SHORT COMMUNICATIONS

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NUTRIENT MEDIA FOR A PURE CULTURE OF FUNGI OF THE GENUS *Pleurotus* OBTAINING *in vitro*

T. V. Ivanova[1], *I. S. Otkidach*[1], *N. O. Kuziomko*[1], *A. V. Zarutskaya*[1], *A. A. Mamontova*[1], *K. V. Yuronka*[2], *M. D. Melnychuk*[3]

[1]National University of Life and Environmental Sciences of Ukraine, Kyiv
[2]National University of "Kyiv-Mohyla Academy", Ukraine
[3]Kyiv Regional State Administration

E-mail: tivanova1@ukr.net

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The aim of the study was modification of the nutrient medium for basidiomycetes of the genus *Pleurotus* and its linear velocity of the growth determination. Biotechnological (obtaining and subcultivation of the strain NC-35 *in vitro*) and statistical methods were used. It was found that previously known media do not provide sufficient speed of mycelium growth and necessary biomass yield. There were investigated the new components of nutrient medium for greater biomass of pure culture of higher basidiomycetes of the genus *Pleurotus* obtaining. The nutrient medium for pure cultures of higher basidiomycetes, which contains agar-agar and water, additionally included the oat grain and oak bark decoctions in the following ratio per 1 l: agar-agar 15 g, oat grain decoction 600 ml, oak bark decoction 250 ml, distilled water — up to 1 l. The results of the linear growth rate analysis of *Pleurotus ostreatus* on the test and the control nutrient media, the calculation of the correlation coefficient indicate that for isolation and cultivation of oyster mushroom strains the most suitable medium is medium with the content of oat grain and oak bark decoctions. The obtained results will be used in the future for pure cultures of fungi of the genus *Pleurotus* obtaining *in vitro*.

Key words: optimized culture medium, basidiomycetes of the genus *Pleurotus*.

Mushrooms mycelium is sowing material of fungi, the vegetative body of which consists of thin branched filaments (hyphae), sterile grown in different media and intended for planting into the prepared substrate for harvest production [1]. There are many cultivated strains, which differ from each other in appearance, productivity, amount of allocated spores and the requirements for growing conditions [2, 3]. Strain of mushrooms (in German *Stamm* means the trunk, the base) is a pure culture isolated from a particular source or obtained by mutation or selection. Modern strains are characterized by high homogeneity and quality of mushrooms, high productivity in combination with a short loop of substrate colonization and fruiting [2, 3].

In Ukraine, the strain of oyster mushroom (*Pleurotus ostreatus*) NC-35 has proved itself the best. It is a hybrid strain, is one of the most popular for oyster mushrooms cultivation (Fig.1). Data on the origin and development of the strain are given in the tables 1 and 2 [4–9].

The choice of source material. The study was carried out during 2013–2015 at the ecobiotechnology and biodiversity Department of National University of Life and Environmental Sciences of Ukraine, Kyiv. Sowing material of oyster mycelium is presented by the strain NC 35. Mycelium is white with characteristic odor. Fruiting body is of medium size (50–80 mm), cap with concave smooth edges, color from dark gray to light gray, depending on the temperature of cultivation, at natural light –taupe or brown. The lower temperature in the chamber, the darker color. Caps size varies from 50 to 120 mm in diameter. Mushrooms have a meaty texture, especially at low temperature, are well preserved and transported.

Obtaining the pure culture of fungi. For pure culture of oyster mushroom obtaining

Fig. 1. **Fruit bodies of oyster mushroom strain R-2 (NC-35)**
Author of the photo: I. Otkidach, 2015

Table 1. **Passport of oyster mushroom strain R-2 (NC-35)**

The origin of the oyster mushroom strain	Fruitage temperature, °C	Color of mushroom fruit body
Hungary	5–22 (opt 14–16)	Gray

Table 2. **Growth and development parameters of oyster mushroom mycelium of the strain R-2 (NC-35)**

Temperature	22 °C in the room (24–28 °C in the block)
Relative humidity	60–65 %
Fouling duration	14–16 days
CO_2	5 000–20 000 ppm
Air exchange	No
Lighting	No

the follow media are used: meat-peptone agar, potato-glucose agar, potato plantlets and *Elaeagnus angustifolia* decoction agar medium.

