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THE USE OF THERMOPHILIC MICROMYCETES IN THE PREPARATION OF SUBSTRATES FOR THE CULTIVATION OF EDIBLE MUSHROOM BICUSPID

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Abstract

The paper describes the results of research in using the thermophilic mashroom Thielavia terrestris (Apinis) Malloch et Cain in the practice of preparing compost substrate for cultivation of Agaricus bisporus (J.E. Lange) Imbach. Industrial production of poultry produces a large amount of waste - litter and litter materials, potentially suitable for use in champignon composts. However, the litter mass contains a large number of ligno-cellulose inclusions that adversely affect the development of champignon mycelium and are poorly amenable to bioconversion processes. The solution to the problem may be in preliminary implementation of thermophilic mushroom with a rich spectrum of ligno- and cellulolytic enzymes. The use of Thielavia terrestris accelerated compost ripening by 3-5 days, increased the content of sugars available for champignon mycelium in compost by 12-18%, improved the compost structure and increased champignon yield on the converted substrate by 20-22%. The methods proposed in the current article provide the possibility of bioconversion of significant waste amounts in the form of litter and litter mass into valuable mushroom protein.

Key words: *biotechnology, thermophilic mushroom, compost substrate, bioconversion processes.*

INTRODUCTION

Cultivated edible mushrooms are of great consumer importance. It is a source of valuable protein, vitamins, trace elements, and biologically active substances (Ramos et al., 2019). Investigations are being actively conducted in the world on optimizing the cultivation of champignon in a culture and increasing its nutritional value (Rzymski et al., 2016; McGee, 2018).

In recent years, researchers have been paying their attention to reducing of the biological hazard in the resulting product, as well as the problems of the interaction of microorganisms living in compost (Singh et al., 2012; Šantrić et al., 2018; Qiu et al., 2019).

The scientific articles related to the study of the compost microorganisms role in the development of mushrooms are of great interest for our research. It has been shown that *Mycothermus thermophilus* and a number of other thermophilic microorganisms can stimulate the development of champignon (Kertesz et al., 2018).

Studies on cellulose destruction and substrate adaptation for the cultivation of an edible fungus using a complex of microorganisms are noteworthy for our research (Zhang et al., 2014; Zhang et al., 2019). A number of researchers point to the fact that most of the compost carbohydrates remain unused in the process of growing mushrooms, noting the feasibility of searching for *A. bisporus* strains that can utilize components that are poorly used by commercial strains (Pontes et al., 2018). However, the high productivity of commercial strains remains an important factor.

In this context, studies relevant to the investigation the thermophilic microorganisms role that have cellulolytic potential and are able to provide preliminary degradation of materials intended for the production of compost are relevant.

MATERIALS AND METHODS

The current research was conducted on the basis of the biotechnology laboratory in the institution of higher education - Penza State Agrarian University (Russia). In the experiments we used the commercial strain *Agaricus bisporus* 512, which is widespread in Russian mushroom farms, as well as the culture of the *Thielavia terrestris* mushroom strain F144, obtained from the VKPM collection. The cultivation of the strains was carried out according to standard methods.

As a substrate for composting the utilized underlay materials were used such as waste products generated during the industrial rearing of turkeys. These materials contain a significant amount of cellulose, a material difficult to assimilate champignon mycelium.

The biological efficiency of growing basidiomas was determined as the ratio of the fresh mass of mushrooms to the dry mass of the substrate. The conversion coefficient was calculated as the ratio of the dry mass of mushrooms to the dry mass of the substrate. All experiments were performed in triplicate. Statistical processing was carried out using the program for processing and analysis of data "Statistica 10.0".

RESULTS AND DISCUSSIONS

The following compost options were prepared for the research: option 1 - based on broiler underlay (control); option 2 - based on underlay material from laying hens; 3 - based on turkey underlay material.

The prepared composts were placed in the 6 liter plastic crates, inoculated with champignon mycelium (*Agaricus bisporus* 512 strain) and incubated in laboratory conditions to obtain growing mushrooms. Compost based on broiler droppings was mastered first of all on 12 ± 2 days. The emergence of the rudiments of growing mushrooms was noted on 7 ± 2 days after applying the integumentary earth. Compost based on dropping materials from laying hens was mastered more slowly, although the differences were within the statistical error. The third version of compost, based on the litter material of the turkey was mastered by mycelium at a longer time by 18 ± 3 days, the emergence of the rudiments of growing mushrooms was weakly expressed. They were formed singly.

The first wave of uprising had been lasting for 4-8 days in different versions of the experiment. The maximum yield in the first uprising wave was 6.9 ± 0.7 kg/m^2 and was obtained on the control compost. In the second and third variants, using litter and laying materials of laying hens and turkeys, 5.7 ± 0.7 kg/m^2 and 4.4 ± 0.1 kg/m^2 of biomass of growing champignons, relatively, were obtained in the first uprising wave.

During the second wave of uprising, the yield was proportionally lower. The total yield for two waves of growing is presented in Table. 1.

Table 1. The yield of mushrooms on different composts, kg/m^2 ($p < 0.05$)

Variants	The first wave of uprising	Total yield
Option 1 (control)	6,9±0,7	9.8±0.2
Option 2	5.7±0.7	7.9±0.2
Option 3	4.4±0.1	6.7±0.1

Thus, from the studied compositions, compost with turkey litter materials was the least effective.

The conversion of substances of various composts is ambiguous. The conversion rate of compost prepared on the basis of broiler litter was 8.9%; based on litter materials from laying hens 5.7%; and compost based on turkey litter materials was only 2.4% (Figure 1).

