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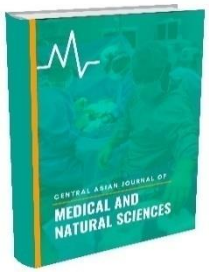

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Basidial Mushrooms and Prospects for their use in the Biotechnology

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Abstract: The biosynthetic abilities of some basidiomycetes Pleurotus ostreatus, strain UzBI 108, Agaricus bisporus, strain 12, A.bisporus, strain -2, Fomes fomentarius, Inonotus hispidus, strain T-18 on various plant wastes for the formation of protein, proteolytic enzymes on waste were studied alcohol industry. Selected under conditions of maximum accumulation of protein and enzyme by Pleurotus ostreatus UzBI 108. Agaricus bisporus pcs. sp 12 and Agaricus bisporus pcs. sp.2, which depends on the cultivation time, the concentration of vinasse in the medium and the type of mushrooms. The formation of a sufficient amount of protein with essential amino acids and proteases opens up prospects for the use of the studied basidiomycetes in the bioconversion of wastes from the fermentation industry into useful products of microbial synthesis.

Keywords: basidiomycetes, grain stillage, bioconversion, biomass, proteins, enzymes, amino acids, valuable products.

INTRODUCTION

In connection with the rapid growth of the population and industry, with the deterioration of environmental indicators and the health of the population, the preparation of feed products for food safety is becoming increasingly relevant. For, meat, milk, eggs and other products of primary, everyday consumption should be of high quality, balanced, and most importantly safe. Therefore, fodder products for farm animals and birds having a full composition of nutrition elements, consisting of natural substances obtained from non-pathogenic mushrooms and waste from the fermentation industry are of particular relevance.

Basidiomycetes, thank to a powerful enzyme system, penetrating into highly crystalline, water-insoluble biopolymers, up to synthetic ones, are active destructors of many polysaccharides (cellulose, hemicellulose, pectin) and aromatic polymers of natural and synthetic origin, which makes them effective in biotechnological processes used in various sectors of the national economy and industry.

Therefore, research related to utilization and biotransformation of annually and daily renewable biopolymers is in demand and relevant.

In turn, large-tonnage industrial wastes and drains are the main sources of environmental pollution of rivers, lakes, seas, soils and can affect not only the quality of water, microbial, aquatic flora, but also the health of the population [1]. Promising alternatives for solving the disposal of these wastes into biologically valuable products can be obtained as a result of the introduction of new, scientifically based technologies not only for treating industrial effluents, but also for converting their wastes into useful products by fermentation with mushrooms. Obviously, anthropogenic interference with the environment has revealed the effectiveness of microorganisms in the process of adaptation to the effects of damaging agents that make up industrial effluents and waste, which makes them a powerful tool for waste destruction and environmental protection.

Vinasse is a waste product obtained from the production of alcohol after the fermentation of wheat wort and distillation of alcohol. For every liter of alcohol produced, 8 to 15 liters of stillage are produced, which is characterized by a sufficient amount of organic matter, acidic nature (low pH) and a high concentration of untreated solids [2,3].

The production of ethanol from hydrocarbon raw materials is accompanied by the formation of large-scale waste - a post-maintenance Vinasse, the amount of which in many sizes exceeds the volume of the main product and product produced 135–150 m3 per 1000 decaliters of ethanol. At present, distillery is most often a burdensome vinasse that poses a threat to the environment. Due to the high content of organic substances, vinasse cannot be cleaned in aeration tank using classical technology. The need to ensure the proper environmental level of alcohol production requires the mandatory disposal of vinasse.

An analysis of world experience indicates that vinasse has the greatest prospects as a raw material for the production of protein-containing feed additives [2, 3, 4,5]. The resulting feed products are in demand on the market and are able to ensure the profitability of microbiological processing of vinasse, and most importantly, to ensure the high needs and demand of feed products containing proteins, enzymes, amino acids, carbohydrates and other biologically valuable products of microbial synthesis.

RESEARCH MATERIALS AND METHODS

Objects of study: plant wastes, existing lignocellulosic residues (wheat, corn, wood, rice), vinasse, yeast in the fermentation industry, basidiomycetes: *Pleurotus ostreatus, strain UzBI 108, Agaricus bisporus, strain 12, A.bisporus, strain -2, Fomes fomentarius, Inonotus hispidus, strain T-18.*

Research Methods. The cultivation of mushrooms was carried out in deep conditions on a synthetic medium Chapek (control) and (50%) vinasse (experience).

We studied the activity of enzymes, the formation of proteins and biomass in the dynamics of mushroom growth for 144 hours, taking samples for analysis every 6 hours. The activity of cellulose, xylanase was determined by the method of M.L. Rabinovich, Phenicsova and others [6].

