INHIBITORY EFFECT OF SUCROSE ESTER OF LAURIC ACID ON THE GROWTH OF *ESCHERICHIA COLI*

Akiko Kato and Kei Arima

Laboratory of Microbiology
Department of Agricultural Chemistry
University of Tokyo, Tokyo, Japan

Received December 4, 1970

Summary: Effect of fatty acids and their derivatives on the growth of *E. coli* was studied. Lauric acid showed the strongest inhibition among the fatty acids tested. Most of the derivatives of lauric acid were less effective than lauric acid but its sucrose ester inhibited the bacterial growth to the greater extent than lauric acid itself. Addition of sucrose ester immediately stopped the bacterial growth independent of the time of addition.

INTRODUCTION

During our screening study for antitumor antibiotics produced by fungi, we found that fatty acids and monoglycerides isolated from acetone extract of some fungal mycelia showed antitumor activity against Ehrlich ascites tumor in mice (1, 2). Subsequent studies on the antitumor activity of commercial fatty acids and their derivatives indicated that some fatty acids as lauric and linoleic acids showed strong activity and that among the fatty acid esters tested, sucrose esters exhibited the strongest antitumor activity (3, 4, 5).

In order to clarify the mechanism of action of antitumor activity of fatty acids and their derivatives, we studied the effect of these compounds on animal and bacterial cells. Sucrose esters of fatty acids were mainly used through these studies because they are very soluble in water and showed strong antitumor activity *in vivo*. The result of our study on the effect of fatty acids and their derivatives on animal cells will be reported elsewhere (in preparation). The present report describes the effect of sucrose ester of lauric acid on the growth

of gram negative bacterium, Escherichia coli.

MATERIALS AND METHOD

Fatty acids and their derivatives: Sucrose monoester of lauric acid was synthesized according to the method described by Osipow et al (6). All the fatty acids and other derivatives were commercially available.

Strain and culture: E. coli K-12 was used through out the present study. E. coli K-12 was grown at 37°C in a Monod tube containing 10 ml of the medium. The composition of the medium is as follows (gram per liter): $Na_2HPO_4 \cdot 2H_2O$, 8.8; KH_2PO_4, 3.0; NH_4Cl, 1.0; $MgSO_4$ $7H_2O$, 0.02; $FeSO_4 \cdot 7H_2O$, 0.0005; glucose, 4.0. The bacterial growth was measured by the optical density at 550 mμ. Seed culture was prepared by inoculating one loopful of the agar slant culture into 10 ml of the medium and shaken at 37°C overnight. The culture thus obtained was inoculated into the medium containing the test compound, the inoculum size being 2.0 %.

RESULTS AND DISCUSSION

First, we examined the effect of long chain fatty acids on the growth of E. coli. Fatty acids with carbon atoms between ten and eighteen were tested: namely, capric (C_{10}), undecanoic (C_{11}), lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}), stearic (C_{18}), oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids. The result indicated that capric, undecanoic and lauric acids showed significant inhibitory effect on the bacterial growth, and that lauric acid exhibited the strongest activity.

All the fatty acids with long chain and most of their esters are insoluble in water, which is a serious problem in carrying out biochemical experiments. In the course of our study of anti-tumor activity of fatty acids, we searched for water soluble derivatives and found that sucrose monoesters were with such

character. We synthesized them and examined their antitumor activity. It was found that they were more active than fatty acids or other esters in vivo (5). So we compared the activity of sucrose laurate with lauric acid and other esters against the bacterium. The result was summarized in Table 1. While the methyl ester and Tween 20 (polyoxyethylene sorbitan monolaurate) showed no inhibitory effect at the final concentration below 1 mg/ml, lauric acid and its sucrose ester inhibited the growth at this concentration. When the concentration was reduced to 100 μg/ml lauric acid showed no more inhibition but sucrose ester did inhibit the growth of E. coli even at that concentration. Therefore we concluded that the sucrose ester is more active than the fatty acid itself against the bacterium.

Table 1. Effect of Lauric Acid and Its Derivatives on the Growth of E. coli

Addition	Concentration (mg/ml)	O. D. at 550 mu 4 hr.	6 hr.	8 hr.
Lauric Acid	1.0	0.072	0.062	0.105
	0.1	0.160	0.550	0.630
Methyl Ester	1.0	0.162	0.499	0.660
	0.1	0.185	0.610	0.700
Sucrose Ester	1.0	0.050	0.050	0.050
	0.1	0.015	0.010	0.010
Tween 20	1.0	0.150	0.520	0.600
	0.1	0.128	0.480	0.630
None		0.160	0.565	0.650

Fig. 1 shows dose responses of sucrose laurate and Tween 20. Sucrose laurate at the concentration higher than 200 μg/ml completely inhibited the growth and 100 μg was still inhibitory.

When concentration was lowered to 10 μg, however, the bacterium could grow with the same rate as the untreated control. Tween 20 did not show inhibitory effect even at the concentration of 10 mg/ml.

