Chapter 3
Bioactive Products from Fungi

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1 Introduction

The microbial drug era began back in 1928 when Alexander Fleming discovered in a petri dish seeded with *Staphylococcus aureus* that a compound produced by a contaminating mold killed the bacterium. The active compound, produced by *Penicillium notatum*, was named penicillin. By using the same strategy, other antibiotics such as streptomycin and chloramphenicol were later isolated from different bacterial and fungal fermentations. Antibiotics can be produced by fermentation, an old technique that was utilized for beer and wine production almost 8000 years ago, during the ancient Egypt and Mesopotamia era. Similarly, cheese production by *Penicillium roqueforti* can be traced back for almost 4000 years. Additional examples of traditional fermentations are soy sauce in Asia and bread production (Hölker et al. 2004; Seviour et al. 2013). Bread production was common in Egypt in 4000 BC. Beer production using the non-filamentous fungus *Saccharomyces cerevisiae* began in 7000 BC by the Sumerians and Chinese. Wine was made in Iran in 5000 BC and in Egypt in 3000 BC.

Natural products (NPs) with high commercial value can be produced by microbial primary or secondary metabolism. Thanks to the technical improvements in screening programs and techniques for separation and isolation, the number of natural compounds discovered surpasses one million (Berdy 2005). Among them, 50–60% are produced by plants (alkaloids, flavonoids, terpenoids, steroids, carbohydrates, etc.), and 5% of these plant products have a microbial origin.

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About 20–25% of the reported natural products show biological activity and of these, approximately 10% have been obtained from microbes.

Microorganisms produce many compounds with biological activity. From 22,500 bioactive compounds so far obtained from microorganisms, about 9000 are produced by fungi (Berdy 2005; Brakhage and Schroekh 2011). Therefore, the role of fungi in the production of antibiotics and other drugs for treatment of non-infective diseases has been crucial (Demain et al. 2004).

With less than 5% of the fungal world having been cultured, there have been significant advances in microbial techniques for growth of uncultured organisms as a potential source of new chemicals (Kaeberlein et al. 2002). As more genomes are sequenced, it is found that filamentous fungi grasp the genetic capacity to produce an arsenal of secondary metabolites. In fungi, biosynthetic genes are present in clusters coding for large, multidomain, and multimodular enzymes such as polyketide synthases, prenyltransferases, non-ribosomal peptide synthases, and terpene cyclases. Genes adjacent to the biosynthetic gene clusters encode regulatory proteins, oxidases, hydroxylases, and transporters. Aspergilli usually contain 30–40 secondary metabolite gene clusters. Most of these clusters coding for secondary metabolites are still cryptic or silent under standard culture conditions (Hertweck 2009). Therefore, mining for these cryptic secondary metabolites can be an excellent source of new drugs by awakening cryptic clusters for secondary metabolism. In addition, recent knowledge on cluster regulation has unlocked many hidden fungal bioactive compounds. Regulation of fungal secondary metabolism has been reviewed by Brakhage (2013). Emphasized are the regulatory elements that control gene transcription, including the targeted activation of silent gene clusters (Brakhage and Schroekh 2011). A method to predict secondary metabolite gene clusters in filamentous fungi has been devised (Anderson et al. 2013).

In addition, metagenomics, i.e., the extraction of DNA from soil, plants, and marine habitats and its incorporation into known organisms, allow access to a vast untapped reservoir of genetic and metabolic diversity (Colwell 2002; Gaudilliere et al. 2001). Thus, the potential for discovery of new fungal secondary metabolites with beneficial use for humans is great.

2 Antibiotics

Of the 12,000 antibiotics known in 1955, filamentous fungi produced 22% (Verdine 1996; Strohl 1997). The beta-lactams are the most important class of antibiotics in terms of use. They constitute a major part of the antibiotic market. Included are the penicillins, cephalosporins, clavulanic acid, and the carbapenems. Of these, fungi are responsible for the production of penicillins and cephalosporins (Fig. 1). The natural penicillin G and the biosynthetic penicillin V had a market of $4.4 billion by the late 1990s. Major markets also included semi-synthetic penicillins and cephalosporins amounting to $11 billion. In 2006, the market for cephalosporins was $9.4 billion and that for penicillins was $6.7 billion.

Fig. 1 Chemical structures of penicillin (*left*) and cephalosporin (*right*)
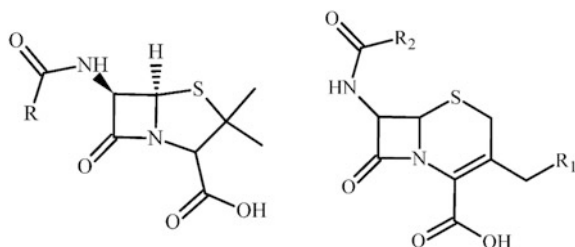
Production of all beta-lactams in 2003 had reached over 60,000 tons. The titer of penicillin is over 100 g L^{-1} and that for cephalosporin C is more than 35 g L^{-1} (Masurekar 2008; Yang et al. 2012). Recovery yields are more than 90%. There have been over 15,000 molecules based on penicillin that have been made by semi-synthesis or by total synthesis.

Important in penicillin biosynthesis are the regulatory factors. *Penicillium chrysogenum,* the producer of penicillin G, contains global regulatory factor PcRFX1, which positively regulates three beta-lactam biosynthetic genes, i.e., *pcbAB, pcbC, and penDE* (Dominguez-Santos 2012). This regulatory factor not only controls secondary metabolism but also controls primary metabolism. Related factor CPCR1 is a global regulator found in the cephalosporin C producer *Acremonium chrysogenum*, binding to at least two sequences of the *pcbAb-pcbC* intergenic region and regulating cephalosporin C biosynthesis.

1,3-Diaminopropane (1,3-DAP) is secreted by *P. chrysogenum* and *A. chryso-genum.* This and spermidine (which contains 1,3-DAP) increase the transcription levels of the penicillin biosynthetic genes *pcbAB, pcbC,* and *penDE* (Martín et al. 2012). They thus stimulate the production of penicillin G. The mechanism appears to involve stimulation of the expression of *laeA*, a global regulator that acts epigenetically on the expression of secondary metabolism genes via heterochromatin reorganization. 1,3-DAP also stimulates the production of cephamycin in *Amycolatopsis lactamdurans*. Spermidine's activity appears to be due to 1,3-DAP.

By the mid-1990s, 160 antibiotics and their derivatives were already on the market (Strohl 1997; Brown 1996). The market in 2009 was $79 billion dollars. Despite these impressive figures, more antibiotics are needed to combat evolving pathogens, naturally resistant microbes, and bacteria and fungi that have developed resistance to current antibiotics. A new and approved cephalosporin is ceftobiprole, which is active against methicillin-resistant *S. aureus* (MRSA) and is not hydrolyzed by a number of beta-lactamases from gram-positive bacteria (Shang et al. 2010). Another antibiotic of note is cerulenin, an antifungal agent produced by *Acremonium caerelens*. It was the first inhibitor of fatty acid biosynthesis discovered (Vance et al. 1972). It alkylates and inactivates the active-site nucleophilic 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.

Parafungin from *Fusarium lavarum* is a recently discovered antifungal agent inhibiting poly(A) polymerase in *Candida albicans* as well as in a broad range of pathogenic fungi (Harvey et al. 2015).

3   Additional Pharmacological Agents

Over the years, non-infectious diseases were mainly treated with synthetic compounds. Despite testing thousands of synthetic chemicals, only a handful of promising structures was obtained. As new synthetic lead compounds became extremely difficult to find, microbial products came into play. Since microorganisms are such a prolific source of structurally diverse bioactive metabolites, over the years, the pharmaceutical industry extended their antibiotic screening programs to look for additional applications of antibiotics in medicine and agriculture (Cardenas et al. 1998; Kremer et al. 2000). As a result of this move, some of the most important products of the pharmaceutical industry were obtained. For example, the immune suppressants have revolutionized medicine by facilitating organ transplantation (Verdine 1996). Other products include anti-tumor drugs, hypocholesterolemic agents, enzyme inhibitors, gastrointestinal motor stimulators, ruminant growth stimulants, insecticides, herbicides, antiparasitics versus coccidia and helminths, and other pharmacological activities. Stimulated by the use of simple enzyme assays for screening, prior to testing in intact animals or in the field, further applications are emerging in various areas of pharmacology and agriculture. In 2013, there were more than 15 secondary metabolites derived from marine fungi in clinical trials (Bhatnagar and Kim 2013). Many of the new natural products from marine sources are polyketides.

S. cerevisiae and *Pichia pastoris* are used for the production of biopharmaceuticals (Berlec and Strukelj 2013). Biopharmaceuticals have the fastest growth rate of products on the market. *S. cerevisiae* produces 20% of these. Of 211 biopharmaceuticals approved by 2011, 31 were produced by yeasts, 30 by *S. cerevisiae,* and one by *P. pastoris*. The production of biopharmaceuticals by *S. cerevisiae* has been reviewed by Nielsen (Nielsen 2013). The yeast is used to make insulin and insulin analogs. The insulin market was $12 billion in 2011. Other products are human serum albumin, hepatitis vaccines, and virus-like particles used for vaccination against human papilloma virus. The advantages of *S. cerevisiae* include proper folding of human proteins and their secretion into the extracellular medium, facilitating purification and proper post-translational modification of the protein. This includes proteolytic processing of signal peptides, disulfide bond formation, subunit assembly, acylation, and glycosylation. Human serum albumin is produced at 3 g L^{-1}.

4 Anticancer Drugs

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

Taxol *(paclitaxel)* was first isolated from the Pacific yew tree, *Taxus brevifolia* (Wall and Wani 1996), and later found to be a fungal secondary metabolite (Stierle et al. 1993). It is a steroidal alkaloid diterpenoid that has a characteristic N -benzoylphenyl isoserine side chain and a tetracycline ring (Fig. 2). It inhibits rapidly dividing mammalian cancer cells by promoting tubulin polymerization and interfering with normal microtubule breakdown during cell division. The benzoyl group of the molecule is particularly crucial for maintaining the strong bioactivity of taxol. The drug also inhibits several fungi (species of *Pythium, Phytophthora,* 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 (Newman and Cragg 2007). A formulation in which paclitaxel is bound to albumin is sold under the trademark Abraxane®. Taxol sales amounted to $1.6 billion in 2006 for Bristol-Myers Squibb, representing 10% of the company's pharmaceutical sales and its third largest selling product. It has reached $3.7 billion annual sales in international markets.

