利用蛋白質工程及固定化技術改善幾丁聚糖酶之表現量及酵素特性

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摘要

幾丁聚糖酶 (chitosanase) 為一種可以水解幾丁聚糖 (chitosan) 上 β-1,4-糖苷鍵，進而形成幾丁寡糖 (chitooligosaccaride) 或是葡萄糖胺 (glucosamide) 之酵素，而本篇報告探討利用不同方式改善幾丁聚糖酶之表現量及酵素特性。將 *Aspergillus fumigatus* 來源之幾丁聚糖酶基因選殖入大腸桿菌中表現，在 25°C 下以 10 g/L 乳糖誘導並添加 1% 甘胺酸 (glycine) 時，酵素活性為 6015 U/mL，此外進行批式發酵 (fed-batch) 兩天，酵素活性更可達至 14000 U/mL。另外，利用定位突變方式增加 *Bacillus ehimensis* 來源之幾丁聚糖酶 EAG1 (chitosanase EAG1) 之雙硫鍵，以提高其熱穩定性。在所有突變型酵素中，以 *Mut5* 表現最好，半衰期由原本 10.5 分鐘增加至 69.3 分鐘，且其催化效率約為原生型酵素之 2 倍。最後，將幾丁聚糖酶固定於矽膠 (silica-gel) 上用以生產幾丁寡糖並進行其特性分析，在 37°C 時，其固定化酵素活性最佳，雖然固定化酵素活性僅有非固定化酵素的 82.3%，但酵素經固定化後能增加其穩定性及連續催化幾丁聚糖能力皆更加良好，因此具有連續生產之優勢。綜合上述結果，不論是以批式發酵、定位突變增加雙硫鍵或是固定化技術，皆能分別有效增加幾丁聚糖酶之表現量、熱穩定性或是重複利用性，使得在生產幾丁寡糖之應用上更加具有優勢。

參考文獻

Cheng, C. Y., Chang, C. H., Wu, Y. J., & Li, Y. K. (2006). Exploration of glycosyl hydrolase family 75, a chitosanase from *Aspergillus fumigatus. J Biol Chem, 281*, 3137-3144.

Cheng, C. Y., & Li, Y. K. (2000). An *Aspergillus* chitosanase with potential for large-scale preparation of chitosan oligosaccharides. *Biotechnol Appl Biochem, 32 (Pt 3)*, 197-203.

Clarke, J., Hounslow, A. M., Bond, C. J., Fersht, A. R., & Daggett, V. (2000). The effects of disulfide bonds on the denatured state of barnase. *Protein Sci, 9*(12), 2394-2404.

Dag, Y. W., Yu, J., Pan, X., Wang, P., Lan, W., & Tao, S. (2007). Antimicrobial effect of chitooligosaccharides produced by chitosanase from Pseudomonas CUY8. *Asia Pac J Clin Nutr, 16*, 174.

Ding, R., Li, Z., Chen, S., Wu, D., Wu, J., & Chen, J. (2010). Enhanced secretion of recombinant a-cyclodextrin glucosyltransferase from *E. coli* by medium additives. *Process Biochemistry, 45*, 880-886.

Ferdjani, S., Ionita, M., Roy, B., Dion, M., Djeghaba, Z., Rabiller, C., & Tellier, C. (2011). Correlation between thermostability and stability of glycosidases in ionic liquid. *Biotechnol Lett, 33*, 1215-1219.

Huang, L., Wang, Q., Jiang, S., Zhou, Y., Zhang, G., & Ma, Y. (2016). Improved extracellular expression and high-cell-density fed-batch fermentation of chitosanase from *Aspergillus Fumigatus* in *Escherichia coli*. *Bioprocess Biosyst Eng, 39*(11), 1679-1687.

Kouichi, A., Fujita, T., Kuroshima, K., Sakane, T., Yakota, A., & Takata, R. (1999). Purification and gene cloning of a chitosanase from Bacillus ehimensis EAG1. *J Biosci Bioeng, 87*, 383-385.

Montilla, A., Ruiz-Matute, A. I., Corzo, N., Giacomini, C., & Irazoqui, G. (2013). Enzymatic generation of chitooligosaccharides from chitosan using soluble and immobilized glycosyltransferase (Branchzyme). *J Agric Food Chem, 61*(43), 10360-10367.

Sheng, J., Ji, X., Zheng, Y., Wang, Z., & Sun, M. (2016). Improvement in the thermostability of chitosanase from *Bacillus ehimensis* by introducing artificial disulfide bonds. *Biotechnol Lett, 38*(10), 1809-1815.

Sinha, S., Dhakate, S. R., Kumar, P., Mathur, R. B., Tripathi, P., & Chand, S. (2012). Electrospun polyacrylonitrile nanofibrous membranes for chitosanase immobilization and its application in selective production of chitooligosaccharides. *Bioresour Technol, 115*, 152-157.

Song, J. Y., Alnaeeli, M., & Park, J. K. (2014). Efficient digestion of chitosan using chitosanase immobilized on silica-gel for the production of multisize chitooligosaccharides. *Process Biochemistry, 49*, 2107-2113.

Su, L., Yu, L., Xu, C., & Wu, J. (2015). Extracellular expression of Thermobifida fusca cutinase with pelB signal peptide depends on more than type II secretion pathway in Escherichia coli. *J Biotechnol, 204*, 47-52.

Suzuki, K., Tokoro, A., Okawa, Y., Suzuki, S., & Suzuki, M. (1985). Enhancing effects of N-acetyl-chito-oligosaccharides on the active oxygen-generating and microbicidal activities of peritoneal exudate cells in mice. *Chem Pharm Bull (Tokyo), 33*(2), 886-888.

Thadathil, N., & Velappan, S. P. (2014). Recent developments in chitosanase research and its biotechnological applications: a review. *Food Chem, 150*, 392-399.

Tokoro, A., Tatewaki, N., Suzuki, K., Mikami, T., Suzuki, S., & Suzuki, M. (1988). Growth-inhibitory effect of hexa-N-acetylchitohexaose and chitohexaose against Meth-A solid tumor. *Chem Pharm Bull (Tokyo), 36*(2), 784-790.