ISSN 0003-6838, Applied Biochemistry and Microbiology, 2015, Vol. 51, No. 4, pp. 419–424. © Pleiades Publishing, Inc., 2015.
Original Russian Text © O.A. Mogil'naya, N.O. Ronzhin, S.E. Medvedeva, V.S. Bondar', 2015, published in Prikladnaya Biokhimiya i Mikrobiologiya, 2015, Vol. 51, No. 4, pp. 395–401.

Total Peroxidase and Catalase Activity of Luminous Basidiomycetes *Armillaria borealis* and *Neonothopanus nambi* in Comparison with the Level of Light Emission

O. A. Mogil'naya, N. O. Ronzhin, S. E. Medvedeva, and V. S. Bondar'

Institute of Biophysics, Siberian Branch, Russian Academy of Sciences, Krasnoyarsk, 660036 Russia

e-mail: ol_mog@mail.ru

Received November 25, 2014

Abstract—The peroxidase and catalase activities in the mycelium of luminous basidiomycetes *Armillaria borealis* and *Neonothopanus nambi* in normal conditions and under stress were compared. An increase in the luminescence level was observed under stress, as well as an increase in peroxidase and catalase activities. Moreover, the peroxidase activity in extracts of *A. borealis* mycelium was found to be almost one and a half orders of magnitude lower, and the catalase activity more than two orders of magnitude higher in comparison with the *N. nambi* mycelium. It can be suggested that the difference between the brightly luminescent and dimly luminescent mycelium of *N. nambi* is due to the content of H_2O_2 or other peroxide compounds.

Keywords: basidiomycetes, luminescence, peroxidase, catalase

DOI: 10.1134/S0003683815040110

INTRODUCTION

The luminescence systems of numerous live organisms are currently well studied; the enzymes catalyzing light-emitting reactions (luciferases) and their substrates (luciferins) have been isolated and characterized [1]. Nevertheless, the mechanisms of light emission by higher fungi—basidiomycetes—have remained a mystery for researchers working in this area for more than a hundred years. Until now, neither the molecular organization of the luminescent system of higher fungi not the mechanism of light-emitting reaction have been elucidated. First, the enzyme (or enzymatic complex) that plays the role of luciferase in fungi and the structure of the light-emitting reaction substrate—luciferin—have not been identified. In the middle of last century it was shown in experiments with fungi extracts that two thermolabile protein components—soluble NAD(P)H-dependent reductase and luciferase present as insoluble particles—and thermostable luciferin and NAD(P)H participated in the light-emitting reaction [2–4]. This result was later confirmed by two other groups of researchers [5–8]. However, until now the structure and properties of luciferase, NAD(P)H-dependent reductase, and luciferin of fungi have been unknown, because these components have not been isolated in their pure form.

It was suggested earlier that the reactive oxygen species (ROS) and enzymes with oxidase functions participated in the mechanism of luminescence in higher fungi [1, 9, 10]. The results of the investigation of the luminous basidiomycete *Neonothopanus nambi* also indicate the participation of ROS and oxidase enzymes in the mechanism of the fungal luminescence [11–13]. It was shown in these studies that the addition of hydrogen peroxide to samples of the fungal mycelium at millimolar concentrations stimulated their luminescence. A similar effect of H_2O_2 was demonstrated in a work with luminous extracts that were obtained from the *N. nambi* mycelium and contained the luminescent system of this fungus [14]. On the other hand, stimulation of the luminescence of the *Armillaria borealis* basidiomycete under the action of hydrogen peroxide was not demonstrated.

It is known that ROS, including H_2O_2, are formed in the cell continuously, but their level is quite low normally, and the cell inactivates them with the help of the antioxidant system [15]. The higher fungi have a large set of enzymes participating in the metabolism of ROS, with superoxide dismutases, catalases, and peroxidases being the most prevalent [16–18]. The fungal peroxidases can be secreted, cytosolic, microsomal, or localized in the cell organelles. The majority of them are heme-containing enzymes. Heme-containing peroxidases from basidiomycetes have been studied in sufficient detail [19–26]. They play an important role in fungi metabolism and are of interest for biotechnological applications. Catalases from the lower fungi and ascomycetes were the most characterized [27, 28]. It was shown that these enzymes function extracellularly or intracellularly, demonstrating the highest catalytic activity at high H_2O_2 concentrations.

