Filamentous fungi as emerging cell factories for the production of aromatic compounds

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REVIEW

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Filamentous fungi as emerging cell factories for the production of aromatic compounds

Pavithra Umashankar[1] and Yvonne Nygård[1,2*]

Abstract

Microbial production of aromatic compounds from renewable feedstocks has gained increasing interest as a means towards sustainable production of chemicals. The potential of filamentous fungi for production of aromatic compounds has nonetheless not yet been widely exploited. Notably, many filamentous fungi can naturally break down lignin and metabolize lignin-derived aromatic compounds. A few examples where a fungal cell factory, often of *Aspergillus* spp., is used to produce an aromatic compound, typically through the conversion of one compound to another, have already been reported. In this review, we summarize fungal biosynthesis of biotechnologically interesting aromatic compounds. The focus is on compounds produced from the shikimate pathway. Biorefinery-relevant efforts for valorizing residual biomass or lignin derived compounds are also discussed. The advancement in engineering tools combined with the increasing amounts of data supporting the discovery of new enzymes and development of new bioprocesses has led to an increased range of potential production hosts and products. This is expected to translate into a wider utilization of fungal cell factories for production of aromatic compounds.

Keywords Aromatic compounds, Filamentous fungi, *Aspergillus*, Shikimate pathway, Lignin conversion, AROM, Bioconversion, Cell factory

Introduction

Aromatic compounds are widely used for diverse industrial applications, ranging from pharmaceuticals, fragrances, pigments, and flavors to bio-based polymers and specialty chemicals [1]. Traditionally aromatic compounds are derived from petroleum and their production is associated with environmental concerns and limited sustainability [2]. While some aromatic compounds can be extracted from plants, this is often hampered by low yields and expensive downstream processes [3]. Hence, there is a growing imperative to explore and establish more sustainable production processes that utilize renewable resources to produce aromatic compounds. The global aromatics manufacturing market size value was 233.6 billion US$ in 2021 and has been anticipated to reach 382.4 billion US$ by 2030 [4].

Aromatic compounds are characterized by flat, conjugated six-carbon cyclic structures. Prokaryotes, microbial eukaryotes, and plants produce aromatic compounds via the ubiquitous shikimate pathway. Some aromatic compounds such as aromatic amino acids, ubiquinone, and vitamin K are vital to all species' cellular metabolism [5]. The metabolism of aromatic compounds is, however, still not fully understood. Notably, the degradation of lignin and lignin-derived aromatic compounds has been studied quite extensively, also in filamentous fungi. Lignin is the second most abundant plant biomass on earth and comprises various aromatic molecules.

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Valorization of lignin in biorefinery efforts has gained a lot of attention but remains technically challenging [6]. So-called white-rot fungi, a heterogeneous group of the basidiomycetes division, can naturally degrade lignin into various aromatic chemicals using laccases. In addition, some brown-rot fungi have been shown to modify lignin-derived compounds, but only a few brown rot laccases have thus far been characterized [7]. Heterologous laccases have successfully been expressed in well-established cell factories such as *Trichoderma reesei* [6, 7]. While soft rot fungi of the ascomycetes division to which *T. reesei* belongs, are well-known to break down cellulose and hemi-cellulose from biomass, less is known about their potential for degrading or modifying lignin derived compounds. Microbial breakdown of lignin results in a variety of aromatic compounds. Consequently, there is a need to engineer effective microbial platforms that can channel these aromatic compounds into a target product. A few recent studies have focused on conversion of lignin derived, aromatic compounds into distinct, more valuable molecules, namely vanillic acid, methoxyhydroquinone or protocatechuic acid by *Aspergillus niger* [8, 9]. These studies showcase the potential of established fungal cell factories for aromatic compound production. Importantly, CRISPR/Cas based genome engineering has now made an increasing number of fungal species genetically accessible. This not only diversifies the range of potential production hosts but also expands the fungal product portfolio.

Filamentous fungi have long been exploited for the production of e.g. enzymes and various secondary metabolites, not the least for the production of antibiotics and other bioactive compounds. Lately, the interest in fungal pigments as natural coloring alternatives has gained increased attention. Many interesting fungal pigments are flavonoids, alkaloids, quinones or similar aromatic compounds that are derived from the shikimate pathway [10]. In addition to being used as food or textile colorants, many pigments also find applications in pharmaceuticals or cosmetics. Substantial efforts have been put into isolating fungi that naturally produce pigments, but achieving high enough yield, stability, and purity of naturally produced fungal pigments remains challenging [11]. In this review, we highlight fungal production of aromatic compounds with a focus on those derived from the shikimate pathway. Given the current interest in the biorefinery concept, we also describe conversion of aromatic compounds derived from lignin.

