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Studies on Marine Bacteria Producing Lytic Enzymes-V
Culture Condition for the Production of Lytic Enzyme

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The production of lytic enzyme by strain V 37 was investigated using polypeptone-yeast extract medium or chemically defined medium under various culture conditions.

Strain V 37 grew well and also produced lytic enzyme at pH 5.0, 6.0, 7.0 and 8.0 in polypeptone-yeast extract medium containing 0.5 mole/l of NaCl.

The high yield of lytic enzyme was observed in the media containing $NaNO_3$ (0.1-1.0 mole/l) or NaSCN (0.2-0.3 mole/l). Chloride(Cl^-) and nitrate (NO_3^-) ions were more effective than thiocyanate (SCN^-), dihydrogenphosphate ($H_2PO_4^-$) and sulfate (SO_4^{2-}) ions for the production of lytic enzyme. The elevated $MgCl_2$ in the presence of NaCl rather inhibited the production of lytic enzyme.

Strain V 37 could not grow in a chemically defined medium proposed by MACLEOD (1968) during 24 hours of cultivation.

Key words: marine bacteria, lytic enzyme.

In the proceeding paper (SUGAHARA *et al.* 1980), strain V 37 isolated from coastal regions considerably produced bacteriolytic enzyme in the medium containing NaCl or KCl as inorganic salts. The salt requirement for the enzyme production could be partially satisfied by the substitution of LiCl, Na_2SO_4 or K_2SO_4 for NaCl or KCl.

The present paper deals with culture condition for the production of lytic enzyme by strain V 37.

Methods

Assay of bacteriolytic activity

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

Culture of strain V 37 producing lytic enzymes

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

Results

Effect of pH value on the production of lytic enzyme

In order to determine the effect of pH value of culture medium on the production of lytic enzyme, the following medium was used: polypeptone, 1.0%; yeast extract, 0.5%; NaCl, 0.5 mole/l, pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0, respectively. A loopful of strain V 37 was incculated to the preculture medium without added NaCl, pH 7.0, and incubated at 30°C for 24 hours on reciprocating shakers. Each 1.0 ml of V 37 culture fluids from preculture medium was inoculated to the medium containing 0.5 mole/l of NaCl. The inoculated flasks varying from pH 4.0 to pH 10.0 were incubated at 30°C for 48 hours on reciprocating shakers.

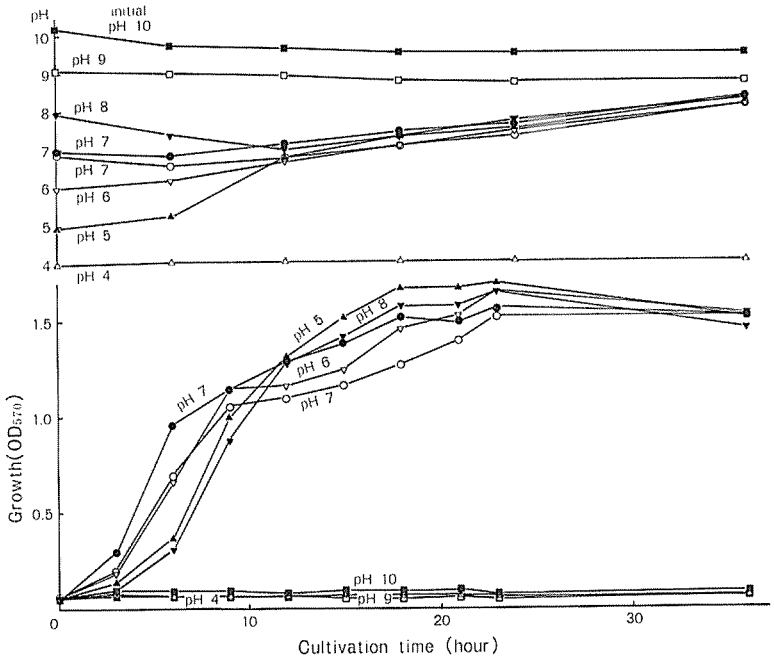

Fig. 1. Effect of pH value of culture medium on the growth of strain V 37. open circle; medium containing 0.5 mole/l of NaCl, filled-in circle; medium without added NaCl.

As shown in Fig. 1, no bacterial growth was observed at pH 4.0, 9.0 and 10.0. Strain V 37 grew well at pH 5.0, 6.0, 7.0 and 8.0 in the medium containing NaCl. The pH value of the medium changed to about 7.0 after 12 hours of cultivation, increased gradually, and finally reached to near pH 8.0 after 36 hours.

