Review

Research Progress on Fungal Sesterterpenoids Biosynthesis

Ping Zhang 1,†, Jianzhao Qi 1,2,†, Yingce Duan 1, Jin-ming Gao 2,* and Chengwei Liu 1,*

1 Key Laboratory for Enzyme and Enzyme-like Material Engineering of Heilongjiang, College of Life Science, Northeast Forestry University, Harbin 150040, China
2 Shaanxi Key Laboratory of Natural Products & Chemical Biology, College of Chemistry & Pharmacy, Northwest A&F University, Yangling 712100, China
* Correspondence: jinminggao@nwuaf.edu.cn (J.-m.G.); liuchw@nefu.edu.cn (C.L.)
† These authors contributed equally to this work.

Abstract: Sesterterpenes are 25-carbon terpenoids formed by the cyclization of dimethyl allyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) as structural units by sesterterpenes synthases. Some (not all) sesterterpenoids are modified by cytochrome P450s (CYP450s), resulting in more intricate structures. These compounds have significant physiological activities and pharmacological effects in anti-inflammatory, antibacterial, antitumour, and hypolipidemic communities. Despite being a rare class of terpenoids, sesterterpenoids derived from fungi show a wide range of structural variations. The discovered fungal sesterterpenoid synthases are composed of C-terminal prenyltransferase (PT) and N-terminal terpene synthase (TS) domains, which were given the name PTTSs. PTTSs have the capacities to catalyze chain lengthening and cyclization concurrently. This review summarizes all 52 fungal PTTSs synthases and their biosynthetic pathways involving 100 sesterterpenoids since the discovery of the first PTTSs synthase from fungi in 2013.

Keywords: sesterterpenes; sesterterpene synthase; terpene biosynthesis; cytochrome P450

1. Introduction

Over 80,000 terpene natural products have been identified in nature [1], making them one of the most abundant and structurally diverse natural product families. Terpenoids are synthesized from the universal C5 precursors, DMAPP and IPP. DMAPP and IPP can be generated by the mevalonate (MVA) or 2-C-methyl-D-erythritol-4-phosphate (MEP) pathways, depending on the species. Subsequently, PT assembles IPP and DMAPP into varying linear isoprenoid diphosphate chains including geranyl-farnesyl pyrophosphate (GFPP). Terpene synthase (TPS) generates these chains into terpene backbones with multiple chiral centers. Various enzymes modify the core backbone to produce structurally and functionally distinct terpenoids. Terpenoids are further classified according to the number of isoprene units employed in their formation as monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), and triterpenes (C30). Compared to monoterpenes, sesquiterpenes, diterpenes, and triterpenes, sesterterpenes are the rarest terpenoids found to date, with approximately 1000 diterpenes isolated [2], mainly from fungi, bacteria, lichens, higher plants, insects, and various marine invertebrates [3].

Fungi-derived sesterterpenoids exhibit a diverse set of physiological and pharmacological properties. Fusaproliferin (I), discovered from the eggplant-disease fungus Fusarium solani, demonstrated powerful and rapid cytotoxic effects on both pancreatic and breast cancer cells, paving the way for the creation of more potent anti-pancreatic cancer medicines [4]. The metabolites of Aspergillus flavus, 14,15-dehydro-6-epi-ophiobolin K (2), 14,15-dehydro-6-epi-ophiobolin K (3), 14,15-dehydro-6-epi-ophiobolin G (4), 14,15-dehydro-ophiobolin G (5), and 14,15-dehydro-(Z)-14-ophiobolin G (6) were extremely cytotoxic against six cancer cell lines, HCT-15, NUGC-3, NCI-H23, ACHN, PC-3, and MDA-MB-231, with GI50 values ranging from 0.14 to 2.01 µM [5]. The antibacterial effects of
ophiobolin derivatives 3-anhydro-6-hydroxy-ophiobolin A (7) and Ophiobolin T (8) against *Bacillus subtilis*, Bacille Calmette–Guerin strain, *Staphylococcus aureus*, and Methicillin-resistant *S. aureus* are promising molecules, and they are expected to be the lead compounds for the development of new bacteriostatic agents [6,7]. Bipolarin E (9), derived from the phytopathogenic fungus *Bipolaris* sp. TJ403-B1, showed significant antibacterial action against *Enterococcus faecalis* and *Pseudomonas aeruginosa* [8]. The ophiobolin-type sesterterpenoids, Asperophobolins H–J (10–12) and 3-anhydro-6-epi-ophiobolin A (13), isolated from *Aspergillus* sp., showed significant inhibition of lipopolysaccharide-induced nitric oxide production by macrophages, and hence, had excellent anti-inflammatory activity [9]. Further investigations revealed that 12 substantially suppressed the macrophage production of IL-1, RANTES, MIP-1, and TNF-α, and stimulated the release of IL-13 via blocking HO-1 induction and the NF-kB pathway, providing a scientific basis for anti-inflammatory treatment [10]. Compound 12 [10] and Bipolarolides A (14) [11], isolated from the fungus *Bipolaris* sp. TJ403-B1, had a strong inhibitory effect on HMG-CoA reductase (Figure 1). Compound 14 was also found to have a hypolipidemic effect on HepG2 cells, with an effect comparable to that of the cholesterol-lowering drug pravastatin. As a result, compound 14 is a potential HMGR antagonist that merits further investigation [11]. These discoveries could pave the way for the development of novel HMG-CoA reductase inhibitors and anti-hyperlipidaemic drugs.

**Figure 1.** Representative natural sesterterpenes of fungi.

The results of sesterterpenes biosynthesis research from various species indicate that the PT domain and the TS domain are required functional modules for sesterterpene synthesis, and they, together, constitute bifunctional chimeric sesterterpene synthases. In plants, the two functional domains of PT and TS that comprise sesterterpene synthase are encoded by two genes independently [12], such as AtGFPS2-AtTPS18 from *Arabidopsis thaliana* (Figure 2A) [13]. In a few plants, the enzymes responsible for the synthesis of sesterterpenes harbor two functional domains, PT and TS domains, which are bifunctional chimeric enzymes, such as LcTPS2 from *Leuceopterum canum* [14] (Figure 2B). Sesterterpenes are produced in bacteria by two distinct genes, each with a PT domain and TS domain, such as GFPPS-SmTS1 from *Streptomyces mobaraensis* (Figure 2C)—the former is responsible for the formation of GFPP, and the latter for the cyclization of GFPP to form a sesterterpene backbone [15]. In contrast, sesterterpene synthases in fungi are all chimeric enzymes with PT and TS functional domains, such as AcOS from *A. clavatus* [16] (Figure 2D).
With the advancement of sequencing technology in recent years, more and more genes with biological functions have been discovered. To date, 52 PTTSs (Table 1) from fungi have been identified, and their functions have been functionally validated via heterologous expression in *Escherichia coli*, *Saccharomyces cerevisiae*, or *A. oryzae*. Parts of the backbone generated by 52 PTTSs were further modified by other post-modifying enzymes on their gene cluster, yielding a total of 100 sesterterpenoids. This review provides a detailed summary of these enzymes and their biosynthetic pathways.