The shikimate pathway – the aromatic compounds synthesis route

The shikimate pathway for *de novo* synthesis of aromatic compounds is well conserved among microorganisms (Fig. 1) [12]. It is composed of seven cytosolic reactions that combine phosphoenolpyruvate (PEP) from the Embden-Meyerhof-Parnas pathway and erythrose 4-phosphate (E4P) from the pentose phosphate pathway to chorismate. The first reaction in the shikimate pathway is the condensation of E4P and PEP to generate 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP). In fungi, the DAHP synthase activity that accelerates the input reaction is under tight feedback regulation, controlled by the aromatic amino acids, namely phenylalanine, tyrosine, or tryptophan that are end products of the

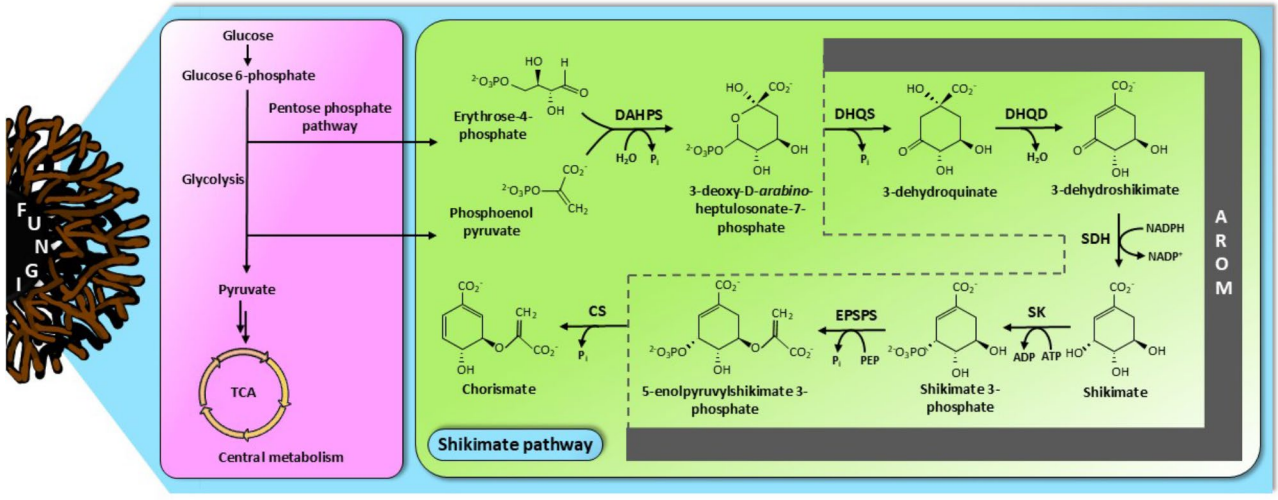

Fig. 1 Enzymes and metabolites of the shikimate pathway in fungi. The first and last step in the shikimate pathway is catalyzed by 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (DAHPS) and chorismate synthase (CS). The AROM pentafunctional polypeptide catalyzes five steps in the shikimate pathway converting 3-deoxy-D-arabino-heptulosonate-7-phosphate to 5-enolpyruvylshikimate 3-phosphate, indicated in grey. The domains of AROM are the following: 3-dehydroquinate synthase (DHQS), 3-dehydroquinate dehydratase (DHQD), shikimate dehydrogenase (SDH), shikimate kinase (SK) and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)

shikimate pathway. In *Neurospora crassa*, three DAHP synthase isoenzymes have been identified, each regulated by one of the aromatic amino acids [13]. In *Aspergillus nidulans*, two DAHP synthases have been isolated, and tyrosine was shown to be a competitive inhibitor of its aroF DAHP synthase [14].

While bacteria express separate enzymes for each reaction of the shikimate pathway, in fungi (and protists) the AROM multifunctional enzyme catalyzes five central reactions, i.e., the second to sixth step for DAHP to 5-enolpyruvylshikimate 3-phosphate (EPSP) conversion (Fig. 1). The AROM protein appears to be the result of gene fusions resulting in a mosaic pentafunctional polypeptide encoded by a single gene that is well conserved across fungal species [15]. The AROM protein of *A. nidulans* [16] (encoded by *AroM*) has been characterized and AROM homologous are found in other fungi; encoded by *aro-1* in *N. crassa*, by *ARO1* in *T. reesei* and by *FOXG_13955P0* in *Fusarium oxysporum* to name a few [17, 18]. Overexpression of the two C-terminal domains of the AROM protein [19] as well as the whole AROM protein has been demonstrated in *A. nidulans* [20, 21]. The AROM protein is recognized to be 'leaky', allowing branching at intermediate steps within the pathway [22]. Therefore, each intermediate within the shikimate pathway can also serve as a branchpoint for the biosynthesis of specialized metabolites. The shikimate pathway ends in the conversion of EPSP to chorismate, the precursor for the aromatic amino acids.

Production of shikimate pathway derived compounds

The shikimate pathway is responsible for the production of aromatic amino acids and a diverse array of other molecules. This includes precursors for essential processes e.g. isoprenoid quinones which serve important roles in electron transport and as antioxidants [23]. Aromatic amino acids are used for protein synthesis but also incorporated into co-factors, peptides, pigments, alkaloids and organic polymers. The flux through the shikimate pathway is tightly regulated, thus not typically resulting in the accumulation of high amounts of intermediates. Still, some fungi are known to accumulate shikimate pathway derived aromatic chemicals.