The level of lytic enzyme increased to parallel the growth curve. The high production of lytic enzyme by strain V 37 was observed at initial pH 5.0, 6.0, 7.0 and 8.0(Fig. 2). However, the activity of lytic enzyme produced in the media with pH 5.0 and 8.0 decreased considerably at 39 hours' cultivation.

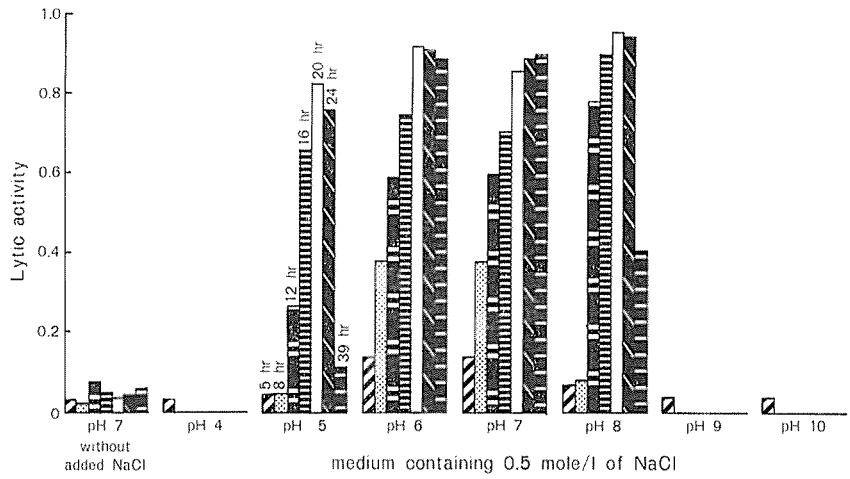

Fig. 2. Effect of pH value of culture medium on the production of lytic enzyme during the growth of strain V 37.

Effect of $NaNO_3$ concentration on the production of lytic enzyme

As shown in Figs. 3 and 4, the duration of the lag phase depended on the concentration of $NaNO_3$ added into V 37 culture medium. Strain V 37 grew well after small lag in the medium containing 1 mole/l of $NaNO_3$. High yield of lytic enzyme was observed in the media comprising $NaNO_3$ (0.1–1.0 mole/l). However, the lytic enzyme elaborated in the culture fluids of media having 0.1–0.2 mole/l of $NaNO_3$ was inactivated at 36 hours of cultivation.

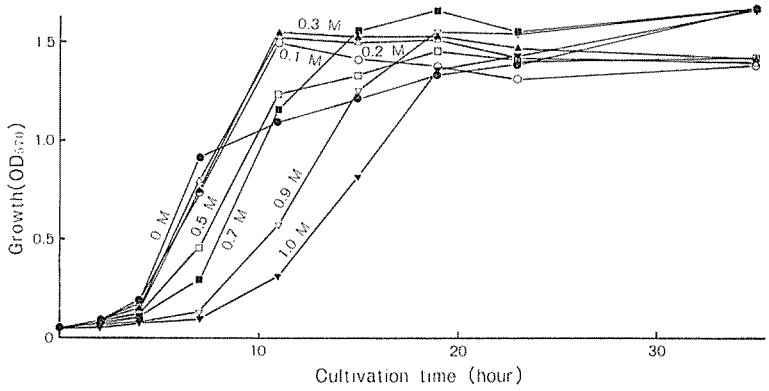

Fig. 3. Effect of $NaNO_3$ concentration on the growth of strain V 37.

Effect of NaSCN concentration on the production of lytic enzyme

The growth of strain V 37 in the media containing 0.1–0.5 mole/l of NaSCN was not inhibited (Fig. 5). The culture having 0.7 mole/l of NaSCN showed a lag of about 24 hours. However, no growth was observed in the media including 0.9–1.0

