Effects of Cations on Thermal Death of a Marine Bacterium during the Mild Heat Treatment

Mem. Fac. Fish., Kagoshima Univ.
Vol. 25, No. 1, pp. 67~75 (1976)

Effects of Cations on Thermal Death of a Marine Bacterium during the Mild Heat Treatment

Taizo SAKATA,* Sow Eng SON** and Daiich KAKIMOTO*

Abstract

The lysis points of a marine *Pseudomonas* sp. for NaCl, KCl and $MgCl_2$ were 0.3, 0.8, and 0.02 M, respectively.

The effect of NaCl on preventing thermal death of the organism at 40 C increased in paralell with NaCl concentration of suspending solution up to 8% (1.4 M). The additional effects of Mg^{++} to Na^+ as well as those of Na^+ to Mg^{++} were observed in preventing the thermal death. In the coexistence of Na^+, Mg^{++} had a marked effect on preventing the thermal lesion, while the capacity of Mg^{++} was lowered extremely without cooperation of Na^+.

It is well known that many marine bacteria lyse when suspended in fresh water or the greatly diluted sea water.

Salts in sea water have long been considered to protect marine bacterial cells against lysis by osmotic action. However, evidence that there might be a specific function for the ions of sea water in the prevention of the cell lysis have been provided by various investigators.[1,2,3] BUCKMIRE and MACLEOD[4] reported that the capacity of NaCl to prevent lysis of a marine pseudomonad could not be due to its osmotic action and that NaCl prevented the lysis by interacitng with, and thereby maintaining the integrity of, the cell envelope of the organism. UNEMOTO et al[5] stated that Na^+ prevented cooperatively with Mg^{++} the cell lysis of a slightly halophile, *Vibrio alginoliticus* by providing the sufficient mechanical strength of the envelope and that K^+ did it by balancing the internal osmotic pressure of the cells.

Furthermore, MORITA and coworkers[6,7] reported that salinity of the growth medium had a marked effect of the maximal growth temperature for marine isolates and that this effect was shown to be due to the presence of various cations in the medium. They also observed that at the maximum growth temperature and above, intracellular materials such as protein, DNA, RNA and amino acids were released from thermally shocked cells.

These works indicate the important roles of salts in maintenance of cell envelope of marine bacteria.

To clarify the roles of salts in the growth and survival of marine bacteria, this investigation was initiated to determine the effect of various salts on thermal death

* Laboratory of Microbiology, Faculty of Fisheries, Kagoshima University.
** Present address: 17, Jalan Lumut, Sitiawan, Perak, Malaysia.

of a marine bacterium during the mild heat treatment.

Materials and Methods

Organism. Marine *Pseudomonas* sp. I–4 used for this study was originally isolated from Kinko Bay in 1970. This organism is belonging to "marine type", which has a specific requirement for sea water for its growth, according to HIDAKA's typing method.[8] It forms a smooth and opalescent colony.

Media and growth conditions. The culture was maintained by monthly transfer on slants of ZoBELL 2216E modified medium containing 0.5% polypeptone (Daigoeiyo), 0.1% yeast extract (Daigoeiyo), HERBST's artificial sea water (ASW) and 1.5% agar (pH 7.6). The liquid medium used contained the same constituents without agar and the inoculated culture was incubated on a reciprocal shaker at 25 C. In the growth experiment on salts media, various concentrations of NaCl with or without $MgCl_2$ (10 mM) were used instead of ASW in ZoBELL 2216E modified medium. Growth was followed by measuring optical density at 540 nm using spectrophotometer (Tokyo photoelectric Co., Model IV).

Preparation of cell suspension. One hundred milliliters of log phase cells in ZoBELL medium were harvested by centrifugation at 7,500 r.p.m. for 15 min at 4 C, washed by centrifugation in cold sterile salt solution (1.0 M NaCl or 0.5 M $MgCl_2$ solution) and resuspended in a small volume of the same salt solution.

