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Studies on Marine Bacteria Producing Lytic Enzymes—VIII
Release of Lytic Enzyme from Bacterial Cells

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The release of lytic enzyme was studied by washing and suspending the cells in solutions containing inorganic salts or organic anions.

The ratio of lytic enzyme released into culture fluids to the total lytic enzyme produced during the growth of strain V 37 had a tendency to increase with progression of the culture age.

Inorganic salts and organic anions used in this study exhibited no significant effect on the release of lytic enzyme from strain V 37 cells.

More than 70 % of the lytic enzyme liberated seemed to be instantaneously released from strain V 37 cells.

Key words: lytic enzyme, marine bacteria, enzyme release

In a previous paper (SUGAHARA *et al.* 1981), the effect of inorganic salts on the release of lytic enzyme was investigated using bacterial cells of strain V 37 grown for 6 or 8 hours in polypeptone-yeast extract medium containing NaCl (0.5 M). Inorganic salts (0.5 M) used were not effective for the liberation of lytic enzyme.

The present paper deals with the effect of different concentrations of inorganic salts and organic anions on the liberation of lytic enzyme from strain V 37 cells.

Methods

Assay of lytic activity

Lytic activity was determined as described previously (SUGAHARA *et al.* 1976, 1978, 1979, 1980, 1981, 1982).

Culture of strain V 37 producing lytic enzyme

Culture of strain V 37 capable of producing lytic enzyme was carried out as described previously (SUGAHARA *et al.* 1978, 1980, 1981, 1982).

Release of lytic enzyme from strain V 37 cells

Lytic enzyme released from strain V 37 cells was assayed as described previously (SUGAHARA *et al.* 1981).

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 minutes at 0°C with a sonicator (Ultrasonics W-375). After removing the cell debris by centrifugation, lytic activity of the supernatant was determined.

Total lytic activity

Total lytic activity was determined by adding lytic activity associated with the cells to lytic activity of the culture supernatant per 1 ml of culture fluids.

Results

Extracellular production of lytic enzyme by strain V 37

Fig. 1 shows the ratio of lytic activity released into culture fluids to the total lytic activity produced by strain V 37 during the growth in polypeptone-yeast extract medium containing NaCl (0.5 M). The ratio was found to increase with progression in the

