Genome Mining of Secondary Metabolites From a Marine-derived Aspergillus Terreus B12

xinyang du (1451189146@qq.com)
China Pharmaceutical University College of Life Science and Technology https://orcid.org/0000-0003-0318-9200

Huanhuan Li
China Pharmaceutical University College of Life Science and Technology

Jiangfeng Qi
China Pharmaceutical University College of Life Science and Technology

Chaoyi Chen
China Pharmaceutical University College of Life Science and Technology

Yuanyuan Lu
China Pharmaceutical University College of Life Science and Technology

Ying Wang
China Pharmaceutical University College of Life Science and Technology https://orcid.org/0000-0002-5706-5361

Research Article

Keywords: Aspergillus terreus, BGCs, SMs, whole genome sequence

Posted Date: May 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-541300/v1

Genome mining of secondary metabolites from a marine-derived Aspergillus terreus B12

Xinyang Du[1], Huanhuan Li[1], Jiangfeng Qi, Chaoyi Chen,Yuanyuan Lu, Ying Wang*

School of Life Science and Technology, China Pharmaceutical University, Nanjing 211100, China.

*Corresponding author. Tel: +86-25-86185219. fax: +86-25-8321249. E-mail: waying@cpu.edu.cn

Abstract

As an important saprophytic filamentous fungus, *Aspergillus terreus* is ubiquitously distributed, including soil rhizospheres and marine environments. Due to the prominent capabilities of bioconversion and biosynthesis, *A. terreus* has become attractive in biotechnical and pharmaceutical industry. In this work, an *A. terreus* strain, B12, was isolated from sponge in South China Sea, which demonstrated broad bacteriostatic effects against a variety of pathogenic bacteria. The whole genome was sequenced, showing a genetic richness of BGCs, which might underpin the metabolic plasticity and adaptive resilience for the strain. Genome mining identified 67 biosynthetic gene clusters (BGCs), among which, 6 gene clusters could allocate to known BGCs (100% identity), corresponding to diverse metabolites like clavaric acid, dihydroisoflavipucine /isoflavipucine, dimethylcoprogen, alternariol, aspterric acid and pyranonigrin E. However, instead of the putative compounds, several other products were obtained from the B12 fermentation, including terrein, butyrolactone I, terretonin A&E, acoapetaline B and epi-aszonalenins A. Of note, acoapetaline B and epi-aszonalenins A, discovered natural products recently with little information, unexpectedly were reported in this *A. terreus* strain. The genomic and heterogeneity observed in strain B12, should be at least partially attributed to the genetic variability and biochemical diversity of *A. terreus*, which could be an interesting issue open to future efforts.

Keywords: Aspergillus terreus；BGCs；SMs；whole genome sequence

Abbreviation List:

BGCs: biosynthetic gene clusters
SMs: secondary metabolites
MH: Mueller-Hinton
MEA: Malt Extract Agar
MeOH: Methanol
LPCB: lactophenol cotton blue
ITS: internal transcribed spacer
ML: maximum-likelihood
GO: Gene Ontology
KEGG: Kyoto Encyclopedia of Genes and Genomes
KOG: euKaryotic Orthologous Groups
CAZy: Carbohydrate-Active enZYmes Database
NRPS: nonribosomal peptide synthase
PKS: polyketide synthase

Introduction

As a valuable producer of secondary metabolites (SMs), marine fungi represent an

underestimated source of biological and chemical diversity, although their distribution and ecological roles often remain scarce[1]. Owing to the particularity of marine environment, many SMs are structurally unique and possess promising biological and pharmacological properties, in comparison with their terrestrial origin counterparts[2]. For decades, despite the significant increase in the number of structures discovered from marine fungi, the species diversity and the large potential of SMs is not yet adequately represented[3].

As filter feeders in marine ecosystem, sponges form close associations with diverse groups of microbes, potentially involved in a variety of ecological functions including SMs generation, which could contribute to an ecological success, to themselves and to the host in niche competition[4, 5]. In our previous work, a range of filamentous fungal strains were isolated from sponges in the South China Sea, typically dominated by *Aspergillus* and *Penicillium* sp.[6].

Hereon, we present the draft genome of B12, an *A. terreus* strain with broad antibacterial activities in preliminary screening. Subsequently, genome mining, metabolites separation and structure determination were performed, partially presenting the biosynthetic potential and chemical diversity of the strain, which highlighted the need for further study and shed some lights on the ecological and biochemical properties of *A. terreus* strains.

Materials and methods

conditions of fermentation

The B12 was activated in the PDB liquid medium, and then inoculated onto corn medium (corn 100g, MgSO4 0.2g, sea salt 1.5g, malt sugar 2g, sorbitol 2g; yeast extract 0.3g, tryptophane 0.05g, sodium glutamate 1g, K2HPO4 0.05g,water 1L) to culture at 28 ℃ for 15 days. The fermented products were extracted by methanol (MeOH) followed by decompressing distillation to acquire a crude extract. The crude extract was dissolved in 2 mL MeOH and test for antibacterial activity by the agar diffusion method.

Escherichia coli ATCC 25922, *Klebsiella aerogenes* ATCC 700603, P*seudomonas aeruginosa* PA101, *methicillin-resistant Staphylococcus aureus* (MRSA) USA300, *methicillin-resistant Staphylococcus epidermidis* (MRSE) ATCC 35984, *Micrococcus luteus* ACCC11001, and *Acinetobacter baumannii* ATCC 19606 were utilized as control strains. All the above strains were stored in the Marine Pharmaceutical Laboratory of China Pharmaceutical University.

single colonies of indicator bacteria was inoculated into Mueller-Hinton (MH,Solarbio, China) broth and cultured to logarithmic phase. The bacterial inoculum(OD600nn≈0.1 ） was spread onto MH agar (Solarbio, China). Wells measuring 6 mm in diameter were punched onto the surface of the agar using a sterile

hole puncher. 30 μL crude extract was added to the wells and incubated for 24 h at 28 °C. Eachassessment was developed in triplicate. The standard antibiotics (0.1 mM chloramphenicol) were used as a positive control,MeOH was used as a vehicle control. The diameters (in mm) of the inhibition zone were recorded to estimate antimicrobial activities, which were expressed by the ratio of the inhibition zone relative to that of the positive control. The bacteriostatic activities were considered strong if the ratio was greater than 1.0, moderate when the scale was between 0.5 and 1, and weak if it was less than 0.5.

species identification

The spore suspensions of B12 was inoculated and grown on Malt Extract Agar (MEA, OXOID, UK) which adding 3% sea malt,28 ℃ for 5 days. The colony was observed and characterized, such as size, texture, color, soluble pigments, and exudates. Next, microscopic examination was performed on spores and hyphae followed by lactophenol cotton blue (LPCB) staining[7]under a BA210 light microscope (Motic, Xiamen, China).

Molecular markers have been widely approved as crucial for the taxonomic identification of fungi, such as ITS[8, 9]. In our work, the phylogenetic status of the B12 was analyzed based on the ITS sequences. Phylogenetic trees were constructed by dataset of ITS sequences and concatenated sequences using maximum-likelihood (ML) analysis in MEGA7.

Genome sequencing

Due to the relatively wide anti-bactericidal activity of B12 ferment, suggest that the vital role played by SMs and the generation process. In order to explore the potential of produce the SMs of B12, we sequenced the whole genome of B12.In brief, the construction of DNA libraries was performed using 100 ng genomic DNA, which was randomly fragmented to 500 bp by sonication (Covaris S220, USA). Sequencing was subsequently performed using a 2×150-bp paired-end (PE) configuration; image analysis and base calling were performed using HiSeq Control Software. The adapter and low-quality sequences were removed from the raw sequencing data by cutadapt (v1.9.1). The ideal reads were assembled and gap-filled using Velvet[10], SSPACE[11] and GapFiller[12], respectively. All genome sequencing data have been submitted to the NCBI SRA database. The SRA accession numbers of B12 is PRJNA714189.

The genomic sequences of B12 was analyzed with the antiSMASH[13] online server with the ClusterFinder algorithm to identify the potential biosynthetic gene clusters (BGCs) based on homology analysis.

Gene prediction and functional annotation

The software Augustus (version 3.3)[14] was used to predict coding genes and high-GC regions. Through a homology-based approach, the gene structures were mapped to the reference genome Aspergillus terreus NIH2624. Next, the coding genes were annotated with the NCBI nr database by BLAST, and the functions of genes were annotated by the Gene Ontology (GO) [15]and Kyoto Encyclopedia of Genes and Genomes (KEGG)[16] databases. In addition, the predicted proteins were classified by the Cluster of Orthologous Groups of proteins (KOG) database[17].

Results

Antibacterial activities screening

A range of pathogenic bacteria were hired to assess the antimicrobial property of B12 (Fig.1). Relative to equivoluminal antibiotics (0.1 mM chloramphenicol), the fermented products of strain B12 displayed a considerable and broad-spectrum growth inhibition against almost all of tested bacteria. More precisely, the crude extract from B12 fermentation exhibited more potent effects against Gram-positive bacteria (MRSA, MRSE, *M.luteus*, *S.aureus*) than against Gram-negative bacteria (*E.coli*, *A. baumannii*, *K.aerogenes*, *P.aeruginosa*), specified by the bacteriostatic efficacy relative to standard antibiotics (0.1 mM chloramphenicol). In view of the unique ecological fitness, further genetic and physiological investigations are necessary on the strain to unveil the underlying bioactive components and possible synthetic profiles.

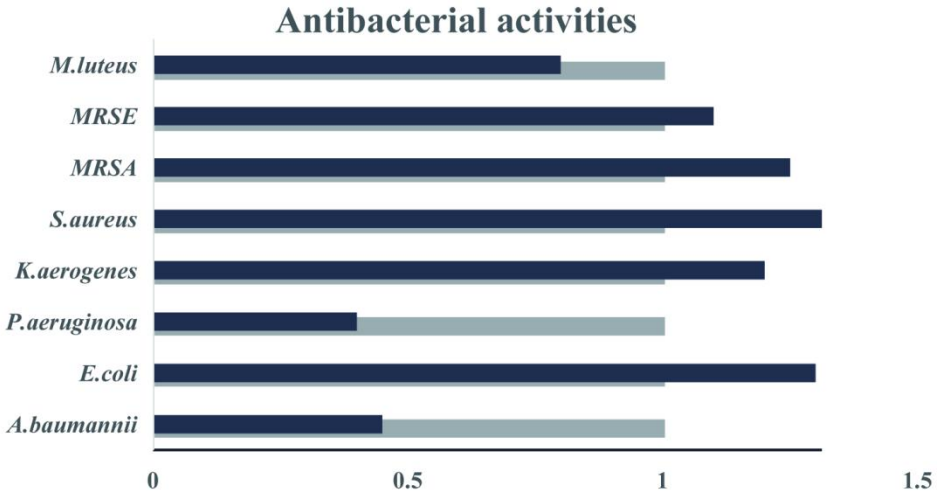


Figure 1. the antibacterial activities of the extractive of B12. The standard antibiotics (0.1 mM chloramphenicol) were used as a positive control, MeOH was used as a vehicle. The diameters (in mm) of the inhibition zone were recorded to

estimate antimicrobial activities, which were expressed by the ratio of the inhibition zone relative to that of the positive control （0.1 mM chloramphenicol）. The bacteriostatic activities were considered strong if the ratio was greater than 1.0, moderate when the scale was between 0.5 and 1, and weak if it was less than 0.5.the blue bar indicated as 1, the black-blue indicated as the ratio of the inhibition zone relative to that of the positive control. *E. coli: Escherichia coli; P. aeruginosa: Pseudomonas aeruginosa; K. aerogenes: Klebsiella aerogenes; MRSA: methicillin-resistant Staphylococcus aureus; MRSE: methicillin-resistant Staphylococcus epidermidis; M. luteus: Micrococcus luteus; A. baumannii: Acinetobacter baumannii*.

