Halophilic and thermo-stable enzymes from halophilic bacteria.

Masao Tokunaga, Matsujiro Ishibashi, Hiroko Tokunaga and Mayumi Miyauchi Faculty of Agriculture, Kagoshima University

## Summary

We have attempted to characterize halophilic enzymes, aiming at expansion of their industrial applications. Extremely halophilic archaea and moderately halophilic bacteria require more than 2.5 M and 0.5 M NaCl for growth, respectively and accumulate high concentration of ions and compatible solutes inside the cells. Thus, the industrial application of halophilic enzymes is very attractive, since these enzymes can function under the extreme conditions where most of the 'normal' enzymes cannot. In addition, we have found that many halophilic enzymes showed obvious thermo-stability against heat treatment of enzymes. This property is another advantage of halophilic enzymes for industrial applications. Nucleoside diphosphate kinase from extremely halophilic archaea was stable up to 70 °C, and a certain hydrolytic enzyme from moderately halophilic bacteria maintained 70% activity after boiling for 10 min.

In this study, we attempted to screen thermo-stable proteases from halophilic bacteria, which we have isolated from the natural habitat and stocked in this laboratory. Crude homogenates from these strains were treated at 70  $^{\circ}$ C or 90  $^{\circ}$ C for 5 min, and protease activities were assayed for Azocoll, Azocasein, and Hammerstein casein as well as several synthetic protease substrates. Several strains contain thermostable protease activities. We partially purified proteases from one of these strains, and confirmed that these proteases remained fully active after boiling for 10 min.