. Activation of 'silent' gene clusters in *A. nidulans* has revealed many new fungal secondary metabolites. This application of 'genome mining' revealed that the *A. nidulans* genome contains 56 potential secondary metabolism core genes including the following: 27 polyketide synthase (PKS) genes, two polyketide synthase-like genes, 11 nonribosomal peptide synthetase (NRPS) genes, 15 NRPS-like genes and one hybrid NRPS–PKS gene.

Echinocandins

The echinocandins, which are often called the 'penicillin of antifungals', are lipopeptide molecules that inhibit β-(1,3)-glucan synthesis, an integral component of the fungal cell wall.[170] Although they lack activity against Zygomycetes, *Cryptococcus neoformans* or *Fusarium* spp., *in vitro* and *in vivo* studies have demonstrated that echinocandins are fungicidal against most *Candida* spp. but are fungistatic against *Aspergillus* spp.[170] Caspofungin was the first echinocandin to be approved, for the indication of refractory invasive aspergillosis. It is a semisynthetic derivative of pneumocandin B0, a lipophilic cyclic peptide isolated from the fungus, *Glarea lozoyensis*.[171] The sales of this antifungal, which was sold under the name Cancidas by Merck, was $681 million in 2014 and $573 million in 2015. The second antifungal from the echinocandin class that was licensed was micafungin, sold under the trade name Fungard/Mycamine by Astellas Pharma. It achieved annual sales of 38.8 billion yen or about $352 million in 2014, and in 2015 the sales reached 41.6 billion yen or $346 million. Patients who have received either of these antifungal agents have reported mild adverse events that include local phlebitis, fever, abnormal liver function tests and hemolysis.[170] Echinocandins are usually given once daily, via the parenteral route, because of poor absorption after oral administration. Caspofungin has shown promising results in studies of candidemia and invasive candidiasis, with nearly the same efficacy as amphotericin B, but markedly less toxicity. Because of the absence of antagonism and very minimal drug interactions with other antifungal agents, the echinocandin class represents a promising therapeutic area that may potentially be used in combination for the treatment of invasive aspergillosis.

CONCLUDING REMARKS

For the past 85 years, microbes have provided significant contributions to the fields of medicine and agriculture, especially in the area of antibiotic production. The compounds they have produced have helped save millions of lives, alleviated pain and suffering and increased human life expectancy. In addition, they have also played a vital role in the animal industry and agricultural operations. However, for a variety of reasons, pathogenic microbes have become resistant to many antibiotics, creating a dangerous situation. Therefore, the need for new and effective antibiotics is imperative. Unfortunately, most of the large pharmaceutical companies have abandoned the search for new antimicrobial compounds. Because of 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; that is, termination of antibiotic development while resistance continues to increase.[172] 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.[173] Another exciting possibility is that of genome mining.[174] 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 nonantibiotics such as antitumor agents and other microbial products. In addition, natural products must continue to be

tested for desirable therapeutic activities. We 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.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 Hölker, U., Höfer, M. & Lenz, J. Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. *Appl. Microbiol. Biotechnol.* **64**, 175–186 (2004).
2 Berdy, J. Bioactive microbial metabolites. A personal view. *J. Antibiot.* **58**, 1–26 (2005).
3 Brakhage, A. A. & Schroekh, V. Fungal secondary metabolites. Strategies to activate silent gene clusters. *Fungal Genet. Biol.* **48**, 15–22 (2011).
4 Demain, A. L., Velasco, J., Adrio, J. L. in *Handbook of Industrial Mycology* (ed. An, Z.) 1–25 (Marcel Dekker, New York, 2004).
5 Kaeberlein, T., Lewis, K. & Epstein, S. S. Isolating 'uncultivable' microorganisms in pure culture in a simulated natural environment. *Science* **296**, 1127–1129 (2002).
6 Colwell, R. R. Fulfilling the promise of biotechnology. *Biotechnol. Adv.* **20**, 215–228 (2002).
7 Gaudilliere, B., Bernardelli, P., Berna, P. in *Annual Reports in Medicinal Chemistry* Vol. 36: (ed. Doherty, A. M.) 293–318 (Academic, Amsterdam, 2001).
8 Anderson, M. R. *et al.* Accurate prediction of secondary metabolite gene clusters in filamentous fungi. *Proc. Natl Acad. Sci. USA* **110**, E99–E107 (2013).
9 Adrio, J. L. & Demain, A. L. Fungal biotechnology. *Int. Microbiol.* **6**, 191–199 (2003).
10 Wiemann, P. & Keller, N. P. Strategies for mining fungal natural products. *J. Ind. Microbiol. Biotechnol.* **41**, 301–313 (2014).
11 Brakhage, A. Regulation of fungal secondary metabolism. *Nat. Rev. Microbiol.* **11**, 21–32 (2013).
12 Cardenas, M. E., Sanfridson, A., Cutler, N. S. & Heitman, J. Signal-transduction cascades as targets for therapeutic intervention by natural products. *Trends Biotechnol.* **16**, 427–433 (1998).