2. Characteristics and Classification of Fungal PTTSs

Fungal PTTSs are bifunctional enzymes with two domains, PT and TS. PT is responsible for catalyzing the combination of IPP and DMAPP to generate C25 GFPP, while TS catalyzes the cyclization of GFPP to generate various sesterterpenes. Phylogenetic analysis revealed that fungal diterpene synthases could be divided into two broad categories, clades I and II, which catalyze the formation of various ring systems according to their cyclization patterns [17].

2.1. Characteristics of Fungal PTTSs

Terpene synthases are typically classified into two classes, class I and class II, based on their initial carbon cation formation strategy. The former catalyzes an olefin cation cyclization reaction initiated by diphosphate (PPI) cleavage, while the latter catalyzes an olefin protonation reaction [18]. The N-terminus of class I terpene synthase sequences typically contain DDxxD and NSE/DTE-conserved motifs (Figure 3A), while the C-terminus contains two aspartic acid-rich-conserved regions, DDXXD and DDXXN (Figure 3A). A class II cyclase uses aspartic acid from the DXDD motif as a catalytic acid to protonate the polisoprenoid’s terminal olefinic bond, resulting in a tertiary carbocation [19].

Sequence alignment revealed that all identified fungal PTTSs had the N-terminal motifs DDxxD and NSE/DTE, as well as the C-terminal motifs DDXXD and DDXXN, which are common features of class I terpene synthases [20], and on this basis, it is assumed that all known fungal PTTSs are class I terpene synthases. Analysis of the cyclization mechanism for sesterterpenes shows that the binding of three metal ions (frequency, Mg$^{2+} >$ Mn$^{2+} >$ Co$^{2+}$) to GFPP induces a conformational change in GFPP, resulting in the closure of the enzyme active site and ionization of GFPP, which becomes an allylic cation and releases PPI [18].

Three-dimensional structural AcOS, the first sesterterpene synthase identified in a fungus, modeled by the trRosetta website (http://yanglab.nankai.edu.cn/trRosetta/, accessed on 1 September 2022), revealed two distinct structural domains, with sequence analysis indicating that the TS structural domain is at the N-terminal, while the PT structural domain is located at the C-terminal (Figure 3B).

---

Figure 2. Schematic diagram of functional domains (PT and TS) associated with the biosynthesis of sesterterpene from plants (A, B), bacteria (C), and fungi (D). The prediction of functional domains was performed by PFAM (https://pfam.xfam.org (accessed on 1 September 2022)).
Figure 3. Motif analysis of fungal PTTSs (A) and domain analysis of AcOS (B). The DNAMAN package was used to compare 12 protein sequences of characteristic PTTSs from six clusters, whose GenBank accession no. are shown in Table 1. “*” indicates positions which have a single, fully conserved residue.

2.2. Phylogenetic Analysis of Fungal PTTSs

A phylogenetic analysis of 52 fungal-derived PTTSs revealed six distinct groups (clusters A–F). According to the cyclization pattern, clusters A, E, and F belong to clade I, while clusters B–D belong to clade II (Figure 4).

Figure 4. Phylogenetic tree analysis of fungal PTTSs with the maximum-likelihood method. The phylogenetic tree was constructed by IQ-TREE v 1.6.9 based on the full-length sequences of 52 PTTSs and presented in Figtree v1.4.4. A–F denote six subfamilies of PTTSs, where A, E, and F are in clade I, and B, C, and D are in clade II.

2.3. Catalytic Characteristics of Fungal PTTSs

Clades I and II, respectively, catalyze type A (C1-IV-V) and type B (C1-III-IV) cyclization [21]. All TS domains in clusters A, F, and E initiate cyclization by forming a 15-5-membered ring system [22]. The type A ring system is formed by the sequential cyclization reaction of the C1 cation, the C14-C15 alkene (IV), and the C18-C19 alkene (V) of GFPP (C1-IV-V). In the initial cyclization step, all known TS structural domains in clusters B, C, and D produce an 11-5-membered ring system [23]. The cyclization reaction between the C1 cation, the C10-C11 alkene (III), and the C14-C15 alkene (IV) of GFPP produces the B-type ring system (C1-III-IV). The structures of compounds from 52 fungal PTTSs and their possible cyclizations are shown in Figure 5.
3. Fungal Sesterterpene Biosynthesis Pathway

The biosynthetic pathways of sesterterpenes in fungi are all confirmed by a heterologous expression, with *S. cerevisiae*, *A. oryzae*, and *E. coli* being common hosts. In fungi, the majority of sesterterpenes are synthesized by single PTTSs with no post-modification. A small number of sesterterpenes on the gene cluster modifies the final compound via CYP450s. There are also very few functional genes encoding flavin-dependent oxidases on the biosynthetic gene clusters of sesterterpenes.

3.1. Sesterterpene Yielded by a Single PTTS

The first PTTS, AcOS, was discovered serendipitously by the Hideaki Oikawa lab in 2013 during the identification of possible diterpene synthase coding genes in public databases using the *A. oryzae* expression system [16]. The *A. oryzae* transformant of AcOS produced four sesterterpenoids, but in the enzymatic reaction in vitro, AcOS produced diterpene in addition to sesterterpenes. These four sesterterpenoids include Ophiobolin F (15), Ophiobane 1 (16), Ophiobolane 2 (17), and Clavaphyllene (18). Of these four metabolites, 15–17 are 5/8/5-fused tricyclic sesterterpenes [16]. AcOS contains two domains, the N-terminal PT domain and the C-terminal GFPP synthase domain, which are identical to diterpene synthases, according to the protein sequence analysis of the cDNA expressed in *E. coli* [16]. With the discovery of the first PTTS, the pathways of sesterterpenes derived from plants, fungi, and bacteria have been gradually identified.
Sequence analysis indicated that the C-terminus of AcOS contains the αα domain responsible for the generation of linear GFPP, while the N-terminus contains the cyclase domain responsible for the formation of Ophiobolin F (15) [24]. PaFS, a diterpene synthase found in the phytopathogenic fungus *Phomopsis amygdali* [25], is 41% identical with AcOS. They both catalyze the cyclization reactions of geranylgeranyl pyrophosphate (GGPP) and GFPP via a common 5–11 ring system intermediate. Sequence alignment showed that the majority of residues in PaFS and AcOS are conserved, but two pairs of residues, W225 and V228 in PaFS and L217 and A220 in AcOS, differ significantly in size [24]. The α-domain at C-terminal of PaFS, which is responsible for GGPP synthase, was swapped with the α-domain of EvSS, which is responsible for GFPP synthesis, but no sesterterpenes were produced. Therefore, it is speculated that the active pocket of the PaFS cyclase domain is too small to accommodate GFPP [24].

The N-terminal cyclase domain of the chimera PaFS underwent a double mutation (W225L/V228A), yielding three unknown sesterterpene products. The chimera obtained by replacing the GFPP synthase domain on the C-terminal of EvSS with the GGPP synthase domain of PaFS produced only diterpenes and sesquiterpenes. A double mutant L217W/A220V was made on the N-terminal cyclase structural domain of this chimera AcOS, which produced not sesterterpenes, but diterpenes and sesquiterpenes [24]. Thus, the substitution of crucial amino acid residues can change the size of the active packet, resulting in the formation of the corresponding compounds.

