Genome sequencing and analysis reveal the biosynthetic mechanism of anticancer drug terpenoid in *Aspergillus tubingensis*

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Abstract

In recent years, *Aspergillus tubingensis* is extremely rich in secondary metabolites, which is a valuable medical fungus needs to be further explored. So far, many small molecular compounds with novel structure and significant biological activity have been discovered from *A. tubingensis*, among which terpenoids account for about 20%, showing great research potential in anti-cancer. Although more and more new terpenoids have been discovered from *A. tubingensis* and their structures have been identified, few studies have investigated the biosynthetic pathway of terpenoid. In order to further elucidate the biosynthetic mechanism of terpenoid, the key genes and enzymes involved in terpenoid biosynthesis were successfully mined and further analyzed based on genome sequencing analysis. Subsequently, hydroxymethylglutaryl-CoA synthase, mevalonate kinase, phosphomevalonate kinase as well as diphosphomevalonate decarboxylase were annotated, which played important roles in terpenoid biosynthesis of *A. tubingensis*. Furthermore, the biosynthetic pathway of terpenoid in *A. tubingensis* has been constructed, which could be applied to develop the metabolic regulation of *A. tubingensis*. This study would provide more sufficient scientific basis and new ideas for the genetic regulation of anticancer drug terpenoid biosynthesis in *A. tubingensis*.

Introduction

The deep sea is one of the most mysterious and unexplored extreme environments in the world, characterized by lack of light, low temperatures, anaerobic conditions, and hydrostatic pressures that could reach more than 1000 atmospheres (Thornburg et al., 2010). Over the past 50 years, there have been about 24,000 natural products of marine origin, but less than 2% of them come from deep-sea sources. Generally, deep-sea microorganisms have unique physiological metabolic processes by the adaptation to extreme environmental conditions, and thus exhibit the chemical structure diversity, novelty, and significant biological activity of their metabolites, such as anticancer (Skropeta and Wei, 2014). It has been reported that over 75% of natural products from deep-sea sources are bioactive, and more than half show significant cytotoxic activity against human tumor cell lines (Skropeta, 2008). As an important group of deep-sea microorganisms, deep-sea fungi are well known for their diversity of secondary metabolites, which have the potential to treat cancers and find new biological targets, providing important support for anticancer drug research and development (Xu et al., 2018).

Aspergillus tubingensis, as a valuable deep-sea-derived fungus needs to be further explored, is extremely rich in secondary metabolites (Koch et al., 2014). At present, anthraquinones, alkaloids, terpenoids and other compounds have been isolated from *A. tubingensis*, and many of the secondary metabolites have the activities of anticancer, antibacterial, anti-inflammatory and antiviral, which have a certain potential as drugs (Carboue et al., 2019; Ottoni et al., 2019). Terpenoids, which have a wide range of sources and varieties, are one of the hot anti-cancer drugs currently studied (Batool et al., 2021). There are many mechanisms of these drugs against cancer, among which the inhibition of tumor angiogenesis to inhibit the growth and metastasis of tumor cells is one of the hot research directions at present (Sun et al., 2021).

Terpenoids refer to derivatives with $(C_5H_8)_n$ general formula, oxygen content and different saturation levels, which can be regarded as a class of natural compounds linked in various ways by isoprene or isopentane (Hillier and Lathe, 2019). Although terpenoids are not the dominant components in *A. tubingensis*, they play an important role among the active ingredients because of their anticancer, antiviral and anti-inflammatory activities. It was reported that five terpenoids of 20-norisopimarane were isolated from *Aspergillus* from deep-sea sediments in the South China Sea, which showed significant antibacterial activity against *Fusarium graminearum* (Li et al., 2016). Furthermore, a new indole diterpene Penicindopene A was isolated from *Penicillium* YPCMAC1 from deep sea of western pacific, which showed moderate cytotoxicity to A549 and HeLa cell lines (Liu et al., 2019). Subsequently, two unique phenol-sesquiterpenoids, phomeroid A-B, were isolated from *Phomopsis tersa* FS441 from deep-sea sediment samples in the Indian Ocean, which showed moderate inhibitory activity against human glioblastoma SF-268, breast cancer MCF-7, liver cancer HepG-2 and lung cancer A549 (Chen et al., 2020). The above studies indicated that more and more novel chemical structures derived from medical microorganisms have been discovered, with the development of deep-sea microbial sample collection, separation and purification technology and compound structure analysis technology, which is beneficial to the excavation of medicala fungal resources (Li et al., 2015).

