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Metabolic Engineering and Synthetic Biology – Mini-Review

Towards engineering agaricomycete fungi for terpenoid production

Riccardo Iacovelli[1], Dominik Mojzita[1], Peter Richard[1], Yvonne Nygård[1,2]

[1]VTT Technical Research Centre of Finland, 02150 Espoo, Finland
[2]Chalmers University of Technology, Department of Life Sciences, 412 96 Gothenburg, Sweden
Correspondence should be addressed to: Riccardo Iacovelli at riccardo.iacovelli@vtt.fi

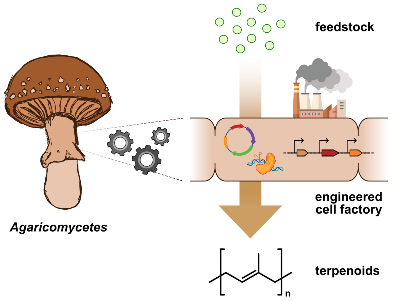
Abstract: Since ancient times, humans have harnessed the vast metabolic abilities of fungi to produce food, beverages, and medicines. Biotechnology and genetic engineering have opened new avenues to tailor and enhance these abilities, transforming fungi into powerful industrial workhorses.

In this minireview, we focus on the biotechnological potential of *Agaricomycetes*, a class of basidiomycete fungi that includes the so-called 'true' mushrooms. Although many species are widely used in the food sector, their broader potential in biotechnology remains largely untapped. These fungi naturally produce a diverse array of metabolites with promising applications across various industries. Here, we highlight their ability to synthesize a wide range of terpenoids, many unique to this taxon, and we present recent advancements in genomics and genetic engineering tools developed for *Agaricomycetes*. We anticipate that continued progress in tailored genetic engineering tools and improved cultivation technologies will facilitate the establishment of these fungi as robust cell factories for producing valuable terpenoids, with significant contributions to the food, biotech, and pharmaceutical sectors.

One-Sentence Summary: This minireview highlights the potential of mushroom-forming fungi to be engineered into cell factories for producing terpenoids—valuable compounds with diverse applications in food, medicine, and biotechnology.

Keywords: *Agaricomycetes*, Terpenoids, Biotechnology, Mushrooms, Cell factories

Graphical abstract



Introduction

Fungi were among the first complex organisms to make land, and it is now a broadly accepted view that they helped plants establish themselves in terrestrial habitats and essentially kickstart life as we know it (Jermy, 2011). Throughout our planet's history, fungi have thrived in all Earth's ecosystems. Therefore, it is not surprising that our relationship with them is thousands of years old. Microscopic fungi like baker's yeast and moulds (typically belonging to the *Ascomycota* division) have been used for centuries to produce food and beverages (Money, 2016). Modern fungal biotechnology was born in the 20th century, when food chemist James Currie pioneered a process to produce citric acid with the common mould *Aspergillus niger* (Cairns et al., 2018). Since then, ascomycete fungi have exponentially gained traction and became established industrial cell factories used to produce enzymes, food additives, pharmaceuticals, and more (Nielsen & Nielsen, 2017; Punt et al., 2002; Yang et al., 2017).

Macroscopic fungi from the *Basidiomycota* division have been equally important to mankind. Notably, nearly all edible mushrooms (aside from morels and truffles) belong to the class *Agaricomycetes* within this division. Besides their value as food, agaricomycete fungi are particularly attractive from a biotechnological perspective due to other features such as their unmatched ability to degrade lignin. Furthermore, *Agaricomycetes* produce a plethora of hydrolytic enzymes with numerous applications in industry (Janusz et al., 2017) and they can also be used to produce packaging and even construction materials (Meyer et al., 2020). Lastly, a wide variety of bioactive metabolites have been isolated from their fruiting bodies, in particular terpene and terpenoid compounds that are unique to *Agaricomycetes* (Gressler et al., 2021;

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Schmidt-Dannert, 2015). However, heterologous production hosts are often required to access valuable metabolites and enzymes from *Agaricomycetes*. Indeed, many mushroom species form symbiotic relationships with specific host trees (Sharma, 2017) and cannot be readily cultivated in laboratory conditions, nor genetically modified. Unfortunately, heterologous production of *Agaricomycetes* metabolites in well-established ascomycete hosts has so far proven very challenging. This may be due to differences in gene structure and genetic regulatory elements across taxa, abundance of proteases that degrade basidiomycete enzymes, limited availability of essential precursors (e.g. due to competing metabolic pathways), and the absence of appropriate auxiliary proteins and cellular machinery required for correct processing of the products (e.g. membrane transport and secretion) (Casado López et al., 2016; Fischer et al., 2016). Thus, it would be beneficial to establish genetically amenable *Agaricomycetes* hosts and expand the currently limited set of suitable genetic tools. Universal transformation protocols and synthetic biology tools, such as CRISPR-based systems and toolkits containing easy-to-assemble genetic parts (e.g. promoters, selection markers, reporters, etc.), need to be developed. These advancements are expected to pave the way for a broader application of *Agaricomycetes* in biotechnology and related industries.