Nutrient media. The basis of nutrient media creation for tissue cultures preparing is a mixture of mineral salts and, as the nutrition of cultured tissue is heterotrophic, the source of carbon is added into the medium in the form of sucrose or glucose. In addition to carbon, oxygen and hydrogen, for the tissues growth the nitrogen is required in the form of nitric or ammonium salt, the phosphorus — in phosphate form, and the sulfur — in sulfate form. The total concentration of mineral elements is the highest in meat-peptone agar medium.

Carbohydrate nutrition. In most media, the source of carbon and energy is sucrose or glucose at a concentration of 20–40 g/l.

pH of the medium. In native conditions the cell operates in a narrow range of fluctuations of hydrogen ions concentration. The relative stability of the pH value inside the cell and in the environment that surrounds it is supported by buffer systems, in which the protein molecules — ampholytes — are of great importance. Relatively stable pH in the medium is supported by chelating reagents and appropriate compounds. Most of the stationary isolated cultures of mushrooms grow on media with pH 7–7.8 [10–14].

The fruit bodies of the mushroom are collected during maturation. They are taken away from fruiting bodies according indicators that correspond to the strain. Spores are sown in Petri dishes. After spores germination and complete fouling of medium, the stock culture is kept in the refrigerator at a temperature of 0–2 °C [10–14]. Introduction to the pure culture is carried out by general methods [15, 16].

We investigated the linear rate of fungi growth (LRG) [15]. For experiment with the rate of growth *Pleurotos ostreatus* estimation the suspension of mushrooms (in the ratio1:2) was placed on the studied nutrient media. Then drop of fungi spores suspension (0.01 ml) was applied to the center of the Petri dish on control medium with meat-peptone agar (MPA). Then, the dishes were incubated in thermostat at (27 ± 2) °C and humidity of 90 %. The growth of colonies was assessed by measuring the diameter in two perpendicular directions every 2 days for 2 weeks. Based on the obtained data the graphs of the growth rate of the studied strains on time were plotted.

The closest analogue of modified medium is a nutrient medium for the work with pure cultures of higher Basidiomycetes — the objects of industrial growth, which involves the following stages of its implementation. 250 g of ethylized potato plantlets boil on low heat in a conical flask containing 500 ml of water. The decoction is filtered through a cotton-gauze filter on a glass funnel after cooking for 15–20 min; the volume of filtrate is made up to 500 ml. Air-dry fruits of *Elaeágnus angustifólia* (50 g) are cooked in another flask in 500 ml of water during the same time. Decoction is filtered on a glass

funnel through a cotton-gauze filter. Decoctions of potato plantlets and *Elaeágnus angustifólia* are united, 20 g of agar-agar is added, filtrate is made up to 1 000 ml and agar is melted over low heat. Prepared culture medium is poured into tubes of size 20–200 mm, autoclaved at a pressure of 1.5 MPa for 1 h and poured on sloping agar [11, 12]. After 3 days (control of medium sterility) the sloping agar is inoculated with pure cultures of fungi of the genus *Pleurotus* strains. Incubation was lasted round the clock in an incubator at (37 ± 2) °C. Pure cultures are obtained at the 7^{th} — the 8^{th} day.

The study task is a modification of nutrient media for higher basidiomycetes — promising producers of physiologically active substances. We have found that this medium does not provide a sufficient rate of mycelium growth and necessary biomass yield as compared to the analyte. The disadvantages are the lack of data on the content of sugars and proteins — the main sources of carbon and nitrogen of macromycetes nutrition. Performing the task was facilitated by the fact that nutrient medium for higher basidiomycetes pure cultures, which contains agar-agar and water, additionally includes oat grain and oak bark decoctions in the components ratio per 1 liter: agar-agar 15 g, oat grain decoction 600 ml, oak bark decoction 250 ml, others — distilled water — up to 1L. Experiments were performed in 3 repetitions.

The experimental data on linear growth rate of edible mushroom strains R-2 (NC-35) of oyster mushroom (*Pleurotus ostreatus)* were obtained in such nutrient media (NM): a culture medium with potato plantlets and *Elaeágnus angustifólia* decoctions (NM №1), modified growth medium with grain oats and oak bark decoctions (NM №2), control was the meat-peptone agar (NM №3). Figure 2 shows the linear growth rate of colonies grown from the spores for 14 days.

It was necessary to test the presence of connection between the variables at the calculation of selective correlation coefficient. For this purpose, the master of functions Microsoft Exel was used. Values of r for NM № 1 = 0.977536, for NM № 2 = 0.98615, for NM № 3 = 0.985223. 0.98615 was optimal index, it is nearly 1, indicating the close linear connection between the parameters.

