Asian Journal of Mycology 3(1): 408–418 (2020) ISSN 2651-1339

www.asianjournalofmycology.org **Article**

Doi 10.5943/ajom/3/1/13

Production of extracellular oxidases in the mycelium of the bioluminescent *Neonothopanus nambi* (Omphalotaceae, Basidiomycota) grown in submerged culture in different media

Mogilnaya OA*, Ronzhin NO, Posokhina ED and Bondar VS

Institute of Biophysics, Siberian Branch of Russian Academy of Sciences, Federal Research Center Krasnoyarsk Science Center SB RAS, 660036 Krasnoyarsk, Russia

Mogilnaya OA, Ronzhin NO, Posokhina ED, Bondar VS 2020 – Production of extracellular oxidases in the mycelium of the bioluminescent *Neonothopanus nambi* (Omphalotaceae, Basidiomycota) grown in submerged culture in different media. Asian Journal of Mycology 3(1), 408–418, Doi 10.5943/ajom/3/1/13

Abstract

Comparative investigations have been conducted to study extracellular peroxidase and alcohol oxidase activities of *Neonothopanus nambi* grown in submerged culture of different nutrient media. The activities of the enzymes were determined both in the pellets and in the enzyme concentrates. For determining the activity in pellets, mycelial pellets were removed before measuring the nutrient media. The enzyme concentrates were produced by treating the pellets with β-glucosidase. The composition of the nutrient medium affects the biosynthesis rate of fungal extracellular enzymes and the effectiveness of their secretion to the external medium. The most effective release of extracellular oxidases to the external medium was observed in the experiment with the fungus cultivated in the medium based on malt extract (ME). Treatment of *N. nambi* mycelial biomass with β-glucosidase can be recommended as a technique for extracting pool of extracellular enzymes (including oxidases) from basidiomycetes. This technique can be employed to derive extracts rich in extracellular fungal enzymes and containing low amounts of ballast impurities from basidiomycetes, using a relatively mild treatment, which does not involve the destruction of the biomass.

Key words – β-glucosidase – fungi – mycelial pellets – nutrient media

Introduction

Basidiomycetes have a huge biotechnological potential for manufacturing diverse, valuable target products required by medicine, biology, pharmaceuticals, food and chemical industries and environmental monitoring. For example, the biomass of basidiomycetes (mycelium and fruiting bodies) is used as a source for fibrous polymer compound synthesis (polysaccharides, chitin) (Haneef et al. 2017) compounds with pharmacological activity (such as antioxidant and antitumor compounds) (Lim et al. 2014, Li et al. 2015, Prasad et al. 2015, Wu & Xu 2015, Greeshma et al. 2016, Kalaras et al. 2017, Lin et al. 2017), and enzymes and enzyme systems catalyzing lignin and cellulose degradation (Kersten & Cullen 2007, Goswami et al. 2013, Dubey et al. 2017, Martínez et al. 2017, Sützl et al. 2018).

Scientists are particularly interested in the extracellular oxidases of basidiomycetes such as heme- and FAD-containing ones, as they can be used in analytical applications. These enzymes are

Submitted 17 July 2020, Accepted 9 September 2020, Published 2 October 2020
Corresponding Author: Olga A. Mogilnaya – e-mail – ol_mog@mail.ru

promising peptide biomarkers for constructing effective indication and diagnostic tools, such as multi-enzyme indicators and diagnostic test systems. For example, glucose oxidase, pyranose oxidase, and peroxidases of basidiomycetes are successfully used to construct various analytical systems (Giffhorn 2000, Wong et al. 2008, Wongnate & Chaiyen 2013, Knop et al. 2015, Ji et al. 2016, Farrugia et al. 2017). Extracellular FAD-containing aryl-alcohol oxidase from basidiomycetes has good potential for biotechnological applications, owing to the broad substrate specificity of the enzyme and the simplicity of the reaction catalyzed by that (Hernández-Ortega et al. 2012, Goswami et al. 2013, Galperin et al. 2016, Tamaru et al. 2018).

Researchers continue their search for basidiomycetes that could be used as producers of the currently known oxidases and enzymes with novel properties (Romero et al. 2009, Couturier et al. 2016), and develop approaches for creating recombinant strains capable of producing extracellular oxidases of basidiomycetes (Wang et al. 2016, Vasina et al. 2017). At the same time, many studies are conducted to determine the possibility of increasing the enzyme production in fungal biomass (mycelium, fruiting bodies) at the stage of cultivation. Several studies showed that the carbon to nitrogen ratio in the nutrient medium used to cultivate basidiomycetes influenced the rate at which the fungi produced exopolysaccharide matrix and extracellular enzymes (Dosoretz & Grethlein 1991, Rosado et al. 2003, Fraga et al. 2014). Those data suggest that the composition of the nutrient medium is a major factor that affects the biosynthesis rate of extracellular enzymes by basidiomycetes.

The bioluminescent fungus *Neonothopanus nambi* is a model fungus used to study not only the fundamental aspects of bioluminescence in higher fungi but also their applicability in the biotechnological production of valuable target products, for example, for analytics. A fungal luminescent system has been isolated from this fungus for the first time, which provides high level of long-lasting luminescence and functions *in vitro* (Bondar et al. 2012, 2014). This opens up the possibilities of its application in bioanalytics. Extracellular oxidases of this fungus were used to create indicator systems applicable for glucose detection, and environmental monitoring of phenolic pollution in the aqueous medium (Mogilnaya et al. 2018a, 2019). The luminescence intensity of *N. nambi* correlates with the secondary metabolism related to enzymatic activities of lignin destruction (Mogilnaya et al. 2016). Therefore, optimizing cultivation conditions for *N. nambi* is of primary significance. Many studies aimed at investigating fungal bioluminescence and the production of mushroom biomass with steady glow and high concentration of extracellular enzymes.

