

Effect of methyl oleate and Tween 80 on the antibiotic productivity and the fatty acid composition of the total lipids of *Streptomyces hygroscopicus* CH-7

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The effects of methyl oleate and Tween 80, hexaene H-85 production stimulators, on the fatty acid composition of the total lipids of *S. hygroscopicus* CH-7 were investigated. Besides oleic acid in the biomass, an increase of the branched chain fatty acid contents was observed: from 1.6 to 2.1 fold in the medium with methyl oleate and 2.5–2.9 in the medium with Tween 80 compared to the base medium. These two additives act differently on the growth of the producing strain and the uptake of valine, an essential nutrient. A decreased proteolytic activity and similar cellular fatty acid profiles indicate that the main effect of the added compounds is to facilitate the excretion of antibiotics to the outer medium, thus increasing biosynthesis.

Keywords: *Streptomyces hygroscopicus*, methyl oleate, Tween 80, fatty acid(s), polyene antibiotic(s).

INTRODUCTION

The cellular fatty acid composition in streptomycetes has been studied, with different purposes, as a taxonomic criterion or as parts of studies of the biosynthesis of secondary metabolites.

The *S. hygroscopicus* strain CH-7 isolated from soil samples from Yugoslavia, produces the polyene macrolide antibiotic – hexaene H85.¹ In addition to this polyene antibiotic, this strain also produces the polyether antibiotics nigericin and macrodiolide elaiophylin. The addition of methyl oleate or Tween 80 to the fermentation medium enhances the production of the polyene antibiotic two to three times.²

Salts and esters of fatty acids (particularly methyl oleate) are often used in various fermentation media as secondary carbon sources. The polyoxyethylene sorbitol esters (Tween 20 to 80 series) are used to improve the exoenzyme secretion of bacterial and fungal cultures.³

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The aim of this research was to investigate changes in the cellular fatty acids content caused by methyl oleate and Tween 80 in order to explain the enhanced polyene production and altered metabolic properties of the strain.

EXPERIMENTAL

Microorganism: The strain *Streptomyces hygroscopicus* CH-7 was from the Collection of Microorganisms of the Faculty of Chemistry, Belgrade.

Fermentation: Fermentations were conducted in a basic original medium (BM, composition: 1 % soybean meal, 1.5 % glucose, 0.25 % NaCl, 0.2 % CaCO₃, tap water, pH 6.5), and in BM with 0.5 % methyl oleate (MO medium) or 0.5 % Tween 80 (T-80 medium). The strain was grown using a two stage submerged culture in 500 mL Erlenmayer flasks containing 50 mL of medium on a rotary shaker (250 rev/min) at 28 °C. The flasks were inoculated with 2 % of a 48 h culture grown under the same conditions. At the end of exponential growth phase, the biomass was harvested by centrifugation and washed twice with 0.9 % NaCl.

Analytical methods: The samples were taken in 24 h intervals during fermentation. The dry biomass was estimated gravimetrically. The content of hexaene H-85 was determined spectrophotometrically at 364 nm in *n*-butanol extracts of the whole broth.² The proteolytic activity was measured using BAPA (Merck) as a substrate.⁴ The valine dehydrogenase (VDH) activity was determined using a previously described method.⁵

Fatty acid analysis: The lipids were extracted three times with CHCl₃-methanol (2:1, v/v). The extracts were mixed with a 1 % solution of NaCl, the bottom phase was separated, dried and evaporated under reduced pressure. The lipids were transesterified with BF₃.⁶ The methyl esters of the fatty acids were determined using gas chromatography under the following conditions: Varian GC-3400, Carbowax 20M, 60 m × 0.33 mm ID, oven: 150 to 200–230 °C at 2 °C/min, carrier gas: helium, detection FID, 300 °C. Identification of the detected compounds was done by comparing the retention times with standard Bacterial Acid Methyl Esters CP Mix (Supelco). Free (lipid non-bound) MO was determined by GC using the total lipids extracts without transesterification.

RESULTS AND DISCUSSION

The general characteristics of growth and hexaene production, as well as the proteolytic and VDH enzyme activities in the basic and media supplemented with methyl oleate and Tween 80 are given in Table I. The results shown are maximal values obtained during 6-day fermentation. The increase of the antibiotic yield caused by both additives is accompanied by differences in growth characteristics. While methyl oleate stimulates biomass production, Tween 80 supplementation decreases the growth rate and thus biomass yield. The marked improvement in antibiotic production (up to 2.5 times) in the MO medium results not only from the greater mycelium yield (27 %).

In some strains, the enhanced antibiotic productivity is connected with greater amino acid (valine)^{7,8} uptake which serve as antibiotic precursors or nutrient supply. In this work, the VDH activity is taken as a marker for valine uptake. Valine dehydrogenase (E.C. 1.4.1.8.) is the key enzyme which is responsible for the incorporation of branched chain amino acid in polyketide molecules.⁵ VDH activity is increased in the MO medium and decreased in the T-80 medium. Accordingly, it is evident from the obtained results that there is difference in the uptake of essen-

tial nutrients by the biomass grown in media supplemented with MO and T-80. Proteolytic activity in both media are decreased, indicating a lower degree of lysis and a prolonged antibiotic production phase.