Such results can be explained by a relatively higher dry matter content, as well as low yield of champignon on compost with turkey litter.

In this regard, it becomes necessary to optimize this material in order to change its structure and properties. To do this, the turkey-added substrate was fermented using the thermophilic fungus *Thielavia terrestris* strain F 144, which biodegradable sawdust contributed to a qualitative change in the compost composition.

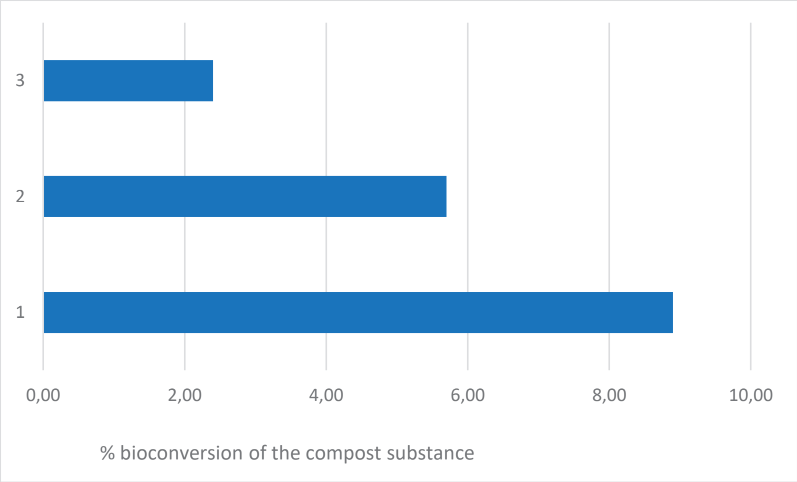

Figure 1. Conversion factors of substances with mycelium on different composts: 1 - compost based on broiler litter; 2 - compost based on litter material from laying hens; 3 - compost based on turkey litter materials ($p < 0.05$)

The mushrooms grown in deep conditions was added to the compost at the stage of layering according to the scheme "layer by layer" in the amount of 10.0 g/kg of compost.

The control material was the compost prepared in the usual way (litter-bedding mixture and wheat straw). Fermentation was carried out for two weeks. Accounting for yield on compost preliminarily fermented with thermophilic mycelial fungus *Thielavia terrestris* showed that the result significantly exceeded the control indicators (Table 2) and amounted to 9.4 ± 0.3 kg/m^2, which is comparable to the yield of champignon on compost using broiler litter.

Table 2. Productivity of champignons on composts based on litter material of turkeys, kg/m^2 ($p < 0.05$)

Compost Features	**1-wave yield**	**Total yield**
Compost without pre-fermentation	4.4±0.1	5.8±0.3
Thielavia terrestris fermented compost	6.7±0.1	9.4±0.3

This is probably due to the fact that the fungal enzymes *Thielavia terrestris* contributed to the conversion of lignocellulosic components of the litter into sugar, which is accessible for assimilation by mycelium.

This is also indicated by the sharply increased coefficient of substrate nutrient conversion, which is 3 times higher than in the control (without using *Thielavia terrestris*) (Figure 2).

It should be noted that the obtained conversion rate of 6.3% was higher than in the variant with the use of litter-laying materials of laying hens, although slightly lower than compost based on broiler litter.

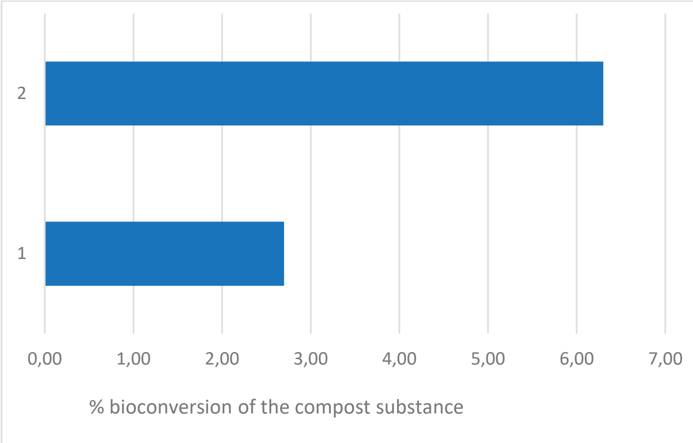

Figure 2. The conversion factors of substances with mycelium on different composts: 1 - compost based on litter materials of turkeys without fermentation *Thielavia terrestris*; 2 - compost based on material previously fermented with *Thielavia terrestris* ($p < 0.05$)

Reliably noted increase in the efficiency of substrate nutrient conversion and an increase in the yield of champignons with the addition of the compost, originally rich in lignocellulosic components, but fermented by the thermophilic cellulosolytic fungus *Thielavia terrestris*. Using the pre-fermentation process with cellulolytic enzymes makes it possible to utilize waste materials that are massively produced in turkey growing – litter and bedding materials containing a significant amount of straw and sawdust as nutrient substrates. Thus, in laboratory conditions, the positive effect of using preliminary fermentation of a substrate rich in cellulose and lignin sources for the subsequent utilization of nutrients by the mycelium of edible champignon fungus and increasing yield was established (Figure 3).

Figure 3. Growing of *Agaricus bisporus*: on the left - compost without preliminary fermentation, on the right - compost pre-fermented with *Thielavia terrestris*

CONCLUSIONS

Thus, in laboratory conditions, the positive effect of using preliminary fermentation of a substrate (turkey litter and bedding materials) rich in cellulose and lignin sources, mycelium of the thermophilic fungus *Thielavia terrestris* for subsequent utilization of nutrients by the mycelium of edible champignon mushroom and increase its productivity was established.

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