Identification and Characterization of A Lanosterol synthase Gene from *Sanghuangporus Baumii*

XuTong Wang
Northeast Forestry University
TingTing Sun
Harbin University
Jian Sun
Northeast Forestry University
Zengcai Liu
Northeast Forestry University
Li Zou (372138603@qq.com)
Northeast Forestry University

Research Article

Keywords: Lanosterol synthase, Promoter, Sanghuangporus baumii, Triterpenoids

Posted Date: February 18th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-203582/v1

Abstract

Lanosterol synthase (LS) is a key enzyme involved in the mevalonate pathway (MVA pathway) to produce lanosterol, which is a precursor for synthesizing *Sanghuangporus baumii* triterpenoids. To research the characteristics and construction of *LS*, *LS* ORF and promoter were cloned from S. baumii. A 2,445 bp *S. baumii LS* sequence was obtained by rapid amplification of cDNA ends (RACE) technology and recombinant PCR. *S. baumii LS* sequence includes a 5′-untranslated region (129 bp), a 3′-untranslated region (87 bp), and an open reading frame (2,229 bp) encoding a 734 amino acids. The molecular weight of LS is 84.99 kDa, and transcription start site of *S. baumii LS* promoter sequence ranged from 1 740 bp to 1790 bp. *LS* promoter contained 12 CAAT-boxes, 5 ABREs, 6 G-Boxes, 6 CGTCA-motifs, and so on. The *S. baumii* LS protein was expressed in E. coli BL21 (DE3) (84.99 kDa + 21.15 kDa tag protein). The transcription level of *S. baumii LS* was the highest on day 11 in mycelia (1.6-fold).

Introduction

Sanghuangporus baumii, a traditional Chinese medicine, grows on the trunk of *Syringa reticulat* [1]. *S. baumii* used to belong to the genus of *Inonotus* or *Phellinus* and now belongs to the genus of *Sanghuangporus*[2]. Numerous studies have demonstrated that *S. baumii* possesses antitumor, antioxidant, and anti-inflammatory [3]. Besides, *S. baumii* contains many secondary metabolites, such as polysaccharides, flavonoids, and terpenes [1]. Triterpenoids in *S. baumii* are important pharmacological active substances, which have the anti-tumor, anti-inflammatory, anti-bacterial and antiviral effect [4].

Triterpenoids are complex mixtures, formed by six isoprene units and have a variety of structures. Because of the diversity of the triterpenoids structure, they have a wide range of pharmacological activities [5]. Triterpenoids are synthesized by the mevalonate (MVA) pathway and 1-deoxy-D-xylulose-5-phos-phate pathway (DXP) pathway [6, 7]. Fungi triterpenoids in which are synthesized mainly through the MVA pathway (Fig. 1) [8].

Lanosterol is a common intermediate of triterpene and ergosterol biosynthesis [9, 10]. The squalene synthase (SQS) catalyzes reaction from the isoprenoid pathway toward to sterol and triterpenoids biosynthesis, and lanosterol synthase (LS) catalyzes the cyclization of 2, 3-oxidosqualene to lanosterol [11]. The precursor of triterpenoids is lanosterol, but how lanosterol syntheses triterpenoids are still unknown [7].

Lanosterol synthase, a member of the OSC ((3S)-2, 3-oxidosqualene cyclase) family, is not only a key enzyme in cholesterol and steroid synthesis in animals but also in sterol and triterpenoids synthesis in plants and fungi. The enzyme activities are determined by a few key amino acids in the active site [5]. Therefore, amino acids structure and activities are of great importance for the study of enzyme function and catalytic mechanism. In *Sacchar cerevisiae*, a variety of cyclization products are produced by mutating lanosterol synthase His234, which means the lanosterol synthase is related to the deprotonation of cations on the four-ring structure [12]. The swiss-models of OSC were studied in herbal plants, which shows the OSC structure in *Panax ginseng*, *Panax notoginseng*, *Taraxacum mongolicum*, *Cimicifuga racemosa*, and *Lotus*

corniculatus are stable. There are some variations in the random curl, most of them are distributed on the surface of the protein [13]. In *Siraitia grosvenorii*, homology modeling has been used to predict the 3D structure of OSC (Cycloartenol Synthase, CAS). The interaction between CAS and substrates are analyzed by molecular docking, which shows that Asp491, Cys492, Cys570, Tyr540, and His265 are the key catalytic sites in CAS [14].

At present, the *LS* gene has been cloned and examined in other organisms. For example, *Ganoderma lucidum LS* is cloned and transferred in an erg7 yeast strain lacking LS activity, which demonstrates that the cloned cDNA encodes a functional LS [15]. The ergosterol content in deficient mutant decreases to 42% than that of in wild strain after the *Saccharomyces cerevisiae* LS knockout cassette harboring the loxP-Marker-loxP element [16]. *Poria cocos LS* and promoter are cloned and then transformed into ERG7 hybrid diploid *Saccharomyces cerevisiae* strain YHR072W. The results show that the *P. cocos LS* gene mediates the formation of ergosterol in *S. cerevisiae* [17]. In *S. baumii*, acetyl-CoA acetyl transferase gene (*AACT*) [18], 3-hydroxy-3-methylglutaryl-CoA synthase gene (*HMGS*) [19], and squalene epoxidase gene (*SE*) [20] in MVA pathway have been cloned and expresses in *E. coli*, but *S. baumi* triterpenoids synthesis pathway is not fully understood. Therefore, it is highly important to analyze the characteristics of key genes at the beginning of the experiment.

S. baumii LS and promoter was analyzed for the first time in this study. And we detected the transcription level of *LS* by real-time quantitative PCR further. Then, *LS* was constructed in pET-32a (+) and expressed in *E. coli*.

Materials And Methods

Strain and plasmid

S. baumii was authenticated by visual observation and Internal Transcribed Spacer (ITS) identification. The pET-32a(+) vector was used as expression vectors. *E. coli* DH5α and BL21 (DE3) strains (Tiangen, Beijing, China) were purchased to expand reproduction and express recombinant vectors.

RNA, cDNA and DNA extraction

S. baumii mycelia were collected and washed by distilled water. Then ground to powder by using liquid nitrogen. Next, *S. baumii* RNA, cDNA, and DNA were extracted according to Wang's description [18].

Amplification of the full length of LS

To obtain the full length of *LS*, the *LS* gene fragment should be cloned firstly. Primers (*LS*-S, *LS*-A; Table 1) were designed according to *S. baumii* transcription data [21]. Using cDNA as a template, *LS* 3 'and 5' gene fragments were amplified [18]. The 3’ and 5’ RACE PCR amplification products were verified by AGE (agarose gel electrophoresis) and sequencing (Boshi, Harbin, China). Subsequently, A 468 bp *LS* 5’ fragment and a 2,011 bp 3’ fragment were obtained, and the 5’ and 3’ cDNA fragments were spliced using software, a 2,445 bp *S. baumii LS* sequence was obtained.