TABLE I. Effects of methyl oleate and Tween 80 addition on the growth and metabolic properties of *S. hygroscopicus* CH-7

	BM	MO	T-80
Biomass yield/(g/L)	8.2	10.5	7.3
Hexaene H-85/(mg/L)	135	329	389
VDH/(nkat/mL)	50.7	67.6	39.2
Proteolytic activity/(mU/mL)	6.2	4.2	3.1

The fatty acids generally found in streptomycetes are in the range C₁₂ – C₂₀, both saturated, straight or branched (iso, anteiso) chain, and unsaturated, with one or more double bonds. Neither hydroxylated nor cyclopropane fatty acids are observed in this class of microorganisms. The production of antibiotics by streptomycetes (nigericin,⁸ neomycin,⁹ monensin¹⁰ and streptomycin¹¹) is mainly observed when *i* 16:0 is the dominant compound among mycelium total lipids.

In strains of *S. hygroscopicus* isolated from Bulgarian soil, which produce similar mixtures of polyketides as *S. hygroscopicus* CH-7 (polyether complex, polyene macrolide, nonpolyene macrolide), high levels of 18:2 (12.57–22.95 %), 18:1 (5.74–14.21 %) and 16:0 (7.34–10.75 %) were found.¹²

Table II lists the fatty acids content per total lipid extracts of *S. hygroscopicus* CH-7 (only the main compounds with content greater than 2 % are presented). In the base medium branched chain fatty acids *ai* 15:0, *i* 16:0 and *ai* 17:0 are the most abundant.

TABLE II: Fatty acid composition of the total lipids of *S. hygroscopicus* CH-7 grown in basic medium, as well as medium containing methyl oleate and Tween 80 (concentrations are given in g/100 g dried cells)

Fatty acid	BM	MO	T-80
18:2	0.08±0.03	0.06±0.02	0.07±0.02
18:1	0.11±0.01	5.27±0.23	1.77±0.07
18:0	0.06±0.01	0.08±0.01	0.10±0.01
17:0	0.03±0.01	0.03±0.01	0.02±0.01
<i>ai</i> -17:0	0.19±0.03	0.39±0.02	0.52±0.03
<i>i</i> -17:0	0.08±0.01	0.15±0.01	0.23±0.02
16:0	0.14±0.02	0.18±0.01	0.21±0.02
<i>i</i> -16:0	0.23±0.03	0.37±0.02	0.56±0.03
15:0	0.02±0.01	0.02±0.01	0.01±0.01
<i>ai</i> -15:0	0.20±0.02	0.36±0.04	0.50±0.04
<i>i</i> -15:0	0.11±0.03	0.21±0.01	0.15±0.02

Methyl oleate and Tween 80 caused similar changes in the cell fatty acid composition. The addition of MO and Tween 80 causes an increase of the oleic acid content in the biomass, with free methyl oleate (nonlipid) being about 6 % in the BM, 20 % in the T-80 and 60 % in the MO culture. The level of 18:2, 17:0 and 15:0 is the same in all media but a slight increase in the 16:0 and 18:0 content is observed. During fermentation in the MO medium, the acid content of *i* 16:0, 17:0, 15:0 and *ai* 17:0, 15:0 is about 1.6 to 2.1 times higher, while in the T-80 medium it is about 2.5–2.9 times higher.

David *et al.*⁸ reported that the stimulating effect of MO on nigericin production by *S. hygroscopicus* NRRL B-1865 is accompanied by an increase of all the individual fatty acid contents of the mycelium, particularly *i* 16:0. Tween 80 is often added together with fatty acid esters in fermentation media. The stimulating effect of Tweens alone on the production of antibiotic has been confirmed only for monensin¹³ and oleandomycin¹⁴ producing strains. There are no published data concerning the fatty acid composition change of, influenced by Tweens, antibiotic producing strains.

It is known that the branched-chain fatty acids are the initiation precursors for the biosynthesis of units of polyketide antibiotics, including the polyene antibiotics.^{10,15} Although this paper does not include investigations of this particular aspect, it is assumed that a secondary effect—permeability of the cellular membrane is of crucial significance. Upon completion of their biosynthesis, the polyene antibiotics were localized on the mycelium surface in the form of colloid structures,¹⁵ so it is apparent that such a change of lipid components (higher proportion of unsaturated and branched chain fatty acids) facilitates the excretion of the antibiotics to the outer medium, thus increasing biosynthesis. The protection of the mycelium from toxic antibiotics is also supported by the decreased production of proteolytic enzymes. In the case of Tween 80, although one part is metabolized (increase of 18:1 fraction) the effect of the intact detergent molecule on the solubilization of the polyene complex should be considered as well.

Further research directed towards investigating the changes of the mycelium topology under the influence of methyl oleate and Tween 80, as well as towards isolating and characterizing the individual phospho- and glyco-lipids of the hexaene H85-producing strain, is underway.

ИЗВОД

ДЕЈСТВО МЕТИЛ ОЛЕАТА И ТВЕЕН-а 80 НА ПРОДУКЦИЈУ АНТИБИОТИКА И
САДРЖАЈ МАСНИХ КИСЕЛИНА У УКУПНИМ ЛИПИДИМА СОЈА *Streptomyces*
hygroscopicus CH-7

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У раду је испитивано дејство у подлогу додатих метил олеата и Tween-а 80, стимулатора продукције хексаена H-85, на садржај масних киселина у укупним ли-

пидима соја *S. hygroscopicus* CH-7. Поред повећања садржаја олеинске киселине у биомаси при ферментацији на подлози са метил олеатом садржај *i* 16:0, 17:0, 15:0 и *ai* 17:0, 15:0 је од 1,6 до 2,1 пута виши, док на подлози са Tween-ом 80 ово повећање износи 2,5-2,9 пута. Ова два адитива различито делују на раст продукционог соја као и на брзину усвајања валина из подлоге. Смањење протеолитичке активности и сличности у саставу липида указују да је вероватно најважније дејство метил олеата и Tween-а 80 стимулација екскреције полиенског антибиотика у подлогу чиме се повећава његова синтеза.

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