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Changes in Fatty Acid Branching and Unsaturation of *Streptomyces cinnamonensis* as a Response to NaCl Concentration

S. POSPÍŠIL and T. ŘEZANKA

Institute of Microbiology, Academy of Sciences of the Czech Republic, 142 20 Prague

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ABSTRACT. Three different *Streptomyces cinnamonensis* strains (*viz.* standard, regulatory, and non-differentiating) were grown in a synthetic medium containing 0–4 % NaCl and the composition of total cellular fatty acids was analyzed. The increasing salt concentration resulted in an increasing proportion of even-numbered straight-chain fatty acids, mostly at the expense of branched-chain fatty acids. Unsaturated fatty acids, palmitoleic acid in particular, represent the major proportion of straight-chain fatty acids.

Exposure of cells to a high external osmolarity results in several effects, *viz.* passive cell volume regulation, accumulation of compatible solutes (by *de novo* synthesis or by transport from the culture medium) which reduce water activity in the cell interior. Further effects involve changes in composition and properties of cellular membranes. All these changes are genetically based. About 20 genes whose transcription is either enhanced or diminished by osmotic stress have been identified in *Escherichia coli* and *Salmonella typhimurium* (Csonka 1989).

Changes of the cellular lipid composition and membrane fluidity were mostly studied in halophilic and halotolerant microorganisms. Only a limited number of studies in this respect have been devoted to the actinomycetes. The effect of NaCl and exogenous fatty acids on the cellular fatty acid composition and neomycin production (Arima *et al.* 1973) was observed in special mutant strains of *Streptomyces fradiae*; studies with *Streptomyces hygroscopicus* were also reported (David *et al.* 1992). Intracellular solutes in relation to the salt stress were studied in *Streptomyces griseus* (Killham and Firestone 1984).

In the course of an extensive research of physiology of *Streptomyces cinnamonensis*, a producer of the oligoether antibiotic monensin (Haney and Hoehn 1968), we also studied the effect of the osmotic stress caused by NaCl on the total cellular fatty acid composition in three different (standard, regulatory and non-differentiating) *S. cinnamonensis* strains.

MATERIALS AND METHODS

Microorganisms. S. cinnamonensis strain C-100-5 was obtained by a multistep selection from strain ATCC 15413, the subsequent strains are derived from the former. ACB-NLR-13 is a double resistant regulatory mutant (Pospíšil *et al.* 1984) and TAR-34 is a spontaneous non-differentiating mutant.

Media and cultivation. Submerged cultivation was carried out in 500 mL flat-bottomed flasks (fitted with a 13 mm diameter stainless steel spring) filled by 50 mL of minimal liquid growth medium containing (g/L): $NaNO_3$ 10, glucose 25, $MgSO_4 \cdot 7H_2O$ 1, K_2HPO_4 0.5, $FeSO_4 \cdot 7H_2O$ 0.01; $MnCl_2 \cdot 4H_2O$ 1 mg, $ZnSO_4 \cdot 7H_2O$ 1 mg, $CaCl_2$ 1 mg; pH 7.2. The cultures were incubated at 34 °C on a rotary shaker at 2.7 Hz. NaCl concentrations were at 0–4 % (*W/V*). NaCl was added before sterilization. The seed culture was grown for 3 d in a basal medium without NaCl and after concentration by centrifugation, it was used for inoculation (2 %, *V/V*). Cultures with various NaCl concentrations (from 3–5 flasks) were harvested after 3 d by centrifugation at 6 000 *g*, and frozen.

Fatty acid analysis. Fatty acids were prepared from frozen cells by alkaline hydrolysis, and after esterification, were analyzed as methyl esters by GC–MS on capillary column according to Vančura *et al.* (1987).

