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Physico-chemical characteristics of melanins culture medium *Inocutis dryophila* (Berk) Flasson & Niemela

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Abstract. *The purpose of the study was to investigate the effect of light treatment on the synthesis of endomelanins from the mycelium of Inocutis dryophila in a liquid culture medium. To identify the effect of light on the synthesis of melanins, the mycelium of Inocutis dryophila was cultivated in a liquid medium in the dark and using blue light treatment. As a result, two types of melanins IDM-1 and IDM-2 were obtained. It has been established that the light factor influences the quantitative release of melanins from the mycelium of Inocutis dryophila into the culture liquid. The use of blue light resulted in lower melanin content in the culture fluid than in the dark. Ultraviolet and infrared spectrometry showed that both types of melanins have typical spectra and graphs for fungal melanins. Infrared spectrometry showed that treatment of mycelium with blue light led to deformation of IDM-1. It was revealed that IDM-2 melanins exhibit greater antiradical activity than IDM-1 melanins. Thus, light treatment of Inocutis dryophila mycelium in a liquid medium affects the quantitative release of melanins into the culture liquid, promotes a change in the structure, as well as manifestation of a biological effect.*

Keywords: *Inocutis dryophila, culture medium, melanin, blue light, UV-, IR-spectrometry.*

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КРАТКИЕ СООБЩЕНИЯ

УДК 582.284

Физико-химическая характеристика меланинов культуральной среды *Inocutis dryophila* (Berk) Flasson & Niemela

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Аннотация. *Целью настоящего исследования явилось изучение влияния световой обработки на синтез эндомеланинов мицелия Inocutis dryophila в жидкую культуральную среду. Для выявления действия света на синтез меланинов проводили культивирование мицелия Inocutis dryophila на жидкой среде в темноте и с применением обработки синими светодиодами. В результате были получены два вида меланинов IDM-1 и IDM-2. Установлено, что световой фактор оказывает влияние на количественный выход меланинов из мицелия Inocutis dryophila в культуральную жидкость. Применение синего света приводило к меньшему содержанию меланина в культуральной жидкости по сравнению с количеством меланина, полученного в темноте. Методом ультрафиолетовой и инфракрасной спектрометрии выявлено, что оба вида меланинов имеют типичные спектры и графики для*

грибных меланинов. В ходе инфракрасной спектрометрии было обнаружено, что обработка мицелия синим светом приводила к деформации молекул меланинов IDM-1. Установлено, что меланины IDM-2 проявляли большую антирадикальную активность, чем меланины IDM-1. Таким образом, при глубинном культивировании световая обработка мицелия Inocutis dryophila оказывает влияние на количественный выход меланинов в культуральную жидкость, вносит вклад в изменение структуры, а также проявление биологического эффекта.

Ключевые слова: *Inocutis dryophila, культуральная жидкость, меланины, синий свет, ультрафиолетовая и инфракрасная спектрометрия*

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INTRODUCTION

Melanins are biopolymers, widespread in nature, having diverse origins, structures and functions, and are currently actively used and introduced into various areas of industry [1, 2]. Considering the complexity of the melanins structure of, currently the number of fungi used to obtain melanins remains small, so the search and characterization of new species of fungi as a potential source of melanins is an urgent task. Basidial fungi have already proven themselves as a source of highly active melanins [3, 4]. The most extensively studied genera include the melanins of *Inonotus obliquus* [5–7], revealing a wide range of activities [7–9]. The genus *Inocutis* is a closely related taxon to *Inonotus* [10], whose representatives have been little studied, but they may probably be no less promising objects for obtaining biologically active substances. We previously discovered that *Inocutis dryophila* is characterized by a high rate of mycelial growth on solid and liquid media. This study examines the possibility of obtaining fungal melanins from the culture liquid of the basidiomycete fungus *I. dryophila* using lighting.

EXPERIMENTAL

We used a pure culture of the basidiomycete fungus *Inocutis dryophila* (Berk) Flasson & Niemela strain 1422. Mycelium cultivation was carried out in 250 ml flasks containing 160 ml of wort-agarized liquid medium of the following composition: water 120 ml, unhopped light wort 40 ml, sucrose 1,6 g, glucose 1,6 g. The duration of cultivation was 30 days at a temperature of 25 °C, on a shaker at 140 rpm, under continuous illumination with blue LEDs with a luminous flux intensity of 12,8 W/m^2, and in the dark. Afterwards, filtration was carried out using a vacuum pump to separate the mycelium from the culture medium, followed by the isolation of melanin from the culture medium, as described in the method [11] and obtaining two types of melanins: IDM-1 – melanin from the culture liquid of *I. dryophila*, obtained on blue light and IDM-2 – melanin from the culture liquid of *I. dryophila*, obtained in the dark.