Heterologous expression of LS in E. coli

Trelief™ SoSoo Cloning Kit was used to structure the expression vector to express in *E. coli*. Primers (*LS*-*EcoR*I, *LS*-*Hind*III; Table 1) (containing 20 bp homologous flanks, which complementary with the ends of the pET-32a(+) linearized vector) were designed for PCR amplification [18]. The PCR product with homologous flanks was purified using a MiniBEST DNA Fragment Purification Kit Ver.4.0 (TaKaRa). Then, the purified product and linearized vector were mixed according to the instructions. The recombinant vector was transferred to *E. coli* DH5α, and a single positive clone was inoculated in LB medium for extracting plasmid (pET-*LS*).

pET-*LS* and pET-32a (+) were transferred into the *E. coli* BL21 (DE3) competent cell. A single positive clone was selected and cultivated to OD600 = 0.5–0.8 in LB medium, respectively. Then, 1 mL bacteria solution from two kinds of vectors was fetched as controls. Whereafter, bacteria solution was induced using 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and incubated (shaking at 200 r/min, 28°C) [22].

Bacterial liquid and gel were treated according to Wang's description [18]. At the end of running, the gel was stained with Coomassie Brilliant Blue Fast Staining solution (Solarbio).

Amplification of the LS promoter

LS promoter primers (*LS*-P-f, *LS*-P-r; Table 1) were designed based on LS sequencing analysis and *S. baumii* genomic DNA. The PCR product was amplified and sequenced (Boshi, Harbin, China).

Sequence analysis

The LS ORF was obtained using ORF Finder (https://ncbiinsights.ncbi.nlm.nih.gov/tag/orffinder/), and the *LS* sequence was compared using the NCBI database (https://www.ncbi.nlm.nih.gov/). Phylogenetic trees were constructed using MEGA 6.0 with the neighbor-joining method, and LS sequences from other species were downloaded from NCBI.

The transmembrane region was predicted by TMHMM Server v.2.0 (https://www.hsls.pitt.edu/obrc/index.php?page=URL1164644151). The theoretical isoelectric point (pI), molecular weight (MW), amino acid composition and protein transmembrane structures were calculated by ExPASy ProtParam (https://web.expasy.org/protparam/). The solubility of LS was analyzed by Protein-Sol (http://protein-sol.manchester.ac.uk). Subcellular localization was predicted through the POSRT II server (https://psort.hgc.jp/cgi-bin/runpsort.pl). LS sequences were aligned through ESPript (http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi). Domain architectures were analyzed by SMART (http://smart.embl.de/smart/job_status.pl?jobid=601520980332911588497123qEJfXLclpn).

The secondary structure of the LS was determined by the PredictantProtein tool (https://www.predictprotein.org/). The three-dimensional structure of the LS was modeled by SWISS-MODEL (https://www.swissmodel.expasy.org/interactive). The three-dimensional structure model was evaluated by PROCHECK (https://servicesn.mbi.ucla.edu/PROCHECK/). Prediction of signal peptide was performed using SignalP 4.0 software (http://www.cbs.dtu.dk/services/SignalP/).

The *LS* promoter was analyzed using PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and the Berkeley Drosophila Genome Project tool (http://www.fruitfly.org/seq_tools/promoter.html).

Transcription analysis of LS in the different development stage

Total RNA from mycelia, primordia, and young fruiting bodies were extracted, and cDNA was produced using a PrimeScript RT Reagent Kit with gDNA Eraser (Takara). Quantitative real-time PCR (qRT-PCR) was performed on an Mx3000P Sequence Detection System (Agilent Technologies, California, USA). The reaction system was mixed as follow: 10 μL of SYBR GreenMaster Mix (Takara), 0.4 μL of *LS*-T-f (Table 1), 0.4 μL of *LS*-T-r (Table 1), 1 μL of cDNA from the different development stage and 8.2 μL of ddH_2O. Each sample was analyzed in triplicate and repeated three times. Mycelia from day 9 served as a control sample, and *β*-tubulin was used as an internal reference for all qRT-PCR analyses. Relative transcription levels were calculated using the $2^{-\Delta\Delta CT}$ method[23]. Variance (ANOVO) was used to analyze data, and $P < 0.05$ was considered statistically significant.

Data analysis

LS transcription levels data was compared with total triterpenoids content [18]. Line chart was drawn using Excel software.

Results

Sequence analysis of the LS sequence

The *S. baumii LS* sequence we obtained includes a 129 bp 5' UTR, an 87 bp 3' UTR, and a 2,229 bp ORF encoding a 734 amino acids. Next, it was submitted to NCBI GenBank to get accession number (Genbank accession number: MT108416). *S. baumii* LS sequence shared 89% identity and 99% query cover with *Inonotus obliquus* (Genbank accession number: QEP49720.1). The 20 homologous LS sequences from NCBI were used for phylogenetic tree construction, which showed that *S. baumii* LS was most similar to *I. obliquus*, but more distantly related to *Amanita thiersii LS* and *Amanita muscaria LS* (Fig. 2). The molecular weight was 84.99 kDa and the theoretical isoelectric point was 5.87. Alanine (Ala) was the most common (8.5%), and valine (Val) was the least (5.3%) in the amino acid sequence. Instability index of protein was 48.38, which means LS was unstable [24]. LS did not exist transmembrane helix and signal peptide through TMHMM Server (v.2.0) and SignalP 4.0 Server analysis. And LS was a hydrophilic protein by ProtScale software analysis. Prediction by solubility and subcellular localization showed LS was an insoluble protein in the cytoplasmic. When compared LS sequences with other fungi (Fig. 3) and predicted conserved domains, they were relatively conservative. The SQ Hop_cyclase_N was located in the 76–362 amino acid region of *S.baumii* LS, and the SQ Hop_cyclase_C was located in the 382–716 amino acid region of *S.baumii* LS.

The secondary structure of LS contained 46.36% α-helix, 2.56% β-sheet, and 51.08% random coil [25]. The three-dimensional structure of LS had 47.8% similarity with other LS by X-ray prediction [26–29] (Fig. 4). The three-dimensional structure model typically had 92% of residues in the allowed regions by PROCHECK soft, which suggested that the LS protein is theoretically reliable.

Analysis of the LS promoter

A 1,854 bp sequence of *LS* upstream was amplified and submitted in NCBI (Genbank accession number: MT108416). Promoter sequence was analyzed by software, which found sequence transcription start site ranged from 1,740 bp to 1,790 bp. *LS* promoter contained many acting elements (Table 2).