Even so, few studies have investigated the biosynthetic pathway of anticancer drug terpenoid, and it is becoming more and more urgent to investigate the hereditary information or functional genes of *A. tubingensis* by omics sequencing technology (Lin et al., 2019). Therefore, the functional genes involved in the biosynthetic pathway can be obtained by genome sequencing analysis, and the biological processes in different states can be revealed (Xiong and Zhao, 2018). In the present study, to better understand the molecular factors and their regulatory genes involved in accumulation of terpenoid, the genomic profiles of *A. tubingensis* were analyzed. We gain insights into the terpenoid accumulation mechanism of *A. tubingensis*, particularly the functional genes and enzymes involved in terpenoid biosynthesis. Physiological observations such as growth and terpenoid biosynthesis were linked to genomic data obtained by genome sequencing. These results would provide novel insight into understanding the molecular mechanisms of anticancer drug terpenoid accumulation and aid in understanding its biosynthesis, and developing future studies on the metabolic regulation of *A. tubingensis*.

Materials And Methods

Strains and genomic data

The Draft Genome Sequence of *A. tubingensis* WU-2223L, a Citric Acid-producing Filamentous Fungus Belonging to Aspergillus Section Nigri, has been submitted to NCBI (BLWE00000000). The Genome Coverage was 163x, which was applied by HiSeq X; Nanopore MinION. The Assembly Method was MaSuRCA v. 3.3.3. The genomic profiles of *A. tubingensis* were downloaded for further bioinformatics analysis.

Functional gene mining of anticancer drug terpenoid biosynthesis in A. tubingensis

The files of genome sequencing results were downloaded on computer. Subsequently, the annotation result files of assembled Unigenes from the genome sequencing results were obtained. Noteworthy, paired-end reads were used again for gap filling of scaffolds to obtain sequences with least Ns and cannot be extended on either end. Such sequences were defined as Unigenes. Unigene annotation provides information of expression and functional annotation of Unigene. The “annotation files” path was opened, and “map 00900” in the search bar was entered. Then, terpenoid backbone biosynthesis (map00900) in the KEGG classification of annotation files was searched. Furthermore, the functional genes such as hydroxymethylglutaryl-CoA synthase, mevalonate kinase, phosphomevalonate kinase and diphosphomevalonate decarboxylase, were mined and analyzed to confirm whether annotated in *A. tubingensis* genome.

Bioinformatic analysis of functional genes involved in anticancer drug terpenoid biosynthesis

The fungus *A. tubingensis*, along with the annotation results of Unigenes, which has been submitted on NCBI (BLWE00000000). Open reading frame (ORF) of functional genes was analyzed by ORF program (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Theoretical molecular mass and isoelectric point of functional enzymes was calculated by ProtParam tool (http://us.expasy.org/tools/protparam.html), and catalytic domain was identified by InterProScan (http://www.ebi.ac.uk/Tools/InterProScan/). Moreover, protein secondary structure was predicted by Predict Protein (http://www.predictprotein.org/) online. In addition, the amino acid sequences of 18SRNA from different fungi were aligned using the Clustal X program (http://www.clustal.org/). Finally, phylogenetic tree was carried out by MEGA 4.0 (https://www.megasoftware.net/mega4/).

Construction of biosynthetic pathway of anticancer drug terpenoid in A. tubingensis

Based on the KEGG Pathway annotation information by genome sequencing and short read sequence assembly, the biosynthetic pathways of terpenoid in *A. tubingensis* were analyzed. The metabolic pathways of terpenoid were searched and screened from KEGG PATHWAY Database (http://www.genome.jp/kegg/pathway.html). Subsequently, the KEGG Pathway annotation Database was used to find the annotation pathways corresponding to the Pathway numbers obtained from the KEGG Pathway Database. The online KEGG Pathway database and genomic KEGG Pathway annotation information were compared and analyzed to study the biosynthetic pathways of terpenoids, as well as the corresponding Unigene sequences of related enzymes.