Samples for IR spectroscopy were dissolved in the 10% NH_3 solution, the solution was applied to thallium-bromine-iodine glass plates (KRS-5) and dried in vacuum. IR spectra were recorded on a Spectrum 100 IR-Fourier spectrometer (Perkin-Elmer, USA) in film in the range of 4000–450 cm^{-1}.

Antiradical activity was determined with ABTS radical according to the method [12].

The experiment was carried out in three biological replicates. Data were expressed as the mean and their standard deviation. The results were processed using Microsoft Excel. Differences between experimental data were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

As a result of isolating mycelium from the culture medium, melanin IDM-1 and IDM-2 were obtained, which is an amorphous substance of dark brown color, insoluble in water, acids and organic solvents, but highly soluble in aqueous solutions of alkalis.

It was shown that the light factor affects the yield of melanin in the culture liquid of *I. dryophila*, so the use of blue light led to a decrease in melanin content compared to darkness during mycelium cultivation, the yield of IDM-1 and IDM-2 was 1,443 and 1,663 g/l, respectively. Regulation of melanin synthesis was previously shown for *I. obliquus*; however, the opposite pattern in its accumulation is observed [13].

The UV spectrum of melanins alkaline solutions is typical for representatives of this class of compounds; it lacked absorption bands in the visible wavelength range with a characteristic linear dependence of the optical density logarithm on wavelength (Fig. 1).

Regression analysis showed that in the equations of this dependence ($\log D = a\lambda + b$) the values of the coefficient of determination r^2 were in the range of 0,9706–0,9759, and the values of the regression analysis were – 0,0039 to -0,0042, this is consistent with previously published data on fungal melanins [14]. The chromatic coefficients E465/E665 of melanins were 6,24 and 6,59, indicating the presence of aliphatic and O-containing functional groups.

During the analysis of IR spectrometry, it was found that the graphs typical for fungal melanins are in the wavelength ranges from 3600 to 3000 cm^{-1}, from 1650 to 1600 cm^{-1} and from 1500 to 1400 cm^{-1} [15, 16] (Fig. 2).

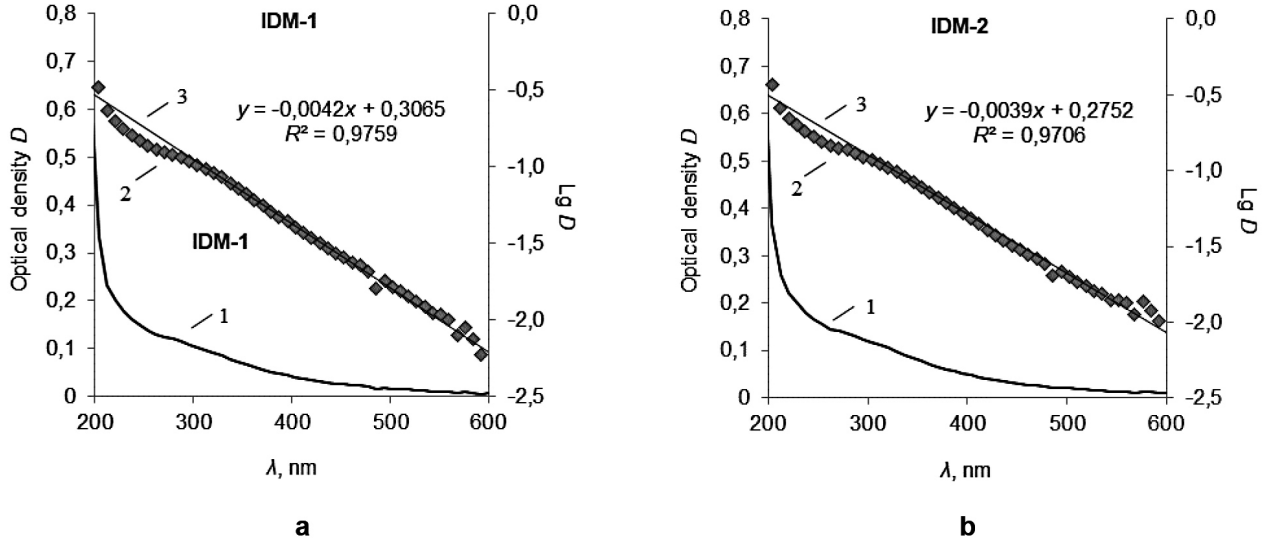

Fig. 1. Dependency graphs for melanins *Inocutis dryophila* IDM-1 (blue light) (a) and IDM-2 (dark light) (b): 1 – absorption spectrum of 0,002% solutions of *Inocutis dryophila* melanins; 2 – dependence of the logarithm of optical density on wavelength; 3 – linear regression graph for the λ – log *D* dependence