Chai et al. conducted a systematic study on the biosynthesis of ophiobolin in *A. ustus* in 2016 [26]. Au8003 was confirmed to be the synthase responsible for ophiobolin F (15) in *A. ustus* by gene deletion and complementation experiments [26]. Subsequently, Yuan et al. attempted to increase the yield of ophiobolin F (15) by optimizing the codons of synthase, adding substrates, and optimizing the pathway of precursor supply [27].

Qin et al. and Matsuda et al. reported two bifunctional chimeric terpene synthases, EvVS [28] and EvSS [29] from *Emericella variecolor* in 2015. The heterologous expression of EvVS in *A. oryzae* only produced the diterpene varidiene, whereas the in vitro reaction of EvVS produced not only varidiene, but also sesterterpenes (2E)-α-Cericene (19). The TS domain of EvVS was combined with the PT domain of EvSS to create a chimeric enzyme that was expressed in *A. oryzae* to produce 19 and diterpene varidiene [28]. Matsuda et al. confirmed to mine the genome of *E. variecolor* in 2016 and discovered another PTTS, EvAS, 196 and its heterologous expression in *A. oryzae* produced a new sesterterpene astelliadiene 197 (20) with a fused 6-8-6-5 ring [30].

Narita et al. discovered four sesterterpene synthase genes from phytopathogenic fungi in 2017, including BmTS1, BmTS2, and BmTS3 from *B. maydis*, as well as PbTS1 from *P. betae*. The four sesterterpenes Bm1-3 (21–23) and Pb1 (24) were produced by heterologous expression of these four genes in *A. oryzae* and *E. coli*, respectively [31].

In 2017, Bian et al. discovered two new sesterterpenes, mangidiene (25) and varicoteltraene (26), via the heterologous expression of FgMS from *F. graminearum* using an optimized terpene substrate supplying *S. cerevisiae* as a chassis. The active sites F65L and F159G on FgMS were then mutated, yielding compound 27 and five possible sesterterpenes [32].

A survey of 34 possible PTTS-encoding genes derived from Ascomycete identified 28 PTTSs and 15 sesterterpenes, and these PTTSs were characterized via high-throughput automated platforms and efficient yeast chassis in 2021 [33]. These enzymes and compounds included Sesterfisherol (28) from AaSS; Bm3 (23) from MpBS and CfsB; Pb1 (24) from ChPS, CoFS, CiGS, and CsPS; sesterbiculene (29) from GiSS, CoSS, CgSS; fusaproliferene (30) from CoFS; (-)-variculatriene B (31) from LmVS, PoVS1, ChVS, CsVS, and PoVS2, variculatriene A (32); clavaphyline (18) from PfVS; ophiobolin F (15) from AuOS, and BmOS; preasperterpenoid A (33) from TvPS; sesterbrasiliatriene (34) from PaSS, brassiteraene A (35), brassiteraene B (36) from ChBS; sesterevisene (37) from ZbSS; β-geranylfernesene (38) from CiGS, and PfVS; geranylfernesol (39) from AnGS, PgGS, TaGS, and AaGS. Among the above compounds, sesterevisene (37) and sesterbiculene (29) are two new cyclic sesterterpenoids [33].
In 2022, Jiang et al. discovered two novel PTTSs, CsSS and NnNS, from *Cytospora schulzeri* 12, 565; and *Nectria nigrescens* 12, 199, respectively, and identified their functions through heterologous expression in *A. oryzae* and *S. cerevisiae*. CsSS generates 5/12/5 tricyclic A-type sesterterpene schultriene (40), while NnNS produces the first 5/11 bicyclic B-type sesterterpene nigtetraene (41) to date [34].

