Available online at www.sciencedirect.com
ScienceDirect
Enzyme and Microbial Technology 40 (2007) 1531–1537
ENZYME AND MICROBIAL TECHNOLOGY
www.elsevier.com/locate/emt

Co-expression of L-arabinose isomerase and D-glucose isomerase in *E. coli* and development of an efficient process producing simultaneously D-tagatose and D-fructose

Moez Rhimi, Ezzedine Ben Messaoud, Mohamed Ali Borgi, Khalifa Ben khadra, Samir Bejar *

Laboratoire d'Enzymes et de Métabolites des Procaryotes, Centre de Biotechnologie de Sfax BP "K"3038 Sfax, Tunisie

Received 31 May 2006; received in revised form 25 October 2006; accepted 30 October 2006

Abstract

To develop a feasible enzymatic process for the concomitant D-tagatose and D-fructose production, the thermostable L-arabinose isomerase of *Bacillus stearothermophilus* US100 (L-AI US100) and the mutant D-glucose isomerase obtained from that of *Streptomyces* SK (SKGI-A103G) were successfully co-expressed in *Escherichia coli* HB101 strain. The recombinant cells were immobilized in alginate beads and showed, similarly to the free cells, optimal temperatures for D-galactose and D-glucose isomerisation of 80 and 85 °C, respectively. The two isomerases were optimally active at pH 7.5. Cell entrapment significantly enhanced the acidotolerance of the two isomerases, as well as their stability at high temperatures. To perform simultaneous isomerisation of D-galactose and D-glucose at 65 °C and pH 7.5 in packed-bed bioreactor, cells concentration, dilution rate, productivity and bioconversion rate were optimized to be 32 g/l, 2.6 h^{-1}, 3 g/l h and 30%, respectively.
© 2006 Elsevier Inc. All rights reserved.

Keywords: L-Arabinose isomerase; Glucose isomerase; Co-expression; Immobilization; D-Fructose; D-Tagatose

1. Introduction

The D-tagatose, an isomer of D-galactose, is a novel natural ketohexose having a taste and physical properties similar to sucrose [1]. It is also an anti-biofilm agent, which can be used as a cytoprotective supplement for the storage of organs to reduce the reperfusion injury [2,3]. Interestingly, D-tagatose is an anti-hyperglycemiant factor with a very low calorie carbohydrate and bulking agent [4–6]. It was the subject of recent interests in food and drug industries and was considered as safe and low calorie substrate in the United States [7].

The D-fructose ketohexose is twice as sweet as D-glucose playing an important role as a diabetic sweetener, according to its slow intestinal absorption. Therefore it does not influence the glucose level in blood [8]. D-fructose production cost, in HFCS using xylose isomerase commonly known as glucose isomerase (EC 5.3.1.5), was 10–20% lower than that of sucrose. Additionally, this natural sweetener is preferred in the food industry since it does not provoke the crystallisation problem as it is the case with sucrose [8].

The use of the D-tagatose was limited due to its high cost. Recently, an enzymatic procedure of D-tagatose production has been developed as an easy feasible and environmentally clean procedure. This process was based on the use of a combined immobilized β-glycosidase derived from *Sulfolobus solfataricus* and L-arabinose isomerase (EC 5.3.1.4) of *Thermoanaerobacter mathranii*, in the same reactor, allowing the direct conversion of lactose into D-tagatose and D-glucose [9]. In fact, lactoserum is considered as cheaper source for the D-galactose production at industrial scale due to its large abundance in the by-milk products such as: cheese and whey.

Actually, the D-tagatose production procedure from lactoserum leads to a mixture of D-tagatose and D-glucose followed by a separation step of D-tagatose from D-glucose. Alternatively, it will be more profitable to convert the residual D-glucose into D-fructose using the xylose isomerase, which is a commercial enzyme with high relevance, having the ability to convert D-glucose into D-fructose [10,11]. Therefore, the co-expression

Abbreviations: B, *Bacillus*; L-AI, L-arabinose isomerase; GI, glucose isomerase; HPLC, high performance liquid chromatography; HFCS, high fructose corn syrup

* Corresponding author. Tel.: +216 74 44 04 51; fax: +216 74 44 04 51.
E-mail address: Samir.bejar@cbs.rnrt.tn (S. Bejar).

