（学位第３号様式）

学 位 論 文 要 旨

氏　名	Fina Amreta Laksmi
題　目	Study on industrial application of enzymes from extremophiles (極限環境微生物由来酵素の産業利用に関する研究)

Enzymes isolated from extremophilic microorganisms has gained great interest as an industrially important enzymes that can adapt with the conditions employed in industrial process. In this study, a thermophilic L-arabinose isomerase (L-AI) and a halophilic alkaline phosphatase from *Geobacillus stearothermophilus* and *Halomonas* sp. 593, respectively, were used and their properties were improved to cope with industrial application. L-AI is an enzyme that used as the catalyst in the production of D-tagatose, a low-calorie sugar-substituting sweetener, with D-galactose as the substrate. D-Galactose isomerization requires a high temperature to overcome the energy barrier and to shift the reaction equilibrium to D-tagatose. Thus, L-AI from *Geobacillus stearothermophilus* is expected to be a thermostable L-AI, which is desirable for the D-tagatose production. However, for industrial demands, several modifications were necessary, for example improving its substrate specificity for D-galactose. Among the selected residues, mutation at residue 18 produced a mutant strain, H18T, which exhibited increased activity for D-galactose compared with the wild-type (WT) enzyme. Analysis of the substrate specificity of H18T showed a 45.4% improvement for D-galactose. Replacing histidine with threonine at residue 18 resulted in approximately 2.7-fold and 1.8-fold higher substrate binding and catalytic efficiency, respectively, for D-galactose. Further enhancement of the specific activity and catalytic efficiency of H18T for D-galactose by up to 2.7-fold and 4.3-fold, respectively, was achieved by adding borate during L-arabinose isomerase catalysis. Moreover, H18T showed thermostability and no destabilization was detected. In addition to improve the substrate specificity, the activity of L-AI under acidic condition needs to be improved. Reaction under slightly acidic condition is desirable to minimize the Maillard reaction when done at alkaline pH and high temperature. Here we successfully obtained a potential variant of the H18T protein, H18TY234C, which achieved improved activity at pH 6.0, based on random mutagenesis using error-prone PCR around binding pocket area of H18T. This double H18TY234C mutant possessed 1.8-fold and 3-fold higher activity at pH 6.0 than the parent H18T and the wild-type, thereby broadening the optimal pH range to 6.0–8.0. Halophilic enzymes contain a large number of acidic amino acids and marginal large hydrophobic amino acids, which make them highly soluble even under strongly hydrophobic conditions. However, halophilic enzymes easily degrade when used for industrial applications compared with enzymes from other extremophiles because of their instability in low-salt environments. We aimed to clarify the stabilization mechanism of halophilic enzymes. We previously attempted to express halophilic alkaline phosphatase from *Halomonas* (HaALP) in non-halophilic *E. coli*. However, the expressed HaALP showed little activity. Therefore, we overexpressed HaALP in Gram-positive non-halophilic *Brevibacillus choshinensis* in this study, which was successfully expressed and purified in its active form. HaALP was denatured in 6 M urea, refolded using various salts and the non-ionic osmolyte trimethylamine N-oxide (TMAO), and assessed by native polyacrylamide gel electrophoresis. HaALP refolded in 3 M NaCl or 3 M TMAO containing Na^+ ions. Hydrophobic interactions due to a high salt concentration or TMAO enhanced the formation of the folding intermediate (the monomer precursor), and only Na^+ ions activated the dimer form.