| Entry | Genebank No./Gene | Producer | Host/Chassis | Product | Reference |
|-------|------------------|----------|--------------|---------|-----------|
| AcOS  | ACLA_76850       | *Aspergillus clavatus* | *A. oryzae, E. coli* | Ophiobolin F(15), Ophiobolane 1 (16), Ophiobolane 2 (17), Clavaphylene (18) | [16] |
| Au8003| QHJ97826.1       | *Aspergillus ustus* | *E. coli* | Ophiobolin F(15) | [26] |
| BmOS | MW798226             | *Bipolaris maydis* | *S. cerevisiae* | Ophiobolin F (15) | [30] |
| AsOS | MW798208             | *Aspergillus ustus* | *S. cerevisiae* | Ophiobolin F (17) | [33] |
| PIVS | MW798216             | *Pestalotiopsis fici* | *S. cerevisiae* | Clavaphylene (18), | [33] |
| EvVS | LC063849            | *Emericella variecolor* | *A. oryzae, E. coli* | Variculatriene A (32), β-Geranylfernesene (38) (2E)-a-cericerene (19) | [28] |
| EvAS | LC113889            | *Emericella variecolor* | *NBR 32302* | Aspegillidene (20) | [30] |
| BmTS1| EMD84919            | *Bipolaris maydis ATCC48331* | *A. oryzae* | Bm1 (21) | [31] |
| BmTS2| EMD93209            | *Bipolaris maydis ATCC48331* | *E. coli* | Bm2 (22) | [31] |
| BmTS3| EMD93704            | *Bipolaris maydis ATCC48331* | *A. oryzae* | Bm3 (23) | [31] |
| CbBS | MW798209             | *Colletotrichum fiorina* | *S. cerevisiae* | Bm3 (23) | [33] |
| MpBS | MW798229             | *Macrophominaphaseolina* | *S. cerevisiae* | Bm3 (23) | [33] |
| PbTS1| LC274619            | *Phoma betae PS-13* | *E. coli* | Pb1 (24) | [31] |
| BtAc1 | N4V6D4.1             | *Colletotrichum orbiculare* | *A. oryzae* | Pb1 (24) | [33] |
| ChPS | MW798213             | *Colletotrichum higginsianum* | *S. cerevisiae* | Pb1 (24) | [33] |
| CsPS | MW798219             | *Colletotrichum orbiculare* | *S. cerevisiae* | Pb1 (24) | [33] |
| CoFS | MW798210             | *Colletotrichum orbiculare* | *S. cerevisiae* | Pb1 (24), Fusaproliferene (30) | [33] |
| CiGS | MW798200             | *Colletotrichum inanum* | *S. cerevisiae* | Pb1 (24), 1,β-Geranylfernesene (38) | [33] |
| FgMS | AQQ56777             | *Fusarium graminearum* | *S. cerevisiae, A. oryzae, E. coli* | Mangicidiene (25), Variecolletraene (26) | [32,36] |
| NiFS | EA16201              | *Necatoria fischeri* | *A. oryzae, E. coli* | Sesterfisbolen (28) | [22] |
| AzSS | MW798204             | *Alternaria alternata* | *S. cerevisiae* | Sesterfisbolen (28) | [35] |
| CsBS | MW798201             | *Colletotrichum inanum* | *S. cerevisiae* | Sesterbroculicene (29) | [33] |
| CoSS | MW798211             | *Colletotrichum orbiculare* | *S. cerevisiae* | Sesterbroculicene (29) | [33] |
| GcSS | MW798218             | *Colletotrichum glaucoporaides* | *S. cerevisiae* | Sesterbroculicene (29) | [33] |
| CrVS | MW798212             | *Colletotrichum higginsianum* | *S. cerevisiae* | (-)-Variculatriene B (31) | [33] |
| PoVS | MW798215             | *Pyricularia oryzae* | *S. cerevisiae* | (-)-Variculatriene B (31) | [33] |
| LnVS | MW798221             | *Laphontoma macroclum* | *S. cerevisiae* | (-)-Variculatriene B (31) | [33] |
| CsVS | MW798223             | *Colletotrichum sultilema* | *S. cerevisiae* | (-)-Variculatriene B (31) | [33] |
| PoVS | MW798227             | *Pyricularia oryzae* | *S. cerevisiae* | (-)-Variculatriene B (31) | [33] |
| TIPS | MW798214             | *Thermotheliales terrestres* | *S. cerevisiae* | Preasperterpenoid A (33) | [33] |
| PIPS | LC228602             | *Penicillium verruculosum* | *A. oryzae, E. coli* | Preasperterpenoid A (33) | [33] |
| AsTC | MK14062              | *Talaromyces wortmannii* | *A. oryzae, E. coli* | Preasperterpenoid A (33) | [33] |
| TyPS | MW798225              | *Talaromyces verruculosus* | *S. cerevisiae* | Preasperterpenoid A (33) | [33] |
| PsBS | MW798222              | *Penicillium arizonicum* | *S. cerevisiae* | Preasperterpenoid A (33) | [33] |
| PhBS | LC228601              | *Penicillium brasiliunam* | *A. oryzae, E. coli* | Sesterbrasiliatetraene (34) | [37] |
| ChBS | MW798232              | *Colletotrichum higginsianum* | *S. cerevisiae* | Sesterbrasiliatetraene (34) | [37] |
| ZbSS | MW798202              | *Zymoseptoria brevis* | *S. cerevisiae* | Sesteresiene (37) | [33] |
| AnGs | MW798203              | *Aspergillus niger* | *S. cerevisiae* | Geranylfernesol (39) | [33] |
| PgGs | MW798206              | *Penicillium griseofulmum* | *S. cerevisiae* | Geranylfernesol (39) | [33] |
| PgFS | MW798217              | *Pyricularia grisea* | *S. cerevisiae* | Geranylfernesol (39) | [33] |
| ItAS | MW798220              | *Thielena arenaria* | *S. cerevisiae* | Geranylfernesol (39) | [33] |
| AaSS | MW798231              | *Aspergillus aculeatus* | *S. cerevisiae* | Geranylfernesol (39) | [33] |
| CsSS | MW685620              | *Cytospora schulzeri 12,565* | *A. oryzae, E. coli* | Schultriene (40) | [34] |
| NnNS | MW685621              | *Nectria nigrescens 12,199* | *A. oryzae, E. coli* | Nigtetraene (41) | [54] |
| EvSS | LC037904              | *Emericella variecolor* | *A. oryzae, E. coli* | Stellate-2,19-Triene (42) | [29] |
| EvQS | LC152100              | *Emericella variecolor* | *A. oryzae, E. coli* | Quanattuene (44) | [39] |
| AcdAS | CEL06489.1            | *Asterigladium clavatus* | *A. oryzae* | Asperterpenoid A (47) | [40] |
| AuAS | MW387950             | *Aspergillus ustus 094102* | *A. oryzae* | Aspegillidene A (49) | [41] |
| FoFS | MW446505             | *Fusarium oxysporum* | *A. oryzae, E. coli* | Aspegillidene A (49) | [41] |
| PaTB | KX49366               | *Aspergillus terreus* | *A. oryzae* | Aspegillidene A (49) | [41] |
| PsTA | NA                  | *Penicillium hongii TY403-A1* | *A. oryzae, E. coli* | Preasperterpenicid I (62) | [42,43] |

NA indicates not available.
3.2. Sesterterpenoid Yielded by PTTS and CYP450

In addition to PTTSs, the biosynthetic gene cluster (BGC) of a small number of sesterterpenes contains CYP450, which is responsible for post-cyclization modifications. Matsuda et al. discovered the PTTS, Stl-SS (EvSS), in the E. variecolor genome using AcOS as a probe in 2015, and expressed it heterologously in A. oryzae NSAR1 to generate a sesterterpene stellata-2,6,19-triene (42) with an 11-5 tricyclic ring. The CYP450 monoxygenase Stl-P450 located near Stl-SS converts this compound to stellatic acid (43) (Figure 6A) [29].

Figure 6. The BGCs composed of PTTSs and CYP450s and their biosynthesis. (A–F) represent six fungal sources of BGCs composed of PTTSs and CYP450s. Okada et al. discovered another PTTS, EvQS, from E. variecolor through genome mining in 2016, and expressed it in A. oryzae to produce quannulatene (44). Compound 44 was then oxidized to quannulatic acid (45) by CYP450 enzymes located near the EvQS gene (A). Feeding experiments with isotopically labeled acetates and in vitro experiments with GFPP isotopoisomer revealed that 44 cyclizes in an unprecedented manner to form a unique fused 5-6-5-5-5 ring [39].

Sato et al. discovered a PTTS NfSS from Neosartorya fischeri using a genome mining strategy in 2015. In A. oryzae, the heterologous expression of this gene resulted in sesterfisherol (28) with the 5-6-8-5 tetracyclic ring. CYP450 monoxygenase (NfP450) converts 28 to sesterfisheric acid (46) (Figure 6B). Compounds 28 and 46 have not been identified in N. fischeri metabolites, indicating that this BGC is silent. F191A, a site-directed mutagenesis of NFSS, produced novel sesterterpenes, but not 28, confirming that phenylalanine F191 is a critical amino acid residue for the production of 28 [45].

The regioselectivity and stereoselectivity in terpene formation reactions are determined by the conformations of carbocation intermediates that mirror the initial conformation of GFPP [46]. By calculating the intrinsic atomic mobility of the carbon positive ion intermediate during the enzymatic catalysis of sesterterpenes (28 and 44), Sato et al. found that the two methyl groups (C20 and C23) remained unchanged during the first half of the cyclization cascade. It is also suggested that the 28 and 44 enzyme-catalyzed process is divided into three stages: (I) formation of the 5/12/5 tricycles, (II) conformational change and hydrogen transfer, and (III) ring rearrangement to form the 5-6-8-5 ring system [46].

With the help of genome mining, Quan et al. found another sesterterpene BGC in A. calidoostus in 2020. The cluster contains two genes: AcldAS, a PTTS; and AcldA-P450,
a CYP450 family member. AcldAS was discovered through heterologous expression and in vitro enzymatic assays to produce the sesterterpene asperterpenol A (47), which was then oxidized by AcldA-P450 to asperterpenol B (48) (Figure 6C). Asperterpenol A have a unique 6/6/8/5 tetracyclic ring system, and isotope labeling was used to determine the absolute configuration and cyclization mechanism of 47 [40].