Although synthetic methods for taxol production have been tried, the chemical molecular structure is so complex that commercial synthetic production is unfeasible.

Currently, Italy, the UK, the Netherlands, and other Western countries are engaged in the production of taxol by plant cell fermentation technology. Taxol production by a plant cell culture of *Taxus* sp. was reported to be at 67 mg L^{-1} (Sabater-Jara et al. 2010). However, the addition of methyl jasmonate, a plant signal transducer, increased the production to 110 mg L^{-1}.


Fig. 2 Chemical structure of taxol. The dotted section corresponds to the molecule N-benzophenyl isoserine side chain
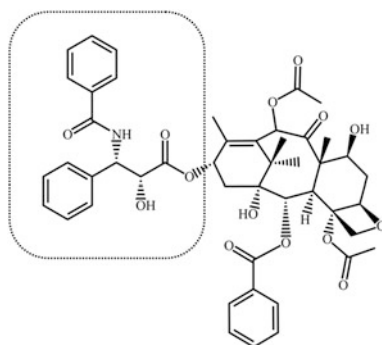
As stated above, taxol has also been found to be a fungal metabolite (Stierle et al. 1993; Jiang et al. 2012). Fungi such as *Colletotrichum gloeosporoides*, *Colletotrichum capsici, Fusarium maire*, *Nodulisporium sylviforme*, *Pestalotiopsis microspora*, *Pestalotiopsis versicolo*r, *Phyllosticta citricarpa*, *Taxomyces andreanae*, and *Tubercularia* sp. are taxol producers (Stierle et al. 1993; Flores-Bustamante et al. 2010; Gangadevi and Muthumary 2008; Kumaran et al. 2010, 2011; Li et al. 1996; Wang et al. 2000; Xu et al. 2006; Zhao et al. 2004]. *C. gloeosporoides* produced 163 μg L^{-1} of taxol (Gangadevi and Muthumary 2008), and the endophyte *F. maire* made 225 μg L^{-1} (Xu et al. 2006). The production by *P. citricarpa* amounted to 265 μg L^{-1} (Kumaran et al. 2008) and 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 and taxadiene synthase (Wei et al. 2012). The endophyte *P. versicolor*, from the plant *Taxus caspodata,* produced 478 μg L^{-1} (Kumaran et al. 2010). *C. capsici* from *Capsicum annuum* made 687 μg L^{-1} (Kumaran et al. 2011). Another endophytic fungus, *Phoma betae*, isolated from the medicinal tree *Ginkgo biloba* produced taxol at 795 μg L^{-1} (Kumaran et al. 2012). *Colletotrichum annutum* from *Capsium annuum* *Cladosporium cladosporoides*, an endophyte of the *Taxus media* tree, produced 800 μg L^{-1} of taxol (Zhang et al. 2009). *Metarhizium anisopiliae* H-27, isolated from the tree *Taxus chinensis*, yielded 846 μg L^{-1} (Liu et al. 2009). 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} (Zhou et al. 2010), 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} (Duan et al. 2008).

Another important antitumor agent is camptothecin (Fig. 3), a modified monoterpene indole alkaloid produced by certain plants (angiosperms) and by the endophytic fungus, *Entrophospora infrequens*. The fungus was isolated from the plant *Nathapodytes foetida* (Wall and Wani 1996). 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 μg L^{-1} of camptothecin in the presence of the elicitor methyljasmonate and XAD adsorbent resin (Pu et al. 2013). 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

Fig. 3 Chemical structure of camptothecin
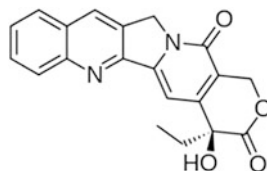
cancer (Amna et al. 2006). Colon cancer is the second-leading cause of cancer fatalities in the USA and the third most common cancer among the US citizens.

Camptothecin is known commercially as Camptosar and Campto and achieved sales of $1 billion in 2003 (Lorence and Nessler 2004). 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 (2010).

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

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

Inhibitors of farnesyltransferase (FTIs) have anticancer activity because farnesylation is required for the activation of Ras, a necessary step in cancer progression. They also induce apoptosis in cancer cells. The fungus *Phoma* sp. FL-415 produces an FTI known as TAN-1813 (Bernardes et al. 2010).

5 Immunosuppressant Drugs

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

Microbial compounds capable of suppressing the immune response have been discovered as fungal secondary metabolites. Cyclosporin A was originally discovered in the 1970s as a narrow-spectrum antifungal peptide produced by the
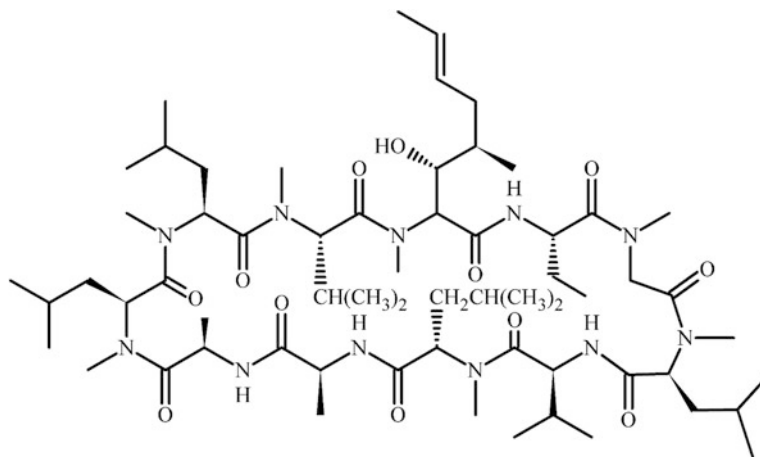
Fig. 4 Chemical structure of cyclosporin A

mold, *Tolypocladium nivenum* (previously *Tolypocladium inflatum*) in an aerobic fermentation (Borel et al. 1976). Cyclosporins (Fig. 4) are a family of neutral, highly lipophilic, cyclic undecapeptides containing some unusual amino acids, synthesized by a non-ribosomal peptide synthetase, cyclosporin synthetase. Discovery of the immunosuppressive activity of this secondary metabolite led to use in heart, liver, and kidney transplants and to the overwhelming success of the organ transplant field (Borel 2002). Cyclosporin was approved for use in 1983. It is thought to bind to the cytosolic protein cyclophilin (immunophilin) of immuno-competent lymphocytes, especially T lymphocytes. This complex of cyclosporin and cyclophilin inhibits calcineurin, which under normal circumstances is respon-sible for activating the transcription of interleukin-2. It also inhibits lymphokine production and interleukin release and therefore leads to a reduced function of effector T cells. Annual world sales of cyclosporin A are approximately $2 billion. Cyclosporin A also has activity against corona viruses (de Wilde et al. 2011).

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 acti-vation 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 the activation of T cells.

Pleuromutilin, a tricyclic terpenoid inhibitor of protein synthesis, was originally isolated in 1951 from the basidiomycete *Pleurotis* sp. (Kirst 2012). Although it was rapidly metabolized and had unfavorable pharmacokinetics, its semi-synthetic derivatives tiamalin and valnemulin have been successful for control and treatment

of swine and poultry diseases. Also, retapamulin (Altabax®) was approved for topical treatment of human skin diseases.

A very old broad-spectrum antibiotic, actually the first antibiotic ever discovered, is mycophenolic acid, which has an interesting history. Bartolomeo Gosio (1863–1944), an Italian physician, discovered the compound in 1893 (Bentley 2001). Gosio isolated a fungus from spoiled corn, which he named *Penicillium glaucum*, which was later reclassified as *P. brevicompactum*. He isolated the crystals of the compound from culture filtrates in 1896 and found it to inhibit the 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 (1913) and given the name mycophenolic acid. They used a strain originally isolated from spoiled corn in Italy called *Penicillium stoloniferum*, a synonym of *P. brevi-compactum*. The chemical structure was elucidated many years later (1952) by Birkinshaw et al. (1952) 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 (Sebastian et al. 2011). 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 (Lee et al. 1990). The ester is called mycophenolate mofetil (CellCept) and is a prodrug that is hydrolyzed to mycophenolic acid in the body. It is sometimes used along with cyclosporin in kidney, liver, and heart transplants. Mycophenolic acid also appears to have anti-angiogenic activity (Chong et al. 2006).

6 Hypocholesterolemic Agents

Only about 30% of cholesterol in humans comes from the diet. The rest is synthesized by the body, predominantly in the liver. Many people cannot control their level of cholesterol at a healthy level by diet alone and require hypocholesterolemic agents. High blood cholesterol leads to atherosclerosis, which is a chronic, progressive disease characterized by continuous accumulation of atheromatous plaque within the arterial wall, causing stenosis and ischemia. Atherosclerosis is a leading cause of human death. The last two decades have witnessed the introduction of a variety of anti-atherosclerotic therapies. The statins form a class of hypo-lipidemic drugs, formed as secondary metabolites by fungi, and used to lower cholesterol by inhibiting the rate-limiting enzyme of the mevalonate pathway of cholesterol biosynthesis, i.e., 3-hydroxymethyl glutaryl-CoA (HMG-CoA) reductase. Inhibition of this enzyme in the liver stimulates low-density lipoprotein (LDL) receptors, resulting in an increased clearance of LDL from the bloodstream and a decrease in blood cholesterol levels. They can reduce total plasma cholesterol by 20–40%. Through their cholesterol-lowering effect, they reduce the risk of

Fig. 5 Chemical structure of lovastatin
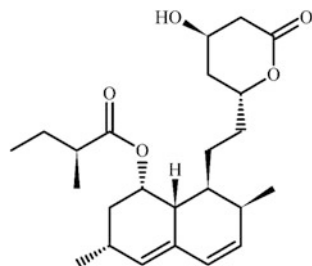
cardiovascular disease, prevent stroke, and reduce development of peripheral vascular disease (Nicholls et al. 2007).

Currently, there are a number of statins in clinical use. They reached an annual market of nearly $30 billion before one became a generic pharmaceutical. The history of the statins has been described by Akira Endo, the discoverer of the first statin, compactin (mevastatin; ML-236B) (Endo 2010). This first member of the group was isolated as an antibiotic product of *P. brevicompactum* (Brown et al. 1976). At about the same time, it was found by Endo and coworkers as a cholesterolemic product of *Penicillium citrinum* (Endo et al. 1976). Although compactin was not of commercial importance, its derivatives achieved strong medical and commercial success. Lovastatin (monacolin K; mevinolin; Mevacor TM) was isolated in broths of *Monascus rubra* and *Aspergillus terreus* (Alberts et al. 1980; Endo and Monacolin 1979). Lovastatin, developed by Merck & Co. and approved by the US Food and Drug Administration (FDA) in 1987, was the first commercially marketed statin. In its chemical structure, lovastatin has a hexahydronaphthalene skeleton substituted with a *p*-hydroxy-lactone moiety (Fig. 5).

