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

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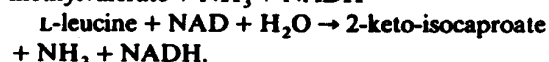
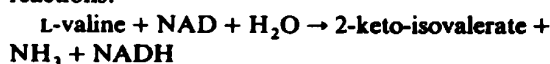
1. SUMMARY

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 C₂–C₄ 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:



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; CaCO₃, 0.3; MgSO₄ · 7 H₂O, 0.05; K₂HPO₄, 0.1; (NH₄)₂SO₄, 0.33; Tris-(hydroxymethyl)-aminomethane (Trizma base) 1.21. To 1 litre of the medium 1 ml of a solution of trace elements containing FeSO₄ · 7 H₂O; MnCl₂ · 4 H₂O; ZnSO₄ · 7 H₂O; CuSO₄ · 5 H₂O; CoCl₂ · 2 H₂O (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; (NH₄)₂SO₄, 0.17 (25 mM NH₄⁺); K₂HPO₄, 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 (NH₄)₂SO₄ 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 C₁₄–C₁₈ 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 NH₄⁺ 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 · s⁻¹ · (mg⁻¹ protein).

^c Presented values of dry cell weight are in g · 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.

terminated 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 am-
monium ions. Tylosin production was also in-
hibited under these conditions. Repression and
inhibition of valine dehydrogenase by ammonium
ions is probably a mechanism responsible for both
phenomena. This assumption is strongly sup-
ported by the findings of Tanaka et al. [4] that
catabolism of branched-chain amino acids but not
of branched-chain α -keto acids is under control
exerted by ammonium ions. The enzymes synthe-
sizing branched-chain α -keto acids could be prin-
cipally also regulated by ammonium ions, al-
though as far as we know it has not been proven
in any microorganism and it appears to be un-
probable.

Valine dehydrogenase has a relatively low sub-
strate 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 cyto-
plasmic membrane [19,20]. It indicates that valine

dehydrogenase is not only involved in the bio-
synthesis of protylonolide but plays also an im-
portant role in the regulation of other cell func-
tions. As it is induced by valine and isoleucine and
repressed by ammonium ions it has rather a cata-
bolic 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.

6. REFERENCES

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