Microbial transformation of bikaverin, a metabolite produced by fungi

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Summary. Five of 40 screened cultures of microorganisms transformed bikaverin added into their cultivation media. Aspergillus sclerotiorum was used in large-scale experiments. Five metabolites were detected in a chloroform extract of A. sclerotiorum. One of them was isolated and identified as (Z)-5-methyl-2-methoxy-4-oxopent-2-enoic acid *3*. Theoretically, metabolite *3* can arise directly by transformation of bikaverin by A. sclerotiorum. But, we do not exclude also the second alternative that the metabolite *3* is formed by A. sclerotiorum via biosynthesis and that this process can be induced by bikaverin.

Bikaverin *1* is a microbial metabolite isolated from Fusarium oxysporum [2, 7, 10], Fusarium lycopersici and Fusarium vasinfectum [3, 11] as well as from Gibberella fujikuroi [1, 3, 5] and Mycogone jaapii Lindau [12]. Bikaverin inhibits the growth of protozoa Leishmania braziliensis [1], biochemical functions and growth of various type of tumors [4, 5] and also influences morfogenesis of fungi [3]. In HeLa and P388 leukemic cells *1* inhibits mitochondrial functions, synthesis of nucleic acids, while in animal erythrocytes bikaverin caused damage of membrane of cells and their lysis [5, 9]. In animals *1* caused serious disease ill-trift.

Because *1* was found very often in nature and there is a possibility to be transfered into food-chain we studied its transformation by microorganisms.

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Material and methods

Bikaverin was isolated from G. fujikuroi in our laboratories [1, 5].

Fermentation methods. In our screening programm we evaluated 40 cultures. Five of them: Aspergillus sclerotiorum (ATCC 11395), Sepedonium chrysospemum (ATCC 13378), Aspergillus niger (X-172), Streptomyces griseus (ATCC 10137) and Micrococcus gypseum (ATCC 11395) transformed bikaverin present in cultivation media. A. sclerotiorum was used in large scale for biotransformation. The microorganism was grown in a two-stage fermentation procedure [6] in a soybean meal-glucose medium of the following composition: soybean meal 5 g, glucose 20 g, K_2HPO_4 5 g, NaCl 5 g, yeast extract 5 g, water 1000 ml. The medium was adjusted to pH 7.0 before being autoclaved at 121 °C for 15 minutes. Fermentations were conducted in 500 ml cotton-plugged Erlenmayer flaks holding 100 ml of medium on rotary shaker operating at 200 rpm at 28 °C. Bikaverin as a substrate was added to 24-hr old second-stage cultures as a 10 % solution in dimethylformamide (DMF) to a final concentration in the medium of 0.5 $mg.ml^{-1}$. Samples (4 ml) of substrate-containing cultures were taken at various time intervals, extracted with 1 ml of chloroform, pH adjusted to 2.5, and 30 μl of the extracts were examined for metabolites by TLC.

Conversion of bikaverin to **2** and **3**. A total of 1.0 g of bikaverin in 10 ml of DMF was evenly distributed into of 20—500 ml flasks containing 100 ml of 24-hr old stag-two cultures, and the formation of metabolites was investigated by TLC analysis. After 168 hrs, the fermentation beer including mycelia was combined, pH adjusted to 2.0 and metabolites were exhaustively extracted with chloroform. The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness. The resulting solids were dissolved in a minimum volume of chloroform, applied to a silica gel column and eluted gradually with chloroform, chloroform : methanol and methanol alone, in 10 ml fractions. Metabolites **2** and **3** were finaly purified by TLC and crystallized from benzene : n-hexane.

Chromatography (TLC). Thin-layer chromatography was performed on nonactivated silica gel (Silufol UV 254, Kavalier, ČSSR) plates which were developed in chloroform : methanol : acetic acid (94 : 1 : 4) and chloroform : : methanol (9 : 1). The metabolites were detected in UV light at 254 and 366 nm or visualised by spraying with $FeCl_3$ solution (1 % in methanol) and (or Dragendorff reagent.

