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Study of the Induction of Secondary Growth of Alcoholic Yeast Saccharomyces Cerevisiae

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ABSTRACT
The results of the study of the effect of MgSO4, CuSO4 and ultraviolet irradiation on the induction of secondary growth of the colonies of alcohol yeast Saccharomyces cerevisiae are presented. It was found that secondary growth induces CuSO4 when introduced into the nutrient medium in an amount of 0.05-0.0125 mg / ml, as well as irradiation with ultraviolet rays for 180-300 seconds. The maximum number of secondary growth colonies is observed under the influence of ultraviolet irradiation for 300 seconds.
Keywords: Saccharomyces cerevisiae, secondary growth, ultraviolet irradiation, magnesium sulfate, copper sulfate.

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I. INTRODUCTION

Currently, the yeast is widely used in all sectors of the food industry, as they have a high rate of growth and rapid accumulation of biomass [1]. They can also be viewed as a source for a number of very valuable products for medical and veterinary purposes, feed additives and production of ethanol [2]. At the moment, research scientists focus on intensifying yeast growth, increasing their metabolic activity and resistance to extreme impact under condition of biotechnological production [3-5]. Among the factors limiting the growth of microorganisms leading role is given to change in the composition of the substrate and the accumulation of various metabolic products, as a result the growth of secondary colonies is observed in some types of bacteria: actinomycetes, fungi and yeast. Cells produced in secondary growth, may have impaired differentiation, increased frequency of fission, dramatically changed antibiotic and enzyme activity [6].

The aim of study was to study the effect of MgSO4, CuSO4 and ultraviolet irradiation on induction of secondary growth in alcoholic yeast *Saccharomyces cerevisiae*.

II. MATERIALS AND METHODS

The object of the study was a strain of alcoholic yeast *Saccharomyces cerevisiae*, which showed increased readings of fermentation activity and tolerance to osmotic pressure, resistance to low pH of the medium and an elevated temperature of cultivation when applied to substrates used in the production of alcohol at an alcohol plant.

The study of the induction of secondary growth of yeast was carried out by culturing on a solid nutrient medium Reader. The medium was prepared with an agar content of 15 g / l to avoid drying out the nutrient medium layer during prolonged cultivation. For the same purpose, spilling media over the cups was performed in a thicker layer (1 cm) than in the case of short-term cultivation of microorganisms. The cultures were performed on variants of medium with different concentration of CuSO4 (0.1 mg / ml, 0.05 mg / ml, 0.025 mg / ml, 0.0125 mg / ml, 0.0062 mg / ml) and MgSO4 (0.2 g / l). When choosing the concentration of copper sulfate and magnesium sulfate, they were guided by literature data on their significance for inducing secondary growth in microorganisms [8].

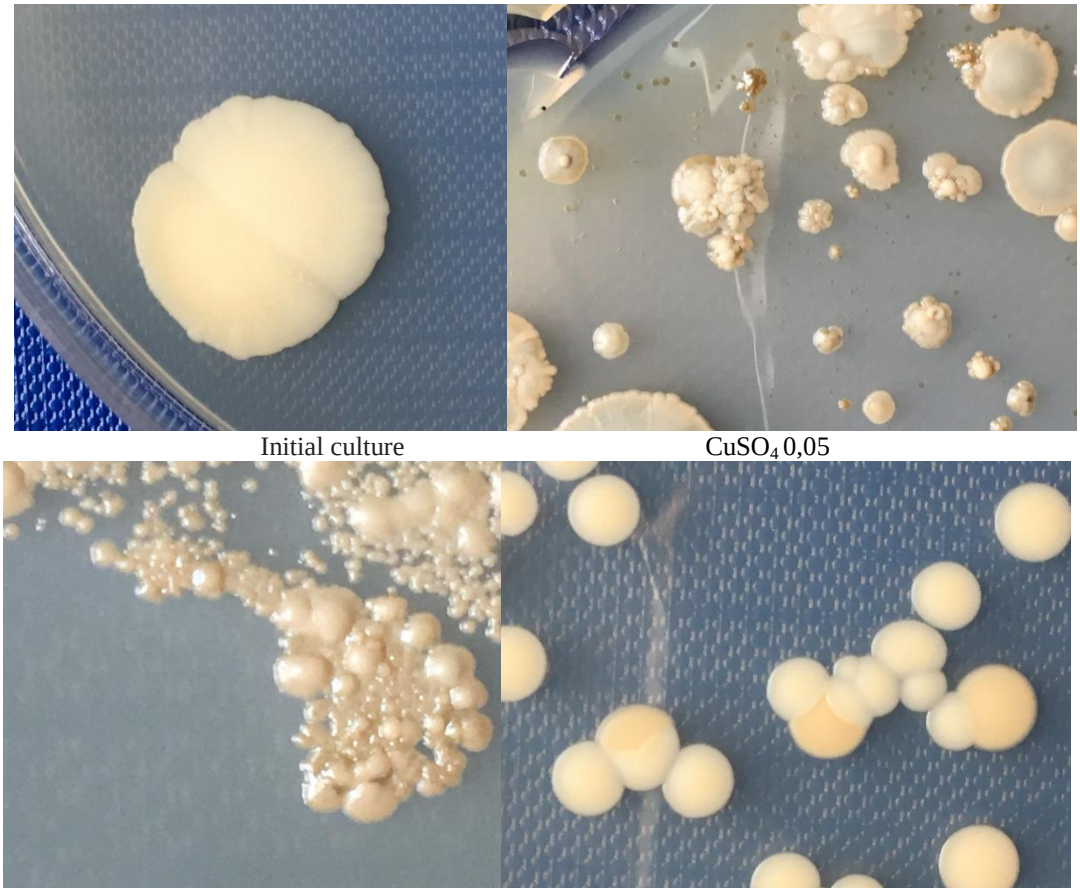
Also secondary growth of alcoholic yeast was induced by irradiation with ultraviolet rays. For the experiment, a yeast culture incubated for 18 hours at 28°C was taken. The grown culture was diluted 10 times with a liquid complete nutrient medium of Reeder and incubated for another 2 hours. The yeast cells were then washed from the nutrient medium by centrifugation and resuspended in the initial volume of saline.The irradiation of the culture was carried out in open Petri dishes with a DBM-30 bactericidal lamp (for 180, 240, 300 seconds) at a distance of 40 cm from the source [9]. For each dose, a separate cup was used, into which 5 ml slurry was poured, unirradiated suspensions

