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# Effect of ammonium ions on the composition of fatty acids in *Streptomyces fradiae*, producer of tylosin

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## 1. SUMMARY

The regulation of fatty acid composition by ammonium ions and amino acids was studied in *Streptomyces fradiae*, a tylosin producer. The quantity of branched-chain fatty acids in *S. fradiae* cells decreased significantly in cultures cultivated in a medium containing high concentrations of ammonium ions, in which the biosynthesis of tylosin was also strongly inhibited. Amino acids stimulating the tylosin production most pronouncedly, also substantially modified the composition of cell fatty acids.

## 2. INTRODUCTION

*Streptomyces fradiae* produces a macrolide antibiotic tylosin. Protylonolide, a precursor of its aglycone, is formed from 2 acetates, 5 propionates and 1 butyrate [1]. Tylosin biosynthesis is inhibited by ammonium ions [2–4], the mechanism of their action consisting in the inhibition of formation of $C_2$–$C_4$ protylonolide subunits from amino acids, succinate and occasionally lower fatty acids (FA) [2,3]. At the enzymic level, repression and also inhibition of valine dehydrogenase (EC 1.4.1.8) by ammonium ions have been described [5]. Valine dehydrogenase catalyzes the following reactions:

L-valine + NAD + $H_2O$ → 2-keto-isovalerate + $NH_3$ + NADH

L-isoleucine + NAD + $H_2O$ → 2-keto-3-methylvalerate + $NH_3$ + NADH

L-leucine + NAD + $H_2O$ → 2-keto-isocaproate + $NH_3$ + NADH.

It is probably the first enzyme of the metabolic pathway producing *n*-butyrate, an immediate precursor for the protylonolide biosynthesis, from L-valine [5,6]. Branched-chain α-keto acids formed from respective branched-chain amino acids in reactions catalyzed by valine dehydrogenase are then transformed to corresponding acyl-CoAs by dehydrogenase of branched-chain α-keto acids (EC

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1.2.4.3, EC 1.2.4.4) [7]. These acyl-CoAs, i.e. isobutyryl-CoA formed from valine, isovaleryl-CoA formed from leucine and 2-methylbutyryl-CoA formed from isoleucine [7,8] can then serve as starting primers in the synthesis of branched-chain iso- and anteiso FA. The relative amount of these branched-chain FA in the lipid fraction of streptomycetes is important with respect to a number of cell functions [9–13].

In the present paper the regulation of cell FA profile by concentration of ammonium ions in the medium is described for this first time.

## 3. MATERIALS AND METHODS

### *3.1. Strains and cultivation conditions*

*Streptomyces fradiae* 30/3 was obtained from the collection of the Research Institute of Biofactors and Veterinary Drugs in Kouřim near Prague. Description of this strain has been published previously [14]; in a complex medium it produced 8.000 μg of tylosin/ml. The first generation was cultivated in a synthetic medium containing (%, w/v): sucrose, 2.0; NaCl, 0.5; $CaCO_3$, 0.3; $MgSO_4 \cdot 7\ H_2O$, 0.05; $K_2HPO_4$, 0.1; $(NH_4)_2SO_4$, 0.33; Tris-(hydroxymethyl)-aminomethane (Trizma base) 1.21. To 1 litre of the medium 1 ml of a solution of trace elements containing $FeSO_4 \cdot 7\ H_2O$; $MnCl_2 \cdot 4\ H_2O$; $ZnSO_4 \cdot 7\ H_2O$; $CuSO_4 \cdot 5\ H_2O$; $CoCl_2 \cdot 2\ H_2O$ (each at 0.1%) was added. The first generation was cultivated for 48 h, the second generation was inoculated by 5% of the first generation and cultivated in a medium containing (%, w/v): sucrose, 2.5; $(NH_4)_2SO_4$, 0.17 (25 mM $NH_4^+$); $K_2HPO_4$, 0.05; concentration of the other compounds was as described above. pH of both media was adjusted to 7.3 with 1 M HCl before thermal sterilization. Solutions of amino acids and $(NH_4)_2SO_4$ were always sterilized separately and added at required concentrations before the inoculation. The cultivation was in 500 ml flasks containing 70 ml of the medium on a reciprocal shaker (1.7 Hz). At indicated time intervals the biomass from 10–20 flasks was harvested by centrifugation (4°C).