0141-0229/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2006.10.032

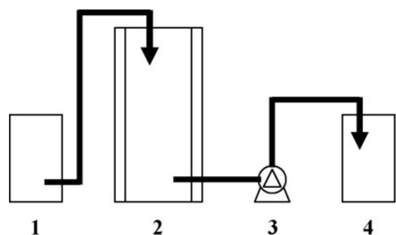

Fig. 1. Schematic diagram of enzymatic tagatose and fructose production process: (1), feed tank containing substrates solution; (2), thermostated column reactor containing the alginate beads; (3), peristaltic pump sucking up the fluid through the column; (4) products recuperation tank.

of these two isomerases offers an excellent opportunity toward innovative aspect for the concomitant production of D-fructose and D-tagatose (Fig. 1).

In a previous work, we have reported the gene cloning and the characterization of the L-arabinose isomerase extracted from *Bacillus stearothermophilus* US100 strain (LAI-US100). This thermoactive metallic ions independent enzyme had an optimal activity at 80 °C and pH 7.5 [12]. In addition to the study of the structure-function relationship of the glucose isomerase (SKGI) extracted from thermophile *Streptomyces* SK strain, we have obtained the SKGI-A103G mutant enzyme [13]. The SKGI-A103G showed approximately the same biochemical characteristics, compared to the LAI-US100, with a maximal activity at pH 7.5 and 85 °C [12,13].

The present paper concerns the construction of a recombinant *Escherichia coli* strain, efficiently co-expressing the L-AI US100 and the SKGI-A103G genes. A comparative study between free and immobilized cells was investigated, as well as the optimization of operating conditions for the production of D-tagatose and D-fructose, using immobilized cells in packed-bed bioreactor.

2. Materials and methods

2.1. Bacterial strains, plasmids and media

E. coli HB101 (F^- *hsdS20 ara-1 recA13 proA12 lacY1 galK2 rpsL20 mtl-1 xyl-5*) was used in this work as host strain. Culture of *E. coli* strains was done in Luria Bertani (LB) medium [14]. McConkey agar medium from Sigma (Steinheim, Germany) was used for the identification of colonies having L-AI and GI activities [15]. These media were supplemented, when necessary, with ampicillin (100 μg/ml) and IPTG (isopropyl-β-D-thiogalactopyranoside) at 160 μg/ml. pBMA5 is the plasmid harboring the *xylA*-A103G gene encoding the mutated glucose isomerase SKGI-A103G under the control of its own promoter [13]. pMR5 contains the encoding gene of L-AI US100 downstream of the P*lac* promoter [12]. pMR20 construction, carrying both genes encoding the L-AI US100 and SKGI-A103G is described in this work.

2.2. DNA manipulation and co-expression procedures

Plasmid DNA preparation, digestion with restriction endonucleases and separation of fragments by agarose gel electrophoresis were performed as described by Sambrook et al. [14]. The DNA purification was carried out using the GFX™ PCR DNA and Gel Band Purification Kit (Amersham Bioscience), on the basis of the manufacturer's instructions.

In order to co-express the two enzymes, the pMR5 plasmid was digested by *Sal*I and *BamH*I located downstream of the L-AI US100 encoding gene. Taking advantage of the compatibility of *Sal*I and *Xho*I restriction enzymes, the *Xho*I-*BamH*I DNA fragment, harboring the *xylA*-A103G gene from pBMA5, was cloned into the linearized pMR5/*Sal*I/*BamH*I plasmid. This leads to pMR20 plasmid, containing the *araA* US100 gene under the control of the inducible P*lac* promoter and the *xylA*-A103G gene downstream of its own constitutive promoter. *E. coli* HB101 strain was transformed by pMR20 plasmid and recombinant cells were selected on McConkey agar plates.