Results

Phylogenetic tree analysis of 18S rRNA

The medical fungi have the characteristics of species diversity. Phylogenetic tree analysis of the genetic relationships could be carried out to classify the characteristics at a higher level based on molecular biology and ITS region sequencing. The phylogenetic tree of *A. tubingensis* and other strains in Genbank was shown in Fig. 1, which indicated that *A. tubingensis* is closely related to *Aspergillus*

magaliesburgensis (MK450649.1) and *Aspergillus alliaceus* (EF661548.1) in evolutionary relationship, forming a relatively independent branch. These diverse species of medicalsea fungi provided abundant secondary metabolites for humans.

Draft genome sequence of A. tubingensis

The *A. tubingensis* whole genome shotgun (WGS) project has the project accession BLWE00000000, which has been submitted to NCBI(Yoshioka et al., 2020). This version of the project has the accession number BLWE01000000, and consists of sequences BLWE01000001-BLWE01000015. We downloaded the genomic profiles from NCBI for further bioinformatics analysis, and the genome features of *A. tubingensis* were shown in Table 1.

Table 1
Genome features of *A. tubingensis*

Characteristics	***A. tubingensis***
Genome Coverage	163x
Contigs	15
Proteins	11,479
Total length	35,047,229 bp
BioProject	PRJDB9560
BioSample	SAMD00216538

Mining functional proteins involved in anticancer drug terpenoid biosynthesis of A. tubingensis

Paired-end reads were used for gap filling of scaffolds to obtain Unigenes with least Ns that cannot be extended on either end. Furthermore, the files of annotation result of assembled Unigenes from the genome sequencing were obtained for further analysis. Subsequently, the functional proteins involved in terpenoid biosynthesis of *A. tubingensis* were shown in Table 2. Among them, hydroxymethylglutaryl-CoA synthase, mevalonate kinase, phosphomevalonate kinase as well as diphosphomevalonate decarboxylase were annotated, which played important roles in terpenoid biosynthesis of *A. tubingensis*.

Table 2
Functional proteins involved in terpenoid biosynthesis of *A. tubingensis*

Protein Name	Protein Accession	Protein Length	Molecular weight	Theoretical pI
hydroxymethylglutaryl-CoA synthase	GFN16044.1	460	50832.65	5.98
	GFN17970.1	451	49879.84	5.72
mevalonate kinase	GFN12941.1	539	58477.35	6.77
phosphomevalonate kinase	GFN11149.1	463	50637.27	5.21
diphosphomevalonate decarboxylase	GFN12862.1	762	82702.22	8.98
	GFN12877.1	404	43399.13	5.68

Subsequently, bioinformatics analysis of the above proteins was developed, and the molecular weight and theoretical pI were shown in Table 2, respectively. Among them, diphosphomevalonate decarboxylase (GFN12862.1) was alkaline protein, mevalonate kinase (GFN12941.1) was neutral protein, and the rest were acidic proteins.

Secondary and tertiary structures of functional proteins involved in anticancer drug terpenoid biosynthesis

In order to further understand the function of these proteins, secondary and tertiary structures of the functional proteins involved in terpenoid biosynthesis of *A. tubingensis* were predicted. The predicted secondary structure composition of functional proteins was shown in Table 3, which showed the ratio of strand to helix in each protein, respectively. Subsequently, the predicted tertiary structures of functional proteins involved in terpenoid biosynthesis were shown in Fig. 2, which could indicate the protein family to which the protein belongs and the preference of the substrate.

Table 3
Predicted secondary structure composition of functional proteins involved in terpenoid biosynthesis of *A. tubingensis*.

Protein Name	Protein Accession	Helix (%)	Strand (%)	Loop (%)
hydroxymethylglutaryl-CoA synthase	GFN16044.1	41.52	13.26	45.22
	GFN17970.1	43.33	13.11	43.56
mevalonate kinase	GFN12941.1	26.16	15.77	58.07
phosphomevalonate kinase	GFN11149.1	36.50	19.01	44.49
diphosphomevalonate decarboxylase	GFN12862.1	12.86	0.66	86.48
	GFN12877.1	25.50	13.86	60.64

Constructing the predicted biosynthetic pathway of anticancer drug terpenoid in A. tubingensis