Agaricomycetes are Terpenoid Super-Producers

Fungi are incredible producers of secondary metabolites (SMs)—organic molecules not involved in primary metabolic processes such as growth or reproduction, but that play important roles in shaping interactions with other organisms and with the environment. At the time of writing, 23,040 unique SMs from fungi have been described and recorded in the Natural Product Atlas (van Santen et al., 2021). Of these, about 94% originate from fungi belonging to either *Ascomycota* (17,519, 76%) or *Basidiomycota* (4,206, 18%). In fungi, SMs are typically biosynthesized by specialized enzymes that are encoded by physically clustered sets of genes, so-called biosynthetic gene clusters (BGCs). These can be predicted directly from genetic sequences, which allows researchers to easily gauge the biosynthetic potential of an organism. That is, the specific set of BGCs that the genome encodes, which is directly correlated with the capacity of an organism to produce secondary metabolites. In accordance with the numbers mentioned above, kingdom-wide analyses suggest that the majority of the biosynthetic potential of fungi is concentrated within the two sister divisions *Ascomycota* and *Basidiomycota* (Mosunova et al., 2020; Zhang et al., 2024). However, it is worth mentioning that natural product research and fungal genomics have focused mainly on these two divisions, leaving others (e.g. *Zoopagomycota* and *Mucoromycota*) substantially under-sampled. Actual differences in abundance of BGCs across the fungal kingdom might thus be more nuanced. Even so, ascomycetes undoubtedly possess the highest number of BGCs per genome, with their biosynthetic repertoire largely represented by polyketides and non-ribosomal peptides. Instead, the genomes of basidiomycetes carry less BGCs in total, but they encode a higher number and a wider diversity of terpene BGCs (Mosunova et al., 2020; Zhang et al., 2024). This is particularly true for the *Agaricomycetes*, which produce many unique terpenoids (Table 1) (Schmidt-Dannert, 2015; Steindorff et al., 2024). While the term ‘terpenoids’ more accurately refers to oxygen-containing compounds derived from terpenes (which consist only of carbon and hydrogen atoms) (Moss et al., 1995), we use it throughout this article to collectively refer to both groups for simplicity.

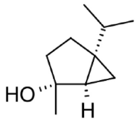
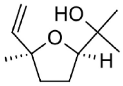
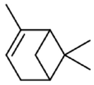
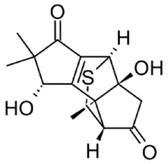
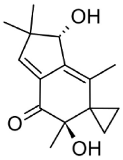
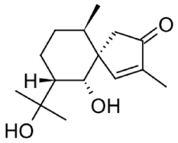
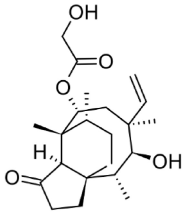
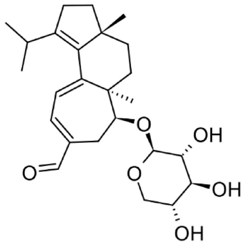
With over 80,000 known terpenoids, this is the largest and most diverse group of natural products (Luo et al., 2024). The core structure of all terpenoids derives from the condensation of the functional 5-carbon isoprene units isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). Based on the number of condensation reactions, and the resulting length of their linear precursors, terpenoids can be classified into several groups (Fig. 1). The biosynthesis of different types of terpenoids is achieved through the activity of class-specific synthases and/or cyclases such as sesquiterpene synthases, diterpene synthases, and oxidosqualene/lanosterol cyclases (Pan et al., 2024; Yang et al., 2017). These are further diversified by a variety of tailoring enzymes, such as P450 monooxygenases and reductases. Examples from all major terpenoid groups shown in Fig. 1 have been found in *Agaricomycetes*, with sesquiterpenoids (C15) being particularly abundant and diverse (Gressler et al., 2021; Schmidt-Dannert, 2015; Wang et al., 2021). Humulyl-derived bioactive sesquiterpenoids (such as hirsutane and illudanes) from *Agaricomycetes* are an especially attractive group because they show potent anti-inflammatory, cytotoxic, antiviral, and antibacterial activities (Schmidt-Dannert, 2015). In addition, several bioactive meroterpenoids have been isolated from *Agaricomycetes*, including medicinal fungi such as *Ganoderma* and *Hericium* (Friedman, 2015; Li et al., 2015; Niu et al., 2006; Wang et al., 2020). Meroterpenoids are diverse molecules with a partial terpenoid structure, and in agaricomycete fungi they are often functionalized phenolics and orsellinic acid derivatives, such as hericenones and erinacerins (Friedman, 2015), terreumol (Yin et al., 2013), and cloquetin (Braesel et al., 2017). Unique cytotoxic and anti-inflammatory lanostane-triterpenoids (C30) such as ganorbiformins (Isaka et al., 2013), ganoderic acids (Paterson, 2006), and inotodiol (Ma et al., 2013) have also attracted considerable attention. Lastly, ectomycorrhizal species of the genus *Lactarius* have long been known to produce large amounts of cis-polyisoprene, a natural rubber with similar properties to that extracted from the rubber tree (Ohya et al., 1998; Tanaka et al., 1994).

Interestingly, most building blocks necessary for the construction of terpenoid compounds in *Agaricomycetes* ultimately derive from the central metabolite acetyl-CoA. While secondary metabolism in ascomycetes channels acetyl-CoA almost entirely to polyketides biosynthesis routes, agaricomycete fungi instead redirect it to terpene synthesis (Mosunova et al., 2020; Schmidt-Dannert, 2015; S. Zhang et al., 2024). This suggests that—though both *Ascomycota* and *Basidiomycota* share central metabolic routes and possess abundant pools of acetyl-CoA—they evolved different chemical arsenals to mediate interactions which are specific to their ecological niches. *Agaricomycetes* are therefore specialized terpenoid producers, which makes them particularly attractive for the biotechnological production of bioactive terpenoids and terpenoid-based materials.