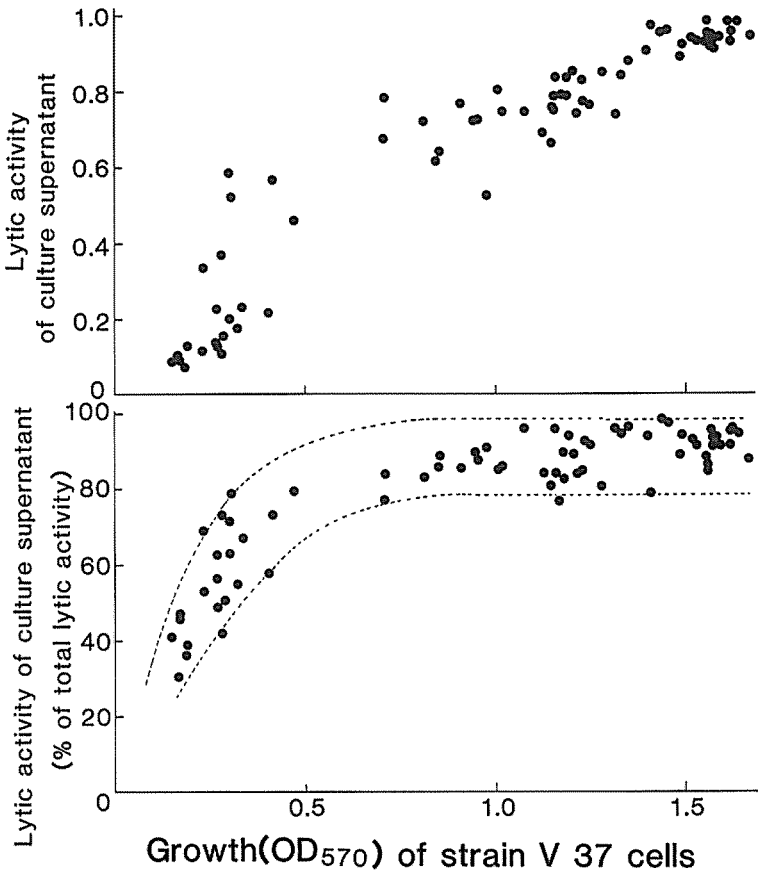

Fig. 1. Extracellular production of lytic enzyme during the growth of strain V 37.

growth phase of strain V 37, and to reach a maximum value at the middle of the exponential growth phase. This fact indicates that lytic enzyme produced in the cells was liberated with the lapse of cultivation time.

Time course of release of lytic enzyme

In the previous paper (SUGAHARA *et al.* 1981), it was found that strain V 37 cells grown for 6 or 8 hours maximally liberated lytic enzyme into cell suspension within 1 hour at 30 °C.

As shown in Fig. 2, lytic enzyme released from the cells grown for 6 or 8.5 hours exhibited a maximum level at 20-30 minutes' incubation. Therefore, for the release experiment in this study, incubation was carried out at 30 °C for 30 minutes with shaking, unless otherwise noted.

However, it is noticeable that the amount of lytic enzyme released at 0 minute was about 70-80 % of the maximal enzyme liberation.

Effect of inorganic salts concentration on the release of lytic enzyme

NaCl: As shown in Fig. 3, no additional effect of NaCl (0.1-0.7 M) was observed on the release of lytic enzyme from strain V 37 cells grown in polypeptone-yeast extract medium containing NaCl (0.5 M) for 6, 8.5 and 23 hours, respectively.

Sodium chloride was not only effective for the release of materials absorbing at 280

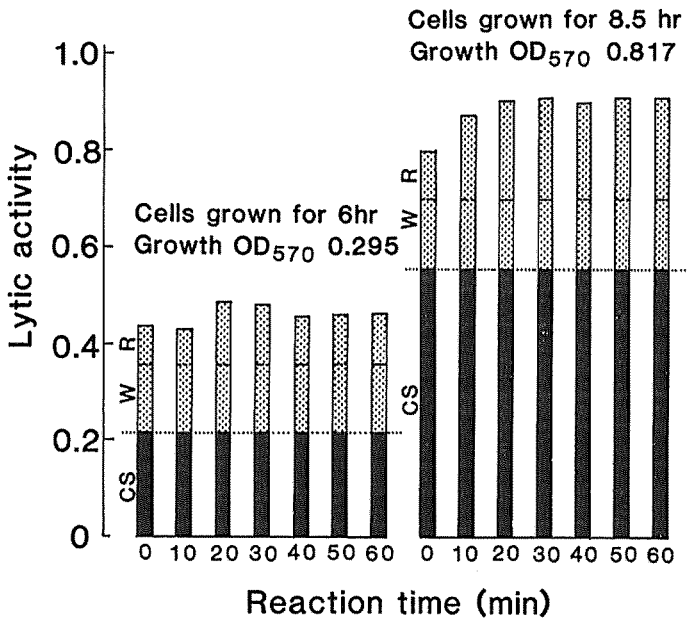

Fig. 2. Time course of release of lytic enzyme from strain V 37 cells grown in polypeptone-yeast extract medium containing NaCl (0.5 M).
CS: lytic activity of culture supernatant,
W: lytic activity liberated from cells during washing,
R: lytic activity released from cells during incubation.