Heat treatment of cell suspension. The washed cells were added to temperature-equilibrated test tubes containing 10 ml of each suspending solution and optical density at 540 nm of the cell suspensions was adjusted to 0.5 approximately. Then the cell suspensions were heated in a water bath at 40 C. The samples were taken at time intervals, being subjected to measurement of viable count on agar plate.

Results

The relationship between the growth temperature and salt concentration

The growth response of marine *Pseudomonas* sp. I–4 in various concentrations of NaCl at 25, 30 and 37 C is illustrated in Fig. 1. This organism started to grow at 25 C in the basal medium containing above 3% NaCl and at 30 C above 4% NaCl, respectively, but could not grow at 37 C in any NaCl concentrations. NaCl requirement for growth in the medium containing $MgCl_2$ was less than that without $MgCl_2$. With the addition of 10 mM $MgCl_2$ to the basal medium, the rate of growth became higher than that without $MgCl_2$.

Effects of salts on the lysis of organism

Fig. 2 shows the relationship between salt concentrations and the turbidity of cell

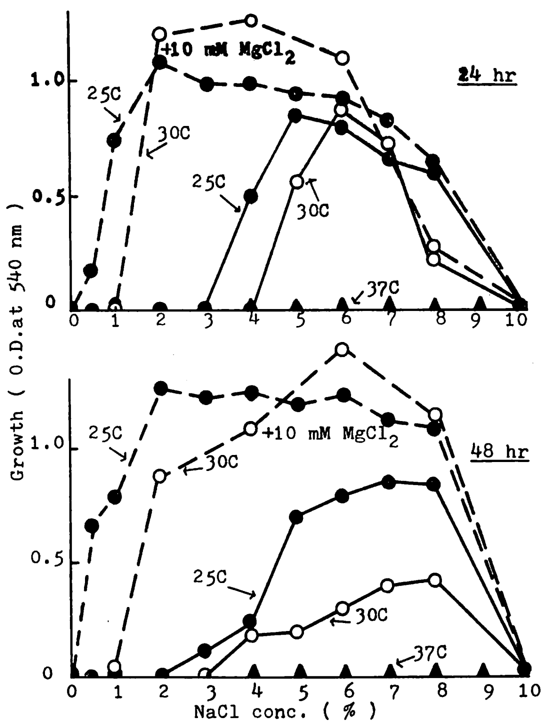

Fig. 1. The relationship between growth temperature and salt concentration of marine Pseudomonas sp. I–4.

suspensions. The minimum concentrations of salts (lysis point), below which a very marked decrease of O. D. was observed, were 0.3, 0.8 and 0.02 M for NaCl, KCl and $MgCl_2$, respectively. When the concentration of NaCl or KCl was lowered in the presence of 10 mM $MgCl_2$ the decrease in O. D. was smaller than that without $MgCl_2$. In this experiment as well, divalent cation was found to prevent the lysis of marine bacteria effectively as reported by many investigators.

Effects of salts on the thermal death of organism

To test the relationship between the heat resistance and salt concentration on test organism, the cell suspensions were exposed to 40 C for 20 min. As shown in Fig. 3, survival percentage of cell suspensions after heat treatment in NaCl solution or ASW with NaCl at various concentrations became higher as NaCl concentration increased. In the case of ASW, NaCl acted more protectively against heat treatment. There was no marked difference in 8% NaCl concentration and higher.

