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У збірнику представлено наукові праці вітчизняних та зарубіжних спеціалістів, написані спеціально для даного видання, присвяченого 200-річчю від дня народження Ч. Дарвіна та 125-річчю від дня народження І.І. Шмальгаузена. В оглядових і експериментальних статтях наведено дані з основних напрямів генетико-біотехнологічного розширення генетичної мінливості живих організмів, генетики господарсько-цінних ознак рослин і тварин, сучасних методів біотехнології і генетичної інженерії при створенні нового покоління сортів і гібридів культурних рослин, ДНК-технологій і молекулярних маркерів у селекції рослин і тварин, генетики людини та медичної генетики; результати аналізу та оцінки генетичних ресурсів.

Для спеціалістів у галузі генетики, селекції, біотехнології, екології, а також викладачів і студентів вищих навчальних закладів III–IV рівнів акредитації.

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RHIZOPUS sp. 2000 FM — THE ACTIVE FUNGI EXOLIPASE PRODUCER

Some physiological-biochemical properties of the new fungus strain Rhizopus sp. 2000 FM being active source of exolipase. The highest levels of lipolytic activity (LA) were obtained at the fungus cultivation at the substrate with: sun-flower oil, sucrose and with starch. The LA evaluation was performed with the use of the spectrophotometric method and n-nitrophenyl-palmitate as a chromogenic substrate. This method was approved experimentally in the Department of Physiology and Systematic of Micromycetes, D.K. Zabolotny Institute of Microbiology and Virology National Academy of Sciences of Ukraine (NASU). We have not established the direct correlation between the level of biomass

accumulation and LA level. The data obtained on the studies of carbon nutrition on the growth and LA of the new perspective source of lipase allows to optimize its nutrition substrate in future. Selection work on the fungi cultures creation and modification to use them as the sources of the ferments with the new properties is one of the most significant biotechnology direction. Among the variety of the known ferments only few have the same perspectives as the lipases do.

The lipolytic ferments could be determined as he hydrolases of the fat acids' esters with the long chain. The substrates for the lipases action are the lipids. According to the Ferments Nomenclature, the lipase has the name of triacylglycerolhydralase (KF 3.1.1.3). Lipases are the ferments of the surface action and activize being localized on the surface of the sub-strate non-dissolvable in water [1]. Being the natural, fats' splitters, the lipolytic ferments are very interesting for those branches of industry, where the total or partial hydrolysis of fats and oils is needed and for the medical and industrial branches of application [2]. Lipases are widely used in food industry (cheese production and non-alcoholic drinks); in confectionery for chocolate and caramel production: in flour-milling and bakery for bread quality amelioration and its storage term prolongation. Lipases will find application in the technology creation of essential fat acids for food and drugs production [2]. Lipolytic ferments could be used as well for esterification and re-estenfication of fat acids in glycerins, what, in turn, opens wider possibilities for creation of the fat products with needed functional properties. New technologies with the use of immobilized microbial lipases are being introduced. Lipases are actively used in medicine as a therapeutic means for the gastroenterological diseases and in medical diagnostics [4].

The lipases' significance is high both in cosmetics, in fur and skin industries for bettering the elasticity of the products and excellent natural appearance, in natural silk production for fat removal through its hydrolysis with the use of lipolytic fermentative preparations. The need in thermophilic lipases' sources of microbial origin is high in textile industry as well where the wax type substances are removed by lipases under the temperatures in the range from 40 °C to 60 °C. The introduction of the thermostable lipases into national industry of washing products is not less perspective for the fats removal from waste waters, especially for the canalization communications and wastes processing. Lipases could be successfully used in agriculture for the development of animal food preparation with the scope of metabolism bettering [1, 3, 5].

Nowadays, the lipolytic ferments production in Ukraine is not organized, and, thus, the creation of the competitive national technology of these compounds is an actual. The determining and limiting factor in the lipolytic ferments' production is the lack of the stable and productive microorganisms — the producents, which could be introduced into technological circle effectively. The fungi, in difference from bacteria, which accumulate mainly the inner cell lipase produce mainly the out-cell ferment [6].

The significant input to the experimental mycology was made by the staff members of the Department of Physiology and Systematic of Micromycetes, D.K. Zabolomy Institute of Microbiology and Virology, NASU, (DPSM-

Zabolotny-NASU) who perform the research on the extraction and identification of fungi isolates from various substrates [7]. One of the main directions of the biologically active fungal metabolites studies is the estimation of the conditions of their effective biosynthesis and demonstration their properties.