Morphological and phylogenetic analysis

In microbial taxonomy, classic species features such as morphological, physiological and genetic characteristics have become essential and canonical approaches. The purified fungus strain B12 was first characterized based on morphological, microscopic and molecular characteristics.

As shown by morphological features, including conidiophores, hyphae, pigments, and colony appearance, the strain grows slowly after 5 days at 25 °C on MEA, with white, circular and flocculose colonies. The colonies are slightly convex, margin filiform, lanose; brownish orange reverse center becomes towards the grey margin pale without soluble pigment or exudate diffusing to the agar. Microscopy shows that mycelium grows filiform, while small and globose conidia stack to form compact conidial heads (Fig.2 A). Macroscopically or microscopically, the strain B12 could be identified as Aspergillus sp. by those morphological features, which certainly requires further identification by molecular techniques.

Among the regions of the ribosomal cistron, internal transcribed spacer (ITS) region provides the highest resolving power for discriminating closely related species, having been adopted as the primary barcode marker for fungi[18]. In our study, based on the ITS sequence, the phylogenetic tree was constructed among fungi, which clearly delineated species delimitation between *Cladosporium* and *Aspergillus*. Consistent with morphological characterization, B12 was clustered into *Aspergillus* clades, supported by the highest identity scores and 100% bootstrap value. More specifically, the strain closely associated with *Aspergillus terreus*, forming a sister taxon with *Aspergillus niger* clade (Fig.2 B).

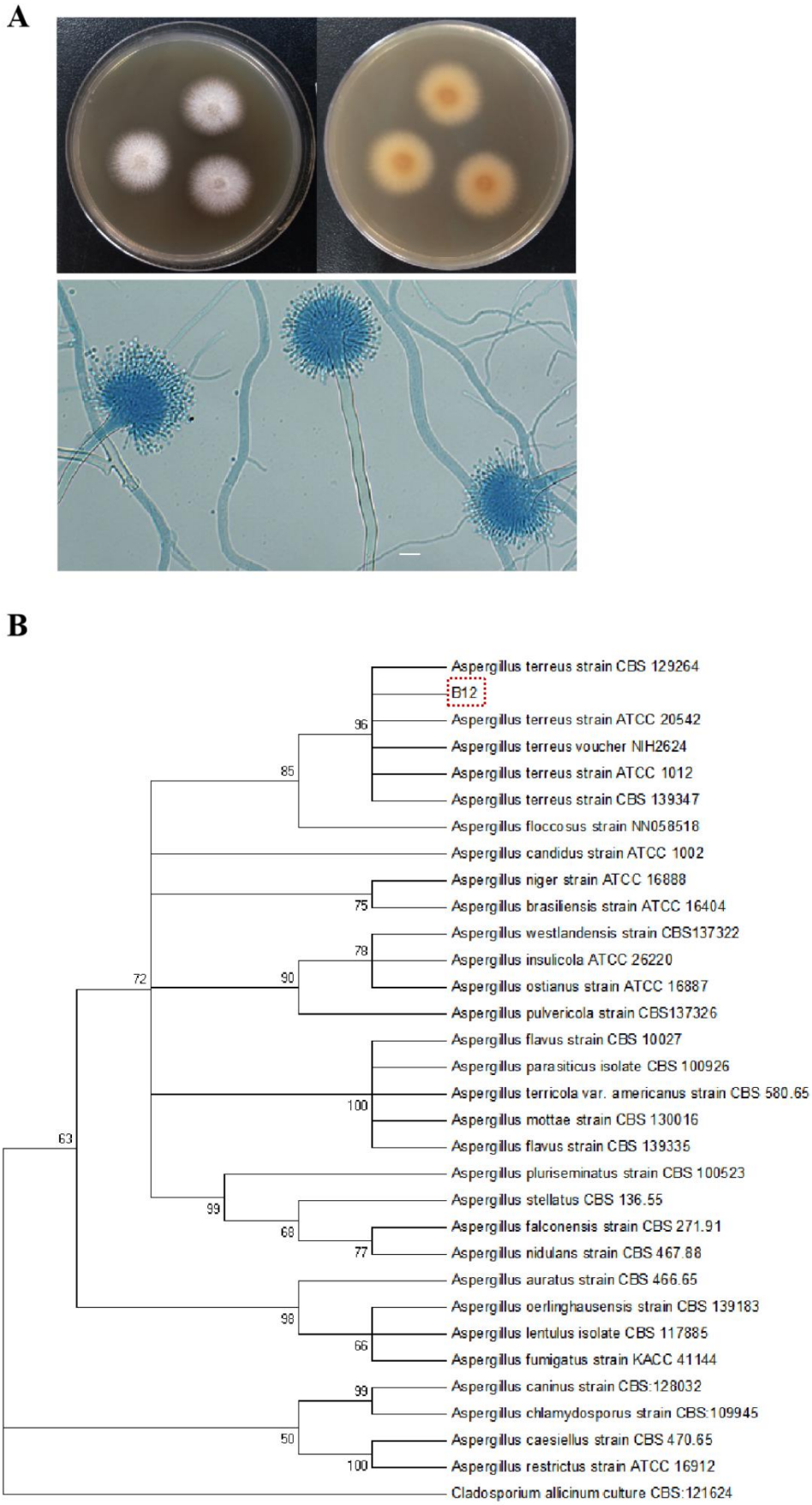


Figure 2. Identification of B12. A. Morphology of characterized strains, Colony

and microscopic morphology after 5 days of incubation. From left to right: obverse colonies on MEA, reverse on MEA, conidiophores and conidiogenous at 40× magnification (scale bar 10 μm). B. Maximum likelihood tree of B12, Multiple sequence alignment was conducted using Clustal W (default settings), and phylogenetic relationships were based on ML analysis with 1000 bootstrap replications in MEGA7.

Genome information of B12

As genome-wide analysis provides first insights into the nature of microbial functioning [19], the genome of B12 was sequenced with a coverage of 173.87 ×. The draft genome was assembled into a total size of 29.51 Mb, with a G+C content of 52.31%, composing 80 scaffolds. The average length of consensus contigs was 368922.8 bp with an N50 of 1603970 bp. The protein-coding regions were predicted via Augustus software, resulting in a total of 10148 protein-coding genes with an average length of 1548.28 bp. The general genomic characteristics of strains B12 is listed in Table 1.

Table 1. General features of the B12 genomes

Genome	Value
Assembly size （Mp）	29.51
G+C（%）	52.31
Assembled scaffolds	80
N50 length（bp）	1603970
average length（bp）	368922.8
predicted protein-coding genes	10148
average length predicted protein-coding genes	1548.28
average depth of reads cover	173.87
sequencing method	Illumina HiSeq

Functional annotation

As far as the structural-functional correlation is concerned, the function of genes, and the presumptive encoding products, can be predicted by sequence similarity[20]. Practically, comparative genomics on B12 genome yielded similar functional annotations in CAZy, KEGG and COG database: a large number of genes involve in metabolic pathways， indicating a vigorous potency in biochemical metabolism. According to the KEGG analyses, 4204 coding genes were annotated, assigned to 332 pathways, which could be classified into 6 functional categories: cellular processes, Environmental Information Processing, Genetic Information Processing, Human Diseases, Metabolism, Organismal Systems(Fig.3). Among the metabolic processes,

besides the primary metabolism (e.g., amino acid, carbohydrate and lipid metabolism), the subcategory of SMs biosynthesis was conspicuous for the significant abundance and diversity(11.94%), which underlined the potential biosynthetic capabilities in the strain.

Figure 3. KEGG classifications of predicted coding genes in the genome of B12. Red: Cellular Processes; Brown: Environment Information Processing; Green: Genetic Information Processing; Cyan: Human Diseases; Blue: Metabolism; Purple: Organismal Systems.

Using CAZy functional classification, the global search assigned 1984 genes associated with carbohydrate active enzyme, accounting for 19.55% of the total protein-coding genes. The putative CAZymes comprised 6 groups: Carbohydrate-Binding Modules (CBMs), Auxiliary Activities (AAs), Carbohydrate Esterases (CEs), Polysaccharide Lyases (PLs), Glycosyl Transferases (GTs), Glycoside Hydrolases (GHs). Among those subfamilies, CBMs (27.92%), GTs (26.66%) and GHs (33.77%) appeared as the majority contribution (~88.36%, Fig.4).

Presumably, the multifarious CAZymes could be an accessible resource for nutrient acquisition and niche adaptation, in term of their potency and versatility in carbohydrates degradation and biotransformation[21].

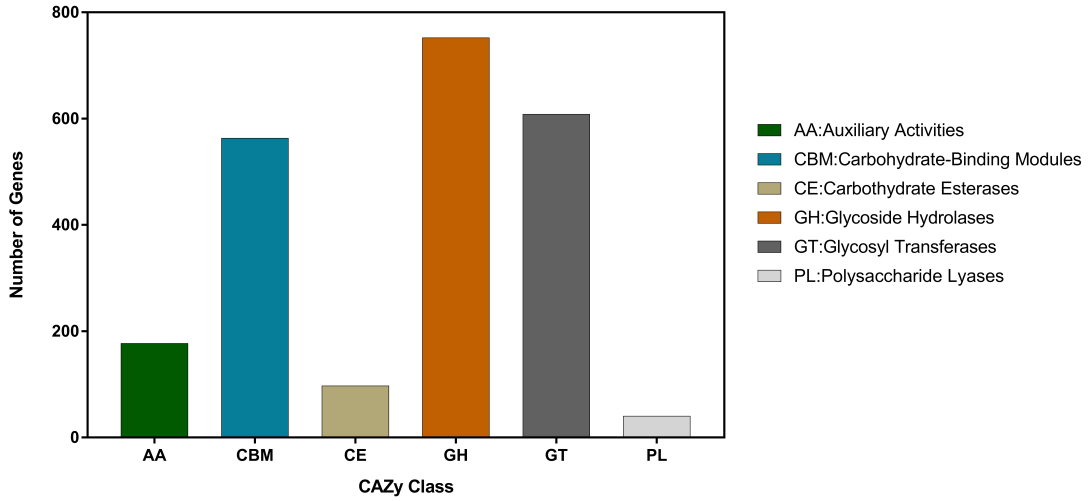


Figure 4. CAZy classifications of putative proteins in the genome of B12.

In COG database, the ortholog query assigned 6886 proteins, accounting for 67.86% of the total protein-coding genes. Concomitantly, the precedence of metabolism was verified by the considerable proportion (57.39%) in functional categories (Fig.5), which was successively followed by poorly characterized function (26.20%), information storage processing (15.93%) and intracellular processes (13.64%). Of note, in the metabolic patterns, the bioprocesses associated with SMs were evident, represented by the relative proportion of this category (4.53%), which might confer a biochemical flexibility and adaptive superiority to the strain.

