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D-Tagatose production by permeabilized and immobilized *Lactobacillus plantarum* using whey permeate

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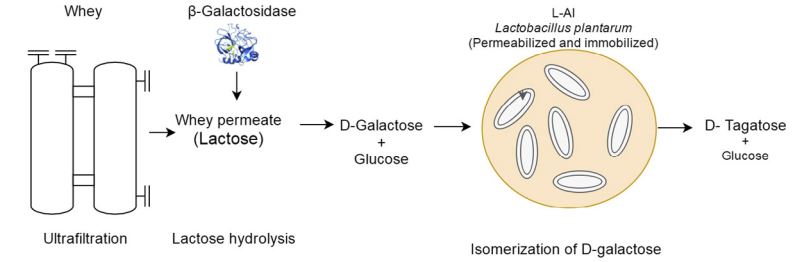
HIGHLIGHTS

- Whey permeate is a compatible source for two-step D-Tagatose production.
- Utilization of whey lactose for production of D-tagatose is proposed.
- β-Galactosidase is used to hydrolyze lactose for D-galactose production.
- Permeabilized and immobilized *Lactobacillus plantarum* is used as catalyst.

GRAPHICAL ABSTRACT



Production of D-Tagatose from whey permeate using permeabilized and immobilized *Lactobacillus plantarum*

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ABSTRACT

The aim of the work is to produce D-Tagatose by direct addition of alginate immobilized *Lactobacillus plantarum* cells to lactose hydrolysed whey permeate. The cells were untreated and immobilized (UIC), permeabilized and immobilized (PIC) and the relative activities were compared with purified L-arabinose isomerase (L-AI) for D-galactose isomerization. Successive lactose hydrolysis by β-galactosidase from *Escherichia coli* and D-galactose isomerization using L-AI from *Lactobacillus plantarum* was performed to investigate the in vivo production of D-tagatose in whey permeate. In whey permeate, maximum conversion of 38% and 33% (w/w) D-galactose isomerization by PIC and UIC has been obtained. 162 mg/g and 141 mg/g of D-tagatose production was recorded in a 48 h reaction time at 50 °C, pH 7.0 with 5 mM Mn^{2+} ion concentration in the initial substrate mixture.

1. Introduction

Global consumption of dairy products escalates with population intensification. About 75% of total production is destined to the generation of high value solid milk products in the dairy industries. Whey is one among the important dairy products that is used less than 5% to obtain other valuable derivates. In 2013, the world whey output was 180 million tonnes containing 1.5 million tonnes of high valuable protein and 8.6 million tonnes of lactose. Whey presents an excellent potential as important carbohydrate source and pre-cursor to prebiotics like galacto-oligosaccharides. Despite being high in carbohydrate (50 g/l) and protein content (10 g/l), whey is under-utilized as raw material in the production of food additives (Panesar and Kennedy, 2011; Patel and Murthy, 2012). Whey poses major disposal problems due to high organic content that demands as high as 40,000–50,000 ppm Biological Oxygen Demand (BOD) when disposed. Therefore, it is important to develop strategies to derive high valued products out of whey (Jin et al., 2016; You et al., 2017). The raw whey generated during cheese production can be subjected to ultrafiltration to obtain protein rich whey retentate and lactose rich whey permeate. Lactose is a major constituent of whey permeate obtained from dairy industries with adequate potential as D-Galactose feedstock.

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D-Galactose is an isomer of D-Tagatose that is extensively being researched as an alternate sweetener (J et al., 2016). D-Tagatose is a low-calorie rare sugar that has similar sweetening properties as sucrose but contains only 30% of the energy contents of sucrose (Fujimaru et al., 2012; Men et al., 2014). D-Tagatose is claimed to be an anti-hyperglycemiant due to its poor absorption and metabolization within the human body (Rhimi et al., 2011a). It is currently investigated as a flavor enhancer in food and in treatment of hemophilia, anemia, obesity and diabetes (Mendoza et al., 2005; Oh, 2007). This natural keto-hexose is proved to be a potential replacement to sucrose like high calorie bulk sweeteners as food additives (Kim, 2004). The compatibility of D-Tagatose in a wide range of food product and dietary supplements with rich health benefits demands immediate commercial bio-production of D-tagatose.

Conventional method of D-tagatose production from lactose involves two-step catalysis. The first involves application of β-galactosidases in breaking lactose to form a D-glucose and a D-galactose moiety (Freitas et al., 2011; Guerrero et al., 2017; Soupioni et al., 2013). The Galactose thus formed is isomerized to D-tagatose by L-arabinose isomerase in the latter step. L-arabinose isomerase is a non-commercially available enzyme with potential application in the production of L-ribulose and D-tagatose. Several attempts were being made to screen the best L-AI producing strains involving recombinant expression of L-AI genes in favorable hosts. Mesophilic L-AIs are often considered to give fair bio-conversion whereas studies on thermophilic L-AI proved better conversion (Choi et al., 2016; Kim et al., 2002; Kim and Oh, 2005). Contrastingly, the need for metallic cofactors to maintain enzyme stability at high temperatures was an inhibitory factory. Added to this, high temperature and harsh alkaline pH conditions introduced undesired products in the process that necessitated a complex down-stream strategy at the end (Grant and Bell, 2012; Rhimi et al., 2010). L-AIs that are independent of metallic cofactors and active at low temperatures are of recent attention. Multivariate analysis showed that under harsh conditions, use of borate can favour D-tagatose production (Salonen et al., 2013). Therefore, an efficient and economical strategy is yet to be developed for bulk production of D-Tagatose. Consequently, development of low-cost efficient bio-catalytic techniques employing L-AI for production of D-Tagatose from lactose is significant and necessary to make the most of whey permeate.

