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Studies on Marine Bacteria Producing Lytic Enzymes—VII
Autolytic Activity of Bacteria Capable of Producing Lytic Enzyme

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The cells harvested at the growth phases from the early log to the middle log exhibited autolytic activity when suspended in Tris-HCl buffer containing the same concentrations of inorganic salt (NaCl) as in the growth medium. However, no significant autolysis was observed in the stationary phase cells.

The log phase cells heated in water at 100°C for 10 minutes also did not show any significant reduction of turbidity.

Key words ; marine bacteria, lytic enzyme, autolytic activity

Autolytic enzyme(s) is indispensable for the growth of bacteria (FORSBERG and ROGERS 1971, HIGGINS and SHOCKMAN 1971). FAN and BECKMAN (1971, 1972) showed that *Bacillus subtilis* autolysins were used during the growth process to open gaps in the cell wall to permit surface expansion and were also used to separate linked daughter cells after septum formation. Similar results were obtained in *Pneumococcus* (TOMASZ 1968), *Streptococcus* (EKSTEDT and STOLLERMAN 1960, LOMINSKI *et al.* 1958), *Staphylococcus* (CHATTERJEE *et al.* 1969) and *Lactobacillus* (COYETTE and GHUYSEN 1970). YOUNG *et al.* (1963) and STEWART *et al.* (1970) demonstrated that autolysin also may be involved in transformation by externally added deoxyribonucleic acid (DNA).

Most autolysins seemed to be closely associated with the cell walls (YOUNG 1966, SHOCKMAN *et al.* 1967, LINDSAY and GLASER 1976), whereas *Escherichia coli* K 12 carboxypeptidase II (one of autolysins) was found to be periplasmic (BECK and PARK 1976).

High concentrations of salt were effective on the release of autolysins from cell walls of *Streptococcus faecalis* and *Bacillus subtilis* (FAN 1970, POOLEY *et al.* 1970). Membrane-bound autolysins of *Escherichia coli* and *Neisseria gonorrhoeae* were liberated by a combination of Triton X-100 and NaCl (HARTMANN *et al.* 1974, HEBELER and YOUNG 1976). However, a *Staphylococcus* (*Diplococcus*) *pneumoniae* autolysin was almost completely removed when cell walls were washed in distilled water (HOWARD and GOODER 1974).

Strain V 37 isolated from coastal regions produced extracellular enzyme(s) capable of lysing bacterial cells when grown in polypeptone-yeast extract medium containing NaCl (SUGAHARA *et al.* 1978, 1980, 1981). Although lytic enzyme(s) was accumulated in the culture filtrate fraction of culture fluids in the course of growth of strain V 37, it has not yet been demonstrated whether intracellular lytic enzyme(s) could be liberated as a result of cell autolysis or whether autolytic enzyme(autolysin) itself could be released from cells during the growth.

The present study was designated to obtain preliminary information concerning autolysis of strain V 37 cells capable of producing lytic enzyme(s).

Methods

Assay of autolytic activity

The culture fluids of strain V 37 were withdrawn periodically and then the cells were collected by centrifugation at 13,000×g for 20 minutes. The harvested cells were washed in distilled water or NaCl solution (0.5mole/l) by centrifugation. The washed cells of strain V 37 were resuspended in Tris-HCl buffer containing 0.5mole/l of NaCl or without added NaCl. The cell concentration of reaction mixture was produced an absorbance of about 1.0 at 570nm. The cell suspension (3ml) was incubated at 40°C for 30 minutes. The autolytic activity was represented as decrease in optical density at 570 nm.

Culture of strain V 37 capable of producing lytic enzyme

The medium used in this study was composed of polypeptone (Daigo Eiyo Kagaku), 10.0g : yeast extract (Nakarai Chemicals), 5.0g : NaCl 0 or 29.3g (0.5mole) and 1,000 ml distilled water. The pH of the medium was adjusted to 7.0. A loopful of strain V 37 was inoculated to the preculture medium without added NaCl, 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 NaCl or without added NaCl. The inoculated flasks were incubated at 30°C for 36 hours on reciprocating shakers.

