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Sequence Analysis and Expression of a Blue-light Photoreceptor Gene, *Slwc-1* from the Cauliflower Mushroom *Sparassis latifolia*

Chi Yang[1,2] · Lu Ma[1,2] · Zhenghe Ying[1,2] · Xiaoling Jiang[1,2] · Yanquan Lin[1,2]

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Abstract Light is a necessary environmental factor for fruit body formation and development of the cauliflower mushroom *Sparassis latifolia*, a well-known edible and medicinal fungus. In this study, we firstly characterized the SP-C strain, which belonged to *S. latifolia*. And then we cloned and sequenced a photoreceptor gene (*Slwc-1*) from *S. latifolia*. The product of *Slwc-1*, SlWC-1 (872 aa residues) contained a coiled-coil region, a LOV domain, and two PAS domains. Phylogenetic tree result showed that SLWC-1 was most close to GfWC-1 from *Grifola frondosa in edible and medicinal fungus*. The *Slwc-1* gene was found to be enhanced by light. This report will help to open the still-unexplored field of fruit body development for this fungus.

Abbreviations

S. latifolia	*Sparassis latifolia*
Slwc-1	*S. latifolia* white color-1
LOV	Light, Oxygen, or Voltage
PAS	Per-Arnt-Sim
RACE	Rapid amplification of cDNA ends
ITS	Internal transcribed spacer
CTAB	Cetyl-trimethyl ammonium bromide
LED	Light Emitting Diode
qPCR	Quantitative real-time PCR
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
PCR	Polymerase Chain Reaction
IBS	Illustrator for biological sequences

Chi Yang and Lu Ma contributed equally to this work.

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✉ Yanquan Lin
lyq-406@163.com

[1] Institute of Edible Fungi, Fujian Academy of Agricultural Sciences, Fuzhou, Fujian Province 350014, China

[2] National and Local Joint Engineering Research Center for Breeding & Cultivation of Featured Edible Fungi, Fujian Academy of Agricultural Sciences, Fuzhou 350014, China

Introduction

Sparassis latifolia (*S. latifolia*) is an edible and medicinal fungus that has been cultivated in Japan, Korea, and China. *S. latifolia* was evaluated as a new species based on macromorphological, microscopic, mating type, and phylogenetic analyses, which was isolated from Asia [1]. *S. latifolia* contains a remarkably high concentration of *β*-glucan [2], which has been reported to have many biological and pharmacologic activities including antiangiogenic activity [3, 4], tumor-suppressing effects [2, 5], and neuroprotection activity [6]. *S. latifolia* also contain lectin, which has antibacterial and antifungal activities [7]. Up to now, there are three factories can cultivated *S. latifolia* in China, and the total fresh fruit production was over 2.5 tons/d [8]. Increasing both yield and quality are principal problems in mushroom commercial cultivation, as it is limited both by high-yield commercial cultivars and improvements of cultivation techniques [9]. Our previous study has shown that light is essential for the fruit body formation of *S. latifolia* [10].

Light is an important environmental signal for diverse organisms from all kingdoms of life, regulating their developmental, physiological and/or behavioral processes [11]. The best characterized fungus is *Neurospora crassa*, that blue light is perceived by the white collar (WC) complex formed by the WC-1 and WC-2 proteins, and

regulates many processes including carotenoid biosynthesis, spore formation, phototropism, photoadaptation, and the circadian clock [12–15]. NcWC-1 contains a LOV (Light, Oxygen, or Voltage) domain and two PAS (Per-Arnt-Sim) domain [16]. There have been only a few studies on the photoreceptors in mushroom-forming fungi, including *Lentinula edodes* [17], *Coprinopsis cinerea* [11], *Schizophyllum commune* [18], and *Cordyceps militaris* [19, 20]. The blue-light receptor complex, WC-1/2, is involved in the fruit body formation of these fungi.

In this study, we analyzed the influence of light on the growth of *S. latifolia* and then cloned the full-length gene of *Slwc-1* from *S. latifolia* by rapid amplification of cDNA ends (RACE). SlWC-1 was possibly involved in the photoreaction and was characterized by the presence of the LOV and PAS domains. Finally, we also briefly described the expression of the *Slwc-1* transcript under the light induce of *S. latifolia*.