In the present study, we investigated the production of extracellular oxidases in the mycelium of the *Neonothopanus nambi* grown in submerged culture using different nutrient media.

Materials & Methods

The bioluminescent fungus *Neonothopanus nambi* IBSO 3293 is available in the Collection of Microorganisms CCIBSO 836 of the Institute of Biophysics, Siberian Branch of Russian Academy of Sciences, Federal Research Center "Krasnoyarsk Science Center SB RAS".

Liquid nutrient media of different compositions that were used for the cultivation of *N. nambi* mycelium include malt extract broth (ME; dextrose 2 g/L, malt extract 20 g/L, mycological peptone 1 g/L), yeast extract-malt extract broth (YM; yeast extract 3 g/L, malt extract 3 g/L, dextrose 10 g/L, mycological peptone 5 g/L), potato dextrose broth (PD; potato extract 4 g/L, glucose 20 g/L), sabouraud broth (S; dextrose 40 g/L, mycological peptone 10 g/L), and potato sucrose medium (PS; freshly cooked potatoes broth 200 g/L, sucrose 20 g/L). All the prepared liquid media were autoclaved at 120°C for 15 min before use.

Active mycelium grown in the petri dishes on potato sucrose agar for 8–10 days was scraped and used as an inoculum for submerged cultivation of the fungus. The volume of the inoculum was 2–5% of the broth volume. Sweet almond β-glucosidase (EC 3.2.1.21) (Serva, Germany) dissolved in 10 mM phosphate buffer (pH 6.0) was used for the enzymatic treatment of the envelope of the hyphal cells of the mycelium.

Fungal cultivation

The mycelia of *N. nambi* were cultivated in 300 ml conical flasks containing 100 ml nutrient medium and incubated at 27°C with constant agitation at 160–180 rpm using an Environmental Shaker-Incubator ES-20 (BIOSAN, Latvia). The process of cultivation was discontinued at day 7 or 8, as our previous study showed that it was the time when the fungus reached the stationary growth phase (Mogilnaya et al. 2018b) (Fig. 1). Pellets of *N. nambi* mycelium in different nutrient media were photographed using a PowerShot S50 camera (Canon, Japan).

Enzymatic treatment of fungal pellets

Extraction of extracellular oxidases from *N. nambi* mycelium was performed as follows (Fig. 1). The mycelial pellets were taken out of the nutrient medium and rinsed multiple times deionized (DI) water to remove residual nutrient media and metabolites. Deionized water was produced using a Milli-Q system (Millipore, USA). Extracellular enzymes (including oxidases) were extracted by treating the mycelium with β-glucosidase. After rinsing, the pellets were placed into DI water containing β-glucosidase at a concentration of 1 IU/ml and incubated at 25°C for 24 hours under continuous stirring at 80 rpm on an OS-10 shaker (BIOSAN, Latvia). Then, the liquid (DI water containing extracellular enzymes extracted from mycelium) was separated from the biomass by passing it through a paper filter. Then, the aqueous extract was subjected to ultrafiltration through a membrane with molecular weight cutoff of 30 kDa (EMD Millipore Amicon, Darmstadt, Germany) in order to concentrate the extracted enzymes and remove low-molecular-weight compounds. The DI water in the sample was replaced three times to remove low-molecular-weight compounds more effectively. The retentate (concentrate of enzymes) was the experimental material.

Measurements of activities of extracellular oxidases

Extracellular oxidase activities were measured in native mycelial pellets grown in different nutrient media, in nutrient media after removal of the pellets, and in enzyme concentrates produced by treating the pellets with β-glucosidase followed by ultrafiltration (Fig. 1). To determine the total activity of the oxidases catalyzing the oxidation of aromatic alcohols, veratryl alcohol was used as a substrate (Ferreira et al. 2005). Total peroxidase activity was determined using the well-known azo coupling reaction (co-oxidation of phenol with 4-aminoantipyrine), catalyzed by peroxidases in the presence of hydrogen peroxide.

Extracellular oxidase activity in native *N. nambi* mycelial pellets was measured as follows. The pellets were taken out of the nutrient medium and rinsed with DI water to remove residual nutrient medium and metabolites. Then, the pellets were used in the test. To determine peroxidase activity, the pellets were placed in 1 ml of DI water containing 5.96 mM phenol (Fluka, Germany), 0.49 mM 4-aminoantipyrine (4-AAP) (Reachim, Russia), and 8 mM H_2O_2 (JSC Pharmaceutical Factory of St. Petersburg, Russia). The samples were incubated for 1 h at 25°C; then the colored solutions were collected, and chromogen (quinoneimine) was measured in a UV-1800 spectrophotometer (Shimadzu, Japan) at a wavelength of 506 nm. To determine alcohol oxidase activity, the pellets were placed in 1 ml of DI water containing 10 mM veratryl alcohol (Sigma, USA) and incubated under conditions described above. The amount of the product (veratryl aldehyde) was determined spectrophotometrically at a wavelength of 310 nm.

In the peroxidase activity test in nutrient media and enzyme concentrates, the reaction mixture (600 µl) contained 5.96 mM phenol, 0.49 mM 4-AAP, 8 mM H_2O_2, and 100 µl of the nutrient medium or enzyme concentrate. In the alcohol oxidase activity test, the reaction mixture (600 µl) contained 10 mM veratryl alcohol and 50 µl of the sample. In both cases, after adding all ingredients, the samples were mixed for 3 sec using a Vortex-Genie 2 g-560E mixer (Scientific Industries, Inc., USA) and incubated for 30 min at 25°C. The yields of reaction products were determined by the spectral method as described above. Each experiment was performed in triplicate.

