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RESEARCH ARTICLE

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# Cloning and Expression Analysis of Bioluminescence Genes in *Omphalotus guepiniiformis* Reveal Stress-Dependent Regulation of Bioluminescence

Mi-Jeong Park, Eunjin Kim, Min-Jun Kim, Yeongseon Jang, Rhim Ryoo and Kang-Hyeon Ka

Forest Microbiology Division, Department of Forest Bio-Resources, National Institute of Forest Science, Suwon, Republic of Korea

**ABSTRACT**

Bioluminescence is a type of chemiluminescence that arises from a luciferase-catalyzed oxidation reaction of luciferin. Molecular biology and comparative genomics have recently elucidated the genes involved in fungal bioluminescence and the evolutionary history of their clusters. However, most studies on fungal bioluminescence have been limited to observing the changes in light intensity under various conditions. To understand the molecular basis of bioluminescent responses in *Omphalotus guepiniiformis* under different environmental conditions, we cloned and sequenced the genes of hispidin synthase, hispidin-3-hydroxylase, and luciferase enzymes, which are pivotal in the fungal bioluminescence pathway. Each gene showed high sequence similarity to that of other luminous fungal species. Furthermore, we investigated their transcriptional changes in response to abiotic stresses. Wound stress enhanced the bioluminescence intensity by increasing the expression of bioluminescence pathway genes, while temperature stress suppressed the bioluminescence intensity *via* the non-transcriptional pathway. Our data suggested that *O. guepiniiformis* regulates bioluminescence to respond differentially to specific environmental stresses. To our knowledge, this is the first study on fungal bioluminescence at the gene expression level. Further studies are required to address the biological and ecological meaning of different bioluminescence responses in changing environments, and *O. guepiniiformis* could be a potential model species.

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## 1. Introduction

Bioluminescence refers to the production and emission of light by living organisms. It differs from fluorescence and phosphorescence as it does not require external light absorption. However, a biochemical process mediates the oxidative exergonic reaction of luciferin (substrate) catalyzed by luciferase (enzyme). Bioluminescence has been reported across the tree of life including bacteria, algae, marine animals, fireflies, and fungi [1–4]. Recent reports have suggested that bioluminescence evolved independently in different luminous species, resulting in diverse luciferins and luciferases [5–8]. Due to the low homology between bioluminescent systems, only eleven luciferin-luciferase pairs have been characterized among forty different bioluminescent systems observed in nature, and the biosynthetic pathways have been genetically uncovered only for bacterial and fungal luciferins [5–8].

The fungal luciferin, 3-hydroxyhispidin, and its precursor, hispidin, were isolated and their chemical structures were determined in 2015 [9]. By examining the yeast cells expressing the cDNA library obtained from a glowing fungus, *Neonothopanus nambi*, the sequences of the fungal luciferase (*Luz*) gene and three other genes involved in fungal luciferin biosynthesis and its recycling were finally identified in 2018 [10]. The fungal luciferin, 3-hydroxyhispidin, is synthesized by converting caffeic acid to hispidin by hispidin synthase (HispS), followed by hydroxylation by hispidin-3-hydroxylase (H3H). The 3-hydroxyhispidin undergoes oxidative decarboxylation by Luz yielding caffeylpyruvic acid and green light. The caffeylpyruvic acid can be recycled into caffeic acid by caffeylpyruvate hydrolase (CPH).

The recent identification of fungal bioluminescence pathway genes has enabled the evolutionary studies on fungal bioluminescence based on comparative genomics [10–12] and the development of novel reporter systems in plants and animals [10,13,14]. The fungal bioluminescence system is highly expected

**CONTACT** Rhim Ryoo rryoo@korea.kr; Kang-Hyeon Ka kasymbio@korea.kr
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to be further applied to plants. Because all plants produce caffeic acid, it is possible to engineer auto-luminescent plants without adding any exogenous chemicals [13,14]. In practice, the auto-luminescence signal was observed by expressing the fungal bioluminescence pathway genes in various plants including the model plant, *Arabidopsis thaliana*, tobacco (*Nicotiana tabacum* and *N. benthamiana*), tomato (*Solanum lycoperisicum*), and the ornamental plants such as Dahlia (*Dahlia pinnata*), periwinkle (*Cataranthus roseus*), petunia (*Petunia hybrida*), and rose (*Rosa rubiginosa*) [13,14].

Despite recent progress achieved in the past decade, there is still limited understanding of the regulatory mechanisms of fungal bioluminescence and its biological significance. Until now, most studies on fungal bioluminescence have been concentrated on observing the changes in bioluminescence intensity according to external conditions such as culture conditions (i.e., light, temperature, pH, and growth medium composition) and abiotic stresses or internal conditions such as developmental age and circadian rhythm [15–22].