Subsequently, terpenoid backbone biosynthesis (map00900) was applied to construct the biosynthetic pathway of terpenoid in *A. tubingensis*. The functional proteins involved in terpenoid biosynthesis of *A. tubingensis* were shown, as well as the predicted biosynthetic pathway of terpenoid in *A. tubingensis* was shown in Fig. 3, which might provide more sufficient scientific basis and new ideas for the genetic regulation of terpenoid biosynthesis in *A. tubingensis*. In this pathway, the biosynthesis up to terpenoid in *A. tubingensis* is different from that in other organisms. Following acetyl-CoA was converted to 3-hydroxy-3-methyl-glutaryl-CoA by hydroxymethylglutaryl-CoA synthase, and 3-hydroxy-3-methyl-glutaryl-CoA was converted to mevalonate by hydroxymethylglutaryl-CoA reductase, mevalonate was subsequently converted to mevalonate-5P by mevalonate kinase, and mevalonate-5P was further converted to mevalonate-5PP by phosphomevalonate kinase. Moreover, mevalonate-5PP was converted to isopentenyl-PP by diphosphomevalonate decarboxylase, and isopentenyl-PP was used as precusor substance for monoterpenoid biosynthesis, diterpenoid biosynthesis and carotenoid biosynthesis and so on.

Discussion

Deep-sea environment is an extreme environment characterized by high pressure, low oxygen, high salt and no illumination. In order to adapt to the survival and reproduction in the deep-sea environment, deep-sea fungi have evolved unique physiological structure and metabolic pathway, and can produce novel structure and significant variety of secondary metabolites with biological activities, which become an important resource for the development of new drugs (Zain Ul Arifeen et al., 2019). However, limited sampling techniques have limited the study of deep-sea fungi and their metabolites (Daletos et al., 2018). In addition, there are hitherto unknown fungal lineages in the deep sea, which have unique ecological and physiological characteristics, and are difficult to grow through conventional culture methods. Fortunately, with the rapid development of deep-sea sampling and multiomics, abundant active natural products will be mined and contribute to the sustainable use of marine microbial resources and drug development. Therefore, it is more and more urgent to investigate the active ingredients of deep-sea fungi.

There are several studies focused on bacteria and archaea in deep sea, involving a wide range of species, including population distribution, separation of new active metabolites and so on (Shin, 2020; Carroll et al., 2021). As marine eukaryotic microorganisms, marine fungi are the main decomposers of low organic matter, which are characterized by diverse species, wide distribution and complex metabolic pathways. In recent years, more than half of the new compounds derived from marine microbes were come from marine fungi (Shin, 2020; Carroll et al., 2021). Secondary metabolites isolated from marine fungi have been found to have many important biological activities, such as anti-tumor, antibacterial, antioxidant, antiviral, anti-adhesion and so on (Moghadamtousi et al., 2015). Although terpenoids account for about 20% of the total active ingredients in *A. tubingensis*, they play an important role because of their anti-tumor, antiviral and anti-inflammatory activities (Khizar et al., 2020). Unfortunately, few studies have investigated the biosynthetic pathway of terpenoid, and the hereditary information or functional genes of *A. tubingensis* by omics sequencing technology is becoming more and more urgent to be investigated. In this study, we investigated the functional genes as well as proteins involved in the biosynthetic pathway

of terpenoid by omics sequencing technology for the first time, which could gain insights into the terpenoid accumulation mechanism of *A. tubingensis*, particularly the functional genes in terpenoid biosynthesis.

So far, there were several studies investigating the pharmacological effects of terpenoid. However, the studies on the functional genes as well as the enzymes involved in terpenoid biosynthesis of deep-sea fungi were few. For instance, the fungal terpenoid biosynthesis from biochemical, genetic, and genomic viewpoints were investigated, as well as the enzymes involved in synthesizing, transferring, and cyclizing the prenyl chains were systematically discussed, which provided the genomic information and functional evidence for biosynthetic mechanism of terpenoid (Schmidt-Dannert, 2015). Furthermore, the characterization and evolution of gene clusters for terpenoid phytoalexin biosynthesis in tobacco were investigated, 1181 metabolic gene clusters with 34 of them potentially being involved in terpenoid biosynthesis were identified, which demonstrated that phytoalexins in tobacco can arise from operon-like gene clusters (Chen et al., 2019). Unfortunately, the key genes involved in terpenoid biosynthesis of deep-sea fungi were lacking in-depth genomic mining and analysis. Therefore, it is certainly needed to conduct the study of genome analysis of the key genes and enzymes involved in terpenoid biosynthesis of deep-sea fungi.