Guo et al. discovered a multi-product sesterterpene biosynthesis gene cluster in *A. ustus* genome in 2021. A bifunctional terpene synthase AuAS and a CYP450 monooxygenase AuAP450 were found in this BGC. By a heterologous expression in *A. oryzae*, AuAS produced five novel sesterterpenes, aspergiltriene A (49), and aspergildiene A–D (50–53). Based on this, the AuAP450-introduced *A. oryzae* producer synthesized four new sesterterpene alcohols, aspergilols A–D (54–57) (Figure 6D). Only 49 has a 5/12/5 tricyclic skeleton, whereas the other four have a 5/6/8/5 tetracyclic skeleton [41]. The compound 49 is thought to be an earlier by-product produced in this pathway.

Jiang et al. identified the fungal chimeric terpene synthases FoFS and AtAS from the plant pathogenic fungi *F. oxysporum* and *A. terreus* in 2021, respectively. AtAS is otherwise known as STtA, as also reported by Clevenger et al. in 2017 [43]. The heterologous expression of the former produces fusoxypenes A–C (58–60) and (-)-astellatene (61), and the heterologous expression of the latter produces preaspterpenacid I (62) [42]. The compounds 58 and 62 are enantiomeric sesterterpenes with a 5–6–7–3–5 ring system, catalyzed by FoFS and AtAS, respectively. The C22 of 62 is then modified oxidatively by CYP450 to generate preaspterpenacid II (63) [42] (Figure 6E). Furthermore, the density functional calculations of FoFS-catalyzed reactions show that the formation of the pentacyclic system is a highly organized process [42].

Qiao et al. established a BGC consisting of a PTTS-encoding gene pstA and a CYP450 gene pstB by the genome mining of *P. herquei* TJ403-A1. The heterologous expression of pstA in *A. oryzae* yielded penissetene (64), and its co-expression with pstB produced two novel penisentene derivatives, penissetenol (65) and penissetone (66) (Figure 6F). Notably, the compound 64 possesses a unique 5/15 cis-fused ring system; in contrast, all other ring systems generated by known PTTSs are in trans. Thus, PsTA is the first PTTS to catalyze sesterterpenoids with a 5/15 cis-fused ring system. This unprecedented mode of ring fusion suggests that PsTA controls the stereochemical product of the initial cyclization in a novel mechanism [44].

### 3.3. Sesterterpene Yielded by the BGC Containing Multiple Genes

The post-modifying enzymes in addition to the typical cytochrome P450 include flavin-dependent oxidases in a minimal number of fungal sesterterpene BGC. Additionally, MFS transporters can occasionally be found in gene clusters involved in sesterterpene biosynthesis.

AcOS was the first report of PTTS [16], and subsequent studies showed that AcOS formed a functional obl BGC in *A. clavatus*, with genes encoding oxidases and transporters clustered around the AcOS-encoding gene [47]. The obl BGC was also found in the genomes of two other filamentous fungi, *B. maydes* and *E. variecolor*, and not only in *A. clavatus*. In addition to TPPCs OblA<sub>Bm/Ac/Ev</sub> and CYP450 monooxygenase (OblB<sub>Bm/Ac/Ev</sub>) in the BGC of ophiobolin compounds from the genome of *A. clavatus*, there are also flavin-dependent oxidases (OblD<sub>Bm/Ac/Ev</sub>) in the obl BGCs (Figure 7A) [47]. AcOS was, therefore, named OblA<sub>Ac</sub>.

These ophiobolin F (15) synthases, OblA<sub>Bm/Ac/Ev</sub> [47], AuOS [33], AU8003 [26], and BmOS [33], showed a high identity of 61% to 100%. The products of OblA<sub>Ac</sub> (AcOS) include 16–18, in addition to 15. The heterologous expression of obl<sub>Bm/Ac/Ev</sub> in *A. oryzae* yielded ophiobolin F (15), and its co-expression with obl<sub>Ac/Ev</sub> produced four oxidized Ophiobolin derivatives (67–70). However, the co-expression of obl<sub>Ac</sub> with obl<sub>Bm</sub> did not result in the production of new compounds. Then, the transporter gene oblD<sub>Bm</sub> was co-expressed with obl<sub>Ac</sub> and obl<sub>Bm</sub>, leading to the three new sesterterpenes—ophiobolin (71), 6-epiophiobolin C (72), and 6-epiophiobolin N (73) [47] (Figure 7B). Combining the co-expression of obl<sub>C</sub> with obl<sub>Ac</sub>, obl<sub>Bm</sub>, and oblD<sub>Bm</sub> in *A. oryzae* led to compound 74.
The compound 75 from B. maydes is presumed to have been further oxidized to form 76 (Figure 7B). Multiple novel sesterterpenes were produced by this combinatorial expression strategy of genes from various origins, revealing novel information about the biosynthetic process for ophiobolins [47].

Figure 7. The obl BGCs containing multiple genes (A) and their biosynthesis (B).

Ophiobolin A (76) is a toxic sesterterpene metabolite produced by the pathogen Cochliobolus miyabeanus and B. maydis. In C. miyabeanus, inactivating the PPT1 gene resulted in a 10-fold increase in the production of 76. This means that inhibiting polyketide or non-ribosomal peptide biosynthesis, or both, promotes the production of sesterterpene metabolites [48].

In 2017, Mitsuhashi et al. predicted two new PTTSs, PbSS and PVPs, from P. brasili anum and P. verruculosum. The heterologous expression of these two genes in A. oryzae produced sesterbrasili triene (34) and preasperterpenoid A (33), respectively [37]. In 2019, Hang et al. discovered a sesterterpene BGC containing three genes from the genome of Talaromyces wortmannii (Figure 8A). The subsequent recombination of this BGC in A. oryzae revealed that AsTC encodes a sesterterpene cyclase that is also capable of synthesizing preasperterpenoid A (33). AsTC has a high degree of homology with TvPS, PvPS, and TtPS, with an identity of at least 37%. The CYP450 oxidase AsTB then multioxidizes compound 33 to produce a new asperterpenoid A (77) and byproduct asperterpenoid B (78). Compound 77 continued to be further oxidized by another CYP450 oxidase, astA, to a new sesterterpenoid, asperterpenoid C (79) (Figure 8B) [38].
Figure 8. The BGC for asperterpenoids (A) and its biosynthetic pathway (B).

Gao et al. discovered two silent BGCs involved in the biosynthesis of betaestacin I (24) in two plant pathogenic fungi, P. betae and C. orbulare, in 2018. Both BGCs contain a PTTS (BtcA\textsubscript{Pb} / BtcA\textsubscript{Do}) and two CYP450 oxidases (Figure 9A), and the heterologous expression of BtcA\textsubscript{Pb} or BtcA\textsubscript{Do} results in the production of betaestacin I (24). There are two pairs of homologous CYP450s in these two BGCs. The first pair of CYP450s, BtcB\textsubscript{Pb} and BtcB\textsubscript{Do}, catalyzed multiple oxidations of 24 with varying degrees of oxidation. The compound 24 is further oxidized by btcB\textsubscript{Pb} to betaestacin II (80) in the Btc BGC pathway from P. betae, whereas 24 is oxidized by btcB\textsubscript{Do} to betaestacin III (81), and further oxidized to IV (82) in the btcC\textsubscript{Do} pathway. The modifications of the second pair of homologous CYP450s, BtcC\textsubscript{Pb} and BtcC\textsubscript{Do}, were different. BtcC\textsubscript{Pb} was unable to oxidize 80 further, whereas btcC\textsubscript{Do} catalyzed the multistep oxidation of 82, yielding four new sesterterpenoids, betaestacinVa-c (83–85) and VI (86) (Figure 9B) [35].