Trichoderma ovalisporum [24], *Penicillium griseofulvum* [25] and *Fusarium decemcellulare* [26] have been reported to produce shikimic acid. The accumulation of shikimic acid in the oyster mushroom (*Pleurotus ostreatus*) was reported to increase upon blue light stimulation, leading to increased amounts of rate-determining enzymes that resulted in increased amounts of shikimate pathway entry compounds [27]. While metabolic engineering to increase shikimate accumulation has been described for various bacteria and yeast, such attempts have to our knowledge not yet been reported for filamentous fungi. Shikimate, today extracted from certain plants or produced by genetically engineered *Escherichia coli*, can be used in the assembly of various bioactive compounds including for production of the viral neuraminidase inhibitor oseltamivir (Tamiflu®) [28]. Tamiflu® is an antiviral compound used in the prevention and treatment of Type A and Type B influenza infections [29]. Initially Tamiflu® was produced from quinic acid, a derivative of 3-dehydroquinate that is an intermediate of the shikimate pathway [30]. Quinic acid is a cyclic polyhydroxy compound used as food additive, cosolvent or as an optical material and finds various application in the medical industry [31]. Certain filamentous fungi, such as *Aspergillus* spp. and *N. crassa* can use quinic acid (or its salt, quinate) as a sole carbon source to produce aromatic amino acids through the shikimate pathway [29]. *A. niger* has been reported to produce quinic acid and caffeic acid by hydrolysis of chlorogenic acid, that is abundant in industrial by-products such as apple marc and coffee pulp [32].

Notably, several recent studies have focused on developing fungal biorefinery schemes to produce aromatic compounds from agro-industrial residues. The production of gallic acid from tannic acid by *A. niger* and *Aspergillus oryzae* was demonstrated in solid-state fermentation of soybean hull and grape pomace [33]. In this study, *A. oryzae* produced 0.36 g of gallic acid/g of tannic acid and reached a titer of 7.2 g/L in 72 h. *A. niger* was reported to produce gallic acid using lye and washing water effluents from green olive processing enriched with tannic acid [34]. Moreover, gallic acid production from tannic acid containing pomegranate peels using tannases extracted from a co-culture of *A. niger* and *Trichoderma viride* has been reported [35]. Gallic acid is typically produced through acid hydrolysis of tannic acid, which is rather costly due to low yields and purity [36]. Microbes can produce gallic acid through dehydrogenation of 3-dehydroshikimic acid [37], the precursor of shikimate. Gallic acid is an important pharmaceutical intermediate due to its potent antioxidant and anti-inflammatory activity. The current gallic acid demand exceeds 10,000 tons per year [35]. A conversion of 71.4% tannic acid to gallic acid was reported using *Aspergillus fischeri* as the cell factory [36]. *A. fischeri* produced 7.35 g gallic acid/g biomass when tannic acid was the sole carbon source [36]. *A. niger* was reported to produce 36 g/l gallic acid when supplemented with 100 g/l tannic acid [34]. *Phycomyces blakesleeanus* was reported to produce small amounts of gallic acid when grown on glucose [37]. In comparison, a production of 51.57 g/L of gallic acid with a yield of 0.45 g/g glucose and a productivity of 1.07 g/L/h was demonstrated with recombinant *E. coli* demonstrating that screening efficient pathway enzymes, balancing the carbon flux and strengthening the shikimate pathway

can lead to great improvements in efficiency [38]. Such efforts would need to be made in order to develop improved fungal cell factories for the production of aromatic chemicals and to expand the product range.

Production of chorismate and aromatic amino acid derived compounds

The shikimate pathway ends in the formation of chorismate (Fig. 1). Chorismate is the substrate of five distinct enzymes, responsible for the production of prephenate, anthranilate, aminodeoxychorismate, *p*-hydroxybenzoate and isochorismate [39] (Fig. 2). These metabolites serve as intermediates for the synthesis of folate, ubiquinone, menaquinones, salicylic acid and enterobactin, as well as aromatic amino acids such as phenylalanine, tyrosine, tryptophan [40]. Aromatic amino acids are today commercially produced through chemical synthesis or bacterial fermentation. The production strains have been developed through classical strain improvement or metabolic engineering for increased shikimate pathway flux. Such efforts have not yet been reported for fungi. Nonetheless, a recent study highlighted tyrosine production with a new isolate of *Rhizopus oryzae* [41].

Tyrosine biotransformation to 3,4-dihydroxyphenylalanine (L-Dopa) has been achieved by *A. niger* [42]. Moreover, enzymatic conversion of tyrosine to L-Dopa using immobilized tyrosinase originating from mushrooms was demonstrated in a continuous membrane reactor [43]. L-Dopa is used as a drug for Parkinson's disease [44], today commonly produced chemically, using environmentally unfriendly catalysts [43]. The biotechnological synthesis of L-Dopa using the bacterium *Erwinia herbicola* was commercialized already in 1993, but the current production scheme has been reported to be challenged by poor productivity [45].