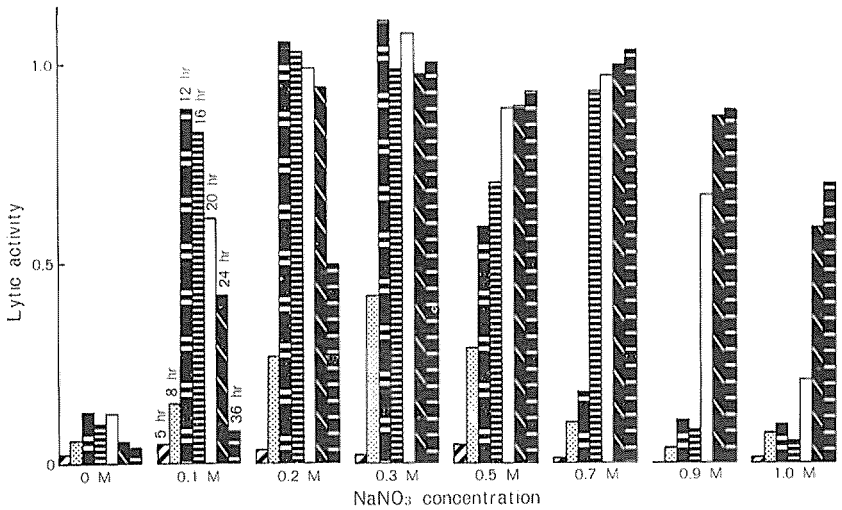

Fig. 4. Effect of $NaNO_3$ concentration on the lytic enzyme production during the growth of strain V 37.

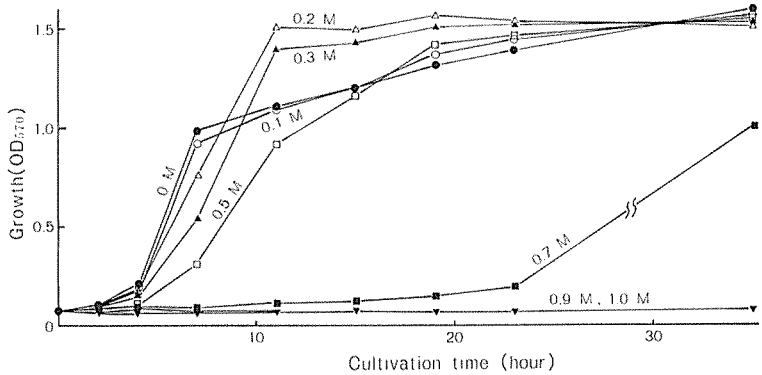

Fig. 5. Effect of NaSCN concentration on the growth of strain V 37.

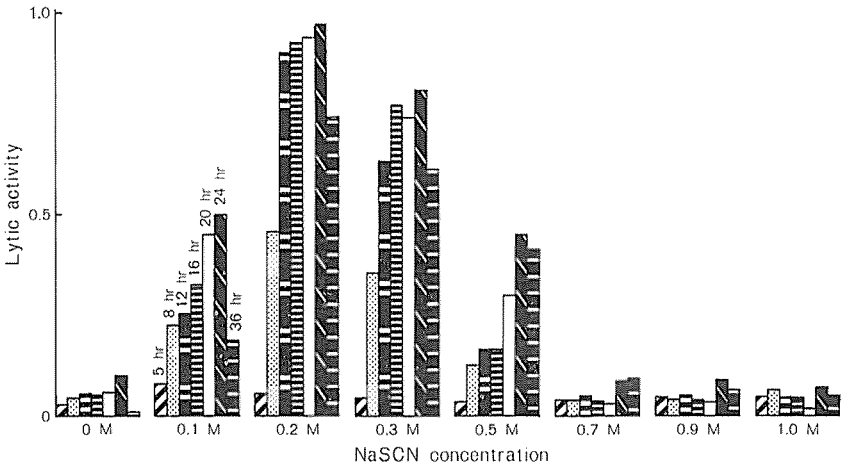

Fig. 6. Effect of NaSCN concentration on the lytic enzyme production during the growth of strain V 37.

mole/l at 36 hours' cultivation.

The high production of lytic enzyme was detected in the media containing 0.2-0.3 mole/l of NaSCN. However, lytic enzyme elaborated seemed to be unstable in the culture fluids (Fig. 6).

Effect of $MgCl_2$ addition on the production of lytic enzyme

The addition of 0.1-0.5 mole/l of $MgCl_2$ into the medium had no inhibitory effect on the growth of strain V 37. However, lytic enzyme production by strain V 37 was not enhanced considerably by the addition of $MgCl_2$ into the medium (SUGAHARA *et al.* 1980).