13 Kremer, L., Douglas, J. D., Baulard, A. R., Morehouse, C. & Guy, M. R. Thiolactomycin and related analogues as novel anti-mycobacterial agents targeting KasA and KasB condensing enzymes in Mycobacterium tuberculosis. *J. Biol. Chem.* **275**, 16857–16864 (2000).
14 Verdine, G. L. The combinatorial chemistry of nature. *Nature* **384**, 11–13 (1996).
15 Borel, J. F., Feurer, C., Gabler, H. U. & Stahelin, H. Biological effects of cyclosporine A: a new anti- lymphocytic agent. *Agents Action* **6**, 468–475 (1976).
16 Borel, J. F. History of the discovery of cyclosporin and of its early pharmacological development. *Wien Klin Wochenschr.* **114**, 433–437 (2002).
17 De Wilde, A. H. Cyclosporin A inhibits the replication of diverse coronaviruses. *J. Gen. Virol.* **92**, 2542–2548 (2011).
18 Bentley, R. Bartolomeo Gosio, 1863-1944: an appreciation. *Adv. Appl. Microbiol.* **48**, 229–250 (2001).
19 Alsberg, C. L. & Black, O. F. *USDA Bur Plant Ind., Bull No. 270* (Government Printing Office, Washington, 1913).
20 Birkinshaw, J. H., Raistrick, H. & Ross, D. J. Studies in the biochemistry of micro-organisms. 86. The molecular constitution of mycophenolic acid, a metabolic product of Penicillium brevicompactum Dierckx. Part III. Further observations on the structural formula for mycophenolic acid. *Biochem. J.* **50**, 630–634 (1952).
21 Sebastian, L., Madhusudana, S. N., Ravi, V. & Desai, A. Mycophenolic acid inhibits replication of Japanese Encephalitis Virus. *Chemotherapy* **57**, 56–61 (2011).
22 Lee, W. A. *et al.* Bioavailability improvement of mycophenolic acid through amino ester derivatization. *Pharm. Res.* **7**, 161–166 (1990).
23 Chong, C. R. *et al.* Identification of type 1 inosine monophosphate dehydrogenase as an antiangiogenic drug target. *J. Med. Chem.* **49**, 2677–2680 (2006).
24 Fleck, W. in *Journal of Basic Microbiology* edsKothe, E. & Umezawa, H.) 244 (University of Tokyo Press, Tokyo, 1972).
25 Paterson, R. R. M. Fungal enzyme inhibitors as pharmaceuticals, toxins and scourge of PCR. *Curr. Enzyme Inhib.* **4**, 46–59 (2008).
26 Nicholls, S. J. Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis. *JAMA* **297**, 499–508 (2007).
27 Endo, A. A historical perspective on the discovery of statins. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* **86**, 484–492 (2010).
28 Brown, A. G., Smale, T. C., King, T. J., Hasenkamp, R. & Thompson, R. H. Crystal and molecular structure of compactin: a new antifungal metabolite from Penicillium brevicompactum. *J. Chem. Soc. Perkin 1* **(11)**, 1165–1170 (1976).
29 Endo, A., Kuroda, M. & Tsujita, Y. ML-236A, ML-236B, and ML-236C, new inhibitors of cholesterolgenesis produced by Penicillium citrinin. *J. Antibiot.* **29**, 1346–1348 (1976).
30 Alberts, A. W. *et al.* Mevinolin: a highly potent competitive inhibitor of hydroxylmethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proc. Natl Acad. Sci. USA* **77**, 3957–3961 (1980).
31 Endo, A. & Monacolin, K. A new hypocholesterolemic agent produced by Monascus species. *J. Antibiot.* **32**, 852–854 (1979).
32 Liu, Z. Q. *et al.* Simvastatin has beneficial effect on pulmonary artery hypertension by inhibiting NF-kB expression. *Mol. Cell Biochem.* **354**, 77–82 (2011).
33 Bader, T. *et al.* Fluvastatin inhibits hepatitis C replication in humans. *Am. J. Gastroenterol.* **103**, 1383–1389 (2008).
34 Makris, G. C., Geroulakos, G., Makris, M. C., Mikhailidis, D. & Falagas, M. E. The pleiotropic effects of statins and omega-3 fatty acids against sepsis: a new perspective. *Expert Opin. Investig. Drugs* **19**, 809–814 (2010).
35 Menge, T., Hartung, H. P. & Stueve, O. Statins—a cure-all for the brain? *Nat. Rev. Neurosci.* **6**, 325–331 (2005).
36 Puttananjaiah, M. K., Dhale, M. A., Gaonkar, V. & Keni, S. Statins: 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors demonstrate anti-atherosclerotic character due to their antioxidant capacity. *Appl. Biochem. Biotechnol.* **163**, 215–222 (2011).
37 Fonseca, A. C., Proenca, T., Resende, R., Oliviera, C. R. & Pereira, C. M. Neuroprotective effect of statins in an in vitro model of Alzheimer's disease. *J. Alzheimers Dis.* **17**, 503–517 (2009).