The conversion of tryptophan to 2,3-dihydroxybenzoate and further to catechol was noted in *A. niger* [46]. Hydroxylation of salicylate to 2,3-dihydroxybenzoate was observed in *A. niger*, *A. nidulans*, and *Trichoderma lignorum* [47]. Enzymatic cleavage of the aromatic ring of catechol using catechol 1,2-dioxygenase (CrcA) to form *cis*, *cis*-muconic acid was demonstrated in *A. niger* [47]. Efficient conversion of salicylic acid into *cis*, *cis*-muconic acid through catechol as an intermediate, has been shown in *A. niger* through the concerted action of recombinant salicylate hydroxylase (ShyA) and CrcA [47]. C*is*, *cis*-muconic acid can be used for production of adipic acid and terephthalic acid, which find applications in food, medicines, cosmetics, and textiles [48]. Catechol is a commercially significant aromatic compound, used as a precursor for artificial fragrances and flavors [49].

Filamentous fungi are widely known to produce secondary metabolites using non-ribosomal peptide synthetases that condensate amino acids and other small monomers into larger bioactive compounds such as alkaloids and siderophores. Many ascomycota such as *Aspergillus*, *Fusarium*, *Penicillium* and *Trichoderma* spp. showcase a notable abundance of tryptophan and

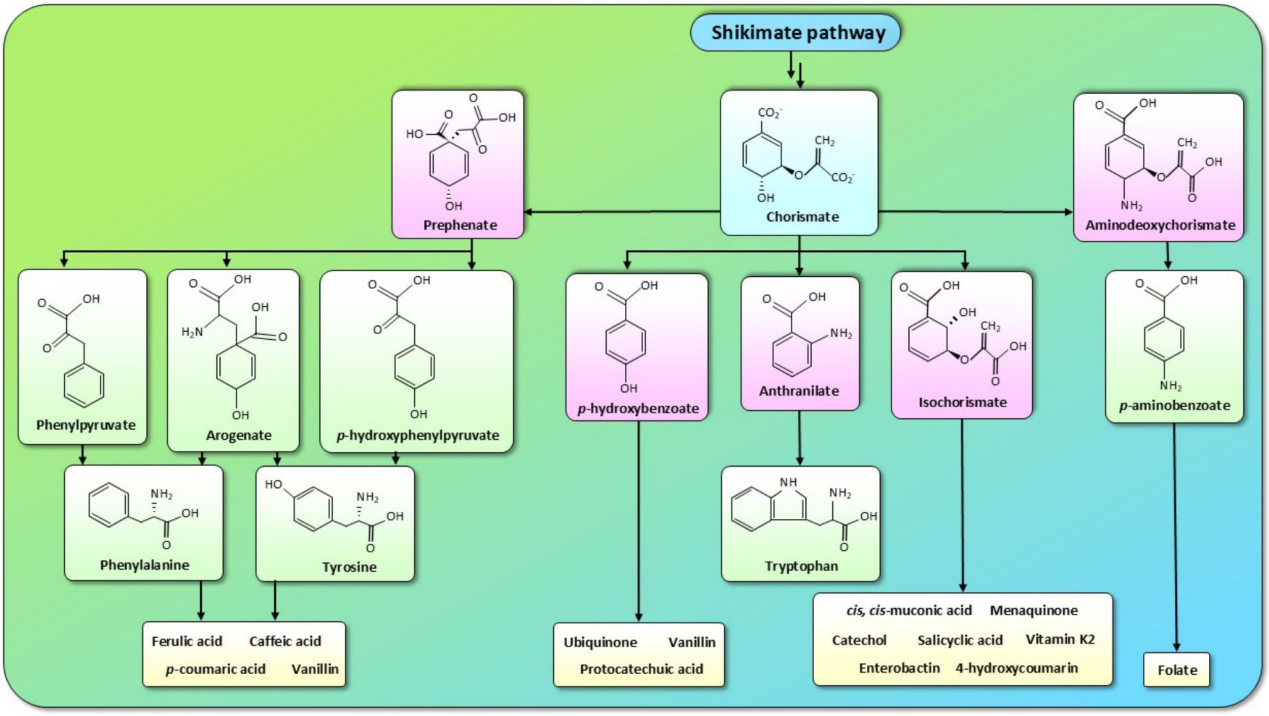

Fig. 2 Examples of fungal aromatic compounds produced from the shikimate pathway

anthranilate-derived alkaloids [50]. Chorismate also serves as the precursor for a broad array of polyketides, including macrolides and strobilurins [51]. Fungal macrolides possess antifungal or antibiotic properties and are utilized in antimicrobial therapy [52]. Strobilurins are antifungal metabolites isolated from various basidiomycota that are used in fungicides for crop protection [53]. Furthermore, chorismate is the precursor for many aromatic pigments, including various quinones, alkaloids, flavonoids and folates. For a review on pigment synthesis in filamentous fungi we refer to Kalra et al. 2020 [11]. Recently, several start-ups focused on pigment production using fungi as production hosts have emerged, including Chromologics and Michroma to name a couple.