Materials and Methods

Fungal Strains

The *S. latifolia* strain SP-C was kept in Institute of Edible Fungi, Fujian Academy of Agricultural Sciences (Fuzhou, China) and maintained on potato dextrose agar medium under dark. The identification of the strain was confirmed by molecular sequence analysis of the internal transcribed spacer (ITS) rDNA regions. Genomic DNA was obtained from the mycelia of *S. latifolia* by cetyltrimethyl ammonium bromide (CTAB) method [21]. PCR primers specific to the internal transcribed spacer (ITS) regions of rDNA, ITS1:5′ TCCGTAGGTGAACCTGCG G 3′, and ITS4: 5′ TCCTCCGCTTATTGATATGC 3′, were used for the selective amplification [22, 23]. The PCR product was sequenced and assembled by Biosune Biology (Shanghai, China). The ITS sequence was verified with a blastN search against NCBI database.

Influence of Light on the Growth

For the influence of light on the growth, vegetative mycelia growth, *S. latifolia* were exposed to dark and light (including white, blue light, and red light). The light was produced by Samvol power 12-W LEDs (Light Emitting Diodes, Zhongshan, China). The distance between the LEDs and the agar plates was 30 cm (light intensity was about 300 lx). The growth of each colony was measured after 1 week of culture. Three biological replicates were used.

Amplification of the *Slwc-1* Gene

The *Slwc-1* gene was amplified by SMARTer RACE 5′/3′ Kit (Clontech, USA) according to the manual instruction. The degenerate primer set forward: 5′ **GATTACGCCAAGCTT**GAYATGWSYTGYGCYTTYGT 3′ and reverse: 5′ **GATTACGCCAAGCTT**SWYTCRAACCARGTRTARCC 3′ were designed based on the previous report [19]. Total RNA was extracted from full matured fruiting body by TRIZOL regent (ComWin Biotech, China). The primer for the amplification of the full length of *Slwc-1* gene were Slwc-F: 5′ **GATTACGCCAAGCTT**ATGCCCTTTGAGCGGTATCTCCAG 3′ and Slwc-R: 5′ **GATTACGCCAAGCTT**TTAGGTGGGTGCCCCGTCTATG 3′. The sequence underlined was the infusion adaptor for the pRACE vector supplied by the SMARTer RACE 5′/3′ Kit.

Sequence Analysis

The resulting PCR products were cloned into vector pRACE following recommendations of the supplier by In-Fusion cloning method (Clontech, USA). The nucleotide sequences were sequenced and assembled by Biosune Biology (Shanghai, China) and were assembled using the ContigExpress program (Invitrogen, Carlsbad, CA). The nucleotide sequences were translated into protein by Primer Primier 5.0. Conserved domain analysis was performed by SMART (http://smart.embl-heidelberg.de/). Schematic representation of WC-1 from different species was drawn by Illustrator for Biological Sequences (IBS) Version 1.0 [24]. The molecular weights and isoelectric points (pI) were calculated using the PROTPARAM tool (http://web.expasy.org/protparam/).

Phylogenetic Analysis

Multiple alignments were performed with CLUSTALW using default settings. The evolutionary history was inferred using the Maximum Likelihood method based on the Jones–Taylor–Thornton (JTT) matrix-based model [25]. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method. All positions containing gaps and missing data

were eliminated. Evolutionary analyses were conducted in MEGA6 [26].

Photo Response of *Slwc-1* in *S. latifolia*

Strain SP-C was grown on PDA medium depending on our previous report [27] in the dark until irradiated with 300 lx LED white light up to 1 h. The mycelia were collected and kept at −80 °C before RNA extraction.

