Bull. Fac. Fish., Mie Univ.
No. 10 : 33—39
October 1 , 1983

Studies on Marine Bacteria Producing Lytic Enzymes—IX
Effect of Inorganic Salts on the Autolysis of Bacterial Cells Capable of Producing Lytic Enzyme

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The effect of inorganic salts on the cell lysis was investigated using strain V 37 cells at the early logarithmic phase of growth.

Strain V 37 cells grown in polypeptone-yeast extract medium containing NaCl or KCl (0.5 M) exhibited a remarkable lysis, when the cells were washed and suspended in 0.05 M Tris-HCl buffer (pH 7.0) comprising 0.5 M NaCl, KCl, LiCl, $NaNO_3$ and NH_4Cl, respectively. However, strain V 37 cells grown in the medium containing $MgCl_2$ (0.5 M) showed low autolytic activity, as compared with that of the cells grown in the medium containing NaCl, KCl or $MgSO_4$.

The elevated NaCl at the concentrations from 0.1 to 2.0 M considerably decreased autolysis of strain V 37 cells.

Low concentration (0.05 M) of Mg^{2+} inactivated the autolytic activity of strain V 37 cells.

Key words: autolysis, inorganic salts, marine bacteria

In the previous paper (SUGAHARA *et al.* 1982), strain V 37 cells, harvested at the growth phases from the early log to the middle log, exhibited a remarkable autolytic activity. However, no significant autolysis was observed in the stationary-phase cells.

The present study was designated to demonstrate the effect of inorganic salts on the autolysis of strain V 37 cells capable of producing bacteriolytic enzyme(s).

Methods

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), 5.0g: inorganic salts (NaCl, KCl, $MgCl_2$, or $MgSO_4$), 0 or 0.5 mole and 1,000 ml distilled water. The pH of the medium was adjusted to 7.0. The flasks containing preculture medium without added inorganic salts were inoculated with a loopful of strain V 37, and incubated at 30 °C for 24

hours on reciprocating shakers. Each 1.0 ml of strain V 37 culture fluids from the preculture medium was inoculated to the medium containing inorganic salt or without added inorganic salt. The inoculated flasks were incubated at 30 °C for about 4–7 hours on reciprocating shakers.

Assay of autolytic activity

The culture fluids of strain V 37 were withdrawn, and then the cells were harvested at 0 °C by centrifugation at 13,000 × g for 20 minutes. The harvested cells of strain V 37 were washed once in 0.05 M Tris-HCl buffer (pH 7.0) containing inorganic salt to be tested, and suspended in the same solution so as to produce an absorbance of about 1.0 at 570 nm. The cell suspension (3 ml) was incubated at 40 °C for 30 minutes. The autolytic activity was represented as a decrease in absorbance at 570 nm. The lysis of cells heated at 100 °C for 10 minutes was also determined as control.

Results

Effect of inorganic salts (0.5 M) on the autolysis of strain V 37 cells grown in the medium containing NaCl or KCl (0.5 M)

As shown in Tables 1 and 2, the cells grown in polypeptone-yeast extract medium containing NaCl or KCl (0.5 M) exhibited a remarkable autolytic activity, when the cells were washed and suspended in 0.05 M Tris-HCl buffer (pH 7.0) comprising 0.5 M NaCl, KCl, LiCl, $NaNO_3$ and NH_4Cl, respectively. However, 0.5 M $MgCl_2$, Na_2SO_4, K_2SO_4, Li_2SO_4 and $MgSO_4$ each completely inhibited the autolytic activity of strain V 37 cells.

Table 1. Effect of inorganic salts on the autolytic activity of strain V 37 cells grown in polypeptone-yeast extract medium containing 0.5 mole/l of NaCl.

Culture age of harvested cells (OD_{570}) (Cultivation time)	Cell suspension		Decrease in turbidity(OD_{570}) of cell suspension		
	Inorganic salts	Ionic strength	Living cells	Boiled cells	Autolytic activity
0.212 (5 hr)	0.5 M NaCl	0.5	0.814	0.097	0.717
	0.5 M KCl	0.5	0.691	0.071	0.620
	0.5 M $MgCl_2$	1.5	0	0	0
0.211 (5.5 hr)	0.5 M $NaNO_3$	0.5	0.627	0.018	0.609
	0.5 M Na_2SO_4	1.5	0	0	0
	0.5 M K_2SO_4	1.5	0	0	0
	0.5 M $MgSO_4$	2.0	0	0	0
0.221 (4.5 hr)	0.5 M NaCl	0.5	0.706	0	0.706
	0.5 M LiCl	0.5	0.845	0	0.845
	0.5 M NH_4Cl	0.5	0.824	0	0.824
	0.5 M Li_2SO_4	1.5	0	0	0

Table 2. Effect of inorganic salts on the autolytic activity of strain V 37 cells grown in polypeptone-yeast extract medium containing 0.5 mole/l of KCl.