A semisynthetic derivative of lovastatin is Zocor® (simvastatin), one of the main hypocholesterolemic drugs, sold for $7 billion per year before becoming generic. An unexpected effect of simvastatin is its beneficial activity on pulmonary artery hypertension (Liu et al. 2011). Another surprising effect is its antiviral activity (Bader et al. 2008). Simvastatin is active against RNA viruses and acts as monotherapy against chronic hepatitis C virus in humans. It has been shown to act in vitro against hepatitis B virus (HBV). This virus infects 400 million people and is the most common infectious disease agent in the world. The virus causes hepatocellular cancer, which is the leading cause of cancer.

7 Mycotoxins

Fungi produce poisons called mycotoxins, which, strangely enough, have been harnessed as medically useful agents. These agents (e.g., ergot alkaloids) caused fatal poisoning of humans and animals (ergotism) for centuries by the 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

Fig. 6 Chemical structure of zearalenone
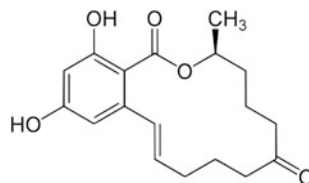
disturbances, inhibition of protein release in agalactorrhea, reduction in bleeding after childbirth, and prevention of implantation in early pregnancy (Bentley 1997; Vining and Taber 1979). Their physiological activities include the inhibition of action of adrenalin, noradrenalin, and serotonin, as 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 (Fig. 6) is an estrogen made by *Gibbberella zeae* (syn. *Fusarium graminearum*) (Hidy et al. 1977). Its reduced derivative zeranol is used as an anabolic agent in sheep and cattle, which increases growth and feed efficiency. Gibberellic acid, a member of the mycotoxin group known as gibberellins, is a product of *Gibberella fujikuroi* and causes "foolish rice seedling" disease in rice (Jefferys 1970). 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 (Tudzinski 1999). More than 25 tons are produced annually with a market of over $100 billion.

8 Inhibitors of Enzyme Activity

Enzyme inhibitors have received increased attention as useful tools, not only for the study of enzyme structures and reaction mechanisms, but also for potential utilization in medicine and agriculture. Several enzyme inhibitors with various industrial uses have been isolated from microbes (Umezawa 1972). Among the most important are the statins and hypocholesterolemic drugs discussed previously. Fungal products are also used as enzyme inhibitors against cancer, diabetes, poisoning, and Alzheimer's disease. The enzymes inhibited include acetylcholinesterase, protein kinase, tyrosine kinase, glycosidases, and others (Paterson 2008).

9 Pigments

Since 800 AD, *Monascus purpurea* has been grown on rice to prepare koji or Angkak (red rice), which is used as a traditional Chinese food and medicine (Ma et al. 2000). Monascorubramine and rubropunctatin are water-soluble red pigments

formed upon the reaction of the orange pigments monascorubrin and rubropunctatin with amino acids in fermentation media (Juzlova et al. 1996). The fungus is used to prepare red rice, wine, soybean cheese, meat, and fish. It is authorized in Japan and China for food use. There are 54 known *Monascus* pigments. They have an amazing number of activities: antimicrobial, anticancer, anti-mutagenesis, anti-diabetes, anti-obesity, anti-inflammatory, cholesterol-lowering, immunosuppressive, and hypotensive (Feng et al. 2012; Lee and Pan 2012). Nutritional control of the formation of the red pigments has been described in a series of publications by Lin and Demain (1991, 1993, 1994, 1995).

Carotenoids are tetra-terpenoid pigments which are excellent anti-oxidants. They are used as nutritional supplements, animal feeds, food additives, pharmaceuticals, food coloring agents, and in cosmetics. They are composed of hydrocarbons (carotenes and lycopene) and oxygenated derivatives (xanthophylls) and are used for protection against cancer, age-related muscular degeneration, and cardiovascular diseases (Roukas 2015). Beta-carotene and lycopene are highly unsaturated isoprene derivatives which stimulate the immune system and prevent degenerative diseases and cancer. Some are made microbiologically. They had a 2010 market of $1.2 billion, and their market is growing by 2.3% per year. Adaptive laboratory evolution was used to increase the 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 due to up-regulation of genes related to biosynthesis of lipid and mevalonate (Reyes et al. 2013). The production amounted to 16 mg g^{-1} dry cell weight. Beta-carotene, a precursor of vitamin A, has a market of $242 million. Although most is made chemically, it can be made by *Blakeslea trispora* at 3 g L^{-1} (Vachali et al. 2012). Lycopene is another carotenoid.

Phaffia rhodozyma (*Xanthophyllomyces dendrorhous*) is a heterobasidiomycetous yeast that has become the most important microbial source for the preparation of the carotenoid astaxanthin (Andrewes et al. 1976; Rodríguez-Saiz et al. 2010). This oxygenated carotenoid pigment (Fig. 7) is used in the feed, food, pharmaceutical, nutraceutical, 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 penreared salmonids with a diet containing this yeast induces pigmentation of the white muscle (Johnson et al. 1980). It is a very good antioxidant, 10 times more active than beta-carotene and 100 times more than alpha-tocopherol. It is the second most important carotenoid. Astaxanthin enhances the immune system and protects skin from radiation injury and cancer. It can be produced synthetically as hydroxyl-astaxanthin from petrochemicals with a selling price of $2500 per kg. However, the natural product is favored because the synthetic product is a mixture of stereoisomers. *X. dendror-hous* produces astaxanthin at 390 mg L^{-1}.

Natural astaxanthin is more stable than the synthetic version and more bioavailable. The natural product is present in algae and fish as mono- and diesters of fatty acids. However, it is difficult to hydrolyze the esters from algae, which limits its usage to trout and salmon. The yeast product is better since it is the 97% free, non-esterified (3R, 3'R) stereoisomer. The natural product is more expensive ($7000 per kg) than synthetic astaxanthin ($2500 per kg). The astaxanthin market
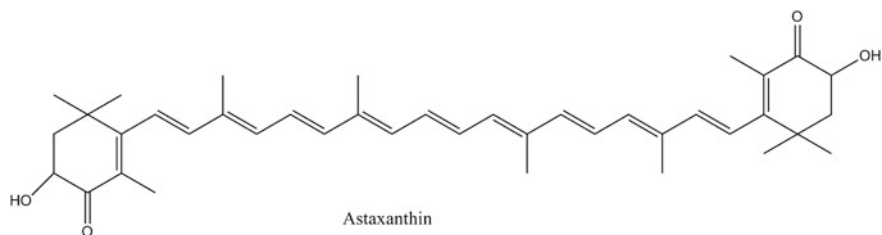
Fig. 7 Chemical structure of astaxanthin

was $219 million in 2007 with 97% being synthetic. Most of the production processes with the yeast yield levels of astaxanthin are lower than 100 mg L^{-1}. However, white light improved production to 420 mg L^{-1} (de la Fuente et al. 2012) and mutant strain UBv-AX2 can make 580 mg L^{-1} (Jacobson et al. 2000).

10 Sweeteners

Thaumatin, a protein produced by the plant *Thaumatococcus danielli*, can also be produced by *P. roqueforti* and *Aspergillus niger* var. *awamori* (Faus 2000). Thaumatin is intensely sweet (i.e., 3000 times sweeter than sucrose) and is approved as a foodgrade ingredient. The 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 (Moralejo et al. 1999). The sweetener xylitol, normally produced by *Pichia stipitis*, can be produced by recombinant *S. cerevisiae* in higher concentrations by transforming the XYL1 gene of *P. stipitis* into *S. cerevisiae*. The gene encodes a xylose reductase (Hallborn et al. 1991).

11 Proteins

Industrial enzymes include detergent enzymes, technical enzymes, food enzymes, and feed enzymes (Hellmuth and Bring 2013). Technical enzymes include those used for textiles, leather, pulp and paper, and fuel ethanol. The largest group is the food enzymes which 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. Fungal producers are a major source, and the main ones are *A. niger* and *Klyveromyces lactis.*

Advances in the production of biopharmaceutical proteins by metabolic engineering have been reviewed by Nielsen (2013). Yeasts are used to produce

recombinant proteins (Celik and Calik 2012). They rapidly reach high levels of growth, produce high amounts of recombinant proteins, and do not contain pyrogens, pathogens, or viral inclusions. About 20% of the biopharmaceuticals on the market are made by *S. cerevisiae*. They include more than 40 different recombinant proteins. This yeast is important for production of FDA-approved insulin and its analogs, hepatitis B surface antigen, urate oxidase, glucagons, granulocyte-macrophage colony stimulating factor (GM-CSF), hirudin and platelet-derived growth factor. The insulin market was $12 billion in 2011 and is still on the increase. Human serum albumin, used as a plasma expander in surgery, is produced by *S. cerevisiae* at 3 g L^{-1}, and human transferrin, used for anemia, is produced at 1.8 g L^{-1}.

Yeasts also are used to make human serum albumin, hepatitis vaccines, and virus-like particles used for vaccination against human papilloma virus. *S. cerevisiae* carries out folding of many human proteins, secretes the proteins, and post-translational modifications, e.g., proteolytic processing of signal peptides, disulfide bond formation, subunit assembly, acylation, and glycosylation. However, *S. cerevisiae* is not favored today because of plasmid instability, low levels of produced proteins, lack of secretion due to retention in the proteins in the periplasm, and hyper-glycosylation of the recombinant proteins including the high-mannose type of N-glycosylation which shortens the in vivo half-life, reduces efficacy, and elicits an immunogenic response to the non-human carbohydrate moiety.