Enzyme activities were expressed in units of optical density per 1 ml of reaction mixture when measuring native pellets, nutrient media or per 1 mg protein in enzyme concentrates. Protein

concentration in the enzyme concentrates was determined by the biuret method using Benedict's reagent and BSA as a standard (Kochetov 1980).

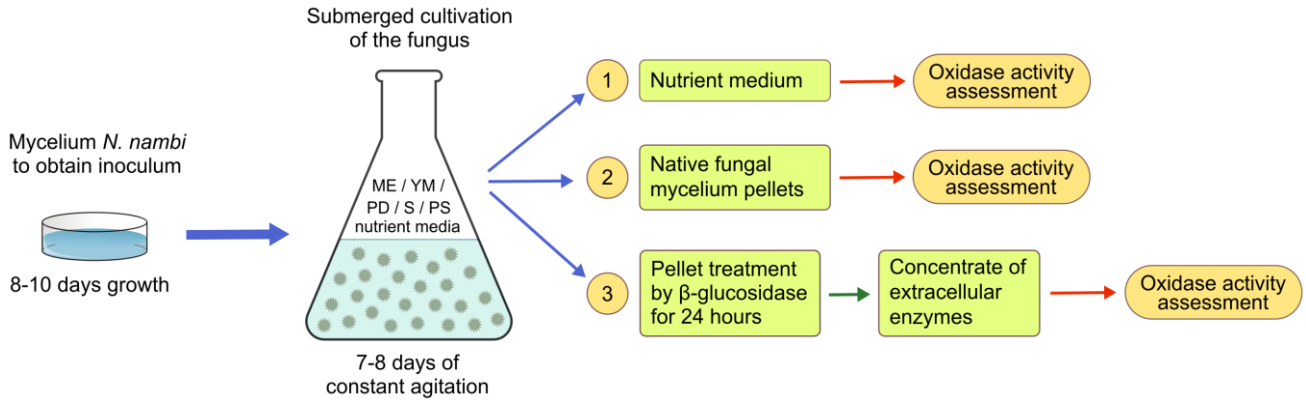

Fig 1 – A schematic representation of the experimental design. Cultivation of the *Neonothopanus nambi* fungal mycelium in different liquid nutrient media and oxidase activity assessment of localized, released, and extracted extracellular enzymes of the fungus.

Results

Morphology of *Neonothopanus nambi* mycelial pellets formed during submerged cultivation of the fungus in different nutrient media

Our research showed that submerged cultivation of the *N. nambi* under continuous orbital stirring produced spherical mycelial pellets of 2–7 mm in diameter with the rough surface created by numerous bundles of surface structures – hyphae. Some of the individual hyphae and bundles of hyphae growing on the surface of the mycelial pellets of this fungus could extend for several millimeters. In the present study, we compared morphological properties of *N. nambi* mycelial pellets (color and size, abundance and length of individual hyphae and hyphal bundles on the surface of the pellets), and found that they varied considerably depending on the composition of the nutrient medium used to cultivate the fungus (Fig. 2). Formation of large pellets with numerous hyphal bundles was observed in the media containing malt extract (ME medium) and starch (PS and PD media) (Fig. 2A–C). At the same time, cultivation of the fungus in the S nutrient medium, which contained mycological peptone, resulted in the formation of a considerable number of small-sized pellets with a practically smooth surface, some of which were not spherical (Fig. 2D). That biomass also contained some large pellets with few short hyphal bundles on their surfaces. Finally, the biomass grown in the YM medium which contained yeast extract were both small- and medium-sized pellets with smooth, almost hyphae-free surface, and larger-sized pellets with short thick hyphal bundles on their surface (Fig. 2E). The hyphae or hyphal bundles growing on the smooth surface of *N. nambi* mycelial pellets formed in the S and YM nutrient media were extremely short, less than 200 µm.

The levels of extracellular oxidase activities in *Neonothopanus nambi* native mycelial pellets and post-cultivation nutrient media

High levels of extracellular peroxidase activity were observed in *N. nambi* native pellets grown in the YM, PD, and PS nutrient media (Fig. 3A). The native mycelial pellets grown in the S and ME media showed considerably lower levels of peroxidase activity. We also revealed peroxidase activity in all nutrient media after cultivation (Fig. 3B). The ME nutrient medium exhibited the highest level of peroxidase activity while in the YM and S nutrient media, the expression lowered by one-third. The levels of peroxidase activity observed in the PD and PS nutrient media were the lowest. It is about 40–45% of the expression level in the ME nutrient medium.