It is possible to stimulate ROS formation in biological objects under the action of physical, chemical, and biological factors [15]. It was established during

the investigation of the *N. nambi* basidiomycete that the action of such stress factors as mechanical damage to the luminous mycelium or its incubation in deionized water resulted in a manifold enhancement of the fungal light emission [11–13]. If the water was replaced regularly, the high level of light emission lasted for several days or more. At the same time, similar actions on the luminous mycelium of the *A. borealis* fungus demonstrated a significantly smaller stimulation effect of the emission [29]. Hence, the prolonged emission of the fungal mycelium could be used as a basis for the development of a new class of bioluminescent sensors.

The facts presented above suggest an interaction between the luminescence of higher fungi, in which the ROS could participate, and the activity of enzymes participating in their metabolism.

The objective of the study was an investigation of the total peroxidase and catalase activities in the mycelium of *A. borealis* and *N. nambi* basidiomycetes in comparison with the changes in luminescence under stress conditions.

METHODS

Cultures and Methods. Two species of luminous higher fungi isolated from different regions were used in this study. Their cultures are available in the CCIBSO 836 collection of the Institute of Biophysics, Siberian Branch of Russian Academy of Sciences. The culture of *N. nambi* (IBSO 2307) was isolated in the subtropical forests of Southern Vietnam and kindly provided by BIO-LUMI Co., Ltd (Vietnam) [30]. The culture of *A. borealis* (IBSO 2328) was isolated from the fruiting body of *Armillaria*, which grows in the surrounding forests in the suburbs of Krasnoyarsk (Russia). Submerged cultivation of the *N. nambi* mycelium was conducted in a liquid potato-sucrose nutrient medium (200 g potato, and 20 g of sucrose in1 L of distilled water) in 250-mL conical flasks containing 100 mL of the medium. A commercial PDB medium (HiMedia Laboratories, India) was used for cultivation of the *A. borealis* mycelium. Suspensions of the ground mycelium of the investigated fungal species, which were first cultivated for 8–10 days in Petri dishes on the nutrient media indicated for each fungus supplemented with 15 g of agar-agar per 1 L of distilled water, were used as inoculum. The inoculum volume was 2–5% of the volume of the nutrient medium. The *N. nambi* mycelium was cultivated for 3–5 days at 26–28°C, and the *A. borealis* – for 10–14 days at 22–23°C. Cultivation was carried out with continuous shaking at a speed of 180–200 rpm on an ES-20 shaker-incubator (Biosan, Latvia). This method of cultivation made it possible to obtain mycelium in the shape of separate spherical globules (pellets). The globules were separated from the nutrient medium at the end of cultivation and incubated in deionized water for several days with continuous mixing.

The level of globule luminescence was recorded with a Glomax 20/20 luminometer (Promega, the United States) calibrated with a Hastings-Weber radioactive standard [31] (one relative light unit corresponded to 2.7×10^3 photons per second). The globules were dried in a vacuum rotary Concentrator 5301 (Eppendorf, Germany) for 1.5 h at 30°C in order to determine dry mass of a globule. The value of the specific luminescent activity of the mycelium was calculated as the ratio of the light-emission intensity to the dry biomass.

Visualization of the globule luminescence was conducted with the help of a ChemiDoc™ XRS+System (Bio-Rad, the United States) in the dark in signal accumulation mode. The exposure was selected experimentally and was 100 or 300 s.

The peroxidase activity of the mycelium was evaluated with the help of reaction of oxidative azo-coupling [32]. To determine peroxidase activity in native globules, three globules of the same size were placed in tubes with 1 mL of deionized water containing 0.56 mg/mL of phenol and 0.1 mg/mL of 4-aminoantipyrine (**4-AA**) and incubated for 1 h at room temperature. Aliquots of the colored solution were taken and the optical density of the colored product formed as a result of the reaction (quinoneimine) was recorded with an UV-1800 Shimadzu spectrophotometer (Japan) at 506 nm.