Results and Discussion

Different types of autolytic enzymes (autolysins) have been found in a wide variety of gram-positive and gram-negative microorganisms : endo-N-acetylmuramidase from *Streptococcus faecalis* (SHOCKMAN *et al.* 1967), *Lactobacillus acidophilus* (COYETTE and GHUYSEN 1970, COYETTE and SHOCKMAN 1973) and *Arthrobacter crystallopoietes* (KRULWICH and ENSIGN 1968), endo-N-acetylglucosaminidase from *Staphylococcus aureus* (TIPPER 1969), glycosidase from *Bacillus subtilis* (FAN and BECKMAN 1973), transglycosidase from *Escherichia coli* (HARTMANN *et al.* 1972), N-acetylmuramyl L-alanine amidase from *Streptococcus* (*Diplococcus*) *pneumoniae* (TOMASZ and WESTPHAL 1971, HOWARD and GOODER 1974), *Neisseria gonorrhoeae* (HEBELER and YOUNG 1976), *Bacillus*

megaterium (CHAN and GLASER 1972) and *Bacillus subtilis* (LINDSAY and GLASER 1976, HERBOLD and GLASER 1975), endopeptidase from *Escherichia coli* (HARTMANN *et al.* 1972, 1974) and carboxypeptidase from *Escherichia coli* (BECK and PARK 1976). The type and characteristics of autolysin(s) of strain V 37 have not been demonstrated.

Table 1 Autolytic activity of strain V 37 cells grown in the media without added NaCl and containing 0.5mole/l of NaCl.

Cultivation time(hour)	Growth OD_{570}	Reduction of turbidity(OD_{570}) of cell suspension			
		0.5M NaCl (medium) ↓ 0M NaCl(cell suspension)	0.5 M NaCl (medium) ↓ 0.5 M NaCl (cell suspension)		
		Boiled cells	Living cells	Boiled cells	Autolytic activity
4	(0.120)	0.148	0.752	0.079	0.673
6	0.400	0.027	——	——	——
8	0.889	0.214	0.695	0.075	0.620
10	1.210	0.104	0.232	0.070	0.162
12	1.464	0.046	0.120	0.016	0.104
16	1.670	0.055	0.136	0.029	0.107
20	1.670	0.062	0.126	0.014	0.112
24	1.625	0	0.128	0	0.128
36	1.625	0.041	0.103	0	0.103
Cultivation time(hour)	Growth OD_{570}	0M NaCl (medium) ↓ 0.5M NaCl(cell suspension)	0 M NaCl (medium) ↓ 0 M NaCl (cell suspension)		
		Boiled cells	Living cells	Boiled cells	Autolytic activity
4	(0.260)	0.083	0.282	0.035	0.247
6	0.604	0.062	0.164	0	0.164
8	0.928	0.037	0.077	0.022	0.055
10	1.089	0.008	0.060	0	0.060
12	1.212	0.040	0.073	0.021	0.052
16	1.451	0.036	0.048	0	0.048
20	1.481	0.012	0.070	0	0.070
24	1.513	0.037	0.031	0.027	0.004
36	1.649	0.015	0.041	0	0.041

As shown in Table 1 and Fig. 1, high level of autolytic activity was observed at 4-8 hours of cultivation when strain V 37 cells grown in the medium containing 0.5 mole/l of NaCl were suspended in the same concentrations of salt solution as in the growth medium. However, autolytic activity decreased considerably at 10 hours' cultivation. Cell autolysis was potential during the middle of exponential growth phase and thereafter the cells became resistant to autolysis. This fact seemed to be coincided