RESULTS AND DISCUSSION

Gas chromatographic analyses of methyl esters of cellular fatty acids from the three *S. cinnamonensis* strains grown in a synthetic medium are shown in Table I . All the identified fatty acids lay in the range $C_{13}-C_{18}$ as commonly found in streptomycetes (Kaneda 1991; Verma and Khuller 1983). Investigated strains also contained typical high quantities of branched-chain fatty acids. Lipids of the strain ACB-NLR-13 (grown without NaCl) contained high amounts of even-numbered *iso*-branched fatty acids as a result of valine overproduction (Pospíšil *et al.* 1985). Differences in the cellular fatty acids between the parent strain C-100 and its bald derivative TAR-34 might be explained in connection with an impaired ability to differentiate. Similar phenomenon was observed with *Streptomyces hygroscopicus* (Gräfe *et al.* 1982). Table I also demonstrates the effect of the increasing salinity on individual cellular fatty acids. That trends of changes are better elicited when fatty acids are grouped according to

Table I. Fatty acid composition (%) of *S. cinnamonensis* after growth at different salinities on synthetic medium

Fatty acid	NaCl concentration, % (*W*/*V*)														
	0	0.1	0.2	0.3	0.4	0.5	1	1.5	2	2.5	3	3.5	4	4.5	
	Strain C-100-5														
i-14:0	5.7	5.7	5.7	5.6	5.3	5.2	5.2	5.2	5.1	5.0	4.9	4.6	3.3	2.9	
14:0	0.6	0.6	0.6	0.6	0.6	0.7	0.9	0.9	1.0	1.1	1.0	1.2	1.5	1.6	
i-15:0	12.4	12.3	12.4	12.3	12.3	11.3	10.5	10.2	9.9	9.6	9.3	9.0	7.4	7.2	
ai-15:0	31.0	31.0	31.1	30.9	28.1	26.5	25.0	24.7	24.8	24.3	24.4	24.1	22.1	20.4	
15:1	0.3	0.3	0.4	0.4	0.3	0.3	0.2	0.2	0.1	0.4	0.3	0.2	0.5	0.6	
15:0	0.6	0.6	0.5	0.6	0.6	0.5	0.6	0.6	0.6	0.8	0.9	0.9	1.1	1.2	
i-16:0	32.3	32.3	32.1	32.1	31.5	29.3	27.2	26.2	25.7	25.3	25.1	24.5	21.8	19.8	
16:1	3.9	3.9	4.0	4.0	7.5	12.0	14.4	15.1	15.4	15.2	15.2	15.7	19.6	22.6	
16:0	4.6	4.6	4.6	4.7	4.6	4.9	5.1	5.4	5.4	5.7	6.2	6.8	8.9	9.6	
i-17:0	1.8	1.9	1.9	1.9	1.9	1.8	1.7	1.7	1.9	2.1	1.7	2.0	2.1	2.1	
ai-17:0	5.8	5.8	5.7	5.9	6.3	6.5	6.9	7.2	7.3	7.3	8.0	8.0	9.2	9.7	
17:1	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	
17:0	0.2	0.2	0.2	0.2	0.2	0.2	0.7	1.0	0.9	1.0	1.1	0.9	1.0	1.0	
18:1	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.6	0.2	0.3	0.4	0.4	
18:1	0.2	0.2	0.2	0.2	0.2	0.2	0.7	0.8	0.9	0.9	0.9	1.0	0.5	0.3	
18:0	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.3	0.4	0.4	0.5	0.5	0.3	0.3	
	Strain ACB-NLR-13														
i-14:0	16.5	16.5	16.4	16.3	15.1	14.6	13.2	12.9	11.8	11.3	8.8	6.9	4.8	–	
14:0	0.9	0.9	0.9	0.9	1.0	1.6	1.9	2.2	2.4	2.5	2.5	2.4	2.5	–	
i-15:0	8.8	8.7	8.6	8.4	7.7	7.1	6.4	5.8	5.1	4.9	4.8	4.7	3.4	–	
ai-15:0	7.5	7.2	7.2	6.8	4.1	3.5	2.3	1.7	1.4	1.1	1.3	1.0	1.1	–	
15:1	0.5	0.5	0.5	0.5	0.7	0.8	0.8	0.9	1.0	1.0	1.1	1.0	1.2	–	
15:0	0.6	0.6	0.6	0.8	1.0	1.3	1.4	1.6	1.8	1.9	2.0	2.2	2.4	–	
i-16:0	47.8	47.6	47.4	47.4	45.6	43.2	40.2	34.4	30.0	27.6	22.4	18.6	16.7	–	
16:1	3.7	3.7	3.8	4.2	8.9	11.6	16.9	23.7	28.6	29.7	35.6	41.4	45.2	–	
16:0	4.9	5.2	5.5	5.5	5.6	5.9	6.2	6.8	8.9	10.8	12.6	13.7	14.3	–	
i-17:0	1.8	1.8	1.8	1.8	1.9	1.8	1.9	1.8	1.7	1.8	1.6	1.5	1.5	–	
ai-17:0	6.2	6.2	6.3	6.1	5.8	5.2	4.7	3.8	2.6	2.3	1.8	1.2	1.3	–	
17:1	0.1	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	–	
17:0	0.2	0.2	0.2	0.2	0.4	0.2	0.4	0.4	0.4	0.2	0.4	0.4	0.2	–	