38 Arnold, D. E., Gagne, C., Niknejad, N., McBurney, M. W. & Dimitroulakos, J. Lovastatin induces neuronal differentiation and apoptosis of embryonal carcinoma and neuroblastoma cells: enhanced differentiation and apoptosis in combination with dbcAMP. *Mol. Cell Biochem.* **345**, 1–11 (2010).
39 Xie, X. & Tang, Y. Efficient synthesis of simvastatin by use of whole-cell biocatalysts. *Appl. Environ. Microbiol.* **73**, 2054–2060 (2007).
40 Serizawa, N. & Matsuoka, T. A two-component-type cytochrome P-450 monooxygenase system in a prokaryote that catalyzes hydroxylation of ML-236B to pravastatin, a tissue-selective inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Biochim. Biophys. Acta* **1084**, 35–40 (1991).
41 Peng, Y. & Demain, A. L. A new hydroxylase system in Actinomadura sp. cells converting compactin to pravastatin. *J. Ind. Microbiol. Biotechnol.* **20**, 373–375 (1998).
42 McLean, K. J. *et al.* Single-step fermentative production of the cholesterol-lowering drug pravastatin via reprogramming of Penicillium chrysogenum. *Proc. Natl Acad. Sci. USA* **112**, 2847–2852 (2015).
43 Alarcón, J. *et al.* Production and purification of statins from Pleurotus ostreatus (Basidiomycetes) strains. *Z. Naturforsch. C* **58**, 62–64 (2003).
44 Panesar, P. S., Kumari, S. & Panesar, R. Biotechnological approaches for the production of prebiotics and their potential applications. *Crit. Rev. Biotechnol.* **33**, 345–364 (2013).
45 Faus, I. Recent developments in the characterization and biotechnological production of sweet- tasting proteins. *Appl. Microbiol. Biotechnol.* **53**, 145–151 (2000).
46 Moralejo, F. J., Cardoza, R. E., Gutierrez, S. & Martin, J. F. Thaumatin production in Aspergillus awamori by use of expression cassettes with strong fungal promoters and high gene dosage. *Appl. Environ. Microbiol.* **65**, 1168–1174 (1999).
47 Hallborn, J. *et al.* Xylitol production by recombinant Saccharomyces cerevisiae. *Biotechnology* **9**, 1090–1095 (1991).
48 Bentley, R. Microbial secondary metabolites play important roles in medicine: prospects to discovery of new drugs. *Perspect. Biol. Med.* **40**, 364–394 (1997).
49 Vining, L. C., Taber, W. A. in *Secondary Products of Metabolism* (ed. Rose, A. H.) 389–420 (Academic, London, 1979).
50 Hidy, P. H., Baldwin, R. S., Greasham, R. L., Keith, C. L. & McMullen, J. R. Zearelanone and some derivatives: production and biological activities. *Adv. Appl. Microbiol.* **22**, 59–82 (1977).
51 Jefferys, E. G. The gibberellin fermentation. *Adv. Appl. Microbiol.* **13**, 283–316 (1970).
52 Tudzinski, B. Biosynthesis of gibberellins in Gibberella fujikuroi: biomolecular aspects. *Appl. Microbiol. Biotechnol.* **52**, 298–310 (1999).
53 Wall, M. E. & Wani, M. C. Camptothecin and taxol: from discovery to clinic. *J. Ethnopharmacol.* **51**, 239–254 (1996).
54 Stierle, A., Strobel, G. & Stierle, D. Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. *Science* **260**, 214–216 (1993).
55 Newman, D. J. & Cragg, G. M. Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.* **70**, 461–477 (2007).
56 Sabater-Jara, A. B., Tudela, L. R. & Lopez-Perez, A. J. *In vitro* culture of Taxus sp.: strategies to increase cell growth and taxoid production. *Phytochem. Rev.* **9**, 343–356 (2010).
57 Flores-Bustamante, Z. R., Rivera-Orduna, F. N., Martinez-Cardenas, A. & Flores-Cotera, L. B. Microbial paclitaxel: advances and perspectives. *J. Antibiot.* **63**, 460–467 (2010).
58 Gangadevi, V. & Muthumary, J. Isolation of Colletotrichum gloeosporiodes, a novel endophytic taxol-producing fungus from the leaves of a medicinal plant. *Mycol. Balc.* **5**, 1–4 (2008).
59 Kumaran, R. S., Kim, H. J. & Hur, B. K. Taxol promising fungal endophyte, Pestalotiopsis species isolated from Taxus cuspidata. *J. Biosci. Bioeng.* **110**, 541–546 (2010).
60 Kumaran, R. S., Jung, H. & Kim, H. J. *In vitro* screening of taxol, an anticancer drug produced by the fungus Colletotricum capsici. *Eng. Life Sci.* **3**, 264–271 (2011).
61 Li, J. Y., Strobel, G., Sidhu, R., Hess, W. M. & Ford, E. J. Endophytic taxol-producing fungi from bald cypress, Taxodium distichum. *Microbiology* **142**, 2223–2226 (1996).
