Folia Microbiol. 29, 306–309 (1984)

The Use of Different Oils for the Cultivation of *Streptomyces cinnamonensis*

T. Řezanka, Z. Vaněk, K. Klánová* and M. Podojil

Department of Biogenesis of Natural Substances, Institute of Microbiology, Czechoslovak Academy of Sciences, 142 20 Prague 4

Received October 12, 1983

ABSTRACT. The addition of 2–4 % oils to the synthetic fermentation medium used for the cultivation of *Streptomyces cinnamonensis* increased the production of monensin three times on the average. When the amount of the added oil was lower than 2 % and higher than 4 % the production sharply decreased. The maximal production preceded the maximal consumption of individual fatty acids of the added oils, the content of oleic acid decreasing most pronouncedly.

Monensin is an efficient coccidiostatic of polyether type (Shumard and Callender 1968) produced by *Streptomyces cinnamonensis*. It was first described in 1967 (Agtarap *et al.* 1967) as a complex of four structurally related compounds, monensins A–D. The original production of the monensin complex in the wild strain reached 125–150 μg/mL. By adjusting cultivation conditions, *e.g.* when supplementing media with ferric sulfate or individual fatty acids in the form of their methyl esters, it was possible to increase the production up to 5 000 μg/mL (Stark *et al.* 1968). However, due to economical reasons, methyl esters of fatty acids cannot serve as components of the cultivation media in a large scale fermentation.

The present communication extends our previous studies concerned with the relationship between monensin production and the type of oil used as a carbon source (Vaněk *et al.* 1982). Four commercially available oils — rape-seed, olive, soya and cod-liver — were tested. To exclude interference of soybean meal fatty acids (soybean meal is a standard component of complex cultivation media) a synthetic medium was used.

MATERIAL AND METHODS

Cultivation and biological assay of monensin. *S. cinnamonensis* EC 100 (Institute of Microbiology, Czechoslovak Academy of Sciences) was cultivated under submerged conditions in a synthetic medium containing (g/L): glucose 50, K_2HPO_4 0.5, $MgSO_4$ 0.5, KNO_3 1.0, NaCl 0.5, $FeSO_4$ 0.01; the medium was supplemented with the tested oil in the amount of 0.062–32 mL per 50 mL of the basic medium (*i.e.* 1.24–640 mL/L). Fermentation con-

* *Present address:* Institute of Hygiene and Epidemiology, 100 00 Prague 10.

ditions were described by Řezanka *et al.* (1984). Biological activity was determined by the plate method using *Bacillus subtilis* as test organism and a standard of monensin.

Analysis of fatty acids. After the cultivation three parallel flasks were combined, the fermentation medium was filtered and the residue on the filter was washed with 50 mL of diethyl ether. The filtrate was extracted with 3 × 50 mL of diethyl ether, dried and evaporated to dryness. Five mL of 2 M MeONa in MeOH were added to the evaporate. After a 1-d standing 2 mL of 6 M HCl in MeOH were added and the mixture was heated to boiling; methyl esters were extracted three times with 5 mL portions of hexane and hexane was evaporated to dryness.

Methyl esters were identified on the basis of retention characteristics and identity of retention times with those of standard mixtures (reference mixture kit RM-7, Supelco, USA) by gas chromatography using the Varian 2740 apparatus with the packed column 2 mm ID × 4.5 m with stationary phase 5 % Silar 10C on Gas Chrom Q AW-DMCS.

RESULTS AND DISCUSSION

The consumption of fatty acids depends on the chain length and degree of their unsaturation. The order of the consumption of fatty acids in individual oils is standard (Fig. 1). The highest decrease is observed in the content of oleic acid (18:1) and linoleic acid (18:2) in all plant oils. In soybean oil and olive oil the above acids are followed by palmitic acid (16:0) and linoleic acid (18:3). In rape-seed oil they are followed by linolenic acid (18:3), palmitic acid (16:0) and erucic acid (22:1).

