Detecting bioluminescence conditions in fruit bodies of two species of Armillaria basidiomycetes

A P Puzyr¹, A E Burov¹,²,³ and V S Bondar¹

¹Institute of Biophysics SB RAS, Federal Research Center “Krasnoyarsk Science Center SB RAS”, Krasnoyarsk 660036, Russia
²Federal Research Center for Information and Computational Technologies, Krasnoyarsk 660049, Russia
³E-mail: aeburov@ict.nsc.ru

Abstract. Mycelia of various Armillaria fungi are bioluminescent while the fruit bodies do not emit light. The presence in fruit bodies of Armillaria species of enzymes involved in the fungal bioluminescence was investigated by treating them with an exogenous analogue of the substrate for the light-emitting reaction. For this, hot extracts from nonluminous fungus Pholiota squarrosa were used. Upon spraying the pristine and transversely cut fruit bodies with the extracts, light emitting regions of different intensity were revealed. This suggests that the fruit bodies of the studied species are nonluminous due to lack of the substrate for light luminescent reaction. The prolonged incubation of the fruit bodies in water elevated the bioluminescence level. A possible mechanism which can explain this phenomenon is discussed.

1. Introduction

Various higher fungi produce light visible at night, i.e. exhibit bioluminescence [1-3]. Almost 100 species of luminous mushrooms were so far discovered around the world [3-4]. Some species of higher fungi have the fruit bodies that are entirely luminous. In some other species of basidiomycetes, visible light is only emitted by parts of the fruit body – caps or stipes [3].

Fruit bodies of species of the fungal genus Armillaria are known to be nonluminous while mycelia growing in natural conditions or cultured on artificial nutrient media emit visible light [2, 4]. Results of studies on different species of Armillaria suggested reasons for this phenomenon [5-7]. Previously, we have assumed that the cold extracts from fruit bodies of Armillaria species lack or contain a negligible amount of enzymes required for bioluminescence [5]. As reported in [6], the fruit bodies of A. mellea contain the luciferase (enzyme that catalyses the substrate) but the synthesis of the hydroxylase enzyme, which catalyses formation of 3-hydroxyhispidin (substrate for bioluminescence reaction) from its precursor, hispidin, is blocked. The bioluminescence of fruit bodies grown from mycelia of North American Armillaria species (A. gallica, A. mellea, and D. tabescens) was measured to be considerably lower (ten times at least) than that of mycelia themselves [7]. At the same time, the light emission in stipe and gills of the fruit bodies was remarkably increased upon addition of the exogenous hispidin. The authors concluded that during the transition stage from the mycelium to the fruit body, synthesis of the substrate’s precursor for bioluminescence reaction, hispidin, is impeded.
In this study, the fruit bodies of the *Armillaria borealis* and *Armillaria* sp. were processed with an exogenous analogue of the substrate of bioluminescence reaction and found regions of detectable bioluminescence.

2. Materials and methods

Fresh fruit bodies of *Armillaria borealis*, *Armillaria* sp., and *Pholiota squarrosa* were harvested near the Krasnoyarsk city, Russia. Before use, the samples were properly washed in distilled water to remove contaminants and impurities. An analogue of the substrate for the luminescent reaction was hot extracted from *P. squarrosa* according to the procedure describe elsewhere [5]. Both mycelium and fruit body of this fungus are considered to be nonluminous.

Imaging study was performed with a GelDoc XR Imaging System (Bio-Rad, U.S.) was used for imaging study. To detect luminous regions, the fruit bodies were dewed with a solution of *P. squarrosa* extract. The following conditions for acquisition of the bioluminescence signals were employed [8]: the diaphragm – maximum open, the signal accumulation time - 999 sec. At low light intensity, the brightness and contrast of images were enhanced using the software transform function. The fruit bodies were also processed with a 1 mM NADPH solution (Serva, Germany). The reduced form of this pyridine nucleotide is known to be involved in the functioning of in vitro light-emitting systems isolated from different species of luminous fungi.