*Omphalotus guepiniiformis*, also known as *O. japonicus* or *Lampteromyces japonicus*, is a luminous fungus native to Korea and Japan. There are several reports on the characteristics related to the bioluminescence of *O. guepiniiformis* [15,23,24]. For example, in the late 1980s, green light emission from its mycelia and gill tissue and the changes in light intensity under different mycelial growth conditions were documented [23]. The dependence of luminescence intensity on monokaryotic or dikaryotic conditions and oxygen concentration was observed [15,23]. Recently, we reported that *O. guepiniiformis* produced more light after wound stress, as observed in *Neonothopanus nambi*, and high- and low-temperature stresses suppressed light production [19,24].

To understand the molecular mechanisms underlying the changes in bioluminescence intensity under different environmental conditions, we cloned and sequenced the genes of crucial enzymes in the bioluminescence pathway of *O. guepiniiformis*, *HispS*, *H3H*, and *Luz*, and then examined their expression levels under different abiotic stress conditions. To our knowledge, this is the first study describing the stress-dependent regulation of fungal bioluminescence at the gene expression level.

## 2. Materials and methods

### 2.1. Strain and culture conditions

The dikaryotic strain of *Omphalotus guepiniiformis*, NIFoS 5378, deposited at the National Institute of Forest Science (NIFoS) was used for experiments. The mycelia were grown on potato dextrose agar (PDA, BD Difco, NJ, USA) plate at 25 °C under dark conditions.

For wound stress treatment, the mycelia grown on PDA at 25 °C for 7 d were mechanically damaged with a surgical blade. For long-term cold/heat stress treatments, the mycelia grown on PDA at 25 °C for 7 d were transferred to 4 °C/35 °C for 24 h. For short-term cold/heat stress treatments, the mycelia grown on PDA at 25 °C for 7 d were exposed to 4 °C/35 °C for 1 h and then transferred to 25 °C.

### 2.2. Imaging

The light and bioluminescence images were obtained using the ChemiDoc XRS + system (BioRad, CA, USA) and processed with the Image Lab 5.2.1 software (BioRad). The light images were acquired under epi-white illumination with an automatically optimized exposure time. The bioluminescence images were obtained under no illumination with an exposure time of 900 s for hispidin addition and wound stress experiments, and 600 s for temperature stress experiments.

### 2.3. Hispidin treatment

To examine the effects of hispidin, also known as a precursor of fungal luciferin, hispidin solutions were added to the *O. guepiniiformis* and *Lentinula edodes* mycelia grown on PDA at 25 °C. Hispidin solutions with two different concentrations, 10 μg/ml and 100 μg/ml, were prepared by dissolving hispidin in 1% of dimethyl sulfoxide (DMSO) and applied on the mycelia by dropping 10 μL of each solution. One percent of DMSO solution was used as a control. Hispidin and DMSO were purchased from Sigma-Aldrich (MO, USA). The bioluminescence images were obtained immediately after dropping the hispidin solution on the mycelia with an exposure time of 900 s.

### 2.4. Gene cloning and sequencing

To obtain gDNA and coding sequence (CDS) clones of bioluminescence pathway genes, gDNA and cDNA were obtained from two-week-old mycelia grown on PDA and used as templates for PCR amplification. The gDNA and total RNA were extracted using DNeasy Plant mini kit (Qiagen, Hilden, Germany) and RNeay mini kit (Qiagen), respectively, according to the manufacturer's instructions.

The primers were designed to target the putative genes predicted from the recently assembled genome

of *O. guepiniiformis* [12]. The sequences of primers used for gene cloning were listed in Supplementary Table S1. PCR amplification was conducted using the AccuPower Pfu PCR premix (Bioneer, Daejeon, Korea). The PCR products were purified using the AccuPrep PCR/Gel DNA Purification kit (Bioneer) and then cloned into a vector using the TOPcloner Blunt kit (Enzynomics, Daejeon, Korea) following the manufacturers' instructions. The obtained clones were sequenced by Macrogen Inc. (Seoul, Korea) using an ABI 3730xl DNA Analyzer (Applied Biosystems, CA, USA). The primers used for sequencing were listed in Supplementary Table S1.