The amount of protein was determined by the Lowry method [7]. Proteolytic activity was determined by the modified method of Anson et al. [8]. The analysis of the total amino acid content contained in the growth culture medium (CS), ie, in mycoproducts, was determined on a high performance liquid chromatography (HPLC) analyzer according to the procedure [9].

By grinding the fungal cells on a disintegrator with alternating treatment with liquid nitrogen, a homogenate was obtained in which the sum of free amino acids and soluble fractions were separated using 10% acetonitrile and centrifuged at 6000 rpm for 30 minutes, then the supernatant was precipitated with 20% trichloroacetic acid. Next, the precipitate was separated by centrifugation at 6,000 rpm, the supernatant was treated with phenyl thio isocyanate to obtain phenyl thiocarbamyl derivatives of amino acids according to the method [10].

Derivatives of amino acids in the protein hydrolyzate were identified by HPLC on an Agilent Technologies 1200 cDAD chromatograph with a detector, column 75 x 4.6 mm, Discovery HSC18, 3µm. Qualitative and quantitative calculation of the concentration of the studied amino acids was carried out by comparing the retention time and calculating the peak areas of the standard and studied FTC - derivatives of amino acids [9].

RESULTS AND DISCUSSION

By comparing the growth and development of basidiomycetes, first by the surface method, then by deep cultivation according to the ability to accumulate biomass and protein, hydrolytic and oxidative enzymes on media containing various plant (wheat straw and bran, corn stalks and cobs, sawdust, rice husk, stalks cotton) and industrial waste (grain stillage, yeast) of fermentation production (JSC Bektemir spirit and Agrofirm Mehnat) were selected highly active polysaccharide destructors (cellulose, hemicellulose) and lignin, which are contained in their composition in concentrations from 28 to 42%. The composition of the nutrient medium was optimized taking into account from 2.0 to 10% of plant waste and from 10 to 50% of production waste and cultivation conditions taking into account the pH of the nutrient medium (in the ranges of 3.5-7.5 and cultivation temperature (25-50 C).

Taking into account the daily large volume and soon spoiling the properties, further study of the biosynthetic activities of fungi was carried out on the medium after the alcohol grain vinasse in experimentally selected concentrations and conditions (50% liquid vinasse, 30 C - cultivation temperature and at pH-5.6).

The enzyme systems and their spectrum formed by fungi on the above medium and cultivation conditions differed among themselves, both in the activity of the enzymes and in the formation of protein and biomass, as well as in the growth rate.

It was shown that, depending on the growth time and the composition of the nutrient medium, fungi form proteins in various concentrations (Fig. 1). The highest protein concentration at 24 hours of cultivation is formed by the fungus *Agaricus bisporius, sp 2* (7.2 mg / ml). At the same time, by 48 hours of growth, the maximum amount of protein was observed both on the Chapek medium (7.3 mg / ml) and on the medium with bard (8.2 mg / ml in the fungus *Pleurotus ostreatus, UZB 108.* The formation of protein occurred in all fungal cultures in during 72 hours of growth, then by 120 hours there is a decrease in the amount of proteins secreted by all fungi into the culture medium.The fungi *Pleurotus ostreatus, UZB 108* and *Agaricus bisporussp, 2* were the most able to form proteins.

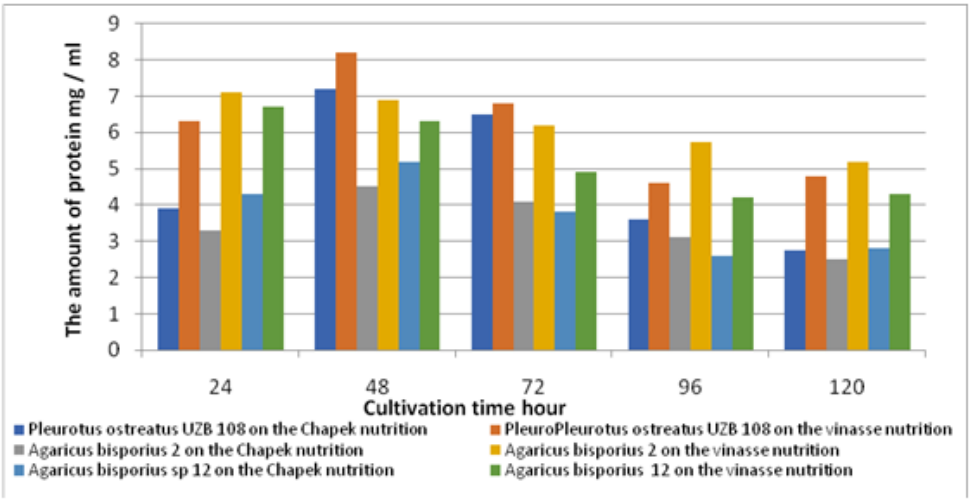

Figure- 1. Dynamics of protein accumulation by basidiomycetes *Agaricus bisporus sp 2, Agaricus bisporus sp 12, Pleurotus ostreatus UZB 108* on test media