To determine the total peroxidase activity in a mycelium extract, the globules were destroyed with ultrasound by an UZTA 0.63/22-OM disintegrator (OOO Tsentr ultrazvukovykh tekhnologii, Russia). The globules were placed into 10 mM of Na-phosphate buffer, pH 6.9, at a ratio 1 : 1.5 (weight of wet biomass : volume of buffer). Treatment with ultrasound was conducted on ice at a power of 97 W several times for 2–3 s each (with 1 min interval between treatments). The destroyed biomass was centrifuged at 16000 g for 20 min at 4°C in a 5415 R centrifuge (Eppendorf, Germany). Supernatants were collected and frozen at –20°C. The frozen samples were thawed at room temperature and centrifuged again under the same conditions. Peroxidase activity was determined in the obtained supernatants with the help of oxidative azo-coupling reaction.

A reaction mixture contained 980 μL of supernatant, 10 μL of 4-AA (0.1 mg/mL), and 10 μL of phenol (0.56 mg/mL). The formation of colored reaction product was assessed after 60 min of incubation from an optical density at 506 nm with a UV-1800 spectrophotometer. The specific peroxidase activity in supernatants was calculated as the ratio of optical density at 506 nm to total protein content.

The protein content in supernatants was determined with the Biuret reagent [33] with BSA as a standard.

The catalase activity in extracts obtained by the methods described above was assessed with a direct spectrophotometric method (www.sigmaaldrich.com), which allowed the determination of an optical density decrease at 240 nm following the addition of H_2O_2 to the extracts. One unit of activity was defined as the

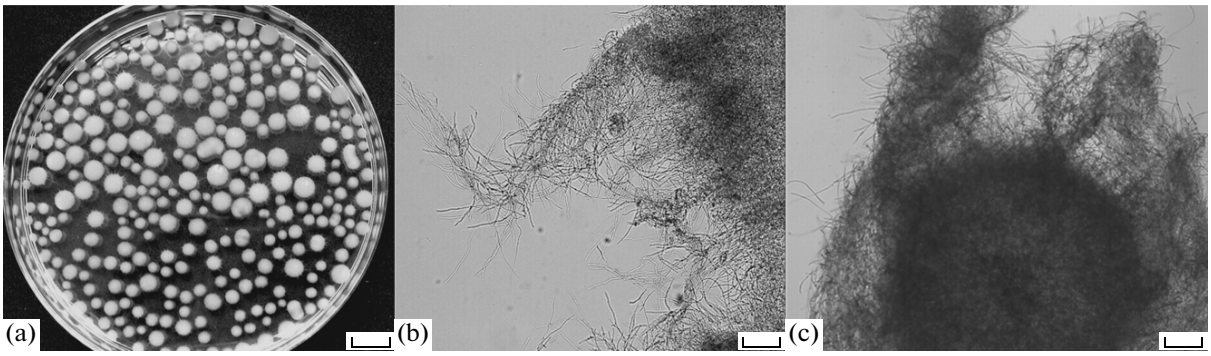

Fig. 1. Microscopic images of smooth and rough globules of *N. nambi* (a, b – edge of the rough globule), and *A. borealis* globule with massive pigmented center and twisted hyphae bundles on the surface (c). Scale bar: 10 mm (a) and 100 μm (b, c).

