Microbiological Research **165** (2010) 384—391

Available online at www.sciencedirect.com

ScienceDirect

Microbiological Research

www.elsevier.de/micres

Purification and characterization of a novel glucansucrase from *Leuconostoc lactis* EG001

Yong-Mo Kim[a], Min Ji Yeon[a], Nack-Shick Choi[a], Young-Hyo Chang[b], Min Young Jung[b], Jae Jun Song[a], Joong Su Kim[a,*]

[a]*Enzyme Fusion Technology Research Team, Molecular Bioprocess Research Center, Jeonbuk Branch Institute, Korea Research Institute of Bioscience and Biotechnology, Jeonbuk 580-185, Republic of Korea*
[b]*Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, Yuseong, Daejeon 305-806, Republic of Korea*

Received 13 July 2009; received in revised form 20 August 2009; accepted 23 August 2009

KEYWORDS
Glucansucrase;
Glycosylation;
L-ascorbic acid;
L-ascorbic acid 2-glucoside

Summary

A gene encoding glucansucrase was identified in *Leuconostoc lactis* EG001 isolated from lactic acid bacteria (LAB) in Kimchi, a traditional Korean fermented food. The *L. lactis* EG001 glucansucrase gene consists of 4503 bp open reading frame (ORF) and encodes an enzyme of 1500 amino acids with an apparent molecular mass of 165 kDa. The deduced amino-acid sequence showed the highest amino-acid sequence identity (75%) to that of dextransucrase of *L. mesenteroides*. The gene was cloned and over-expressed in *Escherichia coli* strain. The recombinant enzyme was purified via Ni-NTA affinity chromatography and its enzymatic properties were characterized. The enzyme exhibited optimum activity at 30 °C and pH 5.0. In addition, the enzyme was able to catalyze the glycosylation of L-ascorbic acid to L-ascorbic acid 2-glucoside. The glycosylated product via EG001 glucansucrase has the potential as an antioxidant in industrial applications.

Introduction

Glycosylation has a crucial role in the biosynthesis of glucosides (Ko et al. 2006). The glycosylation reaction enhances the water solubility, molecular stability and biological activity of sugar acceptors. A variety of glycosylated products have been exploited as bioactive compounds such as antioxidants, antibiotics, and anticancer agents (Kim et al. 2007). L-ascorbic acid (L-AsA) is used in many industrial applications including pharmaceutical preparations, animal feeds, food preservatives, and cosmetic products (Hancock and Viola 2002; Lee et al. 2004; Kyotani et al. 2009). Although,

*Corresponding author. Tel.: +82 63 570 5130; fax: +82 63 570 5109.
E-mail address: joongsu@kribb.re.kr (J.S. Kim)

0944-5013/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.micres.2009.08.005

L-AsA is unstable under oxidative and thermal conditions, it functions well as an antioxidant in industrial applications (Nakamura and Oku 2009). To overcome the instability of L-AsA, researchers have investigated the effects and preparation of glycosyl derivatives of L-AsA (Lee et al. 2004). L-ascorbic acid 2-glucoside (AA-2G), a L-AsA derivative with increased stability, has been enzymatically synthesized by glycosylation for use in numerous applications (Kwon et al. 2007). AA-2G can be synthesized from an acceptor substrate (such as L-AsA) and a donor substrate (such as maltose, sucrose or other α-glucans) by glycosylation with mammalian and rice seed α-glucosidases (Yamamoto et al. 1990), with *Bacillus stearothermophilus* cyclodextrin glucanotransferase (CGTase) (Tanaka et al. 1991), and with *Bifidobacterium longum* sucrose phosphorylase (Kwon et al. 2007).

Glucansucrases (EC 2.4.1.5) can generally catalyze the synthesis of glucans from sucrose and several different reactions according to acceptor substrates (Kang et al. 2009). They catalyze the hydrolysis of glucan by transferring the glucose moiety when water is used as an acceptor. They can also synthesize glucans by glycosyl transfer of the glucose moiety to the growing glucan chains and synthesize oligosaccharide by glycosylation of glucose moiety to other saccharides called acceptor reaction (Meulenbeld and Hartmans 2000).

