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MINI-REVIEW

Polyketides in *Aspergillus terreus*: biosynthesis pathway discovery and application

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Abstract The knowledge of biosynthesis gene clusters, production improving methods, and bioactivity mechanisms is very important for the development of filamentous fungi metabolites. Metabolic engineering and heterologous expression methods can be applied to improve desired metabolite production, when their biosynthesis pathways have been revealed. And, stable supplement is a necessary basis of bioactivity mechanism discovery and following clinical trial. *Aspergillus terreus* is an outstanding producer of many bioactive agents, and a large part of them are polyketides. In this review, we took polyketides from *A. terreus* as examples, focusing on 13 polyketide synthase (PKS) genes in *A. terreus* NIH 2624 genome. The biosynthesis pathways of nine PKS genes have been reported, and their downstream metabolites are lovastatin, terreic acid, terrein, geodin, terretonin, citreoviridin, and asperfuranone, respectively. Among them, lovastatin is a well-known hypolipidemic agent. Terreic acid, terrein, citreoviridin, and asperfuranone show good bioactivities, especially anticancer activities. On the other hand, geodin and terretonin are mycotoxins. So, biosynthesis gene cluster information is important for the production or elimination of them. We also predicted three possible gene clusters that contain four PKS genes by homologous gene alignment with other *Aspergillus* strains. We think that this is an effective way to mine secondary metabolic gene clusters.

Keywords Polyketide · *Aspergillus terreus* · Biosynthesis pathway · Gene cluster · Heterologous expression

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Introduction

Filamentous fungi are talented producers used for thousands of years, and they have attracted increasing attention over the past half century (Papagianni 2004). In East Asia, there are lots of traditional fermented food, for example, *Aspergillus oryzae* is used in rice wine production (Zhang et al. 2012), and *Monascus* is used in the production of fermented bean curd (Jiang et al. 2011). Purified fungal metabolites can be important medicines. Penicillin and cephalosporin (Abraham et al. 1953; Newton and Abraham 1955), two famous antibiotics derived from filamentous fungi, have saved the lives of many infected people. Hypolipidemic agent lovastatin produced by *Aspergillus terreus* (Askenazi et al. 2003) and immunosuppressant cyclosporine A produced by *Tolypocladium inflatum* (Survase et al. 2011) are also typical pharmaceutical products from filamentous fungi. Nowadays, more and more novel natural products have been discovered from filamentous fungi. In the latest report, over 400 new compounds were derived from marine fungi in 2014 (Blunt et al. 2016). However, there is a long way between new compound and pharmaceutical agent. The compound must be stably supplied to meet the need of bioactivity assay and following clinical trial (Leal et al. 2014). And to produce a compound more

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effectively, we should better figure out its biosynthesis pathway. In a word, in the way from new compound to pharmaceutical agent, much more need to be known besides its structure. On the other hand, these information will also be helpful in the re-evaluation of known compounds.

In this review, we take polyketides from *A. terreus* as examples and summarize the information of their bioactivities, biosynthesis pathways, and production enhancement methods. This knowledge is helpful for the utilization of this filamentous fungus. As mentioned above, *A. terreus* has a famous polyketide product lovastatin; moreover, many bioactive polyketides have been isolated from *A. terreus*, such as terreic acid, terrein, geodin, terretonin, citreoviridin, asperfuranone, and so on. Additionally, the strain *A. terreus* NIH 2624 was sequenced by the Broad Institute as part of the Broad Fungal Genome Initiative in 2005, and the data are publicly available through the Broad Institute and National Center for Biotechnology Information (NCBI) website (Guo and Wang 2014). Completed genomic information assists researchers to find out the polyketide pathways in *A. terreus*. It was reported that there were 28 polyketide synthase (PKS) genes, one hybrid PKS/nonribosomal peptide synthetase (NRPS) gene, and two PKS-like genes in *A. terreus* NIH 2624 genome (Khaldi et al. 2010). Here, we will focus on 13 PKS genes, corresponding gene clusters, and their related secondary metabolites (see Table 1).

Lovastatin

Hypolipidemic agent lovastatin works as inhibitor of (3*S*)-hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase (HMGR), that reduces HMG-CoA to mevalonate, which is the key step in cholesterol biosynthesis. Furthermore, statins were found to be anticancer agents (Chan et al. 2003). Malignant gliomas are highly dependent on the mevalonate pathway for the synthesis of lipid moieties critical to cell replication; thus, it is uniquely vulnerable to the growth arrest by lovastatin (Prasanna et al. 1996). In a latest study, lovastatin also exerts anticancer effect on the triple-negative breast cancer cell line MDA-MB-231 (Yang et al. 2016). So, lovastatin may have other applications besides lowering cholesterol.

The lovastatin (**3**) cluster has been well summarized. There are two highly reducing PKSs (HR-PKSs), LovB (lovastatin nonaketide synthase, ATEG_09961) and LovF (lovastatin diketide synthase, ATEG_09968), in its cluster (Hill 2006; Cox 2007; Campbell and Vederas 2010; Chiang et al. 2010a; Chooi and Tang 2012). As shown in Fig. 1a, dihydromonacolin L acid (**1**) is produced by LovB and a trans-acting enoyl reductase (ER) LovC (ATEG_09963), then released by a thioesterase (TE) LovG (ATEG_09962) (Xu et al. 2013). Next, dihydromonacolin L acid is oxidized by LovA (ATEG_09960) twice to form monacolin J acid (**2**). 2-Methylbutyrate moiety of lovastatin is synthesized by another HR-PKS LovF, and the covalent attachment of this moiety to **2** is catalyzed by a transesterase LovD (ATEG_09964) (Guo and Wang 2014). Nowadays, lovastatin is not the most frequently consumed statin; some semisynthetic statins like simvastatin are much more popular. Since simvastatin differs from lovastatin in the side chain and LovD is responsible for the attachment of side chain to monacolin J acid, thus the efforts to engineer LovD were carried out (Jiménez-Osés et al. 2014). A mutant LovD containing 29 point mutations was generated by a directed evolution strategy; consequently, the protein efficiency in the synthesis of simvastatin was improved by about 1000-fold. Therefore, the knowledge of biosynthesis pathway is not only constructive for the production enhancement of its own product but also can facilitate connected semisynthetic drug production.