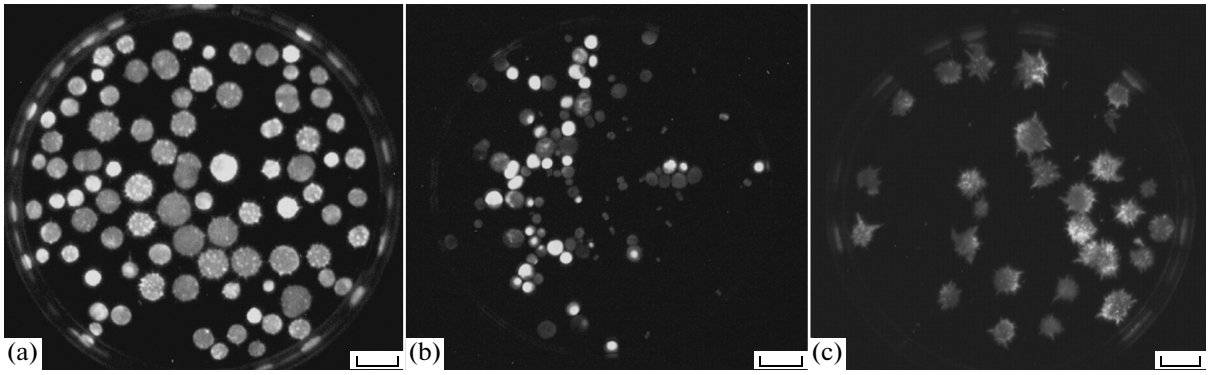

Fig. 2. Luminescence of *N. nambi* globules (a), smooth and rough globules of *A. borealis* with large hyphae bundles (b, c) obtained using signal accumulation mode. Exposure time – 300 s, Scale bar – 10 mm.

amount of the enzyme required to decompose 1 μmol of H_2O_2 per 1 min at pH 7.0 and 25°C at the H_2O_2 concentration of 10 mM. The catalase activity was normalized to 1 mg of total protein per 1 mL of the extract.

Microscopic images of globules were produced with bright field illumination using an AxioImager M2 light microscope (Carl Zeiss, Germany).

RESULTS AND DISCUSSION

Morphology of *N. nambi* and *A. borealis* globules. The cultivation of mycelium submerged in nutrient medium with constant orbital shaking resulted in the formation of spherical globules with a diameter of 2–7 mm that have a smooth or rough surface (Fig. 1). The globule roughness was due to the long (up to a few mm) bundles of the mycelial hyphae. The surface of smooth globules is represented by shorter hyphae located perpendicularly to the globule surface with a length of up to 300 μm. The *A. borealis* globules were often of irregular shape due to the presence on the surface of twisted hyphae bundles larger than those for the *N. nambi*. The central part of the *A. borealis* globules became dense and pigmented (dark brown) as it developed, which was not observed for the *N. nambi*.

Visualization and evaluation of the globule luminescence. Monitoring of the globule luminescence with the ChemiDoc™ XRS+System showed that the globules of both fungal species differed in the intensity of emission and its distribution along the globule surface (Fig. 2). The luminescence intensity of globules was independent of their size. For example, both large and small globules could demonstrate bright luminescence. Heterogeneity in the distribution of emission along the globule surface was also observed. As a rule, the smooth globules demonstrated relatively uniform luminescence along the entire globule surface or its part. Bright spots of luminescence were observed at the base of the bundles in globules with large protruding bundles of the mycelial hyphae, which was especially characteristic for the *A. borealis*.

The measurements of the luminescence levels showed that the globules of *N. nambi* taken from the nutrient medium emitted light at a level of 10^6–10^7 quanta/s. Such globules were considered to be dim. The *A. borealis* globules taken from the nutrient medium demonstrated significantly brighter luminescence than those from *N. nambi*. The intensity of their luminescence could reach up to 10^9 quanta/s. It must be noted that the luminescence intensity of the *N. nambi* globules increased significantly (three to four orders of magnitude) upon the incubation of the mycelium samples in deionized water but only by three to four times in the case of the honey fungus. The

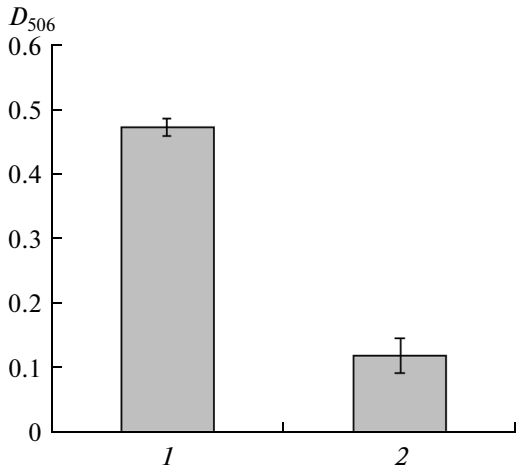

Fig. 3. Formation of colored product in the reaction of oxidative azo-coupling in native *N. nambi* globules with different levels of luminescence: *1* – bright, *2* – dim.