Prokaryotic expression

The prokaryotic expression of the pET-*LS* fusion protein was shown in a gel. Expected protein bands were consistent with software prediction (84.99 kDa + 21.15 kDa tag protein). The LS protease yield was correlated with induction time (Fig. 5). As shown in the figure, LS production was increasing with induction time.

Relationship between LS transcription level and triterpenoids content

The *LS* transcription level up-regulated 1.6-fold for the first time on day 11 in mycelia (Fig. 6), then decreased, and increased again to the primordial stage (1.5-fold). In the young fruit stage, the *LS* transcription level was reduced to a minimum (0.1-fold). The *LS* transcription level was highest on day 11 in mycelia and primordial stage, and it was significantly different from other stages.

The change trend of *LS* transcription level was opposite to that of triterpenoids content after 11 days. *LS* transcription level was lowest, but triterpenoids content was higher in young fruiting bodies phase.

Discussion

At present, the increased yield of *Sanghuangporus* triterpenoids is mainly by optimizing the extraction method and changing the inducer. *Inonotus obliquus* is extracted by 5% (v/w) Viscozyme L, and the total triterpenoids are the highest (24.3 mg/g) [30]. Methyl jasmonate (MeJA) (150 mmol/L) can induce *Inonotus baumii* to enhance triterpenoids yield, which is 4.05-fold higher than that in water[31]. Although these methods can increase triterpenoids yield, they are insufficient for the triterpenoids production in factories. Therefore, improving the triterpene yield by molecular biotechnology is a popular research method.

LS, a key enzyme in the MVA pathway, is a precursor of triterpenoid synthesis. A 2,229 bp *S. baumii LS* ORF sequence was obtained by PCR amplification and BLAST in NCBI. In *Ganoderma lucidum*, *LS* was found to contain a 2,181 bp ORF encoding a 726 amino acids [15]. In *Saccharomyces cerevisiae*, the *LS* gene coding region contains 2,901bp nucleotides [32]. In *Poria cocos*, a 2187 ORF was found out that it codes a 728 amino acid [17]. *S. baumii LS* ORF was longer than *G. lucidum*, *S. cerevisiae*, and *P. cocos*. In this study, we

discovered *S. baumii LS* sequences and analyzed the molecular weight of *S. baumii* LS protein (84.99 kDa), and found the protein was unstable.

According to signal peptide analysis, subcellular localization, and prediction of the transmembrane domain, LS is an insoluble protein in the cytoplasmic, which is consistent with the site of *S. baumii* MVA pathway.

The site of transcription start in *S. baumii LS* promoter sequence ranged from 1 740 bp to 1790 bp. There are responsiveness acting elements contained by *S. baumii LS* promoter, which is similar to other species. In the past, *AACT* promoters in the *S. baumii* MVA pathway were cloned [18]. *LS* and *AACT* promoter all contained ABRE (cis-acting element involved in the abscisic acid responsiveness), CGTCA-motif (cis-acting regulatory element involved in the MeJA-responsiveness), LTR (cis-acting element involved in low-temperature responsiveness), TGACG-motif (cis-acting regulatory element involved in the MeJA-responsiveness), which showed triterpenoids synthesis may relay to abscisic acid, low-temperature, and MeJA. *LS* promoter contained more ARE (essential cis-acting regulatory element for the anaerobic induction), ATC-motif (part of a conserved DNA module involved in light responsiveness), Box 4 (part of a conserved DNA module involved in light responsiveness), GT1-motif (light-responsive element), MRE (MYB binding site involved in light responsiveness) and TGA-element (auxin-responsive element) than *AACT* promoter. *LS* promoter elements are related to anaerobic induction and light responsiveness, so the reaction to catalyzed LS may require anaerobic and light stimuli.

In *Poria cocos*, the LS promoter region contains transcriptional sequesters associated with transcriptional regulation, including acid, light, methyl jasmine, etc[17]. In *G. lucidum*, the potential regulatory elements include the G-box/GT1-motif (light-responsive element), ABRE(cis-acting element involved in abscisic acid responsiveness), AuxRR-core (cis-acting regulatory element involved in auxin responsiveness), MBS (MYB binding site, involved in drought-inducibility), and Box-W1 (fungal elicitor responsive element). But no MeJA responsive element has been found in *G. lucidum*[15].

The *S. baumii* LS protein bands were consistent with software prediction (84.99 kDa + 21.15 kDa tag protein) and whose transcription level first up-regulated 1.6-fold on day 11 in mycelia, then decreased, and increased again in the primordial stage (1.5-fold). The *S. baumii LS* transcription level was the highest on day 11 in mycelia. In *G. lucidum*, gene transcription level is relatively low in the mycelia and then increased to the primordial level, which is also the highest level (about 8.39 fold compared with 10 d-old mycelia) [15]. This result is different from *S. baumii LS* expression, but they all have higher transcription level in the primordial stage.

The variation trend of *LS* transcriptional level was opposite to that of triterpenoids content. This may be because the *LS* transcriptional level was inhibited with the accumulation of triterpenoids in *S. baumii* intracellular. This result is similar to zhang 's[33] research, and the triterpenoids content is the highest in the *S. baumii* mycelia.

To sum up, *S. baumii LS* and promoter were cloned and analyzed for the first time. Subsequently, *LS* was constructed into the vector and expressed in *E. coli* BL21. The transcriptional level of *LS* was explored at different development stages. These studies help us to understand the *LS* as a key enzyme gene in the triterpenoids synthesis pathway. However, to understand the mechanism of triterpenoids synthesis and gene function better, it is necessary to study the overexpression and suppression expression of *LS* in *S. baumii*. Moreover, the transcriptional regulatory factors of *LS* gene upstream may also be the key factors controlling triterpenoids synthesis.

Declarations

Acknowledgements

This research was supplied by the Fundamental Research Funds for the Central Universities, China (2572020AW18).

Authors' Contributions XuTong Wang and Li Zou designed the study. XuTong Wang and Jian Sun performed the experiments. XuTong Wang and Tingting Sun analyzed the results. XuTong Wang and Zengcai Liu wrote the manuscript. All of the authors read and approved the final manuscript. All relevant data are included in this paper. Materials are available upon reasonable request to the corresponding author.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent The research performed did not involve any human or animal subjects. The research did not require informed consent.

References

1. Qi X., Zhang J., Chen Y. (2010) [Comparative analysis of bioactive components in fruit bodies of *Phellinus linteus* growing on six species of trees]. *Food Science* **31**, 199-201. Chinese.
2. Dai Y. C., Cui B. K. (2014) [Progress on the species of medicinal fungus*Inonotus sanghuang*]. *Journal of Beijing Forestry University* **36 (5)**, 1-6. Chinese.
3. Wang C. L., Deng J. S., Huang S. S. (2017) Evaluation of antioxidant, anti-inflammatory and anti-proliferative activities of ethanol extracts from different varieties of Sanghuang species. *Rsc Advance* **7**, 7780-7788.
4. Zhang J. H., Liu W. J., Luo H. M. (2018) [Advances in Activities of Terpenoids in Medicin Plants]. *World Science and Technology/Modernization of Traditional Chinese Medicine and Materia Medica* **20 (3)**, 419-430. Chinese.
5. Shang C. H. (2009) [Molecular cloning characterization and differential expression a lanosterol synthase gene from the fungus *Ganoderma lucidum*]. PhD thesis, *NanJing: NanJing*

Agricultural University. Chinese.