Terreic acid and 6-methylsalicylic acid

Terreic acid shows antibacterial activity (Yamamoto et al. 1980). Bacterial cell wall biosynthetic enzymes UDP-*N*-acetylglucosamine 1-carboxyvinyltransferase (MurA) (Schönbrunn et al. 2010) and *N*-acetylglucosamine-1-

Table 1 Seven reported PKS gene clusters in *A. terreus* NIH 2624

PKS gene	Biosynthetic gene cluster	Downstream metabolites
ATEG_09961 ATEG_09968	ATEG_09960–09964, 09966, 09968	Lovastatin
ATEG_06275	ATEG_06272–06278, 06280	Terreic acid
ATEG_00145	ATEG_00135–00145	Terrein
ATEG_08451	ATEG_08449–08460	Geodin
ATEG_10080	ATEG_10077–10086	Terretonin
ATEG_09617	ATEG_09616–09620	Citreoviridin
ATEG_07659 ATEG_07661	ATEG_07659–07667	Asperfuranone

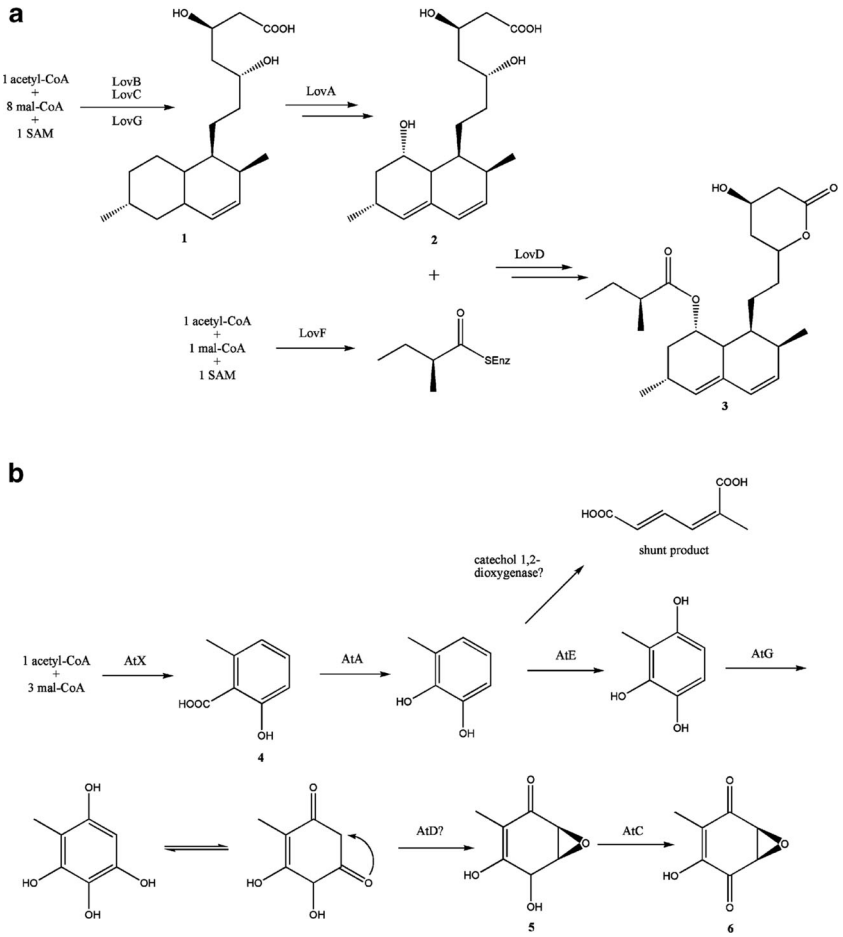

Fig. 1 Biosynthesis of lovastatin (**a**) and terreic acid (**b**) in *A. terreus* NIH 2624

phosphate-uridyltransferase/glucosamine-1-phosphate-acetyltransferase (GlmU) (Sharma et al. 2016) were found to be its molecular targets. But, its selective inhibition against the catalytic activity of Bruton's tyrosine kinase (Btk) attracted more attention (Kawakami et al. 1999), so it could be a chemical probe to examine the function of Btk and a potential agent for rheumatoid arthritis, lymphoma, and other diseases (Pan 2008).

