RECOMBINANT AMINOPEPTIDASE FROM THERMOTOGA MARITIMA: CLONING, EXPRESSION, PURIFICATION AND CHARACTERIZATION

R.M.S. RATNAYAKE^{1,2} AND K. HAYASHI²

¹Department of Botany, Faculty of Science, University of Peradeniya ²Enzyme Laboratory, National Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

Hyperthermophiles are a fascinating group of microorganism that has the remarkable property of growing at a temperature of 70° C or above. Because of the thermostability of the enzymes extracted from these organisms, there is a developing interest of utilizing thermoenzymes as biocatalysts for the industries.

A putative aminopeptidase P gene (TM0042, Swissport Q9WXP9, GeneBank AAD35136) of *Thermotoga maritima* was cloned and expressed in *Escherichia coli* BL21 (RIL). The enzyme was purified by the combination of ion exchange chromatography; Q-Sepharose and Mono-Q column. The purified recombinant

T. maritima aminopeptidase P enzyme gave a homogenous protein band with an apparent molecular weight of 40 kDa in SDS-PAGE analysis. The enzyme was purified 23-fold with the specific activity of 16.5 units/mg and the final recovery of 22%. The enzyme was thermostable up to 90°C for 30 min. An optimal activity was observed at 90°C at pH 7.5. The purified enzyme was stable between pH 6.5 to 8 at 80°C with the pH optimum of 7.5. The enzyme aminopeptidase P showed high thermostability which would possibly be advantageous for biotechnological applications.