Total RNA was isolated from 100 mg of frozen mycelia, primordia, mature fruit body using the TRIZOL reagent and was then treated with gDNA Eraser (Takara, Japan). cDNA synthesis was achieved using PrimeScript™ II 1st Strand cDNA Synthesis Kit (Takara, Japan) and quantitative real-time PCR (qPCR) was performed using the ABI 7500 (ABI, USA) real-time PCR System with the SYBR Premix Ex Taq kit (TaKaRa, Japan). The 20 μL qPCR reactions contained 4.5 μL cDNA, 0.5 uL primers (10 uM) and 12.5 uL 2× SYBR Premix Ex Taq. The thermal cycling conditions were as follows: 95 °C for 1 min; followed by 40 cycles of 10 s at 95 °C, 34 s at 60 °C; and 60 °C for 1 min, 58–95 °C for the dissociation curve analyses. The primers used in qPCR were as follows: WC-1-F 5′ CCAGCATCACCA ACTACAAGAA 3′; WC-1-R 5′ CCTCTGCGGGAGTAT TATTGAC 3′ and GAPDH-F 5′ TCATTACCGCACCCT CTTCC 3′; GAPDH-R 5′ CCACCACGCCAGTCTTTA TG 3′. The GAPDH gene was selected as control (the selection of reference genes please see supplement data).

Statistics Analysis

qRT-PCR data were presented as mean ± SD. The relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method. The statistical significance of differences was assessed using one way ANOVA in Office Excel 2016. The significance was set with *P < 0.05, **P < 0.01.

Results

Characterization of Strain Used in the Study

The morphology of the mycelium to mature fruit body are shown in Fig. 1. The color of the mycelium was white and the fruit body looked like a "Hydrangea." The ITS sequences of SP-C identified by similarity searches in GenBank showed that strain SP-C was mostly close to the *Sparassis* sp. QZ-2012a voucher KFRI 923. Alignment between these two ITS sequence shown that there was only 1 bp difference among 625 bp after cut off the primer part both of 5′ and 3′ in the sequence. Depending on this result, the SP-C strain was belonged to *S. latifolia*. The nucleotide sequences have been deposited in GenBank under accession numbers KX67998.

Influence of Light on the Growth of *S. latifolia*

As shown in Fig. 2a, changes in the morphology of the colonies occurred in different light conditions; the pictures were captured by a microscope with the magnification

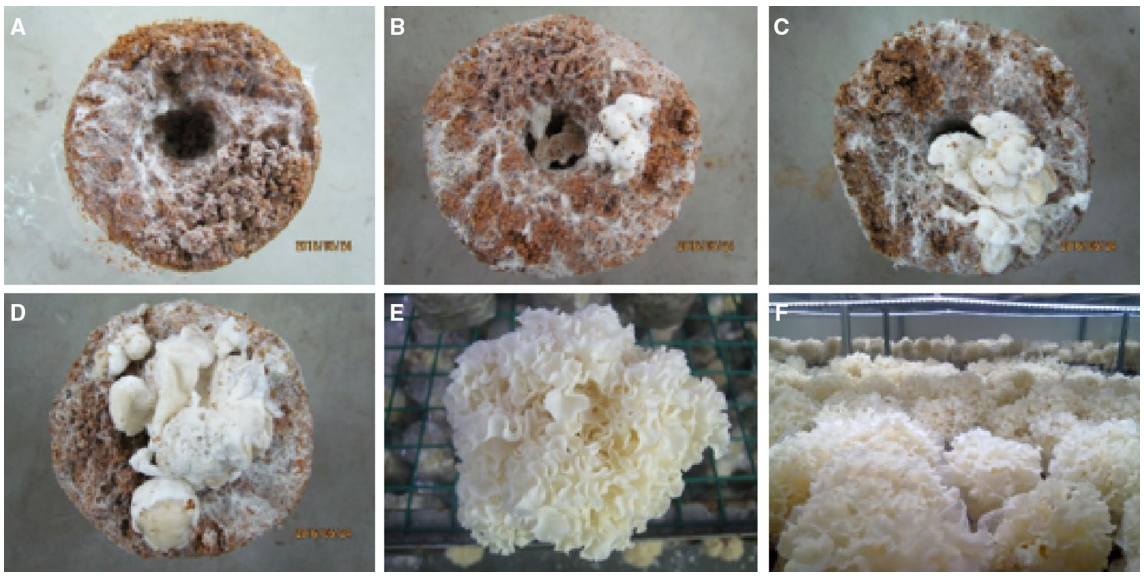

Fig. 1 The morphology of strain used in the study. **a–f** are mycelia, early primordia, middle primordia, late primordia, fruiting body, and production in industry, respectively