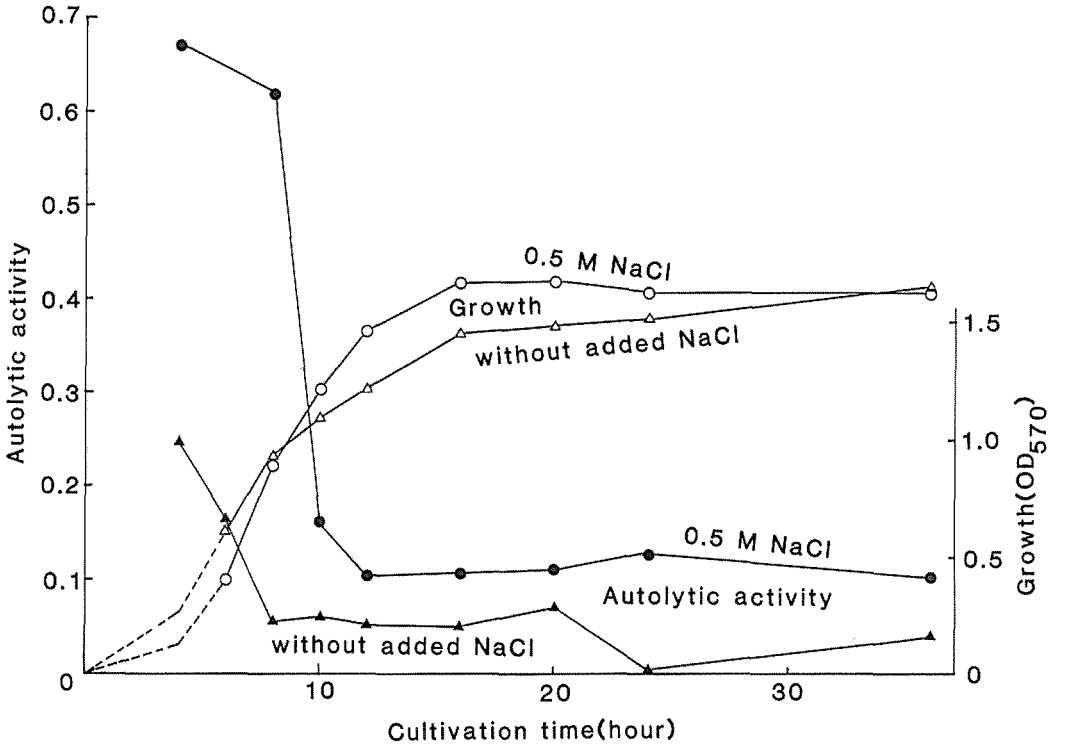

Fig. 1. Autolytic activity of strain V 37 cells grown in the media without added NaCl and containing 0.5mole/l of NaCl.

with the previous findings that autolysins of most bacteria were present during the exponential growth phase or toward the end of exponential growth phase. CHAN and GLASER (1972) reported that the initial rate of N-acetylmuramic acid L-alanine amidase of *Bacillus megaterium* KM from logarithmically growing cells had a 4- to 10-fold higher than that from cells in stationary phase of growth. MONODANE *et al.* (1978) also elucidated that log phase cells of *Micrococcus lysodeikticus(luteus)* IFO 3333 exhibited autolytic activity and lost their autolytic ability as the phases progressed to the stationary phase.

Strain V 37 cells grown in the medium containing 0.5mole/l of NaCl for various intervals did not lyse considerably for the incubation at 40°C for 30 minutes when heated at 100°C for 10 minutes.

On the other hand, cells grown in the medium without added NaCl also showed autolytic activity at the growth phases from the early log to the middle log. However, autolysis was low when compared with that of cells grown in the medium containing NaCl. Autolytic activity seemed to be stimulated by NaCl in the growth medium. GILPIN *et al.* (1972) demonstrated that mutant cells of *Staphylococcus aureus* grown in

the PYK medium contained 1.0 M NaCl enhanced autolysis in the presence of NaCl. The rate and extent of autolysis was dependent on the concentration of NaCl with an optimum at 1.0 M. OGATA and HONGO (1974) also observed similar fact in *Clostridium saccharoperbutylacetonicum*. Growing cells were lysed by sodium ion concentrations above 0.1 M (maximum effect at 0.3 M). The rate of lysis depended on the age of the culture.

From the viewpoint of the resemblance between lysostaphin (bacteriolytic enzyme) and *Staphylococcus aureus* autolysins, IVERSEN and GROV (1973) suggested that strain *Staphylococcus staphylolyticus* may be producing abnormal large amounts of normal autolytic activities or a glycinase with exceptionally high activity. The physiological significance of bacteriolytic enzyme(s) produced by strain V 37 is not well understood. The similarity between lytic enzyme(s) and strain V 37 autolytic enzyme(s) on the NaCl dependence is interesting. Effect of inorganic salts on the autolysis of strain V 37 will be examined in the near future.

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