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Studies on Marine Bacteria Producing Lytic Enzymes−X
Effect of Membrane-modifying Agents on the Production of Lytic Enzyme

Isao SUGAHARA, Koichiro HAYASHI, Toshio KIMURA, Shigekazu YAMANAKA, Hiroyuki NIWA and Nobuyoshi TANIOKA
Faculty of Fisheries, Mie University

The effect of membrane-modifying agents on the production of lytic enzyme by strain V 37 was studied using procaine, cerulenin, Triton X-100, and so on.

At the concentrations where growth (general protein synthesis) was not suppressed, the formation of lytic enzyme by strain V 37 was not inhibited by procaine-hydrochloride, cerulenin, Triton X-100, Tween 80, EDTA-2Na and ouabain, respectively.

Key words: lytic enzyme, marine bacteria, membrane-modifying agents

As previously reported, strain V 37 isolated from coastal waters produced extracellular lytic enzyme(s) when grown in polypeptone-yeast extract medium containing 0.5 M NaCl (SUGAHARA *et al.* 1980). However, NaCl was not effective for the release of lytic enzyme from strain V 37 cells (SUGAHARA *et al.* 1981b, 1983).

In order to demonstrate subcellular location of the lytic enzyme production, the authors investigated the effect of membrane-modifying agents on the production of lytic enzyme using a variety of alcohols (SUGAHARA *et al.* 1984). The formation of lytic enzyme was suppressed by n-alcohols ranging from 8 to 14 carbon atoms, phenethyl alcohol and benzyl alcohol, respectively.

The present paper deals with the effect of membrane-modifying agents, other than alcohols, on the production of lytic enzyme by strain V 37.

Methods

Assay of lytic activity

Lytic activity was determined as described previously (SUGAHARA *et al.* 1976, 1978, 1979, 1980, 1981a, 1981b, 1983).

Culture of strain V 37 capable of producing lytic enzyme

The medium used in this study was composed of polypeptone (Daigo Eiyō Kagaku), 10.0 g: yeast extract (Nakarai Chemicals), 5.0 g: NaCl, 0.5 mole : varying amounts of

membrane-modifying agents to be tested and 1,000 ml distilled water. The pH of the medium was adjusted to 7.0. The liquid medium was dispensed in 250 ml portions into 500 ml Sakaguchi flasks. Sterilization was done by means of autoclaving at 121 °C for 15 minutes. The Sakaguchi flask containing 250 ml of the preculture medium without the addition of any membrane-modifying agent was inoculated with a loopful of strain V 37, and incubated at 30 °C for 24 hours on a reciprocating shaker with a shaking rate of 100 strokes per min. The flasks containing 250 ml of the medium to which had been added varying amounts of membrane-modifying agent were inoculated with each 1.0 ml of strain V 37 culture fluids from the preculture medium. The inoculated flasks were incubated at 30 °C for 24 hours on a reciprocating shaker. The culture fluids were withdrawn after 24 hours of cultivation, and the cells removed by centrifugation at 13,000 × g for 20 minutes. The culture supernatant obtained was dialysed against distilled water at 5 °C for 3 hours. The dialysate of the culture supernatant was used for the assay of lytic activity.

Results

Table 1. Effect of membrane-modifying agents on the production of lytic enzyme

Agents	Concentration	Growth (% of untreated control)	Production of lytic enzyme (% of untreated control)
Procaine-hydrochloride	0.1 mg/ml	97.0	94.9
	0.3	96.0	96.8
	0.5	100.9	71.5
	1.5	94.0	92.9
	3.0	99.5	111.9
	5.0	109.9	98.4
	8.0	104.2	113.1
Cerulenin	1.4 μg/ml	113.2	84.3
	2.0	101.7	109.4
	3.2	99.4	110.9
	6.0	116.8	114.2
	10.0	107.4	104.6
	20.0	148.3	142.7
Triton X-100	0.0005 %(v/v)	98.4	103.1
	0.0010	109.4	113.8
	0.0020	105.7	115.3
	0.0030	104.0	115.4
	0.0040	105.4	113.0
	0.0050	31.9	18.6
	0.0100	0	0

