



**HAL**  
open science

# Focus and Insights into the Synthetic Biology-Mediated Chassis of Economically Important Fungi for the Production of High-Value Metabolites

Pragya Tiwari, Laurent Dufossé

► **To cite this version:**

Pragya Tiwari, Laurent Dufossé. Focus and Insights into the Synthetic Biology-Mediated Chassis of Economically Important Fungi for the Production of High-Value Metabolites. *Microorganisms*, 2023, 11, 10.3390/microorganisms11051141 . hal-04149805

**HAL Id: hal-04149805**

**<https://hal.science/hal-04149805>**

Submitted on 4 Jul 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



Review

# Focus and Insights into the Synthetic Biology-Mediated Chassis of Economically Important Fungi for the Production of High-Value Metabolites

Pragya Tiwari <sup>1</sup> and Laurent Dufossé <sup>2,\*</sup>

<sup>1</sup> Department of Biotechnology, Yeungnam University, Gyeongsan 38541, Republic of Korea; pragyamita02@gmail.com

<sup>2</sup> Chemistry and Biotechnology of Natural Products, CHEMBIOPRO, Université de La Réunion, ESIROI Agroalimentaire, 15 Avenue René Cassin, F-97490 Saint-Denis, France

\* Correspondence: laurent.dufosse@univ-reunion.fr

**Abstract:** Substantial progress has been achieved and knowledge gaps addressed in synthetic biology-mediated engineering of biological organisms to produce high-value metabolites. Bio-based products from fungi are extensively explored in the present era, attributed to their emerging importance in the industrial sector, healthcare, and food applications. The edible group of fungi and multiple fungal strains defines attractive biological resources for high-value metabolites comprising food additives, pigments, dyes, industrial chemicals, and antibiotics, including other compounds. In this direction, synthetic biology-mediated genetic chassis of fungal strains to enhance/add value to novel chemical entities of biological origin is opening new avenues in fungal biotechnology. While substantial success has been achieved in the genetic manipulation of economically viable fungi (including *Saccharomyces cerevisiae*) in the production of metabolites of socio-economic relevance, knowledge gaps/obstacles in fungal biology and engineering need to be remedied for complete exploitation of valuable fungal strains. Herein, the thematic article discusses the novel attributes of bio-based products from fungi and the creation of high-value engineered fungal strains to promote yield, bio-functionality, and value-addition of the metabolites of socio-economic value. Efforts have been made to discuss the existing limitations in fungal chassis and how the advances in synthetic biology provide a plausible solution.

**Keywords:**  $\beta$ -lactam antibiotics; functional food; fungal chassis; genome editing; CRISPR-Cas; *Saccharomyces cerevisiae*



**Citation:** Tiwari, P.; Dufossé, L. Focus and Insights into the Synthetic Biology-Mediated Chassis of Economically Important Fungi for the Production of High-Value Metabolites. *Microorganisms* **2023**, *11*, 1141. <https://doi.org/10.3390/microorganisms11051141>

Academic Editor: Stefan Junne

Received: 23 March 2023

Revised: 21 April 2023

Accepted: 24 April 2023

Published: 27 April 2023



**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

The increasing prevalence of chronic diseases including neurodegenerative, diabetes, and cardiovascular diseases can be addressed by adopting a healthy lifestyle and a balanced diet. Different kinds of less/fat-free diets, low carbohydrate diets [1], and soy-based diets [2] including others are gaining importance and are adopted for good health, together with increased usage of functional food, nutraceuticals, and drugs to delay aging and promote well-being [3–6]. Microorganisms define a key platform to produce different kinds of high-value metabolites/food products for thousands of years, and have garnered attention in commercial initiatives, towards the bio-based production of metabolites for value addition [7]. Several decades have witnessed the consumption of fungi as functional food, majorly including edible mushrooms (*Ascomycota* and *Basidiomycota*), regarded as edible delicacies and key nutritional sources, across the globe. Although several yeasts (*Pichia pastoris*, *Saccharomyces cerevisiae*, and *Yarrowia lipolytica*) and multiple fungal strains have been industrially employed to produce high-value metabolites, filamentous fungi demonstrate key properties, including presence in solid-state cultures, metabolite production, and polymers degradation, and thereby, prospects in commercial applications [8,9].

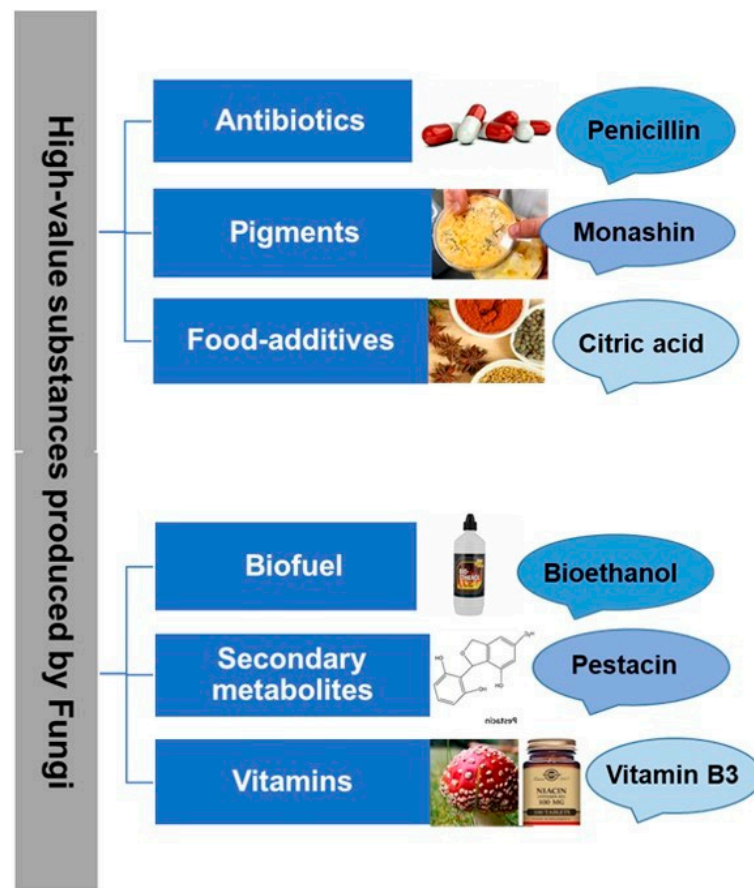
The presence of natural products in fungal species provides a defense against predators, harmful UV radiation, and competitive microbes, ensuring their survival in the ecological niche, while broad-spectrum functions prove beneficial for human health [10]. In the present era, several fungal species (including non-*Basidiomycota* species) are being explored and commercialized as nutraceuticals/supplements, and functional foods attributed to multiple pharmacological properties [11,12], and some well-known examples include *Fusarium*, *Aspergillus*, and *Penicillium* sp. The mycelia of some fungal species comprise healthy lipids, dietary fiber, vitamins, etc., and the consumption of these components defines key health advantages [13,14]. Certain filamentous fungi are used as an alternative protein source (due to higher protein contents) [13] while other species are defined as good sources of food flavors, probiotics, and healthy food lipids, respectively. Recent advances in scientific interventions (e.g., 3D printing in food) are widely employed in improving food product shapes, colors, sizes, and textures [15].

Present decade has witnessed research initiatives on filamentous fungi yielding high-value substances of socio-economic significance, including citrate from *Aspergillus niger* [16], penicillin from *Penicillium rubens* [17], pigments [18], pharmaceuticals (lovastatin and cyclosporine) [19,20], industrially important enzymes from *A. oryzae* and *A. niger* [21], lycopen (carotenoids) from *Blakeslea trispora* [22], organic acids (itaconic acid) and secondary metabolites (lovastatin) from *A. terreus* [23–25],  $\beta$ -lactam antibiotics (cephalosporins) from *Acremonium chrysogenum* [26], riboflavin (vitamin B2) from *Ashbya gossypii* [27],  $\beta$  galactosidase from *Trichoderma* sp. [28], cosmetics ingredients, namely, kojic acid [29], fortified nutraceuticals lipase (lipopan F, folate) from *Rhizopus oryzae* [30], and *S. cerevisiae* [31], polyunsaturated fatty acid (arachidonic acid) from *Mortierella alpina* [32], probiotics (whole cell) of *Saccharomyces boulardii* [33], and fibrinolytic enzymes from *Penicillium* sp. [8], including others.

In recent times, the production of food products from microbial sources has witnessed a tremendous upsurge, attributed to the nutritional content and health benefits of high-value compounds. Microbial biosynthesis not only improves the nutritional content/quality of the food, but it also improves the bio-functionality of the food products via imparting beneficial properties including antimicrobial action, peptide production, probiotic properties, removal of antinutritive substances, and fibrinolytic activity, among others [34,35]. A variety of food products/ingredients have been produced by fungi including food pigments, enzymes, nutraceuticals, and pharmaceuticals, among others [36,37]. Functional food represents an alternative means to decrease the likelihood of chronic diseases by optimizing or addressing any defects in metabolic processes, thereby promoting quality of life. Edible mushrooms are cultivated worldwide, constituting a high protein source in balanced diets [38]. Edible mushrooms comprise a key protein source, with good concentrations of unsaturated fats, fibers, minerals, vitamins, etc. [39]. Nutritional benefits of fungal species, amino acid profiles, and high protein content (some filamentous species) comprise distinct benefits [13,40], while other fungal species are valued for the presence of healthy lipids, probiotics, flavors, etc. [41]. Oligosaccharides produced from copropilous fungi are gaining attention as functional food, comprising significant health-promoting properties [42]. The low molecular weight carbohydrates include xylooligosaccharide, inulooligosaccharide, and fructooligosaccharide and are used as substitutes for low cariogenic sugar in the diet. In this direction, key studies discussed the biological activities of polysaccharides isolated from edible fungi and comprise: antioxidant polysaccharides from *Tremella* and their free-radical scavenging effect, and polysaccharides from *Morchella esculenta* improve the activity of antioxidant enzymes [43]; antitumor polysaccharides from *Agaricus bisporus* controlling cancer cell lines growth [44]; anti-aging polysaccharides from *Tremella* that relieve epidermal bleeding and inflammation [45]; and immunomodulatory polysaccharides from *Ganoderma* that promote the immune function [46], among others. Furthermore, fungal species have been extensively documented to be an invaluable source of functional foods and nutraceuticals and marketed as *Ganoderma* capsules (for increased immunity) [47], Lentinan from *Lentiana edodes* (as chemotherapy adjuvant) [48], Hanqi

edible mushroom products from *Coprinus comatus*, and *A. camphorata* (which reduce high blood pressure) [49], pork sausage from *Pleurotus eryngii* (food ingredient) [50], Canned ground ham from Winter mushroom (a substitute of synthetic nitrite) [51], and Pasta from *L. edodes* (nutritional supplement) [52], among other examples.

Several fungi culture strategies, including solid-state fermentation (SSF) and liquid-state fermentation (LSF), increase the production of fungal biomass, facilitating an easy recovery and increased metabolite production [53]. Furthermore, biotechnological interventions have facilitated the monitoring of variables in edible fungi cultivation, extraction of bioactive constituents, redefining the commercialization of functional food from fungi. Figure 1 provides a schematic outline of high-value substances of socio-economic significance produced from fungi.



**Figure 1.** Schematic outline of high-value substances of socio-economic significance produced from fungi.

Fungal biotechnology aims to define and harness the metabolic functions of filamentous fungi of potential interest and their commercialization in diverse industries, and the health sector [54]. The branch of fungal biotechnology has increasingly tried to promote a bio-based economy and develop alternative biological resources to address the growing global food demands [35,36]. Studies are increasingly investigating the economic potential of fungal species, via metabolic pathway elucidation and optimization of fungal strains, toward the development of efficient biofactories [55,56]. However, multiple bottlenecks/knowledge gaps exist in the complete exploitation of beneficial fungal strains via metabolic engineering. The fungal species *Penicillium chrysogenum*, *Aspergillus terreus*, *Trichoderma reesei*, and *Thermothelomyces* thermophiles are selected for genetic manipulations attributed to their biotechnological potentials [21,57]. Particularly, *A. niger* is extensively used in diverse industrial applications and harnessed to produce food ingredients, metabo-

lites, vegan leather, vitamins, etc. [21]. Besides *A. niger*, many other filamentous strains are used nowadays, in diverse commercial applications, including biofuel, textile, food, pharma, and chemical industries.

While substantial progress has been made in engineering *S. cerevisiae*, the metabolic engineering attempts toward the chassis of filamentous fungi are still limited. The synthetic biology-mediated chassis of fungal strains for introducing a desired trait or enhancing the production of high-value metabolites for biotechnology or therapeutics is gaining momentum. Biotechnological advances in genome editing via CRISPR-Cas, metabolic pathway reconstitution, and expression in a heterologous host, and gene knockouts, have yielded translational success, as exemplified by key examples [10,19,58]. We herein provide a comprehensive insight into the current state of the art of metabolic engineering initiatives in fungi towards enhanced production of high-value metabolites and functional foods.

