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Source of light emission in a luminous mycelium of the fungus *Panellus stipticus*

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ABSTRACT:

Mechanism of bioluminescence and light-emitting sources in higher fungi remain as an open question for a long time. We investigated the mycelium of cultivated luminous *Panellus stipticus* using confocal microscopy. No excitation light was imposed on the sample. Two types of sources of bioluminescence and their location were determined in the substrate mycelium. One were small 0.1-3 µm local formations disposed on the surface of hyphae, the other - relatively vast areas in bulk of the nutrient medium. No luminescence signal was recorded inside the hyphae. This may mean that the components of luminescent reaction are spatially separated within the cells, or the intracellular conditions block the reaction. The origin and formation of the light-emitting structures are discussed.

Keywords:
Bioluminescence, *Panellus stipticus*, luminous mycelium, confocal microscopy.

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INTRODUCTION

Bioluminescence in fungal cells, which involves the emission of light generated by a chemical reaction, has long attracted attention of scientists (Harvey, 1952; Shimomura, 2006; Desjardin *et al.*, 2008). Researchers studying bioluminescence of fungi focus their efforts on three key areas: (i) methods of cultivation under laboratory conditions and characteristics of the light emission (Weitz *et al*., 2001; Prasher *et al*., 2012; Dao, 2009; Mori *et al.*, 2011), (ii) the molecular organization of luminescence system and light emission mechanism (Shimomura, 2006; Airth and McElroy, 1959; Kamzolkina *et al*., 1983; Oliveira and Stevani, 2009; Bondar *et al.*, 2011), (iii) - application of fungal luminescence in analytical techniques (Weitz *et al*., 2002; Mendes and Stevani, 2010).

There has been little research conducted to determine sources of luminescent light in the fungal structures. To the best of our knowledge, only the mycelium of *Panus stipticus* and *Armillaria fusipes*, growing on agar were investigated for light source detection (Berliner and Hovnanian, 1963). The used photographic process allowed to record light from a single hypha.

However, a low resolution of the technique limited by the emulsion grain size denied localizing the source of light. The authors of this, obviously, pioneer work, suggested that the light was emitted over the entire cell. Given the size of the objects under study, such research should employ methods of microscopic investigations. Calleja and Reynolds, who studied *Panus stipticus* and *Armillaria mellea* by optical microscope with EMI 4-stage image intensifier tube, came to the conclusion that light emission in an individual hypha was limited to a segment removed from the apical point (Calleja and Reynolds, 1970). Absence of later works related to structural and morphological studies of mycelium of luminous fungi with microscopy is astonishing as all known microscopic methods are widely used to investigate non-luminous fungi (Riquelme and Bartnicki-Garcia, 2008; Roberson *et al.*, 2011; Steinberg and Schuster, 2011).

In this report the mycelium of luminous *Panellus stipticus* was studied using confocal microscopy to determine and localize the source of light emission. In our opinion it is important to find in luminous fungi structures (or formations), which are the light-emitting sources, and their location. On the one hand, this can provide additional knowledge about morphology of luminous fungi, on the other - might give insight into molecular-cellular organization of fungal luminescent system and mechanism of light emission.

MATERIALS AND METHODS

In this work we studied the culture of *Panellus stipticus* luminous fungus (Bull:Fr.) Karst., IBSO 2301 (Figure 1). The mycelium was grown in plastic Petri dishes at temperature 22°C on a commercial nutrient medium Potato Dextrose Agar (HiMedia Laboratories Pvt., India), or on richer medium containing in 1 liter: 10 g of glucose, 5 g of peptone, 3 g of yeast extract, 3 g of malt extract, 20 g of agar-agar. The specimens exhibiting the highest light intensity were selected for the experiments.

For confocal microscopy, a confocal laser scanning microscope (LSM-780 NLO, Carl Zeiss, Gottingen, Germany) equipped with a high sensitivity GaAsP was used. Bioluminescence was recorded in the accumulation mode with the 491–631 nm filter. The laser was turned off (laser power = 0.0%) so that no excitation light was imposed on the sample. This was done to avoid fungal autofluorescence - emission of light by biological substances such as flavins, lipofuscins and porphyrins when excited by ultraviolet, violet, or blue light (Zizka and Gabriel, 2008).

