Autolysis of Bacterial Cells Capable of Producing Lytic Enzyme

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The autolysis of bacterial cells (strain V37) capable of producing lytic enzyme was investigated using the washed cells grown in polypepton-yeast extract medium.

Strain V37 cells grown in the medium containing 0.5 M NaCl or KCl were conspicuously autolysed than those grown in the medium comprising 0.5 M MgCl₂ or without addition of any inorganic salt. The optimum pH and temperature for the autolysis of strain V37 cells were 7 to 9 and 40°C, respectively. The autolytic activity slightly increased in the presence of 0.2–0.3 M NaCl in 0.05 M Tris-HCl buffer (pH 7.0), but was significantly inhibited by bivalent cations such as Ba²⁺, Ni²⁺, Co²⁺ and Mg²⁺. The presence of NaCl (0.4–0.9 M) in the medium enhanced their ability to autolyse.

Autolytic enzyme (autolysin) is indispensable for the bacterial growth. Autolysins of *Bacillus* sp. are used during the growth process to open gaps in the cell wall in order to permit surface expansion and also used to separate linked daughter cells after septum formation.^{1,2)} The autolysins seem to be involved in transformation by externally added deoxyribonucleic acid,³⁾ in turnover of bacterial wall polymers and also in spore formation *etc.*⁴⁾

The bacterium (named strain V37) isolated from coastal waters extracellularly produced bacteriolytic as well as proteolytic enzymes when aerobically grown at 30°C in polypepton-yeast extract medium containing NaCl or KCl.⁵⁻⁷⁾ On the other hand, strain V37 cells, harvested during the growth from the early log to the middle log phases, exhibited a remarkable autolytic activity, although no significant autolysis was observed in the stationary-phase cells.⁸⁾ High concentration (2.0 M) of NaCl or low concentration (0.05 M) of Mg²⁺ considerably inhibited the autolytic activity of strain V37 cells grown in the medium containing NaCl (0.5 M).⁹⁾

The similarity between extracellular lytic enzyme and autolytic enzyme of strain V37 on the NaCl dependence is very interesting. Although lytic enzyme was accumulated in the culture supernatant fraction of culture fluids in the course of growth of strain V37, it has not yet been demonstrated whether intracellular lytic enzyme was liberated as a result of cell autolysis or whether autolytic enzyme (autolysin) itself was released from cells

during the growth. The physiological significance of extracellular lytic enzyme and autolytic enzyme of strain V37 is not well understood.

The present study was designated to obtain preliminary information concerning autolysis of strain V37 cells capable of producing lytic enzyme.

Methods

Culture of Strain V37 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; inorganic salt (NaCl, KCl or MgCl₂), 0 or 0.5 mol 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. The Sakaguchi flask containing 250 ml of the preculture medium without addition of any inorganic salt was inoculated with a loopful of strain V37, and incubated at 30°C for 24 h on reciprocating shaker with a shaking rate of 100 strokes per min. Each 1.0 ml of strain V37 culture fluids from the preculture medium was aseptically transferred into the medium containing inorganic salt (0.5 M NaCl, KCl or MgCl₂) or having no added inorganic salt. The inoculated flasks were incubated at 30°C on reciprocating shaker.

Assay of Autolytic Activity

After 4–7 h cultivation (OD₅₇₀; about 0.20–0.30)

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Fig. 1. Effect of pH value on the autolytic activity of strain V37 cells grown in polypepton-yeast extract medium containing 0.5 M NaCl.

Fig. 2. Effect of temperature on the autolytic activity of strain V37 cells grown in polypepton-yeast extract medium containing 0.5 M NaCl.

of strain V37, the cells were harvested at 0°C by centrifugation. The harvested cells of strain V37 were washed once in 0.05 M Tris-HCl buffer (pH 7.0) containing inorganic salt to be tested, and suspended in similar solution so as to produce an absorbance of about 1.00 at 570 nm. The assay was carried out at 40°C for 30 min, unless otherwise described. The optical density at 570 nm of the cell suspension was determined at fixed intervals of time. The autolytic activity was represented as a decrease in absorbance at 570 nm in 30 min. The lysis of cells heated at 100°C for 10 min was also determined as a control. The control value was very low in all the autolysis experiments carried out in this study.