Results and discussion

Nine metabolites were detected in a chloroform extract obtained from fermentation beer in which bikaverin was present during the growth of A. sclerotiorum (Table 1). Three metabolites with R_f values 0.08, 0.2 and 0.58 were

Tab. 1. Metabolites detected in a chloroform extract of A. sclerotiorum

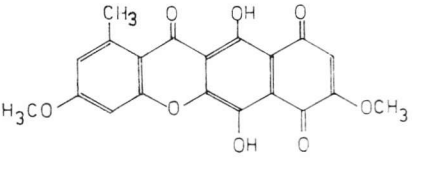
R_f	Metabolites and their colour in UV light at	
	254 nm	366 nm
0.08§	blue-violet	blue fluorescence
0.08	dark gray	dark blue
0.18	dark blue	faintly rose
0.20§	not detected	violet fluorescence
0.23	faintly rose	violet fluorescence
0.29	not detected	gray blue
0.44	dark blue (bikaverin)	red brown
0.52	faintly rose	bright blue fluorescence
0.58§	blue	blue fluorescence

§ Metabolites produced by A. sclerotiorum also in the absence of bikaverin

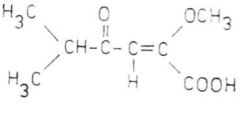
found also in the chloroform extracts obtained from fermentation beer into which bikaverin as a substrate was not added. Two metabolites **2** and **3** were isolated after the solids obtained from evaporated extracts were separated on silica gel column. Metabolite **2** is a substance of faintly orangered colour, m. p. 337—338 °C and was identical with the metabolite having R_f 0.08. The small amount of **2** which was isolated from solids after biotransformation of bikaverin was not sufficient for its structural analysis.

Metabolite **3** is a white substance and it provided the following physical data: m. p. 68—72 °C uncorrected, M. W. 172 for $C_8H_{12}O_4$ calculated: C 58.81, H 7.02, found: 55.76, H 7.08. Using the ^{1}H-NMR, ^{13}C-NMR and mass spectra the metabolite was identified as: (Z)-5-methyl-2-methoxy-4-oxopent-2-enoic acid and its structure is as follows:

1



3



The metabolite **3** was not detected in the fermentation beer into which bikaverin was not added as a substrate. In spite of that it is not possible to pressume unambiquously that **3** is a product arising from bikaverin after its direct spliting by A. sclerotiourm. For this demonstration a labelled molecule of bikaverin had to be used as a substrat. Therefore we also admit that the formation of the metabolite **3** in A. sclerotiorum could be induced by bikaverin added into a cultivation media.

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Микробиальное преобразование бикаверина, метаболита, производимого грибами

Резюме

Пять из сорока проверенных культур микроорганизмов преобразовало бикаверин, добавленный в культурную среду. Для биопреобразования применен в крупном масштабе *Aspergillus sclerotiorum*. В хлороформных экстрактах упомянутой культуры мы обнаружили пять метаболитов. Один из них был изолирован и идентифицирован как (3)-5-метил-2-метокси-4-оксопент-2-еновая кислота (**3**). Теоретически может метаболит **3** возникнуть непосредственным преобразованием бикаверина с помощью *A. sclerotiorum*. Не исключена однако и вторая альтернатива, что метаболит **3** образует *A. sclerotiorum* с помощью биосинтеза, причем этот процесс может индуцирввать бикаверин.

Mikrobiálna transformácia bikaverínu, metabolitu produkovaného hubami

Súhrn

Päť zo 40 overených kultúr mikroorganizmov transformovalo bikaverín, pridaný do kultivačného média. Na biotransformáciu vo väčšom rozsahu sa použil *Aspergillus sclerotiorum*. V chloroformových extraktoch uvedenej kultúry sme detegovali päť metabolitov. Jeden z nich bol izolovaný a identifikovaný ako kyselina (Z)-5-metyl-2-metoxy-4-oxopent-2-énová (*3*). Teoreticky môže metabolit *3* vzniknúť priamou transformáciou bikaverínu pomocou *A. sclerotiorum*. Nevylučujeme však ani druhú alternatívu, že metabolit *3* tvorí *A. sclerotiorum* biosyntézou, pričom tento proces môže byť indukovaný bikaverínom.