The yeasts that are used, having been engineered for more human-type N-glycosylation, include *Pichia pastoris, Hansenula polymorpha, Yarrowia lipolytica,* and *Schizosaccharomyces pombe.* Titers of *P. pastoris* have reached 20–30 g L^{-1}, and it can secrete the proteins. *P. pastoris* has been engineered to produce human-like N-glycosylation that includes terminal addition of sialic acid to the glycoprotein. *P. pastoris* produces ecallantide, which was approved by FDA in 2009 for hereditary angioedema. It also produces plant-derived hydroxynitrile lyase at over 20 g L^{-1} (Hasslacher et al. 1997). *H. polymorpha* has been used for the production of hepatitis B vaccine, interferon alpha-2a, hirudin, insulin, phytase, lipase, hexose oxidase, interleukin-6, serum albumin, glucose oxidase, glycolate oxidase, and catalase; the first four are on the market. This yeast reaches high growth density, secretes proteins as large as 150 kDa, and is highly productive. For example, it produces 13.5 g L^{-1} of recombinant phytase. Other useful yeasts include *K. lactis* for the production of bovine chymosin (rennin), glucoamylase, human serum albumin, interleukin-1 and interleukin-1 beta, and many other recombinant proteins. *S. pombe* has been used to produce human lipocortin I, human papillomavirus type 16 vaccine, and many others. The beauty of these yeasts is their ability to perform post-translational modifications similar to those of higher eukaryotes, e.g., correct folding, disulfide bond formation, N- and O-linked glycosylation, and proteolytic processing of signal sequences. About 70% of all therapeutic proteins are glycoproteins. The production of recombinant microbial enzymes by fungi has been reviewed by Liu et al. (2013a, b).

12 Biofuels

Systems metabolic engineering for the production of biofuels and chemicals by *Aspergillus* and *Pichia* species has been reviewed by Caspeta and Nielsen (2013). Ethanol can be used as a fuel by itself or in combination with gasoline (E10, E15, and E85). It is mainly made in the USA (over 7 billion gallons from corn) and in Brazil. However, corn can only yield 15 billion gallons, and corn prices are rising. Cellulose is a possible source of ethanol but instead of containing only glucose, cellulose also contains C5 sugars such as xylose and arabinose. The best C5 utilizer is *P. stipitis* which can produce ethanol and clean up concentrated toxins liberated from lignocellulose degradation. Its production of ethanol has been reviewed by Agbogo and Coward-Kelly (2008). It can produce ethanol from pretreated sources of biomass such as red oaks, wheat straw, sugarcane bagasse, rice straw, corn cobs, corn stover, aspen wood, pinewood, and poplar wood. From aspen wood such as *Orpinomyces* defined medium, 61 g L^{-1} can be made (Slininger et al. 2006). Attributes of *P. stipitis* include consumption of acetic acid, reduction in the furan ring toxins in HMF, and furfural present in cellulosic biomass conversions.

The production of ethanol via biomass saccharification using fungi has been discussed by Zhang (2011). Saccharification of biomass involves pretreatment, fractionation, and enzymatic hydrolysis. Pretreatment may be the most expensive step, amounting to 40% of total processing costs. Biodelignification of lignocellulose has been carried out by ascomycetes including *Trichoderma reesei*, basidiomycetes such as the white rot fungus *Phaenerochaete* sp. (Chandel et al. 2015). The key enzyme in delignification is manganese peroxidase. Biodelignification is the most expensive step in the conversion of biomass to ethanol mainly due to its slow rate of action. Protein engineering must be applied to make the delignification enzymes better suited to the temperature, pH, and reaction conditions of the industrial process.

Hydrolysis by cellulase is another expensive step costing 50 cents to $1/gallon of produced ethanol. Nearly 100–200 g of cellulase is used per gallon of ethanol produced, where specific activities of fungal cellulases are 0.6–1.5 filter paper units per mg of cellulase. Filamentous fungi can produce native cellulases at levels of more than 100 g L^{-1} (Cherry and Fidanstsel 2003). Novozymes, Genencor, and Iogen produce cellulase from *Trichoderma*, whereas dyadic uses *Chrysosporium lucknowense*. These commercial fungal fermentations produce over 100 g crude cellulase per liter of broth, much higher than that produced by bacteria. An important move is to decrease the amount of cellulase used to produce ethanol. The overall action of *T. reesei* cellulase on cellulosic biomass is limited by a low content of beta-glucosidase. The result is an accumulation of cellobiose which limits further breakdown. By expressing the beta-glucosidase gene of *Pericona sp.* in *T. reesei*, (Dashtban and Qin 2012) were able to increase the level of beta-glucosidase, the overall cellulase activity, and the action on biomass residues.

During pretreatment of biomass, inhibitors are released such as furfural. Tolerance to this inhibitor can be achieved by over-expression of *S. cerevisiae*

genes encoding (a) yeast transcription activator MSN2 (Sasano et al. 2012), (b) ZWF1 of the pentose phosphate pathway (Gorsich et al. 2006), (c) ADH1 encoding alcohol dehydrogenase 1, and (d) TAL1 encoding transaldolase 1 (Hasunama et al. 2014).

Regulation of cellulolytic and hemi-cellulolytic enzyme production by filamentous fungi involves regulatory transcription factors such as xlnR from *Aspergillus* which is involved in D-xylose induction of cellulolytic and xylanolytic enzymes (Tani et al. 2014). Others include C1R-112 from *Neurospora*, ManR, McMA, and C1br from *Aspergillus*, and Bg1R from *Trichoderma* which regulate cellulolytic and/or hemi-cellulolytic enzyme production.

S. cerevisiae is well known for its ability to produce ethanol. Cassava mash-containing sludge was converted to ethanol at 86 g L^{-1} by the *S. cerevisiae* SSF process, employing continuous fermentation (Moon et al. 2012). Volumetric productivity was 2.4 g L^{-1}, and the percent yield was 91%. When immobilized on corn stalks, *S. cerevsiae* can produce 88 g L^{-1} of ethanol from food waste (Yan et al. 2012). Alcohol tolerance in this yeast is increased by adding potassium and raising the pH of the fermentation with KOH (Lam et al. 2014). Under these conditions, 127 g L^{-1} was produced. Using cell cycling of this yeast in very high-gravity fermentations led to an ethanol titer of 142 g L^{-1} with a productivity of 3.5 g L^{-1} h^{-1}. The strain used (PE-2) was obtained from a distillery in Brazil producing ethanol from sugarcane (Pereira et al. 2012).

One hundred billion liters of ethanol are produced each year from sugar cane and corn starch by *S. cerevisiae.* Production at high temperature (ca 40 °C) reduces cooling costs, lowers the effects of contamination, and enables more efficient hydrolysis of feedstocks. This improves the productivity in the simultaneous saccharification and fermentation process. Caspeta et al. (2014), using adaptive laboratory evolution, isolated *S. cerevisiae* strains with improved growth and ethanol production at 40 °C. These strains grew 1.9 times faster and excreted ethanol 1.6 times faster than the parent strain. They noted a change in sterol composition from ergosterol to fenosterol due to mutation in the C-5 sterol desaturase gene and increased expression of sterol biosynthesis genes. Sterols contribute to membrane fluidity. The thermo-tolerant strains were improved in glucose consumption rate which increased by 60% at 40 °C and by 300% at 42 °C.

Jerusalem artichokes produce high levels of biomass, grow rapidly, need only little pesticide, fertilizer, and water, and can grow on marginal land. It could be a good substrate for the production of important products (Li et al. 2013). 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.

Biodiesel is a monoalkyl ester of long-chain fatty acids made by transesterification of feedstocks such as waste animal fats or vegetable oils, e.g., soybean oil. It is a very good fuel, contains less sulfur than conventional fuel, can be used in diesel engines without modification, and can be blended in any ratio with petroleum diesel. It is biodegradable and non-toxic (Lin et al. 2013). The four different methods of biodiesel production include transesterification, blending,

microemulsions, and pyrolysis. Transesterification is the method of choice, the catalyst being chemical (acid or base) or an enzyme. Favored is transesterification via enzymes, i.e., lipases. Microbial lipases are excellent since they are stable in organic solvents, do not need cofactors, have broad substrate specificity and high enantiospecificity. *Candida antartica* is a favored lipase producer. Yields of enzymic transesterification can reach 100%. Maximum enzyme-catalyzed transesterification occurs at 55 °C. The cost of lipase is high, but it can be lowered by the use of enzyme immobilization and recycling of the immobilized enzyme. Adsorption is the best immobilization procedure due to its simplicity, ease, use of mild conditions, and low cost. Genetic engineering has been used to convert *S. cerevisiae* into a biodiesel producer, i.e., one that is oleaginous, supplying fatty acids and alcohols, and converting them to biodiesel. Production of intracellular lipids by yeasts growing on alkali-treated corn stover revealed that *Cryptococcus humicola* produces 15 g L^{-1} lipids in a total biomass weight of 36 g L^{-1} (Sitepu et al. 2014).

2,3-Butanediol is a fuel with a high heating value (27,000 J/g) and is used as a liquid fuel or fuel additive. When compared to acetone, alpha-pinene, 1-butanol, isobutanol, isopropanol, and fatty alcohols, 2,3-butanediol shows lower toxicity. It also is used in the preparation of solvents, anti-freeze agents, synthetic rubber, and plastics. An engineered stain of *S. cerevisiae* can produce it at 96 g L^{-1} (Kim et al. 2013).

13   Additional Compounds

Metabolic engineering has improved yeasts as producers of important metabolites (Liu et al. 2013a, b). Important productivities include *Y. lipolytica,* producing 80 g L^{-1} erythritol, 154 g L^{-1} citric acid from glycerol, 63 g L^{-1} succinic acid, and 27 g L^{-1} mannitol. *S. cerevisiae* produces malic acid at 59 g L^{-1}, 2,3-butanediol at 2 g L^{-1}, and the artemisinin precursor amorpha-4,11-diene at 40 g L^{-1}. L-lactic acid is made by *Candida boidini* at 86 g L^{-1}.

P. pastoris can covert methanol to formaldehyde in a process responsible for the production of 6000 tons per year of formaldehyde (Caspeta and Nielsen 2013).

Erythritol can be produced from glycerol by *Y. lipolytica* at 170 g L^{-1} (Khanna et al. 2012). Mannitol is produced from glycerol at 51 g L^{-1} by *Candida magnolia*.

Alpha-ketoglutaric acid was produced at 195 g L^{-1} by *Y. lipolytica* (*Candida lipolytica*) with a yield of 0.9 g g^{-1} of substrate when grown on *n*-paraffins (Weissbrodt et al. 1988). This acid is used industrially in chemical synthesis of heterocycles or elastomers, as a dietary supplement and as an enhancer of wound healing.

Production of itaconic acid at 90 g L^{-1} was achieved by *A. terreus* with a yield of 0.58 g g^{-1} glucose and a productivity of 0.29 g L^{-1} h^{-1} (Kuenz et al. 2012). Microbial formation of this compound is more productive than by chemical processes. Increasing pH during the production phase was found to increase production

(Hevekerl et al. 2014). A titer of 146 g L^{-1} was reached by raising pH from 4 to 6 or by raising it to 3 after 2.1 days of cultivation. Itaconic acid is used in the production of polymers, coatings, adhesives and textiles. About 80,000 tons are made each year with a selling price of $2 kg^{-1}.

