Available online at www.sciencedirect.com
SCIENCE DIRECT
Gene 376 (2006) 59–67
GENE
SECTION
FUNCTIONAL GENOMICS
www.elsevier.com/locate/gene

Identification and functional characterization of *levS*, a gene encoding for a levansucrase from *Leuconostoc mesenteroides* NRRL B-512 F

Sandra Morales-Arrieta, Maria Elena Rodríguez, Lorenzo Segovia, Agustín López-Munguía, Clarita Olvera-Carranza *

Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, UNAM, Avenida Universidad 2001, Col. Chamilpa, Cuernavaca, Morelos 62250, México

Received 4 October 2005; received in revised form 8 February 2006; accepted 8 February 2006
Available online 24 April 2006
Received by I.K. Jordan

Abstract

A *Leuconostoc mesenteroides* NRRL B-512 F levansucrase gene, (*levS*), was isolated, sequenced and cloned in *Escherichia coli*. The recombinant enzyme was shown to be a fructosyltransferase producing a polymer identified by ^{13}C-NMR as levan. Based on sequence analysis, we found that this levansucrase is a mosaic protein, bearing structural features of glucosyltransferases in the amino and carboxy terminal regions similarly to inulosucrase from *Leuconostoc citreum*. The phylogenetic analysis of the C-terminal region domain of levansucrases from *L. mesenteroides* demonstrates that they group together into a novel putative sub-family of genes and evolved long before all other glucosyltransferases, while their catalytic domain structure is species related.

Keywords: Fructosyltransferase; Levansucrase; Levan; Glucan binding domain; Natural quimeric enzyme; Mosaic protein

1. Introduction

Glycosyltransferases (EC 2.4.) are enzymes that catalyze the transfer of glycosyl residues from a donor molecule to a particular acceptor (Monsan and Paul, 1995). Among them, fructosyltransferases (FTFs) synthesize fructose polymers from sucrose with release of glucose, in a similar way to glucosyltransferases (GTFs) that synthesize glucose polymers from sucrose with release of fructose; both reactions are carried out without the need for cofactors, as the energy required for the reaction is provided by the hydrolysis of the sucrose glycosidic linkage (Funane et al., 1993; Pérez et al., 1996). Up to now, little is known concerning the enzyme structural features responsible for the specificity of the glycosidic linkage in the product, which varies among GTFs. In particular, levansucrase (LS) and inulosucrase (IS) are FTFs that synthesize fructose polymers linked in their main chain by β2-6 and β2-1 bonds respectively, while dextransucrase, alternansucrase, mutansucrase and amylosucrase are GTFs synthesizing polymers with α (1–6), alternated α (1–6) and α (1–3), linear α (1–3) and α (1–4) linkages respectively (Monsan et al., 2001). Although FTFs and GTFs catalyze analogous reactions, they have important biochemical differences, as not only they proceed through a different reaction mechanism, but also their catalytic domains differ completely in structure.

Fructosyltransferases have a molecular weight ranging from 45 to 64 kDa, except for the enzymes from *Lactobacillus reuteri* and *Streptococcus salivarius*, which are larger: 87 and 140 kDa respectively (Jacques, 1993; van Hijum et al., 2002). Bacterial FTFs have a β propeller fold, and have been classified in family 68 of glycoside hydrolases (Pons et al., 2000). Recently, *Bacillus subtilis* levansucrase structure was reported as a single-domain enzyme which folds as a five-bladed β-propeller enclosing a funnel-like central cavity. The crystallographic data shows that D86 and E342 are in contact with sucrose, representing the catalytic nucleophile and the general acid/base residues,

Abbreviations: NMR, Nuclear Magnetic Resonance; FTF, fructosyltransferase; GTF, Glucosyltransferase; LS, Levansucrase; IS, Inulosucrase; aa, Amino Acid; GBD, Glucan binding Domain.

* Corresponding author. Tel.: +52 777 3291609; fax: +52 777 3172388.
E-mail address: clarita@ibt.unam.mx (C. Olvera-Carranza).

0378-1119/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2006.02.007

respectively, while residue D247 was identified as a transition state stabilizer (Meng and Futterer, 2003).

Glucosyltransferases belong to family 70 of glycoside hydrolases and contain in average 1500 amino acids (aa) with a molecular weight of around 170 kDa. Three different domains have been identified in all GTFs: an ~200 aa variable zone with no apparent function is found in the N-terminal region, followed by the catalytic domain, which has the secondary structure of a α/β barrel; finally, a glucan binding domain (GBD) that binds the glucan product is found in the C-terminal region. The GBD is formed by a series of 30 to 50 aa repeat sequences (Monchois et al., 1999).