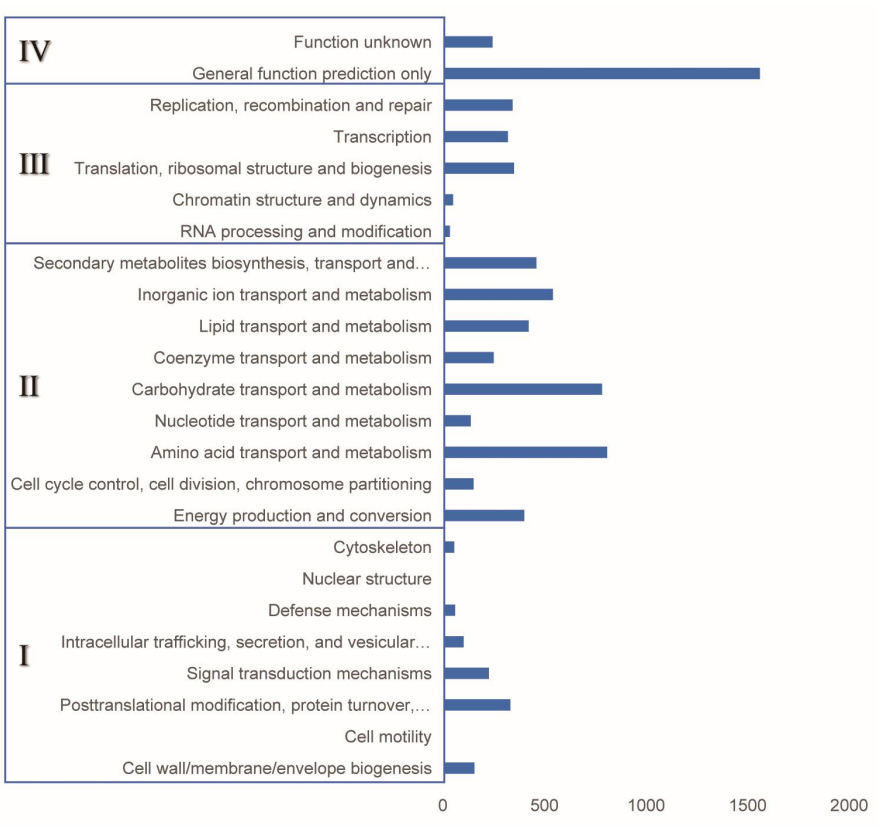


Figure 5. COG classifications of putative proteins in the genome of B12. I: intracellular processes; II: metabolism; III: information storage/processing; IV: poorly characterized function.

Biosynthesis prediction of strain B12

Different functional annotation systems integrated to highlight the biosynthetic potential in strain B12, possibly contributing to its antimicrobial activities. In fungal genomes, the genes that associated with SMs were often clustered, referred to as BGCs [22].

The speculative BGCs in B12 were predicted by antiSMASH database, forwarding 67 BGCs (Fig.6). In terms of biosynthetic pattern, the BGCs were classified into 10 types: NRPS (27), PKS (17), NRPS-indole hybrid (5), NRPS-T1PKS hybrid (5), NRPS-betalactone (1), NRPS-terpene hybrid (1), terpene (5), indole (4), betalactone (1), siderophore (1).

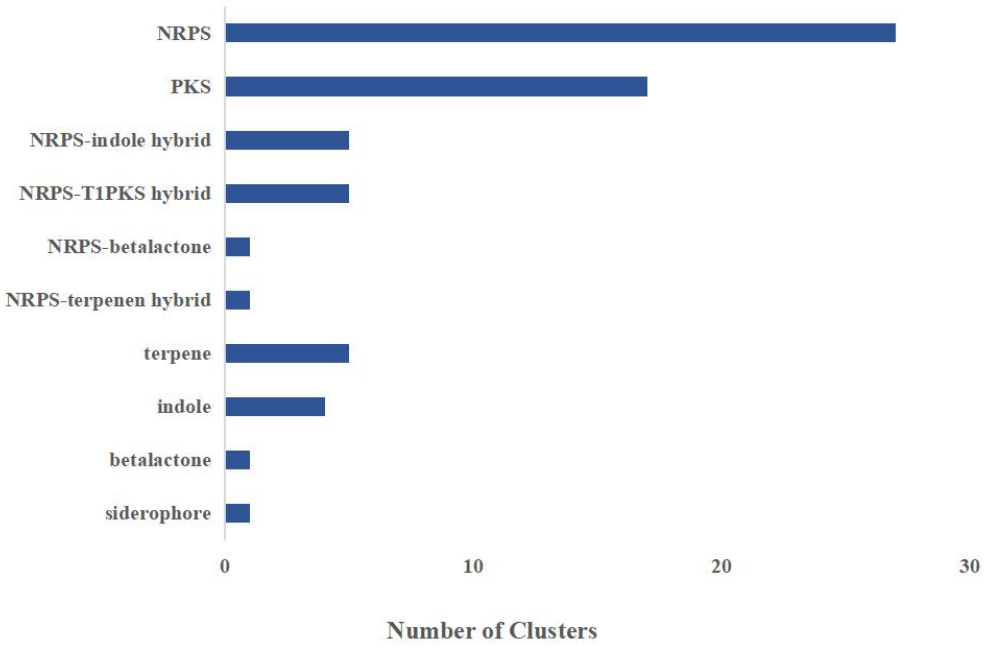


Figure 6. BGCs predicted in the B12 genomes based on antiSMASH

The fertile and multifarious BGCs, supposedly endorse the biosynthetic dexterity to produce novel chemical backbones or natural products, which certainly merits more exploration. Sequence alignment underscored 8 BGCs with 100% similarity to known BGCs, corresponding to various natural products, clavaric acid, dihydroisoflavipucine/ isoflavipucine, dimethylcoprogen, alternariol, aspterric acid and pyranonigrin E (Fig.7). In term of the structural-functional correlations, the chemical diversity of inferred SMs supposed to entail various roles, which have been demonstrated by cumulative reports, comprising antitumor (clavaric acid[23]) and antioxidative (pyranonigrin E[24]) agents, siderophores (dimethylcoprogen[25]), plant growth regulator (aspterric acid[26]) and phytotoxins (alternariol[27], dihydroisoflavipucine/ isoflavipucine[28]). Besides variance in the proposed products, the diversification of biosynthetic patterns was equally inspirational, involving T1PKS (Fig. 7 A-C), NRPS-PKS hybrid (Fig.7 D-E), NRPS (Fig.7 F), terpene (Fig.7 G-H), which might imply a pluripotent biochemical toolkit in this strain, as determined from the current limited genome-mining scope.

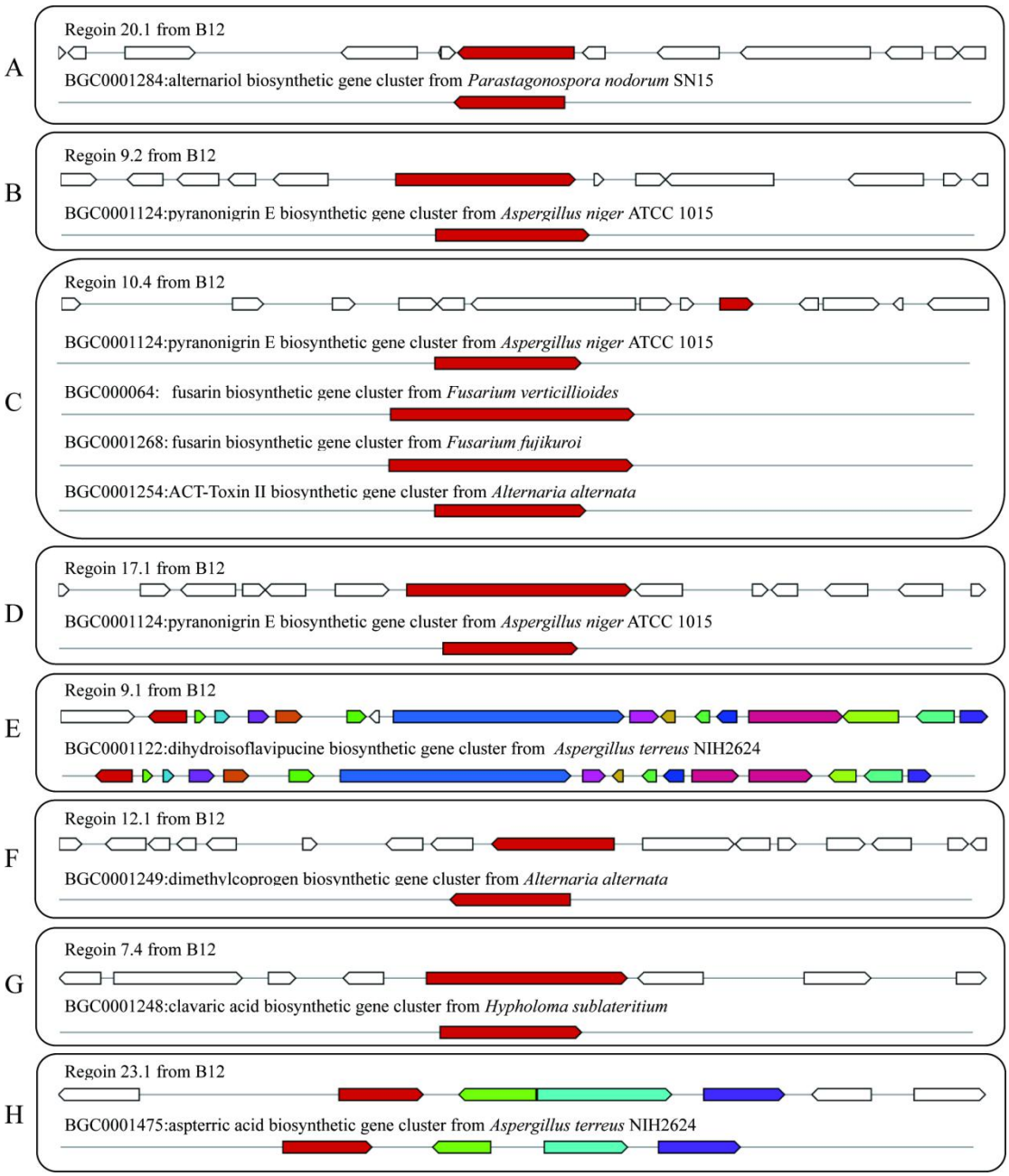
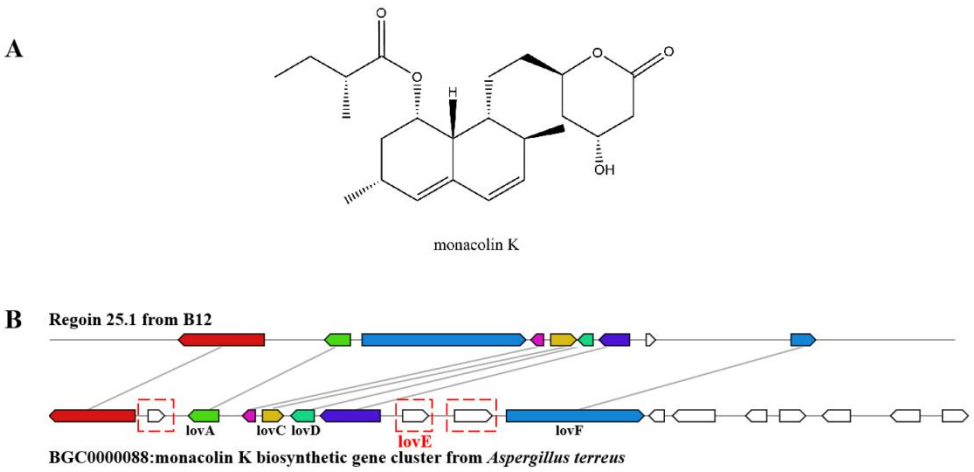


Figure 7. Schematic representation of B12 putative BGCs showing high similarity with genes from characterized BGCs. A-H. The upper part represents the BGC in B12, followed by the known BGCs in the MIBiG database.