### 2.5. Gene expression analysis

Quantitative reverse transcription-PCR (qRT-PCR) was employed to investigate the transcript levels of bioluminescence pathway genes. The qRT-PCR reactions were performed in a 96-well block with the CFX Connect Real-Time System (BioRad) using the SsoAdvanced Universal SYBR Green Supermix (BioRad) in a volume of 20 µL with the following cycling conditions: 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 30 s, followed by fluorescence detection. The primers used for qRT-PCR were listed in Supplementary Table S1. A *β-tubulin* gene was included as the internal control to normalize the variations in the amounts of cDNA used (Supplementary Figure S1). The relative expression levels were calculated by the CFX Manager 3.1 software (BioRad) using the comparative ΔΔCq method. Statistical analysis was conducted by one-way analysis of variation (ANOVA) using SPSS (version 18) software.

## 3. Results

### 3.1. Fungal bioluminescence pathway is conserved in Omphalotus guepiniiformis

Before performing cloning of homologous genes involved in the fungal bioluminescence pathway [10], we wanted to verify whether the recently identified fungal bioluminescence mechanism is functionally conserved in *O. guepiniiformis*. Thus, we decided to examine the effects of hispidin, a phenolic metabolite also known as a precursor of fungal luciferin [9,25], on the bioluminescence of *O. guepiniiformis* mycelia. It was found that the addition of hispidin enhanced the intensity of bioluminescence emitted from the mycelia of *O. guepiniiformis* (Figure 1(A)). Moreover, the increase in intensity was dependent on the concentration of hispidin (Figure 1(A)), supporting the functionality of hispidin in the

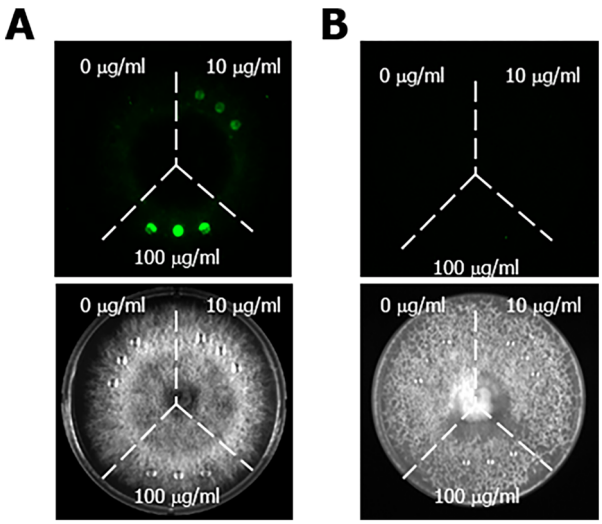


**Figure 1.** Enhanced bioluminescence of *Omphalotus guepiniiformis* with the addition of hispidin. The droplets with different concentrations of hispidin were added to the mycelia of *O. guepiniiformis* (A) and a non-luminous fungus *Lentinula edodes* (B). The light and bioluminescence images were shown on the upper and lower row, respectively. Note that the addition of hispidin increased the intensity of bioluminescence in a dose-dependent manner.

bioluminescence of *O. guepiniiformis*. However, no luminescence signal was detected from a non-luminous fungus, *Lentinula edodes* (Figure 1(B)). Therefore, the previously reported fungal bioluminescence pathway and the involved genes were conserved and active in *O. guepiniiformis*.

Next, we cloned genomic and CDS regions of bioluminescence genes, *HispS*, *H3H*, and *Luz*, using the primers targeting the homologous genes predicted in the genome of *O. guepiniiformis* [12]. The sequence comparison of genomic and CDS regions obtained by molecular cloning showed that *HispS* and *H3H* have a multi-intron structure and *Luz* has a single intron (Supplementary Figures S2–S4). We also compared the amino acid sequences of the enzymes with those of other luminous species in the *Omphalotus* lineage, such as *O. olearius*, *Neonothopanus gardneri*, and *N. nambi* [10,26]. All three enzymes of *O. guepiniiformis* exhibited high similarities (more than 71% identity and 75% similarity) to the enzymes identified in other luminous species (Figure 2), supporting that the mechanism and the involved enzymes in light production are highly conserved in the *Omphalotus* lineage.

### 3.2. Wound stress enhances the intensity of bioluminescence and the expression of bioluminescence pathway genes

Our previous study showed that the intensity of bioluminescence emitted from mycelia depends on the type of stress applied to the mycelia (e.g., the

| | OgHispS | OoHispS | NgHispS | NnHispS |
|---|---|---|---|---|
| OgHispS | | 82.56 | 76.91 | 78.85 |
| OoHispS | 85.67 | | 73.51 | 76.59 |
| NgHispS | 82.64 | 78.75 | | 80.45 |
| NnHispS | 84.58 | 81.52 | 84.62 | |

| | OgH3H | OoH3H | NgH3H | NnH3H |
|---|---|---|---|---|
| OgH3H | | 77.52 | 72.91 | 71.46 |
| OoH3H | 81.84 | | 78.36 | 74.64 |
| NgH3H | 77.80 | 84.37 | | 86.53 |
| NnH3H | 75.79 | 79.38 | 89.66 | |