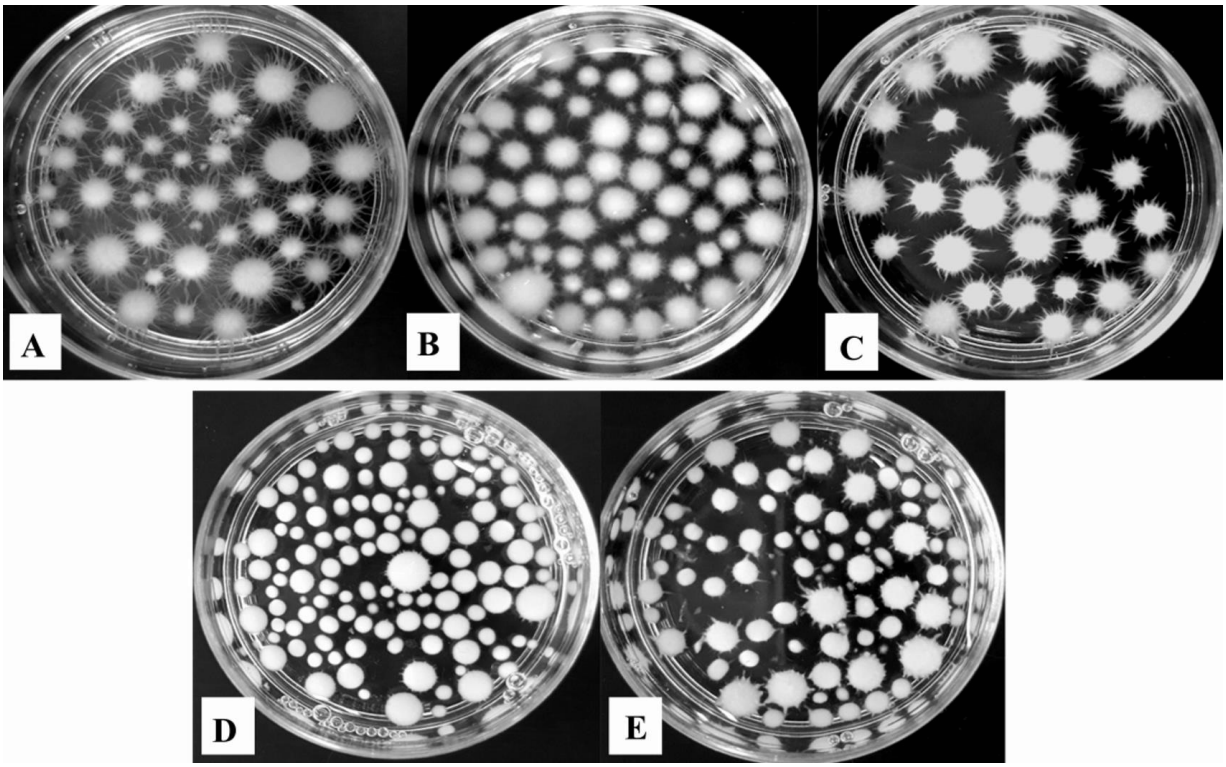

Fig 2 – Morphology of mycelial pellets of *Neonothopanus nambi* produced in submerged culture of the fungus in different nutrient media. A – PD. B – PS. C – ME. D – S. E – YM.

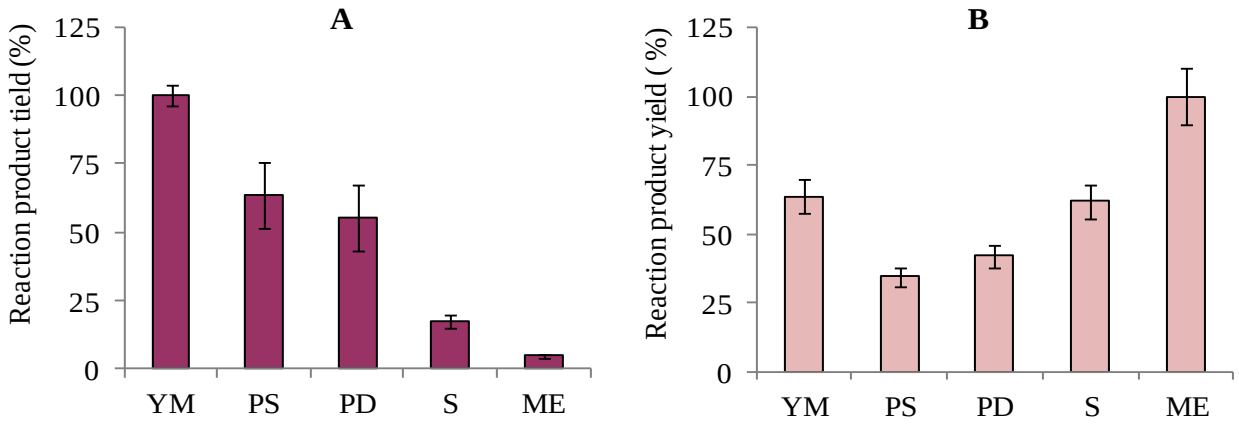

Fig 3 – The yield of the product of oxidative azo coupling reaction, representing the levels of peroxidase activity in *Neonothopanus nambi.* A The yield of the product in native mycelial pellets. B The yield of the product in the nutrient media after cultivation of the fungus. The data are normed on the maximal product yields in measurement series – 0.218 ± 0.04 a.u./ml and 0.046 ± 0.005 a.u./ml.

Comparable levels of alcohol oxidase activity were measured in *N. nambi* native pellets grown in different nutrient media (Fig. 4A) with the exception of the pellets grown in the ME medium, which showed a 20–25% lowered alcohol oxidase activity. We also revealed alcohol oxidase activity in all nutrient media after cultivation (Fig. 4B). The distribution of the levels of alcohol oxidase activity in the nutrient media was generally the same as the distribution of the levels of peroxidase activity (Fig. 3B). The highest levels of alcohol oxidase activity were measured in the YM and ME media, intermediate levels – in the PD and S media, and the lowest level – in the PS medium.