## 2. Nanotechnology and Fungal Biotechnology

The bioactive compounds produced by fungi comprise novel scaffolds and are key bioresources in multi-faceted applications, ranging from bioactive compounds in healthcare to high-value functional food and compounds of commercial/industrial value. Fungal species as a biological platform have the potential for nanoparticle synthesis via extracellular or intracellular enzyme reduction [59]. The prospective application of fungi-synthesized nanoparticles comprises use in medical imaging, in the drug-delivery system, and in cancer treatment, respectively. In addition, nanoparticles from fungi are employed in the management of plant diseases and the production of fungicides in agriculture. The emerging field of nanobiotechnology has opened new avenues in fungal biotechnology, with significant applications in a socioeconomic context [60]. Table 1 discuss representative examples of fungi-mediated nanoparticle biosynthesis and their application in agriculture, healthcare, and industries.

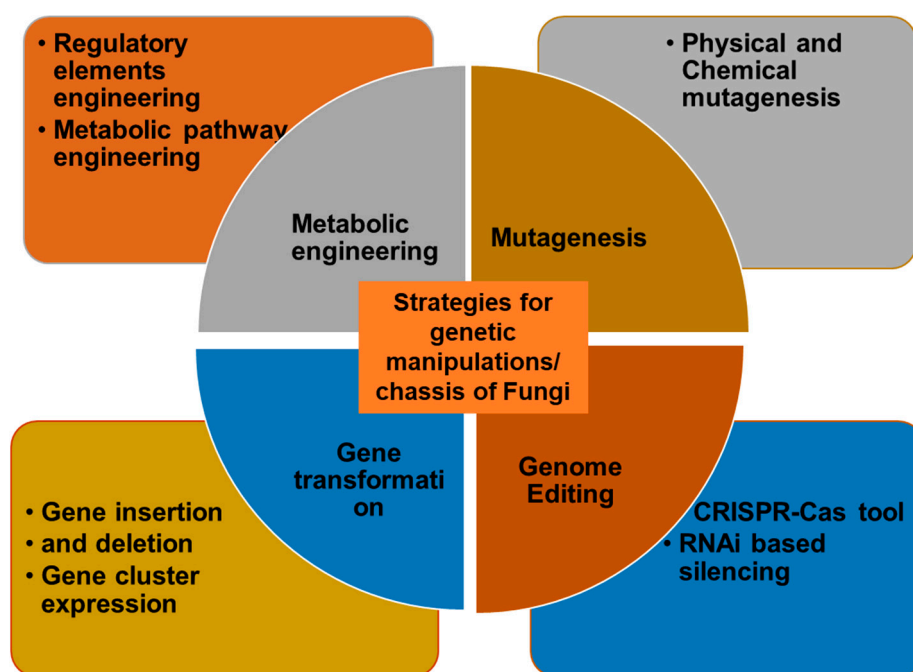
**Table 1.** Discussion of representative examples of fungi-mediated nanoparticles and their application in agriculture, healthcare, and industries.

Nanoparticle	Fungal Species	Socio-Economic Application	Reference
Biomedical and therapeutic applications			
AgNP	<i>Trichoderma viride</i>	Combination with antibiotics produces a synergistic effect	[61]
AuNP	<i>Helmithosporum solani</i>	Anticancer drug	[62]
AgNP	<i>Aspergillus fumigatus</i>	Antiviral against HIV-1	[63]
TiO <sub>2</sub>	<i>Aspergillus flavus</i>	Antimicrobial function	[64]
AuNP	<i>Candida albicans</i>	Liver cancer detection	[65]
Agricultural applications			
AuNP	<i>Rhizopus oryzae</i>	Pesticides	[66]
Ca <sub>3</sub> P <sub>2</sub> O <sub>8</sub> NP	<i>Aspergillus tubingensis</i>	Agriculture	[67]
AuNP	<i>Fusarium semitectum</i>	Optoelectronics	[68]
FeCl <sub>3</sub>	<i>Aspergillus oryzae</i>	Agriculture	[69]
AgNP	<i>Rhizopus stolonifer</i>	—	[70]
TiO <sub>2</sub>	<i>Aspergillus flavus TFR7</i>	Plant nutrient	[71]

## 3. Fungi—An Emerging Biological Platform for Metabolic Engineering

The branch of metabolic engineering aims to modify a biological organism to modulate its metabolism. Genetic manipulation is commonly employed in yeast, plants, or bacteria

for enhanced production of metabolites, demonstrating socio-economic attributes [72,73] (Figure 2). The tremendous prospects of microorganisms in the biotechnological sector cannot be unseen, with non-model organisms being increasingly explored for human welfare [74,75]. The key aims of metabolic engineering comprise biological strain optimization for enhanced production of high-value substances, yield enhancement, decreased by-product formation, defining a broad substrate range, and improving process efficiency [35,76]. This is achieved by the transfer of enzymes or pathway reconstitution in a heterologous system, to achieve maximum results. Other gene editing methods, namely, DNA recombinant technology, CRISPR/Cas9, and RNA interference, alone or in combination, facilitate the production of value-added compounds including flavonoids, terpenoids, non-ribosomal peptides, alkaloids, biofuels, polymers, and enzymes, and others [77]. In this direction, attempts towards metabolic engineering of biological organisms should focus on the optimization of bioprocess technologies and parameters to obtain the yield of the desired products.



**Figure 2.** Genetic manipulation strategies for enhanced production of genetically and metabolically modified food products.

In the past decades, *S. cerevisiae* has been extensively studied as the production platform for metabolites of socio-economic value. Biotechnological interventions have been carried out in yeast including metabolic engineering for fatty acid-derived n-butanol (biofuel) production [78,79], *A. niger* for citric acid and galactaric acid (organic acid) production [80,81], *Y. lipolytica* for omega-3 eicosapentaenoic acid production [82], *Blakeslea tripora* for lycopene (carotenoid) production [22], and other key studies as discussed (Table 2).



**Table 2.** Synthetic biology-mediated engineering of economically viable fungal and yeast strains to produce high-value compounds.

Fungal Strain	High-Value Compounds	Strategy for Metabolic Engineering	Research Outcome	Reference
<i>S. cerevisiae</i>	Naringenin (Flavanones)	Genetic manipulation for SmCHS2 expression in a heterologous system	Increased production of naringenin (648.63 mg/L)	[83]
<i>S. cerevisiae</i>	Rubusoside and Rebaudiosides	Construction of de novo Rubusoside biosynthetic pathway, removal of rate-limiting steps, metabolic model-based prediction of engineerable targets	Improved production of rubusoside (1368.6 mg/L) and rebaudioside (132.7 mg/L)	[84]
<i>S. cerevisiae</i>	Lycopene (carotenoid)	Genome engineering for lycopene pathway optimization and increase in acetyl-CoA pool	Enhanced production of lycopene (56 mg/g)	[85]
<i>S. cerevisiae</i>	Eriodictyol (Flavanones)	A cytochrome P450 F3'H from <i>Gerbera hybrida</i> was functionally expressed in <i>S. cerevisiae</i>	Increased production of eriodictyol (200 mg/L)	[86]
<i>S. cerevisiae</i>	Fatty acid ethyl esters (FAEEs)	Genetic manipulation of <i>S. cerevisiae</i> to utilize glycerol as a substrate for ethanol production	Higher yields of fatty acid ethyl esters	[87]
<i>S. cerevisiae</i>	Ethanol	<i>T. reesei</i> endoglucanase EGLI and <i>Saccharomycopsis fibuligera</i> ( $\beta$ -glucosidase) Bgl1 was introduced into <i>S. cerevisiae</i>	Enhanced ethanol production	[88]
<i>S. cerevisiae</i>	Fatty acid-derived n-butanol (biofuel)	The silencing of <i>ADH1</i> , <i>ADH4</i> , <i>GPD1</i> , and <i>GPD2</i> genes in fungal strain	Increased production of n-butanol (100 mg/L)	[78]
<i>S. cerevisiae</i>	Citrate	Deletion of <i>IDH1</i> and <i>IDH2</i> genes via marker-based homologous recombination	Increased production of citrate	[89]
<i>S. cerevisiae</i>	Free fatty acids (FFA)	Pathway reconstitution and optimization (synthetic citrate lyase pathway), Heterologous expression of ATP citrate lyase, and malic enzyme	Increased free fatty acid production (10.4 g/L)	[90]
<i>S. cerevisiae</i>	Terpenes	Mevalonic acid (MVA) pathway engineering in <i>S. cerevisiae</i>	Enhanced terpene production	[91]
<i>S. cerevisiae</i>	Cis, cis-Muconic acid	Pathway engineering (amino acid synthesis) in yeast and conversion of 3-dehydroshikimate (DHS) into cis, cis-muconic acid	Screening for best heterologous genes catalyzing the conversion of DHS to cis-cis muconic acid	[92]

Table 2. Cont.

Fungal Strain	High-Value Compounds	Strategy for Metabolic Engineering	Research Outcome	Reference
<i>S. cerevisiae</i> WRY2	Fatty acids	ATP citrate lyase was introduced and malate synthase was downregulated in the engineered strain	Increased fatty acid production (460 mg/L)	[93]
<i>S. cerevisiae</i>	Rosmarinic acid (hydroxycinnamic acid ester)	Metabolic engineering of <i>S. cerevisiae</i>	High-level production of rosmarinic acid (5.93 mg/L)	[94]
<i>S. cerevisiae</i>	p-hydroxybenzoic acid (PHBA)	Fungal engineering for gene deletion (for negative feedback), overexpression of chorismite lyase (from <i>E. coli</i> )	Enhanced production of aromatic compounds	[95]
<i>S. cerevisiae</i>	(+)-Valencene and (+)-nootkatone (Sesquiterpenoids)	Combinational engineering comprising promoter change, regulator ROX1 knockout, squalene pathway inhibition, and HMGR overexpression	Enhanced production of $\beta$ -nootkatol and (+)-nootkatone (170.5 and 45.6 mg/mL)	[96]
<i>S. cerevisiae</i>	Fatty acids	Overexpression of <i>ACC1</i> , <i>FAS1</i> , and <i>FAS2</i> genes in the fungal strain	Enhanced fatty acid production	[97]
<i>S. cerevisiae</i>	Chlorogenic acid (phenolic compound)	An optimized de novo biosynthetic pathway for CGA was reconstructed in <i>S. cerevisiae</i> , a multi-module engineering strategy	Increased production of chlorogenic acid (806.8 mg/L)	[98]
<i>S. cerevisiae</i>	Naringenin (Flavanones)	RgTAL (encoding tyrosine ammonia lyase) from <i>Rhodotorula glutinosa</i> , Pc4CL (encoding 4-coumaric acid-CoA ligase) from <i>Petroselinum crispum</i> , PhCHS from <i>P. hybrida</i> , and MsCHI from <i>Medicago sativa</i> in <i>S. cerevisiae</i>	Increased production of naringenin (29 mg/L)	[99]
<i>A. niger</i> , <i>S. cerevisiae</i>	Cheese	Genetic modification with calf rennet gene (chymosin)	Improvement of cheese products	[100]
<i>Y. lipolytica</i>	Taxifolin (flavanonols)	Taxifolin biosynthetic pathway expression in <i>Y. lipolytica</i>	Increased production of taxifolin (48.1 mg/L)	[101]
<i>Kluyveromyces lactis</i>	L-ascorbic acid (vitamin C)	Genetic transformation of <i>K. lactis</i> with a plasmid harboring cloned plant genes	Increased production of L-ascorbic acid (30 mg/L)	[102]
<i>K. lactis</i>	Bioethanol	Construction and characterization of a null mutant ( $\Delta$ klndi1) in the single gene encoding a mitochondrial alternative internal dehydrogenase	Increased bioethanol production	[103]



Table 2. Cont.

Fungal Strain	High-Value Compounds	Strategy for Metabolic Engineering	Research Outcome	Reference
<i>K. lactis</i>	B-galactosidase	A rational mutagenesis strategy by introducing disulfide bonds in the interface between the enzyme subunits was used	Improvement of $\beta$ -galactosidase enzyme for high-temperature industrial applications	[104]
<i>A. niger</i>	Glucoamylase	<i>glaA</i> gene encoding for glucoamylase was expressed in <i>A. niger</i> under the control of the tunable Tet-on system, deletion of the <i>racA</i> gene in the engineered strain	Enhanced glucoamylase secretion in the engineered strain	[105]
<i>A. niger</i>	glucoamylase–glucuronidase (GlaGus) protein	Disruption of ER-associated degradation (ERAD) components, <i>derA</i> , <i>doaA</i> , <i>hrdC</i> , <i>mifA</i> , or <i>mnsA</i> in <i>A. niger</i>	High-viable <i>A. niger</i> strain, enhanced production of (GlaGus) protein	[106]
<i>A. niger</i>	Manganese peroxidase	Heterologous expression of manganese peroxidase (from <i>Phanerochaete chrysosporium</i> ) in <i>A. niger</i> , overexpression of calnexin (chaperone)	Increased production of manganese peroxidase	[107]
<i>A. niger</i>	Human $\alpha$ 1-proteinase inhibitor ( $\alpha$ 1-PI) (antitrypsin)	Heterologous expression of Human $\alpha$ 1-proteinase inhibitor in <i>A. niger</i> , a fusion of $\alpha$ 1-PI with glucoamylase G2, transformants screening	biologically active glycosylated r- $\alpha$ 1-PI with yields of up to 12 mg/L	[108]
<i>A. niger</i>	lignin peroxidase	Heterologous expression of lignin peroxidase (from <i>Phanerochaete chrysosporium</i> ) in <i>A. niger</i> under NOS promoter and terminator	Lignin peroxidase production	[109]
<i>A. niger</i>	Secondary metabolites (enniatins)	Heterologous expression of nonribosomal peptide synthetase ESYN (from <i>F. oxysporum</i> ) under Tet-on hybrid promoter in <i>A. niger</i>	Enhanced production of enniatins	[110]
<i>A. oryzae</i>	Cellulase activity	Integration of multiple copies of genes encoding cellulases (cellobiohydrolase, endoglucanase, and $\beta$ -glucosidase) into the fungal genome	Enhanced activity of cellulases	[111]
<i>A. niger</i>	Citric acid	The system consists of two expression modules jointly targeted to a defined genomic locus	Expression of the desired gene and its function	[80]
<i>A. niger</i>	Galactaric acid (organic acid)	Single- and multiplexed CRISPR approaches for galactarate overproduction	Higher production of galactarate (12 g/L)	[81]

Table 2. Cont.