The bioluminescence response of *Armillaria* fruit bodies before and after their treatment with the hot extracts was recorded with a Glomax® 20/20 luminometer (Promega, U.S.). The fruit bodies placed in Petri dishes were transferred to the luminometer and the initial bioluminescence was recorded. Then, the fruit bodies were sprayed with the hot extract solution, and light emission was again measured. In comparative study, the fruit bodies were additionally sprayed with a 1 mM NADPH solution. Light emission is expressed in relative light units (RLU/s). Additional experiments were carried out to study the light emission produced by the *Armillaria* fruit bodies which were before held immersed in deionized water at room temperature.

3. Results and discussion

To investigate whether the enzymes required for bioluminescence are present in the fruit bodies of *Armillaria* basidiomycetes, we treated them with the hot extract from fruit bodies of the nonluminous *P. squarrosa*. The extract was used as a source of an exogenous analogue of the substrate for light-emitting reaction, and thus to visualize luminous regions. As previously shown, such hot extracts contain hispidin, a precursor of the substrate (preluciferin) for bioluminescence reaction in higher fungi. Addition of the extract to bioluminescence systems isolated from various species of luminous basidiomycetes could stimulate the light emission [10].

Regions exhibiting detectable bioluminescence were found upon spraying the *A. borealis* fruit body with the hot extract of *P. squarrosa*. A weak bioluminescence was observed on the fungus cap, and bioluminescence of higher intensity was detected at the ring on the underside of the cap (figure 1). Examination of the fruit body of *Armillaria* sp. after it was transversely cut and sprayed with the hot extract also revealed luminous regions (figure 2). On the transverse section of fruit body of this fungus, bright luminous regions were detected on the cap surface, exactly on its edge, and on parts of the ring on the stipe. Such location of luminous regions was typical for all samples of *Armillaria* sp. However, no detectable bioluminescence was observed on other parts of fruit bodies after the treatment (figures 1 and 2).

Measurements of light intensity confirmed that treatment with the hot extract from *P. squarrosa* stimulated bioluminescence in *Armillaria* species (figure 3). After the hot extract was added to the *Armillaria* sp. fruit body, the level of bioluminescence quickly (in 3-4 min) increased by almost an order, slowly decreasing afterward. A subsequent treatment of the fruit body with a 1 mM NADPH solution did not significantly influence the kinetics of the light signal initiated by the hot extract addition (figure 3). The likely reason for this is the presence of NADPH in amount sufficient for light emission. The need for reduced pyridine nucleotides to start the bioluminescence reaction in higher
fungi was first established in vitro experiments with cold and hot extracts [11]. As found in the present study, another treatment of the fruit body with the hot extract again increased the light intensity.

![Figures 1 and 2](image-url)

**Figure 1.** The appearance (a, c) and luminous regions (b, d) of the *A. borealis* fruit body sprayed with the hot extract from *P. squarrosa*.

**Figure 2.** A section of the *Armillaria* sp. fruit body (a) and luminous regions detected after the treatment with the hot extract from *P. squarrosa* (b).

Results presented above suggest that there is no substrate for bioluminescence reaction in the fruit bodies of the *Armillaria* species. However, we are not sufficiently certain that hispidin is the only substrate. The hot extract of nonluminous *P. squarrosa* could contain various low-molecular-weight compounds, including hispidin analogues that could initiate the light emission [10]. Moreover, the data reported in [12] suggested that biobrioluminescence of living gills of *Mycena chlorophos* was activated
by trans-p-hydroxycinnamic acid and trans-3,4-dihydroxycinnamic acid, isolated from the mature *M. chlorophos* pilei. We believe that a more accurate interpretation of our results would be the conclusion about the blockage of the synthesis of substrates for bioluminescence reaction in the fruit bodies of the studied species. At the same time, all enzymes required for bioluminescence are present, although they are localized in certain regions of the fruit bodies. This phenomenon needs further research.