GTFs are produced mainly by *Leuconostoc mesenteroides*, *Lactobacillus* and oral *Streptococcus* species (Mooser, 1992). Among all the glycosyltransferases producers, only strains from *Streptococcus*, *Lactobacillus* and *Leuconostoc* genera produce both GTFs and FTFs. Among these microorganisms, the enzymes from *Streptococcus* have received particular attention because of their role in dental caries where both glucans and fructans are involved (Hamada and Slade, 1980). In *L. mesenteroides*, only GTFs have been extensively studied due to their application in the production of industrial dextran. However, since 1979 it has been reported that levansucrase is also present in dextransucrase preparations obtained from the industrial strain *L. mesenteroides* B-512 F (Robyt and Walseth, 1979). In a later report, Miller and Robyt (1986) described two FTFs of 116 and 92 kDa but due to their low activity, their properties have not been studied. Recently, Kang et al. (2005), have reported the isolation of a *L. mesenteroides* B-512 FMC levansucrase gene (*m1ft*). This gene codes for a protein of 424 amino acid residues with a calculated molecular mass of 47.1 kDa. However, as will be discussed later the gene sequence has higher identity with Gram (−) bacteria than with all other levansucrases from Gram (+) bacteria, including those of *Leuconostoc* spp.

In the present study we report the isolation and molecular characterization of a gene (*levS*) encoding for a levansucrase from *L. mesenteroides* B-512 F, as well as the production and the functional characterization of the enzyme. The nucleotide and the predicted amino acid sequence analysis allowed us to conclude that this is a natural chimeric enzyme with a similar construction to inulosucrase, a FTF from *Leuconostoc citreum* (Olivares et al., 2003). Surprisingly, these three domain constructions resemble those found in GTFs with similarities in their N-terminal variable region and the C-terminal GBD, but differing in their catalytic region. Furthermore, this structural scheme was also found in three putative FTFs identified in the *L. mesenteroides* ATCC 8293 genome. Some features concerning these chimeric FTFs are discussed, particularly their catalytic and GBD phylogenetic relationships.

2. Materials and methods

2.1. Bacterial strains, media and growth conditions

The *L. mesenteroides* bacterial strain NRRL B-512 F was cultivated in a New Brunswick incubator shaker at 30 °C and 200 rpm with 20 g/l of sucrose (Lopez and Monsan, 1980). Glucose was used as the carbon source to avoid polymer synthesis that may interfere with DNA extraction. *Escherichia coli* DH5α was used as host for cloning purposes and was grown at 37 °C and 250 rpm in Luria–Bertani (LB) medium supplemented with 50 μg/ml of kanamycin in order to maintain plasmid integrity. Agar plates were made by adding 1.5% agar to LB medium.

2.2. General molecular techniques

L. mesenteroides B-512 F total DNA was extracted from cells with the Ultra clean microbial DNA isolation kit (MO BIO Laboratories). *E. coli* plasmid DNA was isolated with High Pure Plasmid Isolation kit (Roche Diagnostics GmbH). Restriction and DNA-modifying enzymes were purchased from New England Biolabs (Beverly, Mass) and Roche Biochemical (Basel, Switzerland). DNA was amplified by the PCR technique on a DNA Robocycler Gradient 96 (Stratagene, La Jolla, Cal.) using Taq DNA polymerase (Roche Biochemical, Basel, Switzerland or Invitrogen, Carlsbad, CA.); the *levS* gene was amplified with Vent-pol (New England Biolabs). *E. coli* transformations were performed by electroporation in 0.2 mm cuvettes with the Bio-Rad Micropulser equipment (Bio-Rad laboratories, Hercules CA.) with 2.5 kV, 25 μF and 200 Ω. DNA fragments were isolated from agarose gels using a Qiagen gel extraction kit (Qiagen, Inc., Chatsworth, CA). In all cases the biological reagents were applied following the instructions provided by the supplier.

For hybridization, colony DNA was transferred to a Hybond-N+ membrane (Amersham Pharmacia Biotech, Buckinghamshire, England). The probes were labeled with ^{32}P with a Rediprime Kit (Amersham Pharmacia Biotech, Buckinghamshire, England). Hybridizations were performed under both, stringent and non stringent conditions at 65 °C.

Genomic DNA from *L. mesenteroides* B-512 F was extracted and fragmented with a stream of nitrogen for 40 s. The fragmented DNA was blunt ended with T4 DNA polymerase and Klenow fragment. Fragments were size fractionated by agarose gel electrophoresis, and those in the range of 2–5 kbp recovered and cloned using the *Eco*RV site of the pZErO™-2 vector (Invitrogen, Carlsbad, CA.).

The primers used for the isolation of the *levS* gene are *Pep1*-(GCWGATAATATTGCWWSWTTRAATCCAGAT), *Pep2*-(WGTACCTTGWGCYAAAACWGTATCYAAAAC), *1200C*-(AATAAGTGCTGATGGTAGAGC), *43R*-(ACGTAAGTAATATGTGCCAT CATTACC), *Intergenica*-(GTTAACGCCATCATGATTGCG), *Inicio*-(CACCCCATG GAGAAAAAAGTTAT), where R = A or G; W = A or T; S = C or G and Y = C or T. The fragment containing the whole gene was cloned in the pBAD/D-TOPO ThioFusion vector in *E. coli* strain DH5α (Invitrogen, Calsbad, CA).

2.3. Preparation of E. coli cell extracts and enzyme activity assay

E. coli DH5α cells harbouring the recombinant construction were grown until a 0.5 OD_{600} was reached. Afterwards 0.02%