A. borealis globules reached maximum intensity after 1 day of incubation in water, and after that a relatively fast decline of the emission level was observed. The maximal luminescence of the *N. nambi* was observed slightly later (after 2–4 d of incubation in deionized water), and the luminescence level decreased gradually after that.

Investigation of peroxidase activity of native globules. The known reaction of oxidative azo-coupling phenol – 4-AA [32], which is catalyzed by peroxidase in the presence of H_2O_2 and proceeds with the formation of colored product easily detected by spectral methods, was used to evaluate peroxidase activity. The reaction is widely used in medical diagnostics for enzymatic determination of physiologically important compounds (glucose, cholesterol, triglycerides). The use of this reaction in this study was based on two main issues. Firstly, as was mentioned before, the higher fungi contain an extended set of peroxidases, including the secreted enzymes of this group [20–26]. Second, it was shown experimentally that the ROS (H_2O_2 in particular) generated by the fungus during its metabolic development could participate in mechanisms of the fungal bioluminescence [11–14]. It can be expected, then, that the addition of only phenol and 4-AA to the globules (or their extracts) will be accompanied by formation of the colored product.

The rapid coloring (within 5–15 min) of fungal mycelium in pink was observed following the introduction of the brightly luminescent *N. nambi* globules (pre-incubated in deionized water) into the reaction mixture containing 4-AA and phenol. Moreover, globules with multiple long strands of hyphae on the surface were colored most intensively. It must be noted that the globules were colored uniformly, and there was no heterogeneity observed in the chromogen distribution similar to the distribution of emission along the globule surface. The colored product diffused into the solution gradually with time, and the globules became colorless. The *N. nambi* globules demonstrating dim emission (at the level of 10^6–10^7 quanta) produced only slight coloring in the similar experiments, which was accompanied by significantly lower accumulation of the chromogen in the reaction mixture (Fig. 3). The entire set of obtained data indicated that the brightly and dimly emitting globules of the *N. nambi* differed significantly in the amount of H_2O_2 and/or amount of extracellular peroxidases.

Investigation of the *N. nambi* mycelium by light microscopy did not reveal coloring of the intracellular content. The colored solution could be found only between the twisted hyphae (outside the cells). This pattern of the hyphae coloring and the gradual transfer of the chromogen into the reaction mixture indicated that the reaction of oxidative azo-coupling with the formation of the colored product occurred most probably in the space outside the hyphae, where the extracellular fungal peroxidases were located.

Similar studies of peroxidase activity were conducted with the *A. borealis* globules. It was found that the *A. borealis* globules that demonstrated bright luminescence without preincubation in deionized water did not form colored product on introduction into the reaction mixture of phenol and 4-AA. The very insignificant formation of chromogen was observed only after prolonged incubation (1 h or more) in the reaction mixture. The *A. borealis* globules preincubated in deionized water that demonstrated a three- to fourfold increase in the emission level did not form the colored product even after 1–2 h incubation in the reaction mixture.

The obtained data made it possible to propose two suggestions. On the one hand, the lack of the colored product could be related to the fact that the peroxidases of *A. borealis* had low catalytic activity when the mixture of phenol and 4-AA was used as substrates. Such a suggestion does not contradict the notions of the substrate specificity of enzymes. But it is more likely that there is an insufficient amount of extracellular peroxidases and/or of H_2O_2 in the fungus globules, and because of it the formation of the colored product does not occur.

Evaluation of total peroxidase and catalase activities in extracts of mycelium. Since a significant increase in the luminescence intensity of the *N. nambi* and *A. borealis* mycelia following incubation in deionized water was shown during the previous steps of our investigation, in this part of the study we investigated the time course of the level of the globule luminescence in comparison with changes in the total peroxidase and catalase activities in their extracts. As can be seen from the obtained data (Fig. 4), the changes in the total peroxidase activity of the *N. nambi* fungus were in close agreement with the changes in its light emission during the investigated time period of incubation of the globules in deionized water. The catalase activity increased more than one order of magnitude in the process within one day of incubation of the globules in water and remained at the same level for al