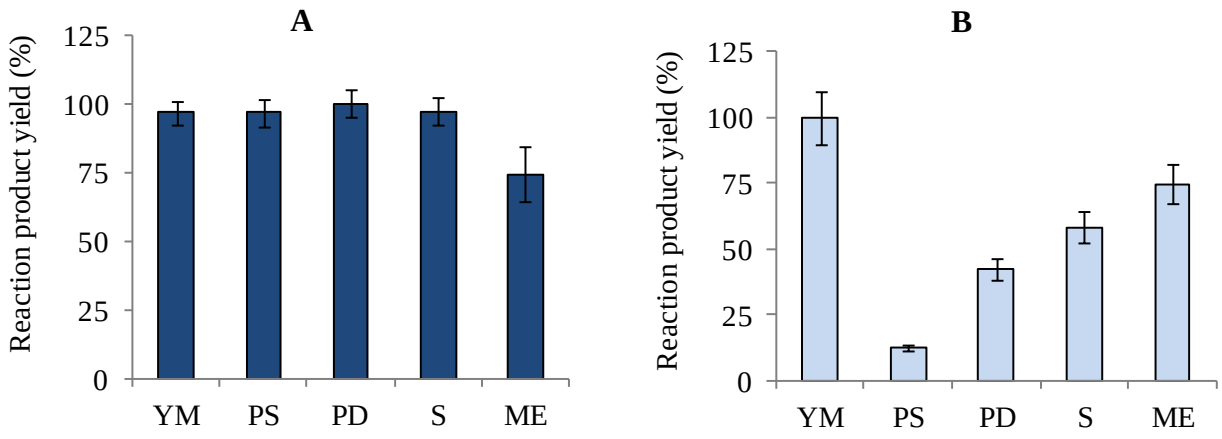

Fig 4 – The yield of the product of veratryl alcohol oxidation reaction, representing the levels of alcohol oxidase activity in *Neonothopanus nambi*. A The yield of the product in native mycelial pellets. B The yield of the product in the nutrient media after cultivation of the fungus. The data are normed on the maximal product yields in measurement series – 0.3 ± 0.05 a.u./ml and 1.39 ± 0.13 a.u./ml.

The levels of extracellular oxidase activities in enzyme concentrates from *Neonothopanus nambi* mycelial pellets treated with β-glucosidase

Experiments showed that all concentrates of extracellular enzymes from *N. nambi* mycelial pellets treated with β-glucosidase exhibited peroxidase and alcohol oxidase activities (Fig. 5). However, the levels of extracellular oxidase activity in the enzyme concentrates varied considerably depending on the nutrient medium used to cultivate the fungus. High levels of peroxidase activity were measured in the enzyme concentrates from the pellets grown in the PD, S, and ME media (Fig. 5A). In the concentrates extracted from the pellets grown in the YM and PS media, the levels of peroxidase activity were significantly lower. The highest level of alcohol oxidase activity was found in the enzyme concentrates extracted from the pellets grown in the ME medium. In the enzyme concentrates extracted from the pellets grown in the PS, PD, and S media, the level of alcohol oxidase activity was reduced by a factor of almost 2 (Fig. 5B). The concentrates extracted from the pellets grown in the YM medium showed the lowest level of peroxidase activity (Fig. 5A) and the lowest alcohol oxidase activity (Fig. 5B).

Discussion

In the present study, we investigated the level of production of extracellular enzymes in the mycelia of *Neonothopanus nambi* based on the composition of the nutrient medium used for submerged cultivation of the fungus. Our research was primarily focused on determining the activity levels of extracellular oxidases of this fungus, total peroxidase activity and alcohol oxidase activity. The choice of this line of research was based on the following facts. First, there is literature suggesting that basidiomycetes are promising sources for producing various enzymes (Goswami et al. 2013, Dubey et al. 2017, Martínez et al. 2017, Sützl et al. 2018). Moreover, extracellular oxidases of basidiomycetes are widely used to create effective tools for indication and diagnostics for analytical applications (Giffhorn 2000, Wong et al. 2008, Wongnate & Chaiyen 2013, Knop et al. 2015, Ji et al. 2016, Farrugia et al. 2017, Li et al. 2018, Mogilnaya et al. 2018a, 2019).

The use of basidiomycetes as sources of enzymes should be based on the research on the rates of enzyme production in fungal biomass and the ways to increase their production during the cultivation of fungi. The effectiveness of the biosynthesis of extracellular enzymes in the mycelium of basidiomycetes can be affected by the composition of the nutrient medium and cultivation

conditions. There is literature showing that the composition of the nutrient medium and conditions for submerged cultivation of mycelia (agitation rate, temperature, aeration with oxygen, etc.) influence biomass concentration, productivity, and the composition of exopolysaccharide matrix in the mycelium (Hsieh et al. 2006, Fraga et al. 2014). Exopolysaccharide matrix is important for vital functions of basidiomycetes, as it contains extracellular fungal enzymes. The polysaccharide slime that covers the outside surface of the cell wall of the hyphae has pores and consists of β-D-glucans, forming a gel-like network in the outer hyphal sheath and intracellular space. The gel-like network contains extracellular enzymes and retains water needed for their function (Daniel et al. 1989, Ruel & Joseleau 1991, Fesel & Zuccaro 2016, Mogilnaya et al. 2018b).