Figs. 7 and 8 show the effect of $MgCl_2$ addition (0.1-0.5 mole/l) into the medium containing 0.5 mole/l of NaCl on the growth and production of lytic enzyme. The increase in the concentration of $MgCl_2$ in the medium containing 0.5 mole/l of NaCl

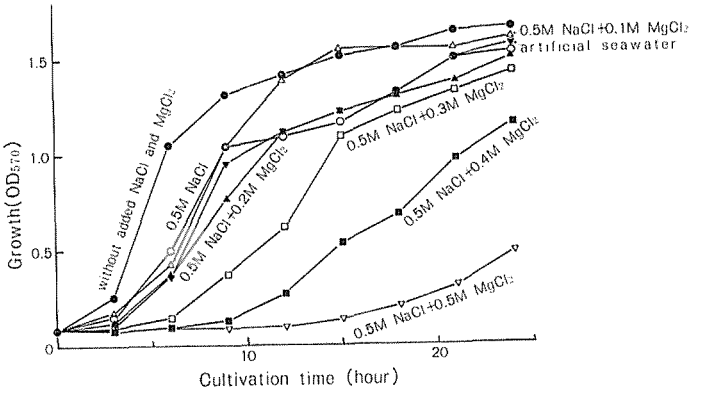

Fig. 7. Effect of $MgCl_2$ addition into the medium containing 0.5 mole/l of NaCl on the growth of strain V 37.

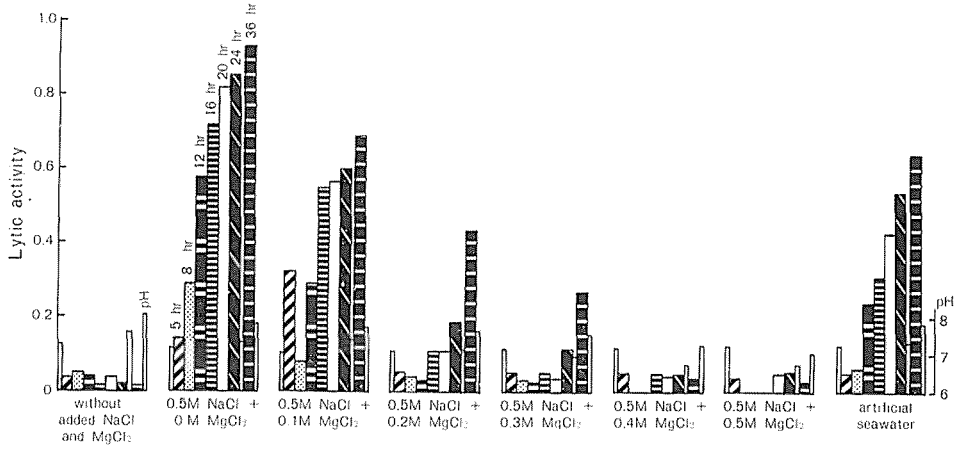

Fig. 8. Effect of $MgCl_2$ addition into the medium containing 0.5 mole/l of NaCl on the lytic enzyme production during the growth of strain V 37.

had a rather inhibitory effect on the production of lytic enzyme as well as the growth of strain V 37. The production of lytic enzyme in the artificial seawater medium was comparable with that in the medium comprising 0.5 mole/l of NaCl and 0.1 mole/l of $MgCl_2$.

Effect of glucose addition on the production of lytic enzyme

In the previous paper (SUGAHARA *et al.* 1978), the presence of glucose had a rather