### *3.2. Analytical methods*

Biomass was determined gravimetrically, cell-free extract was prepared according to Běhal et al. [15] and proteins were determined by an absorbance method [16]. The activity of valine dehydrogenase was assayed according to absorbance changes at 340 nm [17]. Determination of tylosin and fatty acids (capillary GC-MS by means of the apparatus HP 5995 B) has already been described [14].

## 4. RESULTS

The profile of FA extracted from *S. fradiae* cultivated in the presence of individual amino acids or various concentrations of ammonium ions is presented in Table 1. All identified FA are within the region $C_{14}$–$C_{18}$ as generally found in streptomycetes [9]. Fatty acid designated X was not identified; on the basis of its mass spectrum we propose the structure of hexadecanoic acid substituted with a methyl group inside the chain. A lower homologue of tuberculostearic acid is probably involved [18].

Table 2 illustrates homologues iso, anteiso, straight chain FA (derived from Table 1), tylosin production, growth of *S. fradiae* and specific activity of valine dehydrogenase under the given conditions. The addition of L-leucine, L-isoleucine and L-valine increased the relative amount of a corresponding branched-chain FA produced from a given initiation precursor. The relative occurrence of all branched-chain FA decreased significantly with increasing concentration of ammonium ions.

The activity of valine dehydrogenase was highly repressed by ammonium ions (Table 2). In 75 mM and 100 mM $NH_4^+$ medium the activity of the enzyme in a 24-h culture was most repressed, however, during a further cultivation, it increased again. Valine dehydrogenase was induced in the medium with valine and isoleucine; leucine did not act as inducer (Table 2).

Growth of the culture was influenced by concentrations of ammonium ions and by the presence of branched-chain amino acids in the medium to a certain extent (Table 2). Lipid content (de-

Table 1

Composition of FA in *S. fradiae* 30/3 cultivated in a medium containing various concentrations of ammonium ions and amino acids

| Fatty acids [a] | Composition of medium [b,c] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Leu | Val | Ile | 25 | 50 | 75 | 100 |
| 14:0 [d] | 0.9 | 0.9 | 0.3 | 0.9 | 0.8 | 0.7 | 0.8 |
| i-14:0 | 2.5 | 3.2 | 0.5 | 2.1 | 0.8 | 0.2 | 0.1 |
| 15:0 | 0.2 | 0.3 | 0.1 | 3.5 | 3.2 | 2.8 | 2.6 |
| i-15:0 | 28.4 | 4.9 | 1.0 | 4.0 | 2.3 | 1.1 | 0.1 |
| ai-15:0 | 14.8 | 19.9 | 40.3 | 27.3 | 19.3 | 16.1 | 9.9 |
| 15:1 | 1.2 | 4.5 | 1.8 | 2.6 | 2.3 | 2.5 | 3.0 |
| X | 1.1 | 1.7 | 0.9 | 2.4 | 2.4 | 1.3 | 1.2 |
| 16:0 | 9.0 | 6.7 | 3.4 | 12.1 | 16.7 | 20.7 | 24.0 |
| i-16:0 | 0.1 | 2.7 | 0.8 | 2.2 | 2.6 | 1.4 | 0.1 |
| 16:1 | 5.3 | 9.6 | 5.9 | 10.9 | 5.3 | 5.4 | 6.0 |
| 17:0 | 0.4 | 0.5 | 0.5 | 0.3 | 0.3 | 0.5 | 0.4 |
| i-17:0 | 12.6 | 8.4 | 13.0 | 16.4 | 15.2 | 3.9 | 2.2 |
| ai-17:0 | 4.7 | 3.1 | 26.9 | 7.9 | 5.8 | 5.4 | 4.3 |
| 17:1 | 1.9 | 2.7 | 1.9 | 1.5 | 1.5 | 2.2 | 2.0 |
| 18:0 | 0.9 | 1.0 | 0.3 | 1.1 | 10.5 | 15.0 | 19.1 |
| 18:1 | 12.8 | 23.9 | 1.3 | 2.8 | 8.9 | 17.1 | 23.5 |
| 18:2 | 3.2 | 6.0 | 1.1 | 2.0 | 2.1 | 3.7 | 0.7 |

[a] i, isoacid; ai, anteisoacid; X, unidentified acid; the first number denotes number of carbon atoms, the second number of double bonds.

[b] Amino acids (L-leucine, L-valine, L-isoleucine) were added to the medium containing a 25 mM concentration of ammonium ions; the concentration of all amino acids was 5 mM.

[c] Concentration of ammonium ions in mM.

[d] Presented values are given in weight % of total FA.