About half a century ago, isotope labeling experiments showed that the biosynthesis of terreic acid (**6**) originated from 6-methylsalicylic acid (6-MSA, **4**) (Read and Vining 1968). And, 6-MSA synthase (6-MSAS) gene is one of the best characterized fungal PKS gene. This partly reducing PKS (PR-PKS) was first reported in *Penicillium patulum* (Dimroth et al. 1970). In *A. terreus* NIH 2624, the homologous gene is *atX* (ATEG_06275) (Fujii et al. 1996). The heterologous expression of 6-MSA can be a judgment standard for the expression system of fungal polyketides. For instance, in the recombinant *Pichia pastoris* GS115-NpgA-ATX with *Aspergillus nidulans* and phosphopantetheinyl transferase gene *npgA* and *atX*, 2.2 g l^{-1} production of 6-MSA could be obtained, which suggested that *P. pastoris* was a potential host (Gao et al. 2013). In 2014, Boruta and Bizukojc (2014) speculated the terreic acid cluster by bioinformatic analysis, and it was finally experimentally characterized by Guo et al. (2014) by using a targeted gene deletion method in the same year. The cluster contains a total of eight genes: *atA–G* and *X* (ATEG_06272–06278, 06280). As shown in Fig. 1b, AtA (ATEG_06272) is a hypothetical salicylate hydroxylase and catalyzes a decarboxylative hydroxylation of 6-MSA. Sometimes, this catechol-type intermediate will be oxidatively ring-opened and results in a shunt product (2*Z*,4*E*)-2-methyl-2,4-hexadienedioic acid. AtE (ATEG_06277) and AtG (ATEG_06280) are cytochrome P450 monooxygenases, possibly catalyze further hydroxylation. And, the hydroxylation may trigger intramolecular rearrangement like the condition in fungal tropolone biosynthesis (Davison et al. 2012). The function of AtD (ATEG_06276) is still unknown, but terreic acid cannot be biosynthesized without the presence of AtD, so maybe, it is involved in the epoxidation to gain terremutin (**5**). At last, GMC oxidoreductase AtC (ATEG_06274) catalyzes the oxidation of **5** to give the final product terreic acid (**6**). AtF (ATEG_06278) and AtB (ATEG_06273) are transcription factor and major facilitator superfamily (MFS) transporter, respectively.

Terrein

Terrein shows potential anticancer activities in a series of researches, inhibiting multidrug-resistant breast cancer (Liao et al. 2012), cervical carcinoma (Porameesanaporn et al. 2013), prostate cancer (Arakawa et al. 2008), ovarian cancer (Chen et al. 2014), and hepatoma (Zhang et al. 2015). And, it has several different mechanisms, including inhibiting angiogenin secretion, inducing apoptosis, and cell cycle arrest.

Terrein (**9**) is a polyketide metabolite, which was confirmed by isotope labeling study in 1960s (Birch et al. 1965), and its precursor 6-hydroxymellein (6-HM) had also been identified that way (Hill et al. 1981). On the other hand, the terrein cluster (ATEG_00135–00145) was serendipitously characterized in the effort to explore the biosynthesis pathway of conidial pigment in *A. terreus* recently (Zaehle et al. 2014). As shown in Fig. 2, the nonreducing PKS (NR-PKS) gene *terA* (ATEG_00145) has flexibility in controlling the length of the polyketide chain. Three different metabolites could be obtained in the heterologous expression of *terA* in *Aspergillus niger* (Zaehle et al. 2014); among them, 2,3-dehydro-6-HM (**7**) is a pentaketide like 6-HM (**8**). The formation of **8** needs another protein TerB (ATEG_00144), which is a multidomain protein with ketoreductase (KR) and dehydratase (DH) functions. When C-9 ketone is reduced by TerB, **8** can be easily derived by lactonization, while the cyclization in **7** is a bit different, which may need an intramolecular rearrangement that occurs in the biosynthesis of citrinin in *Monascus* (He and Cox 2016). The dehydration may also be catalyzed by TerB. TerC-F (ATEG_00140–00143) may be involved in the unusual contraction of the aryl ring, but the specific roles of these genes await further verification. We speculate that FAD-dependent monooxygenases TerC and TerD (ATEG_00142–143) catalyze hydroxylation and trigger oxidative dearomatization, similar to the key step of sorbicillinoid and tropolone biosynthesis (Davison et al. 2012; Fahad et al. 2014), also somewhat like the aforementioned condition in terreic acid biosynthesis. Hill et al. (1981) suggested that a ring contraction then happened, in contrast to the ring expansion in tropolone biosynthesis (Davison et al. 2012). Lastly, the extra carbon atoms may be removed by decarboxylation. TerG and TerJ (ATEG_00135, 00138) are MFS transporters, and the function of TerH–I (ATEG_00136–00137) is not clear. Terrein production will decrease but not disappear in the absence of them. Amusingly, we find a similar gene cluster (GAQ03937–03946) in the genome of *Aspergillus lentulus* strain IFM 54703, which also contains these unnecessary genes.

Enough supply of glucose and citrate, which are favorable for polyketide biosynthesis, can improve terrein production (Yin et al. 2012, 2013). Furthermore, terrein is thought to play a role in fungus-plant interactions and ecological competition.