Genetic Engineering of *Agaricomycetes*

Several methods for genetic transformation of fungi have been established, the most common being PEG-mediated (PMT) (Case et al., 1979), electroporation-mediated (EMT) (Chakraborty & Kapoor, 1990), and *Agrobacterium*-mediated transformation (AMT) (Michielse et al., 2008). PMT requires the enzymatic digestion of the cell wall to produce fungal protoplasts. Polyethylene glycol and divalent cations (typically Ca^{2+}) are then used to destabilize the plasma membrane of the protoplasts and facilitate DNA uptake. Electroporation employs rapid electric pulses to create temporary pores in the plasma membrane, although the exact

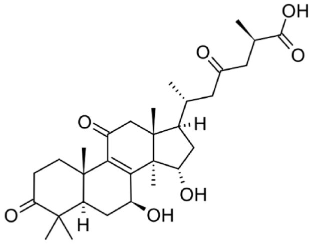
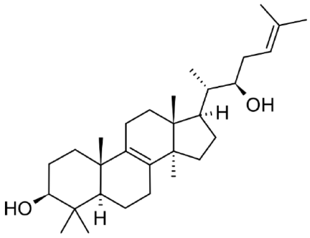
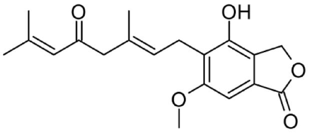
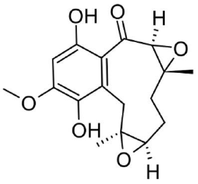
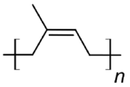
Table 1. Examples of Terpenoids Produced by *Agaricomycetes* (Compounds Unique to This Taxon in Bold) With Applications in the Food, Pharmaceutical, and Chemical Industries

Name	Chemical structure	Type	Producer organisms	Applications/bioactivities
4-thujanol		C10	*Amanita ovoidea* (Rapior et al., 1996)	Flavour and fragrance (Bhatia et al., 2008)
Cis-linalool oxide		C10	*Clitocybe nebularis* (Rapior et al., 1996)	Flavour and fragrance (Maróstica & Pastore, 2007)
α-pinene		C10	*Amanita* spp. (Breheret et al., 1997)	Anti-inflammatory, antimicrobial, cytotoxic (Salehi et al., 2019)
Hirsutenol F		C15	*Stereum hirsutum* (Yoo et al., 2006)	Antioxidant (Yoo et al., 2006)
Illudin M		C15	*Omphalotus olearius* (Wawrzyn et al., 2012)	Cytotoxic (Schobert et al., 2011)
Flammuspirone C		C15	*Flammulina velutipes* (Tao et al., 2016)	Antidiabetic, statin (Tao et al., 2016)
Pleuromutilin		C20	*Clitopilus* spp. (Hartley et al., 2009)	Antibiotic (Hartley et al., 2009)
Erinacine A		C20	*Hericium erinaceus* (Friedman, 2015)	Antioxidant, cytotoxic, neuro-protective (Friedman, 2015)

mechanisms underlying DNA uptake during EMT are poorly understood. AMT makes use of the native ability of the soil bacterium *Agrobacterium tumefaciens* to transfer DNA into wounded plant cells. Domesticated strains of *Agrobacterium* are used for AMT in combination with modified plasmids that promote DNA transfer into fungal cells (Fig. 2). Physical methods such as biolistic DNA delivery (Lorito et al., 1993) and shock-wave–mediated transformation (Magaña-Ortíz et al., 2013) have also been reported for some ascomycetes.

The development of efficient genetic transformation systems in *Agaricomycetes* has proven more challenging compared to ascomycetes. Nonetheless, a few agaricomycete fungi have been genetically manipulated, including the well-studied *Ganoderma lucidum*, *Pleurotus ostreatus*, and *Schyzophyllum commune* (Salame et al., 2012; L. Sun et al., 2001; Van Peer et al., 2009). EMT, PMT, and AMT have all been used in *Agaricomycetes*, although these methods typically need to be extensively optimized and tailored to each specific species. This is likely due to several factors, such as key differences in the composition of the fungal cell wall compared to ascomycetes (Gow et al., 2017) and heterokaryosis (Auxier et al., 2022). Each transformation method has benefits and drawbacks: EMT is a fast method, but it requires extensive parameter optimization and larger numbers of recipient cells, due to substantial cell death caused by the high voltages applied (Hu et al., 2014). AMT can be used with protoplasts or mycelia obtained from different starting material, such as germinating spores (Ford et al., 2016), mycelial pellets (Hanif et al., 2002), and fruiting body fragments (Shi et al., 2017). AMT generally yields genetically

Table 1. Continued

Name	Chemical structure	Type	Producer organisms	Applications/bioactivities
Ganoderic acid A		C30	*Ganoderma lucidum* (Paterson, 2006)	Anticancer (Jia et al., 2023)
Inotodiol		C30	*Inonotus obliquus* (L. Ma et al., 2013)	Anticancer, antiviral, anti-inflammatory, anti-allergic (Maza et al., 2021)
Hericenone A		MT[a]	*Hericium erinaceus* (Friedman, 2015)	Cytotoxic (B.-J. Ma et al., 2010)
Terreumol A		MT[a]	*Tricholoma terreum* (Yin et al., 2013)	Cytotoxic (Yin et al., 2013)
Cis-polyisoprene		Cn[a]	*Lactarius volemus* (Ohya et al., 1998)	Natural rubber

[a] MT = meroterpenoids; Cn = polyisoprenes.

stable transformants, but it is severely limited by complex and long workflows. Furthermore, its success can depend on many variables such as co-culturing conditions and the choice of an appropriate *Agrobacterium* strain (Li et al., 2017). PMT, on the other hand, is cheap, has good transformation efficiencies, and follows rather standard procedures. Therefore, it is often the first approach that is attempted for the transformation of new fungal species. Furthermore, PMT enables CRISPR-based genome engineering using pre-assembled ribonucleoparticles (RNPs) that can be delivered directly into the fungal protoplasts (Boontawon, Nakazawa, Xu, et al., 2021). PMT is nonetheless unfortunately limited to the species for which protoplasts can be generated (Table 2). Most of the successful examples of genetic transformation in *Agaricomycetes* reported the use of protoplasts as recipient cells. The main advantage of using protoplasts is their lack of cell wall, which enables more efficient uptake of exogenous DNA. Indeed, protoplasts have also been used in combination with other DNA delivery methods. For example, protoplasts of *Agaricus bisporus* (van de Rhee et al., 1996), *Ganoderma lucidum* (Sun et al., 2001), and *Flammulina velutipes* (Kim et al., 2010) have been successfully engineered via electroporation. Lastly, AMT of protoplasts has been reported in *F. velutipes* (Shi et al., 2017) and *Hypsizygus marmoreus* (Zhang et al., 2014). However, determining the right conditions and developing protocols to generate protoplasts of new species can be tedious, and commercially available cell-wall degrading enzymes often prove ineffective. Therefore, expanding our knowledge of cell wall biology in *Agaricomycetes* is essential to identify key factors for protoplast formation. This will lead to the development of universal tools and procedures, enabling efficient genetic transformation across multiple agaricomycete species (Li et al., 2017).