Table 2

Effect of concentration of ammonium ions and branched-chain amino acids on valine dehydrogenase activity, growth, FA composition and tylosin production in *S. fradiae* 30/3

| | Culture age (days) | Composition of medium [a] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Leu | Val | Ile | 25 | 50 | 75 | 100 |
| VDH [b] | 1 | 154 | 285 | 302 | 164 | 101 | 58 | 32 |
| | 2 | 138 | 207 | 215 | 143 | 79 | 63 | 48 |
| | 3 | 95 | 202 | 210 | 92 | 85 | 82 | 79 |
| | 7 | 84 | 141 | 160 | 81 | 89 | 87 | 85 |
| Growth [c] | 1 | 0.9 | 1.1 | 1.1 | 0.9 | 1.0 | 1.0 | 1.1 |
| | 3 | 3.9 | 4.0 | 3.8 | 3.5 | 4.8 | 5.2 | 6.1 |
| | 7 | 3.5 | 3.5 | 3.3 | 3.2 | 6.1 | 7.0 | 7.0 |
| FA [d] | 7 | | | | | | | |
| Even straight | | 32.1 | 48.1 | 12.3 | 29.8 | 43.5 | 62.6 | 74.1 |
| Odd straight | | 3.7 | 8.0 | 4.3 | 7.9 | 7.3 | 8.0 | 8.0 |
| Even iso br. | | 2.6 | 5.9 | 1.3 | 4.3 | 3.4 | 1.6 | 0.2 |
| Odd iso br. | | 41.0 | 13.3 | 14.0 | 20.4 | 17.5 | 5.0 | 2.3 |
| Anteiso br. | | 19.5 | 23.0 | 67.2 | 35.2 | 25.1 | 21.5 | 14.2 |
| Tylosin [e] | 7 | 151 | 128 | 142 | 86 | 53 | 25 | 12 |

[a] Symbols as at Table 1.

[b] Activities of valine dehydrogenase (VDH) in cell-free extracts are given in nmol of formed $NADH \cdot s^{-1} \cdot (mg^{-1}$ protein).

[c] Presented values of dry cell weight are in $g \cdot l^{-1}$.

[d] FA composition classified according to structure (derived from Table 1, values are given in % of total FA); br. = branched.

[e] Production of tylosin is given in μg/ml.

termined as material extractable by a $CHCl_3$: MeOH 1 : 1 mixture) was constant in all cultures and did not change during the cultivation. The occurrence of individual classes of fatty acids did not change during the cultivation, values in a 3-day culture (beginning of the stationary phase) and in a 7-day culture (maximum values of tylosin production achieved throughout the fermentation) differed statistically insignificantly. All results were confirmed in 2 separate cultivations.

## 5. DISCUSSION

The amount of branched-chain FA in *S. fradiae* was highly decreased during cultivation in the medium containing high concentrations of ammonium ions. Tylosin production was also inhibited under these conditions. Repression and inhibition of valine dehydrogenase by ammonium ions is probably a mechanism responsible for both phenomena. This assumption is strongly supported by the findings of Tanaka et al. [4] that catabolism of branched-chain amino acids but not of branched-chain $\alpha$-keto acids is under control exerted by ammonium ions. The enzymes synthesizing branched-chain $\alpha$-keto acids could be principally also regulated by ammonium ions, although as far as we know it has not been proven in any microorganism and it appears to be unprobable.

Valine dehydrogenase has a relatively low substrate specificity [5] catalyzing the oxidative deamination of all three branched-chain amino acids. When the enzyme is repressed or inhibited, the amount of branched-chain acyl-CoAs that may serve as starting primers in branched-chain FA biosynthesis is apparently limited.

It was found in the present work that the synthesis of branched-chain FA is regulated by concentration of ammonium ions in the medium affecting the activity of valine dehydrogenase.

As *S. fradiae* does not accumulate lipids as a reserve material, most FA are localized in the cytoplasmic membrane.

Excretion of various metabolites, uptake of nutrients and occasionally cytodifferentiation can be influenced by composition of FA in the cytoplasmic membrane [19,20]. It indicates that valine dehydrogenase is not only involved in the biosynthesis of protylonolide but plays also an important role in the regulation of other cell functions. As it is induced by valine and isoleucine and repressed by ammonium ions it has rather a catabolic function. The regulation of its activity seems to be an interesting model for studying the mutual relationship between the primary metabolism, physiological state of the cell and secondary metabolism.

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