Production of lignin-derived aromatic compounds

Lignin is a complex and highly heterogeneous aromatic biopolymer comprising three primary hydroxycinnamoyl alcohol monomers: coumaryl alcohol (H-unit), sinapyl alcohol (S-unit), and coniferyl alcohol (G-unit) [54]. Depolymerization of lignin, with its intricate structure, presents a great hurdle in the conversion of lignin into value-added compounds. In this regard, various fungi stand out as effective lignin-degrading microorganisms due to their capacity of secreting lignin-degrading enzymes, that break down the lignin polymer into monomers. The fungal aromatic compounds metabolism is very diverse and still today not fully elucidated. Notably, specific parts of aromatic metabolism have been studied in a variety of fungi and diversity among species is to be expected (Fig. 3). A detailed comparison between the aromatic metabolism of fungi and that of bacteria was published some years back [55]; for a review on aromatic metabolism of filamentous fungi we refer to Mäkelä et al. [56].

Aromatic compounds produced from coumaryl alcohol (H-lignin)

Many fungi can convert *p*-coumaryl alcohol to *p*-coumaric acid (4-hydroxycinnamic acid) that can be metabolized to *p*-hydroxybenzoic acid (4-hydroxybenzoic acid) and further to phenolic acids such as protocatechuic acid, hydroxyquinol, hydroxyquinone, gentisic acid and catechol [55]. The conversion of *p*-coumaric acid to caffeic acid has been observed for several fungi, including the *Pycnoporus cinnabarinus*, *Gliocladium deliquescens* and several *Aspergilli*, but the underlying metabolic pathway(s) is still to be discovered [55, 57]. Biotransformation of *p*-coumaric acid into caffeic acid with a molar yield of 21% was demonstrated with *P. cinnabarinus* [57]. Caffeic acid has a range of interesting biological activities, and the compound can be used as a precursor for e.g. flavoring agents and in the production of plastics and rubbers [58]. Today, caffeic acid is extracted from plants and has a growing market.

Schizophyllum commune was reported to transform *p*-coumaric acid into p-hydroxybenzoic acid [59].

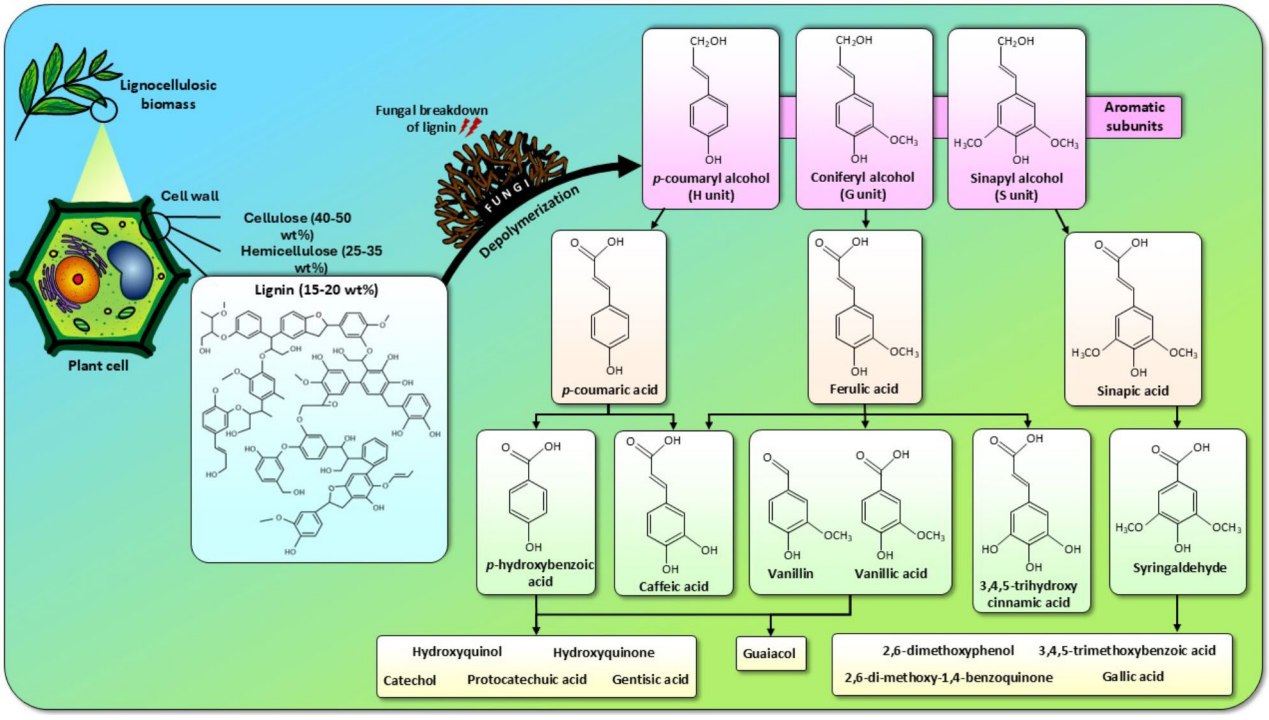

Fig. 3 Examples of aromatic and aromatic-derived compounds obtained from lignin degradation. Structures adapted from Lubbers et al. [55]