Fungal Strain	High-Value Compounds	Strategy for Metabolic Engineering	Research Outcome	Reference
<i>A. niger</i>	Vitamin C	Metabolic engineering of the fungal d-galacturonate pathway	A higher titer of vitamin C (170 mg/L)	[112]
<i>A. niger</i>	Human granulocyte colony-stimulating factor (G-CSF)	Fusion of G-CSF behind a KEX2 cleavage site downstream of glucoamylase	High yields of protein G-CSF in <i>A. niger</i> (5–10 mg/L culture medium)	[113]
<i>P. pastoris</i>	(+)-nootkatone	Chimeric strain co-expressing the premnaspirodiene oxygenase <i>Hyoscyamus muticus</i> and <i>A. thaliana</i> cytochrome P450 reductase. Intracellular production of (+)-valencene by co-expression of valencene synthase	Enhanced production of (+)-nootkatone (208 mg/L)	[114]
<i>P. pastoris</i>	Fatty acid alcohols	Metabolic transformation of the fatty acid cell factory	High-level production of fatty acid derivatives (2.0 g/L)	[115]
<i>P. pastoris</i>	Malic acid	Metabolic engineering and redistribution of metabolic flux	High-level production of malic acid (2.79 g/L)	[116]
<i>Yarrowia lipolytica</i>	$\beta$ -Carotene (carotenoid)	Optimization of promoter–gene pairs of heterologous <i>crt</i> pathway	High yields of $\beta$ -carotene (6.5 g/L)	[117]
<i>Trichoderma reesei</i>	Cellulase	Deletion of the small GTPase <i>rac1</i> in <i>T. reesei</i>	Hyperbranching in <i>T. reesei</i> strain enhanced cellulase production	[118]
<i>Neurospora crassa</i>	Cellulase	Disruption of <i>gul-1</i> decreased culture viscosity <i>gul-1</i> overexpression increased viscosity	Increased cellulase secretion in the engineered strain	[119]
<i>Y. lipolytica</i>	Omega-3 eicosapentaenoic acid	Heterologous expression of $\Delta$ -9 elongase, a $\Delta$ -8 desaturase, a $\Delta$ -5 desaturase and a $\Delta$ -17 desaturase, transformation in <i>Y. lipolytica</i>	High yields of eicosapentaenoic (EPA) acid	[82]
<i>Mortierella alpina</i>	Oleic and Linoleic acids	Heterologous expression of the D12-desaturase (from <i>Coprinopsis cinerea</i> ) in the D6DS activity-defective mutant of <i>M. alpina</i>	Enhanced production of oleic and linoleic acids in the engineered strain	[120]
<i>Xanthophyllomyces dendrorhous</i>	Zeaxanthin	Overexpression of $\beta$ -carotene hydrolase and mutagenesis of astaxanthin synthase	Enhanced production of zeaxanthin (0.5 mg/g)	[121]
<i>T. reesei</i>	Cellulase	Overexpression of $\beta$ -glucosidase in <i>T. reesei</i> under a strong inducible promoter	Enhanced production of cellulase	[122]

Table 2. Cont.

Fungal Strain	High-Value Compounds	Strategy for Metabolic Engineering	Research Outcome	Reference
<i>T. koningii</i>	Cellulase	RNA interference was used to regulate the expression of the <i>cre1</i> gene	Enhanced production of cellulase	[123]
<i>Blakeslea trispora</i>	Lycopene (carotenoid)	Fermentation optimization with lycopene cyclase inhibitor	Enhanced production of lycopene (256 mg/L)	[22]
Filamentous fungi	Austinoids (insecticides)	Combinational engineering and rewiring of austinoid pathway	Production of diverse austinoid derivatives	[124]

Among other genetic studies in yeasts, key studies include: *Pichia pastoris* (*Komagataella* spp.) glycosylated recombinant proteins and showed higher protein secretion [125]; *Kluyveromyces lactis* was used for efficient recombinant protein production [126]; *Yarrowia lipolytica* used hydrocarbon source and synthesized fatty products [127]; *S. cerevisiae* was engineered for isoprenoid synthesis [128]; and *P. pastoris* was manipulated for membrane proteins production [129]. In addition, biopharmaceuticals, namely, growth factors, insulin, vaccines, glucagon, etc., have been produced in yeast and approved for commercial use [130].

#### 4. Fungal Chassis and Production of Functional Foods

Advances in synthetic biology strategies have contributed significantly to enhancing the production of high-value metabolites from diverse fungal strains. Genetic manipulations and mutagenesis aim to address the yield improvement of the desired metabolites from biological systems [131]. Usually, a biological species with a small genome size is preferred for engineering, since it can be handled with precision. For example, *S. cerevisiae* has efficient integration of DNA at desired regions, facilitating easy biological chassis [132]. With an efficient genome editing tool, CRISPR/Cas9, the design, and development of plasmids have considerably improved, adding versatility to the scope of yeast systems as a biotechnological tool [133,134]. Moreover, synthetic biology strategies are adopted to manipulate *S. cerevisiae* and design promoters, cloning plasmids, methods, etc., to benefit their diverse applications in biotechnology. In this direction, efficient transformation protocols, expression systems, and a range of selection markers have been developed [135,136]. Several research initiatives in the chassis of filamentous fungi have created knock-in, knock-out, desired gene expression, and replacement of genes, in a shorter time. Furthermore, the expression of all genes in a biosynthetic pathway via polycistronic expression cassette, in filamentous fungi, has been achieved and used to produce bioactive metabolites [137]. In another initiative, mutant strains of *Monascus* were developed for enhanced pigment production, monashin via enzyme polyketide synthase mutation [138]. The activation of transcription factors DAF-16/FOXO in *M. purpureus* induced thermal shock protein and superoxide dismutase and improved survival in *C. elegans* [139]. A mutant strain of *M. purpureus* was cultivated in fermented rice extract and produced monapurpureusone (a new azaphylone) and monapurpureusin (a natural product), respectively [140]. The filamentous fungi classified in *Basidiomycetes* biosynthesize natural food colorants such as phenazines, melanins, flavins, azaphilones, and quinones [141]. It is significant to optimize the culture conditions in microbial cultures, for maximum recovery of high-value metabolites, particularly in the production of dyes and pigments originating in plants. The biological production of fungal dyes and pigment and their use define safe and healthy food, compared to synthetic colorants [12]. Recent advances in high-throughput methods, strain improvements, and culture parameters optimization have considerably improved the biomass and production of bioactive compounds (Table 1).

#### 4.1. Metabolic Engineering toward Microbial Strain Improvement

High-value metabolites are increasingly identified and isolated from biological organisms, including fungi. However, the amount of these bioactive metabolites is in low concentrations, limiting industrial applications. Moreover, the presence of “cryptic pathways” which are silent and do not produce metabolites is documented and necessitates a requirement of strain improvement approaches [142]. In previous methods, a high-yielding strain was screened among all strains and further improved via mutagenesis and selection [143]. For example, *Penicillium chrysogenum* was screened for penicillin (an antibiotic) production and it produced 100-fold higher titers than the original strain. Further, developments in screening and strain improvement led to industrial production of penicillin (100,000-fold higher than the original strain) [144]. This method is beneficial in the way that no prior knowledge of the microbe’s genetics and the metabolic pathway is required, and the best-screened strain can be employed for metabolite production. Furthermore, multiple strategies can be adopted for the reorientation of metabolic flux toward specific metabolite production, such as improving precursor supply, monitoring gene expression regulation, improving enzyme functions, and metabolic pathway reconstitution in heterologous systems, including others [142].

#### 4.2. Mutagenesis Approaches in Fungi

For strain improvement in fungi, different mutagenesis approaches were adopted, using chemical or physical agents. To induce specific or random alterations, low/controlled levels were used, since they may lead to harmful mutations [145], and screening of mutants to locate the desired mutants was attempted. While the chemical agents used are base analogs causing base deamination, mainly GC→AT and AT→GC transitions, and impairment [145], physical mutagens comprise ionizing radiation ( $\gamma$ , X-rays) that cause DNA strand breakage, structural modifications, and ultraviolet radiation, which may cause frameshift mutations and deletions [145]. In this direction, microwave radiations were also employed for strain improvement [146]. Other mutagens including caffeine lead to frameshift mutations with potent effects in fungi and bacteria, and acridine dyes, and ethidium bromide result in deletions and frameshift mutations [145]. Multiple studies in fungi for strain improvement have employed chemical and physical mutagens in *Trichoderma reesei* Rut C-30, [147], and *Penicillium chrysogenum* [148]. Random mutagenesis was attempted in fungi for the enhanced yield of polyamines [149]. Yang and coworkers [150] employed mutagenesis in *Penicillium oxalicum*, phosphate-solubilizing fungi for improved production of organic acid and phosphate solubilization, respectively, which increased significantly on mutagenesis by radiation. In addition, physical and chemical mutagens were used to produce high-value metabolites from fungi, aimed toward a bio-based economy.

#### 4.3. Pathway Engineering in Fungi-Recent Trends and Initiatives

When the precursor supply does not affect product titer, the pathway enzyme expression may result in key outcomes. For example, a high-yielding strain of *P. chrysogenum* BW1890 (with multiple copies of gene clusters) leads to a 64-fold increase in the production of penicillin [151]. The advances in metabolic engineering facilitate the expression of all biosynthetic pathway enzyme(s), providing a solution. Moreover, the expression of single enzymes can be tuned by regulating transcription [152], and protein engineering [153], including other methods. In another key example, penicillin production in *A. nidulans* showed amino adipyl-cysteiny lvaline synthetase (ACVS) as a limiting enzyme, and the gene overexpression for ACVS resulted in enhanced ACVS expression and increased penicillin production [154]. However, gene overexpression for acyltransferase (ACYT), and iso penicillin N synthetase (IPNS), only slightly improved penicillin production [155]. Malla and coworkers [156] studied enhanced doxorubicin production (anticancer polyketide), aided by gene overexpression for glycosyltransferase and deoxysugar biosynthesis [157]. An important consideration suggests monitoring the toxic effects of a metabolite (if any)

at higher concentrations, in the case of doxorubicin production, the overexpression of resistance genes was essential [158].

In some organisms, the production of desired metabolites has been improved by altering the regulatory components of a metabolic pathway. For example, in *Streptomyces*, gene clusters encode *Streptomyces* antibiotic regulatory protein (SARP) that positively regulates the production of antibiotics [159]. Moreover, the SARP encoding fredericamycin in *Streptomyces griseus* ATCC 49344 was overexpressed and led to higher antibiotic production in the engineered strain [159]. Furthermore, SARP *MtmR* (mithramycin gene cluster in *Streptomyces argillaceus*) overexpression increased mithramycin titer 16-fold, respectively, and the MTMR-activated actinorhodin-producing pathway when expressed in *S. coelicolor* [160].

#### 4.4. Precursor Supply Increase

For all the major classes of natural products, increasing the supply of precursor molecules has been a successful method in both native and heterologous systems. These precursors can be primary metabolites or those derived from primary metabolites. For example, malonyl-CoA comprises a key precursor for polyketide biosynthesis; Ryu and colleagues attempted *S. coelicolor* engineering by overexpression of ACCase genes for enhanced malonyl-CoA production, and the study resulted in enhanced actinorhodin production [161]. Zha and coworkers [162] combined multiple methods for increased malonyl-CoA levels in *E. coli*, including pathway knockouts, gene overexpression, and limiting pathways for malonyl-CoA degradation, resulting in a 15-fold increase in malonyl-CoA [162]. Substantial initiatives in engineering *E. coli* for precursor supply have focused on the heterologous expression of the MVA pathway or its improvement for increased isopentenyl pyrophosphate (IPP) production, a precursor in the generation of terpenoids [163,164]. Research initiatives focusing on MEP pathway engineering have shown that 1-deoxy-D-xylulose-5-phosphate reductase (*dxr*), 1-deoxy-D-xylulose-5-phosphate synthase (*dxs*), and isopentenyl diphosphate isomerase (*idi*) overexpression enhanced production of isoprenoids [165]. In primary metabolism, the shikimate pathway is a key component, generating precursors for the biosynthesis of aromatic amino acids, utilized by several classes of natural products as precursors in the biosynthesis of metabolites. The yield of natural products has been considerably increased via increasing shikimate pathway flux and steps in amino acid biosynthesis [166].