The animal cod-liver oil differs in its characteristics from the three above-mentioned oils containing long, highly unsaturated fatty acids. These acids could not be demonstrated after termination of the cultivation as at oil concentrations higher than 5 mL/L, the acids are degraded due to the increased temperature during the cultivation and due to aeration. The acids are polymerized or oxidized, giving rise to degradation products inhibiting also the growth of the production strain. Also in the case of this oil it can be seen that oleic acid is most effectively consumed, followed by the pair myristic and palmitoleic acid and by the pair palmitic and linoleic acid. Eicosenoic acid is used up much less and erucic acid is again used up least effectively. In the case of the first three plant oils the maximum biological activity precedes the maximum of the consumption of acids, whereas in the case of the animal oil the maxima are almost identical.

The maximum production of monensin (three-fold of the control on the average) is reached, with the exception of the animal cod-liver oil, at a concentration of 40—80 mL/L. The cod-liver oil behaves differently (due to the highly unsaturated fatty acids), in spite of the fact that just in the case of this oil the biological activity reaches highest values (55 μg/mL), already at oil concentrations of 5 mL/L. This fact might be explained by the presence of vitamins (probably vitamins A and D and the B complex) in the oil.

The consumption of oils expressed by the consumption of acids increases in plant oils and cod-liver oil up to 80—160 mL/L and 5 mL/L of the medium, respectively. The growth (metabolism) of the organism culminates apparently after the maximal production of the antibiotic.