Images were processed using ZEN 2010 software (version 6.0; Carl Zeiss). To prepare a specimen for microscopy a fragment of agar with mycelium was cut

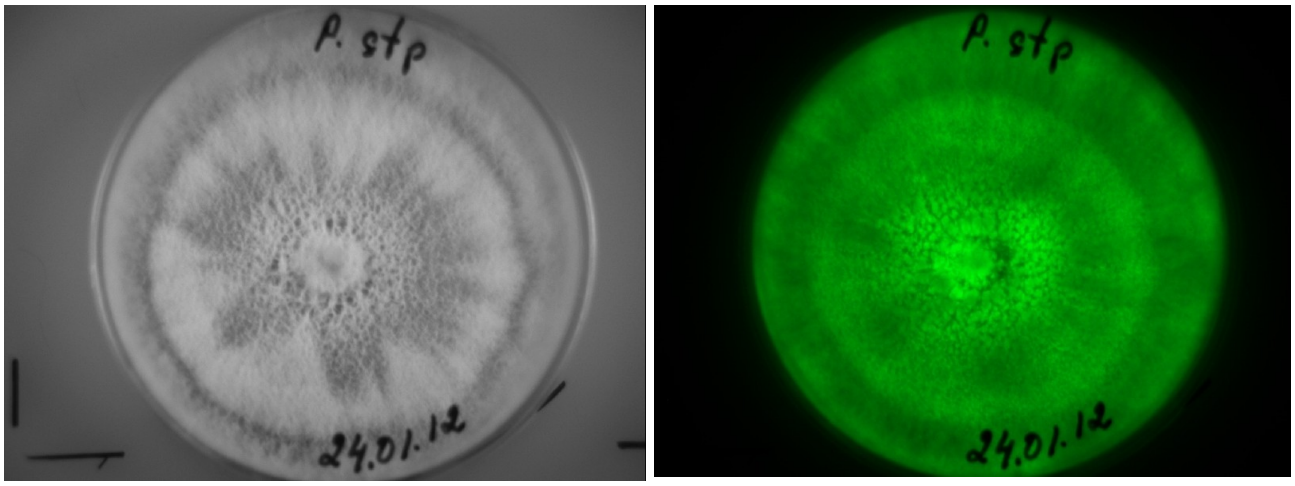

Figure 1 View of culture of *Panellus stipticus* (IBSO 2301) growing on agar in natural light (A) and in the dark (B).

out and transferred to the cover glass.

RESULTS AND DISCUSSION

Figure 2 shows a 3D projection of the mycelium by producing a Z-stack with 82 sections, 0.208 µm thick each. No bioluminescence was detected from the aerial mycelium. The light emission was recorded from the surface of specimen to a depth of ~ 16 µm with maximum intensity localized at the depth of Z= 6-8 µm where the main body of mycelium was located. Only isolated signals were detected at Z=8-16 µm that confirmed that the agar did not contribute to the observed bioluminescence.

Two types of sources emitting luminescent signals could be distinguished. One light source were small 0.1-3 µm local formations, associated with the substrate hyphae, the other – vast areas in bulk of agar (Figure 3). Light intensity recorded in the agar was much higher than that of the local sites in the area of hyphae. The use of the larger magnification (Figure 4) and bright field microscopy (Figure 5a) suggests that the local luminous sites are cellular excretions located on the hyphae surface while vast luminescent areas are formed by their aggregation in agar.

While presence of luminous sites on the surface of hyphae could be assumed, finding of luminescent areas in the agar came as a surprise. It is uncontroversial that the recorded bioluminescent signals result from the interaction of mixing light components synthesized by the fungal cells. Luminescent signals were recorded by the confocal microscope only when these components were outside the cells. No bioluminescence inside hyphae may mean that inside the cells the components of luminescent reaction are spatially separated and do not interact with each other, or the intracellular conditions (pH, oxygen concentration, presence of inhibitors, etc.) block the reaction.

One could argue that the surface of glowing structures should be either hydrophobic or they have a membrane enclosing the internal volume. Only under these conditions components necessary for the luminescent reaction do not mix with the water phase contained within the nutrient medium. This suggestion is based on the sharp boundaries exhibiting by both small local formations on the walls of hyphae and vast areas in the nutrient agar (Figure 5b).