| | OgLuz | OoLuz | NgLuz | NnLuz |
|---|---|---|---|---|
| OgLuz | | 84.92 | 80.55 | 80.95 |
| OoLuz | 88.09 | | 83.14 | 82.75 |
| NgLuz | 87.3 | 87.73 | | 89.39 |
| NnLuz | 86.5 | 85.82 | 91.66 | |

| |
|---|
| >90 |
| 85~90 |
| 80~85 |
| 75~80 |
| 70~75 |

**Figure 2.** Amino acid sequence identity and similarity matrices of bioluminescence pathway genes in the species of the *Omphalotus lineage*. The pairwise amino acid sequence identities and similarities of hispidin synthase (HispS), hispidin-3-hydroxylase (H3H), and luciferase (Luz) of the fungal species in the *Omphalotus* lineage, including *Omphalotus guepiniiformis*, were calculated in Sequence Identity and Similarity (http://imed.med.ucm.es/Tools/sias.html) using the sequence alignment obtained by Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). The percent identities and similarities were presented on the upper right and the bottom left, respectively. Og, *O. guepiniiformis*; Oo, *O. olearius*; Ng, *Neonothopanus gardneri*; Nn, *N. nambi*.

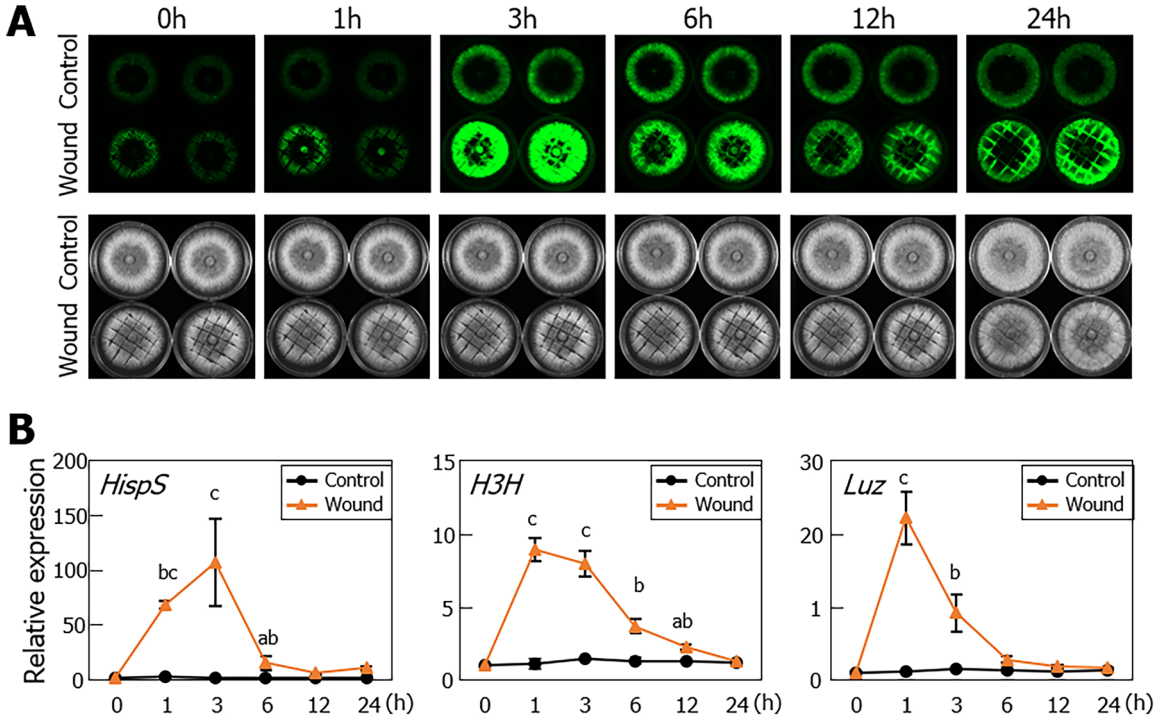


**Figure 3.** Effects of wound stress on the bioluminescence and the expression of bioluminescence pathway genes of *Omphalotus guepiniiformis*. (A) The bioluminescence (upper row) and light images (lower row) of control and wound stress-treated mycelia. For wound stress treatment, mycelia grown on potato dextrose agar media for 7 d at 25 °C under dark conditions were severed with a surgical blade. (B) Expression of bioluminescence pathway genes after wound stress determined by quantitative real-time RT-PCR (qRT-PCR). The mycelia were harvested for total RNA extraction. Biological replicates were averaged. Bars indicate the standard deviation (S.D.). The different letters indicate a significant difference ($p < .05$) according to the one-way ANOVA with Scheffe's *post hoc* test. All unlabeled points belong to group "a".

mechanical stress induced the bioluminescence whereas high and low temperatures reduced the bioluminescence of mycelia) [24]. To elucidate the molecular basis of the differential response of mycelia to environmental changes, we investigated the transcriptional changes in bioluminescence pathway genes, using the nucleotide sequences obtained in this study.