62 Wang, J. F. *et al.* Taxol from Tubercularia sp. strain TF5, an endophytic fungus of Taxus mairei. *FEMS Microbiol Lett.* **193**, 249–253 (2000).

63 Xu, F., Tao, W., Cheng, L. & Guo, L. Strain improvement and optimization of the media of taxol- producing fungus Fusarium maire. *Biochem. Eng. J.* **31**, 67–73 (2006).
64 Zhao, K., Zhou, D., Ping, W. & Ge, J. Study on the preparation and regeneration of protoplast from taxol-producing fungus Nodulisporium sylviforme. *Nat. Sci.* **2**, 52–59 (2004).
65 Kumaran, R. S., Muthumary, J. P. & Hur, B. K. Taxol from Phyllosticta citricarpa, a leaf spot fungus of the angiosperm Citrus medica. *J. Biosci. Bioeng.* **106**, 103–106 (2008).
66 Wei, Y. *et al.* Engineering taxol biosynthetic pathway for improving taxol yield in taxol-producing endophytic fungus EFY-21 (Ozonium sp.). *Afr. J. Biotechnol.* **11**, 9094–9101 (2012).
67 Kumaran, R. S. *et al.* Isolation of taxol, an anticancer drug produced by the endophytic fungus, Phoma betae. *Afr. J. Biotechnol.* **11**, 950–960 (2012).
68 Zhang, P., Zhou, P. P. & Yu, L. J. An endophytic taxol-producing fungus from Taxus media, Cladosporium cladosporoides MD2. *Curr. Microbiol.* **59**, 227–232 (2009).
69 Liu, K., Ding, X., Deng, B. & Chen, W. Isolation and characterization of endophytic taxol-producing fungi from Taxus chinensis. *J. Ind. Microbiol. Biotechnol.* **36**, 1171–1177 (2009).
70 Zhou, X., Zhu, H., Liu, L., Lin, J. & Tang, K. A review: recent advances and future prospects of taxol- producing endophytic fungi. *Appl. Microbiol. Biotechnol.* **86**, 1707–1717 (2010).
71 Duan, L. L., Chen, H. R., Chen, J. P., Li, W. P. & Hong, L. Screening the high-yield paclitaxel producing strain Alternaria alternate var monosporus. *Chin. J. Antibiot.* **33**, 650–652 (2008).
72 Amna, T. *et al.* Bioreactor studies on the endophytic fungus Entrophospora for the production of an anticancer alkaloid camptothecin. *Can. J. Microbiol.* **52**, 189–196 (2006).
73 Lorence, A. & Nessler, C. L. Camptothecin, over four decades of surprising findings. *Phytochemistry* **65**, 2735–2749 (2004).
74 Venditto, V. J. & Simanek, E. E. Cancer therapies utilizing the camptothecins: a review of the in vivo literature. *Mol. Pharm.* **7**, 307–349 (2010).
75 Pu, X. *et al.* Camptothecin-producing endophytic fungus Trichoderma atroviride LY357: isolation, identification, and fermentation conditions optimization for camptothecin production. *Appl. Microbiol. Biotechnol.* **97**, 9365–9375 (2013).
76 Bernardes, N., Seruca, R., Chakrabarty, A. M. & Fialho, A. M. Microbial-based therapy of cancer: current progress and future prospects. *Bioeng. Bugs* **1**, 178–190 (2010).
77 Sanchez, S., Ruiz, B., Rodriguez-Sanoja, R., Flores-Cotera, L. B. in *Microbial Production of Food Ingredients, Enzymes, and Nutraceuticals* (eds McNeil, B., Archer, D., Giavasis, I. & Harvey, L.) 194–223 (Woodhead Publishing, Oxford, 2013).
78 Heider, S. A., Peters-Wendisch, P., Wendisch, V. F., Beekwilder, J. & Brautaset, T. Metabolic engineering for the microbial production of carotenoids and related products with a focus on the rare C50 carotenoids. *Appl. Microbiol. Biotechnol.* **98**, 4355–4368 (2014).
79 Reyes, L. H., Gomez, J. M. & Kao, K. C. Improving carotenoids production in yeast via adaptive laboratory evolution. *Metab. Eng.* **21**, 26–33 (2014).
80 Roukas, T. The role of oxidative stress on carotene production by Blakeslea trispora in submerged fermentation. *Crit. Rev. Biotechnol.* **36**, 424–433 (2016).
81 Ma, J. *et al.* Constituents of red yeast rice, a traditional Chinese food and medicine. *J. Agric. Food Chem.* **48**, 5220–5225 (2000).
82 Juzlova, P., Martinkova, L. & Kren, V. Secondary metabolites of the fungus Monascus: a review. *J. Industr. Microbiol.* **16**, 163–170 (1996).
83 Feng, Y., Shao, Y. & Chen, F. Monascus pigments. *Appl. Microbiol. Biotechnol.* **96**, 1421–1440 (2012).
84 Lee, B. H. & Pan, T. M. Benefit of Monascus-fermented products for hypertension prevention: a review. *Appl. Microbiol. Biotechnol.* **94**, 1151–1161 (2012).
