Practical Production of Oligosaccharides Employing Multiple-enzymes System

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There are many kinds of oligosaccharides which have important functions. The low quantity and difficulty to synthesis limited the utility value of such oligosaccharides. Fortunately, oligosaccharides could be synthesis or exploited by phosphorolytic enzymes (phosphorylase).

D-Galactosyl- β 1→4-L-rhamnose phosphorylase (GRP) could produce Gal-β1,3-Glc, which was a new oligosaccharides, from glucose and Gal-1-P. We named Gal-β1,3-Glc as Novus-Lactose, because the structure of Lactose was Gal-β 1,4-Glc. The Novus-Lactose could be one important candidate Functional Oligosaccharide in food and health products due to its distinctive structure.

For the construction of the enzymatic procedure, it is important to establish a routine method to measure the enzymatic activity. We developed a protocol for the enzymatic colorimetric quantification of orthophosphate (Pi) using pyruvate oxidase and peroxidase. The following relationship was derived from linear regression with a correlation coefficient greater than 0.996: y = 0.46 x (x = [phosphate] (mM); y = ΔAbs505). The calibration curve was not affected by the presence of labile phosphate esters. The method is capable for continuous monitoring of the reaction activity of GRP.
We have attempted the one-pot enzymatic synthesis of Novus-Lactose from sucrose employing multiple-enzymes system: sucrose phosphorylase (SP), UDP-glucose-hexose 1-phosphate urydylal transferase (GalT), UDP-glucose 4-epimerase (GalE), xylose isomerase (XI), and GRP (Fig.1).

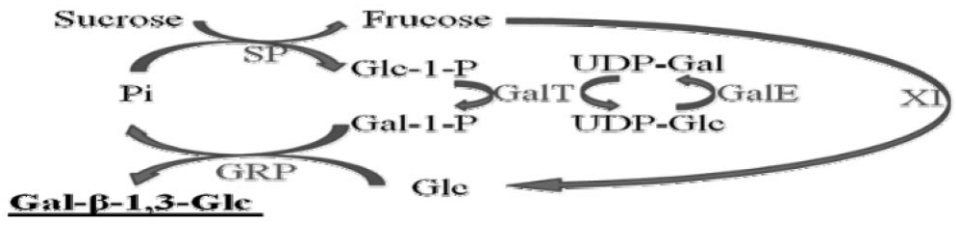

Fig.1 Multiple-enzymes system for synthesizing Gal-β-1,3-Glc

We initially fixed the concentrations of substrates and cofactors as 1.0 M Sucrose, 400 mM phosphate, 10 mM $MgCl_2$, and 1 mM UDPG. Then we fixed the concentration of GalT, GalE, XI, and GRP as optimum concentration 31.2, 81.2, 400, and 100 (μg/ml), respectively. We try to optimize the SP concentration and found it between 31.2 to 125 (μg/ml) The maximum concentration of Gal-β-1,3-Glc reached 322 mM in 14 d at 30 ℃ with SP, GalT, GalE, XI, and GRP at 25, 125, 325, 1600 and 400 (μg/ml), respectively.