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SCREENING OF L. EDODES STRAIN PRODUCING BIOMASS
WITH HIGH CONTENT OF POLYSACCHARIDES

Abstract. Lentinus edodes (shiitake) is a mushroom with medicinal and functional properties. Widely sold as
nutritional agents, these mushrooms are helpful to human health and contain various bioactive compounds including
terpenoids, steroids, phenols, alkaloids, lectins, ergosterols. At present, 70%-80% of all medicinal mushroom
products are derived from fruiting bodies and 20%-30% of all products are based on extracts from mycelia and
culture filtrates. However, the production of medicinal mushrooms’ fruiting bodies usually takes several months, and
it is difficult to control the quality of the final product. By contrast, the growth of pure mushroom cultures in
submerged conditions in a liquid culture media permits acceleration of the growth speed, resulting in high biomass
yield in several days. Commercially grown shiitake currently are cultivated on synthetic media. In this article
Lentinus edodes strain, producing a significant amount of endo-polysaccharides was selected during submerged
cultivation. The maximum endo-polysaccharides production in fungal biomass was observed both in wort and
glucose-peptone-yeast nutrient media. It was stated that L. edodes strain 2541 showed the best endo-polysaccharides
production and was selected for further study.

Key words: Basidiomycetes, Lentinus edodes, endo-polysaccharides, submerged cultivation.

Introduction

Shiitake is the common Japanese name for Lentinus edodes, and is also the common name now used
in the West. Indigenous to Asia, shiitake is now cultivated and is the second most commonly produced
edible mushroom in the world [1-5]. Besides being a culinary delicacy, there is a long tradition of use of
shiitake as medicine in Asia, dating back >2000 years. Shiitake contains protein (26% of dry weight), lipids (primarily linoleic acid); carbohydrate; fiber; minerals; vitamins B-1, B-2, and C; and
ergosterols [6, 7].

Biological activity of most mushrooms has been determined by carbohydrate compounds, which
contains 60% of dry fungal biomass [8, 9]. They are represented by free and bound sugars, as well as by
polysaccharides. These substances isolated from shiitake have immunomodulatory, lipid-lowering,
and antimicrobial properties. In 70s of the last century, a group of Japanese scientists established the
oncostatic effect of polysaccharides isolated from the fruit bodies of some basidiomycetes, which led to
active study of these compounds, as well as the search for their producers [10, 11].

Polysaccharides are a group of biological macromolecules widely distributed in nature. They consist
of repeated structural units - monosaccharide residues, connected by glycosidic bonds. In comparison to
proteins and nucleic acids, polysaccharides have a higher ability to transfer biological information since
they have the greatest potential for structural variability.

At present, 70%-80% of all medicinal mushroom products are derived from fruiting bodies and 20%-30%
of all products are based on extracts from mycelia and culture filtrates [12-16]. However, the
production of medicinal mushrooms’ fruiting bodies usually takes several months, and it is difficult to control the quality of the final product. By contrast, the growth of pure mushroom cultures in submerged conditions in a liquid culture media permits acceleration of the growth speed, resulting in high biomass yield in several days. For most substances, this mycelium biomass obtained by submerged cultivation also has higher nutritional value. The culture media in which mycelium grows is made of chemically pure and ecologically clean substances. Submerged cultivation of mushrooms has significant industrial potential, but its success on a commercial scale depends on increasing product yields and development of novel production systems that address the problems associated with this technique of mushroom cultivation. The production of polysaccharides, as well as other components, is determined both by the biological characteristics of the fungal strain and cultivation conditions such as the content of nutrient components media, aeration, temperature, pH, and other factors.

The present study focuses on *L. edodes* biomass and endo-polysaccharides production capabilities in different nutrient media at submerged cultivation.

Materials and Methods
The objects of the study were 31 strains of *L. edodes* fungi from the collection of Basidiomycetes of the Institute of Botany of Ukraine. The cultures were maintained on wort agar (4°C), stored at 4°C. For biomass production, following nutritional media were used: (i) wort (W); (ii) synthetic glucose-peptone-yeast medium (GPY): (g/l) glucose - 30; peptone - 3; KH$_2$PO$_4$ - 1.0; K$_2$HPO$_4$ - 1.0; MgSO$_4$ x 7H$_2$O - 0.25; yeast extract – 20.

The inoculum preparation was comprise several steps: (i) inoculation of 100 ml of synthetic medium with 25 mycelia discs (Ø 0.5 cm, from 7-day-old culture from wort agar); (ii) incubation at room temperature (22 ± 2°C), on a rotary shaker (180 rpm), for 7 days; (iii) washing of obtained biomass (3 times) by sterile distilled water; (iv) biomass homogenization with 100 ml of sterile water in a laboratory blender. Mycelia biomass was assessed after 7 days of submerged cultivation in 250 ml flasks containing 50 ml of medium. The fungal biomass was separated by centrifugation (4°C, 3000 rpm, 30 min), washed by dH$_2$O, dried at 50°C until constant weight will be obtained, and measured as g L-1 of the medium.

To extract polysaccharides from the deep mycelium it was destroyed in a homogenizer, then poured in distilled water at a ratio of 1:10 and boiled in water bath for 18 hours. The removal of cytoplasmic contents was carried out by repeatedly suspending the destroyed mycelium in distilled water by centrifugation at 3000g for 15 min. The washing was stopped only when the optical density of the supernatant did not exceed 0.1 at 280 nm [17, 18]. The extracts were concentrated 2-3 times on a rotary evaporator, treated with 96% ethyl alcohol in a ratio of 1:1 by volume and left at 4°C until complete precipitation. The precipitate (polysaccharides) was separated by centrifugation and dialyzed against distilled water for 3 days. The dialysed polysaccharides were precipitated with ethyl alcohol at ratio of 1:2, washed with ethanol or acetone and dried at 37°C. The homogeneity of the polysaccharides was checked by gel filtration on Sephadex G-200. The detection of the polysaccharide was carried out using the phenol-sulfuric acid method [19]. The content of polysaccharides was calculated in % to dry biomass (a.s.m.).