For further examination on salt effect, various suspending solutions that were composed of different combinations of NaCl and $MgCl_2$ were used in the following experiments. As shown in Figs. 4 and 5, the addition of $MgCl_2$ prevented the thermal death of this organism more effectively than that of NaCl if NaCl was used as basal

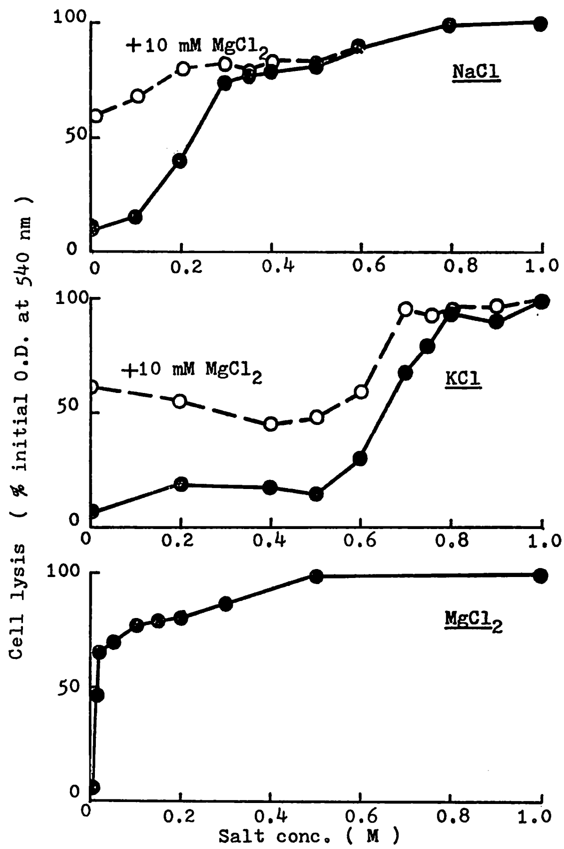

Fig. 2. Effect of salt concentration on the lysis of marine *Pseudomobas* I–4 during the incubation at room temperature for 30 min.

component. On the other hand, the addition of NaCl was more effective than that of $MgCl_2$ in the presence of 0.5 M $MgCl_2$ as the basal. These data were obtained when 1.0 M NaCl was used as the washing and suspending solution. In this case the contamination of a minute amount of salt, which used as the washing solution, was inevitable.

Then, to examine the effect of the washing solution on the thermal death the harvested organism was washed with either 1.0 M NaCl or 0.5 M $MgCl_2$ solution and the concentrated cell suspension in the same solution was added to the test tubes containing 10 ml of various salt solutions. Survival of this organism which had been treated in $MgCl_2$ at 40 C after washed in 0.5 M $MgCl_2$ solution decreased more rapidly in comparison with that washed in 1.0 M NaCl solution (Fig. 6). As indicated in Fig. 7, after the cells washed with 0.5 M $MgCl_2$ in 0.05 or 0.15 M $MgCl_2$ were heated for 10 min, any survival fraction did not be detected. If cells were washed with 0.5 M $MgCl_2$ and heated in 0.5 M $MgCl_2$, viable count decreased with increase of the treatment temperature above 30 C, while viability of the cells after

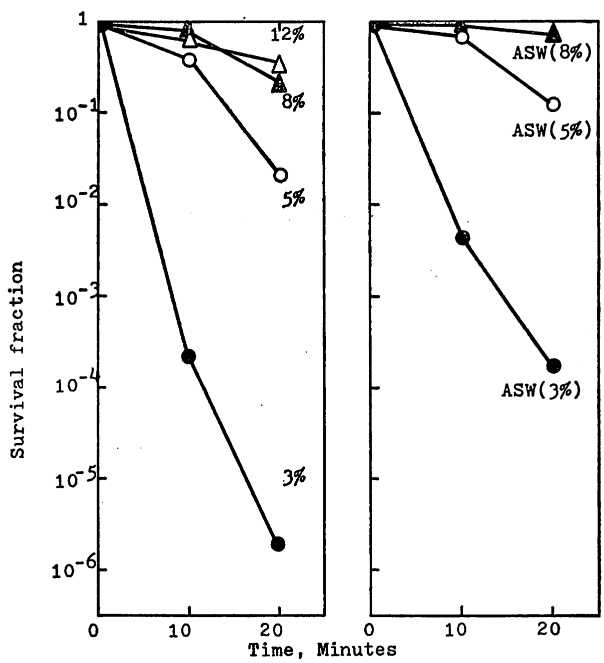

Fig. 3. Cell survival of *Pseudomonas* I–4 suspended in various salt concentration at 40.5 C.