Results

Optimum pH for Autolytic Activity

Strain V37 cells grown in the medium containing 0.5 M NaCl were washed once in pH buffer to be tested, and resuspended in similar buffer as that used for washing cells. The pH buffers used were as follows: 0.2 M acetate buffer for pH 4.0, 5.0 and 6.0, 0.05 M Tris-HCl buffer for pH 7.0, 8.0

Table 1. Effect of inorganic salts on the autolysis of strain V37 cells grown in polypepton-yeast extract medium containing 0.5 M NaCl, KCl or MgCl₂, and in the medium containing no inorganic salt

Inorganic salt in cell suspension	Autolytic activity of cells grown in medium containing			
	0.5 M NaCl	0.5 M KCl	0.5 M MgCl ₂	None
None	0.837	0.824	0.354	0.309
0.5 M NaCl	0.633	0.668	0.251	0.225
0.5 M KCl	0.620	0.791	0.320	0.212
0.5 M LiCl	0.845	0.852	0.154	0.234
0.5 M CsCl	0.760	0.751	—	0.294
0.5 M RbCl	0.612	0.649	0.360	0.245
0.5 M NaNO ₃	0.609	0.736	0.292	0.196
0.5 M NH ₄ Cl	0.824	0.848	0.116	0.137
0.5 M MgCl ₂	0	0	0	0
0.5 M CaCl ₂	0	0	0	0
0.5 M BaCl ₂	0	0	0	0
0.5 M Na ₂ SO ₄	0	0	0.091	0.020
0.5 M K ₂ SO ₄	0	0	0.085	0.059
0.5 M Li ₂ SO ₄	0	0	0.099	0.026
0.5 M MgSO ₄	0	0	0	0

and 9.0, 0.2 M sodium borate-NaOH buffer for pH 10.0. The cell suspension (3.0 ml) was incubated at 40°C for 30 min. As shown in Fig. 1, an optimum pH for the autolytic activity was near pH 7–9.

Optimum Temperature for Autolytic Activity

Strain V37 cells grown in the medium containing 0.5 M NaCl were washed once in 0.05 M Tris-HCl buffer (pH 7.0), and resuspended in similar buffer. The cell suspension was incubated at various temperatures ranging from 10°C to 70°C for 30 min. The autolytic activity showed an optimum temperature of near 40°C (Fig. 2).

Effect of Inorganic Salt on the Autolysis of Strain V37 Cells Grown in the Medium Containing 0.5 M NaCl, KCl, MgCl₂ or No Inorganic Salt

Strain V37 cells were grown in the medium containing 0.5 M NaCl, KCl or MgCl₂ and also in the medium without addition of inorganic salt. The cells were washed once in 0.05 M Tris-HCl buffer (pH 7.0) containing 0.5 M inorganic salt to be tested, and resuspended in similar buffer. The cell suspension was incubated at 40°C for 30 min. It can be seen in Table 1 that autolysis of strain V37 cells slightly decreased with the addition of 0.5 M monovalent cations, such as Na⁺, K⁺, Li⁺,

Fig. 3. Effect of low concentrations of divalent cations on the autolytic activity of strain V37 cells grown in polypepton-yeast extract medium containing 0.5 M NaCl.

Fig. 4. Effect of low concentrations of divalent cations on the autolytic activity of strain V37 cells grown in polypepton-yeast extract medium without inorganic salt.

Cs⁺, Rb⁺ and NH₄⁺. However, 0.5 M divalent cations, such as Mg²⁺, Ca²⁺ and Ba²⁺ completely inhibited autolysis.

The cells grown in the medium containing 0.5 M NaCl or KCl exhibited higher autolytic activity than the cells grown in the medium containing 0.5 M MgCl₂ or without addition of inorganic salt.

Effect of Concentrations of Divalent Cations on the Autolytic Activity of Strain V37 Cells

Low concentration (0.05 M) of MgCl₂ significantly inhibited the autolytic activity of strain V37 cells grown in the medium containing 0.5 M NaCl.²⁾

In order to demonstrate the inhibitory effect of concentrations of divalent cations on the autolysis

Fig. 5. Autolysis of strain V37 cells grown in polypepton-yeast extract media containing different concentrations of NaCl.

of strain V37 cells, strain V37 cells were grown in the medium containing 0.5 M NaCl or without inorganic salt. The cells were washed once in 0.05 M Tris-HCl buffer (pH 7.0) containing various concentrations of NiCl₂, CoCl₂, BaCl₂ or MgCl₂, and resuspended in similar buffer. Autolytic activity was expressed as % of the activity of the cell suspension without addition of inorganic salt. Autolysis of the cells grown in the medium containing 0.5 M NaCl was inhibited by Ba²⁺, Mg²⁺, Co²⁺ and Ni²⁺ at more than concentration of 10⁻³ M (Fig. 3). However, the cells grown in the medium without inorganic salts were more sensitive to lower concentrations of Mg²⁺, Ni²⁺ and Ba²⁺, for preventing autolysis (Fig. 4).

Autolysis of Strain V37 Cells Grown in the Media Containing Different Concentrations of NaCl

In order to compare the ability of autolysis of the cells grown in the media containing different concentrations of NaCl, strain V37 cells were grown in the different media containing 0–2.0 M NaCl. The cells were washed once in 0.05 M Tris-HCl buffer (pH 7.0), and resuspended in similar buffer. As shown in Fig. 5, the cells grown in the media containing 0.4–0.9 M NaCl exhibited high autolytic activity.