85 Lin, T. F. & Demain, A. L. Effect of nutrition of Monascus on formation of red pigments. *Appl. Microbiol. Biotechnol.* **36**, 70–75 (1991).
86 Lin, T. F. & Demain, A. L. Resting cell studies on formation of water-soluble red pigments by Monascus sp. *J. Ind. Microbiol. Biotechnol.* **12**, 361–367 (1993).
87 Lin, T. F. & Demain, A. L. Leucine interference in the production of water-soluble red Monascus pigments. *Arch. Microbiol.* **162**, 114–119 (1994).
88 Lin, T. F. & Demain, A. L. Negative effect of ammonium nitrate as nitrogen source on the production of water-soluble red pigments by Monascus sp. *Appl. Microbiol. Biotechnol.* **43**, 701–705 (1995).
89 Andrewes, A. G., Phaff, H. J. & Starr, M. P. Carotenoids of Phaffia rhodozyma, a red-pigmented fermenting yeast. *Phytochemistry* **15**, 1003–1007 (1976).
90 Rodriguez-Saiz, M., de la Fuente, J. L. & Barredo, J. L. Xanthophyllomyces dendrorhous for the industrial production of astaxanthin. *Appl. Microbiol. Biotechnol.* **88**, 645–658 (2010).
91 Johnson, E. A., Villa, T. G. & Lewis, M. J. Phaffia rhodozyma as an astaxanthin source in animal diets. *Aquaculture* **20**, 123–134 (1980).
92 De La Fuente, J. L. *et al.* High-titer production of astaxanthin by the semi-industrial fermentation of Xanthophyllomyces dendrorhous. *J. Biotechnol.* **145**, 144–146 (2010).
93 Jacobson, G. K., Jolly, S. O., Sedmak, J. J., Skatrud, T. J. & Wasileski, J. M. Astaxanthin over-producing strains of Phaffia rhodozyma. Method for their cultivation and their use in animal feeds. US Patent 6015684 (1999).
94 Berdy, J. Are actinomycetes exhausted as a source of secondary metabolites? Proceedings of 9th International Symposium on the Biology of Actinomycetes, Part 1 (Allerton press, New York, 1995).
95 Strohl, W. R. in *Biotechnology of Antibiotics* (ed. Strohl, W. R.) 1–47 (Marcel Dekker, New York, 1997).
96 Masurekar, P. Nutritional and engineering aspects of microbial process development. *Prog. Drug Res.* **65**, 292–328 (2008).
97 Yang, Y. *et al.* A novel impeller configuration to improve fungal physiology performance and energy conservation for cephalosporin C production. *J. Biotechnol.* **161**, 250–256 (2012).
98 Brown, K. S. Pharmaceutical and biotech firms taking on drug-resistant microbes. *Scientist* **10**, 8–9 (1996).
99 Martín, J. The inducers 1,3-diaminopropane and spermidine produce a drastic increase in the expression of the penicillin biosynthetic genes for prolonged time, mediated by the LaeA regulator. *Fungal Genet. Biol.* **49**, 1004–1013 (2012).
100 Shang, S. *et al.* Activities of TMC207, rifampin, and pyrazinamide against Mycobacterium tuberculosis infection in guinea pigs. *Antimicrob. Agents Chemother.* **55**, 124–131 (2011).
101 Vance, D. *et al.* Inhibition of fatty acid synthetases by the antibiotic cerulenin. *Biochem. Biophys. Res. Commun.* **48**, 649–656 (1972).
102 Harvey, A. L., Edrada-Ebel, R. & Quinn, R. J. The re-emergence of natural products for drug discovery in the genomics era. *Nat. Rev. Drug Discov.* **14**, 111–129 (2015).
103 King, A. M. *et al.* Aspergillomarasmine A overcomes metallo-β-lactamase antibiotic resistance. *Nature* **510**, 503–506 (2014).
104 Du Toit, A. Bacterial toxins: a 'pain-relieving' toxin. *Nat. Rev. Microbiol.* **12**, 530–531 (2014).
105 Meziane-Cherif, D. & Courvalin, P. Antibiotic resistance: to the rescue of old drugs. *Nature* **510**, 477–478 (2014).
106 Kierek-Pearson, K. & Karatan, E. Biofilm development in bacteria. *Adv. Appl. Microbiol.* **57**, 79–111 (2005).
107 Fotie, J. in *Bioactive Natural Products* (ed. Brahmachari, G.) 223–271 (World Scientific Publishing Company, Singapore, 2012).
108 Liu, L., Redden, H. & Alper, H. S. Frontiers of yeast metabolic engineering: diversifying beyond ethanol and Saccharomyces. *Curr. Opin. Biotechnol.* **24**, 1023–1030 (2013).
109 Sandstrom, A. G. *et al.* Saccharomyces cerevisiae: a potential host for carboxylic acid production from lignocellulosic feedstock? *Appl. Microbiol. Biotechnol.* **98**, 7299–7318 (2014).
