

# L-阿拉伯糖異構酶生產 D-塔格糖的特性及固定化條件探討

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## 大綱

一、前言

二、源自 *Lactobacillus fermentum* CGMCC2921 之 L-阿拉伯糖異構酶生產 D-塔格糖之特性探討

三、利用海藻酸鹽固定化 *Lactobacillus fermentum* CGMCC2921 以生產 D-塔格糖

四、源自 *Geobacillus thermodenitrificans* 之突變型 L-阿拉伯糖異構酶特性探討

五、結論

## 摘要

D-塔格糖為 D-半乳糖之異構物，其甜度為蔗糖的 92%，熱量只有蔗糖的 38%，在食品上可作為甜味劑，可減輕第二型糖尿病、高血壓、貧血、血友病等症狀，近年來，多以酵素法生產 D-塔格糖。L-阿拉伯糖異構酶 (L-arabinose isomerase；L-AI；EC 5.3.1.4) 是一種胞內酵素，可將 D-半乳糖 (D-galactose) 轉變為 D-塔格糖 (D-tagatose)。源自 *Lactobacillus fermentum* CGMCC2921 之 L-AI 可在 pH 值 6.5 時具最高的酵素活性，且在 1 mM 的  $Mn^{2+}$  中，D-半乳糖轉換成 D-塔格糖的轉換率為 55%。固定化方面，將含有 L-阿拉伯糖異構酶基因的 *L. fermentum* 菌株進行固定化，並進行其特性探討，發現在 50-80 °C、pH 值 5-7 之間，仍然具有 90% 的相對活性。生產方面，固定化菌體經過反應 192 小時後，仍保有約 90% 的產物產量，使 L-阿拉伯糖異構酶更適用於工業量產。接著利用基因工程技術將 *Geobacillus thermodenitrificans* 來源之 L-AI 進行突變，發現在相同條件下反應 30 分鐘，三突變酵素之產物產率高於雙突變酵素三倍，150 分鐘後，三突變酵素轉換率高於雙突變 24.8%，突變後對 D-塔格糖轉換更為提升。

## 参考文献

- Cheetham P, Wootton A (1993) Bioconversion of D-galactose into D-tagatose. *Enzyme and Microbial Technology* 15: 105-108.
- Jørgensen F, Hansen O, Stougaard P (2004) Enzymatic conversion of D-galactose to D-tagatose: heterologous expression and characterisation of a thermostable L-arabinose isomerase from *Thermoanaerobacter mathranii*. *Applied Microbiology and Biotechnology* 64: 816-822.
- Kim B-J, Hong S-H, Shin K-C, Jo Y-S, Oh D-K (2014) Characterization of a F280N variant of L-arabinose isomerase from *Geobacillus thermodenitrificans* identified as a D-galactose isomerase. *Applied microbiology and biotechnology*: 1-11.
- Kim H-J, Oh D-K (2005) Purification and characterization of an L-arabinose isomerase from an isolated strain of *Geobacillus thermodenitrificans* producing D-tagatose. *Journal of Biotechnology* 120: 162-173.
- Kim P (2004) Current studies on biological tagatose production using L-arabinose isomerase: a review and future perspective. *Applied Microbiology and Biotechnology* 65: 243-249.
- Levin GV (2002) Tagatose, the new GRAS sweetener and health product. *Journal of Medicinal Food* 5: 23-36.
- Lim BC, Kim HJ, Oh DK (2007) High production of D-tagatose by the addition of boric acid. *Biotechnology Progress* 23: 824-828.
- Liu S-Y, Wiegel J, Gherardini FC (1996) Purification and cloning of a thermostable xylose (glucose) isomerase with an acidic pH optimum from *Thermoanaerobacterium* strain JW/SL-YS 489. *Journal of Bacteriology* 178: 5938-5945.
- Manjasetty BA, Chance MR (2006) Crystal Structure of *Escherichia coli* L-arabinose Isomerase (ECAI), The putative target of biological tagatose production. *Journal of Molecular Biology* 360: 297-309.
- Mendoza MR, Olano A, Villamiel M (2005) Chemical indicators of heat treatment in fortified and special milks. *Journal of Agricultural and Food Chemistry* 53: 2995-2999.
- Oh D-K (2007) Tagatose: properties, applications, and biotechnological processes. *Applied Microbiology and Biotechnology* 76: 1-8.
- Oh D-K, Kim H-J, Ryu S-A, Rho H-J, Kim P (2001) Development of an immobilization method of L-arabinose isomerase for industrial production of tagatose. *Biotechnology Letters* 23: 1859-1862.
- Radestock S, Gohlke H (2011) Protein rigidity and thermophilic adaptation. *Proteins*:

- 1           Structure, Function, and Bioinformatics 79: 1089-1108.
- 2    Xu Z, Li S, Fu F, Li G, Feng X, *et al.* (2012) Production of D-tagatose, a functional
- 3           sweetener, utilizing alginate immobilized *Lactobacillus fermentum*
- 4           CGMCC2921 cells. *Applied biochemistry and biotechnology* 166: 961-973.
- 5    Xu Z, Qing Y, Li S, Feng X, Xu H, *et al.* (2011) A novel l-arabinose isomerase from
- 6           *Lactobacillus fermentum* CGMCC2921 for D-tagatose production: Gene
- 7           cloning, purification and characterization. *Journal of Molecular Catalysis B:*
- 8           Enzymatic 70: 1-7.