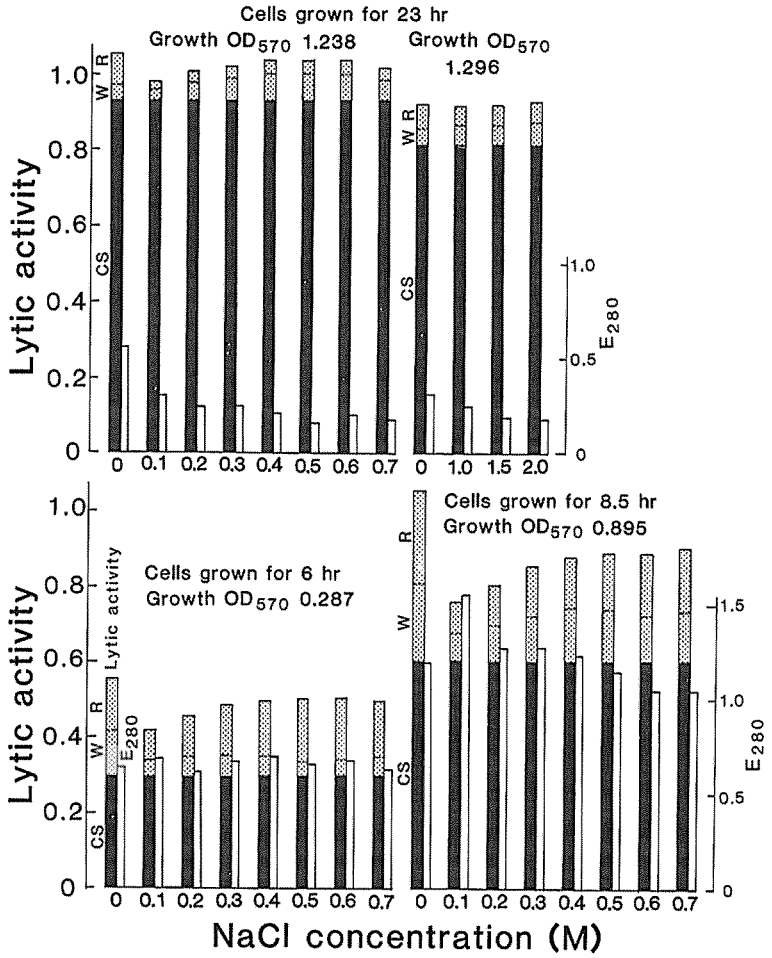

Fig. 3. Effect of NaCl concentration on the release of lytic enzyme from strain V 37 cells grown in polypeptone-yeast extract medium containing NaCl (0.5 M).

nm from the cells grown for 6 and 8.5 hours, respectively, but exhibited an inhibitory effect on that from the cells grown for 23 hours. The amount of 280 nm absorbing-materials released from strain V 37 cells grown for 8.5 hours was more abundant than that grown for 6 or 23 hours. This fact indicates that the level of enzyme liberation was not always proportional to the amount cf materials absorbing at 280 nm.

LiCl, $CaCl_2$, K_2SO_4, H_3BO_3: As shown in Fig. 4, the liberation of lytic enzyme from strain V 37 cells grown for 6-6.5 hours was not stimulated by the addition of LiCl (0.1-0.7 M), $CaCl_2$ (0.1-0.7 M), K_2SO_4 (0.1-0.5 M) and H_3BO_3 (0.1-0.5 M), respectively.

$MgCl_2$, $MgSO_4$, KCl, NaH_2PO_4: It can be seen in Fig. 5 that $MgCl_2$ or KCl stimulated the release of lytic enzyme from strain V 37 cells grown for 6.5 or 7 hours.