So far it is not clear whether the luminous structures containing components necessary for the emission are formed within the fungal hypha or on/in their surface. In the first case it requires a transport system providing for the mechanism excreting the

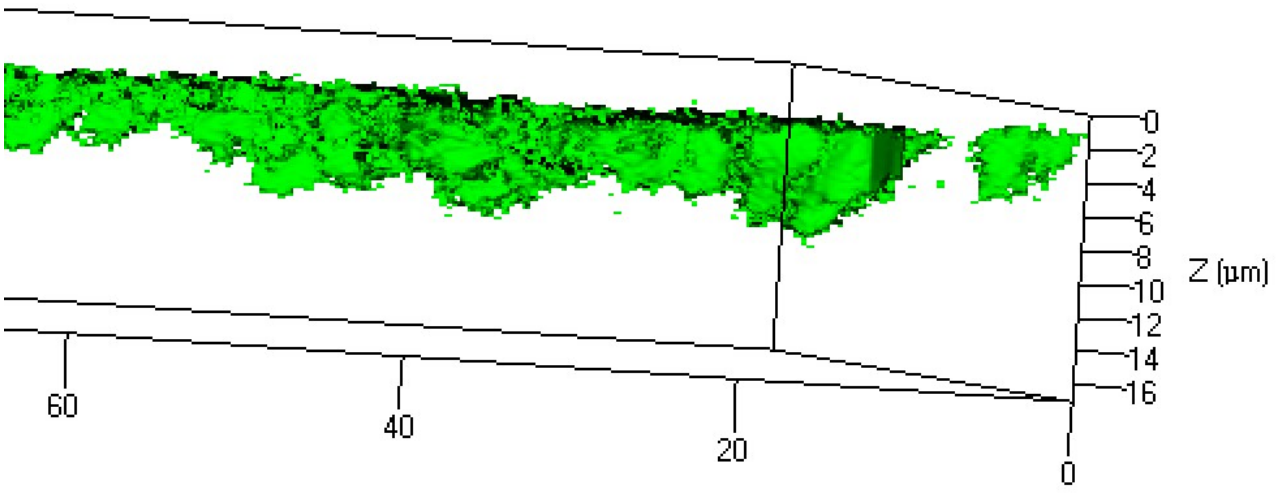

Figure 2 Fragment of 3D pattern of bioluminescence produced by *P. stipticus*.

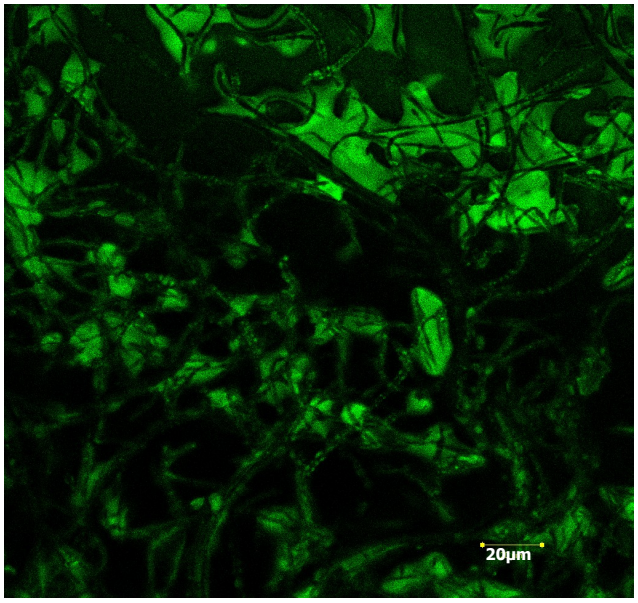

Figure 3 Confocal luminescence image of the *P. stipticus* mycelium.