First, we examined the effects of wound stress, which increased the bioluminescence of mycelia in *Armillaria* species and *N. nambi* as well as *O. guepiniiformis* [19,24]. As shown in Figure 3(A) and Supplementary Figure S5A, the luminescence emitted from mycelia was strongly enhanced within 1 h, peaked at 3 h, and lasted at least until 24 h after damaging the mycelia mechanically. Interestingly, even the region of mycelia, where no direct stimuli were applied but adjacent to the damaged cells, produced higher bioluminescence than the control (Figure 3(A)), suggesting the presence of wound stress signal transduction pathway to other cells.

Consistent with the changes in bioluminescence, wound stress also markedly increased the transcription of bioluminescence pathway genes such as *HispS*, *H3H*, and *Luz* (Figure 3(B)). Considering that these genes encode enzymes, the protein catalysts that could react with large amounts of substrates in small amounts of itself, the increased transcription of fungal luciferin biosynthetic genes and luciferin

oxidase would largely contribute to the increase in bioluminescence after wound stress.

### 3.3. Temperature stress suppresses the intensity of bioluminescence not by reducing the transcription of bioluminescence pathway genes

We investigated the effects of temperatures on the intensity of bioluminescence and the transcription of related genes. It was found that the intensity of bioluminescence emitted from mycelia was significantly decreased during the incubation of mycelia at 4 °C for 24 h (Figure 4(A) and Supplementary Figure S5B). Meanwhile, the suppressed bioluminescence at low temperatures was restored at the level of control 2 h after transferring the mycelia to 25 °C. Therefore, it seemed that short-term cold stress affected the light production pathway in a reversible manner. Although wound stress significantly changed the transcription of *HispS*, *H3H*, and *Luz* genes, cold stress did not affect their transcription except for the marginal increase in *Luz* transcription with prolonged incubation at cold temperatures (Figure 4(B)). However, the transcriptional change of *Luz* was opposed to the changes in bioluminescence intensity, so it could not explain the reduction of bioluminescence intensity upon cold stress.

The response of mycelia to high temperatures was like that to low temperatures in terms of the intensity of bioluminescence; the intensity of light was wholly suppressed at 35 °C for 24 h, while the suppressed light was restored at the level of control 2 h after transferring the mycelia from 35 °C to 25 °C (Figure 5(A) and Supplementary Figure S5C). However, the transcriptional changes in the bioluminescence pathway genes under high temperatures were quite different from those under low temperatures. Exposure of mycelia to 35 °C for 1 h and then recovering at 25 °C resulted in a slight increase of transcription of *HispS*, *H3H*, and *Luz* genes, but the effect did not last for more than 5 h after transferring the mycelia to 25 °C (Figure 5(B)). Continued incubation of mycelia to 35 °C raised the expression of the *Luz* gene with a peak at 3 h but did not significantly affect the transcription of *HispS* and