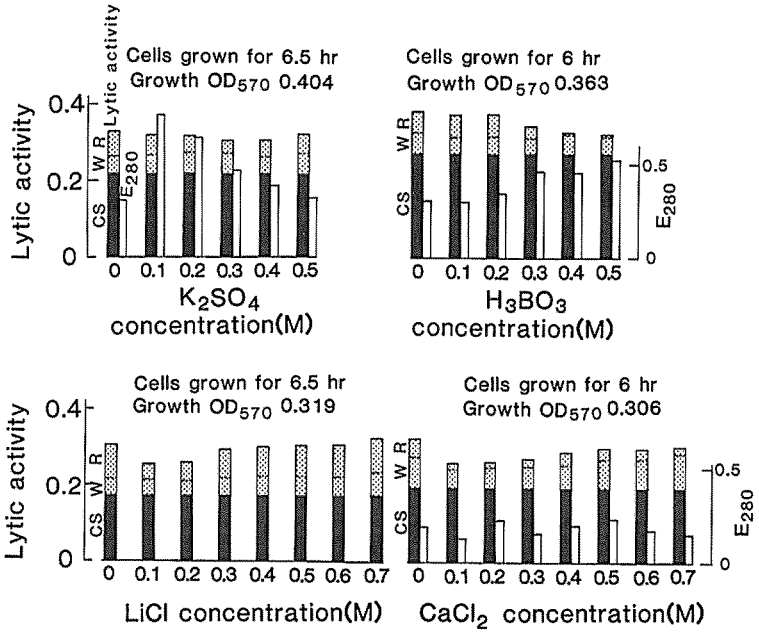

Fig. 4. Effect of inorganic salts (LiCl, $CaCl_2$, K_2SO_4, H_3BO_3) on the release of lytic enzyme from strain V 37 cells grown in polypeptone-yeast extract medium containing NaCl (0.5 M).

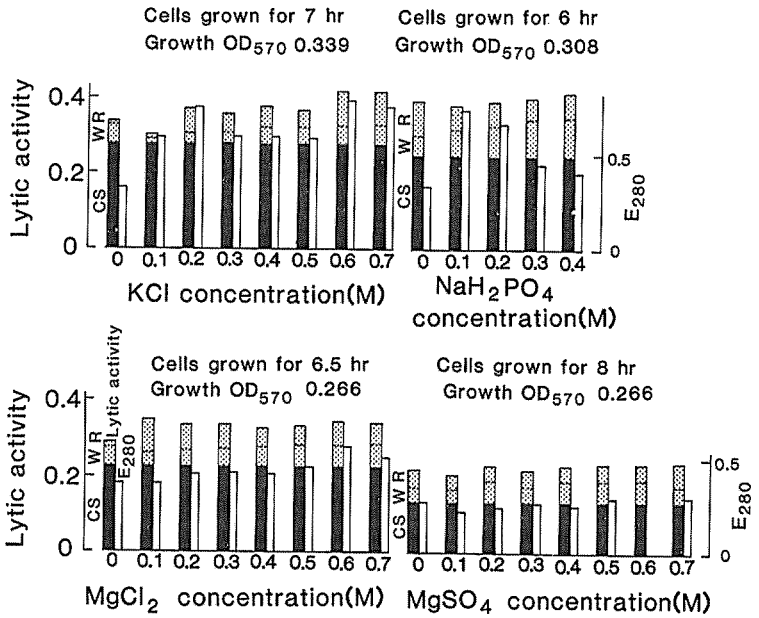

Fig. 5. Effect of inorganic salts ($MgCl_2$, $MgSO_4$, KCl, NaH_2PO_4) on the release of lytic enzyme from strain V 37 cells grown in polypeptone-yeast extract medium containing NaCl (0.5 M).

Slight stimulative effect of NaH_2PO_4 or $MgSO_4$ was also observed on the liberation of lytic enzyme from the cells grown for 6 or 8 hours.

Remarkable liberation of materials absorbing at 280 nm was observed by the addition of KCl or NaH_2PO_4.

Effect of organic anions on the release of lytic enzyme

As shown in Fig. 6, when the cells were suspended in every organic acid solution used in this study at the concentrations of 0.01, 0.05 and 0.10 M, respectively, the level of lytic enzyme released from the cells was not exceeding, as compared with control without added organic acid. This means that organic acid was not effective for the release of lytic enzyme.