6. Zhang C. B., Sun H. X., Gong Z. J. (2007) 植物萜类化合物的天然合成途径及其相关合酶 [Plant terpenoid natural metabolism pathways and their synthases]. *Plant Physiology Journal* **43 (4)**, 779-786. Chinese.
7. Rodríguez-Concepción M, Boronat A (2002) Elucidation of the Methylerythritol Phosphate Pathway for Isoprenoid Biosynthesis in Bacteria and Plastids. *A Metabolic Milestone Achieved through Genomics*, *Plant physiology* **130 (3)**, 1079-1089.
8. Tan Y., Tang C. H., Feng J. (2019) 灵芝三萜生物合成及调控研究进展[Current progress in the Study on Biosynthesisand Regulation of *Ganoderma* Triterpenoids]. *Acta Edulis Fungi* **26 (3)**, 125-140. Chinese.
9. Buckner F. S., Nguyen L. N., Joubert B. M, Matsuda S. P. T. (2000) Cloning and heterologous expression of the trypanosoma brucei lanosterol synthase gene. *Molecular & Biochemical Parasitology* **110(2)**, 399-403.
10. E. J. Corey, Seiichi P. T. Matsuda, Bonnie Bartel (1994) Molecular cloning, characterization, and overexpression of ERG7, the Saccharomyces cerevisiae gene encoding lanosterol synthase. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 2211-2215.
11. Abe I, Rohmer M, Prestwich GD (1993) Enzymatic cyclization of squalene and oxidosqualene to sterols and triterpenes. *Chemical Reviews* **93**, 2189-2206.
12. Wu T. K., Liu Y. T., Chang C. H., Yu M. T., Wang H. J. (2006) Site-saturated mutagenesis of histidine 234 of saccharomyces cerevisiae oxidosqualene-lanosterol cyclase demonstrates dual functions in cyclization and rearrangement reactions. *Journal of the American Chemical Society***128(19)**, 6414-6419.
13. Zhang Z. B. Hou L. Cui Q. H. (2015) 中药三萜皂苷合成通路的生物信息学分析[Bioinformatics analysis of triterpenoid saponins biosynthesis pathway in herbal medicines]. *Chinese Traditional Patent Medicine* **37(6)**, 1255-1261. Chinese.
14. Qiao J., Cui S. R., Shi H. W. (2019) 罗汉果环阿屯醇合酶的同源建模、分子对接及催化环化的机理推测[Homology Modeling and Molecular Docking of Cycloartenol Synthase in *Siraitia grosvenorii* and Speculated Mechanism of Catalytic Cyclization]. Biotechnology Bulletin **35(2)**, 101-108. Chinese.
15. Shang C. H., Shi L., Ren A., Qin L., Zhao M. W. (2010) Molecular cloning, characterization, and differential expression of a lanosterol synthase gene from ganoderma lucidum. Bio Biotechnol Biochem **74(5)**, 974-978.
16. Gao L. L., Wang Q. H., Liang H. C. (2014) 酿酒酵母羊毛甾醇合酶基因单倍体缺陷型突变株的构建[Construction of *Saccharomyces cerevisiae* haploid mutant deficient in lanosterol synthase gene]. *Acta Pharmaceutica Sinica* **49 (5)**, 742-746. Chinese.
17. Zhou Z. (2017) 茯苓羊毛甾醇合酶基因PcLSS克隆及其功能验证[Cloning, expression and functional verification of Lanosterol synthase gene PcLSS from *Poria locos*]. PhD thesis, *WuHan: Huazhong Agricultural University*. Chinese.
18. Wang X. T., Wang S. X., Xu X. R., Sun J., Ma Y. S., Liu Z. C., Sun T. T., Zou L. (2020) Molecular cloning, characterization, and heterologous expression of an acetyl-CoA acetyltransferase gene from *Sanghuangporus baumii*, *Protein Expression and Purification* **170**, 105592.

19. Sun T. T., Liu Z. C., Wang X. T. (2019) 桑黄HMGS基因克隆、分子特性及差异表达分析 [Cloning, molecular properties and differential expression analysis of HMGS gene from *Sanghuangporus baumii*]. *Chinese Traditional and Herbal Drugs* **50 (23)**, 5823-5829. Chinese.
20. Sun T. T., Sun X. Y., Wang X. T. (2018) 桑黄鲨烯环氧酶基因原核表达及过表达载体的构建 [Construction of prokaryotic expression an over-expression vectors of squalene epoxidase gene from *Sanghuangporus baumii*]. *Chinese Traditional and Herbal Drugs* **49 (11)**, 2632-2639. Chinese.
21. Zou L., Sun T. T., Li D. L., Tan Y., Zhang J. (2016) De novo transcriptome analysis of *Inonotus baumii* by RNA-seq. *Journal of Bioscience and Bioengineering* **121 (4)**, 380-384.
22. Wagschal K. C. , Rose Stoller J. , Chan V. J. , et al. (2017)Expression and Characterization of Hyperthermostable Exopolygalacturonase RmGH28 from *Rhodothermus marinus*. *Applied Biochemistry & Biotechnology*, 183, 1503–1515.
23. Qi Y., Liu C., Sun X, Qiu L., Shen J. (2017) The identification of transcriptional regulation related gene of laccase, poxc, through yeast one-hybrid screening from *Pleurotus ostreatus*. *Fungal Biology* **121 (11)**, 905-910.
24. Guruprasad K, Reddy BV, Pandit MW (1990) Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. *Protein Eng* **4 (2)**, 155-161.
25. Garnier B J, Gibrat J F, Robson B (1996) GOR secondary structure prediction method version IV. *Methods in Enzymology* **266**, 540-553.
26. Waterhouse A., Bertoni M., Bienert S., Studer G., Tauriello G., Gumienny R. (2018) Swiss-model: homology modeling of protein structures and complexes. *Nucleic Acids Research* **46**, 296-303.
27. Guex N, Peitsch M C, Schwede T (2010) Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: a historical perspective. *Electrophoresis* **30 (1)**, 162-173.
28. Bienert S., Waterhouse A., de Beer T.A.P. (2017) The SWISS-MODEL Repository-new features and functionality. *Nucleic Acids Research* **45 (1)**, 313-319.
29. Benkert P, Biasini M, Schwede T (2011) Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics* **27 (3)**, 343-350.
30. Hwang A. Y., Chang Yang, S. Kim, J. Lim, Cho H., Hwang K. T. (2019) Effects of non-traditional extraction methods on extracting bioactive compounds from chaga mushroom (*Inonotus obliquus*) compared with hot-water extraction. *LWT-Food Science and Technology* **110**, 80-84.
31. Sun T. T., Zou L., Zhang L., Zhang J., Wang X. T. (2017) Methyl jasmonate induces triterpenoid biosynthesis in *Inonotus baumii*. *Biotechnology & Biotechnological equipment* **31 (2)**, 312-317.
32. Wang Q. H., Gao L. L., Liang H. C. (2015) 利用反义RNA技术抑制酿酒酵母羊毛甾醇合酶基因的表达 [Downregulation of lanosterol synthase gene expression by antisense RNA technology in *Saccharomyces cerevisiae*]. *Acta Pharmaceutica Sinica* **50 (1)**, 118-122. Chinese.
33. Zhang L. F., Sun T. T., Zou L. (2015) 鲍姆纤孔菌总三萜的提取及其体外抗乳腺癌细胞MCF-7活性 [Extraction of total triterpenoids from *Inonotus baumii* and its inhibitory activity on breast cancer cells (MCF-7) in vitro]. *Drug Evaluation Research* **38 (5)**, 497-502. Chinese.