Latest Advancements in Genomics and CRISPR-Based Genome Editing of *Agaricomycetes*

Genetic engineering of fungi (and of organisms in general) to study gene function or to enable biotech applications requires technologies that allow precise manipulation of DNA sequences. These include, for example, targeted deletions of genes of interest (Santiago et al., 2008), functional expression of heterologous genes, or selected modifications of native metabolic pathways (Cho et al., 2022). These technologies depend, first and foremost, upon the availability of high-quality genome sequencing data. Fortunately, dramatic reductions in costs and technological advancements in the last decade—particularly the development and spread of long-read sequencing platforms—have made it relatively easy to obtain complete high-quality assemblies of many fungal genomes (Agustinho et al., 2024). The number of genomes in the JGI MycoCosm database alone has multiplied tenfold in the past 10 years, reaching 2,650 at the start of 2025 (Grigoriev et al., 2014). Combined with those in the NCBI genome database (www.ncbi.nlm.nih.gov/genome), a total of 23,105 fungal genomes are available at the time of writing (Table 3).

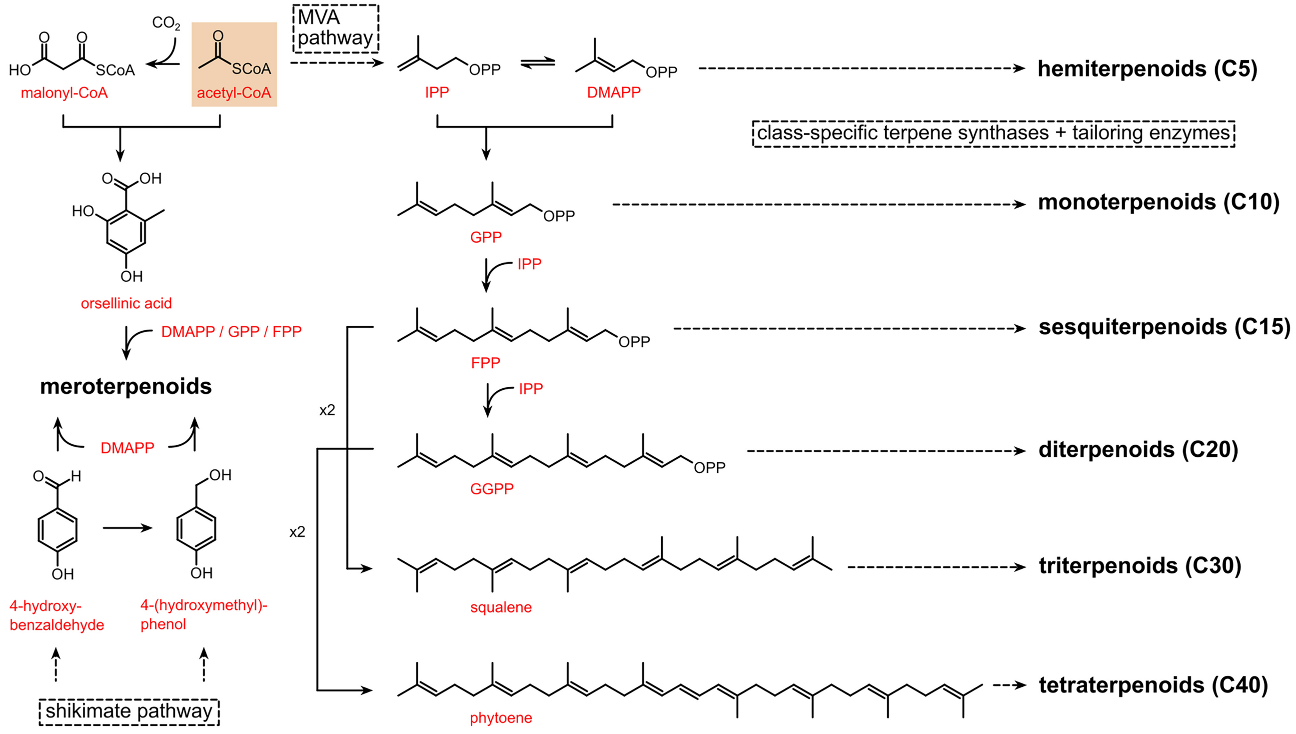


Fig. 1. Major biosynthetic routes of terpenoids in *Agaricomycetes*. Acetyl-CoA is the central precursor for terpenoids in these fungi: it is used for the biosynthesis of the scaffold of polyketide-derived meroterpenoids, and to generate isoprene units IPP and DMAPP through the mevalonate (MVA) pathway. *Note.* IPP = isopentenyl pyrophosphate; DMAPP = dimethylallyl pyrophosphate; GPP = geranyl pyrophosphate; FPP = farnesyl pyrophosphate; GGPP = geranylgeranyl pyrophosphate.