In *Paecilomyces variotii* degradation of *p*-coumaric acid resulted in the synthesis of *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde and protocatechuic acid (3,4-dihydroxybenzoic acid) [60]. Deletion of *phyA* and *prcA*, encoding a protocatechuate hydroxylase and a protocatechuate 3,4-dioxygenase, resulted in accumulation of protocatechuic acid in *A. niger* grown on different H-lignin related aromatic compounds [9]. Protocatechuic acid is a chemical building block for polymers and plastics that also has interesting pharmaceutical properties [9]. The so-called protocatechuate branch of the β-ketoadipate pathway was recently described in *A. niger* [61]. This pathway, consisting of five enzymes, converts protocatechuic acid into β-ketoadipic acid, that can be further metabolized to acetyl-CoA and succinyl-CoA. β-ketoadipic functions as a building block for nylon-6,6 analogs. Deletion of the β-ketoadipate-CoA transferase, led to β-ketoadipate accumulation when *A. niger* was grown on quinic acid [61]. Protocatechuate metabolism through the β-ketoadipate pathway has also been reported for *N. crassa* [62] and *A. nidulans* [63].

Aromatic compounds produced from coniferyl alcohol (G-lignin)

Coniferyl alcohol is used as a precursor for the biosynthesis of silymarin, ferulic acid, and vanillin [54, 64]. Silymarin is a mixture of flavonolignan and flavonoid polyphenolic compounds that exhibit various beneficial properties including antioxidant, anti-inflammatory, anti-cancer, and anti-viral activities, making these compounds potentially useful in the treatment of various liver and neurodegenerative disorders [65]. Ferulic acid (4-hydroxy-3-methoxycinnamic acid) has a wide range of applications in the pharmaceutical industry because of its antioxidant, anti-inflammatory, cholesterol-lowering, anti-cancer, and antimicrobial properties [66]. In the food industry, it is used as a preservative and as a precursor for vanillin production.

Vanillin is a popular flavor and fragrance compound extensively utilized in the food, beverage, cosmetic, and pharmaceutical industries [67]. Vanillin has been produced from ferulic acid in *Aspergillus luchuensis* [68]. Vanillin derivatives like vanillic acid and methoxyhydroquinone find applications in polymer production, such as epoxy resins [69]. The white rot fungi *S. commune* and *Sporotrichum pulverulentum* were reported to transiently accumulate vanillic acid during ferulic acid degradation [56, 70]. Similarly, in *Myceliophthora thermophila*, the decarboxylation of ferulic acid to *p*-vinylguaiacol resulted in the synthesis of vanillic acid. The conversion of ferulic acid into vanillic acid has also been observed in a range of ascomycota, namely in *A. niger* and *Botrytis*, *Cephalosporium*, *Penicillium*, *Trichoderma*, and *Verticillium* spp. [55]. *P. cinnabarinus* can degrade ferulic acid into vanillin [71, 72].

Both vanillic and isovanillic acid are produced during lignin degradation of spruce wood in *Phanerochaete chrysosporium* [73]. A range of fungi, including *Aspergillus japonicus* and *S. commune* have been reported to demethylate vanillic acid to protocatechuic acid [74–76]. Non-oxidative decarboxylation of vanillic acid to guaiacol was reported for *P. variotii*, *M. thermophila* and some *Aspergilli* [76, 77]. Guaiacol serves as a flavoring agent and guaiacol also has diverse pharmaceutical applications [78]. The biotransformation of vanillic acid into vanillin has been documented in numerous filamentous fungi, including, *P. cinnabarinus* and *T. reesei* [76, 79]. Some filamentous fungi such as, *A. japonicus*, *P. cinnabarinus* and *S. commune* oxidize vanillin to vanillic acid [70, 74, 80]. Similarly, oxidation of vanillyl alcohol to vanillin was observed in *A. japonicus*, *S. commune*, *Penicillium simplicissimum and S. pulverulentum* [74, 75, 81, 82]. Conversely, conversion of vanillin to vanillic acid and partially to vanillyl alcohol has been reported for *Polystictus versicolor*, *P. cinnabarinus*, *S. pulverulentum* and *Fomitopsis palustris* [75, 79, 81, 83]. While numerous reaction pathways for the conversion of vanillin have been reported, only one vanillin converting enzyme, VaO, found in *P. simplicissimum*, has been characterized thus far [84].

