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Biosynthesis of avermectins and lipids in *Streptomyces avermitilis*

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1. SUMMARY

A quantitative and temporal correlation was found between the production of avermectins and lipids in *Streptomyces avermitilis* grown in defined media. The lipid fractions contained mostly triglycerides and phospholipids. A significant increase in the content of avermectins and triglycerides was observed during the stationary phase. The syntheses of both of these compounds stopped simultaneously with the depletion of glucose from medium. The results indicate that systems synthesizing avermectins and fatty acids could be competing for common precursors.

2. INTRODUCTION

Streptomyces avermitilis is known to produce a mixture of eight avermectins, 16-membered macrolides exhibiting anthelmintic and insecticidal activities [1–3]. Avermectin types A₂a, A₁a, B₂a and B₁a are the most common. Their lactone ring

is formed from one methylbutyrate, seven acetate and five propionate units [4]. A disaccharide formed by two oleandrose molecules is glucosidically linked to the C-13 of the lactone ring.

A lipid-rich fraction is obtained during the isolation of avermectins. This fraction contains mostly triglycerides with fatty acids typical of streptomycetes: iso-even and -odd, anteiso-odd and straight-chain fatty acids in the range of C₁₄ to C₁₈ [5]. The regulation of the metabolism of building units of fatty acids and avermectins is a suitable model for providing insights into the relationship between primary and secondary metabolites [6–9] and may give information that can be used for the selection of production strains [10,11].

The present paper describes a quantitative correlation between the production of avermectins and fatty acids and the dynamics of their biosynthesis during cultivation.

3. MATERIALS AND METHODS

3.1. Organism, media and growth conditions

A mutant strain of *S. avermitilis* (C-18) obtained by the improvement of the strain ATCC 31267 was used [12]. The inoculum was prepared by using a defined medium [12] containing glucose

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(3%) and ammonium sulfate (0.2%) as the carbon and nitrogen sources. Glucose and ammonium sulfate concentrations used in the synthetic production medium are mentioned in the text. The cultures were grown in 500-ml flasks containing 50 ml of medium at 28°C on a rotary shaker (2.8 Hz).

3.2. Analytical methods

The dry cell mass and the content of lipids in the extract were estimated gravimetrically. Glucose was determined spectrophotometrically by using an enzyme-based test (Lachema, Brno, Czechoslovakia). The production of avermectins was determined by TLC [12]. The lipids were extracted from 20 ml of the culture filtrate by 20 ml of Folch reagent (chloroform:methanol, 2:1) for 1 h at 28°C by using a reciprocal shaker. Fatty acids in the form of methylesters were determined by capillary gas chromatography-mass spectrometry [13]. The lipids were separated and

quantified using an Iatroscan analyzer (TLC-FID) [14].

4. RESULTS AND DISCUSSION

After a 10-day cultivation of *S. avermitilis* grown in a defined medium containing various concentrations of carbon and nitrogen sources, the content of avermectins and lipids was measured and the composition of fatty acids analyzed. A quantitative correlation between the production of avermectins and of lipids was found; the high yield of both was observed in the medium containing 0.2% ammonium sulfate and 9% glucose (Fig. 1). The main components of the lipid fraction were triglycerides and phospholipids containing fatty acids ranging from C₁₄ to C₁₈ (Table 1). The terminally-branched fatty acids amounted to 60 to 70%, the even straight-chain fatty acids to 20 to 30%, whereas the odd straight-chain fatty acids

Table 1

Composition of the main fatty acids in *S. avermitilis* after 10 days of cultivation in a medium containing various amounts of glucose and ammonium sulfate (as listed in the legend of Fig. 1). Each sample is the average from 3 flasks.

FA ^a	Sample number					
	1	2	3	4	5	6
i-14:0	5.0	4.9	2.6	5.2	2.3	2.8
14:0	1.7	1.8	1.7	1.8	1.3	1.6
i-15:0	7.9	4.8	5.4	5.7	5.6	5.9
ai-15:0	28.0	23.1	24.3	20.0	28.8	19.6
15:0	3.3	5.2	2.6	4.1	2.9	3.2
i-16:0	18.8	21.1	18.1	22.6	16.3	18.8
16:1	5.0	5.2	6.2	6.6	5.9	7.4
16:0	14.5	19.0	14.6	19.7	12.7	18.6
i-17:0	2.7	1.7	4.8	2.2	5.5	3.6
17:1	1.9	1.5	2.2	1.7	1.9	2.8
ai-17:0	9.7	9.3	14.4	8.6	14.8	13.4
17:0	0.7	1.1	1.1	0.8	1.0	1.3
18:1	0.8	0.7	2.0	0.7	1.0	0.6
18:0	0	0.6	0	0.3	0	0.4
FA i _e	23.8	26.0	20.7	27.8	18.6	21.6
i _o	10.6	6.5	10.2	7.9	11.1	9.5
ai	37.7	32.4	38.7	28.6	43.6	33.0
n _e	22.0	27.3	24.5	29.1	20.9	28.6
n _o	5.9	7.8	5.9	6.6	5.8	7.3

^a Relative proportions of the individual fatty acids in the sample (mass %): i, isoacid; ai, anteisoacid; e/o, even/odd. The first number indicates the number of carbon atoms, the second the number of unsaturated bonds in the molecule.

amounted to only about 7%. The relative proportion of the individual groups of fatty acids was influenced by the concentrations of the carbon and the nitrogen sources. While the relative proportions of the iso-even and the even straight-chain fatty acids increased at a higher concentration of glucose, those of the anteiso fatty acids and the iso-odd fatty acids decreased under the same conditions. The proportion of the odd straight-chain fatty acids was almost constant.

