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METHODS OF ISOLATION, IDENTIFICATION AND CULTIVATION OF MICROORGANISMS FOR OBTAINING BIOLOGICALLY ACTIVE SUBSTANCES

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Abstract:

Microorganisms are promising for use in pharmaceutical and food production. Their use can have a significant impact on the development of many biotechnological processes, and is also of practical importance in developing rules for long-term storage of cell cultures at sub-zero temperatures. Microorganisms were isolated, identified and cultivated to obtain biologically active substances. The results showed significant enzymatic potential of the tested microorganisms and demonstrated their suitability for biotechnological applications.

Keywords: *Microorganisms, identification, cultivation, nutrient medium, LB, physiological and biochemical properties, isolation.*

Introduction

Introduction: In general, biotechnology and its separate departments are one of the most priority directions of scientific and technical development and are an example of "high technologies" associated with the prospects of improving many industries. The development of modern society cannot be imagined without various biotechnological processes in which microorganisms, their components or waste are not produced or used [9,10,19].

Microorganisms from extreme habitats with the ability to produce biologically active substances active in a wider temperature and pH range compared to mesophilic prototypes are of particular importance to the microbiology industry and scientific research, and the creation of recombinant superproducers that do this is a valuable source of genes [2,4].

Soil samples (soil, sediment) were homogenized in lime in physiological solution, then ultrasonic treatment of samples (from 10-20 seconds to 3 minutes) was used to better desorb microbial cells from soil particles. The resulting suspension was left to settle for 30 seconds, its tenfold dilution was prepared, sprinkled in containers with nutrients and incubated at different temperatures: from 4 to 60°C, depending on the purpose of the experiment [17].

Research methodology: Conditions for isolation and cultivation of microorganisms. The studied samples were planted in nutrient medium with different content: liquid and agar LA medium (Difco, USA); starch-ammonia agar (KAA, composition (g/l): soluble starch- 10, $(NH_4)_2$ SO_4 - 1, $MgSO_4$ x7H_2O - 1, NaCI - 1, $CaCO_3$ - 3, agar 20). In addition, LB diluted 5-10 times with distilled water was used (liquid or agar) and different pH values of the nutrient medium were used during cultivation (5.0; 7.0; 9.0, etc.). From 6 to 60°C, morphologically distinct colonies grown were used to obtain pure cultures [21].

Work on isolation of microorganisms was carried out with the help of sterilized tools, nutrient media and solutions. The obtained soil samples, in addition to the above-mentioned medium, were planted in a nutrient medium consisting of a suitable water sample sterilized by 0.2 micron pore size nitrocellulose filters and 1.6% agar filtration [8].

Counting of the number of inoculated and cultured colonies was performed within 1-4 weeks. Separate colonies that appeared on the surface of the agar medium and differed from each other in terms of morphological characteristics were transferred to a new nutrient medium and incubated under similar conditions.

Analysis and results: Studying the morphological characteristics of microorganisms. Morphological features of colonies were studied visually and with the help of Stemi 2000-C electric light microscope (Carl Zeiss, Germany). Cell staining was determined by the Gramm staining method (Grcgersen 1978).

Determination of physiological and biochemical properties includes studying the growth of strains at different pH values and ambient temperature, NaCl concentration, presence of lysozyme, aerobic or anaerobic conditions. If necessary, indirect tests for the pathogenicity of the strains were carried out, and the sensitivity to antibiotics was determined by the use of nutrient agar surface discs with the test microorganism produced by the Scientific Research Center of Genomics (Tashkent, Uzbekistan). The amount of antibiotics in 1 disk: for rifampicin - 5 μg, penicillin, oxacillin, ampicillin and gentamicin - 10 μg, oleandomycin, erythromycin, lincomycin - 15 μg, streptomycin, neomycin, monochlorinam, tecin, monocinam - 30 μg, carbenicillin - 100 μg, polymyxin for - 300 pcs.

In the study of biochemical properties, the use of different carbon sources, urease, catalase and oxidase, indole, hydrogen sulfide, acetoin formation, nitrate reduction, casein, gelatin, starch hydrolysis, tyrosine degradation, phenylalanine deamination ability and other distinguishing characteristics according to the determinants of bacteria were revealed.

Screening of strains for lipolytic activity was performed using LB agar medium. The relative activity of the enzyme was determined by the ratio of the size of the colony diameter and the diameter of the lipolytic activity manifestation zone.

Cultivation conditions for BFM-producing strains

Strains were grown in a thermostatic shaker (KT 104, Russia) nutrient media: LB (Difco), PS-1 (peptone - 10 g/l, yeast extract - 5 g/l, NaCl - 10 g/l) and PS-2 (sprate hydrolyzate - 35 g/l, yeast autolysate - 5 g/l), BSPR medium (peptone (Difco) - 20 g/l, yeast extract (Difco) - 10 g/l, K_2HPO_4 - 2 g/l). l l, NaCl - 5 g/l, MgSO4x7H_2O - 2 g/l, glucose up to 1%, as well as PS-3 medium with increased yeast extract (peptone - 10 g/l, other extract - 10) g / l, NaCl - 5 g / l). The pH value of all environments is 7.0-7.2; if necessary, glucose up to 1% was added to the culture medium.

Agar and liquid nutrient media were inoculated using inoculum with a cell concentration of 1-5 < 109 cells/ml. Aeration mode, cultivation time and temperature are selected separately for each culture. The growth rate of the strains in the liquid nutrient medium was determined by the optical density (OD1) of the culture liquid (KS) at 550 nm using an SF-46 spectrophotometer (Russia). Each experimental option was carried out in three parallel repetitions, the average values of the obtained data were used for analysis. Biomasses were collected by centrifugation at 6000 rpm, except for K. pneumoniae cells (14000 rpm) and stored at minus 60°C until use.

Conclusion

In conclusion, this study highlighted the level of viability and release of biologically active substances of microorganisms selected for biotechnological processes. Evaluation of the activity of these microorganisms in the nutrient environment provides valuable information about their suitability for various industrial processes.

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