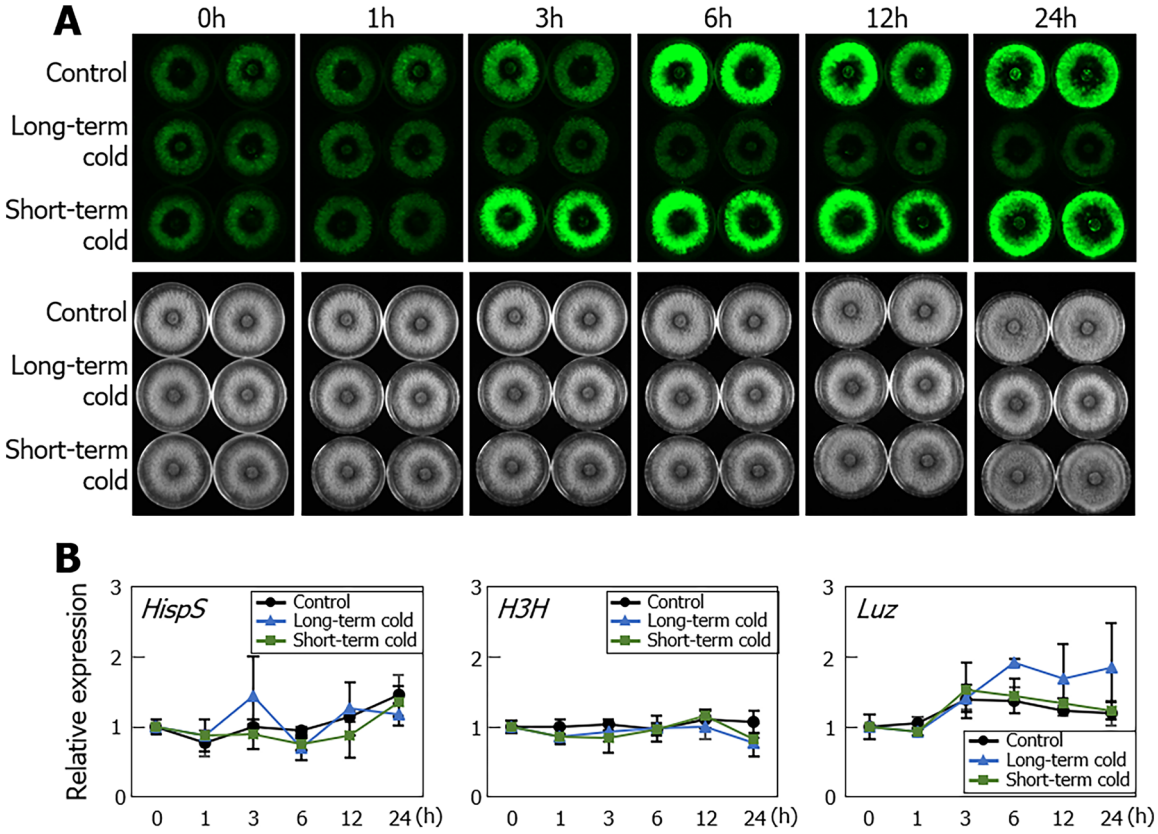


**Figure 4.** Effects of cold stress on the bioluminescence and the expression of bioluminescence pathway genes of *Omphalotus guepiniiformis*. (A) The bioluminescence (upper row) and light images (lower row) of control, long-term cold stress, and short-term cold stress-treated mycelia. The mycelia grown on potato dextrose agar media for 7 d at 25 °C under dark conditions were transferred to 4 °C under dark conditions for cold stress treatment. For long-term cold treatment, the mycelia were incubated at 4 °C for 24 h. For short-term cold treatment, the mycelia were incubated at 4 °C for 1 h and then transferred to 25 °C. (B) Expression of bioluminescence pathway genes under cold stress. Transcript levels were determined by qRT-PCR. ($n = 3$; bars, S.D.) Statistical analysis was conducted using one-way ANOVA with Scheffe's *post hoc* test. All unlabeled points belong to group "a".