110 Karaffa, L. *et al.* A deficiency of manganese ions in the presence of high sugar concentrations is the critical parameter for achieving high yields of itaconic acid by Aspergillus terreus. *Appl. Microbiol. Biotechnol.* **99**, 7937–7944 (2015).
111 Hevekerl, A., Kuenz, A. & Vorlop, K. D. Influence of the pH on the itaconic acid production with Aspergillus terreus. *Appl. Microbiol. Biotechnol.* **98**, 10005–10012 (2014).
112 Purane, N. K. *et al.* Gluconic acid production from golden syrup by Aspergillus niger strain using semiautomatic stirred-tank fermenter. *J. Microbiol. Biochem. Technol.* **4**, 92–95 (2012).
113 Sankpal, N. V. & Kulkarni, B. D. Optimization of fermentation conditions for gluconic acid production using Aspergillus niger immobilized on cellulose microfibrils. *Proc. Biochem.* **37**, 1343–1350 (2002).
114 Kamzolova, S. V. *et al.* Isocitric acid production from rapeseed oil by Yarrowia lipolytica yeast. *Appl. Microbiol. Biotechnol.* **97**, 9133–9144 (2013).
115 Yovkova, V., Otto, C., Aurich, A., Mauersberger, S. & Barth, G. Engineering the α-ketoglutarate overproduction from raw glycerol by overexpression of the genes encoding NADP+-dependent isocitrate dehydrogenase and pyruvate carboxylase in Yarrowia lipolytica. *Appl. Microbiol. Biotechnol.* **98**, 2003–2013 (2014).
116 Brown, S. H. *et al.* Metabolic engineering of Aspergillus oryzae NRRL 3488 for increased production of L-malic acid. *Appl. Microbiol. Biotechnol.* **97**, 8903–8912 (2013).
117 Khan, I., Nazir, K., Wang, Z. P., Liu, G. L. & Chi, Z. M. Calcium malate overproduction by Penicillium viticola 152 using the medium containing corn steep liquor. *Appl. Microbiol. Biotechnol.* **98**, 1539–1546 (2014).
118 Li, S., Chen, X., Liu, L. & Chen, J. Pyruvate production in Candida glabrata: manipulation and optimization of physiological function. *Crit. Rev. Biotechnol.* **36**, 1–10 (2016).
119 Yang, S., Chen, X., Xu, N., Liu, L. & Chen, J. Urea enhances cell growth and pyruvate production in Torulopsis glabrata. *Biotechnol. Prog.* **30**, 19–27 (2014).
120 Morgunov, I. G., Kamzolova, S. V. & Lunina, J. N. The citric acid production from raw glycerol by Yarrowia lipolytica yeast and its regulation. *Appl. Microbiol. Biotechnol.* **97**, 7387–7397 (2013).
121 Thakker, C., Martínez, I., Li, W., San, K. Y. & Bennett, G. N. Metabolic engineering of carbon and redox flow in the production of small organic acids. *J. Ind. Microbiol. Biotechnol.* **42**, 403–422 (2015).
122 Roa Engel, C. A., Straathof, A. J., Zijlmans, T. W., van Gulik, W. M. & van der Wielen, L. A. Fumaric acid production by fermentation. *Appl. Microbiol. Biotechnol.* **78**, 379–389 (2008).
123 Ling, L. B. & Ng, T. K. Fermentation process for carboxylic acids. US Patent 4877731 (1989).
124 Koivistoinen, O. M. *et al.* Glycolic acid production in the engineered yeasts Saccharomyces cerevisiae and Kluyveromyces lactis. *Microb. Cell Fact.* **12**, 82–98 (2013).
125 Xue, Z. *et al.* Production of omega-3 eicosapentaenoic acid by metabolic engineering of Yarrowia lipolytica. *Nat. Biotechnol.* **31**, 734–740 (2013).
126 Wynn, J. P. Taking the fish out of fish oil. *Nat. Biotechnol.* **31**, 716–717 (2013).

127 George, K. W., Alonso-Gutierrez, J., Keasling, J. D. & Lee, T. S. Isoprenoid drugs, biofuels, and chemicals-artemisinin, farnesene, and beyond. *Adv. Biochem. Eng. Biotechnol.* **148**, 355–389 (2015).
128 Chen, Y., Zhou, Y. J., Siewers, V. & Nielsen, J. Enabling technologies to advance microbial isoprenoid production. *Adv. Biochem. Eng. Biotechnol.* **148**, 143–160 (2015).
129 Nielsen, J. Production of biopharmaceutical proteins by yeast: advances through metabolic engineering. *Bioengineered* **4**, 207–211 (2013).
130 Berlec, A. & Strukelj, B. Current state and recent advances in biopharmaceutical production in Escherichia coli, yeasts, and mammalian cells. *J. Ind. Microbiol. Biotechnol.* **40**, 257–274 (2013).