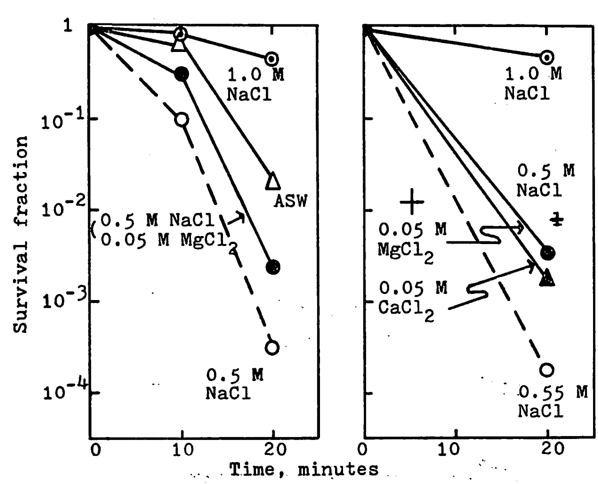

Fig. 4. Effect of cation on cell survival during heating at 40 C for 20 min.

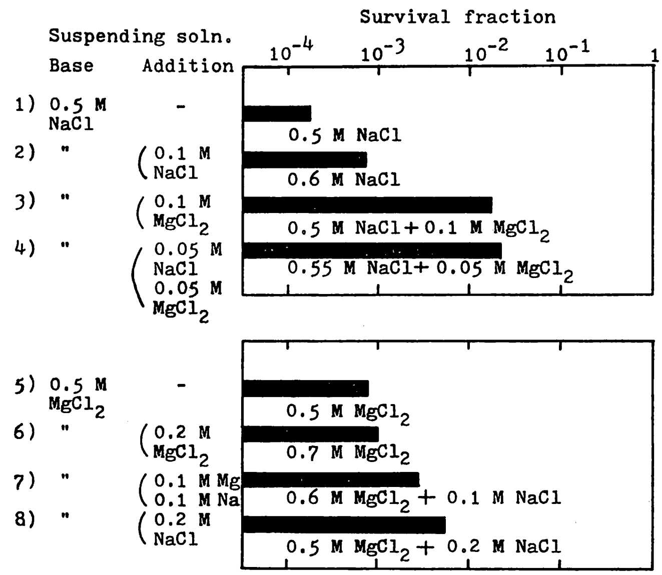

Fig. 5. Effect of cations of suspending solution on cell survival at 40 C for 20 min.

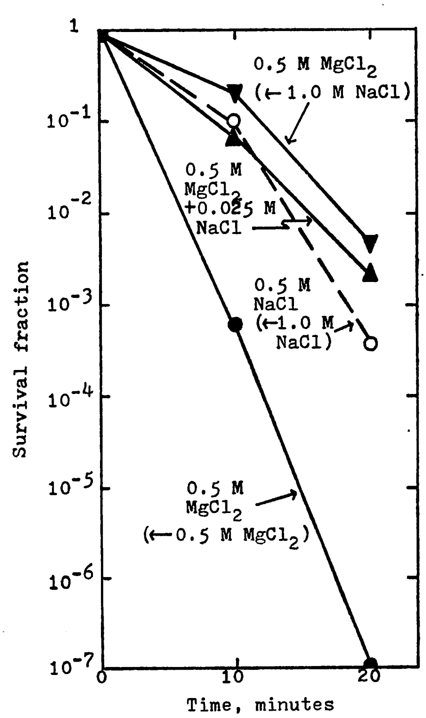

Fig. 6. Cell survival at 40 C in various salt solutions after washed with 1.0 M NaCl or 0.5 M $MgCl_2$ solution.