Tween 80	0.1 %(v/v)	96.7	105.0
	0.2	69.9	84.9
EDTA-2Na	0.1 mM	99.2	82.3
	0.2	81.8	76.4
	0.3	31.9	25.5
	0.4	15.9	10.5
	0.5	16.0	7.3
	1.0	0.7	0
Ouabain	4.0 mM	97.4	101.9
	10.0	98.3	99.4

Procaine—hydrochloride

Table 1 shows the effect of procaine-hydrochloride on the production of lytic enzyme and the growth of strain V 37. Procaine-hydrochloride at the concentrations from 0.1 to 8.0 mg/ml did not inhibit the production of lytic enzyme nor the growth of strain V 37.

Cerulenin

As shown in Table 1, cerulenin (1.4-20.0 μg/ml) also did not have an inhibitory effect on the production of lytic enzyme nor on the growth of strain V 37.

Triton X-100

The production of lytic enzyme and growth of strain V 37 were suppressed by 0.0050 % (v/v) of Triton X-100 (Table 1). Strain V 37 could not grow at concentrations above 0.0100 % (v/v).

Tween 80

Tween 80 (0.2 %, v/v) slightly repressed the production of lytic enzyme and the growth of strain V 37 (Table 1).

EDTA-2Na

The increase in the concentrations from 0.1 to 0.5 mM decreased the production of lytic enzyme as well as the growth of strain V 37 (Table 1).

Ouabain

Ouabain (4.0-10.0 mM) showed no inhibitory effect on the production of lytic enzyme nor on the growth of strain V 37 (Table 1).

Effect of membrane-modifying agents on the lytic activity

As shown in Table 2, none of the membrane-modifying agents at the concentrations used in this study inhibited lytic activity.

Discussion

FEINSTEIN *et al.* (1975) observed alterations in membrane organization and properties by procaine. TRIBHUWAN and PRADHAN(1977) described that procaine inhibited the formation of alkaline phosphatase in *Escherichia coli*. FISHMAN *et al.* (1980) also showed that procaine-

Table 2. Effect of membrane-modifying agents on the lytic activity of strain V 37

Agents	Concentration	Remaining lytic activity
None		100.0 %
Procaine-hydrochloride	3.3 mg/ml	95.1
Cerulenin	20.0 μg/ml	98.4
Triton X-100	0.006 %(v/v)	106.1
Tween 80	0.2 %(v/v)	102.2
EDTA-2Na	0.5 mM	91.8
Ouabain	6.7 mM	95.4
Ethyl alcohol	4.0 %(v/v)	97.8

hydrochloride (1.5-6.0 mg/ml) suppressed the production of alkaline phosphatase and penicillinase from *Bacillus* sp.

Cerulenin is also known to inhibit lipid synthesis and cause changes in membrane composition (OMURA, 1976). The formation of alkaline phosphatase, penicillinase or staphylococcal toxins was susceptible to cerulenin (FISHMAN *et al.* 1977, 1980, BERKELEY *et al.* 1978). According to FISHMAN *et al.* (1977), at low concentrations (5 to 10 μg/ml), cerulenin had little or no effect on the general protein synthesis, but the appearance of active penicillinase of *Bacillus licheniformis* was strongly inhibited. Therefore, they inferred that the preferential suppression of penicillinase can be attributed to the specific effect of cerulenin on the composition of the membrane.

In this study, the production of lytic enzyme by strain V 37 was not inhibited by 0.1-8.0 mg/ml of procaine-hydrochloride or 1.4-20.0 μg/ml of cerulenin (Table 1).