Citric acid production began in England in 1826 by John and Edward Sturge of the city of Selby. It was made from Italian citrus fruits at that time. In 1893, the German microbiologist Carl Wehmer discovered that sugar-growing fungi secreted citric acid. After World War I, the fermentation became the method of choice. John N. Currie had found that *A. niger* was an excellent producer of citric acid and, as a result, the Pfizer company in New York began large-scale fermentation production in 1923. Worldwide production is 1.6 million tons per year. About 95% is used in the food industry. Other uses include chemicals, medicinal, textiles, and metallurgy. Chemicals include surfactants and synthetic detergents (Morgunov et al. 2013). In addition to *A. niger,* another producer is *Y. lipolytica*. Production by the latter is favored by limitation of cell growth via limiting levels of nitrogen, phosphorus, or sulfur with nitrogen limitation being the most useful. This yeast produces high levels of both citric and isocitric acids from rapeseed oil (Kamzolova et al. 2013).

Fumaric acid is used as a food acidulent, a beverage ingredient, and an antibacterial agent in the feed industry (Xu et al. 2012). Its other uses are for the preparation of biodegradable polymers, plasticizers, polyester resins, and as an animal feed supplement to reduce methane emissions (Thakker et al. 2015). *Rhizopus arrhizus* has been used by Pfizer to produce it at 4000 tons per year (Roa-Engel et al. 2008). Other species are also good producers, e.g., *Rhizopus nigricans*, *Rhizopus formosa,* and *Rhizopus oryzae*. *R. nigricans* produced 121 g L^{-1} with a productivity of 1 g L^{-1} h^{-1} and a yield of 0.37 (Ling and Ng 1989). DuPont patented a process using *R. arrhizus* NRRL-1526 with limited dissolved oxygen to produce 130 g L^{-1}.

Glycolic acid can be produced by *S. cerevisiae* and *K. lactis* (Koivistoinen et al. 2013), although it is currently made chemically. 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 which is an excellent packaging material. Glycolic acid can also be used with lactic acid to make a copolymer (PLGA) for medical application in drug delivery. The market for glycolic acid was $93 million for the 40 million kg produced. Glycolic acid is also employed in the textile industry as a tanning and dyeing agent.

Gluconic acid is used in the construction and in the preparation of chemicals, pharmaceuticals, foods, beverages, textiles and leather. It is also used to chelate divalent and trivalent metal ions. About 50,000–60,000 tons are made annually using glucose as substrate. The price varies from $1.20 to $8.50 kg^{-1}. Usually glucose or sucrose is used as fermentation substrate. Golden syrup, a by-product of the process refining sugar cane juice into sugar, or by treating sugar with acid, can be used for fermentation by *A. niger* (Purane et al. 2012). About 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 (Sankpal and Kulkami 2002). Also, Sankpal et al. (1999) reached 135 g L^{-1} with a productivity of 0.09 g L^{-1} h^{-1} using immobilization on cellulose fibers and surface culture. About 80–100 g L^{-1} was obtained using immobilization on waste paper with a productivity of 0.04 g L^{-1} h^{-1} (Singh and Kumar 2007).

Brown et al. (2013) described metabolic engineering of *Aspergillus oryzae* NRRL 3488 to produce malic acid at 154 g L^{-1}. The result was achieved by overexpressing (a) the C-4-dicarboxylate transporter and (b) 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 (Khan et al. 2014). The yield was 1.28 g g^{-1} glucose and productivity was 175 g L^{-1} h^{-1}. Malic acid is a C4 dicarboxylic acid produced at 40,000 tons per year. It is used in the food and beverage industry as an acidulent and taste enhancer/modifier in combination with artificial sweeteners. Additional uses are for the preparation of polyester resins and coatings, in foods and feed, and in the pharmaceutical industry. It is sold for $2–3 kg^{-1} (Thakker et al. 2015).

Torulopsis glabrata (also called *Candida glabrata*) can produce pyruvic acid at 94 g L^{-1} on glucose with a yield of 0.63 g g^{-1} glucose, a high productivity of 1.15 g L^{-1}h^{-1} and high glucose tolerance (Liu et al. 2007, 2013). The organism is an osmotolerant mutant. Production is increased by the use of urea as nitrogen source (Yang et al. 2014). This yeast is used for commercial production of pyruvic acid. The process was industrialized in 1992 by Toray Industries at 400 tons per year.

Erythritol, a polyhydric alcohol, has 60–70% of the sweetness of sucrose and is used to combat obesity. It is non-carcinogenic and non-caloric since 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} glycerol used and a productivity of 0.54 g L^{-1}h^{-1} (Mironczuk and Furgala 2014).

Bioconversion of xylose to xylitol by *Debaryomyces hansenii* amounted to 110 g L^{-1} from 300 g L^{-1} xylose (Misra and Raghuwanshi 2012). The yield was 0.48 g g^{-1}. This sugar alcohol is used in food production, has high activity as a sweetener, is non-cariogenic, and has insulin-independent metabolism properties. It is commercially produced by chemical reduction of D-xylose, but this is an expensive process. Its global market is over 125,000 tons per year. The bioconversion would probably be less expensive than the chemical procedure. Xylitol is an excellent antioxidant. It can be made from lignocellulosic waste (Lima de Albuquerque et al. 2014). 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 produced by bacteria. It is also a contributor to tooth calcification and is active against diabetes, anemia, acute otitis media, and osteoporosis. *Candida* athensensis converts vegetable waste containing 200 g L^{-1} xylose 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} (Zhang et al. 2012).

Coenzyme Q (ubiquinone) is an essential part of the respiratory chain producing ATP. It is composed of a quinonoid nucleus and a side chain of isoprenoids. Best producers include fungi such as species of *Candida, Saitoella, Trichosporon,* and *Sporobolomyces*.

Production of useful products by basidiomycetes includes carotenoids, fragrances, enzymes, astaxanthin, erythritol, lipids, and oils (Johnson 2013). *Trichosporon* sp. produces lipids and is being considered for biodiesel production. *Pseudozyma (Candida) antartica* produces lipase for industrial use and is another 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 the xanthophyll plectaniaxanthin. Some cryptococci utilize glycerol and accumulate 60% of their biomass as triacylglycerols.

Fungi produce long-chain polyunsaturated fatty acids (PUFAs) (Ratledge 2013). They include (a) gamma linoleic acid (GLA; 18:3 omega-6) from *Mucor circinelloides*, (b) docohexaenoic acid (DHA; 22:6 omega-3) from *Crypthecodinium cohnii spp,* (c) arachidonic acid (ARA; 20:4 omega-6) from *Mortierella alpine,* and (d) eicosapentaenoic acid (EPA) from genetically modified *Y. lipolytica* (Xue et al. 2013). 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 is being commercialized by DSM. The yeast was engineered by transformation with 21 heterologous genes encoding five different activities.

PUFAs represent a multibillion dollar industry, mainly ARA 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, which function as anti-inflammatory, anti-arrhythmic, and anti-aggregatory effectors. Many improve cardiovascular health, and certain of them improve eye function and memory in newborn infants and in adults. Microbial oils are produced by 30–40 species of yeast and also by molds. The producers are known as oleaginous microbes. Fungi can accumulate 70% of their biomass as oils. DHA is produced at 2000 tonnes per year and has a market of $317 million. ARA is blended with DHA and used in infant formulas. EPA plus DHA can be used to prevent cardiac problems.

Prebiotics have been reviewed by Panesar et al. (2013). They include fructo-oligosaccharide produced at 116 g L^{-1} from sucrose by beta-fructofuranosidase from *Aspergillus japonicas*. Prebiotics are used in the nutraceutical, pharmaceutical, animal feed, and aquaculture areas. They stimulate the growth of beneficial intestinal bacteria and maintain health of humans by suppression of potentially harmful bacteria, improvement of defecation, eliminating ammonia, preventing colon cancer, stimulating mineral adsorption, and lowering cholesterol and lipids.

Pullulan is produced at 88 g L^{-1} by the yeast *Aureobasidium pullulans* strain RBF 4A3 (Sharma et al. 2013). It is an exopolysaccharide which has potential

application in industries such as medical, food, pharmaceutical, cosmetic, and agriculture.

Some vitamins are produced by fungi (Ledesmo-Amaro et al. 2013). Although vitamin D is derived chemically from cholesterol and ergosterol, it can be made by *S. cerevisiae*, *Saccharomyces uvarum,* and *Candida utilis* at 30 mg g^{-1} of dry cells. Riboflavin (vitamin B$_2$) is made by *Ashbya gossypii*, *Eremothecium ashbyii*, *Candida flaeri,* and *Candida famata*. *A. gossypii* produces 14 g L^{-1} of riboflavin. The increase in production by *A. gossypii* as compared to wild-type strain ATCC 10895 is due to (a) a nine percent increase in flux to pentose-5-phosphate via the pentose phosphate pathway (PPP) and (b) a 16-fold increase in the flux from purine to riboflavin (Jeong et al. 2015). This is due to increased guanosine triphosphate flux through the PPP and the purine synthesis pathway.

Resveratrol (*trans*-3,5,4'-trihydroxystilbene) is a polyphenol found in wine, grapes, berries, and peanuts which can be made by some fungi It is a phytoalexin, i.e., a low molecular weight secondary metabolite. It has beneficial effects against inflammation, carcinogenesis, oxidation, aging, diabetes, and neurodegenerative disease. Recombinant *S. cerevisiae* can produce it at 5.8 mg L^{-1} upon feeding of coumaric acid or L-tyrosine (Shin et al. 2012). *Alternaria* sp. 61, isolated from Merlot cobs, produces 353 μg L^{-1} (Shi et al. 2012).

An improved process for making the anti-malarial compound artemisinin using *S. cerevisiae* was devised by Paddon et al. (2013). The process applies synthetic biology to a previous *S. cerevisiae* process and improves the production of artemisinic acid which is then chemically converted to artemisinin. Whereas the previous process yielded only 1.6 g L^{-1} of artemisinic acid, the new process reaches 25 g L^{-1}.

Genome sequencing of an organism reveals many secondary metabolic pathways that are usually silent. *Aspergillus nidulans* was found to have nearly 50 such loci encoding polyketide synthases (PKs) or non-ribosomal protein synthases (NRPs). Using various types of nutritional limitation in continuous chemostat cultures of *A. nidulans*, (Sarkar et al. 2012) obtained expression of two PKS genes encoding synthases of seven phenolic compounds which were not observed previously under normal growth conditions.