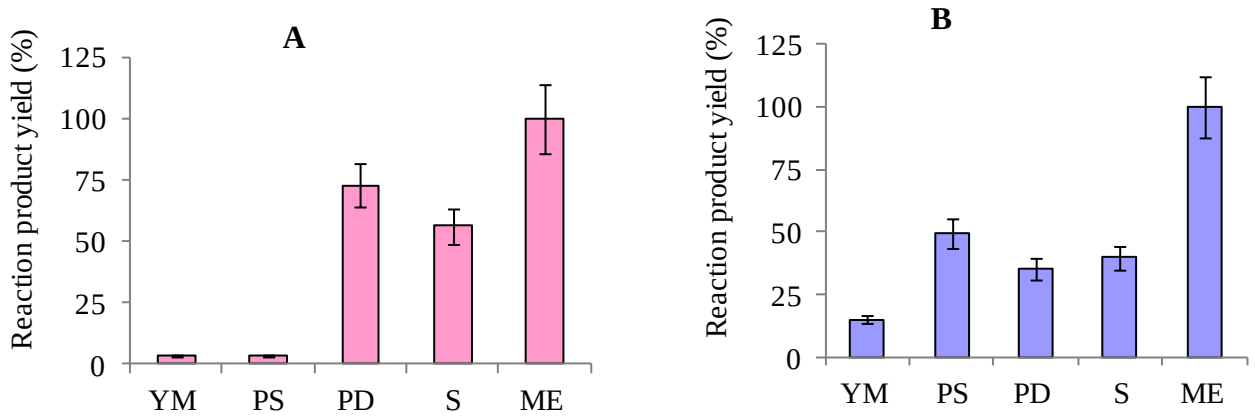

Fig 5 – The levels of activities of extracellular oxidases in the enzyme concentrates of *Neonothopanus nambi* mycelium. A The yield of the product of oxidative azo coupling reaction. B The yield of the product of veratryl alcohol oxidation reaction. Enzyme concentrates were produced by treating the pellets with β-glucosidase in DI water followed by ultrafiltration of the aqueous extracts. The data are normed on the maximal product yields in measurement series – 0.418 ± 0.06 a.u./mg and 1.51 ± 0.18 a.u./mg.

The present study shows that the composition of the nutrient medium used for submerged cultivation of *N. nambi* affects the morphological properties of the mycelial pellets, pellet density and size, abundance and length of individual hyphae and hyphal bundles on the surface of the pellets (Fig. 2). Moreover, results obtained in this study demonstrate that the rate of biosynthesis of extracellular oxidases in mycelium is also influenced by the composition of the nutrient medium used for cultivation. We revealed high levels of extracellular peroxidase activity in the native mycelial pellets produced in the YM, PD, and PS culture media (Fig. 3A). At the same time, the levels of peroxidase activity in the pellets grown in the S and ME nutrient media were considerably (5–10 times) lower. However, we did not find any significant differences in the level of extracellular alcohol oxidase activity between *N. nambi* native pellets grown in different nutrient media (Fig. 4A). Only the pellets grown in the ME medium exhibited a 20–25% lower level of extracellular alcohol oxidase activity.

In our opinion, results obtained in the present study may be of practical value and can be used in biotechnological processes of the production of extracellular oxidases (such as peroxidases and alcohol oxidases) from mycelium of *N. nambi*. The present study demonstrated, for instance, that the YM, PD, and PS nutrient media should be used for submerged cultivation of the fungus to produce mycelium biomass high in extracellular oxidases.

The present study showed that all nutrient media that had been used to grow *N. nambi* mycelium also contained peroxidases and alcohol oxidase after cultivation (Figs. 3B,4B). The levels of peroxidase and alcohol oxidase activities were high in the YM, ME, and S media, while in the nutrient media containing potato starch (PD and PS), they were considerably lower. Comparison of the activities of the enzymes in the nutrient media and the *N. nambi* native mycelial

pellets (Figs. 2,3) suggests the following assumption. The composition of the nutrient medium used to cultivate mycelium of *N. nambi* may determine not only the rate of biosynthesis of extracellular oxidases but also the production of exopolysaccharide matrix by the fungus and the structure of the matrix. This, in turn, may affect morphological properties of the gel-like network in the outer hyphal sheath and intracellular space and, hence, determine the rate of diffusion of the fungus extracellular enzymes to the external medium. This assumption appears valid, as it is consistent with the opinions expressed by other researchers (Dosoretz & Grethlein 1991, Rosado et al. 2003, Qi-he et al. 2011, Lueangjaroenkit et al. 2018). The authors of those studies suggested that the composition of the nutrient medium used to grow mycelial biomass could affect the production of extracellular enzymes by basidiomycetes and secretion of the enzymes to the external medium.

The present study demonstrated the peroxidase and alcohol oxidase activities of extracellular enzymes extracted from *N. nambi* mycelial pellets treated with β-glucosidase (Fig. 5). However, the activity levels of oxidases extracted from the mycelial biomass using this technique differed significantly depending on the composition of the nutrient medium which is used to grow the fungus. Rather high levels of peroxidase and alcohol oxidase activities were measured in the concentrates extracted from the *N. nambi* mycelial pellets grown in the PD and S media, and the highest yield was obtained from the pellets grown in the ME medium. These data may be expected to be useful for the biotechnological processes employed in the production of fungal enzymes from mycelium of *N. nambi*. Among other things, our data show that mycelial biomass of this fungus should be preferably grown in the PD, S, and ME media. Cultivation of the mycelial biomass in these media followed by treatment with β-glucosidase will ensure the production of formulations with the target extracellular enzymes. In our opinion, this method is useful for biotechnology, as extracts rich in extracellular fungal enzymes and containing low amounts of ballast impurities can be derived from basidiomycetes using a relatively mild treatment, which does not involve the complete destruction of the biomass.