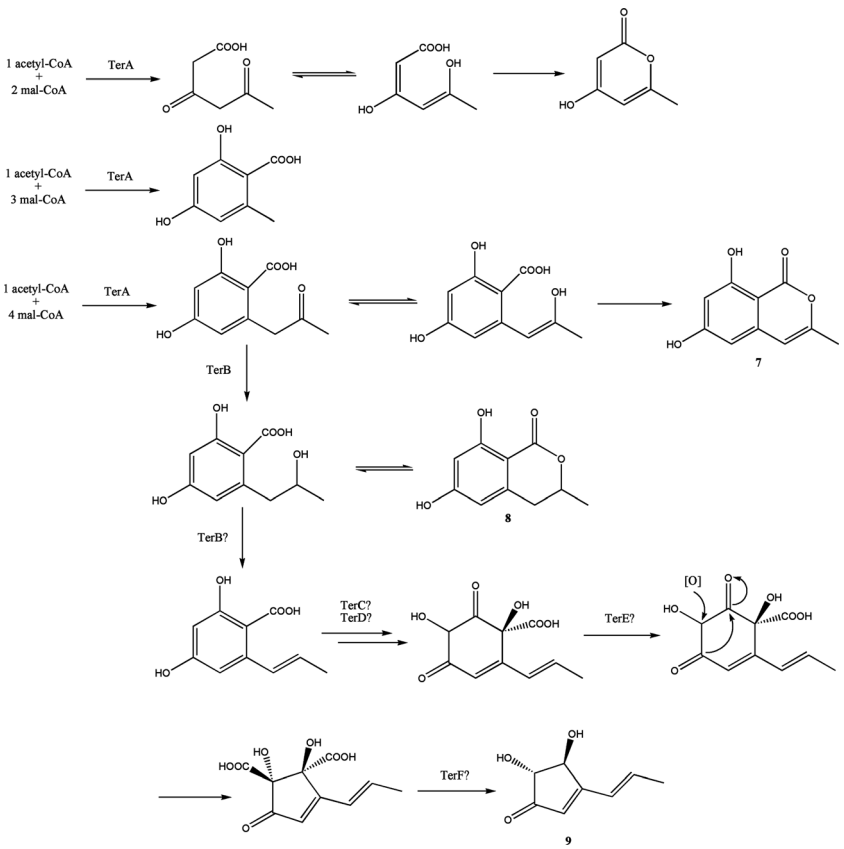

Fig. 2 Biosynthesis of terrein in *A. terreus* NIH 2624

The nitrogen response regulators AreA and AtfA and the iron response regulator HapX are upstream regulators of the terrein transcription factor TerR (ATEG_00139), which can regulate terrein biosynthesis (Gressler et al. 2015a). High production of terrein was achieved in two marine *A. terreus* strains, which were 7~8 g l^{-1} in shake flask (Yin et al. 2015; Zhao et al. 2016) and 9 g l^{-1} in bioreactor (Xiao et al. 2013). Because of the high yield of terrein, the transcription factor TerR is also thought to be a new high-performance heterologous fungal expression system regulatory element (Gressler et al. 2015b).

Geodin and emodin

Geodin is a kind of mycotoxin (Bräse et al. 2009) that always simultaneously biosynthesized with lovastatin (Bizukojc and Ledakowicz 2007; Abd Rahim et al. 2015). Since they are both polyketides, modification of media does not have a good effect on removing geodin; the knowledge of their gene clusters can offer a better guidance.

A. terreus produces series of yellow anthraquinone pigments, such as emodin and questin, but these yellow pigments disappeared when a NR-PKS gene was deleted (Couch and Gaucher 2004). In *A. terreus* NIH 2624, this PKS (ATEG_08451) was then named as atrochrysone carboxylic acid synthase (ACAS), and the polyketide product needs to be released with the aid of atrochrysone carboxyl ACP thioesterase (ACTE) encoded by ATEG_08450 (Awakawa et al. 2009). A small amount of emodin (**11**) could be obtained from in vitro reactions catalyzed by ACAS and ACTE, while the main product was atrochrysone (**10**), which was probably derived by spontaneous decarboxylation of the polyketide product. Much more emodin was gained from in vivo heterologous expression experiment in *A. oryzae*, which suggested that more genes should be involved in emodin biosynthesis.

In fact, emodin is also an intermediate; many other metabolites can be derived from this polyketide pathway, such as questin (**12**), sulochrin (**13**), geodin (**15**), and asterric acid (Couch and Gaucher 2004; Boruta and Bizukojc 2016). In 2013, Nielsen et al. (2013) transferred putative geodin gene cluster (ATEG_08449–08460) from *A. terreus* NIH 2624 to an expression platform in *A. nidulans* and gained geodin successfully. Proposed biosynthesis pathway for geodin is shown in Fig. 3a. Except ACAS (GedC) and ACTE (GedB), dihydrogeodin oxidase GedJ (ATEG_08458) that catalyzed the conversion of dihydrogeodin (**14**) to geodin (**15**) was previously reported (Huang et al. 1995). The functions of the transcription factor GedR (ATEG_08453) responsible for the activation of geodin cluster and the halogenase GedL (ATEG_08460) that catalyzed the conversion of sulochrin (**13**) to dihydrogeodin (**14**) were verified by Nielsen's study. Meanwhile, similar gene clusters are also found in *Aspergillus fumigatus* (Afu4g14450–14580) and *Aspergillus fischerianus* (101790–101920), without the presence of halogenase gene, which further substantiates the delineation of the geodin cluster (Nielsen et al. 2013). As a result, their putative product is trypacidin (Larsen et al. 2007), which differs from geodin only in the absence of chlorines and the presence of an additional methyl group. Furthermore, emodin is also a universal precursor of cladofulvin (Griffiths et al. 2016) and monodictyphenone (Chiang et al. 2010b).