#### 4.5. Downregulation/Deletion of Metabolic Pathways

Another prospective approach in this direction is to delete certain genes for pathway silencing so that associated metabolic pathways and their unnecessary intermediates can be avoided. A key example highlights that squalene synthase in yeast is encoded by *erg9* and utilizes farnesyl-pyrophosphate (FPP), a sesquiterpene precursor, and amorphaadiene production is increased by knocking out the *erg9* gene, respectively. In another example discussing doxorubicin biosynthesis, multiple genes encoded by the *dxr* cluster were removed to improve desired protein production [167]. In addition, the efficiency of the heterologous system can be increased via the deletion of specific genes. NADPH-dependent enzymes are encoded by several natural pathways: for instance, oxidoreductases create metabolic pressure on the cell as the pathway metabolic flux gradually increases [167]. Chemler and coworkers [168] showed NADPH as a limiting factor in flavonoid (+)-catechins production in *E. coli*. In the study, gene knockouts were identified utilizing a metabolic modeling approach, for improving NADPH availability and thereby, flavonoid production [168]. Komatsu and coworkers [169] reported a 'genome-minimized' approach (deletion of non-essential elements) in *Streptomyces avermilitis*, in which the genome was reduced to 83% of its original size, creating space for the introduction of a gene cluster of streptomycin. The minimized genome of *Streptomyces* produced higher amounts of streptomycin, highlighting a prospective approach to enhance the production of biochemicals [169].



#### 4.6. Metabolic Pathway Engineering

In this direction, the existence of divergence and homologies among genes between related metabolic pathways have led to switching genes and modules between interlinked pathways for novel microbial chassis [142]. The metabolic pathways of aromatic polyketide biosynthesis, namely, the macrolides, the teicoplanin, lipopeptides daptomycin/A54145, and aminocourmarins, highlight some examples. Hopwood and coworkers [170] used genes for related polyketides for combinational biosynthesis. In a key study, *Streptomyces* species (producing dihydrogranaticin and medermycin) were engineered by introducing actinorhodin pathway genes, resulting in hybrid antibiotics, dihydrogranatihordin, and mederrhodin, respectively [170]. For pathway engineering in a microbial system, genetic manipulation/switching biosynthetic genes downstream of the pathway defines higher success owing to the involvement of a few downstream enzymes [171]. The biosynthetic enzymes act on similar substrates/intermediates in closely related pathways. The creation of novel chimeras by gene switching showcases higher success potential and defines new research initiatives in the discovery and engineering of natural product pathways [142]. Novel fungal chimeras can be created via the reshuffling of genes and modules among linked pathways aimed at new combinations. Furthermore, a metabolic pathway can be engineered by altering a combination of genes in the biosynthetic pathway, for the creation of new chemical entities, subject to the tolerance of the downstream enzyme to substrate alteration [171]. One key concern in pathway engineering includes the non-disruption of the main scaffold, and the introduction of changes in the latter pathway steps has better chances of success, with the involvement of few downstream enzymes. Moreover, pathway engineering in the native host is carried out by new gene insertion/gene deletion or combinational pathway reconstitution [142].

#### 4.7. CRISPR/Cas Genome Editing in Fungi

An emerging genome editing tool, CRISPR/Cas has witnessed key success in genome editing of filamentous fungi to produce high-value metabolites including pigments, enzymes, secondary metabolites, compounds of industrial importance, and agriculture, respectively. The CRISPR/Cas tool has been widely employed in improving fungal strains including *Aspergillus*, *Trichoderma*, and *Penicillium* sp. having industrial importance. Moreover, studies have documented the genetic manipulation of fungal strains for heterologous protein production. Manganese peroxidase, classified in the family of heme-containing peroxidases, degrades lignin and is produced by white-rot fungi, which has relevance in chemical industries. The two proteins, manganese peroxide and Interleukin 6, were produced in *Aspergillus* species [172]. Besides, socially important fungal strains, namely, *Mortierella alpinis* [173], *Fusarium veneratum* [174], *A. japonicas* [175], *Chrysosporium luc-knowense* [176], have been developed for metabolites and protein production. Genetic manipulation strategies were attempted for *Cordyceps militaris* (edible medicinal mushroom) chassis; codon-optimized cas9 was used with promoter Pcmlsm3, and terminator Tcmura3 was expressed in the system. A CRISPR-Cas9 system comprising a single-strand DNA template, Cas9 DNA endonuclease, and RNA pre-synthesized in vitro was employed for insertion and site-specific deletion. The study aimed at genome editing of edible mushrooms for increasing genomic chassis and rapid development as 'functional food', respectively [177]. Chen and coworkers [178] employed CRISPR/Cas-mediated genome editing tools in *C. militaris* for enhanced ergothioneine production by discovering and regulating the metabolic pathway for ergothioneine biosynthesis [178]. In *Fusarium fujikuroi*, genetic manipulation methods were employed for enhanced gibberellic acid production [179].

#### 4.8. Key Metabolic Engineering Studies in Fungi

Metabolic engineering of microbes has witnessed good translational success, with multiple bacterial and fungal species engineered for food additives production in recent times [180]. For the production of malic acid (used in food and beverages), overexpression of genes was attempted in *A. flavus*, *A. oryzae*, *S. cerevisiae*, etc. [181]. The CRISPR-Cas 9



engineering tool was employed to alter the molecular structure and colors of pigments, by introducing change in the desired sites. Another key study discussed the genetic manipulation of *Y. lipolytica* for  $\beta$ -hydroxylase and  $\beta$ -ketolase production by gene introduction in fungal species and increased astaxanthin production [182]. This study provided key inputs to produce astaxanthin, with a high commercial value. Research initiatives attempted in fungal chassis have substantially enhanced the production of high-value metabolites, thanks to contributions of transcriptome-based analysis, cloning, and mutational approaches in non-yielding species [77]. Moreover, emerging insights into different transcription stages and their manipulations have considerably increased the production of cellulases, amylases, and xylanases in filamentous fungi via gene overexpression [183].

## 5. Conclusions and Future Directions

Harnessing biological resources for high-value products in drug discovery and research is gaining considerable recognition; however, bottlenecks in low product yield in the native organism, tedious procedures in isolation and characterization, and limited knowledge of metabolic machinery make it an ardent task. Chemical modifications of natural scaffolds are challenging, and the major limiting factors have declined the interest in natural products, necessitating a need to address associated concerns. The bio-based production of high-value compounds highlights promising attributes, and recent advances in high-throughput technologies and synthetic biology have contributed immensely to addressing these limitations. Applications of state-of-the-art technologies including omics biology, computational approaches, genome analysis, and genome editing facilitate unprecedented outcomes.

Metabolic engineering approaches coupled with drug discovery and development can be a powerful tool, exemplified and discussed with significant examples of fungal chassis. Furthermore, emerging insights/knowledge of natural product pathways, their regulations, and dynamics have facilitated the screening and development of novel chemical entities, with the potential to impact socio-economic arena. Significant translational success has been achieved in the genetic chassis of the biological organisms; however, it is difficult to predict the biosynthetic categories of both NPs and BGCs and the biological function, attributed to the complexities in the regulation of BGCs. Moreover, gene disruption methods have unraveled multiple biosynthetic pathways including the mycotoxin asirochlorine pathway [184], the protein phosphatase-2 inhibitor rubratoxin A pathway [185], and the mycotoxin cercosporin pathway [186]. In the industrial utilization of economically important fungal strains, a few limitations need to be addressed. For example, it is difficult to get high-density cultures due to pellet formation in the filamentous fungi; *Aspergillus* sp. and genetic engineering is cumbersome. Other limitations with high-throughput sequencing of fungal species with large colony formation and long process of mutant construction (a long time for spore formation in agar culture) further add to the limitations in industrial exploitation.

While significant advances in synthetic biology approaches have substantially contributed to the genetic manipulation of fungal organisms, challenges with precursor availability for natural product biosynthesis, low metabolic flux, and low enzyme activity in microbes add to the limitations [187]. In this direction, challenges concerned with the heterologous production of protein in filamentous fungi can be addressed by codon optimization and strong promoter selection at the transcription level, and other strategies, namely, the inclusion of N-glycosylation sites and signal sequences, and overexpression of chaperones, comprise methods to enhance protein production [188,189]. The heterologous production of terrequinone A and monacolin K polyketide was improved by the co-integration of BGC and universal regulator overexpression [190]. Metabolic engineering approaches individually or in combination, aided with the genomic tools, need to be developed further to address the existing concerns associated with the chassis of fungal organisms. The availability of limited marker genes necessitates the development of molecular genetic tools that would contribute to multidirectional improvement/developments in

fungal biotechnology. CRISPR/Cas9-based genome editing has been quite successful; however, on genome-wide functional analysis, unwanted mutations are caused by microbial cells and they can be addressed by gene modification via homologous recombination [189], although a less feasible method. The application of newer technology, Target-AID, may circumvent this limitation in filamentous fungi. Metabolic engineering-mediated chassis of fungal organisms defines a prospective platform for obtaining high-value metabolites for socio-economic applications, subject to addressing the knowledge gaps/obstacles in fungal biology and engineering.

**Author Contributions:** P.T. and L.D. planned the manuscript. P.T. contributed to the literature collection and writing of the manuscript. L.D. made critical suggestions for improvement. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** The authors extend their gratitude to their respective organizations for their encouragement and support.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Piffieri, F.; Aujard, F. Caloric restriction, longevity, and aging: Recent contributions from human and non-human primate studies. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2019**, *95*, 109702. [[CrossRef](#)] [[PubMed](#)]
2. Zhang, Z.; He, S.; Cao, X.; Ye, Y.; Yang, L.; Wang, J.; Liu, H.; Sun, H. Potential prebiotic activities of soybean peptides Maillard reaction products on modulating gut microbiota to alleviate aging-related disorders in D-galactose-induced ICR mice. *J. Funct. Foods* **2020**, *65*, 103729. [[CrossRef](#)]
3. Tiwari, P.; Bae, H. Endophytic fungi: Key insights, emerging prospects, and challenges in natural product drug discovery. *Microorganisms* **2022**, *10*, 360. [[CrossRef](#)] [[PubMed](#)]
4. Tiwari, P.; Seogchan, K.; Bae, H. Plant-endophyte associations: Rich yet under-explored sources of novel bioactive molecules and applications. *Microbiol. Res.* **2023**, *266*, 1–16. [[CrossRef](#)] [[PubMed](#)]
5. Tiwari, P. Sustainable agriculture and nanotechnologies for food and nutraceutical production—An update. In *Plant and Nanoparticles*; Chen, J., Ed.; Springer Book Series: Berlin/Heidelberg, Germany, 2022; ISBN 978-981-19-2502-3.
6. Karwehl, S.; Stadler, M. Exploitation of fungal biodiversity for the discovery of novel antibiotics. *Curr. Top. Microbiol. Immunol.* **2017**, *398*, 303–338.
7. Tiwari, P.; Srivastava, Y.; Bajpai, M.; Sharma, A. Bioactive metabolites from natural sources: Prospects and significance in drug discovery and research. *Bioingene PSJ* **2021**, *1*, 1–14.
8. Baggio, L.M.; Panagio, L.A.; Gasparin, F.G.M.; Sartori, D.; Celligoi, M.A.P.C.; Baldo, C. Production of fibrinolytic and fibrinolytic enzymes by a strain of *Penicillium* sp. isolated from contaminated soil with industrial eluent. *Acta Sci. Health Sci.* **2019**, *41*, 40606. [[CrossRef](#)]
9. Machida, M.; Yamada, O.; Gomi, K. Genomics of *Aspergillus oryzae*: Learning from the history of Koji mold and exploration of its future. *DNA Res.* **2008**, *15*, 173–183. [[CrossRef](#)]
10. Skellam, E. Strategies for engineering natural product biosynthesis in Fungi. *Trends Biotechnol* **2019**, *37*, 416–427. [[CrossRef](#)]
11. Takahashi, J.A.; Barbosa, B.V.R.; Martins, B.de.A.; Guirlanda, C.P.; Moura, M.A.F. Use of the versatility of fungal metabolism to meet modern demands for healthy aging, functional foods, and sustainability. *J. Fungi* **2020**, *6*, 223. [[CrossRef](#)]
12. Bills, G.F.; Gloer, J.B. Biologically active secondary metabolites from the fungi. *Microbiol. Spectr.* **2016**, *4*. [[CrossRef](#)] [[PubMed](#)]
13. Derbyshire, E.J.; Delange, J. Fungal protein—What is it and what is the health evidence? A systematic review focusing on mycoprotein. *Front. Sustain. Food Syst.* **2021**, *5*, 581682. [[CrossRef](#)]
14. Hashempour-Baltork, F.; Khosravi-Darani, K.; Hosseini, H.; Farshi, P.; Reihani, S.F.S. Mycoproteins as safe meat substitutes. *J. Clean. Prod.* **2020**, *253*, 119958. [[CrossRef](#)]
15. Kewuyemi, Y.O.; Kesa, H.; Adebo, O.A. Trends in functional food development with three-dimensional (3D) food printing technology: Prospects for value-added traditionally processed food products. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 7866–7904. [[CrossRef](#)] [[PubMed](#)]
16. Cairns, T.C.; Nai, C.; Meyer, V. How a fungus shapes biotechnology: 100 years of *Aspergillus niger* research. *Fungal Biol. Biotechnol.* **2018**, *5*, 13. [[CrossRef](#)]
17. Browne, A.G.P.; Fisher, M.C.; Henk, D.A. Species-specific PCR to describe local-scale distributions of four cryptic species in the *Penicillium chrysogenum* complex. *Fungal Ecol.* **2013**, *6*, 419–429. [[CrossRef](#)]
18. Liu, L.; Zhao, J.; Huang, Y.; Xin, Q.; Wang, Z. Diversifying of the chemical structure of native *Monascus* pigments. *Front. Microbiol.* **2018**, *9*, 3143. [[CrossRef](#)]

