Folia Microbiol. 43 (1), 47–50 (1998)

Effect of Salinity on the Formation of Avermectins, Odor Compounds and Fatty Acids by *Streptomyces avermitilis*

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Received June 16, 1997

ABSTRACT. *Streptomyces avermitilis* was cultivated at different concentrations (0–12 %) of NaCl in the medium and the content of fatty acids, production of odorous compounds and avermectins were measured. With increasing medium salinity the content of monoenoic fatty acids, particularly palmitoleic acid, increased while the quantity of branched fatty acids dropped. The production of avermectin was constant up to 0.5 % of the salt in the medium and then it strongly decreased, reaching zero at 2.5 % salt. The biosynthesis of oxolones and geosmin differed: the content of oxolones increased with growing salinity whereas the production of geosmin dropped.

The effect of salinity and salt stress on the physiology of various species of microorganisms was summarized in the reviews by Larsen (1962), Imhoff and Thiemann (1991) and others. The salinity of the medium affects the properties of the cell membrane, composition of cellular lipids and membrane permeability (Olivier *et al.* 1994) of most halophilic and halotolerant microorganisms. It is assumed (Cszonka 1989) that these changes are genetically controlled.

Unfortunately, little attention has been paid to actinomycetes. Arima *et al.* (1973) described the effect of salt on neomycin and intracellular fatty acid production by a mutant of *Streptomyces fradiae*. Recently, similar physiological studies were performed with *Streptomyces hygroscopicus* (David *et al.* 1992), *S. griseus* (Killham and Firestone 1984) and *S. cinnamonensis* (Pospíšil and Řezanka 1994). The production of antibiotics was found to be inhibited and the proportion of aliphatic fatty acids with an even number of carbon atoms in the chain increased at the expense of branched fatty acids with higher concentration of salt in the medium. Monoenoic fatty acids, mainly palmitoleic acid, constitute the greatest part of intracellular aliphatic fatty acids. The production of antibiotics by actinomycetes is usually coupled to formation of earthy odors (Dougherty *et al.* 1966; Gerber 1973). Therefore, the production of odors and antibiotics can be taken as markers of the physiological state to distinguish among growth, production and decay phases of a batch culture.

Based on previous research (Cimburková *et al.* 1988; Vaněk *et al.* 1990; Řezanka *et al.* 1994) we used a strain of *Streptomyces avermitilis* producing a mixture of anthelminthic active avermectins, in our study.

MATERIALS AND METHODS

Strain and cultivation. The mutant strain *Streptomyces avermitilis* C-18 obtained by genetic improvement (Cimburková *et al.* 1988) of ATCC 1267 was used. The inoculum was cultivated for 1 d in a complex medium containing: glucose 0.5 %; soybean meal 1.5 % and yeast extract 0.5 %; fermentation flasks were inoculated with 2 % of the inoculum (1-d old) and cultivated for 10 d. The following synthetic production medium (%) was used for cultivation: glucose 3, $CaCO_3$ 0.5, NaCl 0.2, K_2HPO_4 0.05, $FeSO_4 \cdot 7H_2O$ 0.005, $MnSO_4 \cdot 7H_2O$ 0.005, $ZnSO_4 \cdot 7H_2O$ 0.005, $MgSO_4 \cdot 7H_2O$ 0.01. The cultivation was performed in 500-mL round-bottom flasks on a rotary shaker (2.8 Hz) at 28 °C (Cimburková *et al.* 1988).

Analytical methods. The content of total avermectins in the medium was detected by TLC (Cimburková *et al.* 1988). Individual avermectins were determined by a reverse phase HPLC (*Perkin Elmer* series 3B Liquid Chromatograph) (Čurdová *et al.* 1989). The volatile compounds were determined after steam distillation and extraction by capillary GC–MS (*Jeol* DX 303), as described previously (Řezanka *et al.* 1994). Fatty acid content in the dry matter (with 14–18 C atoms) was determined after alkaline hydrolysis and methyl esters were analyzed by GC–MS (QP1000, *Shimadzu*) as published by Řezanka *et al.* (1991).