Terretonin

Mycotoxin terretonin is a kind of meroterpenoids that originate from both polyketide and terpenoid pathways (McIntyre et al. 1989). The NR-PKS gene *trt4* (ATEG_10080) is responsible for the biosynthesis of 3,5-dimethylorsellinic acid (DMOA, **16**). And, a farnesyl pyrophosphate (FPP) is transferred by a prenyltransferase Trt2 (ATEG_10078) to form farnesyl-DMOA (**17**) (Guo et al. 2012; Itoh et al. 2012). The whole gene cluster contains 10 genes (ATEG_10077–10086); the other genes catalyze following steps, e.g., methylation, epoxidation, and cyclization. The methylation of the carboxyl group catalyzed by Trt5 (ATEG_10081) is an essential step for cyclization (Matsuda et al. 2012). Then, epoxidation and cyclization are catalyzed by epoxidase Trt8 (ATEG_10085) and terpene cyclase Trt1 (ATEG_10077), respectively. Next, short-chain dehydrogenase Trt9 (ATEG_10086) and FAD-dependent monooxygenase Trt3 (ATEG_10079) are involved to yield terrenoid (**18**). The last few steps of the terretonin pathway have been elucidated by Matsuda et al. (2015) recently. They uncovered the unusual D-ring construction in terretonin biosynthesis by collaboration of a multifunctional cytochrome P450 Trt6 (ATEG_10083) and a unique isomerase Trt14 (ATEG_10082). Trt6 catalyzes three successive oxidations to transform **18** into an unstable intermediate, which then undergoes the D-ring expansion and unusual rearrangement of the methoxy group catalyzed by Trt14 to afford the core skeleton of terretonin (**19**). Finally, the nonheme iron-dependent dioxygenase Trt7 (ATEG_10084) accomplishes the last two oxidation steps to complete the biosynthesis (see Fig. 3b). Homologous genes can be also found in *A. nidulans*, but they locate in two different clusters and are responsible for the biosynthesis of austinol and dehydroaustinol (Lo et al. 2012).

Citreoviridin

Citreoviridin is an ATP synthase inhibitor that inhibits mitochondrial oxidative phosphorylation (Suh and Wilcox 1988). It can uncompetitively inhibit ATP hydrolysis and ATP synthesis by binding to the β-subunit of F1-ATPase (Sayood et al. 1989). Ectopic ATP synthase is an accessible molecular target

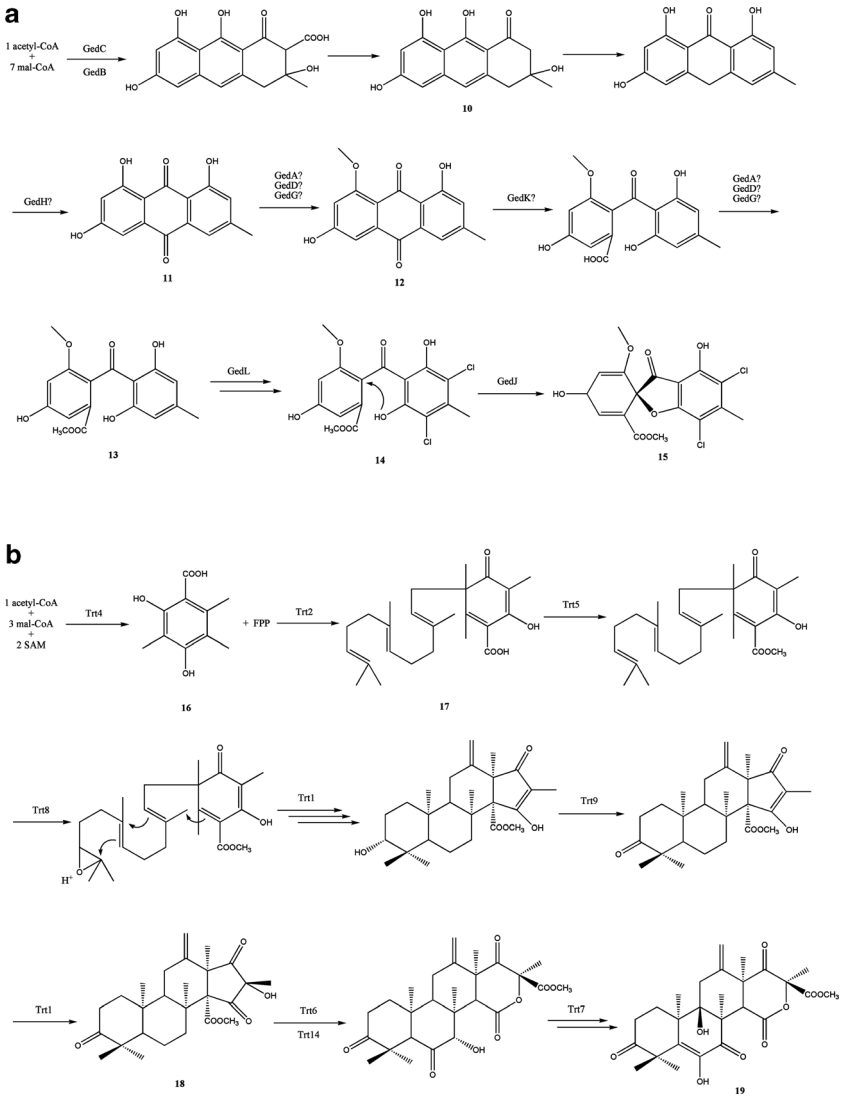

Fig. 3 Biosynthesis of geodin (**a**) and terretonin (**b**) in *A. terreus* NIH 2624

for inhibiting HIV-1 proliferation in vivo, and citreoviridin can completely block antigen-presenting cell (APC)-mediated transfer of HIV-1 at the APC-target cell interaction step (Yavlovich et al. 2012). Ectopic ATP synthase is also a target for lung adenocarcinoma; blockade like citreoviridin can suppresses cancer growth by activating the unfolded protein response (Chang et al. 2012).