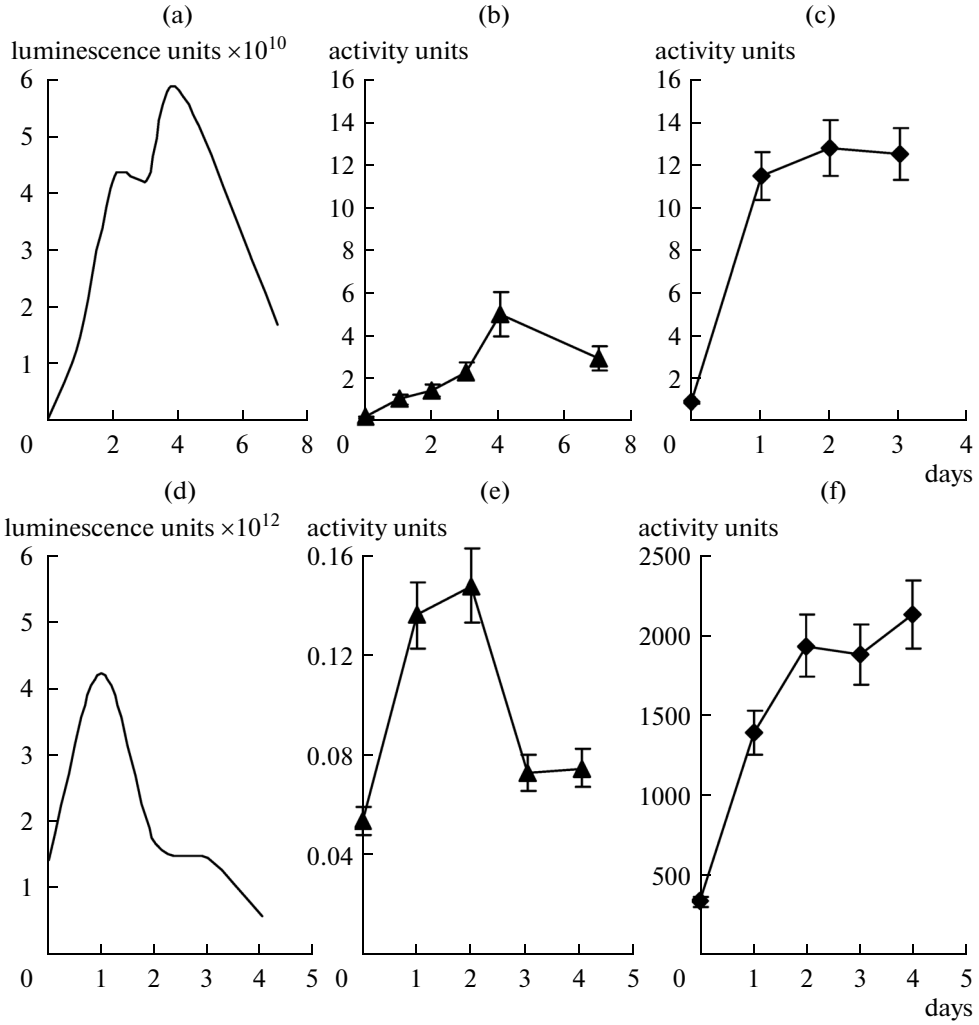

Fig. 4. Changes of luminescence (a, d), total peroxidase (b, e), and catalase (c, f) activities in extracts of *N. nambi* (a–c) and *A. borealis* (d–f) globules on incubation in water for several days. Enzyme activity in globule extracts was calculated per 1 mg of protein per 1 mL of extract.

least two more days. A similar dynamics of changes in the total peroxidase and catalase activities in comparison with the changes in the level of light emission was observed for the *A. borealis* mycelium (Fig. 4). The changes in the total peroxidase and catalase activities in the *A. borealis* globules occurred almost concurrently with the changes in its emission upon incubation in water. A slower dynamics of reaching the maximum level of catalase activity (within 2 days) was observed; it was maintained for a long period, as in the case of *N. nambi*. At the same time, the specific values of the peroxidase and catalase activities indicated significant differences in the amount of these enzymes in the investigated basidiomycetes. It follows from the data obtained for different time points of the experiment that the level of peroxidase activity in the *A. borealis* extracts was almost one and a half orders of magnitude lower than in the *N. nambi* extracts. On the contrary, the level of catalase activity in the *A. borealis* extracts was higher than a similar indicator for *N. nambi* by more than two orders of magnitude.