Results obtained in the present study suggest the following conclusions. Experiments with the cultivation of *N. nambi* showed that the composition of the nutrient medium affected the rate of biosynthesis of extracellular enzymes (oxidases) by the fungus and the effectiveness of their secretion to the external medium. Thus, the production of extracellular enzymes can be increased in the stage of cultivation of basidiomycetes by choosing a proper culture medium. The detection of peroxidase and alcohol oxidase activities in the nutrient media after submerged cultivation of *N. nambi* mycelium offers possibilities for constructing basidiomycete-based bioreactors for producing extracellular fungal enzymes functional as oxidases. Treatment of *N. nambi* mycelial biomass with β-glucosidase can be recommended as a technique for extracting a pool of extracellular enzymes (including oxidases) from the basidiomycete. In addition, the availability of the nutrient media containing extracellular enzymes of basidiomycetes after cultivation of mycelium and extracts produced by treating the biomass with β-glucosidase and rich in these enzymes will contribute to enhancing the effectiveness of processes used to further purify these valuable target products for analytical applications.

References

Bondar VS, Shimomura O, Gitelson JI. 2012 – Luminescence of higher mushrooms. Journal of Siberian Federal University. Biology 4, 331–351.

Bondar VS, Puzyr AP, Purtov KV, Petunin AI, Burov AE, Rodicheva EK, Medvedeva SE, Shpak BA, Tyaglik AB, Shimomura O, Gitelson JI. 2014 – Isolation of luminescence system from the luminescent fungus *Neonothopanus nambi*. Doklady Biochemistry and Biophysics 455, 56–58.

Couturier M, Mathieu Y, Li A, Navarro D, Drula E, Haon M, Grisel S, Ludwig R, Berrin JG. 2016 – Characterization of a new aryl-alcohol oxidase secreted by the phytopathogenic fungus *Ustilago maydis.* Applied Microbiology and Biotechnology 100, 697–706.

Daniel G, Nilsson T, Pettersson B. 1989 – Intra- and extracellular localization of lignin peroxidase during the degradation of solid wood and wood fragments by *Phanerochaete chrysosporium* by using transmission electron microscopy and immuno-gold labeling. Applied and Environmental Microbiology 55, 871–881.

Dosoretz CG, Grethlein HE. 1991 – Physiological aspects of the regulation of extracellular enzymes of *Phanerochaete chrysosporium.* Applied Biochemistry and Biotechnology 28–29, 253–265.

Dubey MK, Zehra A, Aamir M, Meena M, Ahirwal L, Singh S, Shukla S, Upadhyay RS, Bueno-Mari R, Bajpai VK. 2017 – Improvement strategies, cost effective production, and potential applications of fungal glucose oxidase (GOD): current updates. Frontiers in Microbiology 8, 1032.

Farrugia T, Perriman AW, Sharma KP, Mann S. 2017 – Multi-enzyme cascade reactions using protein-polymer surfactant self-standing films. Chemical Communications 53, 2094–2097.

Ferreira P, Medina M, Guillen F, Martinez MJ, van Berkel WJH, Martinez AT. 2005 – Spectral and catalytic properties of aryl-alcohol oxidase, a fungal flavoenzyme acting on polyunsaturated alcohols. The Biochemistry Journal 389, 731-738.

Fesel PH, Zuccaro A. 2016 – β-glucan: Crucial component of the fungal cell wall and elusive MAMP in plants. Fungal Genetics and Biology 90, 53–60.

Fraga I, Coutinho J, Bezerra RM, Dias AA, Marques G, Nunes FM. 2014 – Influence of culture medium growth variables on *Ganoderma lucidum* exopolysaccharides structural features. Carbohydrate Polymers 111, 936–946.

Galperin I, Javeed A, Luig H, Lochnit G, Rühl M. 2016 – An aryl-alcohol oxidase of *Pleurotus sapidus*: heterologous expression, characterization, and application in a 2-enzyme system. Applied Microbiology and Biotechnology 100, 8021–8030.

Giffhorn F. 2000 – Fungal pyranose oxidases: occurrence, properties and biotechnical applications in carbohydrate chemistry. Applied Microbiology and Biotechnology 54, 727–740.

Goswami P, Chinnadayyala SS, Chakraborty M, Kumar AK, Kakoti A. 2013 – An overview on alcohol oxidases and their potential applications. Applied Microbiology and Biotechnology 97, 4259–4275.

Greeshma P, Ravikumar KS, Neethu MN, Pandey M, Zuhara KF, Janardhanan KK. 2016 – Antioxidant, anti-inflammatory, and antitumor activities of cultured mycelia and fruiting bodies of the Elm Oyster mushroom, *Hypsizygus ulmarius* (Agaricomycetes). International Journal of Medicinal Mushrooms 18, 235–244.

Haneef M, Ceseracciu L, Canale C, Bayer IS, Heredia-Guerrero JA, Athanassiou A. 2017 – Advanced materials from fungal mycelium: fabrication and tuning of physical properties. Scientific Reports 7, 41292.

Hernández-Ortega A, Ferreira P, Martínez AT. 2012 – Fungal aryl-alcohol oxidase: a peroxide-producing flavoenzyme involved in lignin degradation. Applied Microbiology and Biotechnology 93, 1395–1410.

Hsieh C, Tseng MH, Liu CJ. 2006 – Production of polysaccharides from *Ganoderma lucidum* (CCRC 36041) under limitations of nutrients. Enzyme and Microbial Technology 38, 109–117.

Ji Q, Wang B, Tan J, Zhu L, Li L. 2016 – Immobilized multienzymatic systems for catalysis of cascade reactions. Process Biochemistry 51, 1193–1203.

Kalaras MD, Richie JP, Calcagnotto A, Beelman RB. 2017 – Mushrooms: a rich source of the antioxidants ergothioneine and glutathione. Food Chemistry 233, 429–433.

Kersten F, Cullen D. 2007 – Extracellular oxidative systems of the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Fungal Genetics and Biology 44, 77–87.

