

Effect of Slats on the Structure and Function of Dehydrogenase

Toshihisa OHSHIMA

Department of Science, Kyoto University of Education

Summary

We have screened thermophilic and halophilic microorganisms for stable and halotolerant NAD dependent dehydrogenases, and isolated a bacterial strain from the sands in Kumihama coast, Kyoto prefecture. This bacterium was able to grow at 65°C and in the presence of 15% NaCl, and identified as a new strain of Bacillus species. The new strain was named as Bacillus sp. TSN9. This strain exhibited high activity of NAD-dependent leucine dehydrogenase (L-Leucine: NAD oxidoreductase, EC 1.4.1.9). Leucine dehydrogenase gene from the Bacillus sp. TSN9 was cloned into E. coli JM 109 with a vector plasmid, pUC18 and sequenced. The enzyme from E. coli cells carrying the recombinant plasmid pKULD66-51 (4.7 kb) was easily purified to homogeneity by heat treatment, ammonium sulfate fractionation and DEAE-Toyopearl column chromatography with a yield of 31%. The molecular mass of the native enzyme was estimated to be 360 kDa. The enzyme consists of eight subunits identical in a molecular mass (43 kDa). The enzyme showed higher thermal stability at high concentrations (1 to 3 M) of NaCl and retained its full activity on heating at 65°C for 1 hour in the presence of 2.5 M NaCl. The enzyme could be stored for more than 6 months in the standard buffer containing 1.0 M at 30°C. The enzyme activity was stimulated by the addition of 1 M NaCl and inactivated with LiCl and NH₄Cl. The enzyme of Bacillus sp. TSN9 is very similar in many aspects to that of Thermoactinomyces intermedius.