Figure 9. The BGC for betaestacins (A) and their biosynthetic pathway (B).
During genome mining of B. maydis in 2018, Narita et al. discovered a BGC consisting of four genes (Figure 10A). The heterologous expression of the bifunctional terpene synthase (TpCA) in A. oryzae produces preterpestacin I (23). The compound 23 is hydroxylated regioselectively by CYP450 (TpCB) to produce preterpestacin II (87), which is then further oxidized to the carboxyl group to form 88. The two-step oxidation of 87 catalyzed by a second cytochrome P450 (TpCC) yields vicinal diol moiety on the α-ring to form preterpinomycin III (89). The oxidation of the two hydroxyl groups on the A-ring of 89 is catalyzed by flavin-dependent oxidase (TpCD) to form a diketone, which forms 90 via a keto-enol interchange [49].

FgMS is a PTTS from F. graminearum responsible for the production of 25 and was identified by Bian et al. in 2017 [32]. Yuan et al. conducted a deeper excavation of the genome to reveal a BGC (Figure 11A) containing FgMS and characterized the BGC with the help of the efficient A. oryzae chassis, showing that this BGC is responsible for the synthesis of mangicols. The co-expression of mgcD (FgMS) and mgcCE produced mangicols A–E and H–J (91–98), and mangicols K–L (99–100) were obtained by introducing mgcF into the transformant of mgcCDE (Figure 11B) [36]. McgE is a multifunctional CYP450 capable of catalyzing hydroxylation, epoxidation, and carbonylation reactions at the isoprene tail (C17–C20) of mangicadiene, and is widely involved in the formation of 91, 93–94, 96–98 (Figure 11B). MgF is another multifunctional P450 that catalyzes hydroxylation at C7 or C8, respectively (Figure 11B) [36]. Given the excellent anti-inflammatory activity of 98, multiple copies of thmGI and mgcCE were randomly inserted into the host chromosome to increase the yield to 12.09 mg/L, a 151-fold increase compared to the initial heterologous expression strain [36].
4. Discussion

A total of 52 PTTSs were identified from 2013–2022, and most of them were functionally validated by heterologous expression using *A. oryzae*, *E. coli*, or *S. cerevisiae*. The heterologous expression enables PTTSs to generate a large number of new sesterterpenes, many of which have novel structural backbones. In fact, there are also numerous biosynthetic pathways for sesterterpenes in fungi that have not yet been identified. Most of these compounds have significant physiological activities, such as 1 with an anti-cancer effect [4], 2–6 with cytotoxicity [5], 7 and 8 with antibacterial activity [6,7], 10–13 with anti-Parkinsonian and anti-inflammatory properties [9], as well as 14 with a cholesterol-lowering effect [11]. These sesterterpenoids have potential applications in clinical treatment.

In recent years, the ongoing advancement of metabolomics and genome mining technologies has made it much easier to find sesterterpene synthases. Additional oxidative modifications such as cytochrome P450 provide further structural diversity of natural sesterterpenes. The rapid advancement of sesterterpene synthase research will be facilitated by the high-efficiency *A. oryzae* heterologous expression system for the targeted insertion of foreign genes into high expression sites [50], and the high-throughput automated protoplast transformation platform [36]. Although the chemical synthesis of some sesterterpenes with distinctive structures and intricate frameworks is challenging, heterologous biosynthesis using microorganisms as the chassis can produce target molecules quickly and effectively. The development of natural product chemistry will also be influenced by synthetic biology, which employs biosynthesis to create highly valuable sesterterpenes in medicine, health, and food chemistry.

Author Contributions: P.Z. and C.L. designed the manuscript; P.Z., J.Q. and Y.D. collected and analyzed data; P.Z. and J.Q. wrote this manuscript; J.-m.G. and C.L. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the National Natural Science Foundation of China (No. 21877089 and 31800031), the Innovation and Development Joint Fund of the Natural Science Foundation of Shandong Province (Project no. ZR2021LSW022), and the Shaanxi Key Laboratory of Natural Product & Chemical Biology Open Foundation (Project no. SXBPCB 2021001).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This review paper is dedicated to Prof. Hideaki Oikawa on the 10th anniversary of his discovery the first sesterterpene synthase. We acknowledge his pioneering work in the field of fungal natural product biosynthesis.

Conflicts of Interest: Not applicable.

References

1. Lauterbach, L.; Rinkel, J.; Dicktschat, J.S. Two Bacterial Diterpene Synthases from *Allokuutzneria albata* Produce Bonnadiene, Phomopsene, and Allokuutznerene. *Angew. Chem. Int. Ed. Engl.* 2018, 57, 8280–8283. [CrossRef] [PubMed]
2. Shirley, H.J.; Jamieson, M.L.; Brimble, M.A.; Bray, C.D. A new family of sesterterpenoids isolated around the Pacific Rim. *Nat. Prod. Rep.* 2018, 35, 210–219. [CrossRef] [PubMed]
3. Li, K.; Gustafson, K.R. Sesterterpenoids: Chemistry, biology, and biosynthesis. *Nat. Prod. Rep.* 2021, 38, 1251–1281. [CrossRef] [PubMed]
4. Hoque, N.; Hasan, C.M.; Rana, M.S.; Varsha, A.; Sohrab, M.H.; Rahman, K.M. Fusaproliferin, a Fungal Mycotoxin, Shows Cytotoxicity against Pancreatic Cancer Cell Lines. *Molecules* 2018, 23, 3288. [CrossRef] [PubMed]
5. Choi, B.K.; Trinh, P.T.H.; Lee, H.S.; Choi, B.W.; Kang, J.S.; Ngoc, N.T.D.; Van, T.T.T.; Shin, H.J. New Ophiobolin Derivatives from the Marine Fungus *Aspergillus flaccidus* and Their Cytotoxicities against Cancer Cells. *Mar. Drugs* 2019, 17, 346. [CrossRef] [PubMed]
6. Wang, Q.X.; Yang, J.L.; Qi, Q.Y.; Bao, L.; Yang, X.L.; Liu, M.M.; Huang, P.; Zhang, L.X.; Chen, J.L.; Cai, L.; et al. 3-Anhydro-6-hydroxy-ophiobolin A, a new sesterterpene inhibiting the growth of methicillin-resistant *Staphylococcus aureus* and inducing the cell death by apoptosis on K562, from the phytopathogenic fungus *Bipolaris oryzae*. Bioorg. Med. Chem. Lett. 2013, 23, 3547–3550. [CrossRef]

7. Wang, Q.X.; Bao, L.; Yang, X.L.; Liu, D.L.; Guo, H.; Dai, H.Q.; Song, F.H.; Zhang, L.X.; Guo, L.D.; Li, S.J.; et al. Ophiobolins P–T, five new cytotoxic and antibacterial sesterterpenes from the endophytic fungus *Ulocladium sp.* Fitorpetra 2013, 90, 220–227. [CrossRef][PubMed]