The liberation of materials absorbing at 280 nm was found to increase considerably by the addition of organic acid, such as fumarate and maleate.

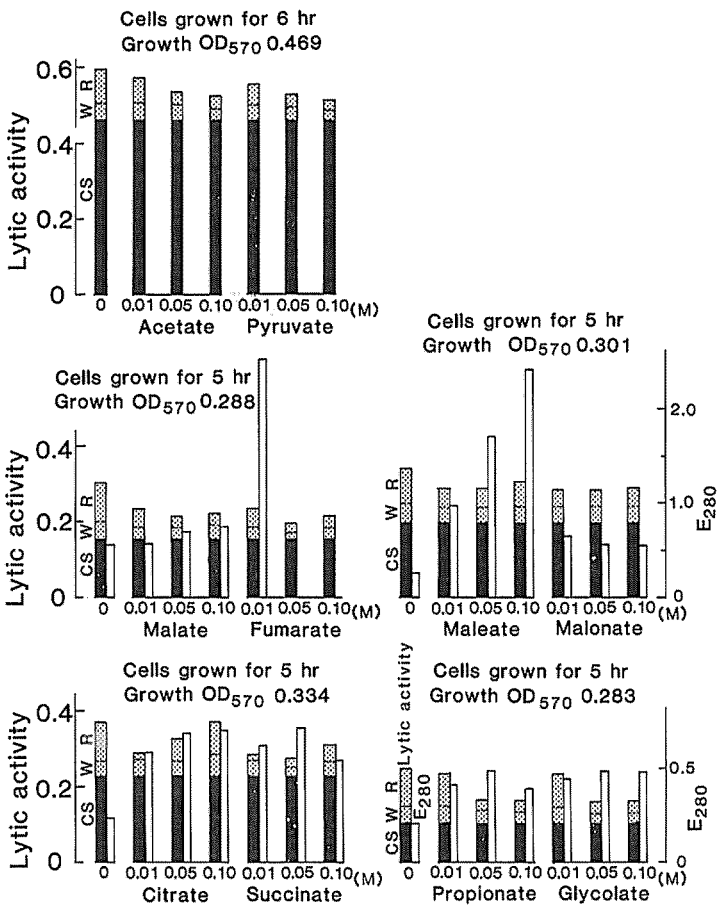

Fig. 6. Effect of organic anions on the release of lytic enzyme from strain V 37 cells grown in polypeptone-yeast extract medium containing NaCl (0.5 M).

Time-independent liberation of lytic enzyme

As shown in Fig. 2, about 70-80 % of maximal enzyme liberation was spontaneously released from strain V 37 cells grown for 6 and 8 hours, respectively. COLES and GROSS (1967b) reported that part of surface-located penicillinase of *Staphylococcus aureus* was liberated instantaneously by inorganic anions, such as phosphate and arsenate. COLES and GROSS (1967a) also demonstrated that citrate was more effective than dicarboxylic or monocarboxylic acids for the time-independent liberation of penicillinase from *Staphylococcus aureus*.

Therefore, an attempt was made to demonstrate whether time-independent liberation of lytic enzyme from the cells grown for 23-24 hours can be observed using 0.4 M NaH_2PO_4 and 0.1 M sodium citrate solutions. As shown in Fig. 7, about 85-100 % of