Citreoviridin (**20**) is a HR-PKS product confirmed by isotope labeling experiments in 1980s (Steyn et al. 1982), and its gene cluster is identified this year by resistance gene-driven genome mining (Lin et al. 2016). Lin et al. search for extra copies of the F1-ATPase β-subunit in the genome of *A. terreus* NIH 2624 and find one gene (ATEG_09616) that is located next to a HR-PKS gene *CtvA* (ATEG_09617). Additionally, a methyltransferase CtvB (ATEG_09618), a hydrolase CtvD (ATEG_09619), and a flavin-dependent monooxygenase CtvC (ATEG_09620) are found in the locus. They are putative tailoring enzymes required for the synthesis of the tetrahydrofuran ring and methylated α-pyrone in citreoviridin (see Fig. 4a). Lin et al. heterologously expressed citreoviridin biosynthetic gene cluster in *A. nidulans* successfully. Similar cluster can be also found in *Metarhizium anisopliae*, which was known to produce analogous toxin aurovertin (Azumi et al. 2008). The self-resistance gene is an effective guidepost; in fact, an extra copy of HMGR can also be found in the lovastatin biosynthetic locus.

Asperfuranone

Asperfuranone was proved to inhibit proliferation of human nonsmall cell lung cancer A549 cells via blocking cell cycle progression and inducing apoptosis (Wang et al. 2010). There are two PKS genes in the asperfuranone cluster just like the lovastatin cluster: One is HR-PKS gene, and the other is NR-PKS gene with a C-terminus reductive domain (NR-PKS-R).

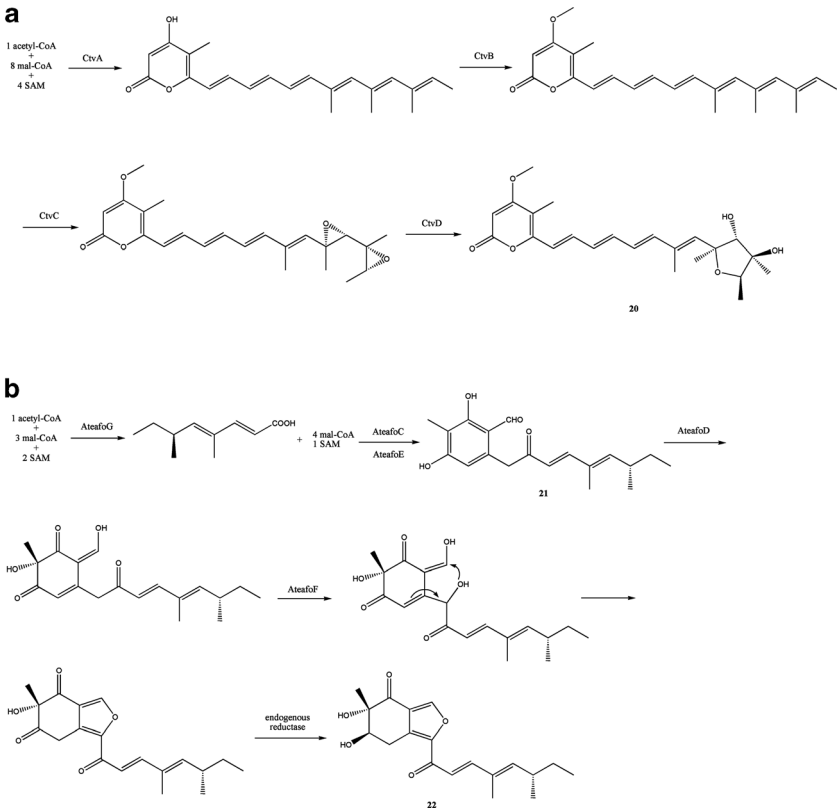

Fig. 4 Biosynthesis of citreoviridin (**a**) and asperfuranone (**b**) in *A. terreus* NIH 2624

In fact, asperfuranone cluster (AN1029–1036) and its products were firstly identified in *A. nidulans* (Chiang et al. 2009). Then, a similar cluster (ATEG_07659–07667) was also found in *A. terreus* NIH 2624, and asperfuranone was successfully produced through heterologous expression of this cluster in *A. nidulans* (Chiang et al. 2013). As shown in Fig. 4b, the polyketide intermediate produced by HR-PKS gene *AteafoG* (ATEG_07659) may be transferred to NR-PKS AteafoE (ATEG_07661) with the aid of AteafoC (ATEG_07663) to give the complete polyketide (**21**). Two steps of hydroxylation are then catalyzed by AteafoD (ATEG_07662) and AteafoF (ATEG_07660), respectively. The hydroxylation of C-3 on the benzene ring results in intramolecular rearrangement, and a furan nucleus is formed by the latter step. Last, the authors inferred that there existed an endogenous reductase in *A. nidulans* to catalyze the final conversion to asperfuranone (**22**).

Three predicted PKS clusters

The gene clusters of another four PKS genes (ATEG_03432, 03446, 03629, 08662) are still not clear, but there are some hints, so we try to give the prediction of their gene clusters in this review. ATEG_03432 and ATEG_08662 are both NR-PKS-R genes like *ateafoE* (ATEG_07661). NR-PKS-R genes are always involved in the biosynthesis of azaphilones, such as citrinin (He and Cox 2016). So, the products of the clusters of ATEG_03432 and ATEG_08662 are probable to be azaphilones. The heterologous expression of NR-PKS gene ATEG_03629 in *A. nidulans* results in the production of 5-methyl orsellinic acid (5-MOA, **25**) (Chiang et al. 2013), similar to the product of *trt4* (ATEG_10080). Moreover, the use of methyl orsellinic acid as a starting material is widely observed in other fungal pathways, such as tropolones (Davison et al. 2012) and xenovulene (Bailey et al. 2007).