The soil fungus *Aspergillus versicolor* produces aspergillomarasmine (AMA) which turns off a bacterial gene that normally leads to antibiotic resistance (King et al. 2014). The gene encodes New Delhi metallo-beta-lactamase (NDM-1). Together with a carbapenem antibiotic, AMA inactivates the gene in *Escherichia coli, Acinetobacter, and Pseudomonas.* NDM-1 requires zinc, and AMA removes zinc from the enzymes. The combination of AMA and the carbapenem has shown its beneficial effect in mice and human cell culture.

Trichoderma species make many valuable secondary metabolites (Keswani et al. 2014) polyketide gliotoxin, an anti-malarial agent and immune system suppressor, (3) harzianolide, an antifungal agent and plant growth promoter, (4) koninginins, which are antifungals and plant growth regulators, (5) 6-pentyl-2H-pyran-2-one, a

plant growth promoter, and coconut aroma used commercially in confectionary products, (6) trichokonins, broad-spectrum antifungals and plant defense inducers, (7) viridofungins, potential anticancer agents, and bacteriocides, (8) viridian, a broad-spectrum antifungal agent, anti-neoplastic, and anti-atherosclerosis agent, and (9) viridiol, a herbicidal and anti-aging agent.

Activation of "silent" gene clusters by genome mining in *A. nidulans* has revealed many new secondary metabolites (Yaegashi et al. 2014). The *A. nidulans* genome contains 56 potential secondary metabolism core genes including 27 polyketide synthase (PKS) genes, two PKS-like genes, 11 non-ribosomal peptide synthetase (NRPS) genes, 15 NRPS-like genes, and one hybrid NRPS-PKS gene.

14   Future Perspectives

Microorganisms have greatly contributed for about 85 years to the development of medicine and agriculture. However, due to different situations, pathogenic microbes have become resistant to many antibiotics creating a dangerous situation and therefore the need for new antibiotics is imperative. Unfortunately, most of the large pharmaceutical companies have abandoned the search for new antimicrobial compounds. Due to economics, they have concluded that drugs directed against chronic diseases offer a better revenue stream than do antimicrobial agents, for which the length of treatment is short and government restriction is likely. Some small pharmaceutical and biotechnology companies are still developing antibiotics but most depend on venture capital rather than sales income, and with the present regulations, face huge barriers to enter into the market. These barriers were raised with the best intentions of ensuring public safety but they are having the opposite effect, i.e., termination of antibiotic development while resistance continues to increase (Livermore 2004). 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 are proceeding and succeeding (Kaeberlein et al. 2002). 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 (Clardy et al. 2006). Another exciting possibility is that of genome mining (Scheffler et al. 2013). In addition to these relatively new techniques, chemical and biological modification of old antibiotics could still supply new and powerful drugs. These comments also apply to non-antibiotics such as antitumor agents and other microbial products. In addition, natural products must continue to be tested for desirable therapeutic activities. I believe that significant progress in identifying new antibiotics, oncology therapeutics, and other useful medicines will

be made, probably not by the big pharmaceutical companies, but by biotechnology companies and small research groups from institutes and universities.

References

Agbogo FK, Coward-Kelly C (2008) Cellulosic ethanol production using the naturally occurring xylose-fermenting yeast, *Pichia stipitis*. Biotechnol Lett 30:1515–1524

Alberts AW, Chen J, Kuron G et al (1980) Mevinolin, a highly potent competitive inhibitor of hydroxylmethylglutarylcoenzyme A reductase and a cholesterol-lowering agent. Proc Natl Acad Sci USA 77:3957–3961

Alsberg CL, Black OF (1913) USDA Bur Plant Ind, Bull No. 270. Government Printing Office, Washington

Amna T, Puri SC, Verma V et al (2006) Bioreactor studies on the endophytic fungus *Entrophospora* for the production of an anticancer alkaloid camptothecin. Can J Microbiol 52:189–196

Anderson MR, Nielsen JB, Klitgaard A et al (2013) Accurate prediction of secondary metabolite gene clusters in filamentous fungi. Proc Natl Acad Sci USA 110:24–25

Andrewes AG, Phaff HJ, Starr MP (1976) Carotenoids of *Phaffia rhodozyma*, a red-pigmented fermenting yeast. Phytochemistry 15:1003–1007

Bader T, Fazili J, Madhoun M et al (2008) Fluvastatin inhibits hepatitis C replication in humans. Am J Gastroenterol 103:1383–1389

Bentley R (2001) Bartolomeo Gosio, 1863–1944: an appreciation. Adv Appl Microbiol 48:229–250

Bentley R (1997) Microbial secondary metabolites play important roles in medicine: prospects to discovery of new drugs. Perspect Biol Med 40:364–394

Berdy J (2005) Bioactive microbial metabolites. A personal view. J Antibiot 58:1–26

Berlec A, Strukelj B (2013) Current state and recent advances in biopharmaceutical production in *Escherichia coli*, yeasts and mammalian cells. J Ind Microbiol Biotechnol 40:257–274

Bernardes N, Seruca R, Chakrabarty AM et al (2010) Microbial-based therapy of cancer. Current progress and future prospects. Bioeng Bugs 1:178–190

Bhatnagar I, Kim S-K (2013) Molecular aspects of fungal bioactive polyketides. In: Brahmachari G (ed) Chemistry and pharmacology of naturally occurring bioactive compounds. CRC Press, Boca Raton, pp 445–457

Birkinshaw JH, Raistrick H, Ross DJ (1952) Studies in the biochemistry of microorganisms. The molecular constitution of mycophenolic acid, a metabolic product of *Penicillium brevicompactum* Dierckx. Part 3. Further observations on the structural formula for mycophenolic acid. Biochem J 50:630–634

Borel JF, Feurer C, Gabler HU et al (1976) Biological effects of cyclosporine A: a new antilymphocytic agent. Agents Action 6:468–475

Borel JF (2002) History of the discovery of cyclosporin and of its early pharmacological development. Wien Klin Wochenschr 114:433–437

Brakhage A (2013) Regulation of fungal secondary metabolism. Nat Revs/Microbiol 11:21–32

Brakhage AA, Schroekh V (2011) Fungal secondary metabolites. Strategies to activate silent gene clusters. Fungal Genet Biol 48:15–22

Brown AG, Smale TC, King TJ et al (1976) Crystal and molecular structure of compactin: a new antifungal metabolite from *Penicillium brevicompactum*. J Chem Soc Perkin Trans 1:1165–1170

Brown KS (1996) Pharmaceutical and biotech firms taking on drug-resistant microbes. The Scientist 10:8–9

Brown SH, Bashkirova L, Berka R (2013) Metabolic engineering of *Aspergillus oryzae* NRRL 3488 for increased production of l-malic acid. Appl Microbiol Biotechnol 97:8903–8912

Cardenas ME, Sanfridson A, Cutler NS et al (1998) Signal-transduction cascades as targets for therapeutic intervention by natural products. Trends Biotechnol 16:427–433

Caspeta L, Nielsen J (2013) Toward systems metabolic engineering of *Aspergillus* and *Pichia* species for the production of chemicals and biofuels. Biotechnol J 8:534–544

Caspeta L, Chen Y, Ghiaci P et al (2014) Biofuels. Altered sterol composition renders yeast thermotolerant. Science 346:75–78

Celik E, Calik P (2012) Production of recombinant proteins by yeast cells. Biotechnol Adv 30:1108–1118

Chandel AK, Gonçalves BCM, Strap JL et al (2015) Biodelignification of lignocellulose substrates: an intrinsic and sustainable pretreatment strategy for clean energy production. Crit Rev Biotechnol 35:281–293

Cherry JR, Fidantsel AL (2003) Directed evolution of industrial enzymes: an update. Curr Opin Biotechnol 14:438–443

Chong CR, Quian DZ, Pan F et al (2006) Identification of type 1 inosine monophosphate dehydrogenase as an antiangiogenic drug target. J Med Chem 49:2677–2680

Clardy J, Fischbach MA, Walsh CT (2006) New antibiotics from bacterial natural products. Nat Biotechnol 24:1541–1550

Colwell RR (2002) Fulfilling the promise of biotechnology. Biotechnol Adv 20:215–228

de la Fuente JL, Rodriguez-Saiz M, Schleissner C et al (2012) High-titer production of astaxanthin by the semi-industrial fermentation of *Xanthophyllomyces dendrorhous*. J Biotechnol 145:144–146

Dashtban M, Qin W (2012) Overexpression of an exotic thermotolerant β-glucosidase in *Trichoderma reesei* and its significant increase in cellulolytic activity and saccharification of barley straw. Microb Cell Fact 11:63–77

de Wilde AH, Zevenhoven-Dobbe JC, van der Meer Y et al (2011) Cyclosporin a inhibits the replication of diverse coronaviruses. J Gen Virol 92:2542–2548

Demain AL, Velasco J, Adrio JL (2004) Industrial mycology: past, present, and future. In: An Z (ed) Handbook of industrial mycology. Marcel Dekker, New York, pp 1–25

Dominguez-Santos R (2012) The regulatory factor PcRFX1 controls the expression of the three genes of β-lactam biosynthesis in *Penicillium chrysogenum*. Fungal Gen Biol 49:866–881

Duan L-L, Chen H-R, Chen J-P et al (2008) Screening the high-yield paclitaxel producing strain *Alternaria alternate* var. *monosporus*. Chin J Antibiot 33:650–652

Endo A, Kuroda M, Tsujita Y (1976) ML-236B and ML-236C, new inhibitors of cholesterol-genesis produced by *Penicillium citrinin*. J Antibiot 29:1346–1348

Endo A, Monacolin K (1979) A new hypocholesterolemic agent produced by *Monascus* species. J Antibiot 32:852–854

Endo A (2010) A historical perspective on the discovery of statins. Proc Jpn Acad Ser B Phys Biol Sci 86:484–493

Faus I (2000) Recent developments in the characterization and biotechnological production of sweet tasting proteins. Appl Microbiol Biotechnol 53:145–151

Feng Y, Shao Y, Chen F (2012) *Monascus* pigments. Appl Microbiol Biotechnol 96:1421–1440

Flores-Bustamante ZR, Rivera-Orduna FN, Martinez-Cardenas A et al (2010) Microbial paclitaxel: advances and perspectives. J Antibiot 63:460–467