Effect of NaCl Concentration on the Autolytic Activity of Strain V37 Cells Grown in the Media Containing Different Concentrations of NaCl

Strain V37 cells were grown in the media containing 0–1.0 M NaCl. The cells were washed once in 0.05 M Tris-HCl buffer (pH 7.0) containing 0, 0.1, 0.2, 0.3, 0.4 or 0.5 M NaCl, and resuspended in similar buffer. Change in the optical density at 570 nm of the cell suspension was followed at 40°C for upto 10 min in a Hitachi spectrophotometer model 330. Autolytic activity was expressed

Fig. 6. Effect of NaCl concentration on the autolytic activity of strain V37 cells grown in pepton-yeast extract media containing different concentrations of NaCl.

Cells grown in the media containing 0 M (●), 0.1 M (▲), 0.2 M (■), 0.3 M (○), 0.4 M (△), 0.5 M (□), 0.6 M (▲), 0.7 M (■), 0.8 M (○), 0.9 M (△) and 1.0 M (□) NaCl.

as a decrease in OD_{570} per min. As shown in Fig. 6, autolysis of the cells grown in the media containing NaCl at concentrations from 0 to 1.0 M was slightly activated by low concentrations of NaCl (0.2–0.3 M).

Discussion

The effect of inorganic salt on the autolysis has been reported for some microorganisms. Ogata and Hongo¹⁰ described that the growing cells of *Clostridium saccharoperbutylacetonicum* were lysed by the addition of univalent cations such as Na^+ , K^+ , Rb^+ , Cs^+ , Li^+ and NH_4^+ . According to them, most rapid lysis of the cells occurred during the middle of the exponential phase, when 0.3 to 0.5 M Na^+ was used. Bivalent cations such as Ca^{2+} , Mg^{2+} , Ba^{2+} , Co^{2+} and Ni^{2+} at above 0.005 M inhibited Na^+ -induced autolysis. Maximum rate of cell lysis was at pH 5.7 to 6.0 and at 35°C.¹¹ They suggested that NaCl-induced lysis was closely related to an autolytic enzyme system.¹¹ Sato *et al.*¹² showed that the logarithmic phase cells of *Escherichia coli* lost their colony-forming ability when the cells were suspended in Tris-HCl buffer containing 0.15 M NaCl. The presence of Ca^{2+} or Mg^{2+} protected the cells from the injurious effect of NaCl. Iijima and Ikeda¹³ also observed a similar sensitivity to 0.15 M NaCl in *Bacillus subtilis*. Gilpin *et al.*¹⁴ demonstrated that mutant cells of *Staphylococcus aureus* were autolysed to the same extent when suspended in 0.05 M Tris-HCl buffer (pH 7.0) containing 1.0 M NaCl, KCl, NaBr or NH_4Cl and incubated at

37°C for 30 min. Equivalent and higher molarities of $NaNO_3$, Na_2SO_4 , $(NH_4)_2SO_4$, NH_4NO_3 , LiCl, urea, sucrose and divalent cations such as $MgCl_2$ had no effect on the autolysis. The NaCl-induced lysis was optimum at pH 6.5 to 7.0, temperature 37°C and NaCl concentration 1.0 M. Exposure to 1.0 M NaCl sensitized the cells so that subsequent autolysis may occur even in the absence of salt. Gilpin *et al.*¹⁴ suggested that NaCl-induced autolysis primarily involved *N*-acyl-muramyl-L-alanine amidase.

No autolysis of strain V37 cells was observed during further incubation when 0.5 M NaCl (final concentration) was added to the growing cultures without NaCl.⁷ The mechanism of autolysis of strain V 37 cells seemed to be different from that reported by Ogata and Hongo.^{10,11} However, a remarkable autolytic activity was detected when the cells grown in the medium containing NaCl or KCl were suspended in 0.05 M Tris-HCl buffer (pH 7.0) (Table 1). The optimum pH and temperature for the autolysis of strain V37 cells were 7 to 9 and 40°C, respectively (Figs. 1 and 2). Autolytic activity of strain V37 cells slightly increased in the presence of 0.2–0.3 M NaCl (Fig. 6), but was rather inhibited by 1.0 M NaCl.⁹ This was also different from the result of Gilpin *et al.*¹⁴ The cells grown in the medium containing 0.5 M NaCl or KCl increased more autolytic activity than those grown in the medium containing 0.5 M $MgCl_2$ or without inorganic salt (Table 1). NaCl-enhanced autolysis (Fig. 5) and protection effect of divalent cations on the cell lysis (Figs. 3 and 4) were similar

to the observations reported up to date.¹⁰⁻¹⁴⁾

It is not yet clear about the relation between production of bacteriolytic enzyme and autolysis of strain V37 cells. Further study will be necessary in order to demonstrate the mechanism of autolysis of strain V37 cells.

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