8. Liu, M.-T.; He, Y.; Shen, L.; Hu, Z.-X.; Zhang, Y.-H. Bipolarins A–H, eight new ophiobolin-type sesterterpenes with antimicrobial activity from fungus *Bipolaris sp.* TJ403-B1. Chin. J. Nat. Med. 2019, 17, 935–944. [CrossRef]

9. Cai, R.; Jiang, H.; Mo, Y.; Guo, H.; Li, C.; Long, Y.; Zang, Z.; She, Z. Ophiobolin-Type Sesterterpenoids from the Mangrove Endophytic Fungus *Aspergillus sp.* ZJ-68. J. Nat. Prod. 2019, 82, 2268–2278. [CrossRef]

10. Liu, M.; Sun, W.; Shen, L.; Hao, X.; Al Anbari, W.H.; Lin, S.; Li, H.; Gao, W.; Wang, J.; Hu, Z.; et al. Bipolaricins A–I, Ophiobolin-Type Tetracyclic Sesterterpenes from a Phytopathogenic *Bipolaris* sp. Fung. J. Nat. Prod. 2019, 82, 2897–2906. [CrossRef][PubMed]

11. Liu, M.; Sun, W.; Shen, L.; He, Y.; Liu, J.; Wang, J.; Hu, Z.; Zhang, Y. Bipolarolides A–G: Ophiobolin-Derived Sesterterpenes with Three New Carbon Skeletons from *Bipolaris* sp. TJ403-B1. Angew. Chem. Int. Ed. Engl. 2019, 58, 12091–12095. [CrossRef]

12. Chen, Q.; Li, J.; Ma, Y.; Yuan, W.; Zhang, P.; Wang, G. Occurrence and biosynthesis of plant sesterterpenes (C25), a new addition to terpene diversity. Plant Commun. 2021, 2, 100184. [CrossRef][PubMed]

13. Shao, J.; Chen, Q.W.; Lv, H.J.; He, J.; Liu, Z.F.; Lu, Y.N.; Liu, H.L.; Wang, G.D.; Wang, Y. (+)-Thalianatriene and (-)-Retigeranin B Catalyzed by Sesterterpene Synthases from *Arabidopsis thaliana*. Org. Lett. 2019, 21, 1816–1819. [CrossRef][PubMed]

14. Chen, Y.G.; Li, D.S.; Ling, Y.; Liu, Y.C.; Zuo, Z.L.; Gan, L.S.; Luo, S.H.; Hua, J.; Chen, D.Y.; Xu, F.; et al. A Cryptic Plant Terpene Cyclase Producing Unconventional 18- and 14-Membered Macrocyclic C25 and C20 Terpenoids with Immunosuppressive Activity. Angew. Chem. Int. Ed. Engl. 2021, 60, 25468–25476. [CrossRef][PubMed]

15. Hou, A.; Dickschat, J.S. The Biosynthetic Gene Cluster for Sestermobaranes-Discovery of a Geranylfernenol Diphosphate Synthase and a Multisubstrate Sesterterpene Synthase from *Streptomyces moehringiae*. Angew. Chem. Int. Ed. Engl. 2020, 59, 19961–19965. [CrossRef]

16. Chiba, R.; Minami, A.; Gomi, K.; Oikawa, H. Identification of Ophiobolin F Synthase by a Genome Mining Approach: A Sesterterpene Synthase from *Aspergillus clavatus*. Org. Lett. 2013, 15, 594–597. [CrossRef]

17. Minami, A.; Ozaki, T.; Liu, C.; Oikawa, H. Sesterterpene Biosynthesis: Cyclization Mechanisms and Oxidative Modifications. In *Comprehensive Natural Products III*, 3rd ed.; Elsevier: Amsterdam, The Netherlands, 2019; pp. 553–576.

18. Shinde, S.S.; Minami, A.; Chen, Z.; Tokiwano, T.; Toyomatsu, T.; Kato, N.; Sassa, T.; Oikawa, H. Cyclization mechanism of pompeosyne synthase: Mass spectrometry based analysis of various site-specifically labeled terpenes. *J. Antibiot.* 2017, 70, 632–638. [CrossRef]

19. Pemberton, T.A.; Chen, M.; Harris, G.G.; Chou, W.K.; Duan, L.; Koskal, M.; Genshaft, A.S.; Cane, D.E.; Christianson, D.W. Exploring the Influence of Domain Architecture on the Catalytic Function of Diterpene Synthases. *Biochemistry* 2017, 56, 2010–2023. [CrossRef]

20. Toyomasu, T.; Tsukahara, M.; Kaneko, A.; Niida, R.; Mitsushashi, W.; Dairi, T.; Kato, N.; Sassa, T. Fusccocins are biosynthesized by an unusual chimera diterpene synthase in fungi. *Proc. Natl. Acad. Sci. USA* 2007, 104, 3084–3088. [CrossRef][PubMed]

21. Chai, H.; Yin, R.; Liu, Y.; Meng, H.; Zhou, X.; Zhou, G.; Bi, X.; Yang, X.; Zhu, T.; Zhu, W.; et al. Sesterterpene ophiobolin biosynthesis involving multiple gene clusters in *Aspergillus ustus*. *Sci. Rep.* 2016, 6, 2781. [CrossRef]

22. Yuan, W.; Lv, S.; Chen, L.; Zhao, Y.; Deng, Z.; Hong, K. Production of sesterterpene ophiobolin by a bifunctional terpene synthase in *Escherichia coli*. *Appl. Microbiol. Biotechnol.* 2019, 103, 8785–8797. [CrossRef]

23. Qin, B.; Matsuda, Y.; Mori, T.; Okada, M.; Quan, Z.; Mitsushashi, T.; Wakimoto, T.; Abe, I. An Unusual Chimeric Diterpene Synthase from *Emergilla variecolor* and Its Functional Conversion into a Sesterterpene Synthase by Domain Swapping. *Angew. Chem. Int. Ed. Engl.* 2016, 55, 1658–1661. [CrossRef]

24. Matsuda, Y.; Mitsushashi, T.; Quan, Z.; Abe, I. Molecular Basis for Stelleral Acid Biosynthesis: A Genome Mining Approach for Discovery of Sesterterpene Synthases. *Org. Lett.* 2015, 17, 4644–4647. [CrossRef]
30. Matsuda, Y.; Mitsuhashi, T.; Lee, S.; Hoshino, M.; Mori, T.; Okada, M.; Zhang, H.; Hayashi, F.; Fujita, M.; Abe, I. Astellifadiene: Structure Determination by NMR Spectroscopy and Crystalline Sponge Method, and Elucidation of its Biosynthesis. Angew. Chem. Int. Ed. Engl. 2016, 55, 5785–5788. [CrossRef] [PubMed]

31. Narita, K.; Sato, H.; Minami, A.; Kudo, K.; Gao, L.; Liu, C.; Ozaki, T.; Kodama, M.; Lei, X.; Taniguchi, T.; et al. Focused Genome Mining of Structurally Related Sesterterpenes: Enzymatic Formation of Enantiomeric and Diastereomeric Products. Org. Lett. 2017, 19, 6696–6699. [CrossRef]

