PRODUCTION OF ANTISTAPHYLOCOCCAL ANTIBIOTIC BATUMIN UNDER CONDITIONS OF BATCH CULTIVATION PROCESS

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Abstract

This work is aimed at studying the cultivation process of Pseudomonas batumici, and particularly on the yield of batumin – secondary metabolite with antibacterial activity. It also focuses on determining the activity of obtained batumin extract, separation of compounds by thin layer chromatography with subsequent bioautography for the identification of said compounds and their activity.

Keywords: *batumin, batch culture process, Pseudomonas, chromatography, bioautography.*

Introduction. Bacteria of the genus *Pseudomonas* are known for the biosynthesis of a number of biologically active compounds that are widely used by humans in agriculture, medicine, food, cosmetics, and for the biodegradation of a number of compounds [1]. The metabolites produced by pseudomonads include peptides, enzymes, lipopeptides such as syringomycin and syringopeptin, polyketides (pseudomonic acid A), fatty acid derivatives, and others [2]. In agriculture, *Pseudomonas* species are a component of biofertilisers due to their antagonistic properties and symbiotic relationships with plants, which significantly increases crop productivity [3].

Metabolites of pseudomonads with antimicrobial properties are of interest to medicine and include mupirocin, batumin, salicylic acid, pyrrolnitrin, safracins, and phenazines. Staphylococci are opportunistic human pathogens, with many species being resistant to penicillins and other antibiotics, which makes it difficult to treat them. Therefore, the search for and study of compounds that show activity against staphylococci is of high priority. Batumin, which is a secondary metabolite of *P. batumici*, is a polyketide antibiotic. It is characterised by high activity and selectivity against staphylococci [4]. Batumin can be obtained by batch cultivation. The aim of this work was to carry out periodic batch cultivation process in order to obtain the batumin extract and to determine its properties.

Materials and methods. The strain *Pseudomonas batumici* UCM B-321, obtained from the Ukrainian Collection of Microorganisms, was chosen as the production strain of the antibiotic compound batumin. It was stored at room temperature on meat peptone agar and subcultured every 7 days at 28 °C for 24 hours. Fermentation was carried out by periodic batch cultivation in 250 ml Erlenmeyer flasks on a shaker set to 150 rpm at 22-24 °C for 96 hours. The medium contained glucose, urea, and salts of microelements. The total volume of the medium was 600 ml with a working volume of 50 ml. After cultivation, the culture fluid was tested for antimicrobial properties by the agar well diffusion method (in 100 and 1000-fold dilutions of the culture fluid). *Staphylococcus aureus* UCM B-918 was used as a test culture. Chloroform (1:2) was used to obtain batumin extract. The obtained extract

was separated by thin-layer chromatography in the system benzene: isopropyl alcohol (5:1). Merck TLC Silica gel 60 F254 plates (USA) were used, and the substances were developed with iodine vapour. Bioautography was performed by applying the plates to meat peptone agar inoculated with *Staphylococcus aureus* B-918. The data obtained was statistically processed (confidence level p = 0.05).

Results and discussion. After cultivation and purification, a brown, odourless extract was obtained. The total extract yield was found to be about 400 mg/l. Previous studies of batumin biosynthesis using a fermenter showed antibiotic yield of 175-180 mg/l [5]. Most likely, this difference in the final concentrations of batumin can be explained by more favourable conditions for the biosynthesis of the antibiotic in flasks on shakers, in particular, the absence of active stirring of the culture liquid with a stirrer and a slightly lowered temperature of cultivation to 22–24 °C.

When testing for antagonistic properties, it was found that the culture fluid diluted 100 and 1000 times inhibited the growth of *S. aureus*, with growth inhibition zones of 27,8 ± 1,62 and 13,2 ± 2,39 mm, respectively. The presence of three compounds with retention factors Rf_1=5.6 Rf_2=7.1 Rf_3=12.5 was determined by TLC. Bioautography confirmed that compound №2 is batumin (growth inhibition zone 42 ± 2,25 mm). Compound №1 also showed antimicrobial properties (growth inhibition zone 23,75 ± 4,38 mm), while compound №3 did not exhibit inhibition. Based on the literature [6], it was concluded that compound №1 is descarbamoylbatumin, natural derivative of biosynthesis and exhibits weaker antimicrobial properties against staphylococci.

Conclusions. In the course of this research, a batch cultivation process of *Pseudomonas batumici* UCM B-321, a producer of the antistaphylococcal antibiotic batumin, was carried out. The obtained antibiotic was characterised by its physicochemical and biological properties.

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