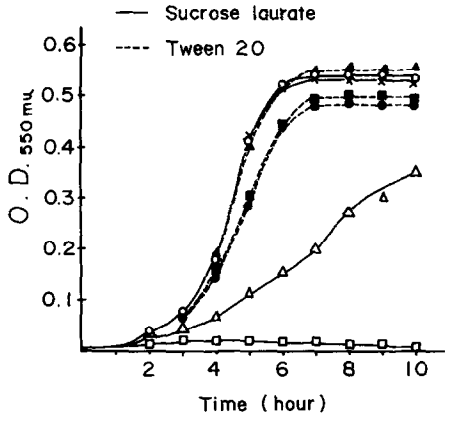

Fig. 1. Effect of sucrose monolaurate and Tween 20 on the growth of E. coli
E. coli K-12 was grown in a medium containing either sucrose monolaurate or Tween 20 at the concentration indicated below.
solid line: sucrose monolaurate
□—□ 200 μg/ml △—△ 100 μg/ml
×—× 10 μg/ml
dashed line: Tween 20
●---● 10 mg/ml ■---■ 1 mg/ml
▲---▲ 100 μg/ml
control ○—○

Fig. 2 presents the effect of sucrose laurate added at the various phases of the growth. The compound was added at 0, 3, 5, and 7 hours after the inoculation in the final concentration of 200 μg/ml. The bacterial growth stopped immediately after the addition of the ester regardless of the bacterial growth phase.

Effects of fatty acids on the growth of various microorganisms had been studied since in 1940's and the results were well reviewed by Nieman in 1954 (7). According to Nieman only gram

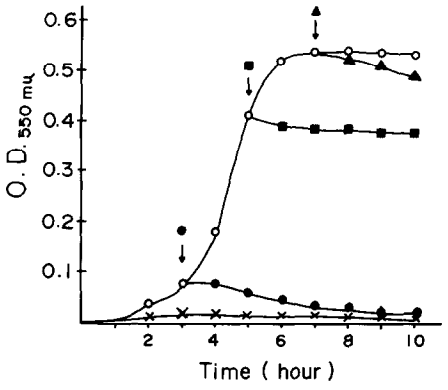

Fig. 2. Effect of sucrose monolaurate added at different growth phases of E. coli
E. coli K-12 was grown in a Monod tube and 0.2 ml of pre-warmed solution of sucrose monolaurate was added in the final concentration of 200 μg/ml. The arrow indicates the time of the addition.

positive organisms were generally susceptible to the action of fatty acids, although effects on gram negative organisms were also reported. D. M. Eisler et al while studying an anti-Pasteurella pestis factor from the organs of normal animals found that some of the fatty acids were effective in killing Pasteurella pestis (8).

Here we reported the effect of some fatty acids and their derivatives on the growth of E. coli. Spector, in 1946, reported the inhibitory effect of the fatty acid mixture from butter fat and corn oil on the growth of E. coli and this was the only report describing the inhibition of E. coli by fatty acids. Utilizing a water soluble derivative of fatty acid, sucrose ester of lauric acid, we studied the effect of fatty acids on the growth of E. coli and found that this bacterium was inhibited by low concentration of sucrose monolaurate in the medium and that its effect was such that its addition immediately stopped the bacterial growth. Inhibitory effect of sucrose

monolaurate was greater than that of lauric acid itself. The effect of the ester was bacteriostatic rather than bactericidal, for the cells began to grow after incubation for more than 20 hours in the medium containing 200 μg/ml of sucrose monolaurate (unpublished data). We don't know yet the mechanism of action of this ester exhibiting bacteriostatic effect on E. coli. In the present report it was also shown that utilization of sucrose esters of fatty acids was a very significant method in studying the mechanism of action of fatty acids without interference by their solubility.

REFERENCES

1. Ando, K., S. Suzuki, K. Kodama, K. Kato, G. Tamura, and K. Arima, J. Antibiotics 22, 18 (1969)
2. Kato, A., K. Ando, K. Kodama, S. Suzuki, K. Suzuki, G. Tamura and K. Arima, J. Antibiotics 22, 67 (1969)
3. Ando, K., A. Kato, S. Suzuki, G. Tamura and K. Arima, Proc. 6th Internatl. Congress Chemotherapy Vol. II, 136 (1970)
4. Kato, A., K. Ando, S. Suzuki, G. Tamura, and K. Arima, J. Antibiotics 22, 83 (1969)
5. Kato, A., K. Ando, S. Suzuki, G. Tamura, and K. Arima, Proc. 6th Internatl. Congress Chemotherapy Vol. II, 142 (1970)
6. Osipow, L., F. D. Snell, W. C. York, and A. Finchler, Indust. Eng. Chem. 48, 1459 (1956)
7. Nieman, C., Bacteriol. Rev. 18, 147 (1954)
8. Eisler, D. M. and E. K. von Metz, J. Bacteriol. 95, 1767 (1968)