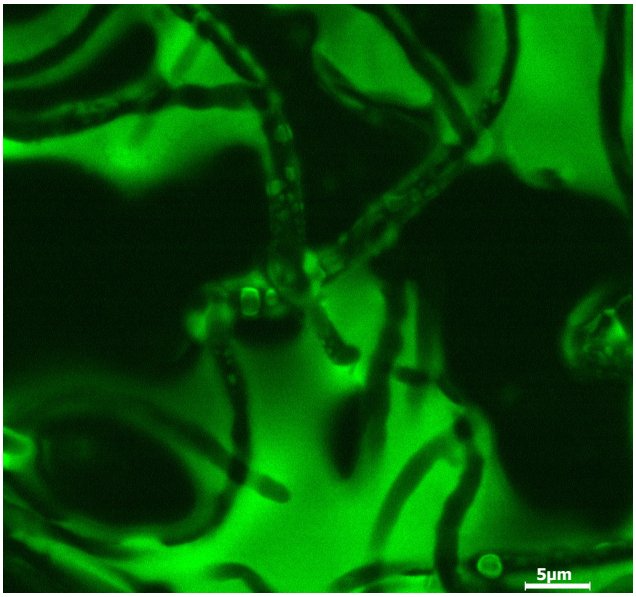

Figure 4 Confocal luminescence image of an individual hyphae.

forming structures outside the cell. This is plausible because the Golgi apparatus, that synthesizes secretory vesicles containing products of vital functions and excretes them from the cell, is well known. In the second case on/in the wall cell there should exist structural elements performing specialized secretory function.

On the basis of the results above we hypothesize the following. Cells of *P. stipticus* synthesize and localize the components required for bioluminescence in structures which can originate within the cell and then are moved on the outside surface of the hyphae by a mechanism analogous to the mechanism of transport via the Golgi complex. They can be also assumed to form directly on/in outside surface of the hyphae by structural elements of the cell possessing secretory function. Such enclosed structures make possible to concentrate the necessary components within a small volume. Separation of luminous structures from the surface of hyphae and their subsequent diffusion into the bulk of the nutrient medium produce the vast

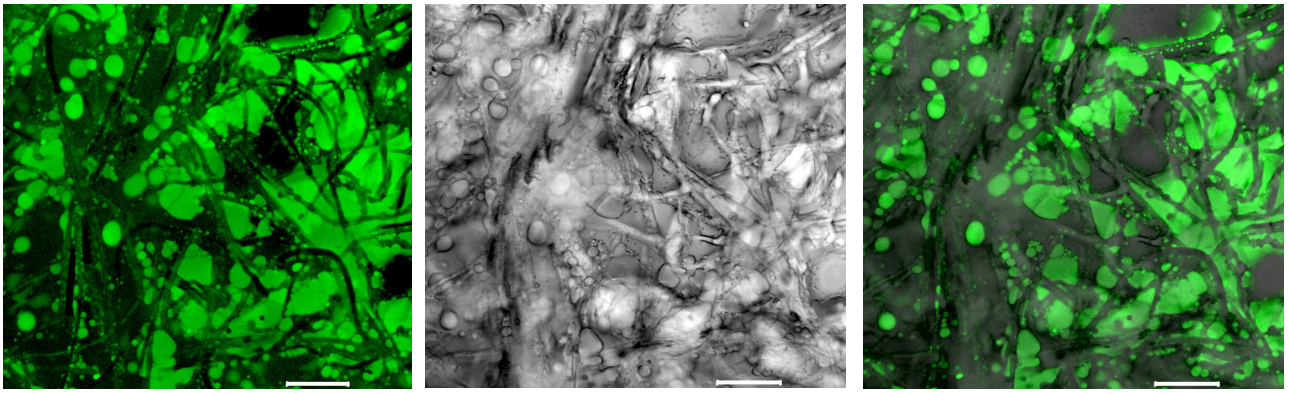

Figure 5 Confocal luminescence (A), bright field (B) and overlay (C) images of the substrate. Scale bar = 20 µm.

areas of luminescence in the agar.

CONCLUSION

Confocal microscopy due to its high resolution and ability to record low light signals offers new opportunities in investigation of fungal bioluminescence system. Using this technique the sources of light emission were identified for the first time in the mycelium of *P. stipticus* (IBSO 2301) cultivated on agar medium. One source were local formations disposed on the surface of the substrate hyphae, the other – vast areas in bulk of agar formed by aggregation of these luminous structures. Further study is required for a detail understanding whether the discovered structures are specific for this fungus or they are common among other luminous fungi.

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