Tables

Table 1 Primers used for PCR amplification

Primers	Sequences (5’- 3’)	Descriptions
LS-S	CACCAAAGGGAGGACGGAGGAT	For *LS* cDNA fragment sequence amplification.
LS-A	AGCTCTCTCCCCATCCTCCGTCCTC	
LS-F	ATGTGGTCACCATTGGATGTCCCGG	For full-length *LS* cDNA isolation.
LS-R	CTAGTGCAGATGTCCATTCCCATTTG	
*LS-EcoR*I	CTGATATCGGATCCGAATTCATGTGGTCACCATTGGATGTCC	For pET-*LS* construction.
*LS-Hind*III	TCGAGTGCGGCCGCAAGCTTCTAGTGCAGATGTCCATTCCCA	
LS-P-f	CCCGACTCTCGTTGCGAAGATACTA	For *LS* promoter isolation
LS-P-r	ATTCCAGGTATTCCCAGACATGACG	
LS-T-f	ACACAGTTCGCCCTTGAGAGCC	For qRT-PCR analysis
LS-T-r	CATCTTCACGGCCCGTTCGATAGGT	
β-tubulin-f	GCTGAATATCGTTCGTGCCC	For qRT-PCR analysis
β-tubulin-r	ATCCGCCTTCCTCCTTACAGT	

Table 2 Cis-acting regulatory elements of the Promoter

Type of cis-acting regulatory elements	LS elements numbers	AACT elements numbers	Function	sequence
A-box	2	1	cis-acting regulatory element	CCGTCC
ABRE	5	6	cis-acting element involved in the abscisic acid responsiveness	ACGTG
ACA-motif	0	1	part of gapA in (gapA-CMA1) involved with light responsiveness	AATCACAACCATA
ARE	1	0	cis-acting regulatory element essential for the anaerobic induction	AAACCA
ATC-motif	1	0	part of a conserved DNA module involved in light responsiveness	AGTAATCT
Box 4	1	0	part of a conserved DNA module involved in light responsiveness	ATTAAT
CAAT-box	12	13	common cis-acting element in promoter and enhancer regions	CCAAT
CAT-box	0	1	cis-acting regulatory element related to meristem expression	GCCACT
CCAAT-box	1	2	MYBHv1 binding site	CAACGG
CGTCA-motif	6	5	cis-acting regulatory element involved in the MeJA-responsiveness	CGTCA
G-Box	4	9	cis-acting regulatory element involved in light responsiveness	CACGTT/TACGTG
GARE-motif	0	1	gibberellin-responsive element	TCTGTTG
GATA-motif	0	1	part of a light responsive element	AAGGATAAGG
GT1-motif	1	1	light responsive element	GGTTAA
GC-motif	0	1	enhancer-like element involved in anoxic specific inducibility	CCCCCG
LTR	1	3	cis-acting element involved	CCGAAA

			in low-temperature responsiveness	
MSA-like	0	1	cis-acting element involved in cell cycle regulation	(T/C)C(T/C)AACGG(T/C)(T/C)A
P-box	0	2	gibberellin-responsive element	CCTTTTG
MBS	1	0	MYB binding site involved in drought-inducibility	CAACTG
MRE	1	0	MYB binding site involved in light responsiveness	AACCTAA
Sp1	2	2	light responsive element	GGGCGG
TCCC-motif	1	1	part of a light responsive element	TCTCCCT
TGA-element	2	0	auxin-responsive element	AACGAC
TGACG-motif	6	5	cis-acting regulatory element involved in the MeJA-responsiveness	TGACG