It was found that high cellulase activity in the hydrolysis of cellulose substrates (sodium salt of carboxymethyl cellulose (Na-CMC, carboxymethyl cellulose (CMC), homogeneous wheat bran cellulose, cotton cellulose) was distinguished by the fungus *Inonotus hispidus-T-18*, whereas The hydrolysis of Spelled oat xylan was most active. The tinder fungus –*Fomes fomentarius -UzBI*-Y-8 showed the greatest amount of phenol-oxidizing enzymes (peroxidase, laccase) in the fungus fungus – *Pleurotus ostreatus-UzBi 108*, then *Agaricus bisporus, sp. 2*.

In view of the fact that proteins are the main source of nitrogen nutrition for farm animals and birds, many feeds include complex and high molecular weight proteins, proteases, along with other enzymes are very important and demand in feed production. Therefore, in parallel with the study of the polysaccharide of hydrolyzing enzymes, analyzes were carried out to determine the proteolytic activity of the studied fungi.

Analyzes showed that along with carbon hydrolysing and phenol-oxidizing enzymes, almost all fungi produced proteolytic enzymes in sufficient concentration, which is an important aspect in the field of their use.

It was found that all the studied strains of basidiomycetes showed sufficient proteolytic activity in both variants of the nutrient medium. At the same time, the maximum proteolytic activity was observed in the strain *Agaricus bisporus, sp 12* in a medium with vinasse (96 h, pH-5.5 0.14 u / ml). (fig. 2).

Among the tested cultures, the most active and productive enzymatic activity and protein accumulation were found to be *Agaricus bisporussp, 2*, then *Pleurotus ostreatus-UzBI 108.*

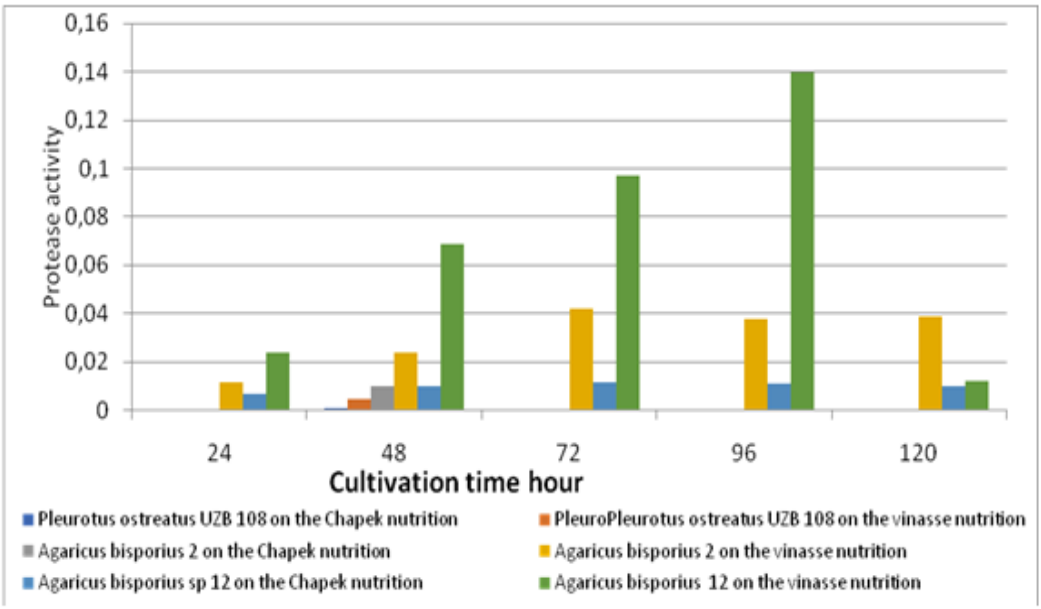

Figure 2. Protease activity of fungi *Agaricus bisporius, sp 2 Agaricus bisporius, sp 12, Pleurotus ostreatus, UZB 108*

Almost all essential amino acids were found in the proteins of the culture medium and fermentolysis products (table). Determination of the amino acid composition of proteins in fungal hydrolysates showed that the fungi *Pleurotus ostreatus, UZBI 108, Agaricus bisporius, sp 2, Agaricus bisporius, 12* possessed the most optimal and valuable amino acid composition.