their type (Fig. 1). Above 0.3 % NaCl abrupt changes in fatty acid composition occur. In all three strains the changes possess a common pattern, which is most pronounced in the derivatives of the parent standard strain C-100-5. NaCl promoted an increasing proportion of even-numbered straight-chain fatty acids, mostly at the expense of all branched-chain fatty acids. Unsaturated fatty acids, palmitoleic acid ($C_{16:1}$) in particular, constitute the highest proportion of straight-chain fatty acids. Odd numbered straight-chain fatty acids form the smallest portion of total fatty acids. Their content grew linearly with the increasing salinity. High NaCl concentration (above 3.5 %) set an extreme conditions accompanied by slow *S. cinnamonensis* growth.

Two different mechanisms, anaerobic and aerobic, participate in the synthesis of unsaturated fatty acids (Fulco 1983). Our findings indicate that the desaturating process or the activity of corresponding enzymes are significantly affected by higher NaCl concentration.

18:1	0.2	0.3	0.3	0.3	0.6	0.9	1.0	1.3	1.3	1.4	1.5	1.5	1.5	–
18:1	0.2	0.2	0.2	0.2	0.7	1.0	1.2	1.4	1.5	1.6	1.7	1.7	1.8	–
18:0	0.1	0.2	0.1	0.3	0.7	1.1	1.2	1.1	1.3	1.5	1.7	1.6	1.9	–
Strain TAR-34														
i-14:0	19.4	18.3	18.3	18.1	16.8	16.2	15.3	15.1	13.3	12.6	9.6	7.5	5.3	5.2
14:0	0.7	1.0	1.0	1.0	1.1	1.8	2.2	2.6	2.7	2.8	2.7	2.6	2.8	2.7
i-15:0	10.1	9.7	9.6	9.3	8.6	7.9	7.4	6.8	5.7	5.5	5.2	5.1	3.7	3.7
ai-15:0	19.0	19.2	18.9	18.7	15.7	15.0	14.3	13.7	12.8	12.4	11.9	10.9	9.9	8.5
15:1	0.7	0.6	0.6	0.6	0.8	0.9	0.9	1.1	1.1	1.1	1.2	1.1	1.3	1.3
15:0	0.9	0.7	0.7	0.9	1.1	1.4	1.6	1.9	2.0	2.1	2.2	2.4	2.6	2.6
i-16:0	18.4	19.5	19.4	19.3	17.3	14.7	11.8	11.5	10.0	8.9	7.6	7.3	6.7	6.1
16:1	3.9	4.1	4.2	4.7	9.9	12.9	15.3	16.0	20.9	22.0	26.1	29.3	31.9	33.8
16:0	15.3	16.9	17.3	17.2	17.3	17.7	18.8	19.7	21.3	22.4	23.9	25.0	26.4	26.9
i-17:0	2.3	2.0	2.0	2.0	2.1	2.0	2.2	2.1	1.9	2.0	1.7	1.6	1.7	1.6
ai-17:0	8.6	6.9	7.0	6.8	6.4	5.8	5.4	4.4	2.9	2.6	2.0	1.3	1.4	1.4
17:1	0.1	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2
17:0	0.2	0.2	0.2	0.2	0.4	0.2	0.5	0.5	0.4	0.2	0.4	0.4	0.2	0.2
18:1	0.1	0.3	0.3	0.4	0.7	1.0	1.2	1.5	1.5	1.6	1.6	1.6	1.7	1.6
18:1	0.2	0.2	0.2	0.2	0.8	1.1	1.4	1.6	1.7	1.8	1.8	1.8	2.0	2.0
18:0	0.1	0.2	0.1	0.3	0.8	1.2	1.4	1.3	1.6	1.8	1.9	1.9	2.2	2.2