Figures

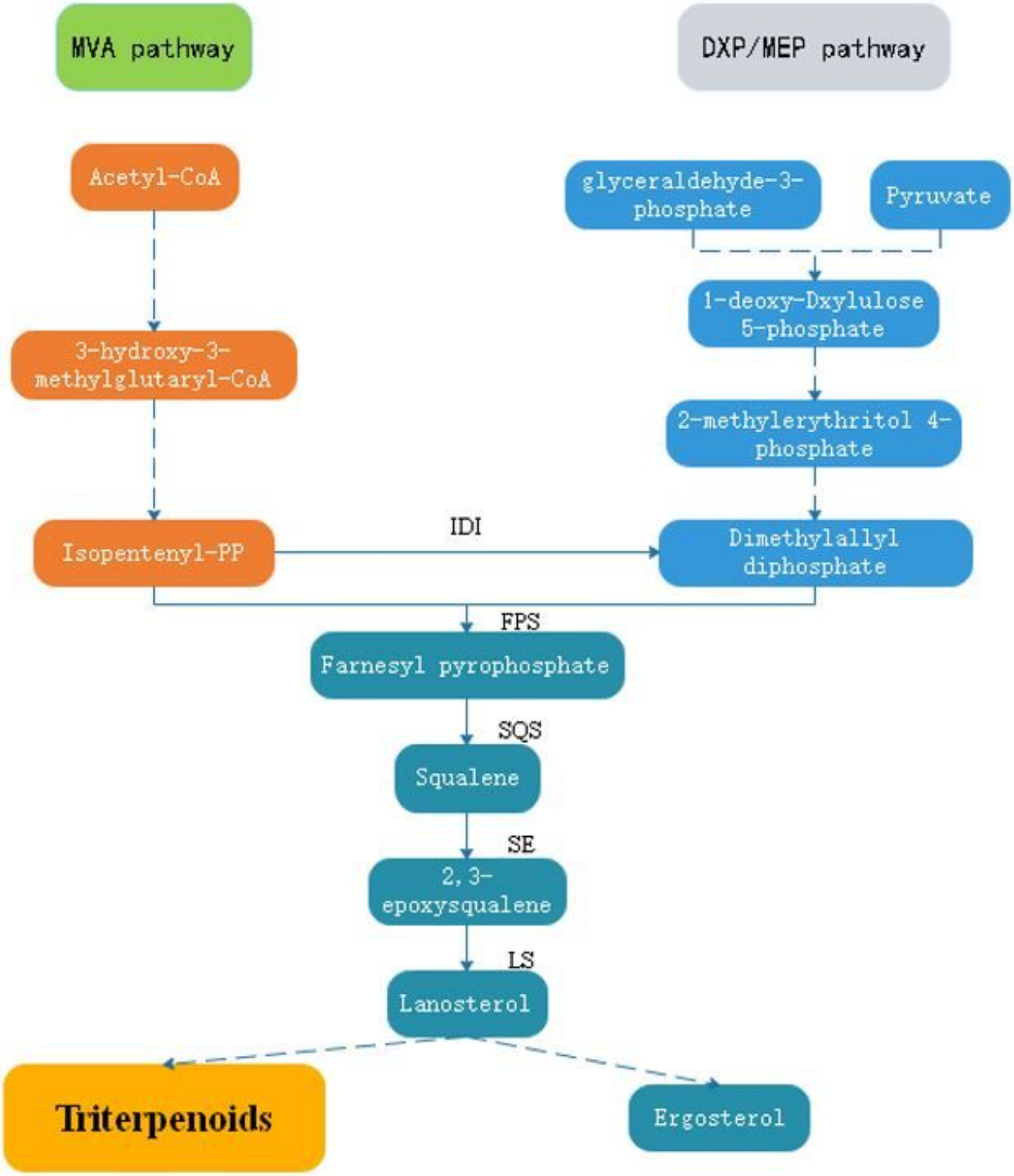

Figure 1

Triterpenoids biosynthetic pathway of S. baumii. Abbreviations: mevalonate (MVA), methylerythritol 4-phosphate (MEP), diphosphate isomerase (IDI), farnesyl-Diphosphate Synthase (FPS), squalene synthase (SQS), squalene epoxidase gene (SE), lanosterol synthase (LS).

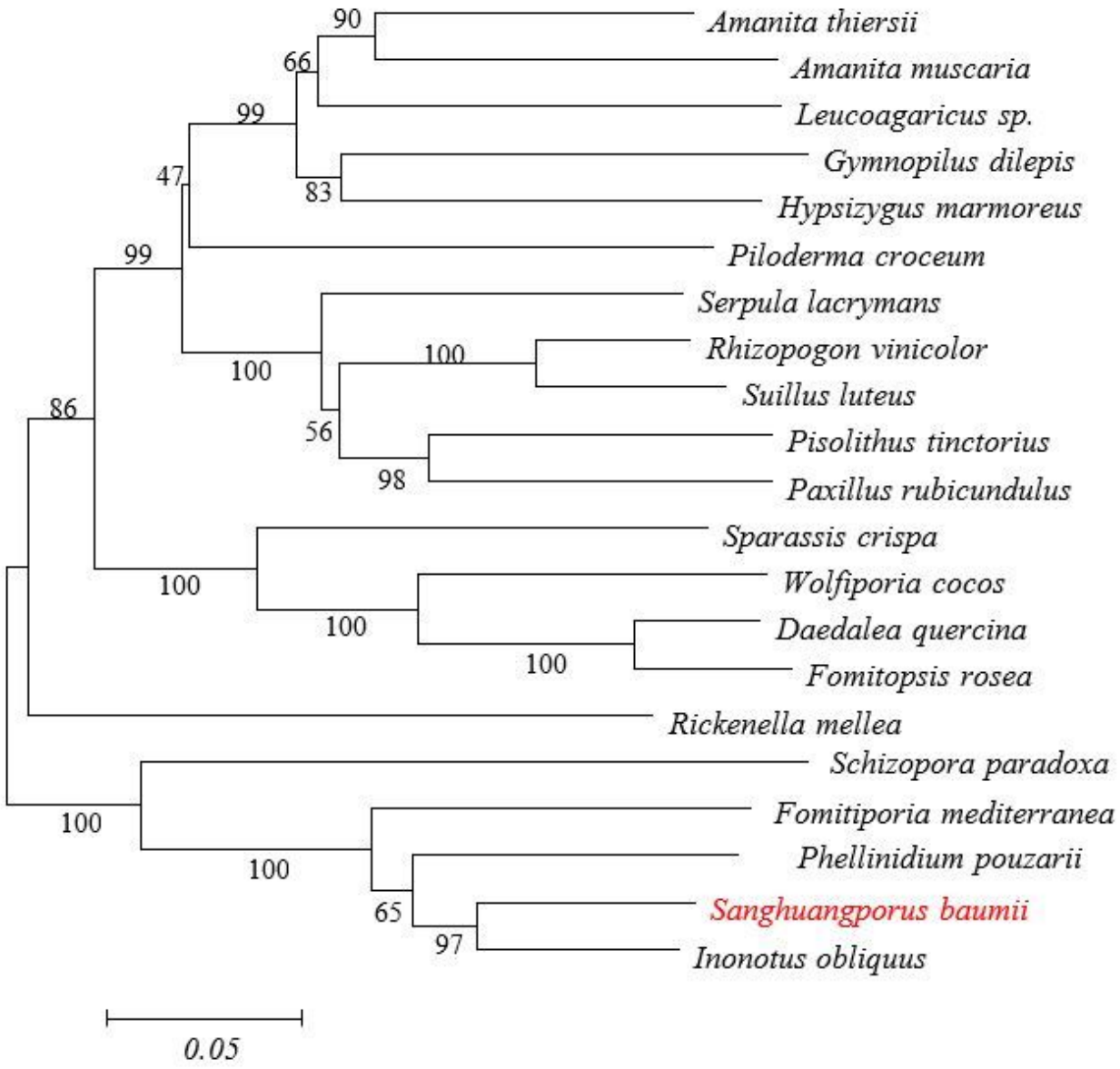

Figure 2

Phylogenetic tree of LS proteins sequences of various species.