Table. Amino acid composition of the fermentation mixture with *Basidiomycetes* and 50 % vinasse grain products

Name of the amino acid	Source bard, mg / ml	*Agaricus bisporius 12*	*Agaricus bisporius sp 2*	*Pleurotus ostreatus УЗБІ 108*
	Concentration of amino acids, mg / ml			
Aspartic acid	0,094583	0,159781	0,162201	0,233215
Glutamine	0,113038	0,838567	1,48174	0,760642
Serine	0,062453	0,545504	0,714961	1,147043
Glycine	0,0822	0,302205	0,477302	0,766537
Asparagine	0,082145	0,303423	0,479123	0,770877
Glutamine	0,070264	4,112774	4,69535	10,68413
Cysteine	0,036051	1,650142	2,358357	3,371105
Threonine	0,037273	0,476066	0,666492	1,552236
Arginine	0,096297	0,561726	0,701623	2,015164
Alanine	0,087764	1,398889	2,481276	1,79512
Proline	0,213914	1,665445	1,700257	2,385489
Tyrosine	0,072814	1,376225	1,870098	0,680147
Valine	0,073361	0,680108	1,136774	1,908667
Methionine	0,024012	0,157219	0,318866	0,10186
Isoleucine	0,054824	0,910451	0,944982	1,953269
Leucine	0,083094	1,26455	0,948854	2,214286
Histidine	0	0	0	0
Tryptophan	0,094143	1,081648	1,024418	2,247234
Phenylalanine	0,011858	0,254677	0,281722	0,223124
Lysine-HCl	0,007209	0,084624	0,184552	1,236046
Total	**1,397052**	**17,82402**	**22,62895**	**36,04619**

Determination of the amino acid composition of proteins in the hydrolyases of fungi showed that the most optimal and valuable composition of aminacids has a mushroom, *Pleurotus ostreatus, UZBI 108, Agaricus bisporius, sp 2, Agaricus bisporiusm 12.*

According to the distribution of the number of amino acids, glutamine was predominant, for example, if the concentration of glutamine in the initial composition of the grain stillage was 0.036051 mg / ml, the fermentation of this substrate by *Agaricus bisporius, 12* increased the concentration to 4.112774, and *Agaricus bisporius, 2* - 4,69535, whereas, with the fungus *Pleurotus ostreatus UZBI* 108 this concentration was - 10.68413 mg / ml. The second position in the accumulation of amino acids is occupied by cysteine, the concentration of which in the original bard was 0.036051 mg / ml, then the fungus *Agaricus bisporius, 12* - 1.650142, *Agaricus bisporius,2* - 2.358357, *Pleurotus ostreatus, UZBI 108* - 3.371105. The concentration of arginine, proline, tryptophan and leucine was almost the same in the fungus *Pleurotus ostreatus UZBI 108*, while the amount of lysine in this fungus was 1.236046 mg / ml.

The total amount of amino acids in the initial composition of the grain stillage and fermented samples on a medium with grain vinasse were as follows:

Grain vinasse > 1.397052

Agaricus bisporius, 12 > 17.82402

Agaricus bisporius, sp 2 > 22.62895

Pleurotus ostreatus, UZBI-108 > 36.04619

The research results showed that the degree of conversion of grain vinasse in the dynamics of growth of macromycetes *Pleurotus ostreatus strains UzBI 108, Agaricus bisporus, strains 12* and *Agaricus bisporus, strains 2* depended on the time of cultivation, the concentration of vinasse in the cultivated medium and the generic affiliation of the fungi. The activity of enzymes (cellulase, xylanase, protease) and the amount of protein formed were maximum during 144 hours of fungal growth. Comparative analyzes between fungi showed that *Agaricus bisporus sp. strain 12*, which forms the largest amount of protein (6.8 mg / ml), had the highest biosynthetic activity on a medium with 50% bard, introduced into the nutrient medium.

Thus, in order to obtain feed products with food safety, edible basidiomycetes and a large tonnage waste of the alcohol industry were used. By deep fermentation of the distillery grain vinasse with mushrooms, the composition of the nutrient medium, physicochemical conditions, and other growth parameters were optimized to maximize the accumulation of biologically valuable substances in the fermented medium: enzymes, proteins, biomass with a high content of amino acids, including almost all essential amino acids.

The formation of a sufficient amount of protein, highly active hydrolytic enzymes, biomass, the belonging of mushrooms to edible mushrooms, the full composition and high content of amino acids allows using the converted biologically valuable food safe food as biological feed in various sectors of agriculture and industry.

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