All the analyses were performed in triplicate, and the results were expressed as mean SD values of the three sets of observations. The mean values and standard deviation will be calculated using STATISTICA 6 [20].

Results and Discussion
Many types of polysaccharides could be produced by mushrooms in submerged cultures. Among them, endo-polysaccharides from mycelia may mediate biological activities. Since the production of polysaccharides is more efficient from mycelia than from fruiting bodies, the influence of culture conditions on mycelial production has drawn much attention. The present study has focused on the biomass and endo-polysaccharides production capabilities of *Lentinus edodes* strains in submerged cultivation on wort and synthetic glucose-peptone-yeast media. The selection of active endo-polysaccharides producers among fungal strains showed that all fungal strains accumulated 5.0 - 13 g/l of mycelial biomass growing on wort medium and 3.5 – 8.5 g/l - on glucose-peptone-yeast medium (Table).
<table>
<thead>
<tr>
<th>Fungal strain</th>
<th>Biomass, g/l</th>
<th>Endo-polysaccharides, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W</td>
<td>GPY</td>
</tr>
<tr>
<td>55</td>
<td>6</td>
<td>3.5</td>
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<tr>
<td><strong>2541</strong></td>
<td><strong>12.5</strong></td>
<td><strong>7.2</strong></td>
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<td>57</td>
<td>7.8</td>
<td>4.4</td>
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<td>65</td>
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<td>503</td>
<td>12</td>
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<td>2082</td>
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<td>2914</td>
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<td>7.7</td>
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<tr>
<td>507</td>
<td>12</td>
<td>7</td>
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</table>

As can be seen from the data presented in the table, fungal strains 2180, 1710, 507 and 2541 were good producers of endo-polysaccharides. Maximum biomass accumulation was reached both in wort and in glucose-peptone-yeast media. Endo-polysaccharides were produced at the highest level by fungal strains 2180, 1710, 507 and 2541; in wort nutrient medium endo-polysaccharides content was 1.1 - 3.5% while on a glucose-peptone-yeast medium – from 1.0 to 4.5%.

Thus, the optimization of growth conditions for enhancing the biomass accumulation by 31 fungal strains was studied. The endo-polysaccharides production by fungal strains was observed both in wort and glucose-peptone-yeast media. L. edodes strain 2541 showed the best endo-polysaccharides production and was selected for further study.

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БИОМАССАСЫНДА ПОЛИСАХАРИДТЕРДІҢ ЖОҒАРЫ МӨЛШЕРЕ БАР L. EODES ШТАМДАРЫНЫҢ СКРИНИНГІ

Аннотация. Lentinus edodes (шитпак) – сомдик және функционалдық қасігерге немесе сыңануыққа. Бұл сыңануққалдардың жемісінің елеңінен қоп тәрізді молшерде орналасқанды және әлі тәрізді терениңді, стерилді, фенолдар, алкалоиддар, лектиндер, зерогстолырдың ырылды болуы мүмкін. Бұл натуралдық қорғау мүмкіндігі менен қатысты, сондықтан, жоғарылықтың ғылымдарының қызметінде өзінің әлі тәрізді молшердегі жоғарылыққа дайын болуы мүмкін. Бұл қазіргі тақырыптағы ұсыныстар мен құралдардың басқа қызметінің әр түрлі, арнайы құралдардың басқаруы менен қорғау барлық құралдар мен қорғау құралдарына қатысты, сондықтан, жоғарылықтың ғылымдарының қызметінің әр түрлі, арнайы құралдардың басқаруы менен қорғау барлық құралдар мен қорғау құралдарына қатысты, сондықтан, жоғарылықтың ғылымдарының қызметінің әр түрлі, арнайы құралдардың басқаруы менен қорғау барлық құралдар мен қорғау құралдарына қатысты.
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СКРИНИНГ ШТАММОВ L. EDODES С ВЫСОКИМ СОДЕРЖАНИЕМ В БИОМАССЕ ПОЛИСАХАРИДОВ

Аннотация. Lentinus edodes (шинтаке) – гриб, обладающий лечебными и функциональными свойствами. Плодовые тела этого гриба выращивают в промышленных масштабах во многих странах мира и содержат различные биологически активные соединения, включая терпеноды, стероиды, фенолы, алкалоиды, лектины, эргостеролы. В настоящее время 70%-80% всех грибных препаратов получают из плодовых тел и 20%-30% - из экстрактов пищевых грибов и культуральной жидкости. Получение препаратов из плодовых тел L. edodes обычно занимает несколько месяцев и, более того, в таких условиях очень трудно контролировать качество производимого продукта. Получение же грибной биомассы в условиях глубинного культивирования по скорости процесса более чем на порядок выше, чем традиционное получение плодовых тел, что позволяет значительно сократить время получения биомассы, увеличить ее количество. Коммерческое выращивание гриба шинтаке в настоящее время предполагает его выращивание на синтетических недорогих питательных средах. Настоящая статья посвящена скринингу штаммов Lentinus edodes с высоким содержанием в грибной биомассе эндополисахаридов. Установлено, что максимальное количество эндополисахаридов отмечено при культивировании изучаемых штаммов как на сусле, так и на глюкозе-пептон-дрожжевой среде. Штамм L. edodes 2524 отобран нами как наиболее перспективный продуцент эндополисахаридов.

Ключевые слова: базидиомицеты, Lentinus edodes, эндополисахариды, глубинное культивирование.

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