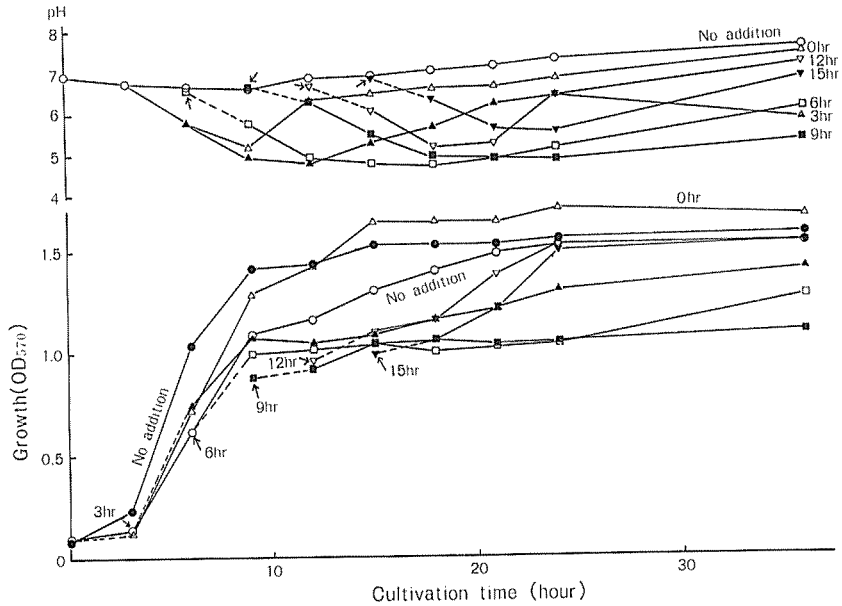

Fig. 9. Effect of glucose addition on the growth of strain V 37. open circle; medium containing 0.5 mole/l of NaCl, filled-in circle; medium without added NaCl.

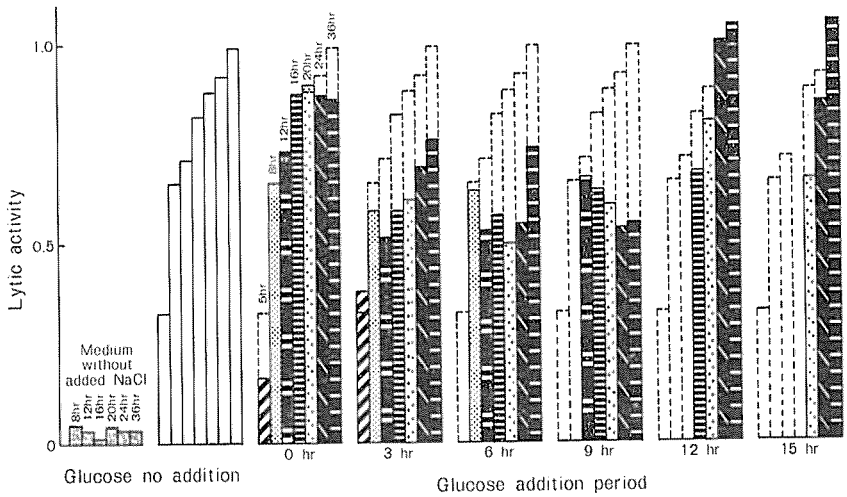

Fig. 10. Effect of glucose addition on the lytic enzyme production during the growth of strain V 37.

inhibitory effect on the production of lytic enzyme in the medium containing 0.02% of yeast extract. However, the inhibitory effect of glucose on the production of lytic enzyme was not observed in the medium having 0.1% of yeast extract(Figs. 9 and 10).

Effect of anions on the production of lytic enzyme

As shown in Figs. 11 and 12, the cation (Na^+ or K^+) concentration necessary for high yield of lytic enzyme rather increased in the case of sulfate than in the case of chloride. The effect of Li^+ on the production of lytic enzyme was observed only in the presence of chloride(Fig. 13).

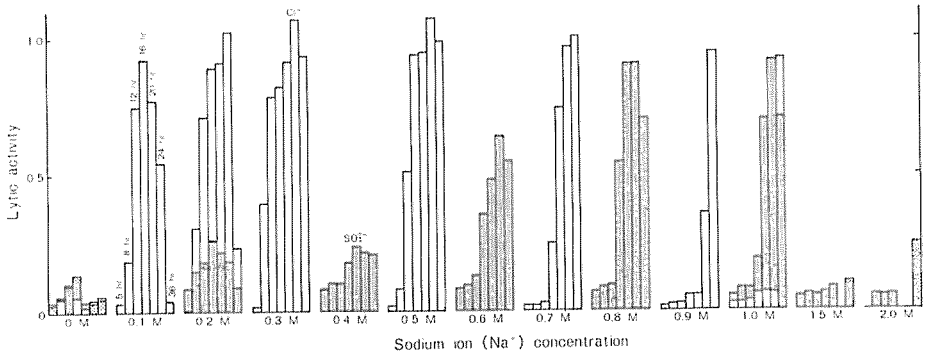

Fig. 11. Effect of sodium ion concentration on the lytic enzyme production during the growth of strain V 37.