Table 2. Protoplast Preparation Conditions for Agaricomycete Fungi

Species	**Starting material**	**Lytic enzyme(s)**[a]	**Digestion buffer**	**Protoplasts yield**	**Ref.**
Pleurotus ostreatus	Mycelium	2% lysing enzymes from *T. harzianum*, 0.05% chitinase from *T. viride*	0.5 M sucrose	5×10^7/mL[b]	Salame et al. (2012)
Ganoderma lucidum	Mycelium	1.5% lywallzyme	0.6 mannitol, 100 mM Na citrate	5×10^7–5×10^8/mL[b]	Sun et al. (2001)
Schizophyllum commune	Mycelium	0.15% lysing enzymes[c]	1 M $MgSO_4$,10 mM malate	1×10^8/mL[b]	Van Peer et al. (2009)
Coprinopsis cinerea	Mycelium or oidia	2% Cellulase Onozuka R-10, 0.1% chitinase	0.5 M mannitol, 50 mM maleate	1–5 $\times 10^8$/mL	Binninger et al. (1987)
Coprinellus congregatus	Mycelium	0.2% Novozyme 234	0.5 M $MgSO_4$, 50 mM maleate	1.25×10^8/mL[b]	Leem et al. (1999)
Ceriporiopsis subvermispora	Mycelium	0.25% lysing enzymes from *T. harzanium*, 0.5% zymolase, 0.1% chitinase	0.5 M mannitol, 25 mM maleate	2×10^7/mL[b]	Honda et al. (2019)
Clitopilus passeckerianus	Mycelium	5% lysing enzymes from *T. harzanium*	1 M $MgSO_4$, 0.6 M phosphate	1×10^8/mL[b]	Kilaru et al. (2009)
Trametes versicolor	Mycelium	0.2% Novozyme 234	0.5 M $MgSO_4$, 50 mM maleate	n/a	Kim et al. (2002)
Lentinula edodes	Mycelium	2.5% cellulase Onozuka RS, 0.1 % chitinase	0.6 mannitol, 50 mM succinate	1×10^8 per 1 g wet biomass	Irie et al. (2003)
Cyclocybe aegerita	Oidia	0.1% lysing enzymes from *T. harzianum,* 0.1% yatalase	1.2 M $MgSO_4$, 50 mM maleate	0.9–1.2 $\times 10^8$/mL[b]	Herzog et al. (2019)
Ganoderma multipileum	Mycelium	0.75% lysing enzymes from *T. harzianum*	0.6 M sucrose in K Phosphate[d]	3×10^7/mL	Chou & Tzean (2016)
Pleurotus eryngii	Mycelium	3% yatalase, 4.5% lysing enzymes from *T. harzianum*	1.2 M $MgSO_4$, 10 mM Na Phosphate	n/a	Wang et al. (2021)
Dichomitus squalens	Mycelium	2% yatalase	0.6 M $MgSO_4$, 50 mM maleate	2×10^6 per 1 g wet biomass	Daly et al. (2017)

[a] Concentration of lytic enzymes in digestion buffer is expressed in weight/volume % (10 mg/mL = 1%).
[b] Protoplasts yield not directly reported. Numbers shown refer to the concentration of protoplasts used for each PEG-mediated transformation.
[c] Organism of origin of lytic enzymes not specified by source institution.
[d] Concentration of buffering agent potassium phosphate not specified.

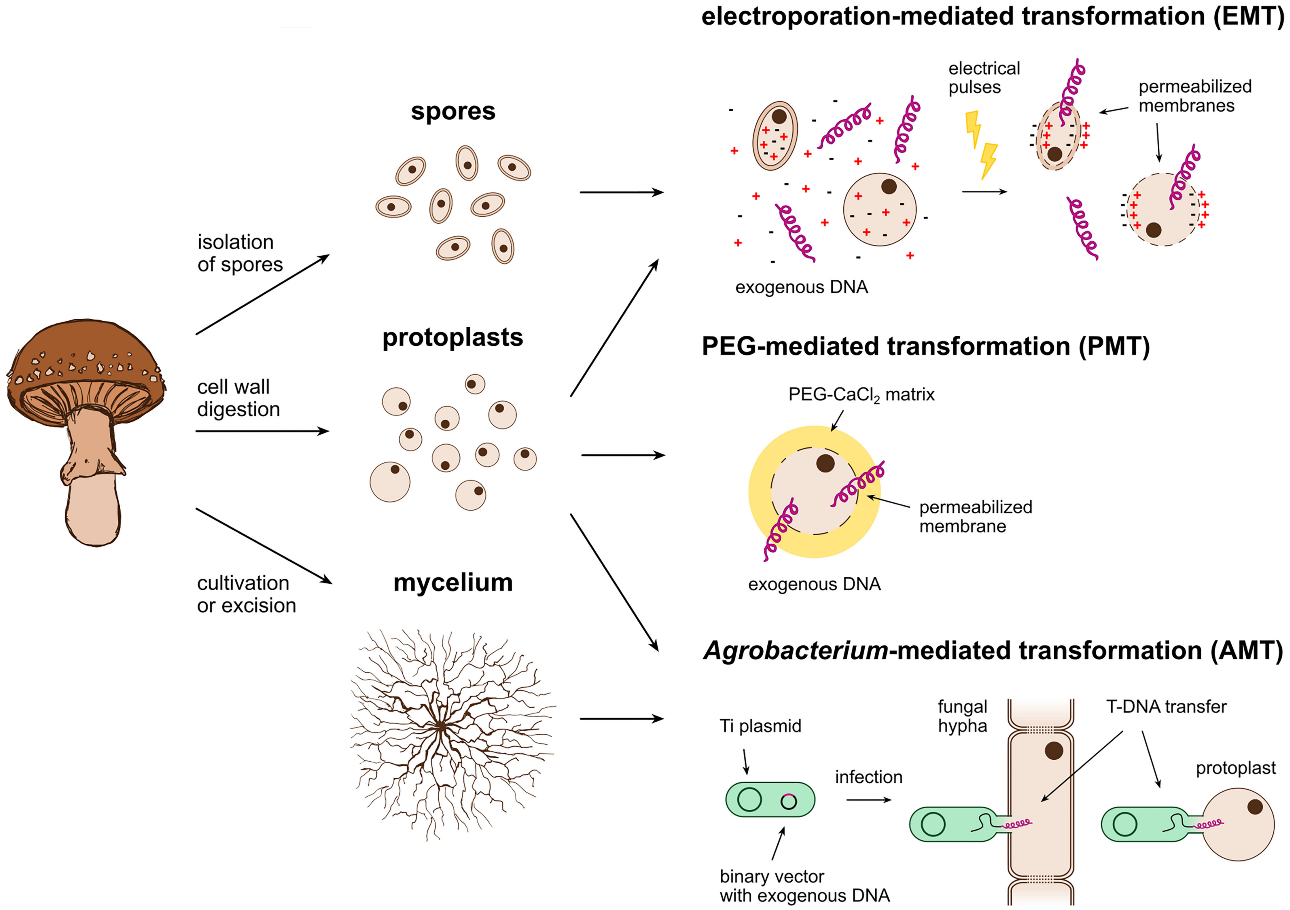


Fig. 2. Established methods for genetic transformation of *Agaricomycetes*. Different types of fungal cells and tissues can be used as recipient material depending on the fungal species.