Gangadevi V, Muthumary J (2008) Isolation of *Colletotrichum gloeosporiodes*, a novel endophytic taxol-producing fungus from the leaves of a medicinal plant. Mycol Balc 5:1–4

Gaudilliere B, Bernardelli P, Berna P (2001) To market, to market-2000. In: Doherty AM (ed) Annual reports in medicinal chemistry, vol. 36, Elsevier Inc Amsterdam, pp 293–318

Gorsich SW, Dien BS, Nichols RE (2006) 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

Hallborn J, Walfridsson M, Airaksinen U et al (1991) Xylitol production by recombinant *Saccharomyces cerevisiae*. Biotechnology 9:1090–1095

Harvey AL, Edrada-Ebel R, Quinn RJ (2015) The re-emergence of natural products for drug discovery in the genomics era. Nat Revs/Drug Disc 14:111–112

Hasslacher M, Schall M, Hayn M et al (1997) High-level intracellular expression of hydroxynitrile lyase from the tropical rubber tree *Hevea brasiliensis* in microbial hosts. Prot Expr Purif 11:61–71

Hasunama T, Ismail KS, Nambu Y et al (2014) 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

Hertweck C (2009) Hidden biosynthetic treasures brought to light. Nat Chem Biol 5:450–452

Hevekerl A, Kuenz A, Vorlop K-D (2014) Influence of the pH on the itaconic acid production with *Aspergillus terreus*. Appl Microbiol Biotechnol 98:10005–10012

Hidy PH, Baldwin RS, Greasham RL et al (1977) Zearelanone and some derivatives: production and biological activities. Adv Appl Microbiol 22:59–82

Hölker U, Höfer M, Lenz J (2004) Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. Appl Microbiol Biotechnol 64:175–186

Hellmuth K, van den Brink JM (2013) Microbial production of enzymes used in food applications. In: McNeil B, Archer D, Harvey L (eds) Microbial production of food ingredients, enzymes and nutraceuticals. Woodhead Publishing, Oxford, pp 262–287

Jacobson GK, Jolly SO, Sedmak JJ et al (2000) Astaxanthin over-producing strains of *Phaffia rhodozyma*. Method for their cultivation and their use in animal feeds. US Patent 6,015,684; 18 Jan 2000

Jeong B-Y Stephanopoulos G, Pfeifer BA et al (2015) Comparative metabolic flux analysis of an *Ashbya gossypii* wild type strain and a high riboflavin-producing mutant strain. J Biosci Bioeng 119:101–106

Jiang M, Stephanopoulos G, Pfeifer BA (2012) Downstream reactions and engineering in the microbially reconstituted pathway for Taxol. Appl Microbiol Biotechnol 94:841–849

Jefferys EG (1970) The gibberellin fermentation. Adv Appl Microbiol 13:283–316

Johnson EA (2013) Biotechnology of non-*Saccharomyces* yeasts—the basidiomycetes. Appl Microbiol Biotechnol 97:7563–7577

Johnson EA, Villa TG, Lewis MJ (1980) *Phaffia rhodozyma* as an astaxanthin source in animal diets. Aquaculture 20:123–134

Juzlova P, Martinkova L, Kren V (1996) Secondary metabolites of the fungus *Monascus*: a review. J Industr Microbiol 16:163–170

Kaeberlein T, Lewis K, Epstein SS (2002) Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. Science 296:1127–1129

Kamzolova SV, Dedyukhina EG, Samoilenko VA et al (2013) Isocitric acid production from rapeseed oil by *Yarrowia lipolytica* yeast. Appl Microbiol Biotechnol 97:9133–9144

Keswani C, Mishra S, Sarma BK et al (2014) Unraveling the efficient applications of secondary metabolites of various *Trichoderma* spp. Appl Microbiol Biotechnol 98:533–544

Khan I, Nazir K, Wang Z-P et al (2014) Calcium malate overproduction by *Penicillium viticola* 152 using the medium containing corn steep liquor. Appl Microbiol Biotechnol 98:1539–1546

Khanna S, Goyal A, Moholkar VS (2012) Microbial conversion of glycerol: present status and future prospects. Crit Rev Biotechnol 32:235–262

Kim SJ, Seo SO, Jin YS et al (2013) Production of 2,3-butanediol by engineered *Saccharomyces cerevisiae*. Bioresour Technol 146:274–281

King AM, Reid-Yu SA, Wang W et al (2014) Aspergillomarasmine a overcomes metallo-β lactamase antibiotic resistance. Nature 510:503–506

Kirst HA (2012) Recent derivatives from smaller classes of fermentation-derived antibacterials. Expert Opin Ther Pat 22:15–35

Koivistoinen OM, Kuivanen J, Barth D et al (2013) Glycolic acid production in the engineered yeasts *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. Microb Cell Factories 12:82–98

Kremer L, Douglas JD, Baulard AR (2000) Thiolactomycin and related analogues as novel anti-mycobacterial agents targeting KasA and KasB condensing enzymes in *Mycobacterium tuberculosis*. J Biol Chem 275:16857–16864

Kuenz A, Gallenmueller T, Willke T et al (2012) Microbial production of itaconic acid: developing a stable platform for high product concentrations. Appl Microbiol Biotechnol 96:1209–1216

Kumaran RS, Choi Y-K, Lee S et al (2012) Isolation of taxol, an anticancer drug produced by the endophytic fungus, *Phoma betae*. Afr J Biotechnol 11:950–960

Kumaran RS, Jung H, Kim HJ (2011) In vitro screening of taxol, an anticancer drug produced by the fungus *Colletotricum capsici*. Eng Life Sci 3:264–271

Kumaran RS, Kim HJ, Hur B-K (2010) Taxol promising fungal endophyte, *Pestalotiopsis species* isolated from *Taxus cuspidata*. J Biosci Bioeng 110:541–546

Kumaran RS, Muthumary JP, Hur B-K (2008) Taxol from *Phyllosticta citricarpa*, a leaf spot fungus of the angiosperm *Citrus medica*. J Biosci Bioeng 106:103–106

Lam FH, Ghaderi A, Fink GR et al (2014) Engineering alcohol tolerance in yeast. Science 346: 71–75

Ledesmo-Amaro R, Santos MA, Jimenez A et al (2013) In: McNeil B, Archer D, Giavasis I et al (eds) Microbial production of food ingredients, enzymes and nutriceuticals. Woodhead Publishing, Oxford, pp 571–594

Lee B-H, Pan T-M (2012) Benefit of *Monascus*-fermented products for hypertension prevention: a review. Appl Microbiol Biotechnol 94:1151–1161

Lee WA, Gu L, Kikszal AR et al (1990) Bioavailability improvement of mycophenolic acid through amino ester derivatization. Pharm Res 7:161–166

Li J-Y, Strobel G, Sidhu R et al (1996) Endophytic taxol-producing fungi from bald cypress, *Taxodium distichum*. Microbiology 142:2223–2226

Li L, Li L, Wang Y et al (2013) Biorefinery products from the inulin-containing crop Jerusalem artichoke. Biotechnol Lett 35:471–477

Li S, Chen X, Liu L et al (2016) Pyruvate production in *Candida glabrata*: manipulation and optimization of physiological function. Crit Rev Biotechnol 36:1–10

Lima de Albuquerque T, da Silva Jr IJ, de Macedo GR et al (2014) Biotechnological production of xylitol from lignocellulosic wastes: a review. Proc Biochem 49:1779–1789

Lin H, Wang Q, Shen Q et al (2013) Genetic engineering of microorganisms for biodiesel production. Bioengineered 4:292–304

Lin TF, Demain AL (1991) Effect of nutrition of *Monascus* on formation of red pigments. Appl Microbiol Biotechnol 36:70–75

Lin TF, Demain AL (1994) Leucine interference in the production of water-soluble red *Monascus* pigments. Arch Microbiol 162:114–119

Lin TF, Demain AL (1995) 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

Lin TF, Demain AL (1993) Resting cell studies on formation of water-soluble red pigments by *Monascus* sp. J Ind Microbiol Biotechnol 12:361–367

Ling LB, Ng TX (1989) Fermentation process for carboxylic acids. US Patent 487731

Liu L, Xu Q, Li Y et al (2007) Enhancement of pyruvate production by osmotic-tolerant mutant of *Torulopsis glabrata*. Biotechnol Bioeng 97:825–832

Liu K, Ding X, Deng B et al (2009) Isolation and characterization of endophytic taxol-producing fungi from *Taxus chinensis*. J Ind Microbiol Biotechnol 36:1171–1177

Liu L, Yang H, Shin HD et al (2013a) How to achieve high-level expression of microbial enzymes: strategies and perspectives. Bioengineered 4:212–223

Liu L, Redden H, Alper HS (2013b) Frontiers of yeast metabolic engineering: diversifying beyond ethanol and *Saccharomyces*. Curr Opin Biotechnol 24:1023–1030

Liu Z-Q, Liu B, Yu L et al (2011) Simvastatin has beneficial effect on pulmonary artery hypertension by inhibiting NF-κB expression. Mol Cell Biochem 354:77–82

Livermore DM (2004) The need for new antibiotics. Clin Microbiol Infect 10:1–9

Lorence A, Nessler CL (2004) Camptothecin, over four decades of surprising findings. Phytochemistry 65:2735–2749

Ma J, Li Y, Ye Q et al (2000) Constituents of red yeast rice, a traditional Chinese food and medicine. J Agric Food Chem 48:5220–5225

Martín J, García-Estrada C, Kosalková K et al (2012) 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 Gen Biol 49:1004–1013

Masurekar P (2008) Nutritional and engineering aspects of microbial process development. Prog Drug Res 65:292–328

Mironczuk AM, Furgala J (2014) Enhanced production of erythritol by *Yarrowia lipolytica* on glycerol in repeated batch cultures. J Ind Microbiol Biotechnol 41:57–64

Misra S, Raghuwanshi S (2012) Fermentation behavior of an osmotolerant yeast *D. hansenii* for xylitol production. Biotechnol Prog 28:1457–1465

Moon SK, Kim SW, Choi GW (2012) Simultaneous saccharification and continuous fermentation of sludge-containing mash for bioethanol production by *Saccharomyces cerevisiae*. J Biotechnol 157:584–589

Moralejo FJ, Cardoza RE, Gutiierez S et al (1999) Thaumatin production in *Aspergillus awamori* by use of expression cassettes with strong fungal promoters and high gene dosage. Appl Environ Microbiol 65:1168–1174