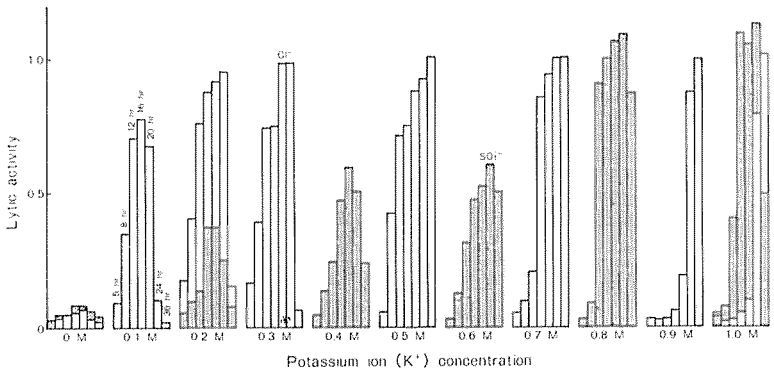

Fig. 12. Effect of potassium ion concentration on the lytic enzyme production during the growth of strain V 37.

Figs. 14, 15 and 16 show the effect of anions at the concentrations of 0.2, 0.3 and 0.5 mole/l on the lytic activity per unit cell density during the growth of strain V 37. Generally, $H_2PO_4^-$ and SO_4^{2-} were not effective for the production of lytic enzyme. However, Cl^- and NO_3^- were more effective than SCN^- at concentration of 0.5 mole/l.

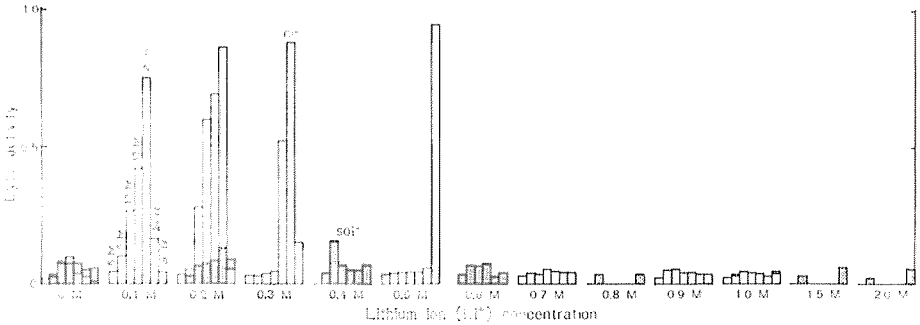

Fig. 13. Effect of lithium ion concentration on the lytic enzyme production during the growth of strain V 37.

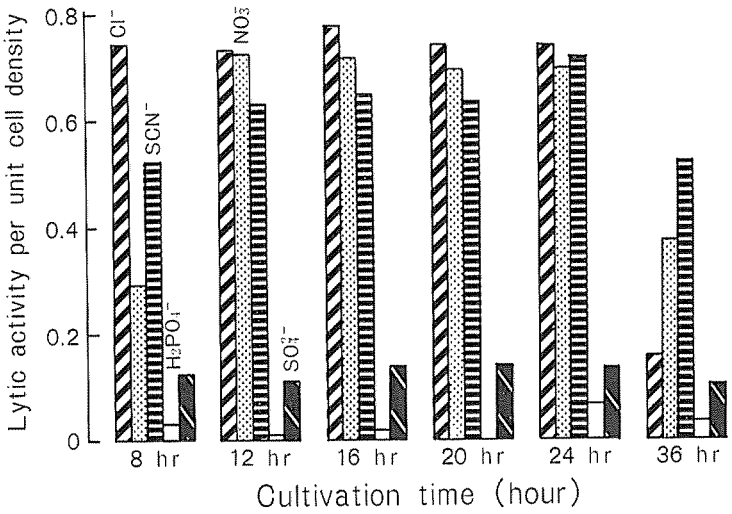

Fig. 14. Effect of anions (0.2 M) on the lytic enzyme production during the growth of strain V 37.

Production of lytic enzyme in the chemically defined medium

In order to examine further organic component responsible for the production of lytic enzyme, a chemically defined medium proposed by MACLEOD(1968) was used in this study (Table 1). Strain V 37 could not grow in chemically defined media without added NaCl and containing 0.5 mole/l of NaCl during 24 hours of cultivation, although strain V 37 grew well in polypeptone-yeast extract media (Table 2). The high production of lytic enzyme was observed only in polypeptone-yeast extract medium containing 0.5 mole/l of NaCl (Table 3).