19. Shi, T.-Q.; Liu, G.N.; Ji, R.Y.; Shi, K.; Song, P.; Ren, L.J.; Huang, H.; Ji, X.J. CRISPR/Cas9-based genome editing of the filamentous fungi: The state of the art. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 7435–7443. [CrossRef]
20. Dutta, B.; Lahiri, D.; Nag, M.; Ghosh, S.; Dey, A.; Ray, R.R. Fungi in pharmaceuticals and production of antibiotics. In *Applied Mycology. Fungal Biology*; Shukla, A.C., Ed.; Springer: Cham, Switzerland, 2022. [CrossRef]
21. Meyer, V. Metabolic engineering of Filamentous fungi. In *Metabolic Engineering: Concepts and Applications*, 1st ed.; Lee, S.Y., Nielsen, J., Stephanopoulos, S., Eds.; WILEY-VCH GmbH: Weinheim, Germany, 2021.
22. Mantzouridou, F.T.; Naziri, E. Scale translation from shaken to diffused bubble aerated systems for lycopene production by *Blakeslea trispora* under stimulated conditions. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 1845–1856. [CrossRef]
23. Saha, B.C.; Kennedy, G.J. Efficient itaconic acid production by *Aspergillus terreus*: Overcoming the strong inhibitory effect of manganese. *Biotechnol. Prog.* **2020**, *36*, e2939. [CrossRef]
24. Patil, R.H.; Krishnan, P.; Maheshwari, V.L. Production of lovastatin by wild strains of *Aspergillus terreus*. *Nat. Prod. Commun.* **2011**, *6*, 183–186. [CrossRef] [PubMed]
25. Huang, X.; Men, P.; Tang, S.; Lu, X. *Aspergillus terreus* as an industrial filamentous fungus for pharmaceutical biotechnology. *Curr. Opin. Biotechnol.* **2021**, *69*, 273–280. [CrossRef] [PubMed]
26. Liu, L.; Chen, Z.; Liu, W.; Ke, X.; Tian, X.; Chu, J. Cephalosporin C biosynthesis and fermentation in *Acremonium chrysogenum*. *Appl. Microbiol. Biotechnol.* **2022**, *106*, 6413–6426. [CrossRef]
27. Kato, T.; Azegami, J.; Yokomori, A.; Dohra, H.; Enshasy, H.A.; Park, E.Y. Genomic analysis of a riboflavin-overproducing *Ashbya gossypii* mutant isolated by disparity mutagenesis. *BMC Genom.* **2020**, *21*, 319. [CrossRef] [PubMed]
28. Jesus, L.F.M.C. Produção de-Galactosidase Por Fungos Filamentosos: Screening, Purificação e Caracterização Bioquímica. Master's Thesis, Universidade Estadual Paulista "Julio Mesquita Filho", UNESP, São Paulo, Brazil, 2020.
29. Terabayashi, Y.; Sano, M.; Yamane, N.; Marui, J.; Tamano, K.; Sagara, J.; Dohmoto, M.; Oda, K.; Ohshima, E.; Tachibana, K.; et al. Identification and characterization of genes responsible for the biosynthesis of kojic acid, an industrially important compound from *Aspergillus oryzae*. *Fungal Genet. Biol.* **2010**, *47*, 953–961. [CrossRef]
30. Huang, Z.; Brennan, C.S.; Zheng, H.; Mohan, M.S.; Stipkovits, L.; Liu, W.; Kulasiri, D.; Guan, W.; Zhao, H.; Liu, J. The effects of fungal lipase-treated milk lipids on bread making. *LWT* **2020**, *128*, 109455. [CrossRef]
31. Hjortmo, S.B.; Hellström, A.M.; Andlid, T.A. Production of folates by yeasts in Tanzanian fermented togwa. *FEMS Yeast Res.* **2008**, *8*, 781–787. [CrossRef]
32. Mamani, L.D.G.; Magalhães, A.I., Jr.; Ruan, Z.; de Carvalho, J.C.; Soccol, C.R. Industrial production, patent landscape, and market trends of arachidonic acid-rich oil of *Mortierella alpina*. *Biotech. Res. Innov.* **2019**, *3*, 103–119. [CrossRef]
33. Anand, S.; Singh, K.S.; Aggarwal, D. Expanding avenues for probiotic yeast. In *Microbial Cell Factories*, 1st ed.; Sharma, D., Saharan, B.S., Eds.; CRC Press: Boca Raton, FL, USA, 2018; pp. 125–141.
34. Mallikarjuna, N.; Yellamma, K. Genetic and metabolic engineering of microorganisms for the production of various food products. *Recent Dev. Appl. Microbiol. Biochem.* **2019**, *60*, 167–182.
35. Tiwari, P.; Khare, T.; Shriram, V.; Bae, H.; Kumar, V. Exploring synthetic biology strategies for producing potent antimicrobial phytochemicals. *Biotechnol. Adv.* **2021**, *48*, 107729. [CrossRef]
36. Tiwari, P.; Srivastava, Y.; Bae, H. Trends of pharmaceutical design of Endophytes as anti-infective. *Curr. Top. Med. Chem.* **2021**, *21*, 1572–1586. [CrossRef] [PubMed]
37. Tiwari, P. *Endophytes: Types, Potential Uses and Mechanisms of Action*; Nova publishers: Hauppauge, NY, USA, 2022; ISBN 979-8-88697-205-4.
38. U.S. Department of Agriculture and U.S. Department of Health and Human Services. *Dietary Guidelines for Americans, 2020–2025*, 9th ed. 2020. Available online: [DietaryGuidelines.gov](https://www.dietaryguidelines.gov) (accessed on 26 August 2021).
39. Vega Oliveros, C. Comparación de la Producción de Metabolitos Secundarios Bioactivos con dos Fuentes de Carbono en la Fermentación Líquida de Una Especie de Pleurotus y su uso Potencial en un Alimento de Tipo Funcional (Thesis). Facultad de Ciencias Agrarias. Posgrado en Ciencia y Tecnología de Alimentos. Ph.D. Thesis, Universidad Nacional de Colombia, Bogotá, DC, Colombia, 2016.
40. Souza Filho, P.F.; Andersson, D.; Ferreira, J.A.; Taherzadeh, M.J. Mycoprotein: Environmental impact and health aspects. *World J. Microbiol. Biotechnol.* **2019**, *35*, 147. [CrossRef] [PubMed]
41. Barzee, T.J.; Cao, L.; Pan, Z.; Zhang, R. Fungi for future foods. *J. Future Foods* **2021**, *1*, 25–37. [CrossRef]
42. Ojwach, J.; Adetunji, A.I.; Mutanda, T.; Mukaratirwa, S. Oligosaccharides production from coprophilous fungi: An emerging functional food with potential health-promoting properties. *Biotechnol. Rep.* **2022**, *33*, e00702. [CrossRef]
43. Fu, L.; Wang, Y.; Wang, J.; Yang, Y.; Hao, L. Evaluation of the antioxidant activity of extracellular polysaccharides from *Morchella esculenta*. *Food Funct.* **2013**, *4*, 871–879. [CrossRef]
44. Jeong, S.C.; Koyyalamudi, S.R.; Jeong, Y.T.; Song, C.H.; Pang, G. Macrophage immunomodulating and antitumor activities of polysaccharides isolated from *Agaricus bisporus* white button mushrooms. *J. Med. Food* **2012**, *15*, 58–65. [CrossRef]
45. Wen, L.; Gao, Q.; Ma, C.W.; Ge, Y.; You, L.; Liu, R.H.; Fu, X.; Liu, D. Effect of polysaccharides from *Tremella fuciformis* on UV-induced photoaging. *J. Func. Foods* **2016**, *20*, 400–441. [CrossRef]
46. Li, R.; Zhang, J.; Zhang, T.H. Immunomodulatory activities of polysaccharides from *Ganoderma* on immune effector cells. *Food Chem.* **2020**, *340*, 127933. [CrossRef]
47. GNC Ganoderma Capsules. Available online: <http://www.vitagou.com/gnc/161.html> (accessed on 23 February 2023).