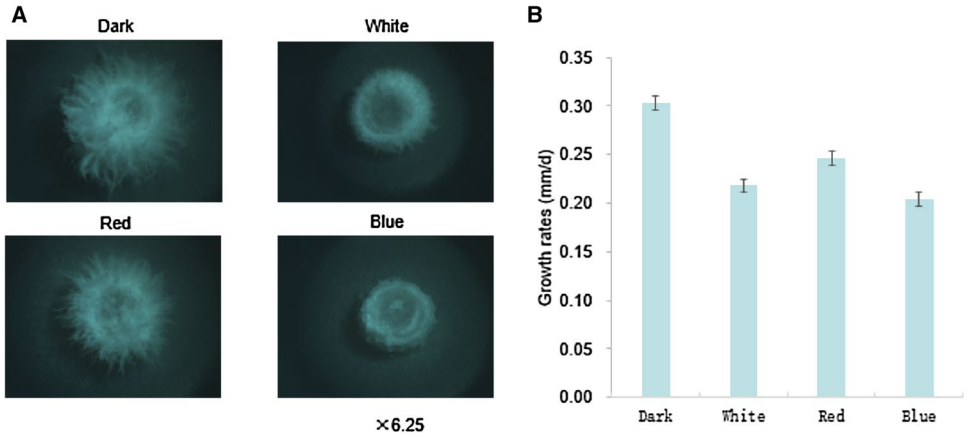

Fig. 2 Inhibition of light on the mycelium growth of *S. latifolia*. (**a**) Effect of light on morphology after light induced for 7 days. (**b**) Effects of light on growth rates

of 6.25. The growth rates were determined by the diameters of their colonies (Fig. 2b). It presented the trend of dark > red light > white light > blue light under the four light conditions.

Amplification of the *Slwc-1* gene and Sequencing Analysis

Using degenerate primers and SMARTer RACE 5′/3′ Kit, a 2619-bp product was amplified from *S. latifolia*. The SlWC-1 protein contained 872 amino acids (isoelectric point: 6.47) with a calculated molecular weight of 94.54 KD. The SlWC-1 protein contained a coiled-coil region, an LOV domain and two PAS domains. It was clearly smaller than other fungal LOV domain-containing blue-light photoreceptors such as *Coprinopsis cinerea* Dst1 (1174 aa), *N. crassa* WC-1 (1131 aa), but similar to *Agaricus bisporus* (880 aa) and *Grifola frondosa* (881 aa) (see Fig. 3). The genomic DNA sequence of *Slwc-1* was also amplified, and the sequence was deposited in GenBank (KX712281, 2016). The gene *Slwc-1* included two introns (51-bp and 52-bp, respectively) that were deduced from genomic and cDNA sequence comparison.

Phylogenetic Analysis

A phylogenetic tree was constructed using the identified SlWC-1 protein sequence, other blue-light receptors from fungi and the model fungi *N. crassa* retrieved from GenBank. Phylogenetic relationship analysis indicated that the WC-1 proteins from *S. latifolia, Fibroporia radiculosa, Fomitopsis pinicola*, and *G. frondosa* were in the same branch (Fig. 4). All these species were belonged to Polyporales and had similar appearance, which were different to Agaricales such as *Agaricus bisporus*, and more different to other order. But SlWC-1 and GlWC-1 from *Ganoderma lucidum* were in different branches although both of them