131 Walsh, G. Biopharmaceuticals approval trends in 2013. *Biopharm. Int.* **26**, 54–56 (2013).
132 Hellmuth, K., Nienburg, C. H., van den Brink, J. M. in *Microbial Production of Food Ingredients, Enzymes, and Nutraceuticals* (eds McNeil, B., Archer, D., Giavasis, I. & Harvey, L.) 262–287 (Woodhead Publishing, Oxford, 2013).
133 Tani, S., Kawaguchi, T. & Kobayashi, T. Complex regulation of hydrolytic enzyme genes for cellulosic biomass degradation in filamentous fungi. *Appl. Microbiol. Biotechnol.* **98**, 4829–4837 (2014).
134 Madzak, C. Yarrowia lipolytica: recent achievements in heterologous protein expression and pathway engineering. *Appl. Microbiol. Biotechnol.* **99**, 4559–4577 (2015).
135 Liu, L. *et al.* How to achieve high-level expression of microbial enzymes: strategies and perspectives. *Bioengineered* **4**, 212–223 (2013).
136 Ahmad, M., Hirz, M., Pichler, H. & Schwab, H. Protein expression in Pichia pastoris: recent achievements and perspectives for heterologous protein production. *Appl. Microbiol. Biotechnol.* **98**, 5301–5317 (2014).
137 Hasslacher, M. *et al.* High-level intracellular expression of hydroxynitrile lyase from the tropical rubber tree Hevea brasiliensis in microbial hosts. *Protein Expr. Purif.* **11**, 61–71 (1997).
138 Ratledge, C. in *Microbial Production of Food Ingredients, Enzymes, and Nutraceuticals* (eds McNeil, B., Archer, D., Giavasis, I. & Harvey, L.) 531–558 (Woodhead Publishing, Oxford, 2013).
139 Tai, M. & Stephanopoulos, G. Engineering the push and pull of lipid biosynthesis in oleaginous yeast Yarrowia lipolytica for biofuel production. *Metab. Eng.* **15**, 1–9 (2013).
140 Blazeck, J., Liu, L., Knight, R. & Alper, H. S. Heterologous production of pentane in the oleaginous yeast Yarrowia lipolytica. *J. Biotechnol.* **165**, 184–194 (2013).
141 Sitepu, I. R. Identification of oleaginous yeast strains able to accumulate high intracellular lipids when cultivated in alkaline pretreated corn stover. *Appl. Microbiol. Biotechnol.* **98**, 7645–7657 (2014).
142 Ledesma-Amaro, R., Santos, M. A., Jiménez, A., Revuelta, J. L. in *Microbial Production of Food Ingredients, Enzymes, and Nutraceuticals* (eds McNeil, B., Archer, D., Giavasis, I. & Harvey, L.) 571–594 (Woodhead Publishing, Oxford, 2013).
143 Schwechheimer, S. K., Park, E. Y., Revuelta, J. L., Becker, J. & Wittmann, C. Biotechnology of riboflavin. *Appl. Microbiol. Biotechnol.* **100**, 2107–2119 (2016).
144 Jeong, B. Y., Wittmann, C., Kato, T. & Park, E. Y. Comparative metabolic flux analysis of an Ashbya gossypii wild type strain and a high riboflavin-producing mutant strain. *J. Biosci. Bioeng.* **119**, 101–106 (2015).
145 Mirończuk, A. M., Furgala, J., Rakicka, M. & Rymowicz, W. Enhanced production of erythritol by Yarrowia lipolytica on glycerol in repeated batch cultures. *J. Ind. Microbiol. Biotechnol.* **41**, 57–64 (2014).
146 Lima de Albuquerque, T., José da Silva, I., Ribeiro de Macedo, G. & Rocha, M. V. P. Biotechnological production of xylitol from lignocellulosic wastes: a review. *Proc. Biochem* **49**, 1779–1789 (2014).
147 Misra, S., Raghuwanshi, S. & Saxena, R. K. Fermentation behavior of an osmotolerant yeast D. hansenii for xylitol production. *Biotechnol. Prog.* **28**, 1457–1465 (2012).
148 Sirisansaneeyakul, S., Wannawilai, S. & Chisti, Y. Repeated fed-batch production of xylitol by Candida magnoliae TISTR 5663. *J. Chem. Technol. Biotechnol.* **88**, 1121–1129 (2013).
149 Zhang, J., Geng, A., Yao, C., Lu, Y. & Li, Q. Xylitol production from D-xylose and horticultural waste hemicellulosic hydrolysate by a new isolate of Candida athensensis SB18. *Bioresour. Technol* **105**, 134–141 (2012).
150 Qi, X. *et al.* Enhanced D-arabitol production by Zygosaccharomyces rouxii JM-C46: isolation of strains and process of repeated-batch fermentation. *J. Ind. Microbiol. Biotechnol.* **42**, 807–812 (2015).
151 Tang, L. *et al.* Three-pathway combination for glutathione biosynthesis in Saccharomyces cerevisiae. *Microb. Cell Fact.* **14**, 139 (2015).
152 De Dieu Ndikubwimana, J. & Lee, B. H. Enhanced production techniques, properties, and the uses of coenzyme Q10. *Biotechnol. Lett.* **36**, 1917–1926 (2014).
