Bioconversion Using Thermostable Enzyme: A Case Study for D-Tagatose Production

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Thermostable enzymes from thermophilic Eubactria and Archaea involved in carbohydrate metabolism are very attractive for industrial applications. L-arabinose isomerase(L-AI), that catabolize the conversion of L-arabinose to L-ribulose is not only important for pentose sugar isomerization *in vivo*, but also very attractive for use in the bioconversion of D-galactose to D-tagatose in vitro.

The ketohexose, D-tagatose, has a sweetness value(92%) equivalent to sucrose but is poorly digested. It has been found to be a safe low calorie sweetner in food products and is approved as GRAS substance by FDA. D-tagatose has potential for use as a non-calorie sweetner, as an intermediate in the synthesis of other optically active compounds, and as an additive in detergent, cosmetic and phamaceutical formulations.

D-galactose isomerization at higher temperature has the advantage because the galactose:tagatose equilibrium favors tagatose as temperature increases. For the development of economic conversion process of D-galactose to D-tagatose, the L-AI should be thermostable. Isomerization at low pH is also advantageous, because it reduces the formation of the colored compounds at higher temperature.

Four *araA* genes encoding L-AI from *Thermotoga neapolitana*, *T. maritima*(hyperthermophile), *Bacillus stearophermophilus*(thermophile), and *B. halodurans*(mesophile) were cloned, sequenced, and expressed in *E. coli*. The biochemical properties of these L-AIs were characterized. A novel acidophilic L-AI was also isolated from the acidophilic bacterium, *Alicyclobacillus acidocaldarius*. Each native enzyme was estimated by gel chromatography to be a homotetramer with a molecular mass of 232*k*Da. Although these enzymes originated from microorganisms that have different growth temperatures and pH, they exhibit high levels of sequence similarity(>92%). Bioconversion of D-galactose to D-tagatose by immobilized L-AI and recombinant *E. coli* cells were studied. In continuous packed-bed cell reactor, 56% tagatose conversion was obtained in 700mM D-galactose