In addition, a gene cluster homologous (~44%) to the BGC of monacolin K (MK/lovastatin) was found in B12 genome (Fig.8 A). As the inhibitor of HMG CoA reductase[29], Lovastatin was originally obtained from a soil-derived *Aspergillus terreus* strain[30], and also produced by other fungal genera like *Penicillium*, *Paecilomyces*, *Trichoderma*, and *Pleurotus ostreatus*[31]. Despite the considerable similarity of genes organization and sequence identity (> 70%), the region in B12 exhibited an obvious different pattern from MK/lovastatin biosynthetic pathway,

reflected by the absence of LovE and distinct truncation of LovF. Insofar as is known, LovE gene encodes a transcription factor that regulates MK/lovastatin gene cluster, whereas lovF encodes a diketide synthase (DKS), one of the two polyketide synthases involved in MK/lovastatin biosynthesis[32], underlying the indispensability of either part. Actually, we failed to obtain lovastatin from the fermented products of B12, appearing plausible from the perspective of biosynthetic mechanism.



Identifiers in *Aspergillus terreus*	Gene code	Product	Query cover	Identity
AAD34550.1	---	esterase	95	90
AAD34551.1	---	unknown	---	---
AAD34552.1	lovA	cytochrome P450 monooxygenase	104	96
AAD34553.1	---	unknown	99	94
AAD34554.1	lovC	enoyl reductase	114	86
AAD34555.1	lovD	transesterase	100	99
AAD34556.1	---	hydroxymethylglutaryl-coenzyme A reductase	81	96
AAD34557.1	lovE	regulatory protein	---	---
AAD34558.1	---	unknown	---	---
AAD34559.1	lovF	polyketide synthase	100	96

Figure 8. Putative monacolin K BGC in B12. A.The structure of monacolin K. B.The homologous genes of region 25.1 in B12 and terrein BGC known in *Aspergillus terreus* have been marked with the same color, and the missing genes are marked with red dotted boxes. Known BGCs description and amino acid homology (query cover and identity) were listed in the table.

Chemical Isolation and Characterization of Metabolites

To interpret the biosynthetic capacities of B12, ethanol extraction of the fermentated product was prepared for SMs isolation. Chemical separation and characterization have identified a range of metabolites differed in panel structure and relative abundance, including Terrein (1), Butyrolactone I(2)，Terretonin E(3), Terretonin A(4), Acoapetaline B(5), epi-Aszonalenins A(6) (Fig 9).

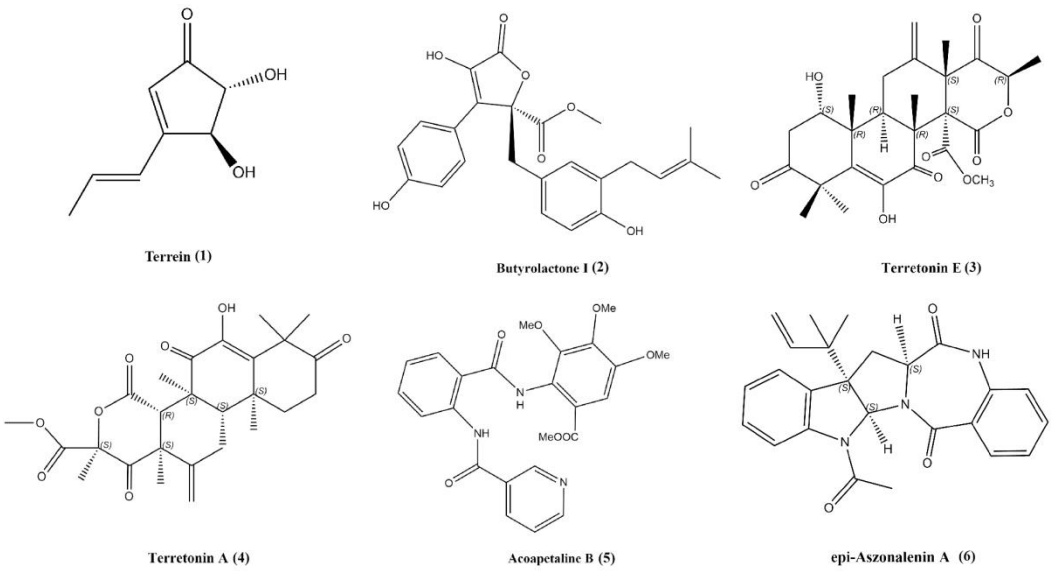
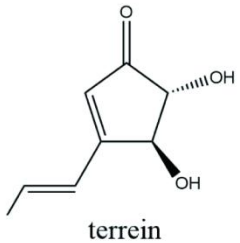


Figure 9 The compounds was isolated from B12

Via chromatographic separation and HNMR determination, the compound terrein (1) was purified as yellow crystal needles, which is addressed as a *A. terreus* metabolite with ecological, antimicrobial, antiproliferative, and antioxidative activities[26] . Based on the chemical clue, a BGC presumably for terrein synthesis was mined in region 9.4 (Fig.10 B), demonstrating a high degree of amino acid sequence homology ($>$ 72%) with the counterpart of *A. terreus* NIH2624[33]. However, despite the approximate gene composition and sequence identity, the assumed BGC was deprived of *ter*H-J genes. In the case of terrein production, a gene locus comprised by *ter*A–J and transcriptional regulator *ter*R, was characterized to encode the biosynthetic process[26]. As *ter*G and *ter*J were depicted as MFS transporters, while function of *ter*H–I was ambiguous, deletion of them putatively would lead to a diminution instead of abolition of terrein generation[33], which seemed to be substantiated in our study.

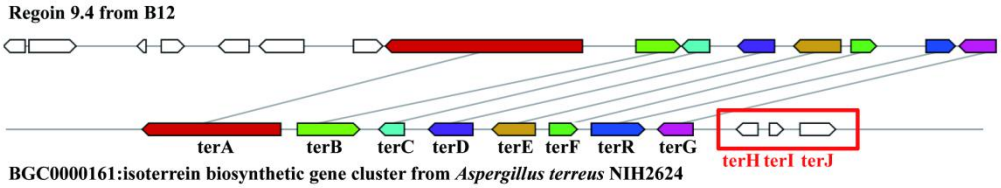
Besides terrein, butyrolactones and terretonins are also frequently reported as typical SMs produced by *A. terreus*[34] . Hence, from the point of SMs production, the presence of those metabolites apparently complied with the species-specific descriptions on biosynthetic profiling. In contrast, as to the latter two, acoapetaline B (5) and epi-aszonalenins A (6), there was little information available, with limited clues in the original organisms and structural characterizations. It was reported that acoapetalines were isolated from plants [35, 36], while epi-aszonalenins derived from *A. novofumigatus*[37], ostensibly divergent from *A. terreus*. The identification of those compounds in strain B12 might be ascribed to the plentiful (33.85%) uncharacterized BGCs to some extent, in term of the pendent and elusive insight of the biosynthetic processes and corresponding products in *A. terreus*[34], which awaits more advance of genome mining and genetic approaches.

A



terrein

B



BGC0000161:isoterrein biosynthetic gene cluster from *Aspergillus terreus* NIH2624

Identifiers in *Aspergillus terreus*NIH2624	Gene code	Description	Query cover	Identity
ATEG_00135	terJ	major facility transporter (MFS)	---	---
ATEG_00136	terI	protein of the superfamily of bleomycin resisitance protein, glyoxalase I, and type I ring-cleaving dioxygenanses	---	---
ATEG_00137	terH	NAD dependent epimerase/dehydratase	---	---
ATEG_00138	terG	major facility transporter (MFS)	100%	100%
ATEG_00139	terR	Zn_2Cys_6 transcriptional regulator	99%	72%
ATEG_00140	terF	protein with kelch motif	123%	80%
ATEG_00141	terE	multicopper oxidase	94%	91%
ATEG_00142	terD	FAD-dependent monooxygenase	99%	99%
ATEG_00143	terC	FAD-dependent monooxygenase	144%	68%
ATEG_00144	terB	DH-KR, multidomain protein with dehydratase and ketoreductase function	74%	73%
ATEG_00145	terA	non-reducing polyketide synthase (NR-PKS)	84%	97%

Figure 8. Putative Terrein BGC in B12. A.The structure of Terrein. B.The homologous genes of region 25.1 in B12 and terrein BGC known in *Aspergillus terreus* have been marked with the same color, and the missing genes are marked with red dotted boxes. B.Known BGCs description and amino acid homology (query cover and identity) were listed in the table.

Discussion

Aspergillus terreus, widely distributed in terrene and marine environment, has become a prolific producer of numerous bioactive agents, such as lovastatin, sulochrin, terrein, itaconic acid, etc[2]. In recent years, this fungus derived from ocean became more attractive, as concerning the structurally unique or biologically active metabolites[38]. In this study, an *A. terreus* strain B12 was isolated from sponges in the South China, which promisingly demonstrated broad bacteriostatic effects against a range of pathogenic bacteria. The whole genome was sequenced followed by comparative genomics analysis and functional annotations, leading to a total of 67 putative BGCs.

However, only a fraction of putative BGCs (18) possessed a high sequence similarity ($\geqslant$50%) with annotated BGCs in the MIBiG database, in contrast with a variety of uncharacterized gene clusters, conjecturally which might provide a biosynthetic blueprint for the products diversification in the strain. The speculation was partially confirmed by chemical separation, which identified several compounds, consisting of SMs commonly in *A. terreus* (terrein, butyrolactone I and terretonins) or not (acoapetaline and epi-aszonalenins).

Besides the excellent ecological adaptability, *A. terreus* is an illustrious strain in the industrial production of lovastatin, a valuable cholesterol-lowering agent for hyperlipidemia treatment[39]. Lovastatin inhibits hydroxyl methyl glutaryl coenzyme A reductase (HMG CoA), the key enzyme of cholesterol biosynthesis, which is supposed as an adaptive strategy to halter fungal ergosterol generation, required for the maintenance of cytoplasmic membrane integrity[40, 41]. The lovastatin biosynthesis is encoded by a BGC (*lov*) containing 18 genes, among which, five genes (*lov*A, B, C, D, and F) have been identified to encode essential enzymes, while *lov*E acts as transcription factor, positively controlling the biogenic route[42, 43]. However, as for the homogenous BGC within B12 genome, the deletion of *lov*E and a significant truncation of *lov*F were detected, which assumedly would abolish lovastatin production. In terms of the synthetic logic of lovastatin, which actually provides a biochemical aid for fungal self-protection[44], the acquisition of *lov* BGC by horizontal gene transfer might actually depend on the niche competitive status. The biosynthetic impuissance due to gene deficits had been reported in an endophytic *A. terreus* strain[45], which possibly should ascribe to the primitive symbiotic environments of the strain above-mentioned or B12, for the comparable but diverse philosophy.

The substantial biosynthetic potential was highlighted by genome mining within B12, depicted by various uncharacterized BGCs, which requested the corresponding linkage with the product. However, compared with lovastatin, many other synthetic processes still remains elusive, even within the best understood genome of *A. terreus* (strain NIH 2624)[34]. For instance, from fermented extract of B12, terretonins and butyrolactone I had been isolated, which were adopted as major metabolites of *A. terreus* and physiological regulators in response to ecological competition [46, 47]. It had been proposed that a compact cluster (*trt*) was responsible for terretonin biosynthesis, although conversion process to the end product (terretonin) remained ambiguous, which might involve other genes in different loci[48]; While a NRPS-like gene *bty*A was presumed to encode the core enzyme for the backbone of

γ-butyrolactone in *A. terreus*[49] . Whereas, according to the sequence homology, the attempt to assign those products to possible BGCs within the B12 genome led to failure, probably due to currently obscure understanding on secondary metabolic pathways, especially given the possibility of isozymes or orthologs presented in the unknown BGCs, which could lead to partly overlapping biosynthetic routes[50, 51].