48. Drugs.com. Lentinan. Available online: <https://www.drugs.com/npp/lentinan.html> (accessed on 23 February 2023).
49. Hanqi Edible Mushroom Products. Available online: <https://www.meipian.cn/1ez6jqn1> (accessed on 23 February 2023).
50. Wang, L.; Li, C.; Ren, L.; Guo, H.; Li, Y. Production of pork sausages using *Pleurotus eryngii* with different treatments as replacements for pork back fat. *J. Food Sci.* **2019**, *84*, 3091–3098. [[CrossRef](#)]
51. Jo, K.; Lee, S.; Jo, C.; Jeon, H.J.; Choe, J.H.; Choi, Y.S.; Jung, S. Utility of winter mushroom treated by atmospheric non-thermal plasma as an alternative for synthetic nitrite and phosphate in ground ham. *Meat Sci.* **2020**, *166*, 108151. [[CrossRef](#)]
52. Lu, X.; Brennan, M.A.; Serventi, L.; Liu, J.; Guan, W.; Brennan, C.S. Addition of mushroom powder to pasta enhances the antioxidant content and modulates the predictive glycaemic response of pasta. *Food Chem.* **2018**, *264*, 199–209. [[CrossRef](#)] [[PubMed](#)]
53. Steudler, S.; Werner, A.; Walther, T. It is the Mix that matters: Substrate-specific enzyme production from filamentous fungi and bacteria through solid-state fermentation. *Adv. Biochem. Eng. Biotechnol.* **2019**, *169*, 51–81.
54. Hussain, H.; Nazir, M.; Saleem, M.; Al-Harrasi, A.; Green, I.R. Fruitful decade of fungal metabolites as anti-diabetic agents from 2010 to 2019: Emphasis on  $\alpha$ -glucosidase inhibitors. *Phytochem. Rev.* **2021**, *20*, 145–179. [[CrossRef](#)]
55. Meyer, V.; Fiedler, M.; Nitsche, B.; King, R. The cell factory *Aspergillus* enters the big data era: Opportunities and challenges for optimizing product formation. *Adv. Biochem. Eng. Biotechnol.* **2015**, *149*, 91–132.
56. Abe, K.; Gomi, K.; Hasegawa, F.; Machida, M. Impact of *Aspergillus oryzae* genomics on industrial production of metabolites. *Mycopathologia* **2006**, *162*, 143–153. [[CrossRef](#)]
57. Jin, F.J.; Hu, S.; Wang, B.T.; Jin, L. Advances in genetic engineering technology and its application in the industrial fungus *Aspergillus oryzae*. *Front. Microbiol.* **2021**, *12*, 644404. [[CrossRef](#)]
58. Jiang, C.; Lv, G.; Tu, Y.; Cheng, X.; Duan, Y.; Zeng, B.; He, B. Applications of CRISPR/Cas9 in the synthesis of secondary metabolites in filamentous fungi. *Front. Microbiol.* **2021**, *12*, 638096. [[CrossRef](#)]
59. Srivastava, A.K. The role of fungus in bioactive compound production and nanotechnology. In *Role of Plant Growth Promoting Microorganisms in Sustainable Agriculture and Nanotechnology*; Woodhead Publishing, Elsevier: Cambridge, UK, 2019; pp. 145–162.
60. Adebayo, E.A.; Azeez, M.A.; Alao, M.B.; Oke, A.M.; Aina, D.A. Fungi as a veritable tool in current advances in nanobiotechnology. *Heliyon* **2021**, *7*, 2405–8440. [[CrossRef](#)] [[PubMed](#)]
61. Fayaz, A.M.; Balaji, K.; Girilal, M.; Yadav, R.; Kalaichelvan, P.T.; Venketesan, R. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: A study against gram-positive and gram-negative bacteria. *Nanomed. Nanotechnol. Biol. Med.* **2010**, *6*, 103–109. [[CrossRef](#)]
62. Kumar, S.A.; Peter, Y.A.; Nadeau, J.L. Facile biosynthesis, separation, and conjugation of gold nanoparticles to doxorubicin. *Nanotechnology* **2008**, *19*, 495101. [[CrossRef](#)] [[PubMed](#)]
63. Alani, F.; Moo-Young, M.; Anderson, W. Biosynthesis of silver nanoparticles by a new strain of *Streptomyces* sp. compared with *Aspergillus fumigatus*. *World J. Microbiol. Biotechnol.* **2012**, *28*, 1081–1086. [[CrossRef](#)]
64. Rajakumar, G.; Rahuman, A.; Roopan, S.M.; Khanna, V.G.; Elango, G.; Kamaraj, C.; Zahir, A.A.; Velayutham, K. Fungus-mediated biosynthesis and characterization of TiO<sub>2</sub> nanoparticles and their activity against pathogenic bacteria. *Spectrochim. Acta Mol. Biomol. Spectrosc.* **2012**, *91*, 23–29. [[CrossRef](#)] [[PubMed](#)]
65. Chuhan, A.; Zubair, S.; Tufail, S.; Sherwani, A.; Sajid, M.; Raman, S.C.; Azam, A.; Owais, M. Fungus-mediated biological synthesis of gold nanoparticles: Potential in detection of liver cancer. *Int. J. Nanomed.* **2011**, *6*, 2305–2319.
66. Das, S.K.; Das, A.R.; Guha, A.K. Gold nanoparticles: Microbial synthesis and application in water hygiene management. *Langmuir* **2009**, *25*, 8192–8199. [[CrossRef](#)]
67. Tarafdar, J.C.; Raliya, R.; Rathore, I. Microbial synthesis of phosphorous nanoparticle from tri-calcium phosphate using *Aspergillus tubingensis* TFR-5. *J. Bionanoscience* **2012**, *6*, 84–89. [[CrossRef](#)]
68. Sawle, B.D.; Salimath, B.; Deshpande, R.; Bedre, M.D.; Prabhakar, B.K.; Venkataraman, A. Biosynthesis and stabilization of Au and Au-Ag alloy nanoparticles by fungus. *Fusarium semitectum*. *Sci. Technol. Adv. Mater.* **2008**, *9*, 035012. [[CrossRef](#)]
69. Tarafdar, J.C.; Raliya, R. Rapid, low-cost, and eco-friendly approach for iron nanoparticle synthesis using *Aspergillus oryzae* TFR9. *J. Nanoparticles* **2013**, 141274. [[CrossRef](#)]
70. Binupriya, A.R.; Sathishkumar, M.; Yun, S.I. Biocrystallization of silver and gold ions by inactive cell filtrate of *Rhizopus stolonifer*. *Colloids Surf. B Biointerfaces* **2010**, *79*, 531–534. [[CrossRef](#)]
71. Raliya, R.; Biswas, P.; Tarafdar, J.C. TiO<sub>2</sub> nanoparticle biosynthesis and its physiological effect on mung bean (*Vigna radiata* L.). *Biotechnol. For. Rep.* **2015**, *5*, 22–26. [[CrossRef](#)]
72. Nature. Available online: <https://www.nature.com/subjects/metabolic-engineering> (accessed on 24 September 2019).
73. Sharma, A.; Mistry, V.; Kumar, V.; Tiwari, P. Production of effective Phyto-antimicrobials via metabolic engineering strategies. *Curr. Top. Med. Chem.* **2022**, *22*, 1068–1092.
74. Podolsky, I.; Seppala, S.; Lankiewicz, T.; Brown, J.; Swift, C.; O'Malley, M. Harnessing nature's anaerobes for biotechnology and bioprocessing. *Annu. Rev. Chem. Biomol. Eng.* **2019**, *10*, 105–128. [[CrossRef](#)]
75. Seppala, S.; Wilken, S.E.; Knop, D.; Solomon, K.V.; O'Malley, M.A. The importance of sourcing enzymes from non-conventional fungi for metabolic engineering and biomass breakdown. *Metab. Eng.* **2017**, *44*, 45–49. [[CrossRef](#)] [[PubMed](#)]
76. de Backe, M.D.; van Dijk, P. Progress in functional genomics approaches to antifungal drug target discovery. *Trends Microbiol.* **2003**, *11*, 470–478. [[CrossRef](#)]



77. Villena Gretty, K.; Kitazono, A.A.; Hernández-Macedo, M.L. Bioengineering fungi and yeast for the production of enzymes, metabolites, and value-added compounds. In *Fungal Biotechnology and Bioengineering*; Hesham, A.E.L., Ed.; Fungal Biology; Springer Nature: Cham, Switzerland, 2020.
78. Lian, J.; Si, T.; Nair, N.U.; Zhao, H. Design and construction of acetyl-CoA overproducing *Saccharomyces cerevisiae* strains. In Proceedings of the Food, Pharmaceutical, and Bioengineering Division 2014-Core Programming Area at the 2014 AIChE Annual Meeting, Atlanta, GA, USA, 16–21 November 2014.
79. Vieira Gomes, A.M.; Souza Carmo, T.; Silva Carvalho, L.; Mendonca Bahia, F.; Parachin, N.S. Comparison of yeasts as hosts for recombinant protein production. *Microorganisms* **2018**, *6*, 38. [[CrossRef](#)] [[PubMed](#)]
80. Meyer, V.; Wanka, F.; van Gent, J.; Arentshorst, M.; van den Hondel, C.A.; Ram, A.F. Fungal gene expression on demand: An inducible, tunable, and metabolism-independent expression system for *Aspergillus niger*. *Appl. Environ. Microbiol.* **2011**, *77*, 2975–2983. [[CrossRef](#)]
81. Kuivanen, J.; Wang, Y.J.; Richard, P. Engineering *Aspergillus niger* for galactaric acid production: Elimination of galactaric acid catabolism by using RNA sequencing and CRISPR/Cas9. *Microb. Cell Fact.* **2016**, *15*, 210. [[CrossRef](#)]
82. Xue, Z.; Sharpe, P.L.; Hong, S.P.; Yadav, N.S.; Xie, D.; Short, D.R.; Damude, H.G.; Rupert, R.A.; Seip, J.E.; Wang, J.; et al. Production of omega-3 eicosapentaenoic acid by metabolic engineering of *Yarrowia lipolytica*. *Nat. Biotechnol.* **2013**, *31*, 8. [[CrossRef](#)]
83. Gao, S.; Lyu, Y.; Zeng, W.; Du, G.; Zhou, J.; Chen, J. Efficient biosynthesis of (2S)-Naringenin from p-Coumaric acid in *Saccharomyces cerevisiae*. *J. Agric. Food Chem.* **2019**, *68*, 1015–1021. [[CrossRef](#)] [[PubMed](#)]
84. Xu, Y.; Wang, X.; Zhang, C.; Zhou, X.; Xu, X.; Han, L.; Lv, X.; Liu, Y.; Liu, S.; Li, J.; et al. De novo biosynthesis of rubusoside and rebaudiosides in engineered yeasts. *Nat. Commun.* **2022**, *13*, 3040. [[CrossRef](#)]
85. Chen, Y.; Xiao, W.; Wang, Y.; Liu, H.; Li, X.; Yuan, Y. Lycopene overproduction in *Saccharomyces cerevisiae* through combining pathway engineering with host engineering. *Microb. Cell Fact.* **2016**, *15*, 113. [[CrossRef](#)] [[PubMed](#)]
86. Amor, I.L.-B.; Hehn, A.; Guedone, E.; Ghedira, K.; Engasser, J.-M.; Chekir-Ghedrira, L. Mohamed Ghoul. 2010. Biotransformation of naringenin to eriodictyol by *Saccharomyces cerevisiae* functionally expressing flavonoid 3' hydroxylase. *Nat. Prod. Commun.* **2010**, *5*, 1893–1898. [[PubMed](#)]
87. Thompson, R.A.; Trinh, C.T. Enhancing fatty acid ethyl ester production in *Saccharomyces cerevisiae* through metabolic engineering and medium optimization. *Biotechnol. Bioeng.* **2014**, *111*, 2200–2208. [[CrossRef](#)]
88. Den Haan, R.; Rose, S.H.; Lynd, L.R.; van Zyl, W.H. Hydrolysis and fermentation of amorphous cellulose by recombinant *Saccharomyces cerevisiae*. *Metab. Eng.* **2007**, *9*, 87–94. [[CrossRef](#)]
89. Tang, X.; Feng, H.; Chen, W.N. Metabolic engineering for enhanced fatty acids synthesis in *Saccharomyces cerevisiae*. *Metab. Eng.* **2013**, *16*, 95–102. [[CrossRef](#)]
90. Zhou, Y.J.; Buijs, N.A.; Zhu, Z.; Qin, J.; Siewers, V.; Nielsen, J. Production of fatty acid-derived oleochemicals and biofuels by synthetic yeast cell factories. *Nat. Commun.* **2016**, *7*, 11709. [[CrossRef](#)] [[PubMed](#)]
91. Paramasivan, K.; Mutturi, S. Progress in terpene synthesis strategies through the engineering of *Saccharomyces cerevisiae*. *Crit. Rev. Biotechnol.* **2017**, *37*, 974–989. [[CrossRef](#)]
92. Weber, C.; Brückner, C.; Weinreb, S.; Lehr, C.; Essl, C.; Boles, E. Biosynthesis of cis, cis-muconic acid and its aromatic precursors, catechol and protocatechuic acid, from renewable feedstocks by *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **2012**, *78*, 8421–8430. [[CrossRef](#)]
93. Ghosh, A.; Ando, D.; Gin, J.; Runguphan, W.; Denby, C.; Wang, G.; Baidoo, E.E.K.; Shymansky, C.; Keasling, J.D.; Martín, H.G. <sup>13</sup>C metabolic flux analysis for systematic metabolic engineering of *S. cerevisiae* for overproduction of fatty acids. *Front. Bioeng. Biotechnol.* **2016**, *4*, 76. [[CrossRef](#)]
94. Babaei, M.; Gheorghie, M.B.Z.; Chen, X.; Christensen, H.B.; Kristensen, M.; Nielsen, J.; Borodina, I. Metabolic engineering of *Saccharomyces cerevisiae* for rosmarinic acid production. *ACS Synth. Biol.* **2020**, *9*, 1978–1988. [[CrossRef](#)] [[PubMed](#)]
95. Krömer, J.O.; Nunez-Bernal, D.; Aversch, N.J.H.; Hampe, J.; Varela, J.; Varela, C. Production of aromatics in *Saccharomyces cerevisiae*-a feasibility study. *J. Biotechnol.* **2013**, *163*, 184–193. [[CrossRef](#)] [[PubMed](#)]
96. Ouyang, X.; Cha, Y.; Li, W.; Zhu, C.; Zhu, M.; Li, S.; Zhuo, M.; Huang, S.; Li, J. Stepwise engineering of *Saccharomyces cerevisiae* to produce (+)-valencene and its related sesquiterpenes. *RSC Adv.* **2019**, *9*, 30171–30181. [[CrossRef](#)] [[PubMed](#)]
97. Runguphan, W.; Keasling, J.D. Metabolic engineering of *Saccharomyces cerevisiae* for production of fatty acid-derived biofuels and chemicals. *Metab. Eng.* **2014**, *21*, 103–113. [[CrossRef](#)] [[PubMed](#)]
98. Xiao, F.; Lian, J.; Tu, S.; Xie, L.; Li, J.; Zhang, F.; Linhardt, R.J.; Huang, H.; Zhong, W. Metabolic engineering of *Saccharomyces cerevisiae* for high-level production of chlorogenic acid from glucose. *ACS Synth. Biol.* **2022**, *11*, 800–811. [[CrossRef](#)] [[PubMed](#)]
99. Santos, C.N.S.; Koffas, M.A.G.; Stephanopoulos, G. Optimization of a heterologous pathway for the production of flavonoids from glucose. *Metab. Eng.* **2011**, *13*, 392–400. [[CrossRef](#)]
100. Pai, J.S. Applications of microorganisms in food biotechnology. *Indian J. Biotechnol.* **2003**, *2*, 382–386.
101. Lv, Y.; Edwards, H.; Zhou, J.; Xu, P. Combining 26s rDNA and the Cre-loxP system for iterative gene integration and efficient marker curation in *Yarrowia lipolytica*. *ACS Synth. Biol.* **2019**, *8*, 568–576. [[CrossRef](#)]
102. Rosa, J.C.C.; Colombo, L.T.; Alvim, M.C.T.; Avonce, N.; Dijck, P.V.; Passos, F.M.L. Metabolic engineering of *Kluyveromyces lactis* for L-ascorbic acid (vitamin C) biosynthesis. *Microb. Cell Fact.* **2013**, *12*, 59. [[CrossRef](#)] [[PubMed](#)]