The correlation field diagram, where the experimental data are presented as a scattering diagram (as correlative field), was constructed that illustrates the relationship between variables. Regression line was also obtained. In our case, the correlation field is elongated indicating the presence of correlation between traits. The regression line has the straight form. Thus the correlation between the features is linear and is evaluated using selective correlation coefficient r. Since the correlation coefficient $t > 0$, the relationship between signs is directly proportional, if r = 0.98615 the correlation is present (Fig. 3).

Analysis results of the linear growth rate of *Pleurotus ostreatus* on investigated and control nutrient media and calculation of correlation coefficient show: for isolation and cultivation of oyster mushroom strains the growth medium with concoctions of oat grains and oak bark is the most suitable. It was

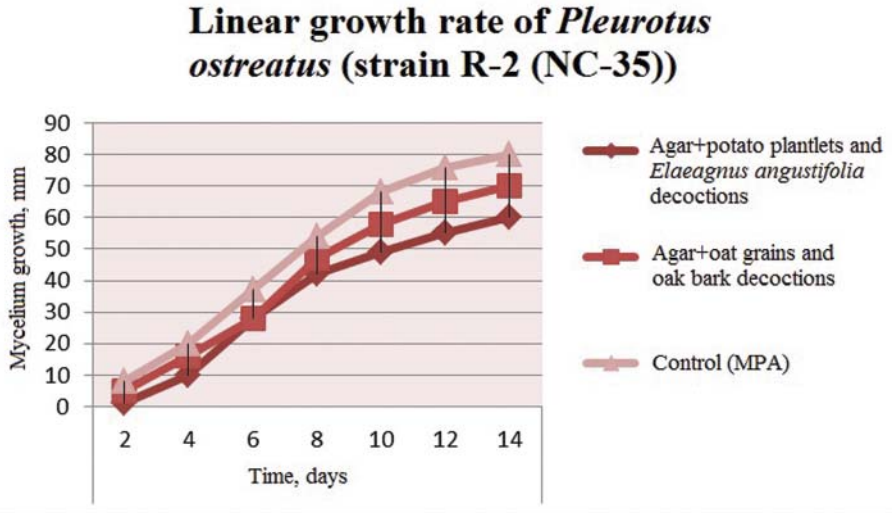

Fig. 2. **The linear growth rate of *Pleurotus ostreatus* strain R-2 (NC-35)**

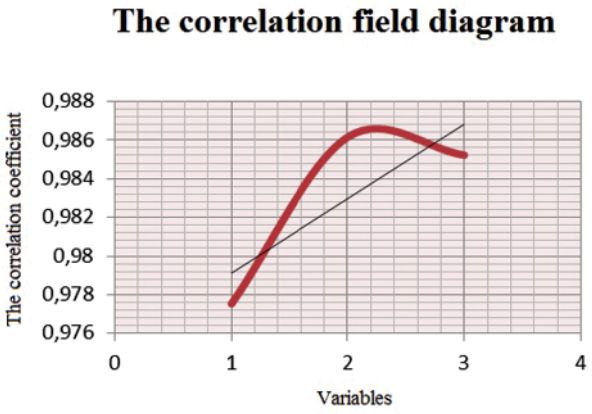

Fig. 3. **Scattering diagram (correlative field) at calculation of linear growth rate of *Pleurotus ostreatus* strain R-2 (NC-35)**

better than the medium which was taken as analogue — with decoction of potato plantlets and *Elaeagnus angustifolia* L.

An example of a particular medium preparation: oat grains and oak bark fill with water in the ratio of 500 g to 1 l and 300 g to 300 ml, respectively; infuse within 24 hours. Unite the infusions together and boil. Filter the decoction through cotton-gauze filter. Add distilled water to a filtered decoction up to the necessary dilution, depending on the type of fungus, and 15 g of agar. Melt agar at low warming of the mixture. Pour the resulting nutrient medium into Petri dishes and sterilize in an autoclave at a pressure of 0.2 MPa and temperature of 130 ± 2 °C for 40 minutes. After sterilization expand and cool the Petri dishes. After three days of media sterility monitoring sow the pure cultures of *Pleurotus*. For sowing take strain R-2 (NC-35) mycelium samples of size 3×3 mm. The pure cultures we get by the stock inoculum re-inoculations on the 7^{th} — the 10^{th} day depending on the linear growth rate and the medium overgrowing rate of the strain.

