Effect of Alcohols on the Production of Lytic Enzyme by a Bacterium Isolated from Coastal Waters

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The suppression of the production of lytic enzyme was investigated using alcohols as membrane-modifying agents.

At the concentrations which growth was not suppressed, the production of lytic enzyme by strain V 37 was inhibited by n-alcohols having a range of 8 to 14 carbon atoms, phenethyl alcohol and benzyl alcohol, respectively.

The formation of extracellular lytic enzyme by the cells from the lag phase to the early logarithmic phase of growth was most susceptible to membrane-modifying agents such as octanol and phenethyl alcohol.

The bacterium (named strain V 37) isolated from coastal regions extracellularly produced bacteriolytic enzyme when aerobically grown at 30°C in polypepton-yeast extract medium containing inorganic salt, such as NaCl, KCl, NaSCN, Na₂SO₄, and K₂SO₄.^{1,2)} Strain V 37 grew well in the medium without added any inorganic salt, but could not produce bacteriolytic enzyme.¹⁾ Among inorganic salts tested, NaCl or KCl was most effective for the production of extracellular lytic enzyme,¹⁾ whereas the release of lytic enzyme from strain V 37 cells was not stimulated by NaCl and KCl, respectively.^{3,4)}

FISHMAN *et al.*⁵⁾ indicated that a variety of alcohols and anesthetics selectively suppressed the formation of exoenzymes produced by various bacterial strains under conditions that allow normal growth.

In order to demonstrate the subcellular location of the enzyme formation, alcohols as membrane-modifying agents were used in this study. This paper deals with the effect of alcohols on the production of bacteriolytic enzyme by strain V 37.

Methods

Culture of Strain V 37 Capable of Producing Lytic Enzyme

The medium used in this study was composed of polypepton (Daigo Eiyō Kagaku), 10.0 g; yeast extract (Nakarai Chemicals), 5.0 g; NaCl, 0.5 mol; varying amounts of alcohol 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 min, except for alcohol component. Alcohol component was added aseptically into the sterile medium. The Sakaguchi flask containing 250 ml of the preculture medium without added any alcohol was inoculated with a loopful of strain V 37, and incubated at 30°C for 24 h on reciprocating shaker with a shaking rate of 100 strokes per min. The flasks containing 250 ml of the medium added varying amounts of alcohol respectively 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 h on reciprocating shaker. The culture fluids were withdrawn at fixed intervals of time, and then the cells were removed by centrifugation at 13,000×g for 20 min. The culture supernatant obtained was dialysed against distilled water at 5°C for 3 h. The dialysate of the culture supernatant was used for the assay of bacteriolytic activity.

Assay of Lytic Activity

Lysis of bacterial suspension was estimated by following the change in optical density at 570 nm (OD₅₇₀) in a Shimadzu Spectronic 20 spectrophotometer. The reaction mixture for the assay of lytic activity was as follows: heat-killed cell suspension of *Micrococcus luteus* 1.0 ml, 0.05 M Tris-HCl

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buffer (pH 7.0) 1.0 ml and dialysate of the culture supernatant or sonicated cell suspension 1.0 ml. The assay was carried out for 30 min at 40°C, and the optical density (OD₅₇₀) of the reaction

mixture was determined at fixed intervals of time. Lytic activity was represented as a decrease in OD₅₇₀ per 1.0 ml of culture fluids for 30 min.

Table 1. Effect of alcohols on the lytic activity of strain V 37

Alcohols	Concentration	Remaining lytic activity
None		100.0%
Octanol	0.0065%(v/v)	102.0
Decanol	0.00083%(v/v)	102.0
Dodecanol	0.00024%(v/v)	104.9
Tetradecanol	0.00048%(w/v)	102.9
Hexadecanol	0.024%(w/v)	95.7
Phenethyl alcohol	0.20%(w/v)	97.2
Benzyl alcohol	0.20%(w/v)	100.6

Lytic Activity Associated with Strain V 37 Cells

Strain V 37 cells, harvested by centrifugation, were suspended in 0.05 M Tris-HCl buffer (pH 7.0), and sonically treated for 4 min at 0°C with a sonicator (Ultrasonics W-375). After removing the cell debris by centrifugation, lytic activity of the supernatant was determined.

Results and Discussion

Effect of Alcohols on the Lytic Activity

As shown in Table 1, all alcohols used in this study did not inhibit lytic activity of strain V 37.