The composition of the lipid fraction isolated at the end of the cultivation is in keeping with the previously published results obtained with another strain [5]. Even in the strain *S. avermitilis* C-18, triglycerides exhibiting a high content of the terminally branched fatty acids were detected as the main component of the lipid fraction. These branched fatty acids are typical of streptomycetes [9,10,15,16].

During cultivation of *S. avermitilis* in the defined production medium (3% glucose, 0.2% ammonium sulfate), growth, production of avermectins, lipids and the proportion of phospholipids and triglycerides were measured (Fig. 2). The concentration of the mycelial biomass and the content of phospholipids increased until 6 days of cultivation and then gradually decreased. The amount of

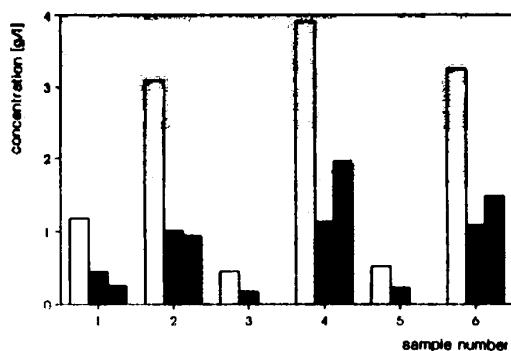


Fig. 1. The production of total lipids(□), ai-FA(▨) (both g/l) and avermectins (■) (g/l $\times$ 10) by *S. avermitilis* grown for 10 days (harvested at the end of production phase) in chemically defined medium under different conditions: Sample nos. 1, 3, 5, 1% glucose; 2, 4, 6, 9% glucose; 1, 2, 0.05% ammonium sulfate; 3, 4, 0.2% ammonium sulfate; 5, 6, 0.5% ammonium sulfate. Each sample is the average from 3 flasks.

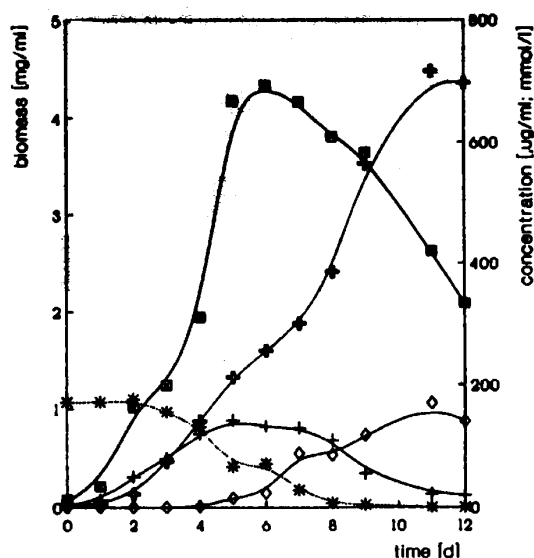


Fig. 2. Growth (■) (mg/ml), production of avermectins (◇) (μg/ml), triglycerides (◆) (μg/ml) and phospholipids (+) (μg/ml) and glucose consumption (●) (mmol/l) during cultivation of *S. avermitilis* in the defined medium (3% glucose, 0.2% ammonium sulfate). Results of one out of two experiments are shown. Samples were taken from 2 to 5 flasks.

triglycerides increased up to 11 days of the cultivation. The major part of triglycerides (65%) was synthesized during idiophase (production phase after 6 days of the cultivation).

Avermectins were detected only after 4 days of cultivation and their synthesis ceased on the 11th day when glucose became depleted from the medium. The content of avermectins tended to decrease during additional cultivation, while the amount of total lipids did not change (J. Novák, unpublished results).

Significant amounts of avermectins and triglycerides were formed under the conditions when nitrogen source was almost completely depleted from the medium but sufficient glucose was still present (J. Novák, unpublished results). Thus it would be unlikely that degradation of anteiso fatty acids provides the 2-methylbutyrate units for the synthesis of "a" type avermectins. Therefore, it was not clear if, for example, 2-methylbutyrate (derivable from isoleucine), is exclusively a de-

gradation product of amino acid catabolism or, more probably, whether it is formed from de novo synthesized amino/keto acids. However, results from preliminary experiments with mutant strains differing in their ability to utilize isoleucine have suggested that at least in defined media (with glucose and ammonium sulfate), the 2-methylbutyrate used as the starting unit of both avermectins and anteiso fatty acids originated from a de novo synthetic pathway (J. Novák, unpublished results).

The fact that triglycerides and avermectins are synthesized at the same time indicates that these two biosynthetic pathways may have to compete for a limited supply of precursors (e.g. 2-methylbutyrate, propionate and acetate building units, etc.). This could have serious implications for the yield of avermectins that can be obtained.

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