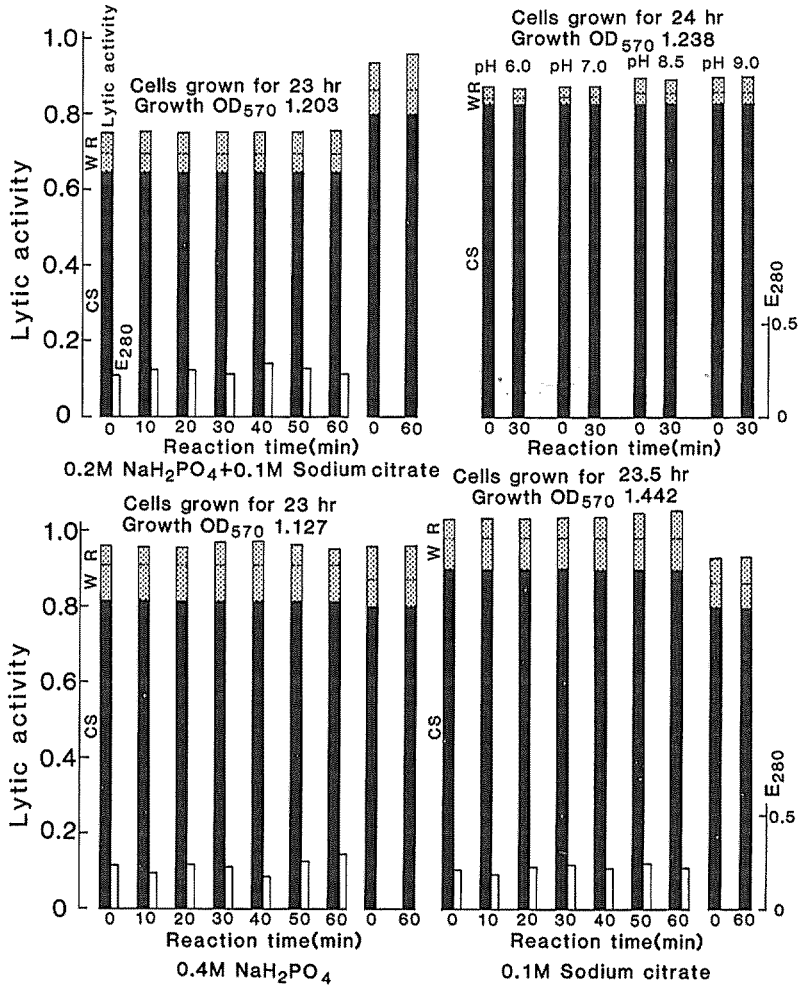

Fig. 7. Time-independent liberation of lytic enzyme from strain V 37 cells grown in polypeptone-yeast extract medium containing NaCl (0.5 M).

maximal enzyme liberation was immediately released from strain V 37 cells grown for 23–24 hours, when the cells were washed and incubated with 0.4 M NaH_2PO_4 and 0.1 M sodium citrate solutions. Spontaneous liberation of lytic enzyme from the cells grown for 23 hours also showed pH dependence.

Discussion

MALVEAUX and SAN CLEMENTE (1967) reported that the amount of loosely-bound acid phosphatase eluted from *Staphylococcus aureus* depended on pH and ionic strength. According to them, elution of the enzyme was maximally effected with 1.0M KCl (pH 7.5) from log-phase cells, but stationary-phase cells required twice the concentration of KCl. They inferred that part of the acid phosphatase of *Staphylococcus aureus* is associated with the cells through electrostatic interactions.

However, POLLOCK (1961) suggested that penicillinase of *Bacillus subtilis* was not eluted from the cells by the treatment of inorganic salt at concentrations up to 0.2 M.

In this study, the amount of lytic enzyme released from strain V 37 cells scarcely increased by the addition of inorganic salts at the concentrations of 0.1–0.7 M (Figs. 3–5).

On the other hand, COLES and GROSS (1967a) suggested that low concentrations (0.01, 0.05 M) of organic acids were effective for the liberation of penicillinase from *Staphylococcus aureus*. However, organic acids did not exhibit a stimulative effect on the release of lytic enzyme from strain V 37 cells (Fig. 6). More than 70 % of the liberated lytic enzyme was instantaneously released from strain V 37 (Figs. 2 and 7).

The mechanism of release of lytic enzyme from strain V 37 cells is not yet clear. Further studies on the release of lytic enzyme are necessary.

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