So optimized by us nutrient medium containing oat grains and oak bark decoctions is suitable for growing the fungi of the genus *Pleurotus*, namely the strain R-2 (NC-35). Development of nutrient medium based on oat grains and oak bark decoctions allows its production directly in mycological laboratories and enterprises engaged in mushroom production.

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ЖИВИЛЬНІ СЕРЕДОВИЩА ДЛЯ ОТРИМАННЯ ЧИСТОЇ КУЛЬТУРИ ГРИБІВ РОДУ *Pleurotus in vitro*

Т. В. Іванова[1], *І. С. Откидач*[1], *Н. О. Кузьомко*[1], *А. В. Заруцька*[1], *А. О. Мамонтова*[1], *К. В. Юронька*[2], *М. Д. Мельничук*[3]

[1]Національний університет біоресурсів і природокористування України, Київ
[2]Національний університет «Києво-Могилянська академія», Україна
[3]Київська обласна державна адміністрація

E-mail: tivanova1@ukr.net

Метою дослідження була модифікація живильного середовища для базидіоміцета роду *Pleurotus* та визначення лінійної швидкості його росту. Використовували біотехнологічні (одержання та субкультивування штаму НК-35 в умовах *in vitro*) і статистичні методи. Показано, що відомі раніше середовища не забезпечують достатньої швидкості росту міцелію і необхідного врожаю біомаси. Вивчено нові компоненти живильного середовища для отримання більшої біомаси чистої культури вищих базидіоміцетів роду *Pleurotus*. Живильне середовище для чистих культур вищих базидіоміцетів, яке містить агар-агар і воду, додатково включало відвари зерна вівса та кори дуба у співвідношенні на 1 л: агар-агар 15 г, відвари — зерна вівса 600 мл, кори дуба 250 мл, дистильована вода — до 1 л. Результати аналізу лінійної швидкості росту *Pleurotus ostreatus* на досліджуваних і контрольному живильних середовищах, розрахунок коефіцієнта кореляції свідчать про те, що для виділення і вирощування штамів гливи звичайної найбільш придатним є середовище зі вмістом відварів зерен вівса і кори дуба. Одержані результати використовуватимуться у подальшому для отримання чистої культури грибів роду *Pleurotus* в умовах *in vitro*.

Ключові слова: оптимізоване живильне середовище, базидіоміцети роду *Pleurotus*.

ПИТАТЕЛЬНЫЕ СРЕДЫ ДЛЯ ПОЛУЧЕНИЯ ЧИСТОЙ КУЛЬТУРЫ ГРИБОВ РОДА *Pleurotus in vitro*

Т. В. Иванова[1], *И. С. Откидач*[1], *Н. А. Куземко*[1], *А. В. Заруцкая*[1], *А. А. Мамонтова*[1], *Е. В. Юронька*[2], *М. Д. Мельничук*[3]

[1]Национальный университет биоресурсов и природопользования Украины, Киев
[2]Национальный университет «Киево-Могилянская академия», Киев
[3]Киевская областная государственная администрация

E-mail: tivanova1@ukr.net

Целью исследования была модификация питательной среды для базидиомицета рода *Pleurotus* и определение линейной скорости его роста. Использовали биотехнологические (получение и субкультивирование штамма НК-35 в условиях *in vitro*) и статистические методы. Показано, что известные ранее среды не обеспечивают достаточной скорости роста мицелия и необходимого урожая биомассы. Изучены новые компоненты питательной среды для получения большей биомассы чистой культуры *Pleurotus*. Питательная среда для чистых культур высших базидиомицетов, содержащая агар-агар и воду, дополнительно включала отвары зерна овса и коры дуба в соотношении на 1 л: агар-агар 15 г, отвары — зерна овса 600 мл, коры дуба 250 мл, дистиллированная вода — до 1 л. Результаты анализа линейной скорости роста *Pleurotus ostreatus* на исследуемых и контрольной питательных средах, расчет коэффициента корреляции свидетельствуют о том, что для выделения и выращивания штаммов вешенки обыкновенной оптимальной является среда, содержащая отвары зерен овса и коры дуба. Полученные результаты будут использоваться в дальнейшем для получения чистой культуры грибов рода *Pleurotus* в условиях *in vitro*.

Ключевые слова: оптимизированная питательная среда, базидиомицеты рода *Pleurotus*.