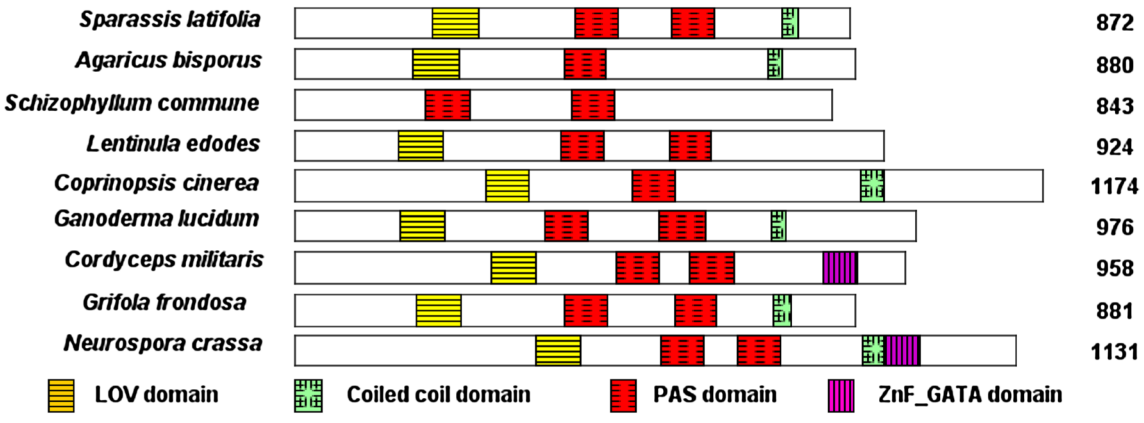

Fig. 3 Schematic representation of WC-1 from several mushrooms and *N. crassa*. Conserved domains of all putative photoreceptors were determined using SMART (http://smart.embl-heidelberg.de/). The size of each protein is indicated on the right. The regions corresponding to the LOV (*Light*-Oxygen-Voltage), PAS (Per-Arnt-Sim) domains, CC (coiled-coil structure), and ZnF (zinc-finger DNA-binding motif) are shown