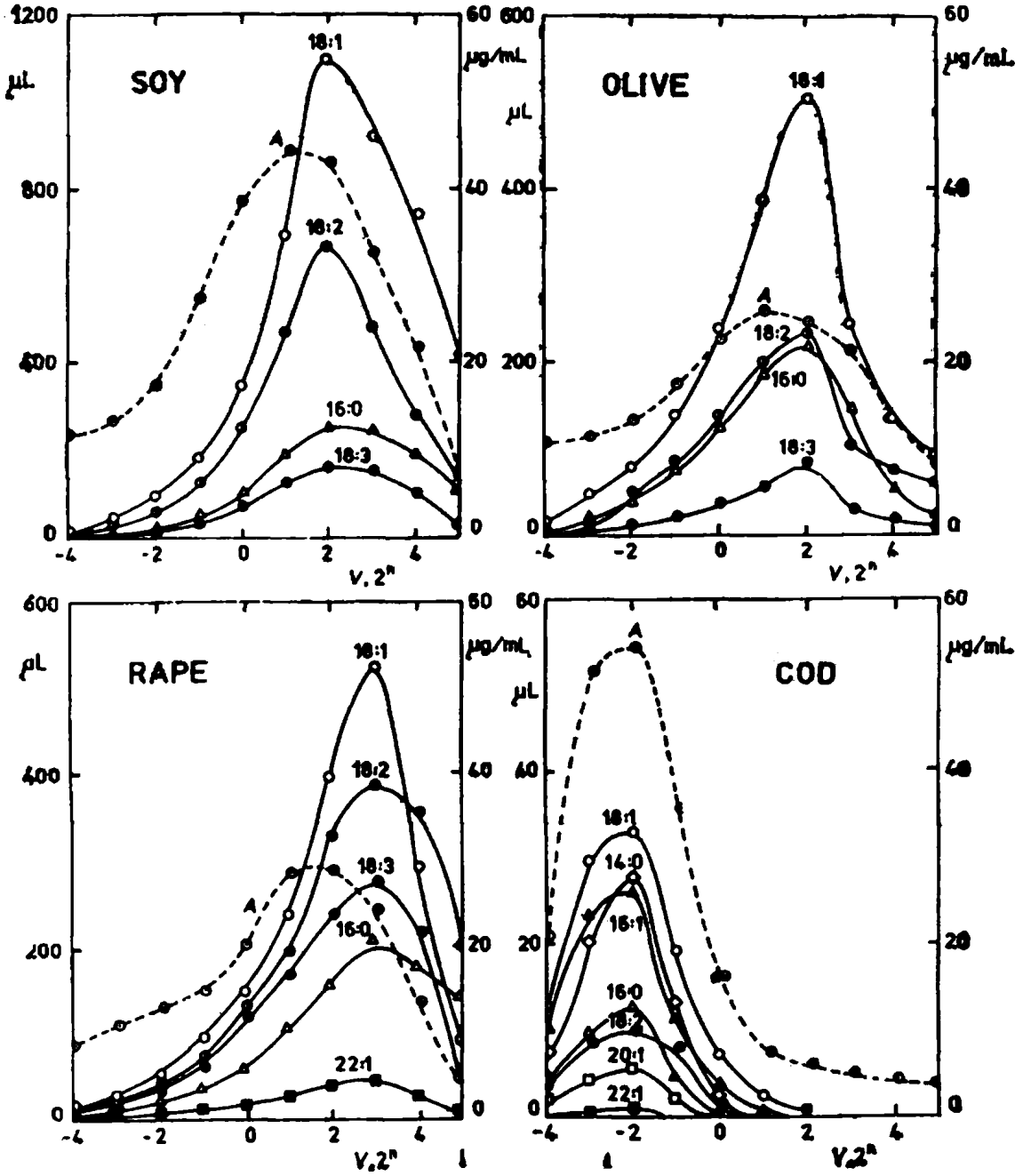

FIG. 1. Relationship between the amount of oils added to the basic medium (V, 2^n; n = mL of oil), consumption of their fatty acids (µL) and monensin production (A, µg/mL);
14 : 0 — myristic (tetradecanoic) acid
16 : 0 — palmitic (hexadecanoic) acid
16 : 1 — palmitoleic (*Z*-9-hexadecenoic) acid
18 : 1 — oleic (*Z*-9-octadecenoid) acid
18 : 2 — linoleic (*Z*-9, *Z*-12-octadecadienoic) acid

The results indicate that the optimum addition of plant oils is 40—80 mL and of the cod-liver oil 5 mL per litre fermentation medium. When these values are exceeded the production of this coccidiostatic sharply decreases, in spite of the fact that the consumption of fatty acids remains practically constant. This is apparently due to the decreased oxygen transfer caused by the high proportion of the added oil.

REFERENCES

AGTARAP A., CHAMBERLIN J.W., PINKERTON M., STEINRAUF L.: The structure of monensic acid, a new biologically active compound. *J. Am. Chem. Soc.* **89**, 5737 (1967).

ŘEZANKA T., VANĚK Z., KLÁNOVÁ K., PODOJIL M.: Fatty acids of *Streptomyces cinnamonensis*, producer of monensin. *Folia Microbiol.* **29**, 217 (1984).

SHUMARD R.F., CALLENDER M.E.: Monensin, a new biologically active compound. VI. Anticoccidial activity. *Antimicrob. Agents Chemother.* **369** (1967).

STARK W.M., KNOX N.G., WHITEHEAD J.E.: Monensin, a new biologically active compound. II. Fermentation studies. *Antimicrob. Agents Chemother.* **353** (1967).

VANĚK Z., KRUMPHANZL V., ŠEVČÍK B., BÉLÍK E., BEDNÁŘ P., STAJNER K., TAX J., ROKOS J., KRÁLOVCOVÁ E., ZAJÍČEK J., KLÁNOVÁ K.: The way of the production of monensin, of the A factor in particular. *Czech. Pat.* 218 032 (1982).

18 : 3 — linolenic (*Z*-9, *Z*-12, *Z*-15-octadecatrienoic) acid
20 : 1 — eicosenic (*Z*-9-eicosenoic) acid
22 : 1 — erucic (*Z*-13-docosenoic) acid