Studies on the halotolerant mechanism of the halotolerant bacterium *Brevibacterium* sp.

Shinichi NAGATA (Kobe University of Mercantile Marine)
Kyoko ADACHI, Katsuhisa SHIRAI, Hiroshi SANO (Marine Biotechnology Institute)

Summary

^{23}Na nuclear magnetic resonance (NMR) spectroscopy was employed for the determination of free sodium ion contents in a halotolerant bacterium *Brevibacterium* sp., extremely halophilic *Halococcus morrhuae*, and *Escherichia coli*. Those of *Brevibacterium* sp. at the stationary phases of growth gradually increased with the increase of NaCl concentrations from 0.1 to 2.5 M. Non-halophilic *E. coli*, on the other hand, accumulated higher concentrations of free sodium ions in the cells than those of *Brevibacterium* sp., *e.g.*, grown in the presence of 0.8 M NaCl, they were 368 and 43 mM for the former and the latter, respectively. Intracellular free Na^+ contents were also analyzed for both strains grown in the presence of osmolyte (proline, glycine betaine, or γ-aminobutyrate) together with NaCl. As a result, those of *Brevibacterium* sp. and *E. coli* were reduced to 40–70 % and unchanged by the addition of osmoprotectants, respectively. In turn, addition of KCl in the medium containing NaCl led to the increase of not total but free sodium contents in the cells, indicating that the sodium ions bound or attached were converted to be free in the cytosol. Internal Na concentrations bound to the cellular structures, invisible fraction by ^{23}Na NMR, in *Brevibacterium* sp. grown in the presence of 0.5 M NaCl were estimated to be 0.23 μmole/mg protein, which were equivalent with 38 % visibility. Internal free Na^+ contents in *H. morrhuae* also increased with the increase of external NaCl concentrations, *i.e.*, they were 384 and 588 mM when it was grown in the presence of 3.5 and 4.0 M NaCl, respectively.