To our knowledge, genome sequencing and analysis could provide information of gene expression and infer the gene functions (Liu et al., 2015; Wu et al., 2020). Next-generation sequencing technology of genome could systematically provide a complete view of expressed genes and their expression levels (Gu et al., 2019). The availability of a draft genome sequence opens new avenues for new exploration, application and improvements of marine fungi (Kumar et al., 2018). It will lead to the identification and manipulation of candidate genes or genomic regions to generate the new ways to synthesize new compounds with potentials in pharmaceuticals (Wang et al., 2010). In order to obtain a comprehensive insight into the biosynthesis of terpenoid in *A. tubingensis*, functional genes screened from the submitted genome database of *A. tubingensis* on NCBI, and their evolutionary relationships has been demonstrated. The availability of genome will facilitate the development of new products, and improve the efficient processes of production. The work present here would aid in understanding and carrying out future research on the genetic basis of biology of this organism and contribute to the further production and application of *A. tubingensis*.

Conclusion

In this study, genome-analysis was efficiently applied for investigating functional gene and enzymes involved in terpenoid biosynthesis of *A. tubingensis*, the key genes and enzymes involved in terpenoid biosynthesis was successfully mined and further analyzed for illuminating the mechanism of terpenoid biosynthesis. Subsequently, bioinformatics analysis of the key genes or enzymes was conducted to investigate the potential functions, as well as the biosynthetic pathway of terpenoid in *A. tubingensis* was constructed. Therefore, the results of this study would provide novel insight into understanding the

molecular mechanisms of terpenoid accumulation and aid in understanding its biosynthetic pathways, and developing future studies on the metabolic regulation of *A. tubingensis*.

Declarations

Author contribution

Shan Lin and Chunhua Xu conceived and designed the article; Shiping Hu and Xiaoni Chen consulted literatures; Shan Lin and Fenfang Wu analyzed the data; Shan Lin and Chunhua Xu wrote and edited the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Ethical approval

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

References

1. Batool R, Rasul A, Hussain G, Shah MA, Nageen B, Sarfraz I, Zahoor MK, Riaz A, Ajaz A, Adem S (2021) Furanodiene: A novel, potent, and multitarget cancer-fighting terpenoid. Current pharmaceutical design, 27(22): 2628–2634. Available from http://www.ncbi.nlm.nih.gov/pubmed/33573548. DOI 10.2174/1381612827666210211125304
2. Carboue Q, Maresca M, Herbette G, Roussos S, Hamrouni R, Bombarda I (2019) Naphtho-gamma-pyrones produced by aspergillus tubingensis g131: New source of natural nontoxic antioxidants. Biomolecules, 10(1). Available from http://www.ncbi.nlm.nih.gov/pubmed/31878243. DOI 10.3390/biom10010029
3. Carroll AR, Copp BR, Davis RA, Keyzers RA, Prinsep MR (2021) Marine natural products. Natural product reports, 38(2): 362–413. Available from http://www.ncbi.nlm.nih.gov/pubmed/33570537. DOI 10.1039/d0np00089b
4. Chen S, Liu Z, Tan H, Chen Y, Li S, Li H, Zhu S, Liu H, Zhang W (2020) Phomeroids a and b: Two novel cytotoxic meroterpenoids from the deep-sea-derived fungus phomopsis tersa fs441. Org Chem Front 7(3):557–562

