新型基因重組Picrophilus torridus R523P 海藻糖合成?之純化與特性分析 = Partial purification and characterization of the tr

林哲弘、張淑微

E-mail: 354786@mail.dyu.edu.tw

摘 要

海藻糖(Trehalose)是一新的多功能醣類，存在於自然界的許多生物體內，除了作為儲備能源與碳源外，還能幫助生物抵抗惡劣環境，且具有穩定生物巨分子的能力，因此能廣泛運用在食品、化妝品、醫藥等工業上。海藻糖合成?(Trehalose synthase)是能將麥芽糖單一步驟轉化成海藻糖的生物觸媒。本研究擬利用新型基因重組Picrophilus torridus海藻糖合成?(PTTS)突變株R523P來進行研究，分析酵素不同的生化特性。 對於純化酵素進行熱穩定性、酵素動力學、反應溫度(20℃~65℃)及pH(3~8)值對於海藻糖產率的影響。實驗結果顯示此酵素(R523P)於40℃下，反應4小時，pH6下，其海藻糖轉換率高於PTTS-wild type達到50.58±0.67%，並且葡萄糖副產物低於7.92±0.06%以下。在溫度穩定性上，70℃依舊維持94.31±0.01%的相對活性。而pH穩定性方面，當pH達到8，相對活性還維持62.49±0.61%，擴大了pH值反應範圍。本實驗發現突變株酵素(R523P)，有降低副產物產生及擴大反應條件範圍，在其未來工業，提高海藻糖生產方面將具有極大的潛力。