Conclusion

In our study, the combination of genome mining and compound separation, although far from comprehensive, have underscored the metabolic potencies of the *A. terreus* strain B12, which might ascribe to the exclusive biological plasticity for niche acclimatization. The peculiar metabolites and BGCs, either characterized or not, presumptively contribute to the defensive mechanisms and survival strategies for the strain, in light of the harshness and competitiveness of marine habitats. Our work shed more light on the genetic and biological profiling of marine-derived *A. terreus*, which might facilitate further investigation and development of the valuable microorganism.

Acknowledgements

This work was supported by funds from the National Key R&D Program of China (2018YFC0311000), the Priority Academic Program Development of the Jiangsu Higher Education Institutions (PAPD).

Reference

1. Shin, H.J.M.d., *Natural Products from Marine Fungi.* 2020. **18**(5).
2. Rateb, M.E. and R.J.N.P.R. Ebel, *Secondary metabolites of fungi from marine habitats.* 2011. **28**(2): p. 290-344.
3. Johannes, I.J.M.D., *Natural Products from Marine Fungi—Still an Underrepresented Resource.* 2016. **14**(1).
4. Taylor, M., et al., *Sponge-associated microorganisms: evolution, ecology, and biotechnological potential.* 2007. **71**(2): p. 295-347.
5. H?Ller, U., et al., *Fungi from marine sponges: diversity, biological activity and secondary metabolites.* 2000. **104**(11): p. 1354-1365.
6. Gao, Y., et al., *Genome sequence of Aspergillus flavus A7, a marine-derived fungus with antibacterial activity.* 2020.
7. Leck, A.J.C.E.H., *Preparation of Lactophenol Cotton Blue Slide Mounts.* 1999. **12**(30): p. 24.
8. Lee, S., J.J.M.b. Taylor, and evolution, *Phylogeny of five fungus-like protoctistan Phytophthora species, inferred from the internal transcribed spacers of ribosomal DNA.* 1992. **9**(4): p. 636-53.
9. Hinrikson, H., et al., *Assessment of ribosomal large-subunit D1-D2, internal transcribed spacer 1, and internal transcribed spacer 2 regions as targets for molecular identification of medically important Aspergillus species.* 2005. **43**(5): p. 2092-103.

10. Zerbino, D. and E.J.G.r. Birney, *Velvet: algorithms for de novo short read assembly using de Bruijn graphs.* 2008. **18**(5): p. 821-9.
11. Boetzer, M., et al., *Scaffolding pre-assembled contigs using SSPACE.* 2011. **27**(4): p. 578-9.
12. Boetzer, M. and W.J.G.b. Pirovano, *Toward almost closed genomes with GapFiller.* 2012. **13**(6): p. R56.
13. Blin, K., et al., *antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline.* 2019. **47**: p. W81-W87.
14. Stanke, M., et al., *AUGUSTUS: ab initio prediction of alternative transcripts.* 2006. **34**: p. W435-9.
15. Consortium, G.O.J.N.A.R., *The Gene Ontology (GO) database and informatics resource.* 2004(suppl_1): p. suppl_1.
16. Kanehisa, M., et al., *New approach for understanding genome variations in KEGG.* 2019. **47**: p. D590-D595.
17. Tatusov, R., et al., *The COG database: an updated version includes eukaryotes.* 2003. **4**: p. 41.
18. Schoch, C., et al., *Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi.* 2012. **109**(16): p. 6241-6.
19. Zhu, B., et al., *Multi-omics analysis of niche specificity provides new insights into ecological adaptation in bacteria.* 2016.
20. Alberts, B., et al., *Integrins - Molecular Biology of the Cell - NCBI Bookshelf.* 2002.
21. Chettri, D., A.K. Verma, and A.K.J.B.R. Verma, *Innovations in CAZyme gene diversity and its modification for Biorefinery applications.* 2020: p. e00525.
22. Bok, J., et al., *Genomic mining for Aspergillus natural products.* 2006. **13**(1): p. 31-7.
23. Godio, et al., *A Squalene Epoxidase Is Involved in Biosynthesis of Both the Antitumor Compound Clavaric Acid and Sterols in the Basidiomycete H. sublateritium.* 2007.
24. Shindo, Riko, and N.J.T.J.o.A.A.I. Journal, *Studies on pyranonigrins-isolation of pyranonigrin E and biosynthetic studies on pyranonigrin A.* 2014. **67**(2): p. 179-181.
25. Jalal, M., S.K. Love, and D.J.B.o.M. Helm, *N α -Dimethylcoprogens Three novel trihydroxamate siderophores from pathogenic fungi.* 1988. **1**(1): p. 4-8.
26. Zaehle, C., et al., *Terrein biosynthesis in Aspergillus terreus and its impact on phytotoxicity.* 2014. **21**(6): p. 719-31.
27. Solhaug, A., et al., *Mechanisms of Action and Toxicity of the Mycotoxin Alternariol: A Review.* 2016.
28. Brock, H.M.J.C. and Biology, *Multifactorial Induction of an Orphan PKS-NRPS Gene Cluster in Aspergillus terreus.* 2011.
29. Alberts, et al., *Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent.* 1980. **77**(7): p. 3957-3961.
30. Mulder, K., et al., *Lovastatin production: From molecular basis to industrial process optimization.* 2015. **33**: p. 648-65.
31. Miranda, R.U., et al., *Reactive oxygen species regulate lovastatin biosynthesis in Aspergillus terreus during submerged and solid-state fermentations.* 2014. **118**(12).
32. Barrios-González, J., et al., *New knowledge about the biosynthesis of lovastatin and its production by fermentation of Aspergillus terreus.* 2020. **104**(21): p. 8979-8998.

33. Yin, Y., et al., *Polyketides in Aspergillus terreus: biosynthesis pathway discovery and application.* 2016. **100**(18): p. 7787-7798.
34. Guo, C. and C.J.F.i.m. Wang, *Recent advances in genome mining of secondary metabolites in Aspergillus terreus.* 2014. **5**: p. 717.
35. Hu, Z.X., et al., *Seven new anthranilamide derivatives from Aconitum apetalum.* 2018.
36. Xie, X.L., et al., *A new prenylated coumarin and a new anthranilamide derivative from Evodia lepta.* 2019: p. 1-5.
37. Rank, C., et al., *epi-Aszonalenins A, B, and C from Aspergillus novofumigatus.* 2006. **47**(34): p. 6099-6102.
38. Zhang, H., Z. Zhao, and H.J.M.d. Wang, *Cytotoxic Natural Products from Marine Sponge-Derived Microorganisms.* 2017. **15**(3).
39. Tobert, J.J.N.r.D.d., *Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors.* 2003. **2**(7): p. 517-26.
40. Rodrigues, M.J.m., *The Multifunctional Fungal Ergosterol.* 2018. **9**(5).
41. Keller, N.J.N.c.b., *Translating biosynthetic gene clusters into fungal armor and weaponry.* 2015. **11**(9): p. 671-7.
42. Campbell, C. and J.J.B. Vederas, *Biosynthesis of lovastatin and related metabolites formed by fungal iterative PKS enzymes.* 2010. **93**(9): p. 755-63.
43. Kennedy, J., et al., *Modulation of polyketide synthase activity by accessory proteins during lovastatin biosynthesis.* 1999. **284**(5418): p. 1368-72.
44. Zhgun, A.A., et al., *The Role of LaeA and LovE Regulators in Lovastatin Biosynthesis with Exogenous Polyamines in Aspergillus terreus.* 2019. **55**(6): p. 639-648.
45. Savitha, J., S.D. Bhargavi, and V.K. Praveen, *Bioinformatic Comparative Analysis of Lovastatin Gene Cluster in Endophytic Fungi and a Soil Fungus, Aspergillus terreus.* 2014.
46. Schimmel, T.G., et al., *Effect of butyrolactone I on the producing fungus, Aspergillus terreus.* 1998. **64**(10): p. 3707-12.
47. Louis, B., et al., *Invasive Aspergillus terreus morphological transitions and immunoadaptations mediating antifungal resistance.* 2017. **10**: p. 425-436.
48. Guo, C.J., et al., *Molecular Genetic Characterization of a Cluster in A. terreus for Biosynthesis of the Meroterpenoid Terretonin.* 2012. **14**(22).
49. Guo, C.J., et al., *Application of an efficient gene targeting system linking secondary metabolites to their biosynthetic genes in Aspergillus terreus.* 2013. **15**(14): p. 3562-3565.
50. Hossain, A.H., et al., *Rewiring a secondary metabolite pathway towards itaconic acid production in Aspergillus niger.* 2016. **15**(1): p. 130.
51. *Spatial regulation of a common precursor from two distinct genes generates metabolite diversity %J Chemical Science.* 2015. **6**(10): p. 5913-5921.

Figures

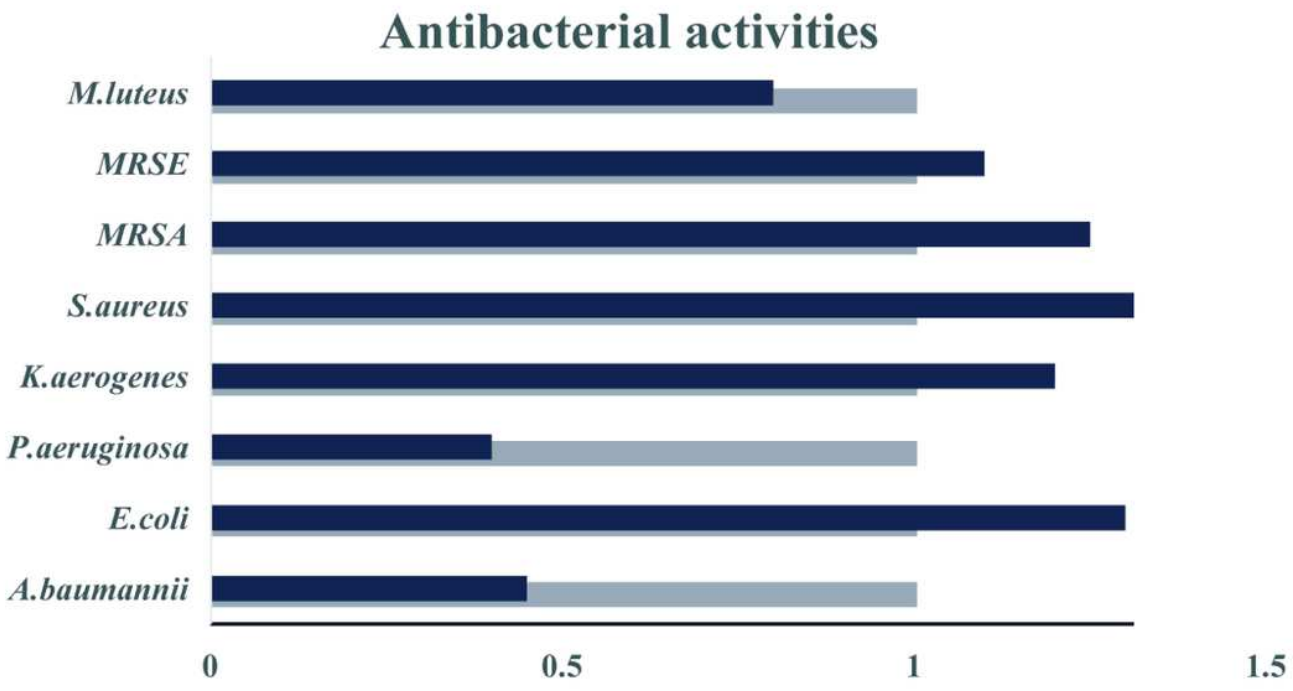


Figure 1

The antibacterial activities of the extractive of B12. The standard antibiotics (0.1 mM chloramphenicol) were used as a positive control, MeOH was used as a vehicle. The diameters (in mm) of the inhibition zone were recorded to estimate antimicrobial activities, which were expressed by the ratio of the inhibition zone relative to that of the positive control（0.1 mM chloramphenicol）. The bacteriostatic activities were considered strong if the ratio was greater than 1.0, moderate when the scale was between 0.5 and 1, and weak if it was less than 0.5.the blue bar indicated as 1, the black-blue indicated as the ratio of the inhibition zone relative to that of the positive control. E. coli: Escherichia coli; P. aeruginosa: Pseudomonas aeruginosa; K. aerogenes: Klebsiella aerogenes; MRSA: methicillin-resistant Staphylococcus aureus; MRSE: methicillin-resistant Staphylococcus epidermidis; M. luteus: Micrococcus luteus; A. baumannii: Acinetobacter baumannii.