5. Chen X, Liu F, Liu L, Qiu J, Fang D, Wang W, Zhang X, Ye C, Timko MP, Zhu QH, Fan L, Xiao B (2019) Characterization and evolution of gene clusters for terpenoid phytoalexin biosynthesis in tobacco. Planta, 250(5): 1687–1702. Available from http://www.ncbi.nlm.nih.gov/pubmed/31414203. DOI 10.1007/s00425-019-03255-7
6. Daletos G, Ebrahim W, Ancheeva E, El-Neketi M, Song W, Lin W, Proksch P (2018) Natural products from deep-sea-derived fungi a new source of novel bioactive compounds? Curr Med Chem 25(2):186–207
7. Gu W, Miller S, Chiu CY (2019) Clinical metagenomic next-generation sequencing for pathogen detection. Annual review of pathology, 14: 319–338. Available from http://www.ncbi.nlm.nih.gov/pubmed/30355154. DOI 10.1146/annurev-pathmechdis-012418-012751
8. Hillier SG, Lathe R (2019) Terpenes, hormones and life: Isoprene rule revisited. The Journal of endocrinology, 242(2): R9-R22. Available from http://www.ncbi.nlm.nih.gov/pubmed/31051473. DOI 10.1530/JOE-19-0084
9. Khizar M, Shi J, Saleem S, Liaquat F, Ashraf M, Latif S, Haroon U, Hassan SW, Rehman SU, Chaudhary HJ, Quraishi UM, Munis MFH (2020) Resistance associated metabolite profiling of aspergillus leaf spot in cotton through non-targeted metabolomics. PloS one, 15(2): e0228675. Available from http://www.ncbi.nlm.nih.gov/pubmed/32049975. DOI 10.1371/journal.pone.0228675
10. Koch L, Lodin A, Herold I, Ilan M, Carmeli S, Yarden O (2014) Sensitivity of neurospora crassa to a marine-derived aspergillus tubingensis anhydride exhibiting antifungal activity that is mediated by the mas1 protein. Marine drugs, 12(9): 4713–4731. Available from http://www.ncbi.nlm.nih.gov/pubmed/25257783. DOI 10.3390/md12094713
11. Kumar A, Sorensen JL, Hansen FT, Arvas M, Syed MF, Hassan L, Benz JP, Record E, Henrissat B, Poggeler S, Kempken F (2018) Genome sequencing and analyses of two marine fungi from the north sea unraveled a plethora of novel biosynthetic gene clusters. Scientific reports, 8(1): 10187. Available from http://www.ncbi.nlm.nih.gov/pubmed/29976990. DOI 10.1038/s41598-018-28473-z
12. Li K, Chung-Davidson YW, Bussy U, Li W (2015) Recent advances and applications of experimental technologies in marine natural product research. Marine drugs, 13(5): 2694–2713. Available from http://www.ncbi.nlm.nih.gov/pubmed/25939037. DOI 10.3390/md13052694
13. Li XD, Li XM, Li X, Xu GM, Liu Y, Wang BG (2016) Aspewentins d-h, 20-nor-isopimarane derivatives from the deep sea sediment-derived fungus aspergillus wentii sd-310. Journal of natural products, 79(5): 1347–1353. Available from http://www.ncbi.nlm.nih.gov/pubmed/27148955. DOI 10.1021/acs.jnatprod.5b01153
14. Lin S, Zou Z, Zhou C, Zhang H, Cai Z (2019) Transcriptome analysis reveals the molecular mechanisms underlying adenosine biosynthesis in anamorph strain of caterpillar fungus. BioMed research international, 2019: 1864168. Available from http://www.ncbi.nlm.nih.gov/pubmed/31915684. DOI 10.1155/2019/1864168