Culture age of harvested cells (OD_{570}) (Cultivation time)	Cell suspension		Decrease in turbidity(OD_{570}) of cell suspension		
	Inorganic salts	Ionic strength	Living cells	Boiled cells	Autolytic activity
0.202 (5.5 hr)	0.5 M NaCl	0.5	0.675	0.007	0.668
	0.5 M KCl	0.5	0.809	0.018	0.791
	0.5 M $MgCl_2$	1.5	0	0	0
0.204 (5.75 hr)	0.5 M $NaNO_3$	0.5	0.763	0.027	0.736
	0.5 M Na_2SO_4	1.5	0	0	0
	0.5 M K_2SO_4	1.5	0	0	0
	0.5 M $MgSO_4$	2.0	0	0.011	0
0.227 (4.5 hr)	0.5 M LiCl	0.5	0.852	0	0.852
	0.5 M NH_4Cl	0.5	0.848	0	0.848
	0.5 M Li_2SO_4	1.5	0	0	0

Effect of inorganic salts (0.5 M) on the autolysis of strain V 37 cells grown in the medium containing $MgCl_2$ or $MgSO_4$ (0.5 M)

As shown in Table 3, NaCl, KCl, LiCl, $NaNO_3$ and NH_4Cl each stimulated autolysis of the cells grown in the medium containing $MgSO_4$ (0.5 M), whereas Na_2SO_4, K_2SO_4 and Li_2SO_4 each inactivated the autolytic activity of strain V 37 cells.

On the other hand, the cells grown in the medium containing $MgCl_2$ (0.5 M) showed low autolytic activity in some degree, when the cells were washed and suspended in 0.05 M Tris-HCl bufer (pH 7.0) containing NaCl, KCl and $NaNO_3$, respectively

Table 3. Effect of inorganic salts on the autolytic activity of strain V 37 cells grown in polypeptone-yeast extract medium containing 0.5 mole/l of $MgSO_4$.

Culture age of harvested cells (OD_{570}) (Cultivation time)	Cell suspension		Decrease in turbidity(OD_{570}) of cell suspension		
	Inorganic salts	Ionic strength	Living cells	Boiled cells	Autolytic activity
0.196 (4 hr)	0.5 M NaCl	0.5	0.527	0	0.527
	0.5 M KCl	0.5	0.655	0.055	0.600
	0.5 M $MgCl_2$	1.5	0	0.021	0
	0.5 M $CaCl_2$	1.5	0	0.021	0
0.208 (4.5 hr)	0.5 M $NaNO_3$	0.5	0.512	0.049	0.463
	0.5 M Na_2SO_4	1.5	0.045	0	0.045
	0.5 M K_2SO_4	1.5	0.096	0	0.096
	0.5 M $MgSO_4$	2.0	0	0	0
0.196 (4.5 hr)	0.5 M LiCl	0.5	0.814	0.027	0.787
	0.5 M NH_4Cl	0.5	0.471	0.013	0.458
	0.5 M Li_2SO_4	1.5	0.040	0.012	0.028

Table 4. Effect of inorganic salts on the autolytic activity of strain V 37 cells grown in polypeptone-yeast extract medium containing 0.5 mole/l of $MgCl_2$.

Culture age of harvested cells (OD_{570}) (Cultivation time)	Cell suspension		Decrease in turbidity(OD_{570}) of cell suspension		
	Inorganic salts	Ionic strength	Living cells	Boiled cells	Autolytic activity
0.196 (6.5 hr)	0.5 M NaCl	0.5	0.279	0.028	0.251
	0.5 M KCl	0.5	0.373	0.053	0.320
	0.5 M $MgCl_2$	1.5	0.028	0.033	0
	0.5 M $CaCl_2$	1.5	0	0.039	0
0.204 (6 hr)	0.5 M $NaNO_3$	0.5	0.339	0.047	0.292
	0.5 M Na_2SO_4	1.5	0.091	0	0.091
	0.5 M K_2SO_4	1.5	0.100	0.015	0.085
	0.5 M $MgSO_4$	2.0	0	0.030	0
0.196 (5.5 hr)	0.5 M LiCl	0.5	0.214	0.060	0.154
	0.5 M NH_4Cl	0.5	0.144	0.028	0.116
	0.5 M Li_2SO_4	1.5	0.115	0.016	0.099

(Table 4). No remarkable autolysis of strain V 37 cells was detected in LiCl, NH_4Cl, Na_2SO_4, Li_2SO_4 and K_2SO_4 solution, respectively.