Table 3. Number of Fungal Genomes Available on Online Databases in 2025

Databases	Fungi	*Ascomycota*	*Basidiomycota*	(*Agaricomycetes*)	Others
MycoCosm	2,650	1,690	679	528	281
NCBI Genome	20,455	16,530	2,971	1,644	954
Total[a] (%)	23,105	18,220 (78.9 %)	3,650 (15.8 %)	2,172 (9.4 %)	1,235 (5.35 %)

[a] Eight hundred fifty-nine entries present in the NCBI database were submitted by JGI and potentially overlap.

Notably, despite ascomycetes continuing to receive the most attention, 2,172 of these genomes belong to *Agaricomycetes*, and the number keeps growing. This facilitates genetic research in these organisms and plays a critical role in bioinformatics studies aimed at deciphering biosynthetic pathways of interest. For example, high-quality genome sequences and annotations were instrumental in elucidating the complete pathways of erinacines (Liu et al., 2019) and pleuromutilin (Wen et al., 2025), identifying key enzymes in the biosynthesis of ganoderic acids (Yuan et al., 2022), and characterizing the first steps of elusive meroterpenoid-producing pathways in *Stereum* and *Hericium* species (Braesel et al., 2017; Iacovelli et al., 2024).

The availability of high-quality genomic data is also essential for the establishment of advanced genome engineering tools such as CRISPR-based systems, as accurate sequence information is required to design guide RNAs and for targeted gene modifications. The first application of CRISPR-Cas9 in an agaricomycete fungus was described in 2017 when a tailored plasmid-based system was used to knock-out the *GFP* gene in a stable *GFP* expression line of *Coprinopsis cinerea* (Sugano et al., 2017). In plasmid-based systems, the codon-optimized Cas9 and guide RNA(s) genes are typically placed under the control of strong promoters on the same vector and co-expressed in vivo (Boontawon, Nakazawa, Inoue, et al., 2021; Nakazawa et al., 2022). Alternatively, the Cas9 system can be delivered via pre-assembled RNPs directly into the fungal protoplasts (Boontawon, Nakazawa, Choi et al., 2023). In both cases, Cas9 needs to be fused to an appropriate nuclear localization signal so that it can effectively localize into the nuclei to access the genomic target (Pohl et al., 2016). Typically, the use of pre-assembled RNPs leads to faster results thanks to their immediate cleavage activity upon transformation. A further advantage of RNPs-based approaches is that they do not require selection pressure to be maintained to ensure that Cas9 remains active at sufficient levels, as plasmid-based systems do. Additionally, continuous plasmid-based expression of Cas9 can increase toxicity and off-target activity. These unwanted side effects are considerably lower with the use of Cas9 RNPs, which are quickly degraded during natural protein turnover (Wang & Coleman, 2019).

CRISPR-Cas systems have been developed for several *Agaricomycetes*, including multiple species of *Ganoderma* (Qin et al., 2017; Tu et al., 2021) and *Pleurotus* (Boontawon et al., 2023; Wang et al., 2021), *Lentinula edodes* (Kamiya et al., 2023), *Agaricus bisporus* (Choi et al., 2023), and *Schyzophyllum commune* (Jan Vonk et al., 2019). CRISPR-based techniques offer a promising solution to improve the efficiency of targeted gene deletions and insertions, which are typically inefficient in agaricomycete fungi due to their strong preference towards non-homologous end joining (NHEJ) over homologous recombination (HR) as DNA repair mechanism (Kim et al., 2015). For example, CRISPR-Cas has successfully been used for the targeted disruption of the orotidine-5′-monphosphate decarboxylase-encoding *pyrG* gene in several species (Boontawon, Nakazawa, Inoue, et al., 2021; Eom et al., 2023; Liu et al., 2022). The resulting strains are auxotrophic for uracil and uridine, while also exhibiting resistance to 5-fluoroorotic acid. This dual positive/negative selection system has been widely applied in the genetic engineering of ascomycete fungi, and it is particularly useful as it enables marker recycling and sequential transformations through the use of 'self-excising' cassettes (Maruyama & Kitamoto, 2008). Establishing this system in *Agaricomycetes* will undoubtedly facilitate genetic engineering efforts in these fungi. CRISPR systems can also be used to generate NHEJ-deficient strains by deleting genes that encode for NHEJ-associated DNA repair proteins, such as the *ku70* homolog (Tu et al., 2021), thereby increasing the probability of HR-mediated events and success rate of targeted modifications. Combining such host strains with CRISPR-based tools in subsequent transformation rounds can yield remarkably high editing efficiencies (Jan Vonk et al., 2019; Tu et al., 2021). For example, a Δ*ku70* mutant of *Ganoderma lucidum* showed 96.3 and 93.1% frequencies of targeted gene insertion and replacement, respectively, using a *ura3* (*pyrG* homolog) expression cassette as donor DNA (Tu et al., 2021).

