

Short Reports

**INFLUENCE OF CONDITIONS OF
INOCULATING MATERIAL PREPARATION
ON ACCUMULATION OF AROMA
BUILDING SUBSTANCES IN CULTURE
OF EREMOTHECIUM ASHBYI GUILL**

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The ascomycete *Eremothecium ashbyi* is known as an overproducer of riboflavin and essential oil, which basic aroma building components are geraniol and β -feniletanol [1, 2, 4, 5]. It is known that the inoculation of fermentative nutrient medium should be made by freshly sprouted fungus spores for achievement of maximal riboflavin efficiency of *E. ashbyi* [3]. The influence of inoculating material morphophysiological state on formation of essential oil components has not been investigated yet.

Material and methods. The strain *E. ashbyi* VKM F-3009 served as an object of researches. An inoculating material was cultivated on liquid nutrient mediums of various composition (g/l): soya-saccharose (a soya flour – 20; sucrose – 20, pH 7,0) and glucose-peptone (glucose – 7,5; peptone – 4,0; sodium succinate – 2,0; K_2HPO_4 – 0,5; inositol – 0,14, pH 6,5) at continuous shaking within 24-72 hours. Fermentation was carried out on a soya-saccharose liquid nutrient medium within 55 hours. The volume of inoculant was from 1 up to 5% (v/v). The aroma building substances were extracted from cultural liquid by diethyl ether and were analyzed by gas-liquid chromatography with use of the internal standard [1].

Results and discussion. The fermentation results, received at cultivation of an inoculating material of various age, are presented in the table 1. It is visible

from this evidence that the cultivation of an inoculating material during 2–3 day was favorable for accumulation aroma building substances. The microscopic analysis has shown that at this moment the fungus mycelium is presented by strongly expanded hyphae (diameter 12-16 microns) with greater number vacuoles and numerous inclusions. This corresponds to a stationary growth phase of culture. Thus, the glucose-peptone inoculating medium provided higher output of essential oil components in comparison with soya-saccharose. The greatest level of accumulation of aroma building substances has been reached in those variants where inoculant was brought in the fermentative medium in quantity of 5% (v/v).

The lead experiments have not revealed connection between spores building activity of inoculating culture and results of fermentation. So, the spores quantity increased from 0,2–0,5 up to 6,0–7,0 million/ml after addition of a barmy extract to glucose-peptone inoculating medium. However, it did not lead strengthening of essential oil accumulation during fermentation (accordingly $121,8 \pm 4,2$ and $109,9 \pm 27,5$ mg/l).

Thus, according to the received data the optimal inoculating material for production of essential oil by *E. ashbyi* is the producer culture which has been grown up on glucose-peptone medium within three days (a stationary growth phase), brought in the fermentative medium in quantity of 5% (v/v). The resulted indexes do not coincide with recommended for industrial conditions parameters of *E. ashbyi* inoculating material for maximal synthesis of riboflavin (freshly sprouted spores) [3]. These data should be considered at elaboration of biotechnology of aromatic products reception on the basis of *E. ashbyi* cultivation.

Influence of period of inoculating material cultivation on biosynthetic activity

Inoculating nutrient medium	Period of inoculant cultivation, day	Content of main aroma building components, mg/l of cultural liquid	
		geraniol	β -feniletanol
Soya-saccharose	1	45,9	4,0
	2	44,3	25,6
	3	46,9	25,0
Glucose-peptone	1	47,2	12,4
	2	52,2	18,7
	3	65,8	28,6

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