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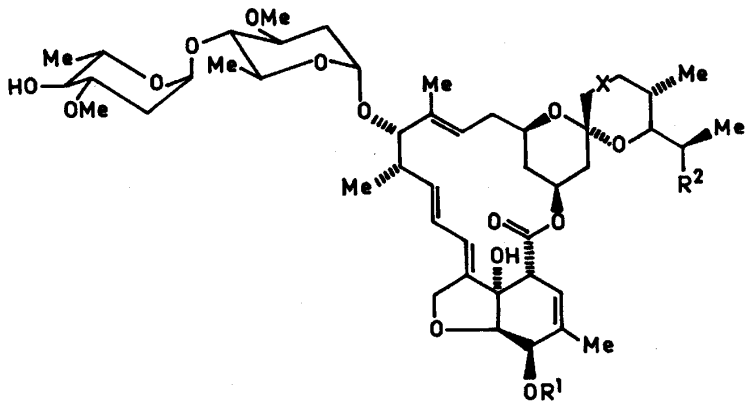
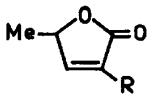
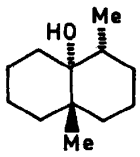


avermectins

	R^1	R^2	X
A_{1a}	Me	Et	-CH=CH-
A_{1b}	Me	Me	-CH=CH-
B_{1a}	H	Et	-CH=CH-
B_{1b}	H	Me	-CH=CH-
A_{2a}	Me	Et	$-CH_2-CHOH-$
A_{2b}	Me	Me	$-CH_2-CHOH-$
B_{2a}	H	Et	$-CH_2-CHOH-$
B_{2b}	H	Me	$-CH_2-CHOH-$



oxolons
R = Me, Et, Pr, Bu, Pe



geosmin

RESULTS AND DISCUSSION

As described earlier (Řezanka *et al.* 1994), the growth of *S. avermitilis* and the production of avermectins is accompanied by the production of specific earthy odors. The first phase of culture development is characterized by fast growth of biomass which is proportional to the consumption of nitrogen source components from the medium (Cimburková *et al.* 1988) and the odor substances include mainly oxolons. The second phase of culture development is characterized by the formation of avermectins. The formation of oxolons ceases and the spectrum of odor substances is dominated by an intensive production of geosmin. The third cultivation phase is characterized by the decay of cell biomass, avermectins and odor components.

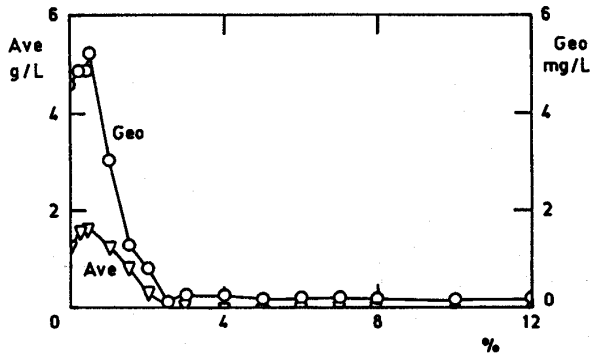


Fig. 1. The effect of salinity (% of NaCl, *W/W*) on the production of avermectins (Ave) and geosmin (Geo) after 10 d of submerse cultivation of *S. avermitilis*.

Fig. 1 shows the dependence of total avermectin production and production of geosmin on the concentration of NaCl in the culture medium. There is a strong linear correlation between the concentration of avermectins and geosmin (r^2 = 0.95). Fig. 2 and 3 summarize the effect of medium salinity on the concentration of oxolons and membrane fatty acids after 10 d of cultivation. The amount of monoenoic fatty acids and acids with an even number of C atoms increases, similarly to oxolons, with the salt content of the medium. The greatest part of aliphatic fatty acids was represented by unsaturated palmitoleic acid, not very common in actinomycetes (Verma and Khuller 1983; Kaneda 1991). On the contrary, the raise of aliphatic acid fraction followed the decrease in the content of anteiso- and isoeven acids. Thus, the increased salinity of the medium changed the ratio between aliphatic and branched fatty acids, known to be an important factor affecting the fluidity of cellular membranes.

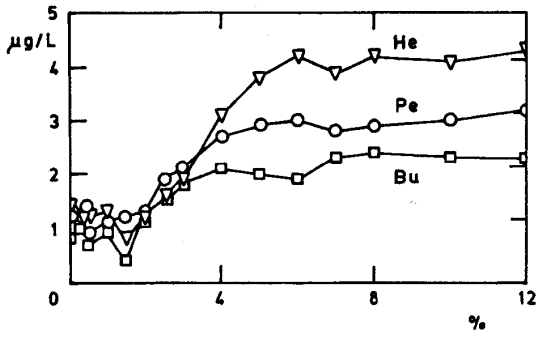


Fig. 2. The effect of salinity (% of NaCl, *W/W*) on the composition of the mixture of oxolons (μg/L); hexyloxolon (He), pentyloxolon (Pe), butyloxolon (Bu).