As shown in Tables 1–4, 0.5 mole/l of divalent cations, such as Mg^{2+} and Ca^{2+}, completely inhibited the autolysis of strain V 37 cells.

Effect of NaCl concentration on the autolytic activity of strain V 37 cells grown in the medium containing NaCl (0.5 M) or without added NaCl

As shown in Table 5, when the cells were suspended in 0.05 M Tris-HCl buffer (pH 7.0) without added NaCl, the autolysis of strain V 37 cells grown in the medium

Table 5. Effect of NaCl concentration on the autolytic activity of strain V 37 cells grown in polypeptone-yeast extract medium containing 0.5 mole/l of NaCl.

Culture age of harvested cells (OD_{570}) (Cultivation time)	Cell suspension		Decrease in turbidity(OD_{570}) of cell suspension		
	Inorganic salts	Ionic strength	Living cells	Boiled cells	Autolytic activity
0.248 (4.4 hr)	0 M	0	0.858	0	0.858
	0.1 M NaCl	0.1	0.824	0	0.824
	0.3 M NaCl	0.3	0.766	0	0.766
	0.5 M NaCl	0.5	0.731	0	0.731
0.221 (4 hr)	0.5 M NaCl	0.5	0.740	0.036	0.704
	0.7 M NaCl	0.7	0.738	0.021	0.717
	0.9 M NaCl	0.9	0.688	0.031	0.657
	1.0 M NaCl	1.0	0.569	0.060	0.509
0.196 (4 hr)	1.0 M NaCl	1.0	0.584	0.015	0.569
	1.5 M NaCl	1.5	0.189	0	0.189
	2.0 M NaCl	2.0	0.074	0.024	0.050

Table 6. Effect of NaCl concentration on the autolytic activity of strain V 37 cells grown in polypeptone-yeast extract medium without added NaCl.

Culture age of harvested cells (OD_{570}) (Cultivation time)	Cell suspension		Decrease in turbidity(OD_{570}) of cell suspension		
	Inorganic salts	Ionic strength	Living cells	Boiled cells	Autolytic activity
0.197 (3.75 hr)	0 M	0	0.313	0	0.313
	0.1 M NaCl	0.1	0.417	0	0.417
	0.3 M NaCl	0.3	0.375	0.032	0.343
	0.5 M NaCl	0.5	0.299	0.035	0.264
0.204 (3.75 hr)	0.7 M NaCl	0.7	0.181	0.020	0.161
	1.0 M NaCl	1.0	0.122	0.023	0.099
	1.5 M NaCl	1.5	0.054	0	0.054
	2.0 M NaCl	2.0	0.057	0	0.057

Table 7. Effect of low concentration of $MgCl_2$, $MgSO_4$ and Na_2SO_4 on the autolytic activity of strain V 37 cells grown in polypeptone-yeast extract medium containing 0.5 mole/l of NaCl.

Culture age of harvested cells (OD_{570}) (Cultivation time)	Cell suspension		Decrease in turbidity(OD_{570}) of cell suspension		
	Inorganic salts	Ionic strength	Living cells	Boiled cells	Autolytic activity
0.196 (4.25 hr)	0.05 M $MgCl_2$	0.15	0	0	0
	0.10 M $MgCl_2$	0.30	0	0	0
	0.15 M $MgCl_2$	0.45	0	0	0
	0.20 M $MgCl_2$	0.60	0	0	0
0.196 (4 hr)	0.05 M $MgSO_4$	0.20	0.080	0	0.080
	0.10 M $MgSO_4$	0.40	0	0	0
	0.15 M $MgSO_4$	0.60	0	0	0
	0.20 M $MgSO_4$	0.80	0	0	0
0.234 (4.5 hr)	0.05 M Na_2SO_4	0.15	0.872	0.025	0.847
	0.10 M Na_2SO_4	0.30	0.837	0	0.837
	0.15 M Na_2SO_4	0.45	0.783	0	0.783
	0.20 M Na_2SO_4	0.60	0.731	0	0.731
0.196 (4.25 hr)	0.30 M NaCl + 0.05 M $MgCl_2$	0.45	0	0	0
	0.30 M NaCl + 0.10 M $MgCl_2$	0.60	0	0	0
	0.30 M NaCl + 0.15 M $MgCl_2$	0.75	0	0	0
	0.30 M NaCl + 0.20 M $MgCl_2$	0.90	0	0	0
*0.248 (4.5 hr)	0.30 M NaCl	0.30	0.766	0	0.766

containing 0.5 M NaCl was found to be at its maximum. The increase in NaCl concentrations from 0 to 2.0 M resulted in a considerable decrease in the autolytic activity of strain V 37 cells.