Agaricomycetes as Cell Factories

So far, most examples of genetic engineering in *Agaricomycetes* concern proof-of-concept studies, for example insertion of a selection marker cassette alone or expression of reporter genes such as GFP (Burns et al., 2005; Ford et al., 2016; Sun et al., 2001). Such studies are particularly useful to provide crucial insights into the mechanisms of gene expression. Notably, it has been shown that the presence of introns may be necessary for the successful expression of genes of interest in several *Agaricomycetes* (Burns et al., 2005; Ford et al., 2016; Lugones et al., 1999). Currently, only a limited number of studies have demonstrated successful engineering of *Agaricomycetes* to overproduce enzymes or other valuable metabolites (Table 4). These efforts have primarily focused on hydrolytic enzymes such as laccases and cellulases, which have applications across several industries (e.g. textile, food, and paper). More recently, the first examples of engineering *Agaricomycetes* for terpenoid production have been reported. For instance, homologous overexpression of core mevalonate and terpenoid pathway genes has been used to generate *Ganoderma lucidum* strains that produce increased titers of ganoderic acids (Fei et al., 2019; Zhang et al., 2017). Similarly, homologous overexpression of the key pathway genes geranylgeranyl diphosphate synthase and pleuromutilin-specific cyclase in the native producer *Clitopilus passeckerianus* led to increased pleuromutilin titers of 6.9 g/L (Wen et al., 2025).

One of the main limitations to the application of higher fungi as cell factories is their complex morphology under submerged cultivation conditions typically used in industrial processes (Meyer, 2021). Fungi are major decomposers and as such they typically favour growth on substrate. When cultivated in liquid media, they often form big clumps or pellets that may have negative effects on biomass accumulation. On the other hand, some species can grow very rapidly as dispersed hyphae, which can cause the cultivation medium to become highly viscous and limit gas-liquid exchanges, and mechanically hamper fermentor parts (Cairns et al., 2019). A limited number of studies show that different morphology types might be desirable for different products. Small pellets might lead to higher production and secretion of secondary metabolites (Cairns et al., 2019), whereas dispersed growth and larger amounts of biomass might work better for the extraction of cell wall components such as glucans (Berovic, 2023). Unfortunately, the progress with characterizing and influencing cultivation morphology has been limited to industrial ascomycete hosts such as *Penicillium*, *Aspergillus,* and *Trichoderma* species. For these fungi, it has been shown that varying inoculum size, medium composition and pH, or physical parameters such as agitation and aeration rates, are all viable routes to achieve a desired morphology (Cairns et al., 2019; Meyer, 2021). So far, fermentative processes with *Agaricomycetes* have been limited to well-studied species like *Ganoderma lucidum* and *Pleurotus ostreatus*, which can grow relatively well in submerged cultivations and reach biomass yields between 20 and 40 g/L in stirred tank reactors (Berovic, 2023). These species should serve as models to enhance our understanding of how cultivation conditions impact the morphological development and productivity of *Agaricomycetes* cell factories. Genetic engineering strategies will help uncover the underlying molecular mechanisms and apply this knowledge to fine-tune morphology for specific biotechnological processes.

The Potential of *Agaricomycetes* for the Biotechnological Production of Terpenoids

The global market for terpenoids is predicted to surpass USD 1.6 billion by 2032, with a compound annual growth rate of 8.26% in the 2024–2032 period (valuemarketresearch.com/report/terpenes-market/, released in January 2025), highlighting the economic potential of these compounds. Terpenoids are used in several products, such as food and beverages, cosmetics, pharmaceuticals, and materials (Leavell et al., 2016). According to the report mentioned above, the most important terpenoids on the market are natural rubber and monoterpenoids used in the food and fragrance industries, such as limonene and linalool. These compounds are currently extracted from their natural plant sources, which limits their supply. While fermentative production can bypass this limitation, terpenoids biosynthesis pathways cannot always be reconstituted in current microbial hosts. Only a few terpenoids have been produced commercially by engineered microbes, most notably β-farnesene and artemisinic acid in engineered yeast strains (Leavell et al., 2016). In many other cases, the production yields are very low and not compatible with commercial processes (Xiao & Zhong, 2016). Additionally, many bioactive terpenoids with potential applications as pharmaceuticals can be toxic to current hosts, which limits compound discovery and greatly reduces the range of compounds that can be produced.

Agaricomycetes are naturally wired to produce commercial terpenoids in larger amounts, for example monoterpenoid aromas

Table 4. Examples of Genetically Engineered *Agaricomycetes* Cell Factories

Organism(s)	Strategy	Product(s)	Reference
Trametes versicolor	Overexpression of native laccase *cvl3* gene	Laccase	Kajita et al. (2004)
Pycnoporus cinnabarinus	Overexpression of native laccase *lac1* gene	Laccase	Alves et al. (2004)
Volvariella volvacea	Heterologous expression of multi-functional cellulase *mfc* gene from *Pomacea maculata*	Fruiting bodies yield	Zhao et al. (2010)
Coprinopsis cinerea	Overexpression of native laccase *Lcc5* gene + silencing (RNAi) of native chitinase *ChiE2* gene	Laccase	Yao et al. (2024)
Ganoderma lucidum	Overexpression of native nicotinamide mononucleotide adenyltransferase *nmnat* gene	Cellulase	Wang et al. (2020)
Ganoderma lucidum	Overexpression of native farnesyl pyrophosphate synthase *FPS* gene	Ganoderic acids	Fei et al. (2019)
Ganoderma lucidum	Overexpression of native HMG-CoA reductase *HMGR* and squalene epoxidase *SQLE* genes	Ganoderic acids	Zhang et al. (2017)
Clitopilus passeckerianus	Overexpresion of native geranylgeranyl diphosphate synthase *ple-ggpps* and diterpene cyclase *ple-cyc* genes	Pleuromutilin	Wen et al. (2025)