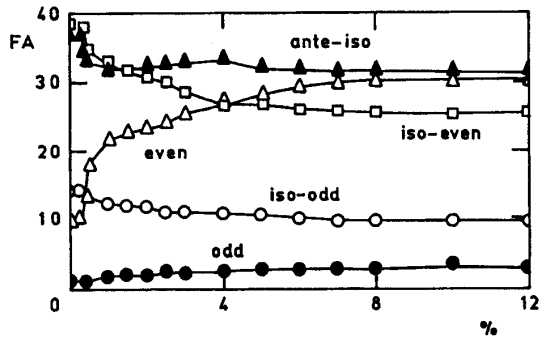


Fig. 3. The effect of salinity (% of NaCl, *W/W*) on the relative composition of fatty acids (FA, %).

To elucidate the effect of salinity on the physiology of *S. avermitilis*, we suggested a simple mathematical model describing the growth and product formation. The course of 10-d cultivation was divided into three physiologically different phases (Fig. 4). The role of nitrogen source limitation on the shift of growth (I) to production phase (II) has been described previously (Cimburková *et al.* 1988; Votruba 1994). The third phase (III) started when sugar became exhausted and was characterized by a decay of cell biomass, avermectins and geosmin.

The time course of biomass (X) and products (P) concentrations may be described by the following balances:

$$dX/dt = \mu X;\ dP/dt = rX$$

where μ and r are specific rates of growth and product formation in the given phase of submersed batch culture development, defined in the following way:

Phase I	$t \le t_1$	$\mu = \mu_{max} K/(K + c_{NaCl})$	$r_{Ave} = 0$	$r_{Geo} = 0$	$r_{Oxo} = k_1\mu$
Phase II	$t_1 \le t \le t_2$	$\mu = 0$	$r_{Ave} = k_2$	$r_{Geo} = k_3$	$r_{Oxo} = -k_D c_{Oxo}$
Phase III	$t_2 \le t$	$\mu = -k_D X$	$r_{Ave} = -k_D c_{Ave}$	$r_{Geo} = -k_D c_{Geo}$	$r_{Oxo} = -k_D c_{Oxo}$

Having applied the model to the experimental data (Figs 1 and 2), we have estimated that the value of inhibition constant K ranges between 0.24 and 0.39 % (*W/W*) NaCl and the value of μ_{max} was about 0.29/d. The duration of phase I increases with the salinity of the medium. The minimum time of its duration (t_1) was about 3.5 d. The length of phase II was independent of salinity and the interval

between t_1 and t_2 was about 5 d. The specific rate constant describing the product decay (k_D) fluctuated between 0.5 to 0.8/d for products and biomass.

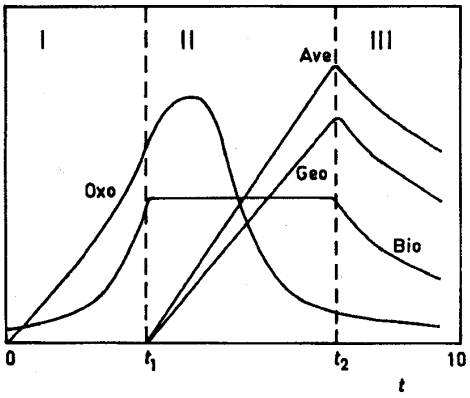


Fig. 4. Physiological phases (I, II, III) of development of *S. avermitilis* batch culture; concentrations of avermectins – Ave, biomass – Bio, geosmin – Geo, oxolons – Oxo, time – *t*.

In conclusion, the salinity of the medium prolonged phase I of culture development. The observed decline in the production of geosmin and avermectins below 0.5–1.5 % (*W/W*) NaCl can be attributed to the decay processes in phase III. The maximum of product formation corresponds to the end of phase II, where time t_2 equals 10 d. The increase of the concentration of oxolons at NaCl upto 1 % (*W/W*) can be ascribed to the extension of phase I due to increased salinity. It should be noted that the relationship between fatty acid composition and salinity (Fig. 3) indicates that the amount of monoenoic acids and fatty acids with even number of C atoms were overproduced in the growth phase I. Relative proportion of iso-odd acids and odd acids was unaffected by increased salinity of the medium.

The mutual relationship among odors, avermectins and fatty acids production is rather complex and requires further study. However, it can be assumed that the biosynthesis of these compounds is closely linked, probably through the existence of common precursors.

The research was supported by grant no. 310/94/1192 of the *Grant Agency of the Czech Republic*.

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