There is a fatty acid synthetase (FAS) gene pair (ATEG_08867 and 08868) that occurs near the NR-PKS-R gene ATEG_08662. Just like the condition of asperfuranone cluster, a highly homologous gene cluster can be found in *A. nidulans* (AN3379–3386) (see Table 2). Ahuja et al. (2012) activated the *A. nidulans* cluster by exchanging the promoters of PKS and FAS genes with controllable *alcA* promoter and isolated a major product, 2,4-dihydroxy-3-methyl-6-(2-oxoundecyl) benzaldehyde (**23**) along with other corresponding products from the mycelium (see Fig. 5a). In particular, a decanoyl starter unit is firstly synthesized by the FAS and then loaded onto PKS. We speculate that similar products can be derived from *A. terreus* cluster. On the other hand, heterologous expression of these three PKS and FAS genes in *A. nidulans* resulted in no product (Chiang et al. 2013). Therefore, loading of the starter unit may need other genes in the predicted cluster. And, the monooxygenase

Table 2 Proposed gene cluster contains PKS gene ATEG_08662

Locus tag	*A. nidulans* homolog	Putative function	Protein identity (%)
ATEG_08662	AN3386	NR-PKS-R	56
ATEG_08663	AN3385	Transcription factor	67
ATEG_08664	AN3384	Condensation	44
ATEG_08665	AN3383	MFS transporter	65
ATEG_08666	AN3382	Salicylate monooxygenase	56
ATEG_08667	AN3381	FASβ	51
ATEG_08668	AN3380	FASα	60
ATEG_08669	AN3379	Unknown	43

(ATEG_08666) may catalyze the benzene ring hydroxylation that results in intramolecular rearrangement.

Boruta and Bizukojc (2014) speculated that ATEG_03432 was responsible for the biosynthesis of citrinin in *A. terreus*. But, the heterologous expression of ATEG_03432 in *A. nidulans* did not result in the precursor of citrinin but the precursor of *Monascus* azaphilone pigment (**24**) (Chiang et al. 2013). Although they have similar structure and biosynthesis pathways, they are different in the length of polyketide chain and the number of methyl groups. Instead of the FAS gene pair that occurred in the *Monascus* azaphilone pigment biosynthesis cluster (Balakrishnan et al. 2013; Balakrishnan et al. 2014), there is a HR-PKS gene ATEG_03446 near to ATEG_03432. Will there be a gene cluster between ATEG_03432 and ATEG_03446? In fact, similar gene clusters *azaA–L* and *R* (EHA28230–28239,

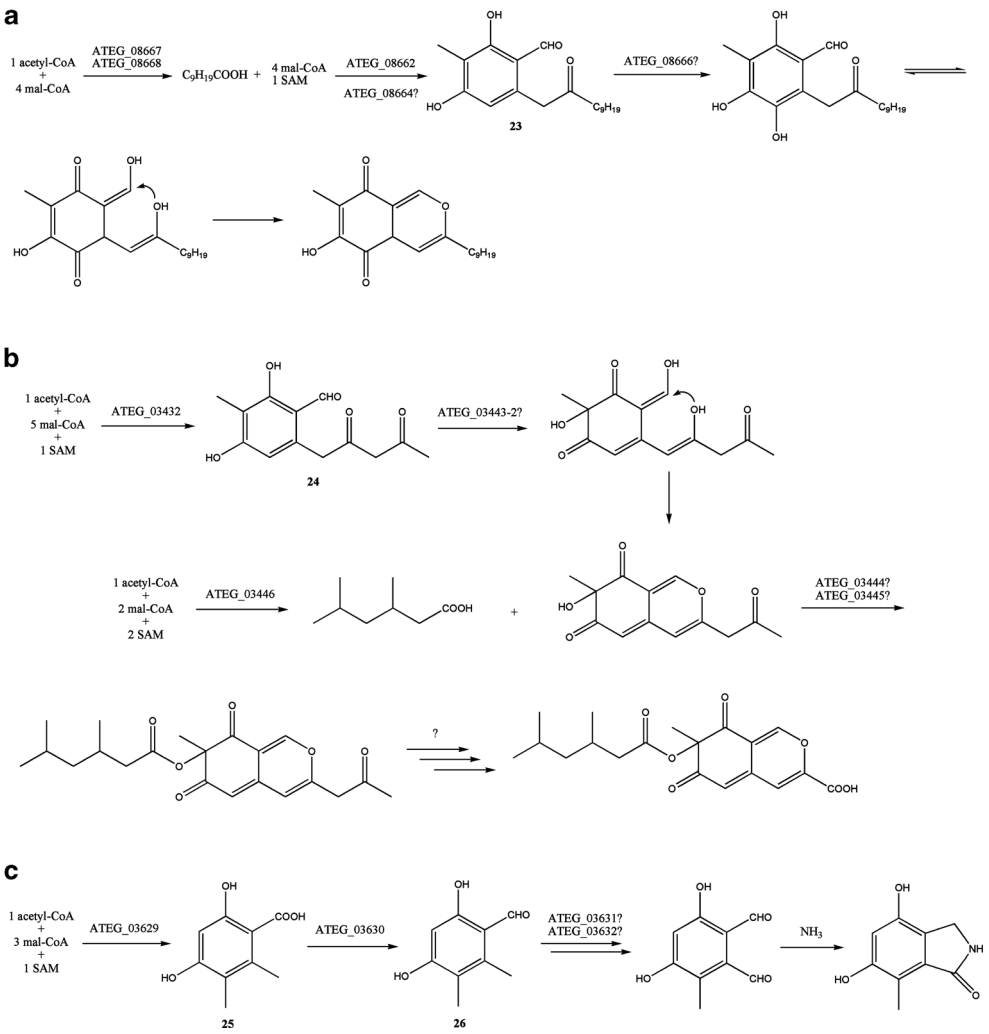

Fig. 5 Predicted biosynthesis processes involve PKS genes ATEG_08662 (**a**), ATEG_03432 and 03446 (**b**), and ATEG_03629 (**c**) in *A. terreus* NIH 2624