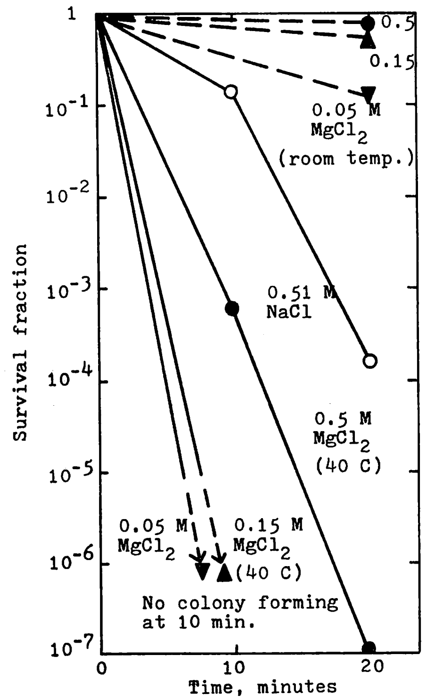

Fig. 7. Survival curves at 40 C in $MgCl_2$ solutions after cells were washed with 0.5 M $MgCl_2$ solution.

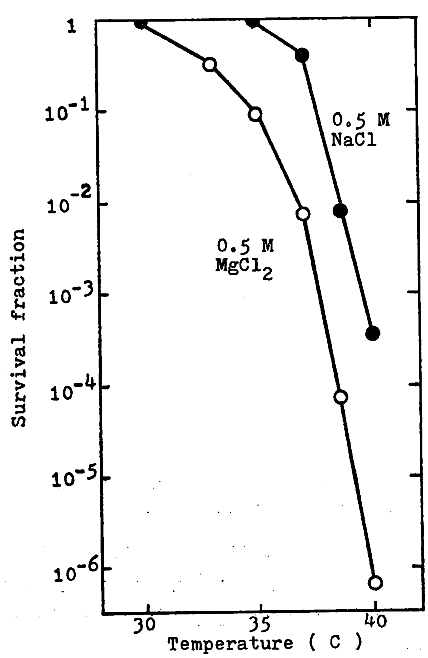

Fig. 8. Cell survival at different temperatures in 0.5 M NaCl or 0.5 M $MgCl_2$ solution.

washed with 1.0 M NaCl began to decrease by heat treatment at higher than 35 C in 0.5 M NaCl solution. (Fig. 8).

It was concluded that $MgCl_2$ possessed higher protective effect against thermal death of a marine bacterium in the presence of NaCl but that $MgCl_2$ alone had little effect on survival during heat treatment.

Discussion

Marine bacteria require inorganic salts in sea water level for normal growth and maintenance of cell integrity. The function of salts has been due to osmotic adjustment, stabilization and activation of enzyme proteins, transport of various substrates and maintenance of cell envelope, but the last should be considered to be the most essential function.

Many investigations have been reported on correlation between the cell lysis phenomenon of marine organisms and salts. Osmotic action, however, alone is not enough to account for salt requirement of marine organisms since individual salts differ markedly in their abilities to prevent lysis of cells. PRATT and RILEY[9] showed that NaCl and LiCl were more effective than KCl or NH_4Cl in preventing lysis. MACLEOD and MATULA[10] also observed that divalent cations were very much more effective than monovalent cations in preventing disruption on the cells of marine bacteria.

In this experiment, it was found that there was a marked difference between the effects of monovalent and divalent cations on preventing the cell lysis and thermal lesion of a marine bacterium. The effect of NaCl on preventing thermal death increased in parallel with NaCl concentration of suspending solution up to 8% (1.4 M). Comparing the additional effect of $MgCl_2$ in the coexistence of 0.5 M NaCl with that of NaCl, the former was found to be more effective in preventing the thermal death. To the contrary, the addition of NaCl to basal solution of 0.5 M $MgCl_2$ was shown to be slightly effective.