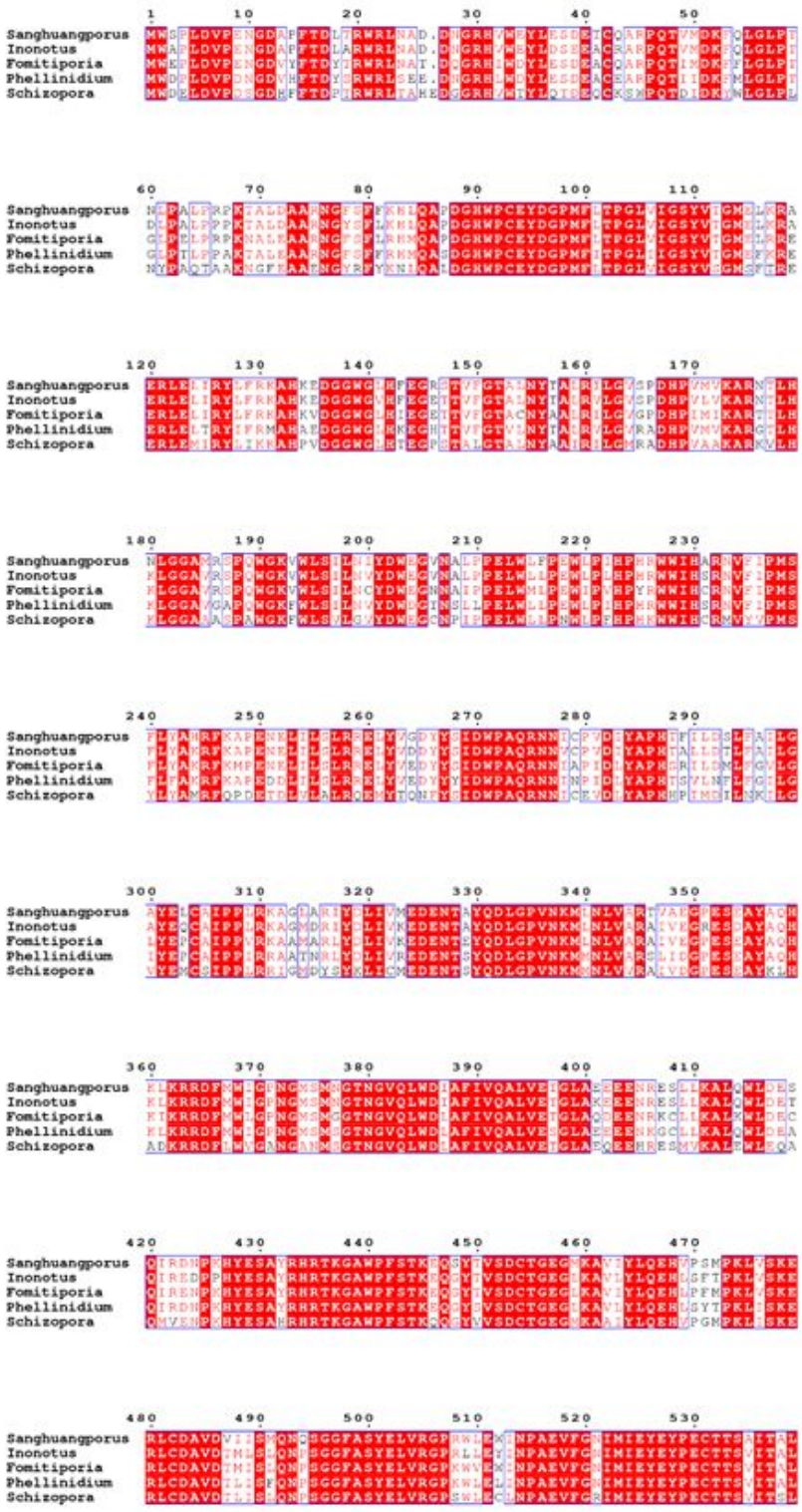

Figure 3

Alignment of the LS amino acid sequences from different species. Sanghuangporus baumii (Sanghuangporus), Inonotus obliquus (Inonotus), Fomitiporia mediterranea (Fomitiporia), Phellinidium pouzarii (Phellinidium), Schizopora paradoxa (Schizopora).

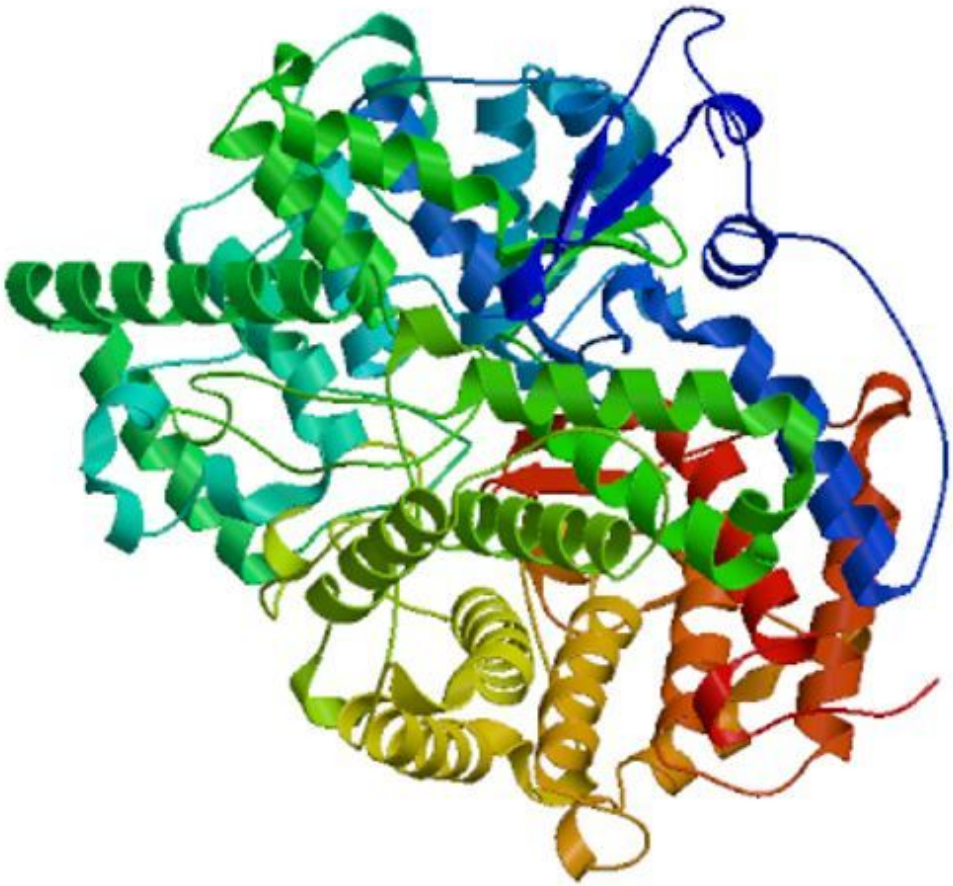

Figure 4

Three-dimensional structure schematic representation of S. baumii LS.