Ouabain (g-strophantin) is known to inhibit both the Na^+, K^+-activated ATPase and Na^+ transport in animal cells. However, ouabain had no effect on either activity in the marine bacterial cells (MACLEOD 1968). At the concentrations where general protein synthesis was not suppressed, the formation of lytic enzyme by strain V 37 was not inhibited by Triton X-100, Tween 80, EDTA-2Na nor ouabain, respectively (Table 1). On the other hand, n-alcohols ranging from 8 to 14 carbon atoms, phenethyl alcohol and benzyl alcohol did have an inhibitory effect on the production of lytic enzyme by strain V 37 (SUGAHARA *et al.* 1984). This may be due to the different interactions between bacterial membranes and membrane-modifying agents.

References

BERKELEY, R. C. W., E. A. PEPPER, M. P. CAULFIED and J. MELLING, 1978. The inhibition of *Staphylococcus aureus* enterotoxin A production by cerulenin and quinacrine: Presumptive evidence for a lipid intermediate, protease release mechanism. FEMS *Microbiol. Lett.*, **4**: 103-105.

FEINSTEIN, M. B., S. M. FERNANDEZ and R. I. SHA'AFI, 1975. Fluidity of neutral membranes and phosphatidylserine and ganglioside dispersions. *Biochim. Biophys. Acta*, **413**: 354-370.

FISHMAN, Y., N. CITRI and S. ROTTEM, 1977. Formation of penicillinase in culture of *Bacillus licheniformis* treated with cerulenin. *Isr. J. Med. Sci.*, **13**: 958.

————, S. ROTTEM and N. CITRI, 1980. Preferential suppression of normal exoenzyme formation by membrane-modifying agents. *J. Bacteriol.*, **141**: 1435－1438.

MACLEOD, R. A., 1968. On the role of inorganic ions in the physiology of marine bacteria. Advances in Microbiology of the Sea (M. R. DROOP and E. J. FERGUSON WOOD eds.), Academic Press, Vol. **1**: 95－126.

OMURA, S., 1976. The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor of fatty acid synthesis. *Bacteriol. Rev.*, **40**: 681－697.

SUGAHARA, I., K. HAYASHI, T. KIMURA, M. MATSUOKA and K. FUJITA, 1976. Studies on marine bacteria producing lytic enzymes-I Lytic activity of culture filtrate. This Bull., **3**: 1－16.

————, ————, ———— and K. KATO, 1978. Studies on marine bacteria producing lytic enzymes-II. Composition of culture medium for the production of lytic enzymes. *Ibid.*, **5**: 25－46.

————, ————, ———— and N. HAZUMI, 1979. Studies on marine bacteria producing lytic enzymes-III. Effects of inorganic salts and enzyme inhibitors on the lytic activity. *Ibid.*, **6**: 49－56.

————, ————, ————, Y. SUZUKI, H. TOYODA and A. MATSUOKA, 1980. Studies on marine bacteria producing lytic enzymes-IV. Effect of inorganic salts on the production of lytic enzyme. *Ibid.*, **7**: 9－27.

————, ————, ————, A. MATSUOKA, C. JINNO and S. YAMANAKA, 1981a. Studies on marine bacteria producing lytic enzymes-V. Culture condition for the production of lytic enzyme. *Ibid.*, **8**: 49－60.

————, ————, ———— and A. MATSUOKA, 1981b. Studies on marine bacteria producing lytic enzymes-VI. Effect of inorganic salts on the release of lytic enzyme from bacterial cells. *Ibid.*, **8**: 61－71.

————, ————, ———— and C. JINNO, 1983. Studies on marine bacteria producing lytic enzymes-VIII. Release of lytic enzyme from bacterial cells. *Ibid.*, **10**: 25－32.

————, ————, ————, S. YAMANAKA, H. NIWA and N. TANIOKA, 1984. Effect of alcohols on the production of lytic enzyme by a bacterium isolated from coastal waters. *Bull. Japan. Soc. Sci. Fish.*, **50**: 1057－1060.

TRIBHUWAN, R. C. and D. S. PRADHAN, 1977. Induction of alkaline phosphatase in *Escherichia coli*: Effect of procaine-hydrochloride. *J. Bacteriol.*, **131**: 431－437.