The analysis of the data obtained in the study makes it possible to suggest several general comments and to draw the following conclusions. It is possible that the differences in formation of the colored product during the reaction of oxidative azo-coupling with native globules of the fungal species is due to the peroxidases to catalases ratio in them. The high catalase activity in the *A. borealis* and, as a consequence, low H_2O_2 content was the reason for the total absence or formation or only minimal amount of the product in the globules of this species. Contrary to the *A. borealis* globules, the levels of total peroxidase and catalase activities in the *N. nambi* globules showed an approximately twofold difference (Fig. 4). It is likely at this ratio of enzymes that the H_2O_2 content even in the samples of the *N. nambi* mycelium not under the action of stress factors was sufficient for the reaction of oxidative azo-coupling to proceed.

It must be noted that the results of the conducted investigations do not provide sufficient grounds for making an unambiguous conclusion on the direct relation between the luminescence intensity of the *N. nambi* and *A. borealis* basidiomycetes and the activity of antioxidant enzymes—peroxidases and catalases. However, the fact that the changes in these parameters were unidirectional for different species of basidiomycetes support the hypothesis that the light emission of higher fungi originated and survived during evolution as a supplementary mechanism for neutralization of ROS (H_2O_2 in particular and, probably, other peroxide compounds). A similar hypothesis stating that luminescence could provide a protective function from damages occurring from active oxygen radicals was suggested in the middle of the last century [34, 35].

ACKNOWLEDGEMENTS

The authors are grateful to N. V. Psurtseva (curator of the collection of basidiomycetes of the Botanical Institute, Russian Academy of Science) for help with the species affiliation of the IBSO 2328 culture. This work was supported by the Program of Interdisciplinary Projects of the Siberian Branch of the Russian Academy of Sciences, project no. 71.