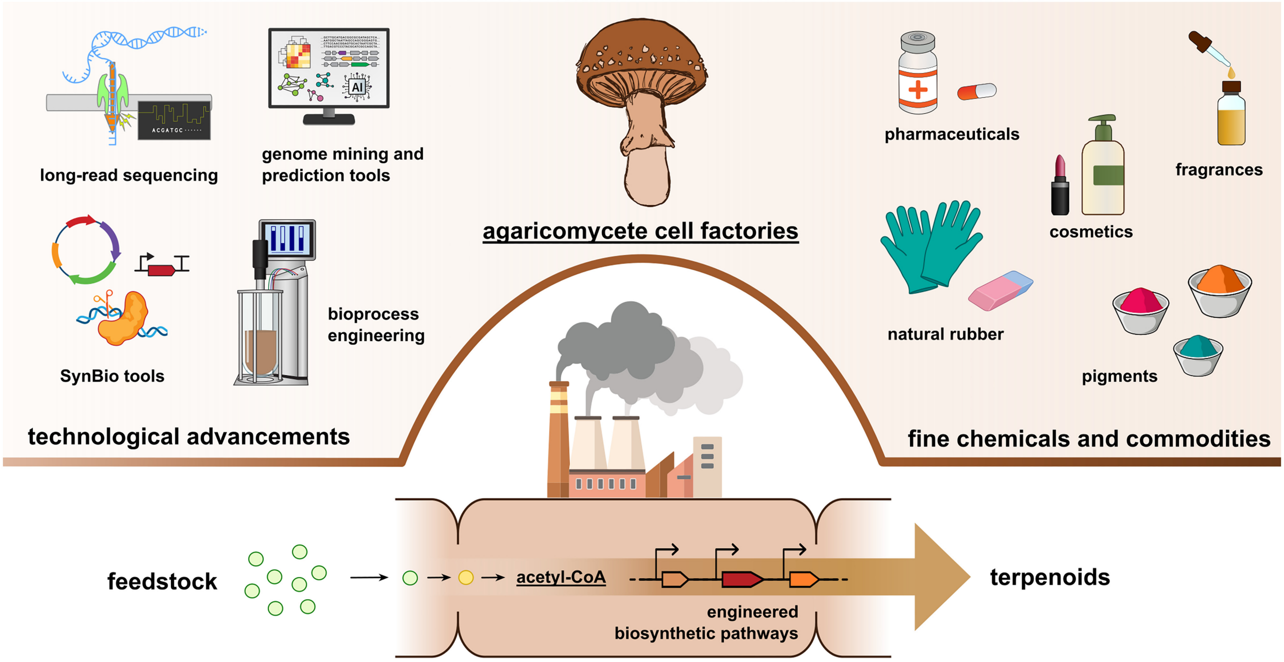


Fig. 3. Envisioned development of *Agaricomycetes*-based cell factories for terpenoid production.

(Breheret et al., 1997) and natural rubber, which can reach up to 7% of the dry weight in certain species of *Lactarius* (Tanaka et al., 1994). Although commercial-scale production of terpenoids using agaricomycete fungi has not yet been realized, small-scale studies demonstrate that certain species can produce native terpenoids in submerged cultures at yields ranging from 0.1 to 10 g/L. For example, bioreactor cultivations of *Hericium erinaceus* reached production of erinacine A of 192 ± 42 mg/L (Krzyczkowski et al., 2010). Similarly, *Ganoderma lucidum* produced up to 367 ± 17 mg/L of ganoderic acids in fed-batch fermentation (Tang & Zhong, 2002). Remarkably, an optimized fermentation process for *Clitopilus mutilus* resulted in pleuromutilin titers of 12 g/L (S. Sun et al., 2017). These examples illustrate the potential of agaricomycete fungi to achieve commercially relevant titers, while also highlighting the substantial room for improvement, which can be achieved through genetic engineering (Wen et al., 2025). Furthermore, *Agaricomycetes* produce and secrete a wide array of bioactive terpenoids, such as anticancer sesquiterpenoids of the illudane family (Schmidt-Dannert, 2015), neuroprotective, anticancer, and antioxidant diterpenes of the erinacine family (Friedman, 2015), and cytotoxic triterpenoids such as ganoderic acids (Paterson, 2006). These capabilities suggest that agaricomycete fungi possess inherent self-resistance mechanisms (Yılmaz et al., 2023) that make them ideal hosts for the discovery and production of otherwise cytotoxic compounds. Thus, it is plausible that engineered *Agaricomycetes* cell factories could serve as robust production platforms for commercially relevant terpenoids from diverse sources, including compounds unique to agaricomycetes that cannot be produced in other hosts. This would significantly advance the biotechnological production of terpenoids.

Future Directions

With the continuous advancements in genetic engineering, synthetic biology, and cultivation technologies, it may be possible to establish *Agaricomycetes* alongside existing industrial hosts as effective cell factories for the biotechnological production of various goods. *Agaricomycetes* with enhanced biodegrading abilities could be used to efficiently recycle agricultural side streams and produce medicines or food (Ahlborn et al., 2019). Their fruiting bodies are already an important part of our diet (Rizzo et al., 2021). Therefore, one could envision engineering easy-to-cultivate 'superfood mushrooms' with higher contents of essential amino acids, vitamins, and fatty acids. These mushrooms could also serve as high-quality and sustainable animal feed, carving out a more significant role in supporting global food security. Importantly, engineered *Agaricomycetes* cell factories will provide a unique platform to produce supply limited terpenoids and discover new fungal terpenoids with applications in the food, cosmetics, pharmaceutical, and biotech industries (Fig. 3). Ultimately, *Agaricomycetes* provide a promising avenue for sustainable biotechnological innovations, strengthening the role of fungi in promoting a fully circular economy.

Author Contributions

Riccardo Iacovelli: Conceptualization, Funding acquisition, Investigation, Visualization, Writing—original draft, Writing—review & editing, Project administration. Dominik Mojzita: Writing—review & editing, Peter Richard: Writing—review & editing; Yvonne Nygård: Supervision; Project administration, Writing—review & editing. All authors have given approval to the final version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability

All data underlying this article are available within the article.

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