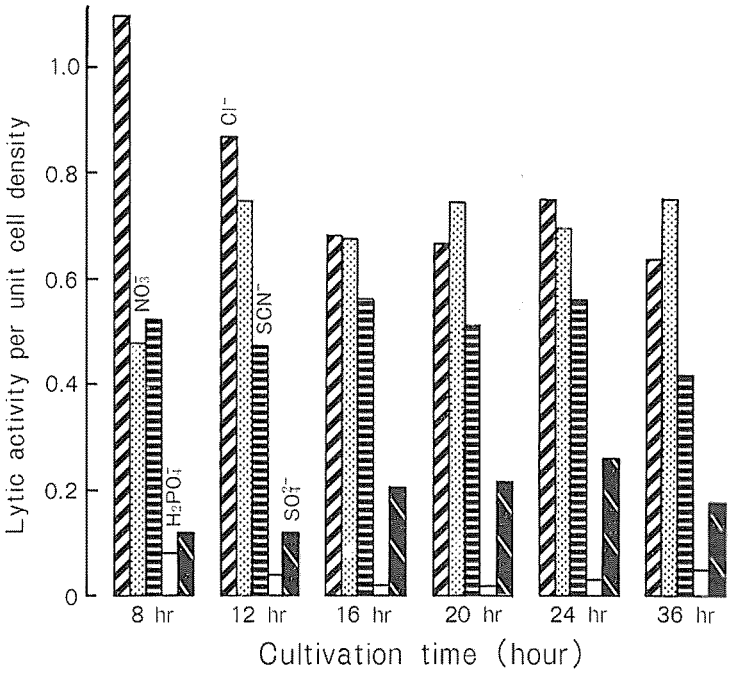

Fig. 15. Effect of anions (0.3 M) on the lytic enzyme production during the growth of strain V 37.

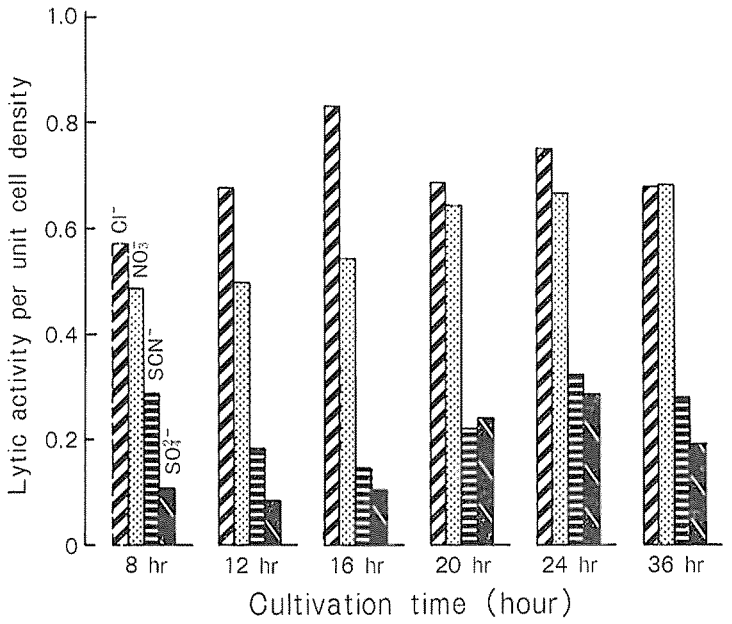

Fig. 16. Effect of anions (0.5 M) on the lytic enzyme production during the growth of strain V 37.

Table 1. Organic components of a chemically defined medium.

Amino acids		Vitamins	
L-Alanine	500 mg/l	p-Aminobenzoic acid	1 mg/l
L-Arginine	200	Biotin	10 μg
L-Aspartic acid	200	Cobalamine	10 μg
L-Cysteine	100	Folic acid	10 μg
L-Glutamic acid	500	Niacin	1 mg
Glycine	100	Pantothenic acid	1 mg
L-Histidine	100	Pyridoxal	1 mg
L-Isoleucine	100	Thiamine	1 mg
L-Leucine	100		
L-Lysine	200		
L-Methionine	100	Glucose	3 g
L-Phenylalanine	100		
L-Proline	100		
L-Serine	100		
L-Threonine	100		
L-Tryptophane	100		
L-Tyrosine	100		
L-Valine	100		

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Table 2. Growth of strain V 37 in the chemically defined medium.