![Figure 3. Bioluminescence from the cap of the *Armillaria* sp.: 1 – initial level; 2-4 – after sequential addition (arrows) of: 2 – hot extract, 3 – 1 mM NADPH, 4 – hot extract.](image)

Our experiments showed that incubation of *Armillaria* fruit bodies in water also increased the bioluminescence intensity (figure 4). The light emission reached its maximum after 5-6 h of incubation in water. The same effect was earlier observed with fruit bodies and mycelia of other luminous fungi [9, 13]. However, the fruit bodies of *Armillaria* basidiomycetes, which did not exhibit visible bioluminescence, were used in this study.

![Figure 4. Light emission detected after incubation of the *A. borealis* fruit body in deionized water.](image)
We propose two possible reasons to explain such incubation-enhanced bioluminescence. First, the incubation may unblock the synthesis of the substrate (substrates) for bioluminescence reaction. In this case, the increase of light intensity was caused by the function of enzymes of the light emission system present in the fungus. On the other hand, the experiments with parts of fruit bodies of various nonluminous higher fungi showed that they exhibited chemiluminescence – low-intensity light emission [14]. Low bioluminescence was also recorded in mycelia of some representatives of the Basidiomycota, Ascomycota, and Zygomycota divisions [15]. The present study revealed low-intensity bioluminescence (2500 RLU/s – 12000 RLU/s) in fruit bodies of A. borealis and Armillia sp. The intensity of chemiluminescence of biological objects is directly related to their contents of reactive oxygen species, whose levels can increase dramatically under stress caused by physical, chemical or biological factors [16]. Thus, we assume that the increase in bioluminescence of the incubated fruit bodies of the Armillaria species was attributed to the increase in their chemiluminescence because of the growing pool of reactive oxygen species, which form in the fungus biomass under hypo-osmotic stress.

4. Conclusion
The fruit bodies of the Armillaria borealis and Armillia sp. basidiomycetes were treated with an analogue of the substrate for bioluminescence reaction extracted from P. squarrosa. Upon spraying the pristine and cut fruit bodies with the extracts, light emitting regions of different intensity were revealed. This suggests that the fruit bodies of these fungi are non-luminous at normal conditions due to the lack of substrate. The prolonged incubation of the fruit bodies in water elevated the bioluminescence. The increase is likely attributed to the change in the level of their chemibioluminescence due to growing pool of reactive oxygen species, which form in the fungal biomass under hypo-osmotic stress.

References
[1] Harvey E N 1952 Bioluminescence (New York: Academic Press)
[2] Shimomura O 2006 Bioluminescence: Chemical Principles and Methods (Singapore: World Scientific)
[3] Oliveira A G, Desjardin D E, Perry BA and Stevani C V 2012 Photochem. Photobiol. Sci. 11 848-52
[4] Mihail J D 2015 Fungal Biol. 119 528-37
[5] Puzov A P, Medvedeva S E and Bondar V S 2017 Mycosphere 8 9-17
[6] Purtov K V, Petushkov V N, Rodionova N S and Gitelson J I 2017 Dokl. Biochem. Biophys. 474 217-9
[7] Mihail J D, Bilyeu L and Lalk S R 2018 Fungal Biol. 122 1064-8
[8] Puzov A P, Medvedeva S E and Bondar V S 2016 Mycosphere 7 1-17
[9] Mogilnaya O A, Ronzhin N O and Bondar V S 2016 Mycosphere 7 499-510
[10] Purtov K V et al 2015 Angew. Chem. Int. Ed. 54 8124-8
[11] Airth R L and McElroy W D 1959 J. Bacteriol. 77 249-50
[12] Teranishi K 2016 Tetrahedron 72 726-33
[13] Mori K, Kojima S, Maki S, Hirano T and Niwa H 2011 Bioluminescence 26 604-10
[14] Gitelson J I, Bondar V S, Medvedeva S E, Rodicheva E K and Vydyrakova G A 2012 Dokl. Biochem. Biophys. 443 105-8
[15] Mihail J D and Bruhn J N 2007 Opera Mycologica 1 28-33
[16] Vladimirov Y A 1994 Free Radicals in the Environment, Medicine and Toxicology (London: Richelieu Press) pp 345-73