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Altered fatty acid composition in regulatory mutants of *Streptomyces cinnamonensis*

(*Streptomyces cinnamonensis*; regulatory mutants; fatty acids; branched amino acids; monensin A; monensin B)

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1. SUMMARY

Fatty acids were analyzed in the producer of monensins A and B, *Streptomyces cinnamonensis* C-100-5, and its regulatory mutants resistant to 2-amino-3-chlorobutyrate, 2-aminobutyrate and norleucine. All resistant strains were found to have a strongly increased content of even iso-branched fatty acids. This change was also reflected in a relative increase of monensin A production, which is affected by branched amino acids.

2. INTRODUCTION

S. cinnamonensis is a producer of polyether antibiotics, monensins, which are oligoketides arising by condensation of C_2–C_4 units [1]. We found that the biosynthesis of monensins is markedly affected by valine and isoleucine [2]. Incorporation experiments showed that valine and its product isobutyrate are precursors of butyric acid, which is a crucial building unit of monensin A [3]. This also provides evidence for a new metabolic pathway involving isomerization of isobutyrate to butyrate. The same finding was made in a study of the biosynthesis of certain macrolide antibiotics [4].

Our subsequent work concerned the preparation, using as selection procedure resistance to amino acid analogues, of regulatory mutants of *S. cinnamonensis* [5]. The present experiments were aimed at analyzing the fatty acid composition of the regulatory mutants, and establishing whether there is any correlation between the production of monensins A and B and the composition of fatty acids. Branched amino acids are known to be precursors of branched fatty acids. In the biosynthesis of these fatty acids the starting acetyl-CoA is replaced by isobutyryl-CoA, isovaleryl-CoA or 2-methylbutyryl-CoA from valine, leucine and/or isoleucine. The literature data concern only the effect of exogenous sources of branched fatty acids [6–8], auxotrophy [9] or a change in the degradation of branched fatty acids [10]. The present work is the first to describe the effect of a change in the biosynthesis of endogenous sources on the composition of fatty acids.

3. MATERIALS AND METHODS

3.1. Organisms

The control strain was *S. cinnamonensis* C-100-5, obtained from the collection strain ATCC 15413.

Regulatory strains ACBR-2 (resistant to 2-amino-3-chlorobutyrate), ABR-37 (resistant to 2-aminobutyrate) and NLR-5 (resistant to norleucine) were obtained from strain C-100-5. The doubly resistant strain ACB-NLR-13 was derived from strain ACBR-2 [5].

3.2. Media and cultivation

Strains were maintained on yeast extract–malt extract agar [11]. The composition of the production medium and the determination of monensins have been described earlier [5,12]. For fatty acid isolation, strains were incubated in a synthetic medium containing (%, w/v): NH_4NO_3, 0.35; $MgSO_4 \cdot 7H_2O$, 0.05; KCl, 0.02; KH_2PO_4, 0.08; $FeSO_4 \cdot 7H_2O$, 0.001; glucose, 0.6. The pH was adjusted to 7.2 before sterilization. Cultivation was carried out in 500 ml boiling flasks containing 80 ml medium. These were inoculated with spore suspensions of the tested strains, and then incubated at 37°C on a rotary shaker for 120 h.

3.3. Isolation and identification

The filtered biomass was used for preparing methyl esters of fatty acids by alkaline saponification and esterification with a methanol–sulphuric acid mixture [13]. Identification of the mixture of methyl esters was carried out using gas chromatography–mass spectrometry (GC–MS) on an LKB 9000 instrument (LKB Produkter AB, Sweden) with glass columns 3000 × 3 mm ID packed with Chromaton N-AW-DMCS (Lachema Brno, Czechoslovakia) with 5% OV-101 or OV-225 phases (Lachema Brno). Quantitative data were obtained on a Chrom-5 instrument coupled with a CI 100 integrator (Laboratorní přístroje, Prague) on a capillary fused silica column, 0.25 mm ID, length 50 m, with OV-101.