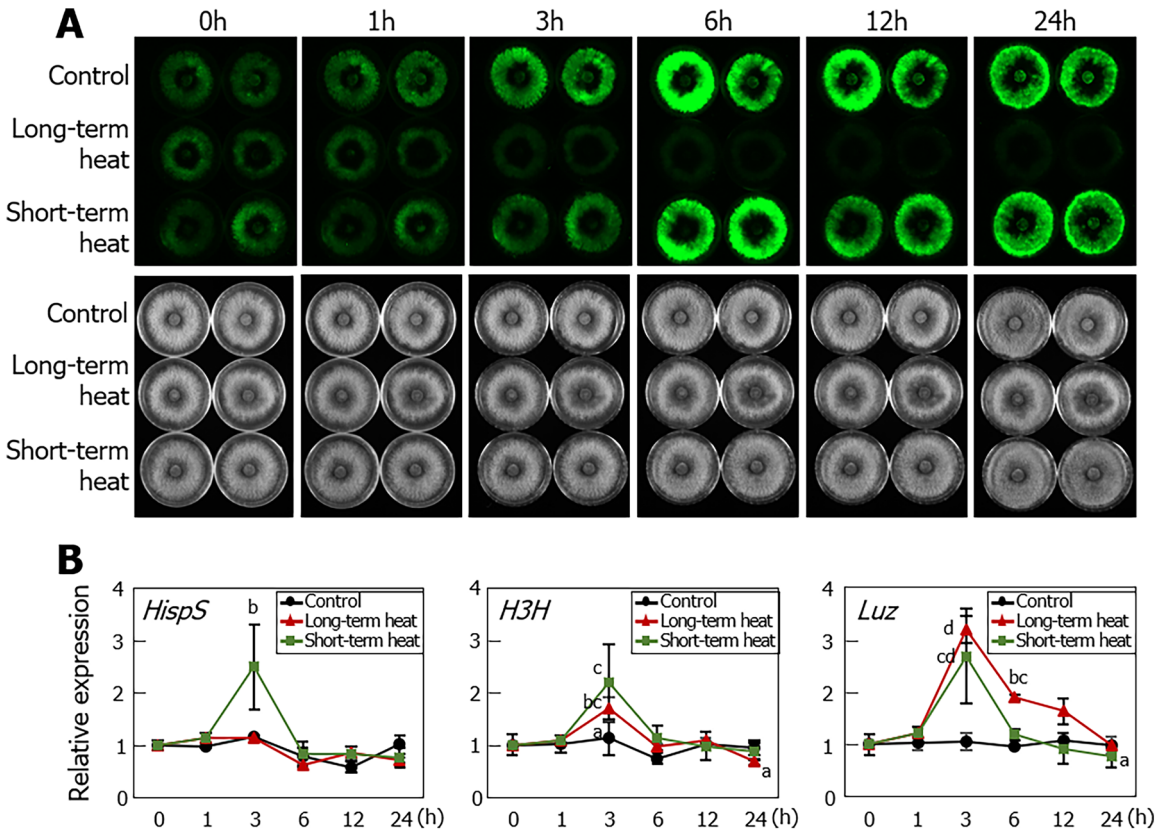


**Figure 5.** Effects of heat stress on the bioluminescence and the expression of bioluminescence pathway genes of *Omphalotus guepiniiformis*. (A) The bioluminescence (upper row) and light images (lower row) images of control, long-term heat stress, and short-term heat stress-treated mycelia. The mycelia grown on potato dextrose agar media for 7 d at 25 °C under dark conditions were transferred to 35 °C under dark conditions for heat stress treatment. For long-term heat treatment, the mycelia were incubated at 35 °C for 24 h. For short-term heat treatment, the mycelia were incubated at 35 °C for 1 h and then transferred to 25 °C. (B) Expression of bioluminescence pathway genes under heat stress. Transcript levels were determined by qRT-PCR. ($n = 3$; bars, S.D.) Statistical analysis was conducted using one-way ANOVA with Scheffe's *post hoc* test. All unlabeled points belong to group "a" for *HispS* and "ab" for *H3H* and *Luz*.

*H3H* genes. The direction of changes in transcription was opposed to that in bioluminescence intensity, suggesting the presence of other mechanisms regulating *HispS*, *H3H*, and *Luz* genes at translational or post-translational levels. One plausible explanation is that the suppression of bioluminescence under heat stress resulted from decreased Luz enzyme activity, as reported on the recombinant protein of Luz encoded in *N. nambi* [10].

## 4. Discussion

### 4.1. Transcriptional and non-transcriptional regulation of bioluminescence under changing environments

The mycelia of *O. guepiniiformis* showed different transcriptional responses of bioluminescence pathway genes according to the type of abiotic stresses (Figures 3–5). Upon wound stress, the prominent increase in transcription of *HispS, H3H,* and *Luz* genes seemed to be a leading factor contributing to increased bioluminescence intensity. Meanwhile, the transcription of *HispS, H3H,* and *Luz* genes was not reduced but bioluminescence intensity was suppressed under long-term cold and heat stresses. Therefore, *O. guepiniiformis* could distinguish wound and temperature stresses and respond differently to those stresses at the transcriptional level.

Studies on transcriptional regulators of mushrooms have been focused on fruiting body development, a significant interest in mushroom science [27–29]. There are several reports on transcription factors related to temperature stresses [30,31], but there are few reports describing transcription factors modulating the mechanical stress response of mushrooms. In a follow-up study, we will conduct RNA-seq analysis to get clues on the upstream regulators of wound stress signaling.

Our data suggested the presence of a post-transcriptional regulatory mechanism in the bioluminescence of *O. guepiniiformis*. Kotlobay et al. showed that the activity of recombinant Luz protein of *N. nambi* was maintained high at 10 to 18 °C but was

markedly suppressed above 30 °C [10]. Therefore, protein inactivation *via* denaturation or modification of HispS, H3H, and/or Luz would result in the suppression of bioluminescence. Otherwise, especially at low temperatures, protein degradation might contribute to the suppression of bioluminescence. Studies on protein abundance and modifications of HispS, H3H, and Luz would allow us to identify the mechanism responsible for the down-regulation of bioluminescence under temperature stresses.