On the other hand, the autolysis of strain V 37 cells grown in the medium without added NaCl reached a maximum when the cells were suspended in 0.05 M Tris-HCl buffer (pH 7.0) containing 0.1 M NaCl (Table 6). Sodium chloride at concentrations from 0.1 to 2.0 M considerably inactivated the autolytic activity of strain V 37 cells.

Inhibitory effect of Mg^{2+} on the autolysis of strain V 37 cells grown in the medium containing NaCl (0.5 M) or without added NaCl

As shown in Tables 7 and 8, very low concentration (0.05 M) of Mg^{2+} strongly inactivated the autolytic activity of strain V 37 cells grown in the medium containing NaCl or without added NaCl.

Table 8. Effect of Mg^{2+} on the autolytic activity of strain V 37 cells grown in polypeptone-yeast extract medium without added NaCl.

Culture age of harvested cells (OD_{570}) (Cultivation time)	Cell suspension		Decrease in turbidity (OD_{570}) of cell suspension		
	Inorganic salts	Ionic strength	Living cells	Boiled cells	Autolytic activity
0.239 (3.75 hr)	0.30 M NaCl + 0.05 M $MgCl_2$	0.45	0.078	0.028	0.050
	0.30 M NaCl + 0.10 M $MgCl_2$	0.60	0.039	0	0.039
	0.30 M NaCl + 0.15 M $MgCl_2$	0.75	0.013	0	0.013
	0.30 M NaCl + 0.20 M $MgCl_2$	0.90	0	0	0
*0.197 (3.75 hr)	0.30 M NaCl	0.30	0.375	0.032	0.343

Discussion

MACLEOD and MATULA (1961) suggested that many marine bacteria required NaCl for preventing cell lysis. Accerding to them, divalent ions, such as Mg^{2+} and Ca^{2+}, most prevalent in seawater at concentrations of 0.05 M and 0.01 M, respectively, eliminated the requirement for other solutes, such as Na^+, for preventing cell lysis.

Strain V 37 isolated from seawater environment is capable of growing in the medium without added NaCl as well as in the medium containing NaCl (0.1–1.0 M) or in seawater medium. The cell lysis of strain V 37 in Tris-HCl buffer without added NaCl may be due to an enzymic action rather than to the change in osmotic pressure.

OGATA and HONGO (1973) reported that the growing cells of *Clostridium saccharoperbutylacetonicum* were lysed by the addition of univalent caticns, such as Na^+, K^+, Rb^+, Cs^+, Li^+ and NH_4^+. According to them, most rapid lysis took place when the

cells were during or toward the middle of the exponential phase of growth and when 0.3 M Na^+ was used. They also suggested that bivalent cations, such as Ca^{2+}, Mg^{2+}, Ba^{2+}, Co^{2+} Ni^{2+} and Zn^{2+}, protected the cells from the lysing action of Na^+. SATO *et al.* (1971) demonstrated that the cells of *Escherichia coli* at the logarithmic phase of growth lost their colony-forming ability when the cells were suspended in 0.15 M NaCl in Tris-HCl buffer. The presence of Ca^{2+} or Mg^{2+} protected the cells from the injurious effect of NaCl. IIJIMA and IKEDA (1969) also observed a similar fact in *Bacillus subtilis*: The cells of *Bacillus subtilis* lost their colony-forming ability in 0.15 M NaCl when harvested at the logarithmic phase of growth.

On the other hand, GILPIN, CHATTERJEE and YOUNG (1972) described that 0.05 M Tris-HCl buffer(pH 7.0) containing 1 M NaCl, KCl, NaBr or NH_4Cl stimulated autolysis of *Staphylococcus aureus* cells to the same extent. According to them, the rate and extent of the lysis of *Staphylococcus aureus* cells was dependent on the concentration of NaCl with an optimum at 1.0 M. 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 $MgCl_2$, were inactive.

In this study, strain V 37 cells grown in the medium without added NaCl were maximally lysed in the presence of 0.1 M NaCl (Table 6). However, the autolysis of strain V 37 cells tended to decrease with increasing NaCl concentrations above 0.1 M. A similar protection effect of divalent cations on the cell lysis was also observed in the case of strain V 37.

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