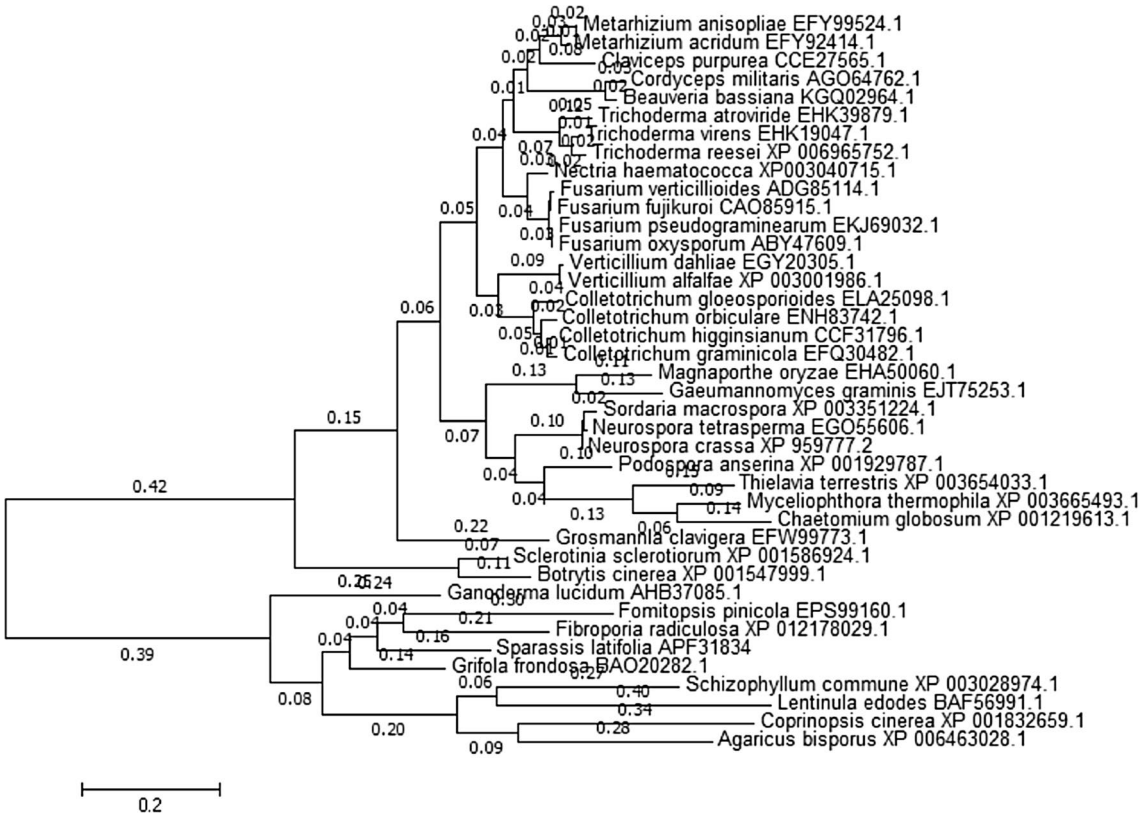

Fig. 4 Evolutionary relationships of Slwc-1

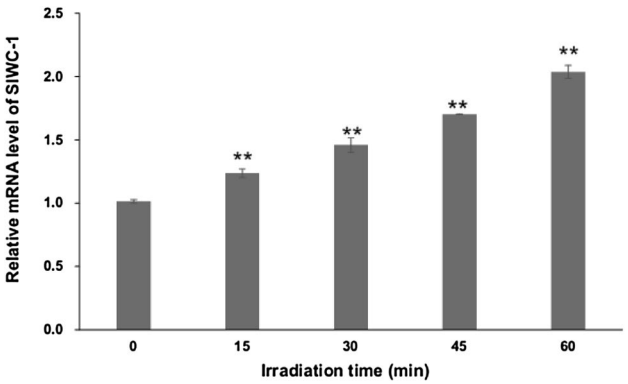

Fig. 5 Relative expression level of *Slwc-1* gene. Relative expression level of *Slwc-1* gene after being irradiated for different times. Data were presented as mean ± SD. *P < 0.05, **P < 0.01

were belonged to Polyporales. The sequence of SlWC-1 was deposited in GenBank (APF31834, 2016).

Slwc-1 mRNA is Light Inducible

Levels of the *Slwc-1* mRNA were ascertained by real-time PCR. The analysis was performed for the same amount of the total cellular RNAs isolated from mycelia obtained by cultivation under dark before being irradiated for different times (Fig. 5). The *Slwc-1* gene was found to be enhanced by light.

Discussion

Mushrooms are considered as an important food for their traditionally famous nutritional and medicinal values, although much information about their potential at the molecular level is unfortunately unknown. *S. latifolia* is an edible mushroom with various medicinal properties, which has been studied for years. *Sparassis* was classified into three groups, *S. crispa* from Europe and eastern North America, *S. radicata* from western North America, and *S. latifolia* from Asia, according to phylogenetic relationships and placement [1]. Based on the ITS sequence, the SP-C strain belongs to species S. latifolia, this result which was consistent with previous reports [1, 22].

Light is essential for the development of stromata of *S. latifolia*. In this study, the influence of light on the growth of *S. latifolia* was analyzed and the gene of *Slwc-1*, the homolog of the blue-light photoreceptor of *N. crassa* is cloned and analyzed. Motif Scan analysis shows that the WC-1 proteins from the eight common edible fungi and *N.*

crassa have similar functional regions of highly conserved co linearity. The SlWC-1 protein contained one coiled-coil region, one LOV domain, and two PAS domains, similar to other edible fungi (Fig. 3). Our phylogenetic results suggest that the sequence of WC-1 can be used as a candidate marker for phylogenetic analysis in fungi, but not a very good one, because the *S. latifolia* and *Ganoderma lucidum* were in different branches although both of them belonged to Polyporales.

There are no relevant effect of light on the amounts of some white color proteins in fungi [28–30], but the results in this study suggest that the *Slwc-1* mRNA is light inducible, as are the genes for other WC-1-like proteins in other edible fungi, such as *Cmwc-1* from *C. militaris* [19], *LephrA* from *L. edodes* [17] and WC-1 from *S. commune* [18].

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest.

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