Many of the enzymatic reactions for conversion of aromatic compounds are reversible. *P. cinnabarinus*, and *P. simplicissimum* have been reported to produce coniferyl alcohol from ferulic acid and eugenol, respectively [79, 82]. Eugenol, a highly biologically active compound found in essential plant oils was reported to be converted into *p*-vinylguaiacol by *S. commune*, *P. variotii* and *Fusarium solani* [77, 85, 86]. *p*-vinylguaiacol is a volatile phenolic compound known for its tobacco flavour, a molecule that is used both as a flavour and as a pharmaceutical intermediate [87]. *Trametes* spp. have been reported to reduce ferulic acid to coniferyl aldehyde and coniferyl alcohol, and to synthesize vanillic acid, vanillyl alcohol, and methoxyhydroquinone [56, 88]. In *Lentinula edodes*, ferulic acid was reported to be hydroxylated to 5-hydroxyferulic acid and further to 3,4,5-trihydroxycinnamic acid, a compound with anti-inflammatory and antioxidant activities [89]. In *Penicillium rubens*, ferulic acid was shown to be demethylated to caffeic acid and further to protocatechuic acid [90].

Aromatic compounds produced from sinapyl alcohol (S-lignin)

Sinapyl alcohol contains two methoxy groups, which makes it more recalcitrant compared to the G- and H-lignin [54]. Some fungi can convert sinapyl alcohol into sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid). Sinapic acid and some of its derivatives, including

sinapine, 4-vinylsyringol, sinapoyl esters, and syringaldehyde, have gained attention due to their diverse biological activities which encompass antimicrobial, antioxidant, anticancer, anti-inflammatory, and anti-anxiety properties [91]. *P. variotii* was reported to convert sinapic acid into syringaldehyde and syringic acid [92]. The demethylation of syringic acid to 3-*o*-methylgallic acid has been documented for *P. variotii*, *P. chrysosporium* and *S. pulverulentum* [92, 93]. On the contrary, *S. pulverulentum*, *Petriellidium boydii*, and *Phialophora mutabilis* have been observed to produce 3,4,5-trimethoxybenzoic acid through syringic acid methylation [93]. 3,4,5-trimethoxybenzoic acid is an antioxidant and a building block compound used in organic synthesis for medical applications. *P. ostreatus* has been demonstrated to oxidize syringic acid to 2,6-di-methoxy-1,4-hydroquinone and 2,6-di-methoxy-1,4-benzoquinone but also to decarboxylate syringic acid to 2,6-dimethoxyphenol [94]. 2,6-dimethoxyphenol possesses antioxidant properties and is used in the pharmaceutical industry [95].

Conclusions and future perspectives

While filamentous fungi have been recognized to play a crucial role in the transition to a circular economy [96], the potential of aromatic chemicals production with fungi remains yet to be realized (Fig. 4). Published work typically reports conversion of one aromatic chemical to another, more valuable compound. Examples of such include tyrosine biotransformation to L-Dopa, conversion of p-coumaric acid to caffeic acid and conversion of ferulic acid to vanillin. Biorefinery initiatives aimed at adding value to industrial side-streams illustrate opportunities such as converting tannic acid into gallic acid, a process successfully achieved using various fungi. Channeling the production into a specific compound of interest and/or required downstream processes to ensure a pure compound are however known to be challenging, especially when a complex biomass is used as feedstock. Unspecific enzymatic and spontaneous reactions within the cells are also to be considered. Enzyme engineering or generation of new enzymes with the help of machine learning are promising future opportunities for improving pathway fluxes and creating new production routes [97]. Furthermore, metabolic models could be exploited to better understand fungal metabolism and how it could