15. Liu L, Xu W, Li S, Chen M, Cheng Y, Yuan W, Cheng Z, Li Q (2019) Penicindopene a, a new indole diterpene from the deep-sea fungus penicillium sp. Ypcmac1. Natural product research, 33(20): 2988–2994. Available from http://www.ncbi.nlm.nih.gov/pubmed/30270680. DOI 10.1080/14786419.2018.1514402
16. Liu ZQ, Lin S, Baker PJ, Wu LF, Wang XR, Wu H, Xu F, Wang HY, Brathwaite ME, Zheng YG (2015) Transcriptome sequencing and analysis of the entomopathogenic fungus hirsutella sinensis isolated from ophiocordyceps sinensis. BMC genomics, 16: 106. Available from http://www.ncbi.nlm.nih.gov/pubmed/25765329. DOI 10.1186/s12864-015-1269-y
17. Moghadamtousi SZ, Nikzad S, Kadir HA, Abubakar S, Zandi K (2015) Potential antiviral agents from marine fungi: An overview. Marine drugs, 13(7): 4520–4538. Available from http://www.ncbi.nlm.nih.gov/pubmed/26204947. DOI 10.3390/md13074520
18. Ottoni CA, Maria DA, Goncalves P, de Araujo WL, de Souza AO (2019) Biogenic aspergillus tubingensis silver nanoparticles' in vitro effects on human umbilical vein endothelial cells, normal human fibroblasts, hepg2, and galleria mellonella. Toxicology research, 8(6): 789–801. Available from http://www.ncbi.nlm.nih.gov/pubmed/32206300. DOI 10.1039/c9tx00091g
19. Schmidt-Dannert C (2015) Biosynthesis of terpenoid natural products in fungi. Advances in biochemical engineering/biotechnology, 148: 19–61. Available from http://www.ncbi.nlm.nih.gov/pubmed/25414054. DOI 10.1007/10_2014_283
20. Shin HJ (2020) Natural products from marine fungi. Marine drugs, 18(5). Available from http://www.ncbi.nlm.nih.gov/pubmed/32349436. DOI 10.3390/md18050230
21. Skropeta D (2008) Deep-sea natural products. Natural product reports, 25(6): 1131–1166. Available from http://www.ncbi.nlm.nih.gov/pubmed/19030606. DOI 10.1039/b808743a
22. Skropeta D, Wei L (2014) Recent advances in deep-sea natural products. Natural product reports, 31(8): 999–1025. Available from http://www.ncbi.nlm.nih.gov/pubmed/24871201. DOI 10.1039/c3np70118b
23. Sun H, Zhang L, Sui B, Li Y, Yan J, Wang P, Wang Y, Liu S (2021) The effect of terpenoid natural chinese medicine molecular compound on lung cancer treatment. Evidence-based complementary and alternative medicine: eCAM, 2021: 3730963. Available from http://www.ncbi.nlm.nih.gov/pubmed/34956377. DOI 10.1155/2021/3730963
24. Thornburg CC, Zabriskie TM, McPhail KL (2010) Deep-sea hydrothermal vents: Potential hot spots for natural products discovery? Journal of natural products, 73(3): 489–499. Available from http://www.ncbi.nlm.nih.gov/pubmed/20099811. DOI 10.1021/np900662k
25. Wang M, Liu S, Li Y, Xu R, Lu C, Shen Y (2010) Protoplast mutation and genome shuffling induce the endophytic fungus tubercularia sp. Tf5 to produce new compounds. Current microbiology, 61(4): 254–260. Available from http://www.ncbi.nlm.nih.gov/pubmed/20232067. DOI 10.1007/s00284-010-9604-7
26. Wu F, Hu S, Ran Y, Chen X, Lin S (2020) Gene mining and sequence analysis of purine nucleosidase based on rna-seq. Journal of visualized experiments: JoVE(164). Available from

http://www.ncbi.nlm.nih.gov/pubmed/33165331. DOI 10.3791/61561

27. Xiong J, Zhao WL (2018) Advances in multiple omics of natural-killer/t cell lymphoma. Journal of hematology & oncology, 11(1): 134. Available from http://www.ncbi.nlm.nih.gov/pubmed/30514323. DOI 10.1186/s13045-018-0678-1
28. Xu JL, Liu HX, Chen YC, Tan HB, Guo H, Xu LQ, Li SN, Huang ZL, Li HH, Gao XX, Zhang WM (2018) Highly substituted benzophenone aldehydes and eremophilane derivatives from the deep-sea derived fungus phomopsis lithocarpus fs508. Marine drugs, 16(9). Available from http://www.ncbi.nlm.nih.gov/pubmed/30208615. DOI 10.3390/md16090329
29. Yoshioka I, Takahashi H, Kusuya Y, Yaguchi T, Kirimura K (2020) Draft genome sequence of aspergillus tubingensis wu-2223l, a citric acid-producing filamentous fungus belonging to aspergillus section nigri. Microbiology resource announcements, 9(33). Available from http://www.ncbi.nlm.nih.gov/pubmed/32817151. DOI 10.1128/MRA.00702 – 20
30. Zain Ul Arifeen M, Ma YN, Xue YR, Liu CH (2019) Deep-sea fungi could be the new arsenal for bioactive molecules.Marine drugs, 18(1)