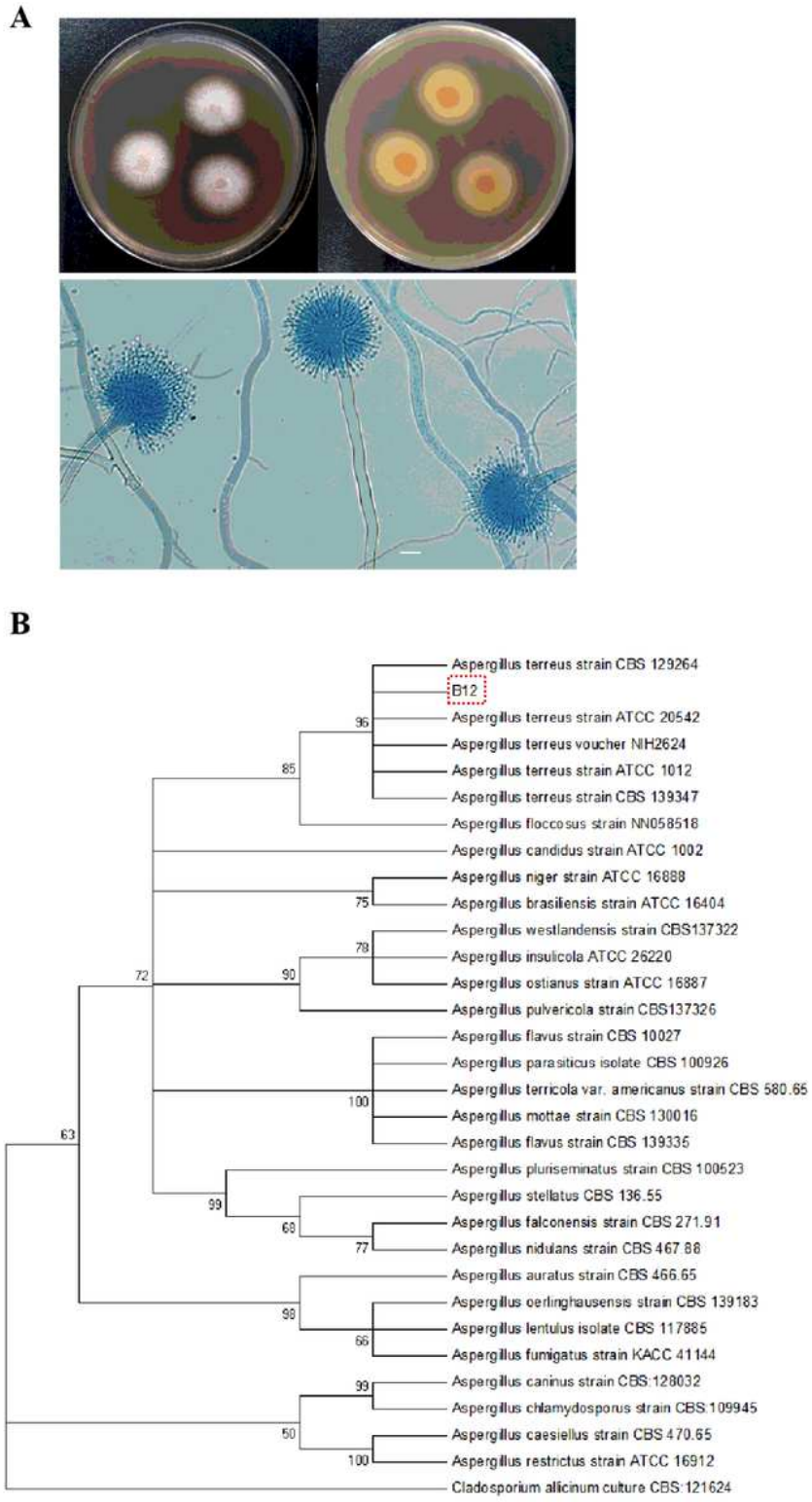


Figure 2

Identification of B12. A. Morphology of characterized strains, Colony and microscopic morphology after 5 days of incubation. From left to right: obverse colonies on MEA, reverse on MEA, conidiophores and conidiogenous at 40× magnification (scale bar 10 μm). B. Maximum likelihood tree of B12, Multiple sequence alignment was conducted using Clustal W (default settings), and phylogenetic relationships were based on ML analysis with 1000 bootstrap replications in MEGA7.

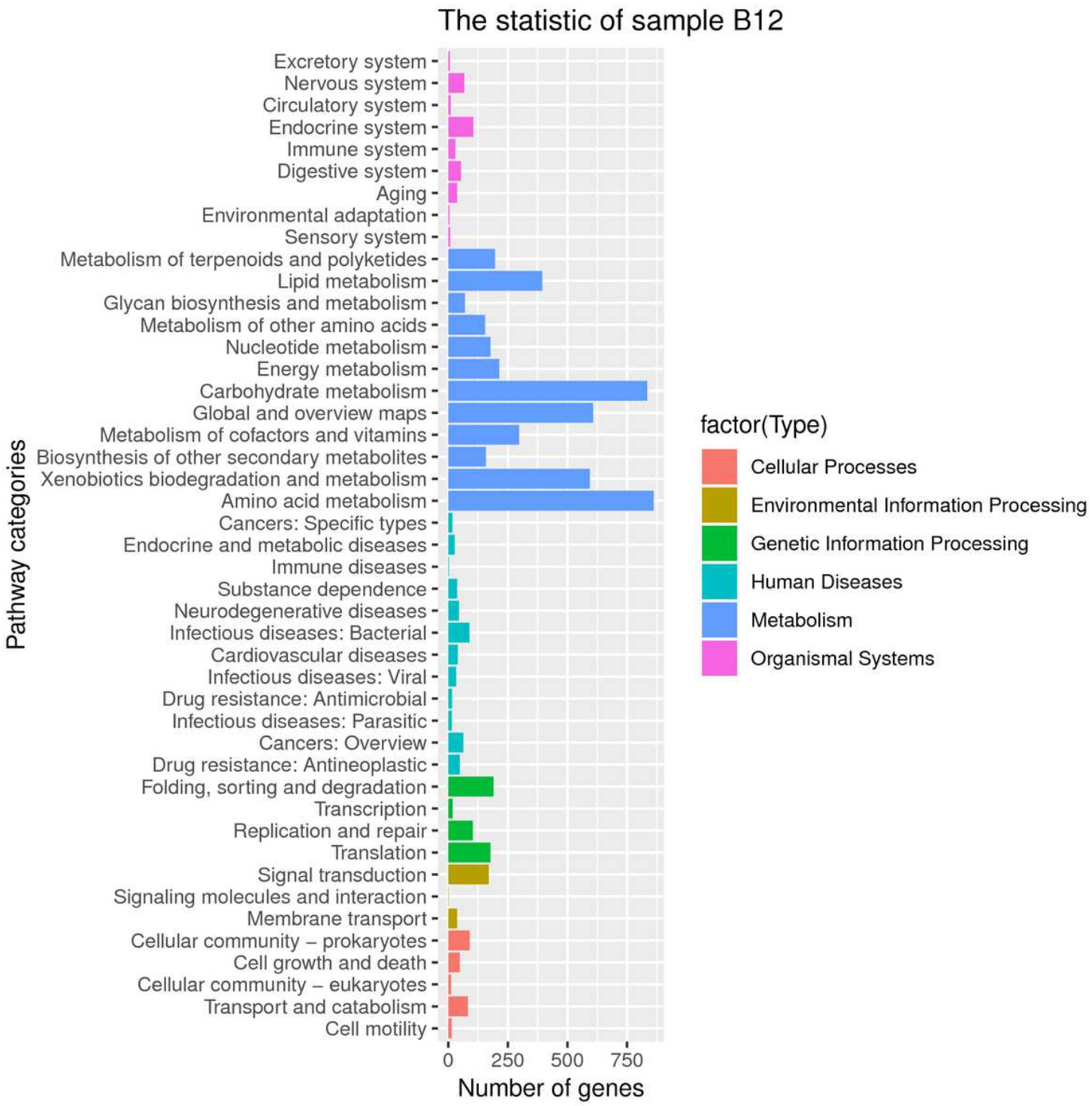


Figure 3

KEGG classifications of predicted coding genes in the genome of B12. Red: Cellular Processes; Brown: Environment Information Processing; Green: Genetic Information Processing; Cyan: Human Diseases; Blue: Metabolism; Purple: Organismal Systems.

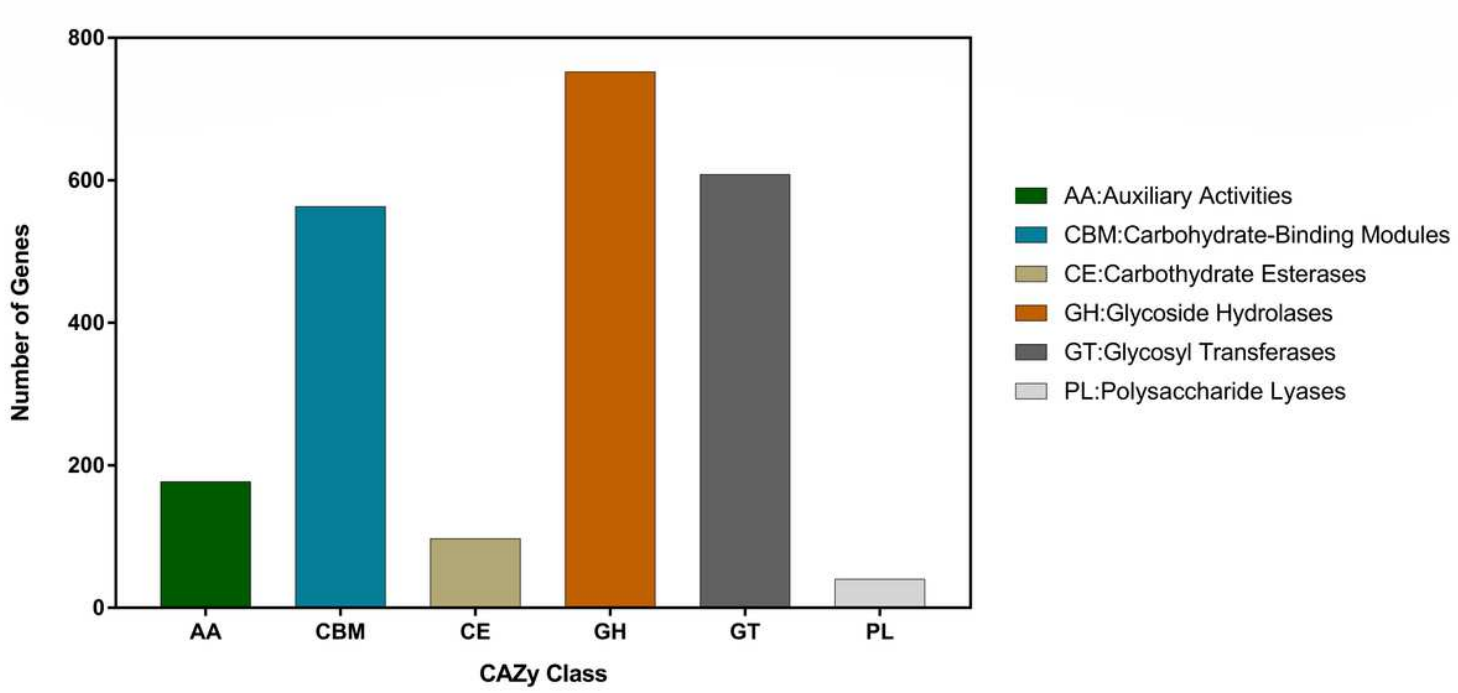


Figure 4

CAZy classifications of putative proteins in the genome of B12.