Meanwhile, a bright light signal at the damaged cells was observed even after 24h of shearing (Figure 3(A)). The increase in bioluminescence intensity at the damaged cells after wound stress could result by not only the increase in the transcription of *HispS*, *H3H*, and *Luz* but also the increase in $O_2$, a substrate of luciferase. It was reported that the supply of $O_2$ increases the luminescence intensity from the aqueous suspension of the gills of *O. guepiniiformis* [23]. Because shearing causes the direct exposure of cells to external air, it could accelerate the oxidative reaction of luciferin by luciferase, by increasing the cellular level of $O_2$. A study on the changes in bioluminescence at the damaged cells in the presence of transcription inhibitors might address the contribution of the increased $O_2$ and transcription of bioluminescence pathway genes in the enhanced bioluminescence at the damaged cells.

### 4.2. Conservation of wound-induced bioluminescence in luminous fungi

Although the degree of response differed between species, wound-induced bioluminescence of mycelia was also reported in *N. nambi*, *Armillaria* sp., and *Panellus stipticus* as well as *O. guepiniiformis* [19]. Taxonomically, they are classified into three distinct evolutionary lineages: *O. guepiniiformis* and *N. nambi* belong to the *Omphalotus* lineage, *Armillaria* sp. belongs to the *Armillaria* lineage, and *P. stipticus* belongs to the *Mycenoid* lineage [10,26]. Therefore, wound-induced bioluminescence of mycelia might be widely conserved in luminous fungi.

If so, what is the benefit of increased light production after wounding for fungi? Considering a report describing that green LED light similar to fungal bioluminescence attracted insects in the natural habitat of *N. gardneri*, a luminous fungus [22], it is supposed that luminous fungi could utilize light for communicating with other organisms. Mycelia in nature are exposed to nematodes and insects, the possible contributors to wound stress. After being damaged by them, mycelia might produce more light to scare away fungivores or to attract predators of fungivores. There were several reports supporting this hypothesis. In 1981, Sivinski showed that more arthropods were attracted by the glowing mycelia of *Mycena* sp. [32]. In 2018, it was reported that low-intensity visible light including green light reduced the lifespan of transparent nematode, *Caenorhabditis elegans*, by creating photooxidative stress [33], supporting the possibility of green light-mediated attack on fungivores. However, it is not yet proven which species induce mechanical damage to luminous fungi and whether the green light emitted from mycelia affects behaviors of them or associated predators. Further studies are required to verify the ecological significance of wound-induced fungal bioluminescence.

### 4.3. Possible biological significance of the reduced bioluminescence under temperature stress

We wondered about the biological meaning of bioluminescence reduction under temperature stress. It had been reported that heat and cold stresses induced the accumulation of reactive oxygen species (ROS) in mushroom species [34–36]. The fungal bioluminescence pathway yields antioxidants and green light. Antioxidant activities of two intermediates in the fungal luciferin biosynthetic pathway, caffeic acid and hispidin, have been reported in many literatures [37–40]. Both phenolic compounds exhibited antioxidant activities against ABTS radicals, DPPH radicals, hydroxyl radicals, and superoxide anion radicals. Therefore, we hypothesized that *O. guepiniiformis* might utilize antioxidant compounds such as caffeic acid and hispidin to reduce ROS under temperature stresses, instead of producing light. To test this hypothesis, further studies on the concentration profile of caffeic acid and hispidin under temperature stress or temperature stress resistance phenotypes of *HispS*-overexpressing and knock-out strains are required.

In summary, we demonstrated that the previously reported fungal bioluminescence system is conserved in *O. guepiniiformis*. Using the sequence information obtained from the cloning of *HispS*, *H3H*, and *Luz* genes, we investigated their transcriptional changes under mechanical and temperature stresses. We found the stress-dependent transcriptional response of bioluminescence and the presence of the non-transcriptional regulatory mechanism of bioluminescence. Upon mechanical stress, *O. guepiniiformis* produces more light by increasing the transcription of *HispS*, *H3H*, and *Luz* genes responsible for the synthesis and oxidation of fungal luciferin. Meanwhile, under high- and low-temperature stresses, *O. guepiniiformis* suppressed the bioluminescence not by

reducing the transcription of *HispS*, *H3H*, and *Luz* genes but perhaps by reducing the accumulation and/or activity of proteins encoded by *HispS*, *H3H*, and *Luz* genes. Taking together our data and previous reports on various responses of fungal bioluminescence to different environmental conditions [15–22], luminous fungi seem to actively regulate bioluminescence *via* diverse molecular mechanisms to cope with environmental stresses. Because not only diverse factors affecting bioluminescence intensity but also high-quality genome information and the conditions for artificial cultivation of fruiting body of *O. guepiniiformis* have been described [12,41], it is possible to investigate the effects of various factors on bioluminescence at the molecular level throughout the whole life cycle, from vegetative mycelium to fruiting body and spores. Therefore, *O. guepiniiformis* can be a model species for studying fungal bioluminescence and fungal signal transduction under various conditions.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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