Figures

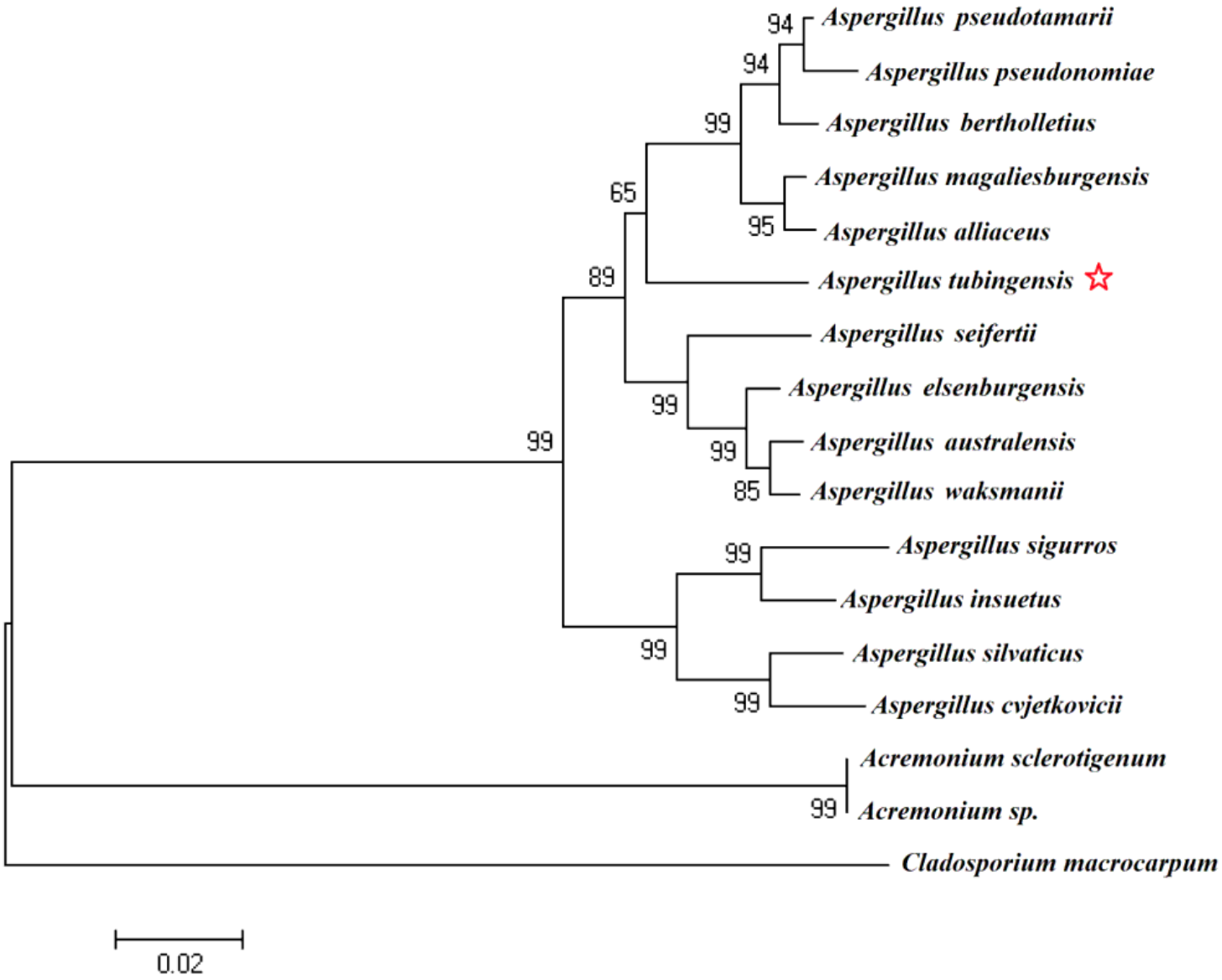

Figure 1

Phylogenetic tree of 18S rRNA of *A. tubingensis*.

Figure 2

The predicted tertiary structures of functional proteins involved in terpenoid biosynthesis. (A). Hydroxymethylglutaryl-CoA synthase: GFN16044.1; (B). Hydroxymethylglutaryl-CoA synthase: GFN17970.1; (C). Mevalonate kinase: GFN12941.1; (D). Phosphomevalonate kinase: GFN11149.1; (E). Diphosphomevalonate decarboxylase: GFN12862.1; (F). Diphosphomevalonate decarboxylase: GFN12877.1.

Figure 3

The predicted biosynthetic pathway of terpenoid in *A. tubingensis*.