153 Yatsyshyn, V. Y., Fedorovych, D. V. & Sibirny, A. A. Metabolic and bioprocess engineering of the yeast Candida famata for FAD production. *J. Ind. Microbiol. Biotechnol.* **41**, 823–835 (2014).
154 Sharma, N., Prasad, G. S. & Choudhury, A. R. Utilization of corn steep liquor for biosynthesis of pullulan, an important exopolysaccharide. *Carbohydr. Polym.* **93**, 95–101 (2013).
155 Keswani, C., Mishra, S., Sarma, B. K., Singh, S. P. & Singh, H. B. Unraveling the efficient applications of secondary metabolites of various Trichoderma spp. *Appl. Microbiol. Biotechnol.* **98**, 533–544 (2014).
156 Caspeta, L. & Nielsen, J. Toward systems metabolic engineering of Aspergillus and Pichia species for the production of chemicals and biofuels. *Biotechnol. J.* **8**, 534–544 (2013).
157 Johnson, E. A. Biotechnology of non-Saccharomyces yeasts-the basidiomycetes. *Appl. Microbiol. Biotechnol.* **97**, 7563–7577 (2013).
158 Caspeta, L. *et al.* Biofuels. Altered sterol composition renders yeast thermotolerant. *Science* **346**, 75–78 (2014).
159 Matsushika, A. & Hoshino, T. Increased ethanol production by deletion of HAP4 in recombinant xylose-assimilating Saccharomyces cerevisiae. *J. Ind. Microbiol. Biotechnol.* **42**, 1623–1631 (2015).
160 Lam, F. H., Ghaderi, A., Fink, G. R. & Stephanopoulos, G. Biofuels. Engineering alcohol tolerance in yeast. *Science* **346**, 71–75 (2014).
161 Lili, L., Li, L., Wang, Y., Du, Y. & Qin, S. Biorefinery products from the inulin-containing crop Jerusalem artichoke. *Biotechnol. Lett.* **35**, 471–477 (2013).
162 Sasano, Y. *et al.* Overexpression of the yeast transcription activator Msn2 confers furfural resistance and increases the initial fermentation rate in ethanol production. *J. Biosci. Bioeng.* **113**, 451–455 (2012).
163 Gorsich, S. W. *et al.* Tolerance to furfural-induced stress is associated with pentose phosphate pathway genes ZWF1, GND1, RPE1, and TKL1 in Saccharomyces cerevisiae. *Appl. Microbiol. Biotechnol.* **71**, 339–349 (2006).
164 Hasunuma, T., Ismail, K. S., Nambu, Y. & Kondo, A. Co-expression of TAL1 and ADH1 in recombinant xylose-fermenting Saccharomyces cerevisiae improves ethanol production from lignocellulosic hydrolysates in the presence of furfural. *J. Biosci. Bioeng.* **117**, 165–169 (2014).
165 Agbogbo, F. K. & Coward-Kelly, G. Cellulosic ethanol production using the naturally occurring xylose-fermenting yeast, Pichia stipitis. *Biotechnol. Lett.* **30**, 1515–1524 (2008).
166 Parekh, S. R., Parekh, R. S. & Wayman, M. Fermentation of xylose and cellobiose by Pichia stipitis and Brettanomyces clausenii. *Appl. Biochem. Biotechnol.* **18**, 325–338 (1988).
167 Slininger, P. J., Dien, B. S., Gorsich, S. W. & Liu, Z. L. Nitrogen source and mineral optimization enhance D-xylose conversion to ethanol by the yeast Pichia stipitis NRRL Y-7124. *Appl. Microbiol. Biotechnol.* **72**, 1285–1296 (2006).
168 Kim, S. J., Seo, S. O., Jin, Y. S. & Seo, J. H. Production of 2,3-butanediol by engineered Saccharomyces cerevisiae. *Bioresour. Technol* **146**, 274–281 (2013).
169 Lin, H., Wang, Q., Shen, Q., Zhan, J. & Zhao, Y. Genetic engineering of microorganisms for biodiesel production. *Bioengineered* **4**, 292–304 (2013).
170 Denning, D. W. Echinocandin antifungal drugs. *Lancet* **362**, 1142–1151 (2003).
171 Balkovec, J. M. *et al.* Discovery and development of first in class antifungal caspofungin (CANCIDAS)-a case study. *Nat. Prod. Rep.* **31**, 15–34 (2014).
172 Livermore, D. M. The need for new antibiotics. *Clin. Microbiol. Infect.* **10**, 1–9 (2004).
173 Clardy, J., Fischbach, M. A. & Walsh, C. T. New antibiotics from bacterial natural products. *Nat. Biotechnol.* **24**, 1541–1550 (2006).
174 Scheffler, R., Colmer, S., Tynan, H., Demain, A. L. & Gullo, V. P. Antimicrobials, drug discovery, and genome mining. *Appl. Microbiol. Biotechnol.* **97**, 969–978 (2013).