served as a control. During irradiation, the contents of the cups were mixed on a magnetic stirrer. Further, the seeds were sowed onto the surface of the agar medium in Petri dishes from dilutions (10^{-8}, 10^{-9},10^{-10}).

Cultures were incubated at 28-30°C. for 4 weeks and the number of secondary growth colonies was counted. The mathematical processing was performed in the standard "Excel 7.0" computer program. All the experiments were performed in triplicate. The data group was considered homogeneous if the standard deviation in it did not exceed 13%. The difference between groups was considered significant at probability criterion P <0.05 [10].

III. RESULTS AND DISCUSSION

Induction of secondary growth of yeast was detected by the addition of CuSO4 in the medium at a concentration of 0.05 mg / ml; 0.025 mg / ml; 0.0125 mg / ml. The number of secondary colonies ranged from 86 to 295 per petri dish. The size of the colonies ranged from point to 4 mm in diameter and was characterized by a high profile, with a uniformly folded crater in the center (Figure 1).



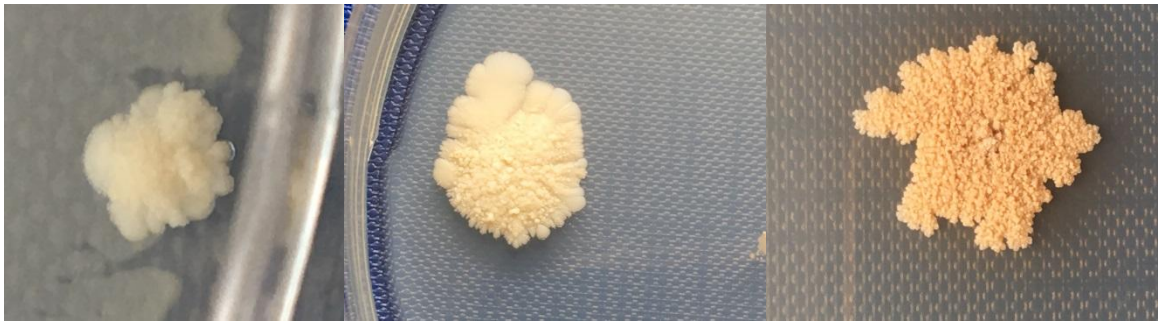
Initial culture $CuSO_4$ 0,05

$CuSO_4$ 0,025 $CuSO_4$ 0,0062

Figure 1 - Colonies of secondary growth of yeast, CuSO4

The folding of the colonies disappears in the CuSO4 medium at a concentration of 0.0062 mg / ml, but a secondary color of the yellow color appears in the presence of CuSO4 (0.1 mg / ml) and MgSO4 (0.2 g / l) in culture medium induction of the secondary growth Yeast and color changes of their colonies are not revealed.

As a result of irradiation of the alcohol yeast culture with ultraviolet rays, the number of secondary growth colonies increases with increasing irradiation time. The sizes of the secondary colonies varied from point to 2 mm in diameter and were characterized by a high folded profile (Figure 2).



180 sec 240 sec 300 sec

Figure 2 - Colonies of secondary growth of yeast, ultraviolet rays

The number of secondary growth colonies formed varied: 96 pcs (180 s), 215 pcs (240 s), 316 pcs (300 s) (Table 1).

Table 1 - Frequency of formation and size of secondary colonies

Inductor of secondary growth of colonies yeast	Number of secondary colonies	
	Размеры, мм	
	1-2	3-4
$CuSO_4$, mg / ml		
0,1	0	0
0,05	24	62
0,025	95	69
0,0125	112	183
0,0062	0	0
Ultra violet rays, sec		
180	96	0
240	215	0
300	316	0

IV. CONCLUSION

As a result of the conducted experiments it was established that the largest number of secondary growth colonies is formed under the action of ultraviolet rays for 300 seconds and makes up 316 colonies per Petri dish. Subsequently, secondary growth cultures isolated in the course of this experiment will be tested for the presence of production-valuable qualities and used for work on intensification of ethanol production in production conditions.

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