32. Bian, G.; Han, Y.; Hou, A.; Yuan, Y.; Liu, X.; Deng, Z.; Liu, T. Releasing the potential power of terpene synthases by a robust precursor supply platform. Metab. Eng. 2017, 42, 1–8. [CrossRef] [PubMed]

33. Chen, R.; Jia, Q.; Mu, X.; Hu, B.; Sun, X.; Deng, Z.; Chen, F.; Bian, G.; Liu, T. Systematic mining of fungal chimeric terpene syntheses using an efficient precursor-providing yeast chassis. Proc. Natl. Acad. Sci. USA 2021, 118, e2023247118. [CrossRef]

34. Jiang, L.; Yang, H.; Zhang, X.; Li, X.; Lv, K.; Zhang, W.; Zhu, G.; Liu, C.; Wang, Y.; Hsiang, T.; et al. Schuiltriene and niglitraene: Two sesterterpenes characterized from pathogenetic fungi via genome mining approach. Appl. Microbiol. Biotechnol. 2022, 106, 6047–6057. [CrossRef]

35. Gao, L.; Narita, K.; Ozaki, T.; Kumakura, N.; Gan, P.; Minami, A.; Liu, C.; Lei, X.; Shirasu, K.; Oikawa, H. Identification of novel sesterterpenes by genome mining of phytopathogenic fungi Phoma and Colletotrichum sp. Tetrahedron. Lett. 2018, 59, 1136–1139. [CrossRef]

36. Yuan, Y.; Cheng, S.; Bian, G.; Yan, P.; Ma, Z.; Dai, W.; Chen, R.; Fu, S.; Huang, H.; Chi, H.; et al. Efficient exploration of terpenoid biosynthetic gene clusters in filamentous fungi. Nat. Catal. 2022, 5, 277–287. [CrossRef]

37. Mitsuhashi, T.; Rinkel, J.; Okada, M.; Abe, I.; Dickshatt, J.S. Mechanistic Characterization of Two Chimeric Sesterterpene Synthases from Penicillium. Chemistry 2017, 23, 10053–10057. [CrossRef]

38. Huang, J.H.; Lv, J.M.; Wang, Q.Z.; Zou, J.; Lu, Y.J.; Wang, Q.L.; Chen, D.N.; Yao, X.S.; Gao, H.; Hu, D. Biosynthesis of an anti-tuberculosis sesterterpenoid asperterpenoid A. Org. Biomol. Chem. 2019, 17, 248–251. [CrossRef]

39. Okada, M.; Matsuda, Y.; Mitsuhashi, T.; Hoshino, S.; Mori, T.; Nakagawa, K.; Quan, Z.; Qin, B.; Zhang, H.; Hayashi, F.; et al. Genome-Based Discovery of an Unprecedented Cyclization Mode in Fungal Sesterterpenoid Biosynthesis. J. Am. Chem. Soc. 2016, 138, 10011–10018. [CrossRef]

40. Quan, Z.; Dickshatt, J.S. Biosynthetic Gene Cluster for Asperterpenols A and B and the Cyclization Mechanism of Asperterpenol A Synthase. Org. Lett. 2020, 22, 7552–7555. [CrossRef]

41. Guo, J.; Cai, Y.S.; Cheng, F.; Yang, C.; Zhang, W.; Yu, W.; Yan, J.; Deng, Z.; Hong, K. Genome Mining Reveals a Multipurpose Sesterterpenoid Biosynthetic Gene Cluster in Aspergillus Ustus. Org. Lett. 2021, 23, 1525–1529. [CrossRef]

42. Jiang, L.; Zhang, X.; Sato, Y.; Zhu, G.; Minami, A.; Zhang, W.; Ozaki, T.; Zhu, B.; Wang, Z.; Wang, X.; et al. Genome-Based Discovery of Enantiomeric Pentacyclic Sesterterpenes Catalyzed by Fungal Bifunctional Terpene Synthases. Org. Lett. 2021, 23, 4645–4650. [CrossRef] [PubMed]

43. Clevenger, K.D.; Bok, J.W.; Ye, R.; Miley, G.P.; Verdan, M.H.; Velk, T.; Chen, C.; Yang, K.; Robey, M.T.; Gao, P.; et al. A scalable platform to identify fungal secondary metabolites and their gene clusters. Nat. Chem. Biol. 2017, 13, 895–901. [CrossRef] [PubMed]

44. Qiao, Y.; Xu, Q.; Huang, Z.; Chen, X.; Ren, X.; Yuan, W.; Guan, Z.; Li, P.; Li, F.; Xiong, C.; et al. Genome Mining Reveals a New Cyclopentane-forming Sesterterpene Synthase with Unprecedented Stereo-control. Org. Chem. Front. 2022, 425–463. [CrossRef]

45. Sato, H.; Narita, K.; Minami, A.; Yamazaki, M.; Wang, C.; Suemune, H.; Nagano, S.; Tomita, T.; Oikawa, H.; Uchiyama, M. Theoretical Study of Sesterfisherol Biosynthesis: Computational Prediction of Key Amino Acid Residue in Terpene Synthase. Sci. Rep. 2018, 8, 2473. [CrossRef]

46. Sato, H.; Mitsuhashi, T.; Yamazaki, M.; Abe, I.; Uchiyama, M. Inherent atomic mobility changes in carbocation intermediates during the sesterterpene cyclization cascade. Beilstein. J. Org. Chem. 2019, 15, 1890–1897. [CrossRef]

47. Narita, K.; Chiba, R.; Minami, A.; Kodama, M.; Fujii, I.; Gomi, K.; Oikawa, H. Multiple Oxidative Modifications in the Ophiobolin Biosynthesis: P450 Oxidations Found in Genome Mining. Org. Lett. 2016, 18, 1980–1983. [CrossRef]

48. Zainudin, N.A.; Condon, B.; De Bruyne, L.; Van Poucke, C.; Bi, Q.; Li, W.; Hofte, M.; Turgeon, B.G. Virulence, Host-Selective Toxin Production, and Development of Three Cochliobolus Phytopathogens Lacking the Sfp-Type 4'-Phosphopantetheinyl Transferase Ppt1. Mol. Plant Microbe. Interact. 2015, 28, 1130–1141. [CrossRef]

49. Narita, K.; Minami, A.; Ozaki, T.; Liu, C.; Kodama, M.; Oikawa, H. Total Biosynthesis of Antiangiogenic Agent (-)-Terpestacin by Artificial Reconstitution of the Biosynthetic Machinery in Aspergillus oryzae. J. Org. Chem. 2018, 83, 7042–7048. [CrossRef]

50. Liu, C.; Minami, A.; Ozaki, T.; Wu, J.; Kawagishi, H.; Maruyama, J.-I.; Oikawa, H. Efficient Reconstitution of Basidiomycota Diterpene Erinacine Gene Cluster in Ascomycota Host Aspergillus oryzae Based on Genomic DNA Sequences. J. Am. Chem. Soc. 2019, 141, 15519–15523. [CrossRef]