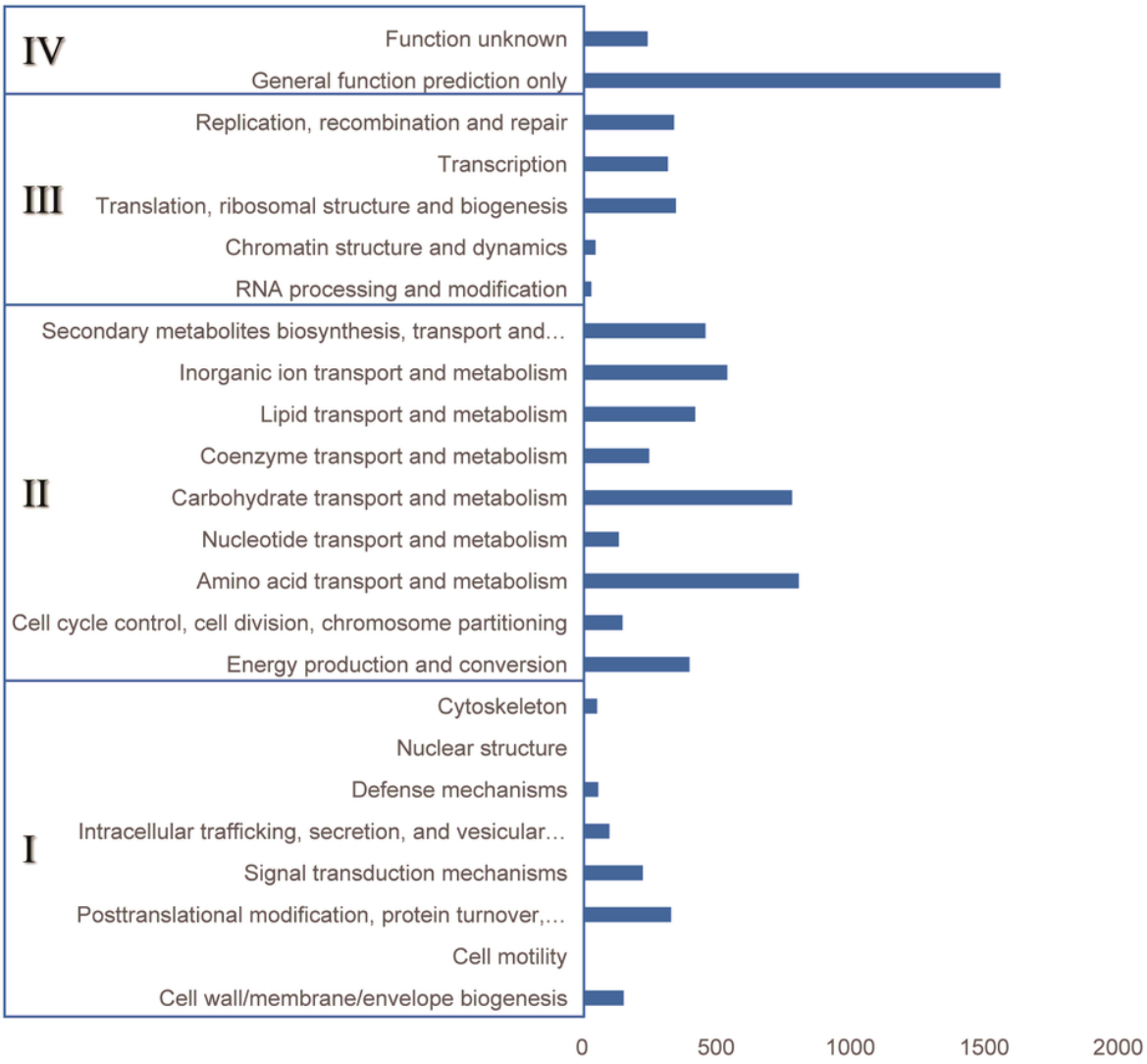


Figure 5

COG classifications of putative proteins in the genome of B12. I: intracellular processes; II: metabolism; III: information storage/processing; IV: poorly characterized function.