Table 2. Effect of alcohols on the production of lytic enzyme by strain V37

Alcohols	Concentration	Growth (% of untreated control)	Production of lytic enzyme
Octanol	0.0001 %(v/v)	101.7	104.0
	0.0010	117.0	136.9
	0.0030	132.5	109.2
	0.0040	132.8	88.8
	0.0050	111.8	49.5
	0.0055	98.7	25.9
	0.0072	87.2	14.7
Decanol	0.00010 %(v/v)	107.3	110.8
	0.00050	95.2	41.5
	0.00060	93.2	56.9
	0.00065	83.7	41.5
	0.00070	69.5	20.9
	0.00100	3.1	11.1
	0.00200	0	0
Dodecanol	0.00001 %(v/v)	93.1	94.9
	0.00010	99.5	52.4
	0.00015	100.2	47.0
	0.00020	81.5	32.8
	0.00030	0	0
Tetradecanol	0.0002 %(w/v)	81.9	41.8
	0.0003	73.7	40.7
	0.0004	80.9	35.5
	0.0007	23.0	23.0
Hexadecanol	0.005 %(w/v)	74.8	78.1
	0.010	91.1	91.2
	0.020	83.3	81.2
Phenethyl alcohol	0.10 %(w/v)	98.7	32.6
	0.20	95.3	5.9
	0.25	69.6	3.0
Benzyl alcohol	0.20 %(w/v)	99.6	9.5
	0.25	100.9	11.9

Effect of Alcohols on the Production of Lytic Enzyme

FISHMAN *et al.*⁵⁾ reported that aliphatic alcohols selectively suppressed the production of penicillinase by *Bacillus licheniformis*. ELIASZ *et al.*⁶⁾ also described that n-alcohols caused a shift in transition temperature of model membrane systems. These facts indicate that the production of exoenzyme was influenced by the changes in membrane organization.

As shown in Table 2, the suppression of lytic enzyme formation by n-alcohols ranging from 8 to 14 carbon atoms was observed in strain V 37. However, hexadecanol did not exhibit significant inhibitory effect on the production of lytic enzyme by strain V 37.

Phenethyl alcohol is known to inhibit the synthesis of unsaturated fatty acids in *Escherichia coli*.⁷⁾ Both phenethyl alcohol and benzyl alcohol caused a broadening of the main lipid transition temperature.⁸⁾ Phenethyl alcohol exerts its effect on the penicillinase formation of *Bacillus licheniformis* by changes in the physical state of membrane lipids rather than by interference with the synthesis of unsaturated fatty acids.⁸⁾

As shown in Table 2, phenethyl alcohol and benzyl alcohol were found to inhibit significantly the production of lytic enzyme by strain V 37.

Fig. 1. Effect of octanol addition on the production of lytic enzyme during the growth of strain V 37.

Each arrow indicates the cultivation time when octanol was added into the medium.

Lytic enzyme in the culture supernatant (■),
Lytic enzyme associated with strain V 37 cells (□).

Fig. 2. Effect of phenethyl alcohol addition on the production of lytic enzyme during the growth of strain V 37.

Each arrow indicates the cultivation time when phenethyl alcohol was added into the medium.

Lytic enzyme in the culture supernatant (■).

Lytic enzyme associated with strain V 37 cells (□).

Effect of Alcohol Addition on the Production of Lytic Enzyme during the Growth of Strain V 37

As shown in Figs. 1 and 2, the production of lytic enzyme was suppressed by the addition of octanol (0.0055%) and phenethyl alcohol (0.020%) respectively into the culture medium after 3 h of cultivation. However, the suppression of the enzyme formation was not complete when octanol or phenethyl alcohol was added into the culture medium after 4–5 h of cultivation. The formation of lytic enzyme by the cells from the lag phase to the early logarithmic phase of growth seemed to be most susceptible to membrane-modifying agents such as octanol and phenethyl alcohol.

References

- 1) I. SUGAHARA, K. HAYASHI, T. KIMURA, Y. SUZUKI, H. TOYODA, and A. MATSUOKA: *Bull. Fac. Fish., Mie Univ.*, **7**, 9–27 (1980).
- 2) I. SUGAHARA, K. HAYASHI, T. KIMURA, A. MATSUOKA, C. JINNO, and S. YAMANAKA: *Bull. Fac. Fish., Mie Univ.*, **8**, 49–60 (1981).
- 3) I. SUGAHARA, K. HAYASHI, T. KIMURA, and A. MATSUOKA: *Bull. Fac. Fish., Mie Univ.*, **8**, 61–71 (1981).

- 4) I. SUGAHARA, K. HAYASHI, T. KIMURA, and C. JINNO: *Bull. Fac. Fish., Mie Univ.*, **10**, 25-32 (1983).
- 5) Y. FISHMAN, S. ROTTEM, and N. CITRI: *J. Bacteriol.*, **141**, 1435-1438 (1980).
- 6) A. W. ELIASZ, D. CHAPMAN, and D. F. EWING: *Biochim. Biophys. Acta*, **448**, 220-230 (1976).
- 7) W. D. NUNN: *Biochemistry*, **16**, 1077-1081 (1977).
- 8) Y. FISHMAN, S. ROTTEM, and N. CITRI: *J. Bacteriol.*, **134**, 434-439 (1978).