Cell permeabilization is carried out using different permeabilizing solutions. An appropriate permeabilizing agent highly permeabilizes the cells facilitating the transport of small molecular components across the cell wall. The macromolecules like proteins and intracellular organelles are retained within the cells allowing the cells to carry out normal metabolism. In this case, L-AI's retained within the cells are easily accessed by D-galactose for isomerization. This work investigates the L-AI activity of Lactic acid bacteria for producing D-Tagatose from Galactose in whey permeate. The lactose in whey permeate is utilized for D-galactose generation by direct addition of purified β-galactosidase. Permeabilized lactic acid bacterial whole cells were applied to carry out D-tagatose isomerization. In brief, in an attempt to make protein rich sweetening liquor from whey as an additive to food materials, whey permeate is investigated as a potential resource for bioconversion of D-tagatose using *Lactobacillus plantarum*.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Lactobacillus plantarum 1407 cells were obtained from MTCC, Chandigargh, India. The lyophilized cells were activated on 50 ml of de Man, Rogosa and Sharpe (MRS) broth with 2% glucose as carbon source initially. The culture is slowly allowed to utilize lactose as sole carbon source by adding 0.2 ml of culture to MRS medium containing 2% lactose and 0.25% L-arabinose as inducer for maximum L-AI activity of the cells (De Man et al., 1960). The cells were obtained from the late exponential phase by centrifugation at 8000 rpm for 10 min. The cells were washed with MOPS (3-(N-morpholino) propane sulfonic acid) buffer and stored at −80 °C for later use. Dry cell weight of the wet cells was obtained by establishing a calibration curve correlating the Optical density of cell culture at 600 nm to the corresponding dry weight of cells. The established correlation is used to attain the wet cells of equivalent dry weight for use in further experimentations.

2.2. Permeabilization of bacterial cells and immobilization

The frozen cells were thawed and suspended in permeabilizing solution at 4 °C for 10 min. About 1 g of cells were taken and treated with different concentrations of permeabilizing solution to investigate the effect of permeabilizing agent with enzyme activity. The influence of factors relating cell permeabilization was determined by varying the concentration of permeabilizing agent and time. The concentration of tween-20 and span-20 was varied from 2 to 10% (v/v) and the concentration of Trition-X-100 was ranging from 0.2 to 1% (v/v). CTAB concentration was varied by making 2–10% w/v solutions in deionized water. The permeabilized cells were washed thrice with 50 mM HEPES buffer (pH 8.5) containing 4-(2-hydroxyethyl)-1-piperazineethanesulfanoic acid to ensure food compatibility and biological stability of the cells.

The prepared permeabilized cells were immobilized using sodium alginate and calcium chloride. Lower the size of the beads, larger is the surface area and number of pores on the bead. 2% w/v sodium alginate and 0.5 M $CaCl_2$ was used to produce beads of good stability. Mixture containing permeabilized cells in 2% sodium alginate solution was dropped on calcium chloride solution with continuous stirring. Higher concentration of biomass in the alginate may lower the enzyme efficiency due to steric hindrance by other proteins (Anisha and Prema, 2008). The optimum cell loading for sodium alginate immobilization was investigated between 1 and 4% (w/v) in 2% sodium alginate and 0.5 M $CaCl_2$. The beads were left to stand on ice cold $CaCl_2$ for at least 8 h to ensure complete polymerization and enhanced physical stability. The beads were washed with distilled water and 50 mM phosphate buffer and stored by suspension in phosphate buffer at 4 °C. The untreated immobilized cells (UIC) and permeabilized-immobilized cells (PIC) were used for D-tagatose production. The L-AI activity of UIC and PIC were determined by performing standard enzyme assays.

2.3. L-AI activity of pure L-Arabinose isomerase (L-AI), untreated-immobilized cells (UIC) and permeabilized-immobilized cells (PIC)

To determine the effect of temperature on the galactose isomerization by L-AI, UIC and PIC, enzyme assays were carried out in 50 mM phosphate buffer at different temperatures and constant pH 7.0. The effect of pH on enzyme activity was determined by conducting enzyme assays at pH between 3 and 10 using 50 mM acetate buffer (pH 3.0–6.0), 50 mM phosphate buffer (pH 6.0–7.0) and 50 mM Tris-HCl (pH 7.0–10.0) at 50 °C. The effect of the concentration of Mn^{2+} ion was studied at 1 mM, 2 mM, 5 mM and 10 mM concentrations at pH 7.0 and 50 °C in 50 mM phosphate buffer. The relative enzyme activity is calculated by the ratio of initial enzyme activity (E_o) to the enzyme activity of treated test sample (E_t).

One unit of L-AI activity corresponds to the quantity of cells contributing to the formation of 1 μmol D-tagatose per minute. L-AI activities of immobilized whole cells were determined by measuring the amount of L-ribulose or D-tagatose produced. 100 mM