Cultivation time (hour)	Amino acids Vitamins	Amino acids Vitamins Glucose	Amino acids Vitamins	Amino acids Vitamins Glucose	Polypeptone Yeast extract	Polypeptone Yeast extract
	Without added NaCl		0.5 M NaCl	0.5 M NaCl	Without added NaCl	0.5 M NaCl
5	0.015	0.014	0.001	0.011	0.127	0.074
8	0.013	0.012	0	0.002	0.719	0.321
12	0.016	0.026	0.007	0.001	1.553	1.442
16	0.029	0.014	0.005	0.008	1.721	1.699
20	0.034	0.028	0.014	0.008	1.769	1.769
24	0.054	0.037	0.009	0.015	1.824	1.796

Table 3. Production of bacteriolytic enzyme during the growth of strain V 37 in the chemically defined medium.

Cultivation time (hour)	Amino acids Vitamins	Amino acids Vitamins Glucose	Amino acids Vitamins	Amino acids Vitamins Glucose	Polypeptone Yeast extract	Polypeptone Yeast extract
	Without added NaCl		0.5 M NaCl	0.5 M NaCl	Without added NaCl	0.5 M NaCl
5	0	0.006	0.020	0.020	0.042	0.014
8	0.058	0.079	0.005	0.063	0.085	0.188
12	0.066	0.074	0.032	0.059	0.403	0.936
16	0.047	0.036	0.051	0.005	0.133	1.061
20	0.047	0.046	0.051	0.050	0.062	1.083
24	0.036 (0.047)	0.037 (0.055)	0.043 (0.031)	0.051 (0.044)	0.043 (0.092)	1.081 (1.058)

( ); Bacteriolytic activity of sonicated culture fluids

Discussion

The high yield of bacteriolytic enzyme was obtained from shake flask cultures of strain V 37 which were grown at 30 °C for 24–48 hours in the medium containing 1 % polypeptone, 0.5% yeast extract and 0.1–1.0 mole/l of NaCl or KCl, pH 7.0. The salt requirement for the enzyme production could be partially satisfied by the substitution of LiCl, Na_2SO_4, K_2SO_4, $NaNO_3$ or NaSCN for NaCl or KCl (SUGAHARA *et al.* 1980, Figs. 4 and 6). Chloride and nitrate could be used interchangeably on an essentially mole for mole basis for the production of lytic enzyme of strain V 37. However, thiocyanate could be substituted for chloride at the concentration of 0.2 mole/l, but not at the concentration of 0.5 mole/l (Figs. 14, 15 and 16).

Magnesium chloride seemed to inhibit the formation of lytic enzyme in the medium containing NaCl (Fig. 8). The production of lytic enzyme in the medium prepared with artificial seawater (NaCl 30.0 g, KCl 0.7 g, $MgCl_2 \cdot 6H_2O$ 10.8 g, $MgSO_4 \cdot 7H_2O$ 5.4 g and $CaCl_2 \cdot 2H_2O$ 1.0 g per 1 liter of distilled water) was slightly inhibited, as compared with that in the medium containing 0.5 mole/l of NaCl. This may be due to the presence of magnesium ion (about 7.5×10^{-2} mole/l) in artificial seawater.

The addition of glucose showed rather inhibitory effect on the production of lytic enzyme when glucose was added into the medium at the cultivation period of 3, 6 and 9 hours.

Various kinds of media for the production of lytic enzymes have been proposed for many species of microorganisms, such as *Pseudomonas* sp. (BURKE and PATTEE. 1967, LACHE *et al.* 1969), *Achromobacter* sp. (WATANABE *et al.* 1976), *Flavobacterium* sp. (KATO *et al.* 1968), *Staphylococcus* sp. (IVERSEN and GROV 1973, SATTA *et al.* 1977), *Bacillus* sp. (MURAO and TAKAHARA 1974) and *Streptomyces* sp. (YOSHIMOTO *et al.* 1975, YOKOGAWA *et al.* 1976). Such media were all semi-synthetic. On the other hand, MCDONALD(1965) used synthetic medium (90 ml) containing 1.0 mg of methionine, 0.5 g of L-glutamic acid, 200 μg of thiamine-HCl, 4 μg of biotin, 0.5 g of NaCl, 0.5 g of KH_2PO_4, 0.1 g of NH_4Cl, 0.1 g of $NaHCO_3$, 0.5 mg of $MgSO_4$ and 0.5 mg of $FeSO_4 \cdot 7H_2O$ for the production of lytic enzyme of *Micrococcus freudenreichii* 407. A chemically defined medium shown in Table 1 could not support the growth of strain V 37. Strain V 37 may require more high level of organic components or inorganic salts other than NaCl for good growth and enzyme production.

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