關鍵詞 : 海藻糖、Picrophilus torridus、海藻糖合成?、突變、熱穩定性

目錄

參考文獻

1.王惠珠、李菁菁、林苑暉、林旺熠、周薰修、康藏文、張永鍾、曹欽玉、邱文貴、黃培安、游銅錫、郭嘉信、劉建功、葉彥宏、陳榮輝、陳陸宏、謝喻文。2005。第534-545頁。食品添加物。華格那出版。台中，台灣。 2.井瑞洁。2009。海藻糖合?特性及固定化條件的研究。山東輕工業學院碩士論文。山東，中國。 3.石東原，活性部位殘基突變後對於海藻糖生成?之活性與基質選擇性的影響。國立海洋大學食品科學系碩士論文。基隆，台灣。 4.李卓。2008。紫丁香蘑液體發酵海藻糖的提取純化及應用研究。吉林農業大學碩士論文。吉林，中國。 5.李俊穎。海藻糖合成?的基因表現及其應用於合成海藻糖。國立中正大學化學工程所碩士論文。嘉義，台灣。 6.尚宏麗。2006。產海藻糖合成?菌株的篩選及其合?的分離純化和性質驗研究。瀋陽農業大學碩士論文。瀋陽，中國。 7.周欣宏。2007。新型基因重組蛋白Picrophilus torridus 海藻糖合成?之蛋白質工程及其固定化酵素之最適反應研究。國立海洋大學生物科技研究所碩士論文。基隆，台灣。 8.邵引剛、李峰、孫輝、劉朝良。2008。海藻糖的應用及基因工程研究。安徽農業科學。36期，第857 - 859頁。安徽，中國。 9.張鴻民、許祥純、傅慧音、田欽仁、洪連欉、楊明華、蔣宗哲、柯文慶、賴盈章、江文德、謝淳仁、江伯源、巢佳莉。2006。第155-166頁。食品生物化學。華格那出版。台中，台灣。 10.張淑微。2001。以反應曲面法研究酵素合成己醇酯類之最優化:8。大葉大學食品工程研究所碩士論文。彰化，台灣。 11.孫薏琳。2008。以大腸桿菌表達系統生產雙功能基因重組熱穩定型Clostridium thermosulfurogenes β-澱粉?及Picrophilus torridus 海藻醣合成?之融合酵素及其催化甘藷澱粉單一步驟轉化海藻糖反應。國立中興大學食品暨應用生物科技研究所碩士論文。台中，台灣。 12.陳怡珊。2006。Picrophilus torridus 海藻糖合成?基因在大腸桿菌之選殖、表現及其重組蛋白質之生化特性分析。國立陽明大學生化暨分子生物研究所碩士論文。台北，台灣。 13.鄭世雄。1999。有機化學（上冊）。第211-212頁。藝軒圖書出版社。台北，台灣。 14.羅英庭。2011。以基因重組Candida rugosa 脂肪同功酵素探討左旋薄荷醇衍生物之最優化合成條件。大葉大學生物產業科技研究所碩士論文。彰化，台灣。 15.曹文正。2005。有機化學。第106頁。新文京開發出版股份有限公司。台北，台灣。 16.聶凌鴻、寧正祥。2001。海藻糖的生物保護作用。生命的化學21卷3期:206-209。廣州，中國。 17.陳雲輝、徐程、余小林、胡卓炎、余凱。2011。海藻糖對荔枝罐頭非?褐變特性的影響。食品與機械27卷1期:15-18。廣東，中國。 18.Anna Zdzieblo, Jozef Synowiecki, 2006. Production of trehalose by intramolecular transglucosylationof maltose catalysed by a new enzyme from Thermus thermophilus HB-8. Food Chemistry,96, 8-13. 19.Ann MacGregor, E., Janecek, S., Svensson, B. 2001. Relationship of sequence and structure to specificity in the α-amylase family of enzymes, Biochimica et Biophysica Acta, 1546, 1-20. 20.Arguelles, J.C. 2000. Physiological roles of trehalose in bacteria and yeasts: acomparative analysis. Arch. Microbiol, 174, 217-224. 21.Behm, C.A. 1997. The role of trehalose on the physiology of nematodes. Int J, Parasitol, 27, 215-229. 22.Benaroudj, N., Lee, D.H., Goldberg, A.L. 2001. Trehalose accumulation during cellular stress protects cells and cellular protein form damage by oxygen radicals. Journal of Biological Chemistry, 276, 24261-24267. 23.Best, M., Koenig, K., McDonald, K., Schueller, M., Rogers, A., Ferrieri, R.A. 2011. Inhibition of trehalose breakdown increases new carbon partitioning into cellulosic biomass in Nicotiana tabacum, 346, 595-601. 