103. González-Siso, M.I.; Touriño, A.; Vizoso, Á.; Pereira-Rodríguez, Á.; Rodríguez-Belmonte, E.; Becerra, M.; Cerdán, M.E. Improved bioethanol production in an engineered *Kluyveromyces lactis* strain shifted from respiratory to fermentative metabolism by deletion of NDI1. *Microb. Biotechnol.* **2015**, *8*, 319–330. [[CrossRef](#)] [[PubMed](#)]
104. Rico-Díaz, A.; Álvarez-Cao, M.E.; Escuder-Rodríguez, J.J.; González-Siso, M.I.; Cerdán, M.E.; Becerra, M. Rational mutagenesis by engineering disulfide bonds improves *Kluyveromyces lactis* beta-galactosidase for high-temperature industrial applications. *Sci. Rep.* **2017**, *7*, 45535. [[CrossRef](#)]
105. Fiedler, M.R.; Barthel, L.; Kubisch, C.; Nai, C.; Meyer, V. Construction of an improved *Aspergillus niger* platform for enhanced glucoamylase secretion. *Microb. Cell Fact.* **2018**, *17*, 95. [[CrossRef](#)]
106. Carvalho, N.D.; Arentshorst, M.; Kooistra, R.; Stam, H.; Sagt, C.M.; van den Hondel, C.A.; Ram, A.F. Effects of a defective ERAD pathway on growth and heterologous protein production in *Aspergillus niger*. *Appl. Microbiol. Biotechnol.* **2011**, *89*, 357–373. [[CrossRef](#)] [[PubMed](#)]
107. Conesa, A.; Jeenes, D.; Archer, D.B.; van den Hondel, C.A.M.J.J.; Punt, P.J. Calnexin overexpression increases manganese peroxidase production in *Aspergillus niger*. *Appl. Environ. Microbiol.* **2002**, *68*, 846–851. [[CrossRef](#)]
108. Karnaukhova, E.; Ophir, Y.; Trinh, L.; Dalal, N.; Punt, P.J.; Golding, B.; Shiloach, J. Expression of human  $\alpha$ 1-proteinase inhibitor in *Aspergillus niger*. *Microb. Cell Fact.* **2007**, *6*, 34. [[CrossRef](#)]
109. Aifa, M.S.; Sayadi, S.; Gargouri, A. Heterologous expression of lignin peroxidase of *Phanerochaete chrysosporium* in *Aspergillus niger*. *Biotechnol. Lett.* **1999**, *21*, 849–853. [[CrossRef](#)]
110. Richter, L.; Wanka, F.; Boecker, S.; Storm, D.; Kurt, T.; Vural, O.; Süßmuth, R.; Meyer, V. Engineering of *Aspergillus niger* for the production of secondary metabolites. *Fungal Biol. Biotechnol.* **2014**, *1*, 4. [[CrossRef](#)] [[PubMed](#)]
111. Wakai, S.; Nakashima, N.; Ogino, C.; Tsutsumi, H.; Hata, Y.; Kondo, A. Modified expression of multi-cellulases in a filamentous fungus *Aspergillus oryzae*. *Bioresour. Technol.* **2019**, *276*, 146–153. [[CrossRef](#)]
112. Kuivanen, J.; Penttilä, M.; Richard, P. Metabolic engineering of the fungal d-galacturonate pathway for l-ascorbic acid production. *Microb. Cell Fact.* **2015**, *14*, 2. [[CrossRef](#)]
113. Kraševc, N.; Milunović, T.; Lasnik, M.A.; Lukančič, I.; Komel, R.; Porekar, V.G. Human granulocyte colony-stimulating factor (G-CSF) produced in the filamentous fungus *Aspergillus niger*. *Acta Chim. Slov.* **2014**, *61*, 709–717.
114. Wriessnegger, T.; Augustin, P.; Engleder, M.; Leitner, E.; Müller, M.; Kaluzna, I.; Schürmann, M.; Mink, D.; Zellnig, G.; Schwab, H.; et al. Production of the sesquiterpenoid (+)-nootkatone by metabolic engineering of *Pichia pastoris*. *Metab. Eng.* **2014**, *24*, 18–29. [[CrossRef](#)]
115. Cai, P.; Wu, X.; Deng, J.; Gao, L.; Shen, Y.; Yao, L.; Zhou, Y.J. Methanol biotransformation toward high-level production of fatty acid derivatives by engineering the industrial yeast *Pichia pastoris*. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2201711119. [[CrossRef](#)] [[PubMed](#)]
116. Guo, F.; Dai, Z.; Peng, W.; Zhang, S.; Zhou, J.; Ma, J.; Dong, W.; Xin, F.; Zhang, W.; Jiang, M. Metabolic engineering of *Pichia pastoris* for malic acid production from methanol. *Biotechnol. Bioeng.* **2021**, *1*, 357–371. [[CrossRef](#)] [[PubMed](#)]
117. Larroude, M.; Celinska, E.; Back, A.; Thomas, S.; Nicaud, J.M.; Ledesma-Amaro, R. A synthetic biology approach to transform *Yarrowia lipolytica* into a competitive biotechnological producer of b-carotene. *Biotechnol. Bioeng.* **2018**, *115*, 464–472. [[CrossRef](#)] [[PubMed](#)]
118. Fitz, E.; Gamauf, C.; Seiboth, B.; Wanka, F. Deletion of the small GTPase rac1 in *Trichoderma reesei* provokes hyperbranching and impacts growth and cellulase production. *Fungal Biol. Biotechnol.* **2019**, *6*, 16. [[CrossRef](#)]
119. Lin, L.; Sun, Z.; Li, J.; Chen, Y.; Liu, Q.; Sun, W.; Tian, C. Disruption of gul-1 decreased the culture viscosity and improved protein secretion in the filamentous fungus *Neurospora crassa*. *Microb. Cell Fact.* **2018**, *17*, 96. [[CrossRef](#)]
120. Sakamoto, T.; Sakuradani, E.; Okuda, T.; Kikukawa, H.; Ando, A.; Kishino, S.; Izumi, Y.; Bamba, T.; Shima, J.; Ogawa, J. Metabolic engineering of oleaginous fungus *Mortierella alpina* for high production of oleic and linoleic acids. *Bioresour. Technol.* **2017**, *247*, 1610–1615. [[CrossRef](#)]
121. Pollmann, H.; Breitenbach, J.; Sandmann, G. Engineering of the carotenoid pathway in *Xanthophyllomyces dendrorhous* leading to the synthesis of zeaxanthin. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 103–111. [[CrossRef](#)]
122. Li, Y.H.; Zhang, X.Y.; Xiong, L.; Mehmood, M.A.; Zhao, X.; Bai, F. On-site cellulase production and efficient saccharification of corn Stover employing cbh2 overexpressing *Trichoderma reesei* with novel induction system. *Bioresour. Technol.* **2017**, *238*, 643–649. [[CrossRef](#)]
123. Wang, S.; Liu, G.; Wang, J.; Yu, J.; Huang, B.; Xing, M. Enhancing cellulase production in *Trichoderma reesei* RUT C30 through combined manipulation of activating and repressing genes. *J. Ind. Microbiol. Biotechnol.* **2013**, *40*, 633–641. [[CrossRef](#)]
124. Mattern, D.J.; Valiante, V.; Horn, F.; Petzke, L.; Brakhage, A.A. Rewiring of the Austinoid biosynthetic pathway in filamentous fungi. *ACS Chem. Biol.* **2017**, *12*, 2927–2933. [[CrossRef](#)]
125. Prielhofer, R.; Barrero, J.J.; Steuer, S.; Gassler, T.; Zahrl, R.; Baumann, K.; Sauer, M.; Mattanovich, D.; Gasser, B.; Marx, H. GoldenPiCS: A Golden Gate-derived modular cloning system for applied synthetic biology in the yeast *Pichia pastoris*. *BMC Syst. Biol.* **2017**, *11*, 123. [[CrossRef](#)] [[PubMed](#)]
126. Rodicio, R.; Heinisch, J.J. Yeast on the milky way: Genetics, physiology, and biotechnology of *Kluyveromyces lactis*. *Yeast* **2013**, *30*, 165–177. [[CrossRef](#)] [[PubMed](#)]
127. Goncalves, F.A.G.; Colen, G.; Takahashi, J.A. *Yarrowia lipolytica* and its multiple applications in the biotechnological industry. *Sci. World J.* **2014**, *2014*, 476207. [[CrossRef](#)] [[PubMed](#)]



128. Vickers, C.E.; Williams, T.C.; Peng, B.; Cherry, J. Recent advances in synthetic biology for engineering isoprenoid production in yeast. *Curr. Opin. Chem. Biol.* **2017**, *40*, 47–56. [CrossRef]
129. Dilworth, M.V.; Piel, M.S.; Bettaney, K.E.; Ma, P.; Luo, J.; Sharples, D.; Poyner, D.R.; Gross, S.R.; Moncoq, K.; Henderson, P.J.F.; et al. Microbial expression systems for membrane proteins. *Methods* **2018**, *147*, 30–39. [CrossRef] [PubMed]
130. Wang, G.; Huang, M.; Nielsen, J. Exploring the potential of *Saccharomyces cerevisiae* for biopharmaceutical protein production. *Curr. Opin. Biotechnol.* **2017**, *48*, 77–84. [CrossRef]
131. Tiwari, P.; Bajpai, M.; Singh, L.K.; Mishra, S.; Yadav, A.N. Phytohormones producing fungal communities: Metabolic engineering for abiotic stress tolerance in plants. In *Agriculturally Important Fungi for Sustainable Agriculture*; Gupta, V.J., Tuohy, M., Eds.; Springer publication: Berlin/Heidelberg, Germany, 2020.
132. Duina, A.A.; Miller, M.E.; Keeney, J.B. Budding yeast for budding geneticists: A primer on the *Saccharomyces cerevisiae* model system. *Genetics* **2014**, *197*, 33–48. [CrossRef]
133. Stovicek, V.; Holkenbrink, C.; Borodina, I. CRISPR/Cas system for yeast genome engineering: Advances and applications. *FEMS Yeast Res.* **2017**, *17*, 1–16. [CrossRef]
134. Schwartz, C.; Wheeldon, I. CRISPR-Cas9-mediated genome editing and transcriptional control in *Yarrowia lipolytica*. *Methods Mol. Biol.* **2018**, *1772*, 327–345.
135. Meyer, V. Genetic engineering of filamentous fungi—Progress, obstacles, and future trends. *Biotechnol. Adv.* **2008**, *26*, 177–185. [CrossRef]
136. Kluge, J.; Terfehr, D.; Kück, U. Inducible promoters and functional genomic approaches for the genetic engineering of filamentous fungi. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 6357–6372. [CrossRef]
137. Schuetze, T.; Meyer, V. Polycistronic gene expression in *Aspergillus niger*. *Microb. Cell Fact.* **2017**, *16*, 162. [CrossRef] [PubMed]
138. Mondal, S.; Pandit, S.G.; Puttananjaiiah, M.H.; Harohally, N.V.; Dhale, M.A. Structural and functional characterization of new pigment molecule monashin from *Monascus purpureus* CFR410-11. *Process. Biochem.* **2019**, *82*, 173–178. [CrossRef]
139. Shi, Y.C.; Pan, T.M.; Liao, V.H.C. Monascin from *Monascus*-fermented products reduces oxidative stress and amyloid-beta toxicity via DAF-16/FOXO in *Caenorhabditis elegans*. *J. Agric. Food. Chem.* **2016**, *64*, 7114–7120. [CrossRef] [PubMed]
140. Wu, H.C.; Cheng, M.J.; Wu, M.D.; Chen, J.J.; Chen, Y.L.; Chang, H.S.; Chen, K.P. Secondary metabolites from the fermented rice of the fungus *Monascus purpureus* and their bioactivities. *Nat. Prod. Res.* **2019**, *33*, 3541–3550. [CrossRef]
141. American Chemical Society (ACS). Dyes, Pigments, and Inks. 2020. Available online: <https://www.acs.org/content/acs/en/careers/college-to-career/chemistry-careers/dyes-pigments-ink.html> (accessed on 8 September 2020).
142. Pickens, L.B.; Tang, Y.; Chooi, Y.-H. Metabolic engineering for the production of Natural products. *Annu. Rev. Chem. Biomol. Eng.* **2011**, *2*, 211–236. [CrossRef]
143. Parekh, S. Strain improvement. In *The Desk Encyclopedia of Microbiology*; Schaechter, M., Ed.; Elsevier Academic Press: San Diego, CA, USA, 2004; pp. 960–973.
144. Rokem, J.S.; Lantz, A.E.; Nielsen, J. Systems biology of antibiotic production by microorganisms. *Nat. Prod. Rep.* **2007**, *24*, 1262–1287. [CrossRef]
145. Parekh, S.; Vinci, V.A.; Strobel, R.J. Improvement of microbial strains and fermentation processes. *Appl. Microbiol. Biotechnol.* **2000**, *54*, 287–301. [CrossRef]
146. Li, X.-H.; Yang, H.-J.; Roy, B.; Park, E.Y.; Jiang, L.-J.; Wang, D.; Miao, Y.-G. Enhanced cellulase production of the *Trichoderma viride* mutated by microwave and ultraviolet. *Microbiol. Res.* **2010**, *165*, 190–198. [CrossRef]
147. Peterson, R.; Nevalainen, H. *Trichoderma reesei* RUT-C30—thirty years of strain improvement. *Microbiology* **2012**, *158*, 58–68. [CrossRef] [PubMed]
148. Ziemons, S.; Koutsantas, K.; Becker, K.; Dahlmann, T.; Kück, U. Penicillin production in industrial strain *Penicillium chrysogenum* P2niaD18 is not dependent on the copy number of biosynthesis genes. *BMC Biotechnol.* **2017**, *17*, 1. [CrossRef]
149. Zhgun, A.A. Random mutagenesis of filamentous fungi strains for high-yield production of secondary metabolites: The role of polyamines. In *Genotoxicity and Mutagenicity—Mechanisms and Test Methods*; Soloneski, S., Larramendy, M.L., Eds.; Intech Open: London, UK, 2020. [CrossRef]
150. Yang, T.; Li, L.; Wang, B.; Tian, J.; Shi, F.; Zhang, S.; Wu, Z. Isolation, mutagenesis, and organic acid secretion of a highly efficient Phosphate-solubilizing fungus. *Front. Microbiol.* **2022**, *13*, 793122. [CrossRef]
151. Smith, D.J.; Bull, J.H.; Edwards, J.; Turner, G. Amplification of the isopenicillin N synthetase gene in a strain of *Penicillium chrysogenum* producing high levels of penicillin. *Mol. Gen. Genet.* **1989**, *216*, 492–497. [CrossRef]
152. Keasling, J.D. Gene-expression tools for the metabolic engineering of bacteria. *Trends Biotechnol.* **1999**, *17*, 452–460. [CrossRef] [PubMed]
153. Leonard, E.; Ajikumar, P.K.; Thayer, K.; Xiao, W.H.; Mo, J.D.; Tidor, B.; Stephanopoulos, G.; Prather, K.L.J. Combining metabolic and protein engineering of a terpenoid biosynthetic pathway for overproduction and selectivity control. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 13654–13659. [CrossRef] [PubMed]
154. Kennedy, J.; Turner, G.  $\delta$ -(L- $\alpha$ -Aminoacyl)-L-cysteiny-D-valine synthetase is a rate-limiting enzyme for Penicillin production in *Aspergillus nidulans*. *Mol. Gen. Genet.* **1996**, *253*, 189–197. [CrossRef] [PubMed]
155. Fernández-Cañón, J.M.; Peñalva, M.A. Overexpression of two Penicillin structural genes in *Aspergillus nidulans*. *Mol. Gen. Genet.* **1995**, *246*, 110–118. [CrossRef]