Morgunov IG, Kamzolova SV, Lunina JN (2013) The citric acid production from raw glycerol by *Yarrowia lipolytica* yeast and its regulation. Appl Microbiol Biotechnol 97:7387–7397

Newman DJ, Cragg GM (2007) Natural products as sources of new drugs over the last 25 years. J Nat Prod 70:461–477

Nicholls SJ, Tuzcu EM, Sipahi I et al (2007) Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis. JAMA 297:499–508

Nielsen J (2013) Production of biopharmaceutical proteins by yeast. Bioengineered. 4:207–211

Paddon CJ, Westfall PJ, Pitera DJ et al (2013) High-level semi-synthetic production of the potent antimalarial artemisinin. Nature 496:528–532

Panesar PS, Kumari S, Panesar R (2013) Biotechnological approaches for the production of prebiotics and their potential applications. Crit Rev Biotechnol 33:345–364

Parekh SR, Parekh RS, Wayman M (1988) Fermentation of xylose and cellobiose by *Pichia stipitis* and *Brettanomyces clausenii*. Appl Biochem Biotechnol 18:325–338

Paterson RRM (2008) Fungal enzyme inhibitors as pharmaceuticals, toxins and scourge of PCR. Curr Enzyme Inhib 4:46–59

Pereira FB, Gomes DG, Guimaraes PMR et al (2012) Cell recycling during very high gravity bio-ethanol fermentations using the industrial *Saccharomyces cerevisiae* strain PE-2. Biotechnol Lett 34:45–53

Pu X, Qu X, Chen F et al (2013) Camptothecin-producing endophytic fungus *Trichoderma atroviride* LY357: isolation, identification, and fermentation conditions optimization for camptothecin production. Appl Microbiol Biotechnol 97:9365–9375

Purane NK, Sharma SK, Salunkhe PD et al (2012) Gluconic acid production from golden syrup by *Aspergillus niger* strain using semiautomatic stirred-tank fermenter. J Microb Biochem Technol 4:92–95

Ratledge C (2013) In: McNeil B, Archer D, Giavasis I et al (eds) Microbial production of food ingredients, enzymes and nutriceuticals. Woodhead Publishing, Oxford, pp 531–558

Reyes LH, Gomez JM, Kao KC (2013) Improving carotenoids production in yeast via adaptive laboratory evolution. Metab Eng 21:26–31

Roa-Engel CA, Straathof AJJ (2008) Fumaric acid production by fermentation. Appl Microbiol Biotechnol 78:379–389

Rodriguez-Saiz M, de la Fuente JL, Barredo JL (2010) *Xanthophyllomyces dendrorhous* for the industrial production of astaxanthin. Appl Microbiol Biotechnol 88:645–658

Roukas T (2015) The role of oxidative stress on carotene production by *Blakeslea trispora* in submerged fermentation. Crit Rev Biotechnol Jul 15 Early Online 1-10

Sabater-Jara AB, Tudela LR, Lopez-Perez AJ (2010) In vitro culture of *Taxus* sp.: strategies to increase cell growth and taxoid production. Phytochem Rev 9:343–356

Sankpal NV, Kulkarni BD (2002) Optimization of fermentation conditions for gluconic acid production using *Aspergillus niger* immobilized on cellulose microfibrils. Proc Biochem 37:1343–1350

Sankpal NV, Joshi AP, Sutar II et al (1999) Continuous production of gluconic acid by *Aspergillus niger* immobilized on a cellulosic support: study of low pH fermentative behaviour of *Aspergillus niger*. Proc Biochem 35:317–325

Sarkar A, Funk AN, Scherlach K et al (2012) Differential expression of silent polyketide biosynthesis gene clusters in chemostat cultures of *Aspergillus nidulans*. J Biotechnol 160: 64–71

Sasano Y, Watanabe D, Ukibe K et al (2012) 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

Sebastian L, Madhusudana SN, Ravi V et al (2011) Mycophenolic acid inhibits replication of Japanese Encephalitis Virus. Chemotherapy 57:56–61

Scheffler R, Colmer S, Tynan H et al (2013) Antimicrobials, drug discovery, and genome mining. Appl Microbiol Biotechnol 97:969–978

Seviour RJ, Harvey LM, Fazenda M et al (2013) In: McNeil B, Archer D, Giavasis I et al (eds) Microbial production of food ingredients, enzymes and nutriceuticals. Woodhead Publishing, Oxford, pp 97–124

Shang S, Shanley CA, Caraway ML et al (2010) Activities of TMC207, rifampin, and pyrazinamide against *Mycobacterium tuberculosis* infection in guinea pigs. Antimicrob Agents Chemother 54:956–959

Sharma N, Prasad GS, Choudary AR (2013) Utilization of corn steep liquor for biosynthesis of pullulan, an important exopolysaccharide. Carbohydr Polym 93:95–101

Shi J, Zeng Q, Liu Y et al (2012) *Alternaria* sp. MG1, a resveratrol-producing fungus: isolation, identification, and optimal cultivation conditions for resveratrol production. Appl Microbiol Biotechnol 95:369–379

Shin S-Y, Jung S-M, Kim M-D et al (2012) Production of resveratrol from tyrosine in metabolically engineered *Saccharomyces cerevisiae*. Enz Microb Technol 51:211–216

Singh OV, Kumar R (2007) Biotechnological production of gluconic acid: future implications. Appl Microbiol Biotechnol 75:713–722

Sitepu IR, Jin M, Fernandez JE et al (2014) Identification of oleaginous yeast strains able to accumulate high intracellular lipids when cultivated in alkaline pretreated corn stover. Appl Microbiol Biotechnol 98:7645–7657

Slininger PJ, Dien BS, Gorsich SW et al (2006) Nitrogen source and mineral optimization enhance D-xylose conversion to ethanol by the yeast *Pichia stipitis* NRRL-Y-7124. Appl Microbiol Cell Physiol 72:1285–1296

Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science 260:214–216

Strohl WR (1997) Industrial antibiotics: today and the future. In: Strohl WR (ed) Biotechnology of antibiotics. Marcel Dekker, New York, pp 1–47

Tani S, Kawaguchi T, Kobayashi T (2014) Complex regulation of hydrolytic enzyme genes for cellulosic biomass degradation in filamentous fungi. Appl Microbiol Biotechnol 98:4829–4837

Thakker C, Martinez I, Li W et al (2015) Metabolic engineering of carbon and redox flow in the production of small organic acids. J Ind Microbiol Biotechnol 42:403–422

Tippmann S, Chen Y, Siewers V et al (2013) From flavors and pharmaceuticals to advanced biofuels: production of isoprenoids in *Saccharomyces cerevisiae*. Biotechnol J 8:1435–1444

Tudzinski B (1999) Biosynthesis of gibberellins in *Gibberella fujikuroi*: biomolecular aspects. Appl Microbiol Biotechnol 52:298–310

Umezawa H (1972) Enzyme inhibitors of microbial origin. University of Tokyo, Tokyo

Vachali P, Bhosale P, Bernstein PS (2012) Microbial carotenoids. In: Barredo J-L (ed) Microbial carotenoids from fungi: methods and protocols. Springer, New York, pp 41–59

Vance D, Goldberg I, Mitsuhashi O et al (1972) Inhibition of fatty acid synthetases by the antibiotic cerulenin. Biochem Biophys Res Commun 48:649–656

Venditto VJ, Simanek EE (2010) Cancer therapies utilizing the camptothecins: a review of the in vivo literature. Mol Pharm 7:307–349

Verdine GL (1996) The combinatorial chemistry of nature. Nature 384:11–13

Vining LC, Taber WA (1979) Ergot alkaloids. In: Rose AH (ed) Secondary products of metabolism, vol. 3. Academic, London, pp 389–420

Wall ME, Wani MC (1996) Camptothecin and taxol: from discovery to clinic. J Ethnopharmacol 51:239–254

Wang JF, Li GL, Lu HY et al (2000) Taxol from *Tubercularia* sp. strain TF5, an endophytic fungus of *Taxus mairei*. FEMS Microbiol Lett 193:249–253

Wei Y, Liu L, Zhou X et al (2012) Engineering taxol biosynthetic pathway for improving taxol yield in taxol-producing endophytic fungus EFY-21 (*Ozonium* sp.). Afr J Biotechnol 11:9094–9101

Weissbrodt E, Barth G, Weber H et al (1988) Production of 2-oxoglutaric acid by yeasts. Patent DD 267999

Xu F, Tao W, Cheng L et al (2006) Strain improvement and optimization of the media of taxol-producing fungus *Fusarium maire*. Biochem Eng J 31:67–73

Xu Q, Li S, Huang H et al (2012) Key technologies for the industrial production of fumaric acid by fermentation. Biotechnol Adv 30:1685–1696

Xue Z, Sharpe PL, Hong SP et al (2013) Production of omega-3 eicosapentaenoic acid by metabolic engineering of *Yarrowia lipolytica*. Nat Biotechnol 31:734–740

Yaegashi J, Oakley BR, Wang CC (2014) Recent advances in genome mining of secondary metabolite biosynthetic gene clusters and the development of heterologous expression systems in *Aspergillus nidulans*. J Ind Microbiol Biotechnol 41:433–442

Yan S, Chen X, Wu J et al (2012) Ethanol production from concentrated food waste hydrolysates with yeast cells immobilized on corn stalk. Appl Microbiol Biotechnol 94:829–838

Yang S, Chen X, Xu N et al (2014) Urea enhances cell growth and pyruvate production in *Torulopsis glabrata*. Biotechnol Prog 30:19–27

Yang Y, Xia J, Li J et al (2012) A novel impeller configuration to improve fungal physiology performance and energy conservation for cephalosporin C production. J Biotechnol 161:250–256

Zhang J, Geng A, Yao C et al (2012) Effects of lignin-derived phenolic compounds on xylitol production and key enzyme activities by a xylose utilizing yeast *Candida athensensis* SB18. Bioresour Technol 121:369–378

Zhang P, Zhou P-P, Yu L-J (2009) An endophytic taxol-producing fungus from *Taxus media*, *Cladosporium cladosporoides* MD2. Curr Microbiol 59:227–232

Zhang YHP (2011) What is vital (and not vital) to advance economically-competitive biofuels production. Proc Biochem 46:2091–2110

Zhao K, Zhou D, Ping W et al (2004) Study on the preparation and regeneration of protoplast from taxol-producing fungus *Nodulisporium sylviforme*. Nat Sci 2:52–59

Zhou X, Zhu H, Liu L et al (2010) A review: recent advances and future prospects of taxol-producing endophytic fungi. Appl Microbiol Biotechnol 86:1707–1717