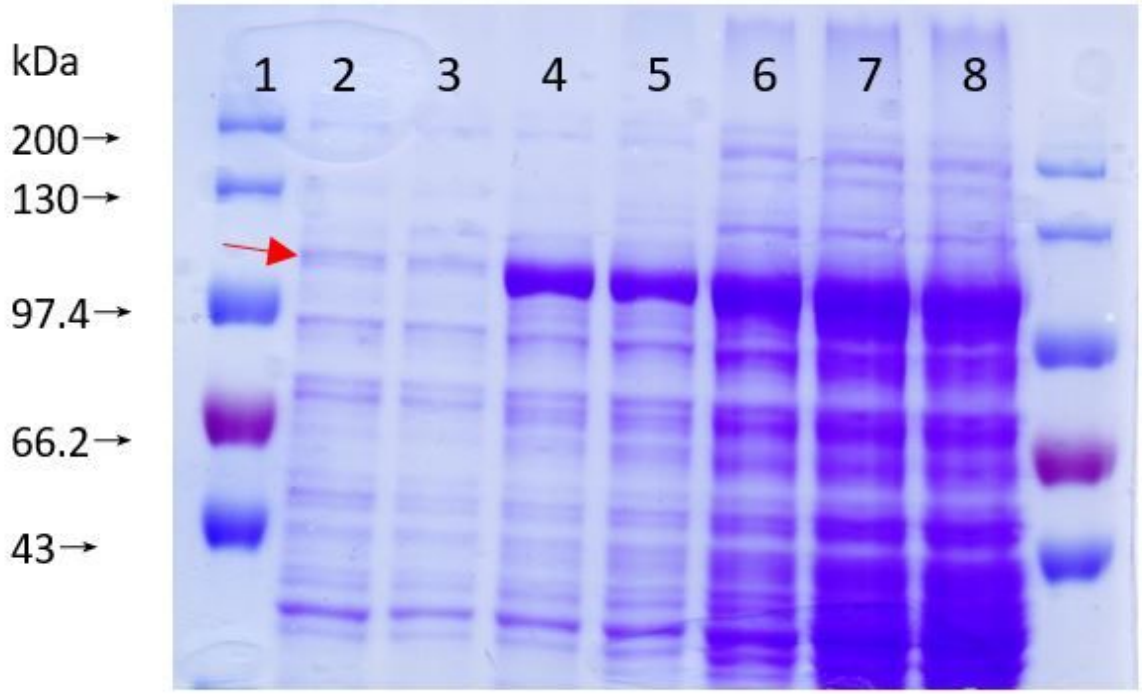

Figure 5

Heterologous expression of S. baumii LS in E. coli via IPTG induction (1 mM) at 37°C for 0, 2, 4, 6, 8 and 10 h. Lane 1, protein markers (43-200 kDa); Lane 2 E. coli harbouring empty vector; Lane 3-8, E. coli harbouring the pET-LS construct induced by IPTG (1 mM) for 0, 2, 4, 6, 8, and 10 h.

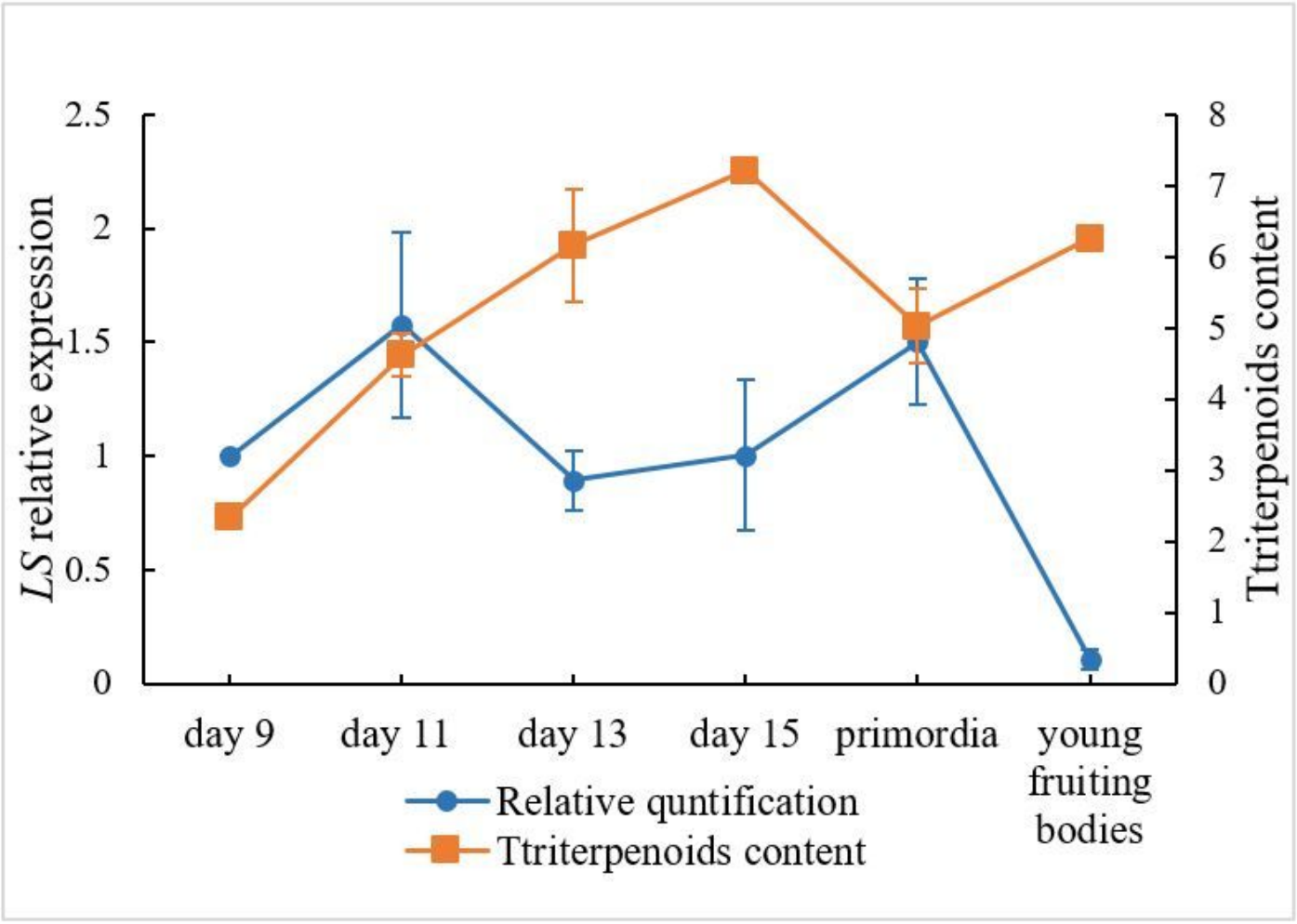

Figure 6

LS transcriptional level and triterpenoids content in S. baumii in different development stages.