Table 3 Proposed gene cluster contains PKS gene ATEG_03432

Locus tag	*A. niger* homolog	Putative function	Protein identity (%)
ATEG_03432	*azaA*	NR-PKS-R	50
ATEG_03433	*azaG*	Dehydrogenase	45
ATEG_03434		Condensation	
ATEG_03435		Acetyltransferase	
ATEG_03436		MFS transporter	
ATEG_03437	*azaC*	Esterase/lipase	49
ATEG_03438		Dehydrogenase	
ATEG_03439		AdoMet_MTases	
ATEG_03440		Enoyl reductase	
ATEG_03441		Hydroxylase	
ATEG_03442	*azaL*	Dehydrogenase	42
ATEG_03443-1	*azaJ*	Enoyl reductase	38
ATEG_03443-2	*azaH*	NADB_Rossmann	55
ATEG_03444	*azaI*	Cytochrome P450	35
ATEG_03445	*azaR*	Zn_2Cys_6 regulator	40
ATEG_03446	*azaB*	HR-PKS	33

28242–28244) can be observed in *A. niger* ATCC 1015 genome, which is responsible for azanigerone biosynthesis (Zabala et al. 2012), another kind of azaphilones. NR-PKS azaA shows 50 % identity with ATEG_03432 and has the same product of ATEG_03432. HR-PKS azaB shows 33 % identity with ATEG_03446. Moreover, homologs of azaC, G–J, L, and R also can be found between ATEG_03432 and ATEG_03446 (see Table 3 and Fig. 5b). Among them, N-terminus of ATEG_03443 shows 38 % identity with azaJ, and its C-terminus shows 55 % identity with azaH, so there may be two genes in ATEG_03443, and we differentiate them as ATEG_03443-1 and ATEG_03443-2. AzaH was proved to be occupied in the pyran-ring formation of azanigerones (Zabala et al. 2012); thus, ATEG_03443-2 may have the same important function, somewhat like the function of AteafoD (ATEG_07662). ATEG_03445 is a Zn_2Cys_6 regulator located next to HR-PKS ATEG_03446 and shows 40 % identity with azaR, the activator of azanigerone cluster; hence, it is suggested to be the transcriptional factor of the cluster. Consequently, we conjecture that there is a gene cluster between ATEG_03432 and ATEG_03446, and its product may be an analog of azanigerone.

Heterologous expression of ATEG_03629 and the next NRPS-like gene ATEG_03630 in *Saccharomyces cerevisiae* showed that ATEG_03630 could reduce 5-MOA to 2,4-dihydroxy 5,6-dimethyl benzaldehyde (**26**) (Wang et al. 2014). The function of the NRPS-like gene is to some extent similar to the R domain in the aforementioned NR-PKS-R genes. Interestingly, engineering of the adenylation domain of this NRPS-like protein can achieve a potential biocatalyst for aldehyde generation (Wang and Zhao 2014). Homologous genes of ATEG_03629 and 03630 can be found in *A. nidulans*, which are AN6448 and AN6444, respectively. Similar gene pairs also exist in the genome of *Stachybotrys* (Li et al. 2016). Furthermore, cytochrome P450 ATEG_03631 shows 49 % identity with AN6449, and short-chain dehydrogenase ATEG_03632 shows 54 % identity with AN6450 (see Table 4). The product of AN6448 is 3-methyl orsellinic acid (3-MOA), different in the location of methyl group. Phytotoxin cichorine is

Table 4 Proposed gene cluster contains PKS gene ATEG_03629

Locus tag	*A. nidulans* homolog	Putative function	Protein identity (%)
ATEG_03629	AN6448	NR-PKS	43
ATEG_03630	AN6444	NRPS-like	42
ATEG_03631	AN6449	Cytochrome P450	49
ATEG_03632	AN6450	Short-chain dehydrogenase	54

thought to be the downstream product of this *A. nidulans* PKS cluster (Ahuja et al. 2012). When 6-methyl is oxidized to aldehyde, the isoindolinone core in cichorine can be formed spontaneously in the presence of ammonium ions (Yuuichi et al. 2012). We predict that ATEG_03631 and 03632 may participate in the oxidation of 6-methyl; thus, related isoindolinone derivatives may be produced by this *A. terreus* PKS cluster (see Fig. 5c).

Conclusions

A. terreus is an important producer of many bioactive agents, and a large part of them are polyketides. In this review, we summarized seven reported polyketide biosynthesis pathways and predicted three possible gene clusters. Most *A. terreus* polyketide biosynthesis pathways are reported in last 5 years, suggesting that the research in this field is speeding up recently. We believe that more polyketide pathways will be characterized in the near future. It is interesting that homologous clusters often can be found in other fungi, especially *Aspergillus* strains, and these clusters are important basis for us to make prediction. We think that this is an effective way to mine secondary metabolic gene clusters.

Many of the aforementioned polyketides are anticancer metabolites, so the mass production of them is of great value. The knowledge of biosynthesis pathways is conducive to improve their production. For instance, native promoters can be replaced by controllable *alcA* promoter; moreover, heterologous expression can be used; unicellular fungi *S. cerevisiae*, *P. pastoris*, and filamentous fungi *A. nidulans* and *A. oryzae* are all optional hosts. On the other hand, some genes such as transesterase gene *lovD*, transcription factor *terR*, and NRPS-like gene ATEG_03630 themselves are potential elements, which can be used in the production of other desired compounds.

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Compliance with ethical standards

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Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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