The scope of the work was the research of physiologyical and biochemical properties of the new active producer of out-cell lipase — Rhizopus sp. 2000 FM, especially, of the influence of the carbon nutrition sources on the activity of the fungi produced ferment.

Materials and methods

The object of the research was the thermotolerant fungus Rhizopus sp. 2000 FM. The deep cultivation was performed on the nutritive substrate under investigation during 72 hours at the temperature of 39–40 °C. For Rhizopus sp. 2000 FM cultivation the nutritive substrates with Chapek ambient were used with the addition of different carbon sources in various concentrations. The influence of the carbon nutrition components on LA and fungus Rhizopus sp. 2000 FM growth was studied experimentally through the change of carbon sources in equivalent relation. The following plants oils were used as the carbon sources: sunflower, olive, lemon, castor, raps, walnut, maize; carbohydrates (glucose, arabinose, maltose, xylose, sucrose, lactose, starch); polyatomic alcohols (inosit, sorbit, douleit) and surface-active compound (SAC) — twin-20.

One of the causes met by researchers studying the microorganisms' ability to exolipase synthesis is the absence of the express and correct method of LA evaluation. Spectrophotometric method (SPM) used in the present work for LA evaluation was approved at the DPSM-Zabolotny-NASU.

The reasoning of the SPM: the micromycetes cultural liquid's lipase action on chromogenic substrate -phenolic ester of palmitate acid with the release of n-nitrophenole (n-NP) as a result of reaction what, in turn, stimulates the changes of optical density of the reaction mixture what is registered by spec-trophotometer at the wavelength of 410 nm. As the unit of LA was taken such quantity of the ferment in 1 ml which catalizes the freeing of 1 nM of n-NP from the emulgated substrate during 1 minute at 3 °C. The level of the biomass accumulation in the cultural ambient was detected by weighting method [9].

Results and discussion

During research, the intensive growth of aerobe culture was detected on mash agare. It is isolated from the soil specimen. The strain creates white colonies with light yellow coloring. The fungus is the thermotolerant one with temperature range of the growth from 19 °C to 50 °C. Temperature optimum of the growth is 36–39 °C speculation 40–42 °C.

The results obtained during the cultivation of the fungus Rhizopus sp. 2000 FM on the nutritive substrate with different oils as the carbon sources in various concentrations have shown that the highest level of LA for the fungus is achieved on the substrate with the sunflower oil (1 %). It was shown that the presence of the oils: sunflower, castor, walnut taken in concentration induces the exolipase synthesis by fungus Rhizopus sp. 2000 FM.

Substantial LA was detected on the substrates with the use of sucrose and starch. During the Rhizopus sp. 2000 FM cultivation on the substrates with polyatomic alcohols and with twin-20 as the carbon sources, the low growth and the LA absence were registered. The fungus Rhizopus sp. 2000 FM strain under the cultivation on the studied sources has demonstrated moderate growth rate. The greatest biomass accumulation was registered during the fungus cultivation on the substrate with the addition of 1% of walnut oil at the comparatively low LA. The direct correlation between the biomass accumulation and the LA was not established. The culture growth on the substrate with the addition of sunflower oil was accompanied by medium oxidation from 7.0 (pH of the medium before cultivation) to 5.0.

The experimental results support the fact that the sunflower oil is the effective inductor of exolipase biosynthesis. The high values of LA demonstrated on the medium with sucrose and starch addition are exponents of the physiological and biochemical properties of the strain Rhizopus sp. 2000 FM.

Maximal activity is registered for the substrate with addition of the 1% sunflower oil. Further increase of this oil caused the decrease of the fermentative activity, thus, one could consider the sunflower oil to be not only the inductor but also the represser of the lipase biosynthesis. What concerns the growth and biomass accumulation we could state that the addition of the significant quantity of the sunflower oil, stimulates the growth of the micromycete Rhizopus sp. 2000 FM.

The data on the growth rate and LA of the fungus strain Rhizopus sp. 2000 ФМ on the carbon sources, along with the detection of other components of the medium, is believed to support the optimization the content of the nutritive medium for the cultivation of the new active exolipase producent — fungus Rhizopus sp. 2000 FM.

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