In this report, we identified a novel glucansucrase in *Leuconostoc lactis* EG001 isolated from lactic-acid bacteria (LAB) in Kimchi, a fermented food. The EG001 glucansucrase was characterized and the ability of glucansucrase to glycosylate from sucrose to glycosyl L-ascorbic acid was investigated.

Materials and methods

Isolation and screening of microorganism

L. lactis EG001 isolated from a mixed culture of lactic-acid bacteria (LAB) found in Kimchi, a traditional Korean fermented food. The LAB was cultured in MRS medium (BD, USA) containing 20 g/L sucrose at 30 °C. The strain was subjected to biochemical and 16S rRNA gene sequences analyses (Chang et al. 2008). Fingerprinting of biochemical characteristics were performed using API 20E and 50CHL galleries (bioMérieux, France) according to the manufacturers' instructions. The reference strains used in the study were *L. mesenteroides* subsp. *mesenteroides* ATCC 8293 (equivalent to NRRL B-1118 and KCTC 3718) and *L. mesenteroides* NRRL B-512F (equivalent to KCTC 3719). The glucans produced by the LAB were assessed using the phenol-sulfuric acid method (Dubiois et al. 1956; Meulenbeld and Hartmans 2000). Briefly, 1 mL of ethanol (75% v/v) was added to 100 μL of culture supernatants. The mixture was precipitated and the pellet was dissolved in 100 μL of deionized water. The sample was mixed with 100 μL phenol (5% v/v) and incubated for 30 s at room temperature. The sample was then incubated at 80 °C for 30 min and cooled. After incubation, the resulting glucan was determined by measuring the absorbance at 490 nm using an Ultrospec 3100-Pro spectrophotometer (Amersham Biosciences, USA).

Identification and cloning of the glucansucrase gene

A partial glucansucrase gene was obtained by PCR amplification of genomic DNA from strain EG001 using degenerate primers based on the conserved amino-acid sequences of glucosyltransferases from several LABs (Kralj et al. 2003). The degenerate primers for PCR were 5′-GAYAAYWSNAAYCCNRYNGTNC-3′ (forward), 5′-ADRTCNCCRTARTANAVNYKNG-3′ (reverse). In degenerate bases, Y=C or T (pyrimidine), R=A or G (purine), W=A or T (weak, 2H-bonds), S=G or C (strong, 3H-bonds), D=A, T or G (not C), V=A, G or C (not T), K=G, T (keto), N=A, T, G or C. PCR reactions were performed in 20 μL mixtures containing 100 ng genomic DNA, 10 × PCR buffer, 10 mM dNTP, 5 μM of each primer, and 1 U EF-*Taq* DNA polymerase (Solgent, South Korea). After an initial denaturation at 95 °C for 3 min, the PCR condition was performed with 30 cycles of denaturation at 95 °C for 30 s, annealing at 42 °C for 45 s, extension at 72 °C for 1 min, and final extension was carried out at 72 °C for 10 min. PCR products were cloned into the pGEM T-easy vector (Promega) and sequenced.

Inverse PCR was used to amplify the glucansucrase gene, which encodes the entire glucansucrase protein. Genomic DNA from strain EG001 was digested using various restriction enzymes and circularized by self-ligation. Primers were designed from the partial nucleotide sequence of the glucansucrase gene. The oligonucleotide primers for first PCR were 5′-CACGATAGTGAAGTGCAAACG-3′ (forward) and 5′-ATCGTTGGCAGTAATCGAGC-3′ (reverse). Primers for secondary PCR were 5′ATAGCTTAGCACCAACAACAGAAC-3′ (forward) and 5′-GTTTCGCTGGCCTTGTTCA-3′ (reverse). PCR reactions were performed in 50 μL volumes with reaction mixtures containing 25 ng of circularized