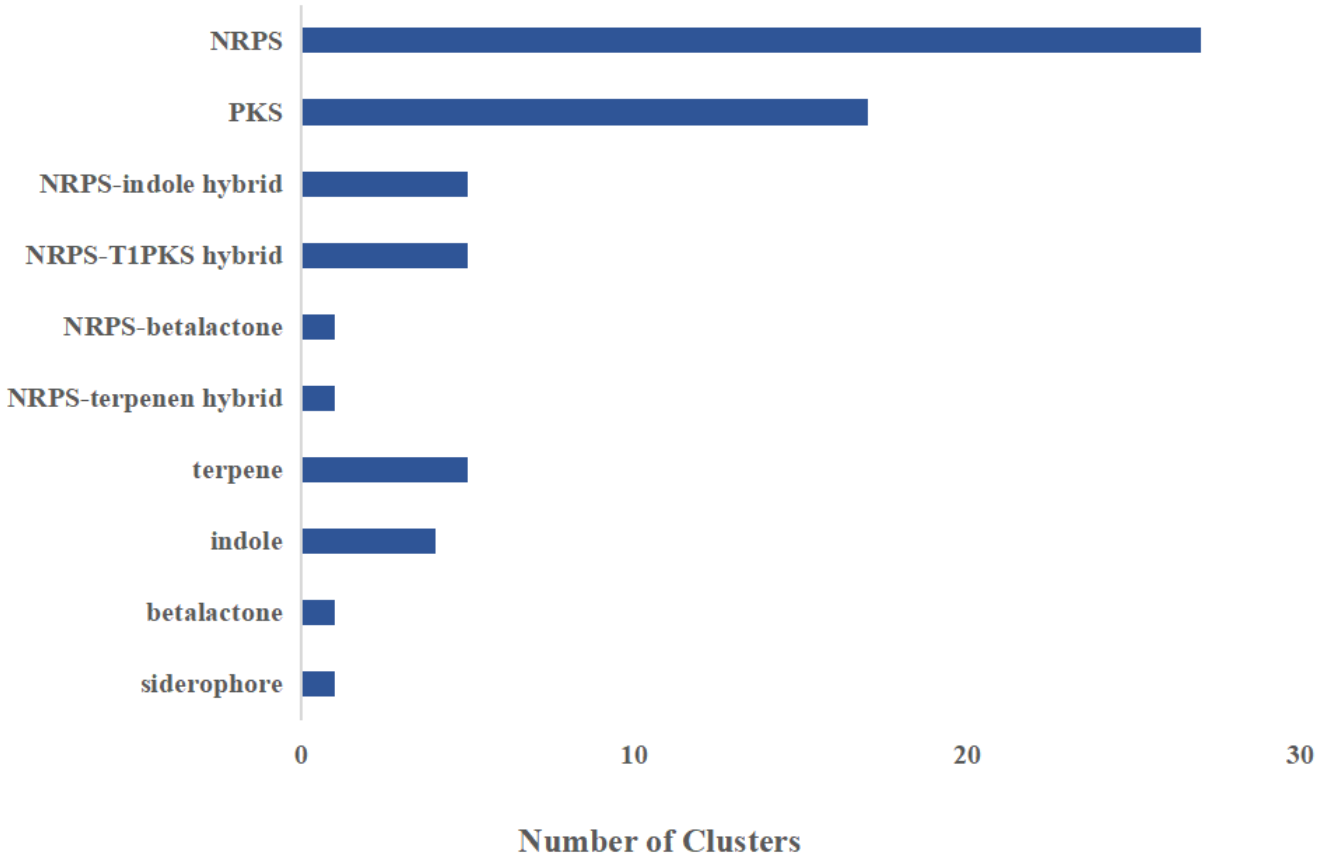


Figure 6

BGCs predicted in the B12 genomes based on antiSMASH

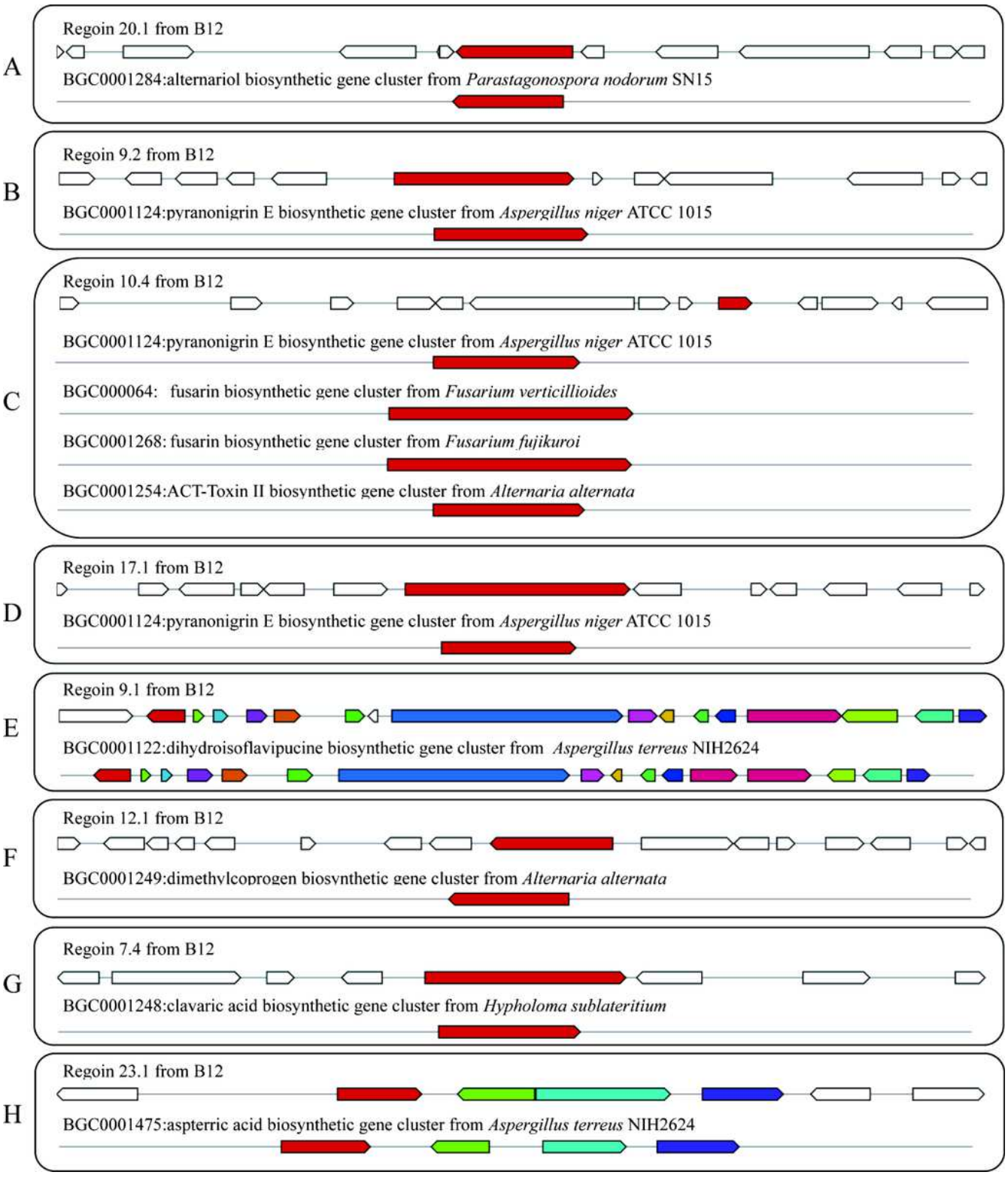
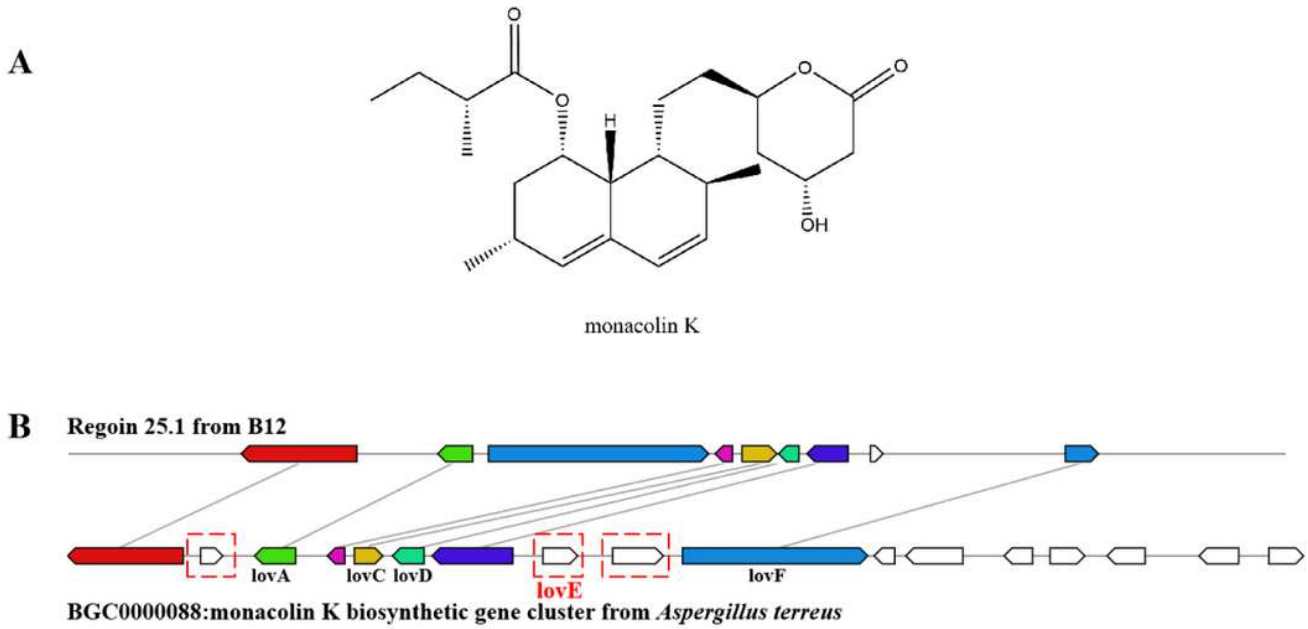


Figure 7

Schematic representation of B12 putative BGCs showing high similarity with genes from characterized BGCs. A-H. The upper part represents the BGC in B12, followed by the known BGCs in the MIBiG database.



Identifiers in *Aspergillus terreus*	Gene code	Product	Query cover	Identity
AAD34550.1	---	esterase	95	90
AAD34551.1	---	unknown	---	---
AAD34552.1	lovA	cytochrome P450 monooxygenase	104	96
AAD34553.1	---	unknown	99	94
AAD34554.1	lovC	enoyl reductase	114	86
AAD34555.1	lovD	transesterase	100	99
AAD34556.1	---	hydroxymethylglutaryl-coenzyme A reductase	81	96
AAD34557.1	lovE	regulatory protein	---	---
AAD34558.1	---	unknown	---	---
AAD34559.1	lovF	polyketide synthase	100	96

Figure 8

Putative monacolin K BGC in B12. A.The structure of monacolin K. B.The homologous genes of region 25.1 in B12 and terrein BGC known in Aspergillus terreus have been marked with the same color, and the missing genes are marked with red dotted boxes. Known BGCs description and amino acid homology (query cover and identity) were listed in the table.

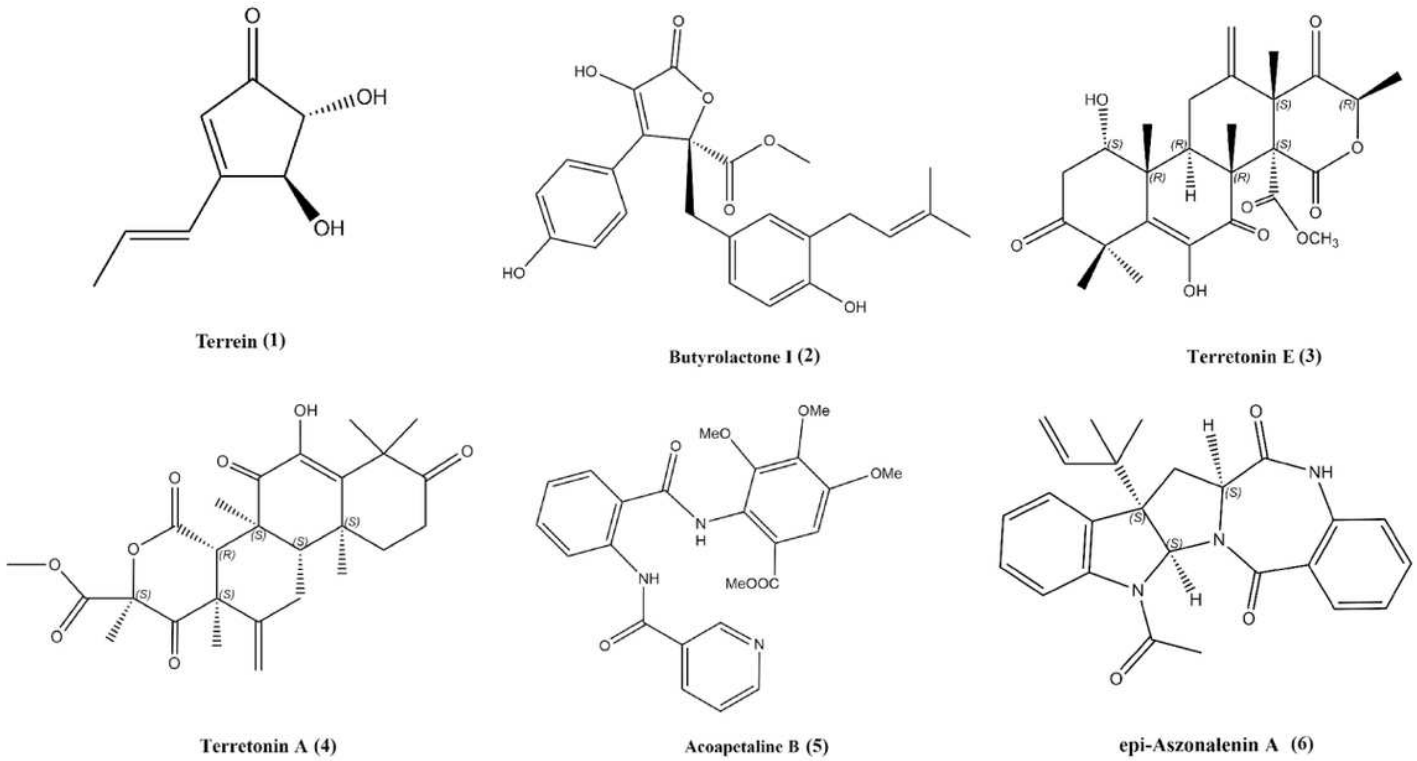
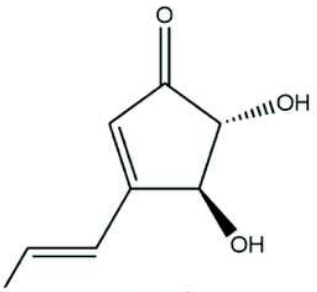


Figure 9

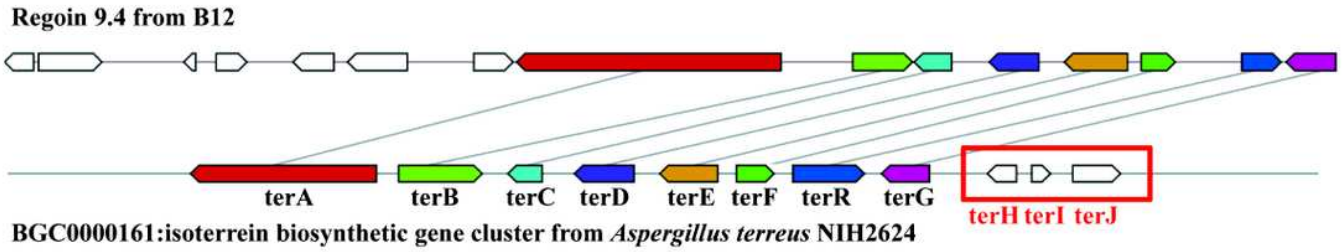
The compounds was isolated from B12

A



terrein

B



BGC0000161:isoterrein biosynthetic gene cluster from *Aspergillus terreus* NIH2624

Identifiers in *Aspergillus terreus*NIH2624	Gene code	Description	Query cover	Identity
ATEG_00135	terJ	major facility transporter (MFS)	---	---
ATEG_00136	terI	protein of the superfamily of bleomycin resisitance protein, glyoxalase I, and type I ring-cleaving dioxygenanses	---	---
ATEG_00137	terH	NAD dependent epimerase/dehydratase	---	---
ATEG_00138	terG	major facility transporter (MFS)	100%	100%
ATEG_00139	terR	Zn_2Cys_6 transcriptional regulator	99%	72%
ATEG_00140	terF	protein with kelch motif	123%	80%
ATEG_00141	terE	multicopper oxidase	94%	91%
ATEG_00142	terD	FAD-dependent monooxygenase	99%	99%
ATEG_00143	terC	FAD-dependent monooxygenase	144%	68%
ATEG_00144	terB	DH-KR, multidomain protein with dehydratase and ketoreductase function	74%	73%
ATEG_00145	terA	non-reducing polyketide synthase (NR-PKS)	84%	97%

Figure 10

Putative Terrein BGC in B12. A.The structure of Terrein. B.The homologous genes of region 25.1 in B12 and terrein BGC known in Aspergillus terreus have been marked with the same color, and the missing genes are marked with red dotted boxes. B.Known BGCs description and amino acid homology (query cover and identity) were listed in the table.