Importance of Mg$^{2+}$ for the Growth of the Halotolerant
*Bacillus* sp. TSK2

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Abstract

The growth rate of *Bacillus* sp. TSK2 increased when Mg$^{2+}$ was externally added to LB medium at 25 °C and showed the maximum value, 0.82 (Doubling time h$^{-1}$), at 50 mM. While the growth rate was repressed to 0.12 in the presence of 1.0 M NaCl in the medium, the value increased to 0.59 at the same concentration of NaCl, and the growth ability expanded to 3.0 M when 50 mM Mg$^{2+}$ was added to the medium. These results indicate that the strain possesses transport system(s) with relatively low affinity to Mg$^{2+}$, and a concentration of Mg$^{2+}$ similar to that in seawater plays an important role in growth under high NaCl stress.

Key Words: Halotolerant *Bacillus* sp. TSK2, Mg$^{2+}$, Growth rate, Glycine betaine

1. Introduction

Halotolerance of bacteria is closely related to the accumulation of K$^+$ as well as compatible solutes. The moderately halotolerant *Bacillus subtilis* cells uptake K$^+$ rapidly as an initial adaptation in order to cope with external osmolarity. For the newly isolated deep sea bacterium, *Oceanobacillus iheyensis*, glycine betaine and proline are the major compatible solutes based on the genome analysis.

Magnesium cation at the physiological level stabilizes membrane fluidity and neutralizes nucleic acids in cytoplasm. The role of Mg$^{2+}$ in osmotic adaptation toward marine halotolerant bacteria, however, has not yet been studied sufficiently. In this report, we examined the importance of the external existence of Mg$^{2+}$ for the growth of the halotolerant *Bacillus* sp. TSK2 under hyper NaCl stress.

2. Materials and Methods

*Bacillus* sp. TSK2 was isolated from seawater in Toyama Bay. The strain colonies on a seawater-based nutrient agar plate containing 2.5 M NaCl and secreted halotolerant extracellular protease. The partial 16S rDNA sequence has appeared in the DNA Data Bank of Japan with accession number AB636270.

Preincubation was carried out at 25 °C in Luria-Bertani (LB) medium (DAIGO, Nihon Seiyaku) for 1.5 days, and then the cell suspension (50 μl) was inoculated to the same medium (50 ml). In this experiment, the concentration of NaCl in the LB medium varied up to 3.0 M from the original concentration of 0.17 M. Doubling time (h$^{-1}$) was calculated from a slope at the exponential phase of growth curve on a semi-log graph.

The concentration of Mg$^{2+}$ in the LB medium was analyzed quantitatively with an ICS-1600 ion chromatography system (Thermo Scientific). Methanesulfonic acid (30 mM) was used as a moving phase at 1.0 ml min$^{-1}$, and the sample volume injected was 250 μl. The analysis was carried out commercially by Sumika Chemical Analysis Service.

Resting cells were prepared from the early stationary phase of growth cells. The cell suspension (1 ml) was harvested and washed twice with 50 mM HEPES-Na$_2$CO$_3$ buffer, pH 7.5, containing 0.50 M NaCl. Washed cells were resuspended with the same buffer (1 ml). Resting cells thus obtained were used for the survival experiment under hyper NaCl stress. Survivability of the cells was estimated by the colony-counting method.

3. Results and Discussion

The concentration of Mg$^{2+}$ in the LB medium alone was 0.28 mM. Growth was examined in the medium containing MgSO$_4$ · 7H$_2$O externally added, the concentration of which varied from 0 to 0.50 M (Fig. 1). Growth was obviously enhanced by the addition of Mg$^{2+}$, and the maximum growth rate, 0.82 (Doubling time h$^{-1}$), was obtained at 50 mM Mg$^{2+}$, indicating that the strain might possess transport system(s) with relatively low affinity to Mg$^{2+}$.

The adaptation period of the growth curve was drastically increased (Fig. 2A), and the doubling times per hour reduced to 0.12 at 1.0 M NaCl (Fig. 2B) when cells were grown in LB medium alone. On the other hand, the growth rate was enhanced by the addition of 50 mM MgSO$_4$ · 7H$_2$O.
The value was almost the same as that obtained at 3.7 M NaCl and reached 0.59 at 1.0 M NaCl. The same result was obtained in the presence of 50 mM MgCl\(_2\) and glycine betaine. These results indicate that Mg\(^{2+}\), rather than SO\(_4^{2-}\) and Cl\(^{-}\), plays an important role in growth under hyper NaCl stress.

Changes in the survivability of resting cells in the presence of 50 mM Mg\(^{2+}\) and a compatible solute, 50 mM glycine betaine, were examined at 3.7 M NaCl (Fig. 3). The initial number of resting cells, 7.4 ± 0.20 (log (CFU) ml\(^{-1}\)), was reduced to 5.6 ± 0.50 after 245.0 h of exposure in the presence of 50 mM Mg\(^{2+}\) as well as 50 mM glycine betaine. The value was almost the same as that obtained at 3.7 M NaCl alone, 5.4 ± 0.40 (log (CFU) ml\(^{-1}\)). The number of surviving cells at 0.50 M NaCl was also reduced to 6.1 ± 0.40 (log (CFU) ml\(^{-1}\)) after 245.0 h. These results indicate that the resting cells have a high ability to cope with hyper NaCl stress without utilizing Mg\(^{2+}\) and glycine betaine externally.

4. Conclusion

The growth rate of \textit{Bacillus} sp. TSK2 was increased by the addition of 50 mM Mg\(^{2+}\), regardless of the presence or absence of NaCl up to 1.0 M. In addition, the strain could grow at 3.0 M NaCl with the addition of 50 mM Mg\(^{2+}\) to LB medium, indicating that Mg\(^{2+}\) plays an important role in the halotolerant growth of the strain.

References