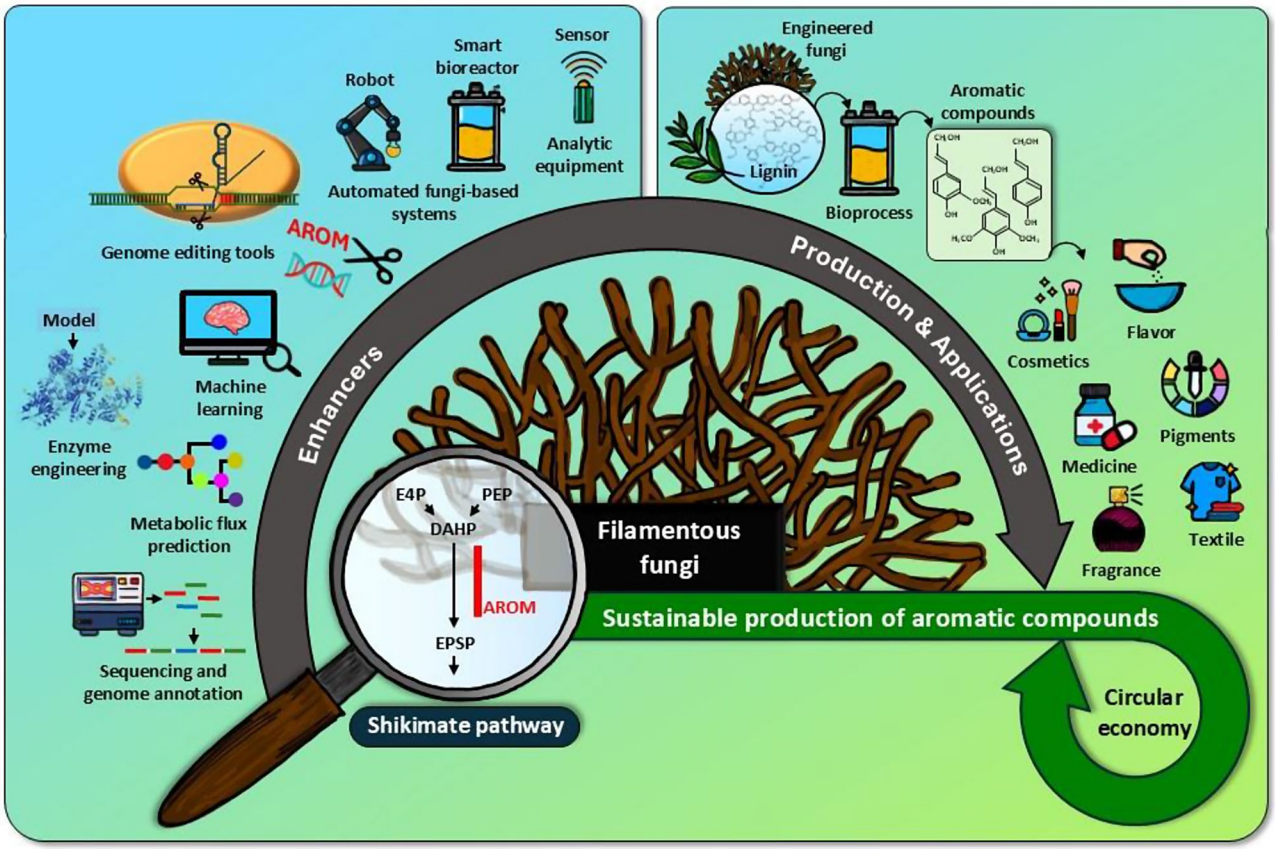

Fig. 4 Overview of the possible drivers to enhance aromatic compound production in filamentous fungi. Production of fungal based aromatic compounds from renewable biomass finds diverse industrial applications and could be part of a circular economy

be altered for increased production. Metabolic models exist for several filamentous fungi and *de novo* construction of genome scale metabolic models is today rather straight forward. In recent years, machine learning methods that facilitate the reconstruction of enzyme-constrained models have appeared [98]. Still, the usability of metabolic models may be constrained by the lack of accurate data. Cultivation of filamentous fungi in controlled conditions to establish accurate measurements of growth and production can be a challenge on its own. While domesticated model fungi can grow very homogenously in submerged culture many filamentous fungi fail to do so. The ongoing digital transformation of the biomanufacturing industry has sparked increased interest in automating fungi-based systems, but further developments, such as new analytic equipment (sensors) and control systems are needed to advance the field [99]. The collection of real-time data and creation of digital twins of fungal bioprocesses has the potential to identify production bottlenecks and create novel understanding of fungal metabolism.

Many of the reaction steps in the aromatic metabolism of fungi remains to be elucidated but the known pathways, including the shikimate pathway and the β-ketoadipate pathway appear to be well conserved across fungal species. The rapidly increasing number of sequenced fungi and the progress in gene annotation and enzyme function prediction can nonetheless be expected to pave the way for a greater understanding of fungal aromatic compound metabolism. Genetic engineering for improving aromatic chemicals production or product spectrum is not yet widely practiced in filamentous fungi. A few earlier studies report the successful engineering of the shikimate pathway in filamentous fungi, through overexpression of, or modification of the AROM pentapeptide [19–21]. The AROM enzyme is known to be subjected to tight feedback control and releasing this through targeted mutagenesis has in other species been a very successful strategy for increasing the flux through the shikimate pathway [100]. Filamentous fungi as production hosts would provide great benefits, such as the ability to transform and metabolize recalcitrant biomasses and lignin, which lessens the necessity for costly pre-treatments and increases carbon conversion efficacy. This, in combination with the recent advances in fungal engineering tools, namely CRISPR/Cas-based systems, leads to a significant potential for leveraging fungi in the synthesis of aromatic compounds.

Abbreviations

TCA cycle	tricarboxylic acid cycle
DAHP	3-deoxy-D-arabino-heptulosonate-7-phosphate
EPSP	5-enolpyruvylshikimate-3-phosphate
DAHPS	3-deoxy-D-arabino-heptulosonate-7-phosphate synthase
CS	chorismate synthase
DHQS	3-dehydroquinate synthase
DHQD	3-dehydroquinate dehydratase
SDH	shikimate dehydrogenase
SK	shikimate kinase
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
CrcA	catechol 1,2-dioxygenase
ShyA	salicylate hydroxylase

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Author contributions
YN initiated this study which was conducted and written by both authors. PU prepared the figures with support of YN.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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