REFERENCES

1. Shimomura, O., *Bioluminescence: Chemical Principles and Methods*, Singapore: World Scientific Publishing, 2006.
2. Airth, R.L. and McElroy, W.D., *J. Bacteriol.*, 1959, vol. 77, no. 2, pp. 249–250.
3. Airth, R.L. and Foerster, G.E., *Arch. Biochem. Biophys.*, 1962, vol. 97, no. 3, pp. 567–573.
4. Airth, R.L. and Forester, G.E., *J. Bacteriol.*, 1964, vol. 88, no. 5, pp. 1372–1379.
5. Kamzolkina, O.V., Danilov, B.C., and Egorov, N.S., *Dokl. Akad. Nauk SSSR,* 1983, vol. 271, no. 3, pp. 750–752.
6. Kamzolkina, O.V., Bekker, Z.E., and Egorov, N.S., *Biol. Nauki*, 1984, no. 1, pp. 73–77.
7. Oliveira, A.G. and Stevani, C.V., *Photochem. Photobiol. Sci.*, 2009, vol. 8, no. 10, pp. 1416–1421.
8. Oliveira, A.G., Desjardin, D.E., Perry, B.A., and Stevani, C.V., *Photochem. Photobiol. Sci.*, 2012, vol. 11, no. 3, pp. 848–852.
9. Shimomura, O., *J. Exp. Bot.*, 1992, vol. 43, no. 256, pp. 1519–1525.
10. Shimomura, O., Satoh, S., and Kishi, Y., *J. Biolumin. Chemilumin.*, 1993, vol. 8, no. 4, pp. 201–205.
11. Bondar, V.S., Puzyr, A.P., Purtov, K.V., Medvedeva, S.E., Rodicheva, E.K., and Gitelson, I.I., *Dokl. Biochem. Biophys.*, 2011, vol. 438, pp. 138–140.
12. Bondar, V.S., Puzyr, A.P., Purtov, K.V., Medvedeva, S.E., Rodicheva, E.K., Kalacheva, G.S., and Gitelson, J.I., *Luminescence*, 2012a, vol. 2, no. 1, pp. 101–102.
13. Bondar, V.S., Shimomura, O., and Gitelson, J.I., *J. Siber. Fed. Univ., Ser. Biol.*, 2012b, vol. 5, no. 4, pp. 331–351.
14. Bondar, V.S., Puzyr, A.P., Purtov, K.V., Petunin, A.I., Burov, A.E., Rodicheva, E.K., Medvedeva, S.E., Shpak, B.A., Tyaglik, A.B., Shimomura, O., and Gitelson, I.I., *Dokl. Biochem. Biophys.*, 2014, vol. 455, pp. 56–58.
15. Vladimirov, Y.A., in *Free Radicals in the Environment, Medicine and Toxicology*, Nohl, H., Esterbauer, H., and Rice-Evans, C., Eds., London: Richelieu Press, 1994, pp. 345–373.
16. Bai, Z., Harvey, L.M., and McNeil, B., *Crit. Rev. Biotechnol.,* 2003, vol. 23, no. 4, pp. 267–302.
17. Gessler, N.N., Aver'yanov, A.A., and Belozerskaya, T.A., *Biochemistry* (Moscow), 2007, vol. 72, no. 10, pp. 1091–1109.
18. De Castro, C., Del Valle, P., Rua, J., Garsia-Armesto, M.R., Gutierrez-Larrainzar, M., Busto, F., and De Arriaga, D., *Fungal Biol.*, 2013, vol. 117, no. 4, pp. 275–287.
19. Conesa, A., Punt, P.J., and Hondel, C.A., *J. Biotechnol.*, 2002, vol. 93, no. 2, pp. 143–158.
20. Martinez, A.T., *Enzyme Microb. Technol.*, 2002, vol. 30, no. 4, pp. 425–444.
21. Gazaryan, I.G., Khushpul'yan, D.M., and Tishkov, V.I., *Usp. Biol. Khim.,* 2006, vol. 46, pp. 303–322.
22. Kersten, F. and Cullen, D., *Fungal Genet. Biol.*, 2007, vol. 44, no. 2, pp. 77–87.
23. Aizenshtadt, M.A. and Bogolitsyn, K.G., *Khim. Rastit. Syr'ya*, 2009, no. 2, pp. 5–18.
24. Hiscox, J., Baldrian, P., Rogers, H.J., and Boddy, L., *Fungal Genet. Biol.*, 2010, vol. 47, no. 6, pp. 562–571.
25. Hofrichter, M., Ullrich, R., Pecyna, M.J., Liers, C., and Lunde, T., *Appl. Microbiol. Biotechnol.*, 2010, vol. 87, no. 3, pp. 871–897.
26. Janusz, G., Kucharzykb, K.H., Pawlika, A., Staszczaka, M., and Paszczynski, A.J., *Enzyme Microb. Tech.*, 2013, vol. 52, no. 1, pp. 1–12.
27. Miroshnichenko, O.S., *Biopolim. Kletka*, 1992, vol. 8, no. 6, pp. 3–25.
28. Hansberg, W., Salas-Lizana, R., and Dominguez, L., *Arch. Biochem. Biophys.*, 2012, vol. 525, no. 2, pp. 170–180.
29. Medvedeva, S.E., Artemenko, K.S., Krivosheenko, A.A., Rusinova, A.G., Rodicheva, E.K., Puzyr, A.P., and Bondar, V.S., *Mycosphere*, 2014, vol. 5, no. 4, pp. 565–577.
30. Vydryakova, G.A., Van, D.T., Shoukouhi, P., Psurtseva, N.V., and Bissett, J., *Mycology*, 2011, vol. 3, no. 2, pp. 89–99.
31. Hastings, J.W. and Weber, G., *J. Opt. Soc. Am.*, 1963, vol. 53, no. 12, pp. 1410–1415.
32. Eryomin, A.N., Semashko, T.V., and Mikhailova, R.V., *Appl. Biochem. Microbiol.*, 2006, vol. 42, no. 4, pp. 399–408.
33. Kochetov, G.A., *Prakticheskoe rukovodstvo po enzimologii* (A Practical Course in Enzymology), Moscow: Vysshaya shkola, 1980.
34. McElroy, W.D. and Strehler, B.L., *Arch. Biochem.*, 1949, vol. 22, no. 3, p. 420.
35. McElroy, W.D. and Seliger, H.H., *Light and Life*, Mc, W.D. and Glass, B., Eds., Baltimore: Johns Hopkins Press, 1961.

Translated by L. Brovko