24.Cai, Y., Liu, S., Liao, X., Ding, Y., Sun, J., Zhang, D. 2011. Puri?cation and partial characterization of antifreeze proteins from leaves of Ligustrum lucidum Ait, Food and bioproducts processing, 89, 98-102. 25.Chang, S.W., Chang, W.H., Lee, M.R., Yang, T.J., Yu N.Y., Chen, C.S., Shaw, J.F. 2010. Simultaneous production of trehalose, bioethanol, and high-protein product from rice by an enzyme process. Journal of Agricultural and Food Chemistry, 58, 2908-2914. 26.Chen, Y.S., Lee, G.C., Shaw, J.F. 2006. Gene Cloning, Expression, and Biochemical Characterization of a Recombinant Trehalose Synthase from Picrophilus torridus in Escherichia coli, Journal of Agricultural and Food Chemistry, 54, 7098-7104. 27.Chou, H.H., Chang, S.W., Lee, G.C., Chen, Y.S., Yeh, T., Akon, C.C., Shaw, J.F. 2010. Site-directed mutagenesis improves the thermostability of a recombinant Picrophilus torridus trehalose synthase and effciency for the production of trehalose from sweet potato starch. Food Chemistry, 119, 1017-1022. 28.Colaco, C., Sen, S., Thangavelu, M., Pinder, S., Roser, B. 1992. Extraordinary stability of enzymes dried in thehalose: simplified molecular biology, Nature Biotechnology,10, 1007-1010. 29.Crowe, J.H., Crowe, L.M. 2000. Preservation of mammalian cells-learning nature ’ s tricks, Nature Biotechnol, 18, 145-146. 30.Dong, C.L., Zhang, M.I., Zhang, Y.Q., Yang, L.L., Liang, G.M., Sun, J., Lin, Z.P., Gon, J.F. 2011. Transformation of trehalose synthase gene (TPS Gene) into corn inbred line and identification of drought tolerance. African Journal of Biotechnology, 10,15253-15257. 31.Elbein, A.D.,Pan, Y.T.,Pastuszak, I.,Carroll, D. 2003. New insights on trehalose: a multifuntional molecule. Glycobiology, 13, 17-27. 32.Foster, J.A., Jenkinson, J.M., Talbot, N.J. 2003. Trehalose synthesis and metabolism are required at different stage of plant infection by Magnaporthe grisea, The EMBO journal, 22, 225-235. 33.Galmarini, M.V., Baeza, R., Sanchez, V., Zamora, M.C., Chirife, J., 2010. Comparison of the viscosity of trehalose and sucrose solutions at various temperature: effect of guar gum addition. Food Science and Technology, 44, 186-190. 34.Garg, K.A., Kim, J., Owents T.G., Ranwala, A.P., Choi, Y.D., Kochian, L.V., Wu, R.J. 2002. Trehalose accumlation in rice plants confers high tolerance levels to different abiotic syresses. Proceedings of the National Academy of Sciences, 99, 15898-15903. 35.Goh, K.M., Voon, C., Chai, Y.Y., Rosli, M.I. 2011. Meiothermus sp. SK3-2: A potential