In these experiment a small amount of NaCl was carried over to test suspensions since cell pellets were washed and prepared with 1.0 M NaCl solution. If the cells were heated in 0.5 M $MgCl_2$ solution after washed with 0.5 M $MgCl_2$, they became extremely heat-sensitive. In this case Na^+ was removed from cells by being exchanged for Mg^{++} during washing process. In the presence of Na^+, Mg^{++} had a marked effect on preventing of the thermal lesion, while the effect of Mg^{++} was lowered without cooperation of Na^+. These results suggest that binding (active) sites on the cell envelope are different to monovalent and divalent cations.

In studies with isolated envelopes of marine bacteria, MACLEOD[11] suggested that cations of the salt could screen the electron-negative charges (polyanion) to permit the units to come close enough together to form cross linkage between adjacent units by for example hydrogen, hydrophobic and Mg^{++} bonding. O'LEARY et al[12] also reported that Mg^{++} and to a lesser extent Na^+ caused the aggregation of lipopolysaccharide molecules from marine bacteria.

However, the differences between individual cations in the maintenance of cell integrity have not still been revealed essentially. An effort is now being made to confirm the roles of different inorganic ions in the marine bacterial growth and survival.

References

1) HIDAKA, T. and D. KAKIMOTO (1968): Studies on the marine bacteria-III. On the effect of minerals on the lysis of bacteria in hypotonic medium. *Bull. Japan. Soc. Sci. Fish.*, **34**, 73–77.
2) ISHIDA, Y., A. NAKAYAMA and H. KADOTA (1972): Temperature-salinity effects upon the growth of marine bacteria. *Proc. Sec. U. S.-Jap. Conf. Marine Microbiol.* p. 80–91. Univ. Park Press, Baltimore.
3) DAIKU, K., Y. FUJITA, Y. EZURA and M. SAKAI (1976): Minimum salt concentration in the cell-suspension required to prevent lysis. *Bull. japan. Soc. Sci. Fish.*, **42**, 307–313.
4) BUCKMIRE, F. L. A. and R. A. MACLEOD (1965): On the mechanism of lysis of a marine bacterium. *Can. J. Microbiol.*, **11**, 677–691.
5) UNEMOTO, T., T. TSURUOKA and M. HAYASHI (1973): Role of Na^+ and K^+ in preventing lysis of a slightly halophilc *Vibrio alginolyticus*. *Can. J. Microbiol.*, **19**, 563–571.
6) MORITA, R. Y. (1968): The basic nature of marine psychrophilic bacteria. *Bull. Misaki Mar. Riol. Ins. Kyoto Unive.*, **12**, 163–177.
7) STANLEY, S. O. R. Y. and MORITA (1968): Salinity effect on the maximal growth temperature of some bacteria isolated from marine environments. *J. Bacteriol.*, **95**, 169–173.
8) HIDAKA, T. and M. SAKAI (1968): Comparative observation of the inorganic salt reguirements of the marine and terrestrial bacteria. *Bull. Misaki Mar. Biol. Ins. Kyoto Univ.*, **12**, 125–149.
9) PRATT, D. B. and W. H. RILEY (1955): Lysis of a marine bacterium in salt solution. *Bact. Proc.* **55**, 26.
10) MACLEOD, R. A. and T. I. MATULA (1962): Some characteristics of the lytic phenomenon. *Can. J. Microbiol.*, **8**, 883–896.
11) MACLEOD, R. A. (1968): On the role of inorganic ions in the physiology of marine bacteria. *Adv. Microbiol. Sea.,* vol **1**. p. 95–126.
12) O'LEARY, G. P., J. D. NELSON, Jr., and R. A. MACLEOD (1972): Requirement for salts for the isolation of lipopolysaccharide from a marine pseudomonad. *Can. J. Microbiol.* **18**, 601–606.