156. Malla, S.; Niraula, N.P.; Liou, K.; Sohng, J.K. Enhancement of doxorubicin production by expression of structural sugar biosynthesis and glycosyltransferase genes in *Streptomyces peucetius*. *J. Biosci. Bioeng.* **2009**, *108*, 92–98. [[CrossRef](#)]
157. Malla, S.; Niraula, N.P.; Liou, K.; Sohng, J.K. Self-resistance mechanism in *Streptomyces peucetius*: Overexpression of *drdB*, *drdB* and *drdB* for doxorubicin enhancement. *Microbiol. Res.* **2010**, *165*, 259–267. [[CrossRef](#)]
158. Yihua, C.; Smanski, M.J.; Shen, B. Improvement of secondary metabolite production in *Streptomyces* by manipulating pathway regulation. *Appl. Microbiol. Biotechnol.* **2010**, *86*, 19–25.
159. Yihua, C.; Wendt-Pienkowski, E.; Shen, B. Identification and utility of FdmR1 as a *Streptomyces* antibiotic regulatory protein activator for fredericamycin production in *Streptomyces griseus* ATCC 49344 and heterologous hosts. *J. Bacteriol.* **2008**, *190*, 5587–5596.
160. Lombo, F.; Brana, A.F.; Mendez, C.; Salas, J.A. The Mithramycin gene cluster of *Streptomyces argillaceus* contains a positive regulatory gene and two repeated DNA sequences that are located at both ends of the cluster. *J. Bacteriol.* **1999**, *181*, 642–647. [[CrossRef](#)]
161. Ryu, Y.-G.; Butler, M.J.; Chater, K.; Lee, K.J. Engineering of primary carbohydrate metabolism for increased production of actinorhodin in *Streptomyces coelicolor*. *Appl. Environ. Microbiol.* **2006**, *72*, 7132–7139. [[CrossRef](#)]
162. Zha, W.; Rubin-Pitel, S.B.; Shao, Z.; Zhao, H. Improving cellular malonyl-CoA level in *Escherichia coli* via metabolic engineering. *Metab. Eng.* **2009**, *11*, 192–198. [[CrossRef](#)]
163. Kajiwara, S.; Fraser, P.D.; Kondo, K.; Misawa, N. Expression of an exogenous isopentenyl diphosphate isomerase gene enhances isoprenoid biosynthesis in *Escherichia coli*. *Biochem. J.* **1997**, *324*, 421–426. [[CrossRef](#)]
164. Kim, S.W.; Keasling, J.D. Metabolic engineering of the non-mevalonate isopentenyl diphosphate synthesis pathway in *Escherichia coli* enhances lycopene production. *Biotechnol. Bioeng.* **2001**, *72*, 408–415. [[CrossRef](#)]
165. Martin, V.J.J.; Pitera, D.J.; Withers, S.T.; Newman, J.D.; Keasling, J.D. Engineering a mevalonate pathway in *Escherichia coli* for the production of terpenoids. *Nat. Biotechnol.* **2003**, *21*, 796–802. [[CrossRef](#)]
166. Thykaer, J.; Nielsen, J.; Wohlleben, W.; Weber, T.; Gutknecht, M.; Lantz, A.E.; Stegmann, E. Increased glycopeptide production after overexpression of shikimate pathway genes being part of the Balhimycin biosynthetic gene cluster. *Metab. Eng.* **2010**, *12*, 455–461. [[CrossRef](#)]
167. Lomovskaya, N.; Otten, S.L.; Doi-Katayama, Y.; Fonstein, L.; Liu, X.-C.; Takatsu, T.; Inventi-Solari, A.; Filippini, S.; Torti, F.; Colombo, A.L.; et al. Doxorubicin overproduction in *Streptomyces peucetius*: Cloning and characterization of the *dnrU* ketoreductase and *dnrV* genes and the *doxA* cytochrome P-450 hydroxylase gene. *J. Bacteriol.* **1999**, *181*, 305–318. [[CrossRef](#)]
168. Chemler, J.A.; Fowler, Z.L.; McHugh, K.P.; Koffas, M.A.G. Improving NADPH availability for natural product biosynthesis in *Escherichia coli* by metabolic engineering. *Metab. Eng.* **2010**, *12*, 96–104. [[CrossRef](#)]
169. Komatsu, M.; Uchiyama, T.; Omura, S.; Cane, D.E.; Ikeda, H. Genome-minimized *Streptomyces* host for the heterologous expression of secondary metabolism. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 2646–2651. [[CrossRef](#)]
170. Hopwood, D.; Malpartida, F.; Kieser, H.M.; Ikeda, H.; Duncan, J.; Fujii, I.; Rudd, B.A.M.; Floss, H.; Omura, S. Production of ‘hybrid’ antibiotics by genetic engineering. *Nature* **1985**, *314*, 642–644. [[CrossRef](#)] [[PubMed](#)]
171. de Boer, A.L.; Schmidt-Dannert, C. Recent efforts in engineering microbial cells to produce new chemical compounds. *Curr. Opin. Chem. Biol.* **2003**, *7*, 273–278. [[CrossRef](#)] [[PubMed](#)]
172. Punt, P.J.; van Biezen, N.; Conesa, A.; Albers, A.; Mangnus, J.; van den Hondel, C. Filamentous fungi as cell factories for heterologous protein production. *Trends Biotechnol.* **2002**, *20*, 200–206. [[CrossRef](#)]
173. Mackenzie, D.A.; Wongwathanarat, P.; Carter, A.T.; Archer, D.B. Isolation and use of a homologous histone H4 promoter and a ribosomal DNA region in a transformation vector for the oil-producing fungus *Mortierella alpine*. *Appl. Environ. Microbiol.* **2000**, *66*, 4655–4661. [[CrossRef](#)] [[PubMed](#)]
174. Royer, J.C.; Moyer, D.L.; Yoder, W.; Shuster, J.R. *Patent Application WO 96/00787*; World Intellectual Property Organization: Geneva, Switzerland, 1996.
175. Berka, R.M.; Feng, X.U.; Thompsom, S.A. *World Patent Application WO 95/15391*; World Intellectual Property Organization: Geneva, Switzerland, 1995.
176. Emalfarb, M.A.; Punt, P.J.; van Zeijl, C.; van den Hondel, C. *World Patent Application WO 00/20555*; World Intellectual Property Organization: Geneva, Switzerland, 2000.
177. Chen, B.-X.; Wei, T.; Ye, Z.-W.; Yun, F.; Kang, L.-Z.; Tang, H.-B.; Guo, L.-Q.; Lin, J.-F. Efficient CRISPR-Cas9 gene disruption system in edible-medicinal mushroom *Cordyceps militaris*. *Front. Microbiol.* **2018**, *9*, 1157. [[CrossRef](#)]
178. Chen, B.-X.; Xue, L.-N.; Wei, T.; Ye, Z.-W.; Li, X.-H.; Guo, L.-Q.; Lin, J.-F. Enhancement of Ergothioneine production by discovering and regulating its metabolic pathway in *Cordyceps militaris*. *Microb. Cell Fact.* **2022**, *21*, 169. [[CrossRef](#)]
179. Wang, H.N.; Ke, X.; Zhou, J.P.; Liu, Z.Q.; Zheng, Y.G. Recent advances in metabolic regulation and bioengineering of gibberellic acid biosynthesis in *Fusarium fujikuroi*. *World J. Microbiol. Biotechnol.* **2022**, *38*, 131. [[CrossRef](#)]
180. Iyyappan, J.; Bharathiraja, B.; Baskar, G.; Kamalanaban, E. Process optimization and kinetic analysis of malic acid production from crude glycerol using *Aspergillus niger*. *Bioresour. Technol.* **2019**, *281*, 18–25. [[CrossRef](#)]
181. Kallscheuer, N. Engineered microorganisms for the production of food additives approved by the European Union—A systematic analysis. *Front. Microbiol.* **2018**, *9*, 1746. [[CrossRef](#)]
182. Tramontin, L.R.R.; Kildegaard, K.R.; Sudarsan, S.; Borodina, I. Enhancement of astaxanthin biosynthesis in oleaginous yeast *Yarrowia lipolytica* via the microalgal pathway. *Microorganisms* **2019**, *7*, 472. [[CrossRef](#)]

183. Sun, X.; Su, X. Harnessing the knowledge of protein secretion for enhanced protein production in filamentous fungi. *World J. Microbiol. Biotech.* **2019**, *35*, 54. [[CrossRef](#)] [[PubMed](#)]
184. Chankhamjon, P.; Boettger-Schmidt, D.; Scherlach, K.; Urbansky, B.; Lackner, G.; Kalb, D.; Dahse, H.M.; Hoffmeister, D.; Hertweck, C. Biosynthesis of the halogenated mycotoxin aspirochlorine in koji mold involves a cryptic amino acid conversion. *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 13409–13413. [[CrossRef](#)] [[PubMed](#)]
185. Bai, J.; Yan, D.; Zhang, T.; Guo, Y.; Liu, Y.; Zou, Y.; Tang, M.; Liu, B.; Wu, Q.; Yu, S.; et al. A cascade of redox reactions generates complexity in the biosynthesis of the protein phosphatase-2 inhibitor rubratoxin A. *Angew. Chem. Int. Ed. Engl.* **2017**, *56*, 4782–4786. [[CrossRef](#)]
186. Newman, A.G.; Townsend, C.A. Molecular characterization of the cercosporin biosynthetic pathway in the fungal plant pathogen *Cercospora nicotianae*. *J. Am. Chem. Soc.* **2016**, *138*, 4219–4228. [[CrossRef](#)] [[PubMed](#)]
187. Li, R.; Li, R.; Li, X.; Fu, D.; Zhu, B.; Tian, H.; Luo, Y.; Zhu, H. Multiplexed CRISPR/Cas9-mediated metabolic engineering of  $\gamma$ -aminobutyric acid levels in *Solanum lycopersicum*. *Plant Biotechnol. J.* **2018**, *16*, 415–427. [[CrossRef](#)] [[PubMed](#)]
188. Gouka, R.J.; Punt, P.J.; Van Den Hondel, C.A.M.J.J. Efficient production of secreted proteins by *Aspergillus*: Progress, limitations, and prospects. *Appl. Microbiol. Biotechnol.* **1997**, *47*, 1–11. [[CrossRef](#)] [[PubMed](#)]
189. Wakai, S.; Arazoe, T.; Ogino, C.; Kondo, A. Future insights in fungal metabolic engineering. *Bioresour. Technol.* **2017**, *245*, 1314–1326. [[CrossRef](#)]
190. Sakai, K.; Kinoshita, H.; Nihira, T. Heterologous expression system in *Aspergillus oryzae* for fungal biosynthetic gene clusters of secondary metabolites. *Appl. Microbiol. Biotechnol.* **2012**, *93*, 2011–2022. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.