source for the production of trehalose from maltose. African Journal of Biotechnology, 10, 12775-12783. 36.Goihberg, E., Dym, O., Tel-Or, S., Levin I., Peretz, M., Burstein, Y. 2007. A single proline substitution is critical for the thermostabilization of Clostridium beijerinckii alcohol dehydrogenase, Structure, Function, and Bioinformatics, 66, 196 – 204 37.Green, J.L., Angell. C.A., 1989. Phase relations and vitrification in saccharide – water solutions and the trehalose anomaly. The Journal of Physical Chemistry, 93, 2880-2882. 38.Harding, T.S. 1923. Histroy of trehalose, its discovery and methods of preparation. Suger, 25, 476-478. 39.Hardy, F.,Vriend, G., Veltman, O.R., Van der Vinne, B., Eijsink, G.H. 1993. Stabilization of Bacillus stearothermophilus neutral protease by introduction of proline. FEBS Lett, 317, 89-92. 40.Herdeiro, R.S., Pereira, M.D., Panek, A.D., Eleutherio, E.C. 2006. Trehalose protects Saccharomyces cerevisiae from lipid peroxidation during oxidative stress. Biochimica et Biophysica Acta, 1760, 340-346. 41.Higashiyama, T. 2002. Novel functions and applications of trehalose, Pure and applied chemistry, 74, 1263-1269. 42.Isabella Di Lernia, Chiara, S., Maddalena, G., Mario De Rosa, 2002. Trehalose production at high temperature exploiting an immobilized cell bioreacter: Extremophiles. 6, 341-347. 43.Kandror, O., DeLeon, A., Goldberg, A.L. Trehalose synthesis is induced upon exposure of Escherichia coli to cold and is essential for viability at low temperatures. Proceedings of the National Academy of Sciences, 99, 9727-9732. 44.Kim, T.K., Jang, J.H., Cho, H.Y., Lee, H.S., Kim, Y.W. 2010. Gene Cloning and Characterization of a Trehalose Synthase from Corynebacterium glutamicum ATCC13032. Food Science Biotechnol, 19, 565-569. 45.Kiutts, S., Pastuszak, I., Edavana, V.K., Thampi, P., Pan, Y.T., Abraham, E.C., Carroll, J.D., Elbein, A.D. 2003. Purification cloning expression and properties of mycobatreial trehalose-phosphate phosphatase, The Journal of Biological Chemistry, 278, 2093-2100. 46.Lee, J.H., Lee, K.H., Kim, C.G., Lee, S.Y., Kim, G.J., Park, Y.H., Chung, S.O. 2005. Cloning and expression of a trehalose synthase from Pseudomonas stutzeri CJ38 in Escherichia coli for the production of trehalose, Applied Microbiology And Biotechnology, 68, 213-219. 47.Li, H.W., Zang, B.S., Deng, X.W., Wang, X.P. 2011.Overexpression of the trehalose-6-phosphate synthase gene OsTPS1 enhances abiotic stress tolerance in rice. Planta, 234, 1007-1018. 48.Lowe Rohan, G.T., Lord, M., Rybak, K., Trengove, Robert, D. 2009. Trehalose biosynthesis is involved in sporulation of Stagonospora nodorum, Fungal Genetics and Biology, 46, 381-389. 49.Ma, Y., Xue, L., Sun, D.W. 2006. Characteristics of trehalose synthase from permeablized Pseudomonas putida cells and its application in converting maltose into trehalose, Journal of Food Engineering, 77, 342-347. 50.Masui, A., Fujiwara, N., Imanaka, T., 1994. Stabilization and rational design of serine protease AprM under highly alkaline and high-temperature conditions. Appl Environ Microbiol. 60, 3584-3597. 51.Maruta, K., Hattori, K., Nakado, T., Kubota, M., Sugimoto, T., Kurimoto, M., 1996. Cloning and sequencing of trehalose bio synthesis genes from Arthrobacter sp.Q36, Biochimica et Biophysica Acta, 1289, 10-13. 52.Nishimoto, T., Nakono, M., Ikegami, S., Chane, H., Fukuda, S., Sugimoto, T., Kurimoto, M., Tsujisaka, Y., 1995. Existence of a novel enzyme converting maltose into trehalose, Bioscience, Biotechnology, and Biochemistry, 11, 2189-2190. 53.Ph. Reignault, Cogan, A., Muchembled, J., Louns-Hadj Sahraoui, A., Durand, R., Sancholle, M. 2001. Trehalose induces resistance to powdery mildew in wheat, New phytologist, 149, 519-529. 54.Purvis, J.E., Yomano, L.P., Ingram, L.O. 2005. Enhanced trehalose production improves growth of Escherichia coli under osmotic stress. Appl Environ. Microbiology. 71, 3761-3769. 55.Richards, A.B., Krakowka, S.,Dexter, L.B.,Schmid, H., Wolterbeek, A.P., Waalkens-Berendsen, D.H., Shigoyuki, A., Kurimoto, M. 2002. Trehalose: a review of properties, history of use and human tolerence, and result of multiple safety studies. Food. Chem. Toxicol, 40, 871-898. 56.Ryu, S.I., Kim, J.E., Huong, N.T., Woo, E.J., Moon, S.K., Lee, S.B. 2010. Molecular cloning and characterization of trehalose synthase from Thermotoga Marititma DSM3109: syntheses of trehalose disaccharide analogues and NDP-glucose. Enzyme and Microbial Technology, 47, 249-256. 57.Saito, K., Yamazaki, H., Onnishi, Y., Fujimoto, S., Takahashi, E., Hroinouchi, S. 1998. Production of trehalose synthase from a basidiomycete Grifola frondosa in Escherichia coli. Applied Microbiology And Biotechnology, 50, 193-198. 58.Schiraldi, C.,Di Lernia, I,. De Rosa, M. 2002. Trehalose production: exploting novel approaches. Trends Biotechnol, 20, 420-425. 59.Singer, M., Lindquist, S. 1998. Multiple effects of trehalose on protein foldong in vitro and vivo. Molecular Cell. 1, 639-648. 60.Suvobrata Chakravarty, Raghavan Varadarajan. 2000. Elucidation of determinants of protein srability through genome sequence analysis. FEBS Lett. 470, 65-69. 61.Wang, D.S., Zhao, S.F., Zhao, M.X., Li, J., Chi, Z.M. 2011. Trehalose accumulation from cassava starch and release by a highly thermosensitive and permeable mutant of Saccheromycopsis fibuligera, Journal of Industrial Microbiology and Biotechnology, 38, 1545 – 1552 62.Yamamoto, T., Maruta, K., Watanabe, H., Yamashita, H., Kubota, M., Fukuda, S., Kurimoto, M. 2001. Trehalose-producing operon treYZ from Arthrobacter ramosus S34. Bioscience, Biotechnology, and Biochemistry, 65, 1419-1423. 63.Zhang, Y., Zhang, T., Chi, Z., Wang, J.M., Liu, G.I., Chi, Z.M. 2010. Conversion of cassava starch to trehalose by Saccharomycopsis fibuligera A11and purification of trehalose. Carbohydrate Polymers, 80, 13-18.