Рис. 1. Графики зависимостей для меланинов *Inocutis dryophila* IDM-1 (синий свет) (a) и IDM-2 (темнота) (b): 1 – спектр поглощения 0,002%-х растворов меланинов *Inocutis dryophila*; 2 – зависимость логарифма оптической плотности от длины волны; 3 – график линейной регрессии для зависимости λ – lg *D*

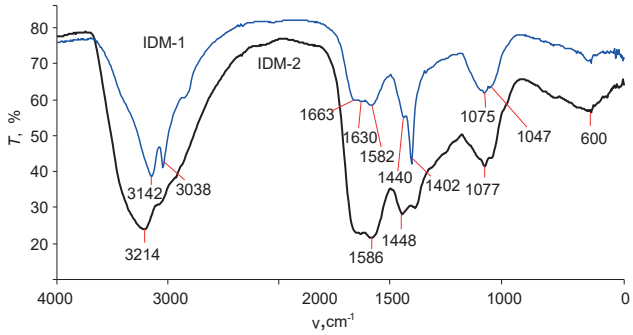

Fig. 2. IR spectrum of *Inocutis dryophila* melanins IDM-1 (blue light) and IDM-2 (dark light)

Рис. 2. ИК-спектр меланинов *Inocutis dryophila* IDM-1 (синий свет) и IDM-2 (темнота)

Broad bands are in the region of 3000–3300 cm^{-1}, due to stretching vibrations of OH bound to NH [17]. The existence of aliphatic fragments (C=H–, CH_2–, CH_3–) was confirmed by bands in the region of 2913–2020 cm^{-1} and 1439–1448 cm^{-1} [6]. Strong bands at 1663–1656 cm^{-1} are corresponding to the stretching vibrations of C=O and NH groups of secondary amides. Vibrations of the aromatic regions of the carboxyl function C=C and C=O are usually associated with a strong, characteristic absorption band between 1650 and 1600 cm^{-1} [18]. The bands in the 1600 cm^{-1} region are associated with vibrations on the CH=CH bond plane [19]. The peak of NH vibration and the peak of stretching vibration of CN– are at 1582–1586 cm^{-1} and 1400 cm^{-1}, which indicates the melanin structure typical of indole [20]. The presence of vibrations of the OH– group (tertiary alcohols) in the region of 1402–1310 cm^{-1} was also characteristic. The bands in the spectral region 1070–1047 cm^{-1} belong to hydroxyl, alcohol and phenol groups, respectively [19]. There are vibrations at 750–650 cm^{-1} OH of the bound group, CS at 700–600 cm^{-1}, O-NO at 600 cm^{-1} [20]. IR spectrometry showed that irradiation with blue light leads to deformation of IDM-1 melanin molecules. During the analysis of the antiradical activity of the obtained melanins using 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS+ method) it was revealed that the light factor influences the degree of manifestation of this type of activity. Melanins content of *I. dryophila* obtained in the dark is higher. It was established that the activity of IDM-2 was IC50 = 1,21 μg/ml, IDM-1 IC50 = 1,51 μg/ml, both values are higher than those indicated in the literature for *I. obliquus* melanins, obtained by the same method [6]. All data indicated for *I. dryophila* are presented for the first time.

CONCLUSION

Thus, the production of melanins from the culture liquid of *I. dryophila* is a promising source of highly active melanin, and it has been shown that cultivation in the dark has greater yield and activity, which is undoubtedly a big advantage for economically beneficial mass production of melanin.

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Contribution of the author

The author performed the research, made a generalization on the basis of the results obtained and prepared the copyright for publication.

Conflict interests

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Вклад автора

Автор выполнил исследовательскую работу, на основании полученных результатов провел обобщение, подготовил рукопись к печати.

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Автор заявляет об отсутствии конфликта интересов.

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