2.3. Cell crude extract preparation

E. coli HB101/pMR20 strain was grown in LB medium with ampicillin (100 μg/ml) and IPTG (160 μg/ml). Cells were harvested by centrifugation (7500 × *g*, for 10 min) and the pellets were suspended in 100 mM 3-[*N*-morpholino] propanesulfonic acid (MOPS) buffer (pH 7.5) supplemented, where indicated, with 1 mM $MnSO_4$ and 0.2 mM $CoCl_2$. Then, cells suspension was incubated for 1 h on ice in presence of 5 mg/ml lysozyme, 100 mM PMSF (phenylmethane-sulfonyl fluoride) and 2 μg/ml pepstatin A. Cell disruption was carried out by sonication at 4 °C for 6 min (pulsations of 3 s, amplify 90) using a Vibra-Cell™ 72405 Sonicator and cell debris were removed by centrifugation (30,000 × *g*, for 30 min at 4 °C). The obtained supernatant constitutes the cytoplasmic crude enzyme extract.

2.4. Preparation of cells and immobilization method

The immobilization was carried out by mixture of 10 ml of cells with 10 ml of 6.0% (w/v) sodium alginate (Fluka). Then, the obtained mixture was dropped slowly within a cooled and stirred 200 mM barium chloride ($BaCl_2$) solution (200 ml). Integrity of beads was improved by renewing the $BaCl_2$ bath for an overnight incubation at 4 °C. Then, beads were washed twice in 100 mM MOPS Buffer (pH 7.5) at 4 °C for 1 h. The average bead size was approximately 0.7 mm.

2.5. Activities assays

In standard conditions, free L-arabinose isomerase activity was measured by the determination of the amount of formed D-tagatose. The reaction mixture contained 50 μl of the crude enzyme preparation at a suitable dilution and 10 g/l of D-galactose in a final volume of 1 ml MOPS buffer 100 mM (pH 7.5). The reaction mixture was incubated at 80 °C throughout 10 min. The free D-glucose isomerase activity was assayed in the same MOPS buffer using 10 g/l D-glucose as substrate and the reaction was incubated during 30 min at 85 °C in presence of 5 mM Mg^{2+}.

Activities in immobilized and free cell preparations were determined in 100 mg of alginate beads or 50 mg of cells, respectively suspended in 100 mM MOPS (pH 7.5). Isomerization was performed in 2 ml reaction mixture under the same assay conditions described above during 30 min.

Samples were then cooled in ice to stop the reactions. The generated ketoses (D-tagatose or D-fructose) were determined by cysteine carbazole sulfuric-acid method, and the absorbance was measured at 560 nm [16].

One unit of glucose isomerase or L-arabinose isomerase activity is defined as the amount of enzyme needed to produce 1 μmol of D-fructose or D-tagatose, respectively, per min under the assay conditions.

In the case of immobilized cells in packed-bed bioreactor, the D-glucose and D-galactose are prepared in the same feed solution. The total produced ketoses (D-tagatose and D-fructose) were determined by means of the cysteine carbazole sulfuric-acid method. The amount of D-fructose was deduced by measuring the residual D-glucose, after isomerisation, by glucose-oxidase (GOD-PAP, kit Biomaghreb). Taking account of the known formed D-fructose quantity, and the total generated ketoses, the amount of the produced D-tagatose was then determined. Conversion rate represent the percentage ratio between the formed ketose concentration obtained at stationary functioning and the feed substrates concentration.

2.6. Cell concentration, temperature, pH and metallic ions effects

To optimize the operating conditions, the effect of cell concentrations, temperature, pH, and metal ion effects were investigated. The cells concentration inside the alginate capsules were varied between 20 and 50 g/l and the GI and L-AI activities were measured. Thermoactivity profiles were studied using free and immobilized cells at temperatures comprised between 50 and 90 °C. pH

Download English Version:

https://daneshyari.com/en/article/18243

Download Persian Version:

https://daneshyari.com/article/18243

Daneshyari.com