4. RESULTS AND DISCUSSION

GC–MS analyses of methyl esters of fatty acids extracted from the standard culture of *S. cinnamonensis* C-100-5 and its derived regulatory mutants are given in Table 1. All the identified fatty acids were in the range C_{13}–C_{18}, as generally found in streptomycetes [14]. All strains exhibited

Table 1

Fatty acid composition of the parent strain *S. cinnamonensis* C-100-5 and its regulatory mutants

Acid [a]	C-100-5	ACBR-2	ABR-37	NLR-5	ACB-NLR-13
i-13:0	0.37	0.11	–	0.05	0.10
ai-13:0	0.19	0.11	–	0.09	0.10
14:0	0.56	0.56	1.52	0.18	0.87
i-14:0	1.81	9.77	9.14	3.66	16.98
14:1	–	0.29	0.17	0.08	0.41
15:0	0.75	0.26	2.29	0.69	0.56
i-15:0	5.28	9.85	3.30	4.94	4.84
ai-15:0	21.28	10.68	9.27	18.43	4.92
16:0	9.82	5.77	19.54	3.01	6.41
i-16:0	17.68	30.09	33.29	30.34	42.65
16:1	9.19	8.06	5.16	3.13	4.33
i-16:1	3.58	5.34	2.34	7.60	5.64
17:0	0.37	0.11	0.34	1.13	0.10
i-17:0	1.70	1.72	1.43	0.36	0.77
ai-17:0	13.08	3.36	3.37	12.62	1.03
c-17:0	1.32	1.46	2.14	2.14	0.62
17:1	2.46	4.16	1.02	3.29	1.64
17:1	4.72	2.55	0.76	6.38	0.93
18:0	0.75	0.47	0.51	1.05	0.62
18:1	2.26	1.02	0.85	0.16	1.29
18:1	1.70	1.09	1.02	0.15	1.59
18:2	1.13	5.18	2.54	0.61	3.60

[a] i, iso-acid; ai, anteiso-acid; c, cyclopropane ring. Number before colon, chain carbon atoms; number after colon, double bonds.

a typically high content of branched fatty acids. The quantitative composition of the individual fatty acids, however, differs substantially from strain to strain. Table 2 shows the distribution of fatty acids with regard to their type or initiation precursors; the differences are thus more pronounced. In all regulatory mutants the content of even iso-branched fatty acids (derived from valine or isobutyryl-CoA) increased, mostly at the expense of anteiso-branched fatty acids; e.g. up to 65.63% of the total fatty acids in the double-resistant strain ACB-NLR-13 compared with 23.07% in the control strain. An exception was strain NLR-5, in which the total amount of anteiso fatty acids was about the same as in the parent strain C-100-5, whilst the content of even nonbranched fatty acids decreased significantly.

Streptomycetes belong among microorganisms which form several groups of fatty acids [15], whose relative proportions may change depending on genetic characteristics, physiological conditions

Table 2

Fatty acid composition of the parent strain *S. cinnamonensis* C-100-5 and its regulatory mutants

Classification according to initiation precursors.

Type of fatty acid	Initiation precursor	Strain				
		C-100-5	ACBR-2	ABR-37	NLR-5	ACB-NLR-13
Even non-branched	Acetyl-CoA [a]	26.73	21.89	33.45	10.51	19.64
Odd non-branched	Propionyl-CoA	8.30	7.08	4.41	11.49	3.13
Even iso-branched	Isobutyryl-CoA	23.07	45.20	44.77	41.60	65.63
Odd iso-branched	Isovaleryl-CoA	7.25	11.68	4.73	5.35	5.71
Anteiso-branched	2-Methylbutyryl-CoA	34.65	14.15	12.64	31.05	6.05

[a] The c-17:0 acid is assumed to arise from 16:0, acetyl-CoA being the initiation precursor.

Table 3

Monensin A/monensin B ratio produced in a complex medium by *S. cinnamonensis* C-100-5 and its regulatory mutants

Strain	C-100-5	ACBR-2	ABR-37	NLR-5	ACB-NLR-13
Ratio $\frac{\text{Monensin A}}{\text{Monensin B}}$	1.0	6.7	3.3	3.5	13.3

and medium. In our strains considerable changes were achieved by genetical means, i.e. by changing the regulation of biosynthesis of the amino acids of the aspartate and pyruvate groups. However, the relative proportions of individual groups of branched fatty acids need not necessarily reflect mutual relationships in the biosynthesis of valine, leucine and isoleucine [16].

Our study of incorporation of [1-^{13}C]isobutyrate into the monensin A molecule showed that this precursor isomerizes to butyrate [3]. The putative isomerase catalyzing this reaction is probably tightly coupled with the biosynthesis of monensin, e.g. as a part of the monensin synthetase in *S. cinnamonensis*. In other compartments this enzyme is either absent or inactive, as documented by the high content of even iso-branched fatty acids, especially in strain ACB-NLR-13. At the same time, this strain produces the highest relative amount of monensin A (Table 3).

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