All *S. cinnamonensis* strains studied exhibited a phenotypic adaptation to changes in NaCl concentration. This adaptation process involved dramatic changes in the relative amount of monounsaturated fatty acids and a concomitant decrease of branched-chain fatty acids. The observed phenomenon casts doubt on the assertion that unsaturated fatty acids play a minor role in microbes containing branched-chain fatty acids (Kaneda 1991). Since virtually all bacterial lipids occur as structural components of membranes, it is evident that changes in fatty acid composition imply corresponding changes of membrane phospholipids. Unsaturation is probably correctly often regarded as one of the important regulators of membrane flexibility and stability (Cronan and Gelman 1975).

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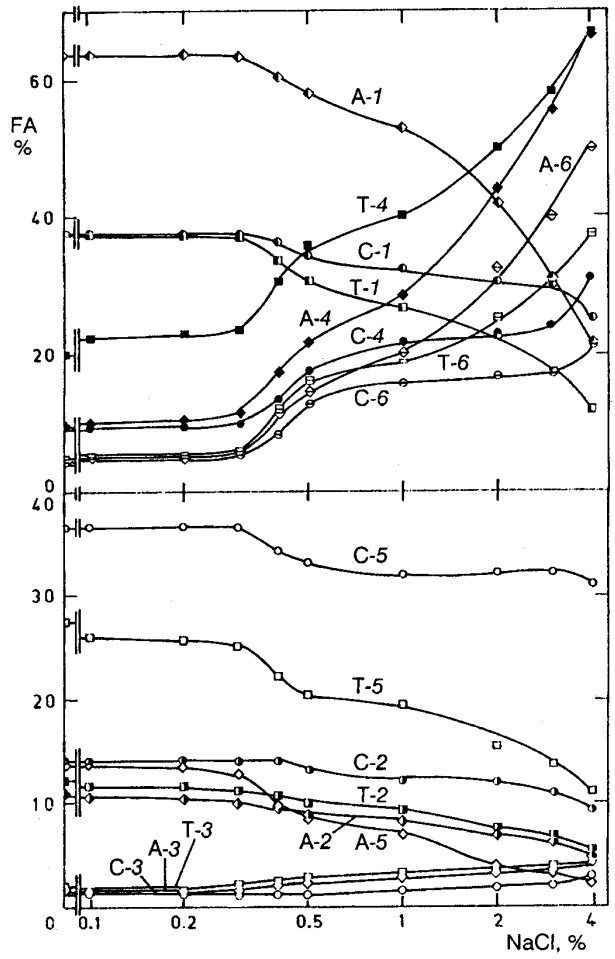

Fig. 1. Influence of the NaCl concentration on the basic cellular fatty acids groups of *S. cinnamonensis* C-100-5 (C, *circles*), ACB-NLR-13 (A, *rhombs*) and TAR-34 (T, *squares*). Fatty acids: *1 iso-*, even-numbered, *2 iso-*, odd-numbered, *3 anteiso-*, *4* straight chain, even-numbered, *5* straight chain, odd-numbered, *6* monoenic.

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