Knop D, Yarden O, Hadar Y. 2015 – The ligninolytic peroxidases in the genus *Pleurotus*: divergence in activities, expression, and potential applications. Applied Microbiology and Biotechnology 99, 1025–1038.

Kochetov GA. 1980. – Practical guide to enzymology (in Russian). Moscow, Russia: Higher School.

Li F, Ma W, Wu X, Wang Y, He J. 2018 – Luminol, horeseradish peroxidase, and glucose oxidase ternary functionalized graphene oxide for ultrasensitive glucose sensing. Analytical and Bioanalytical Chemistry 410, 543–552.

Li N, Zhao L, Ng TB, Wong JH, Yan Y, Shi Z, Liu F. 2015 – Separation and purification of the antioxidant compound hispidin from mushrooms by molecularly imprinted polymer. Applied Microbiology and Biotechnology 99, 7569–7577.

Lim JH, Lee YM, Park SR, Kim DH, Lim BO. 2014 – Anticancer activity of hispidin via reactive oxygen species-mediated apoptosis in colon cancer cells. Anticancer Research 34, 4087–4093.

Lin WC, Deng JS, Huang SS, Wu SH, Lin HY, Huang GJ. 2017 – Evaluation of antioxidant, anti-inflammatory and anti-proliferative activities of ethanol extracts from different varieties of sanghuang species. RSC Advances 7, 7780–7788.

Lueangjaroenkit P, Teerapatsakul C, Chitradon L. 2018 – Morphological characteristic regulation of ligninolytic enzyme produced by *Trametes polyzona.* Mycobiology 46, 396–406.

Martínez AT, Ruiz-Dueñas FJ, Camarero S. 2017 – Oxidoreductases on their way to industrial biotransformations. Biotechnology Advances 35, 815–831.

Mogilnaya OA, Ronzhin NO, Bondar VS. 2016 – Comparative evaluation of total peroxidase and catalase activities during light emission of luminous fungus *Neonothopanus nambi.* Mycosphere 7, 499–510.

Mogilnaya OA, Ronzhin NO, Artemenko KS, Bondar VS. 2018a – Creation of bifunctional indicating complex based on nanodiamonds and extracellular oxidases of luminous fungus *Neonothopanus nambi*. Doklady Biochemistry and Biophysics 480, 135–138.

Mogilnaya OA, Ronzhin NO, Bondar VS. 2018b – Extracellular peroxidase activity and light emission of the mycelium of the basidiomycete *Neonothopanus nambi* in the presence of β-glucosidase. Biophysics 63, 93–99.

Mogilnaya O, Ronzhin N, Artemenko K, Bondar VS. 2019 – Nanodiamonds as an effective adsorbent for immobilization of extracellular peroxidases from luminous fungus *Neonothopanus nambi* to construct a phenol detection system. Biocatalysis and Biotransformation 37, 97–105.

Prasad S, Rathore H, Sharma S, Yadav AS. 2015 – Medicinal mushrooms as a source of novel functional food. The International Journal of Nutrition and Dietetics 4, 221–225.

Qi-he C, Krügener S, Hirth T, Rupp S, Zibek S. 2011 − Co-cultured production of lignin-modifying enzymes with white-rot fungi. Applied Biochemistry and Biotechnology 165, 700–718.

Romero E, Ferreira P, Martínez Á, Martínez M. 2009 – New oxidase from *Bjerkandera* arthroconidial anamorph that oxidizes both phenolic and nonphenolic benzyl alcohols. Biochimica et Biophysica Acta 1794, 689–697.

Rosado FR, Germano S, Carbonero ER, Da Costa SM, Iacomini M, Kemmelmeier C. 2003 – Biomass and exopolysaccharide production in submerged cultures of *Pleurotus ostreatoroseus* SING. and *Pleurotus ostreatus* "florida" (Jack.: Fr.) Kummer. Journal of Basic Microbiology 43, 230–237.

Ruel K, Joseleau JP. 1991 – Involvement of an extracellular glucan sheath during degradation of populus wood by *Phanerochaete chrysosporium*. Applied and Environmental Microbiology 57, 374–384.

Sützl L, Laurent CVFP, Abrera AT, Schütz G, Ludwig R, Haltrich D. 2018 – Multiplicity of enzymatic functions in the CAZy AA3 family. Applied Microbiology and Biotechnology 102, 2477–2492.

Tamaru Y, Umezawa K, Yoshida M. 2018 – Characterization of an aryl-alcohol oxidase from the plant saprophytic basidiomycete *Coprinopsis cinerea* with broad substrate specificity against aromatic alcohols. Biotechnology Letters 40, 1077–1086.

Vasina DV, Moiseenko KV, Fedorova TV, Tyazhelova TV. 2017 – Lignin-degrading peroxidases in white-rot fungus *Trametes hirsute* 072. Absolute expression quantification of full multigene family. PLoS ONE 12(3): e0173813.

Wang N, Ren R, Jia R, Chen W, Sun R. 2016 – Expression of a fungal manganese peroxidase in *Escherichia coli*: a comparison between the soluble and refolded enzymes. BMC Biotechnology 16, 87.

Wong CM, Wong KH, Chen XD. 2008 – Glucose oxidase: natural occurrence, function, properties and industrial applications. Applied Microbiology and Biotechnology 78, 927–938.

Wongnate T, Chaiyen P. 2013 – The substrate oxidation mechanism of pyranose 2-oxidase and other related enzymes in the glucose–methanol–choline superfamily. FEBS Journal 280, 3009–3027.

Wu T, Xu B. 2015 – Antidiabetic and antioxidant activities of eight medicinal mushroom species from China. International Journal of Medicinal Mushrooms 17, 129−140.