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Characterization of glucansucrase and dextran from *Weissella* sp. TN610 with potential as safe food additives

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ABSTRACT

Pear-derived *Weissella* sp. TN610 produced extracellular glycosyltransferase activity responsible for the synthesis of soluble exopolysaccharide from sucrose. Acid and dextranase-catalyzed hydrolysis revealed that the synthesized polymer was a glucan. According to ^{1}H and ^{13}C NMR analysis, the glucan produced by TN610 was a linear dextran made of 96% α-(1→6) and 4% α-(1→3) linkages. Zymogram analysis confirmed the presence of a unique glucansucrase of approximately 180 kDa in the cell-free supernatant from TN610. The crude enzyme, optimally active at 37 °C and pH 5, has promising potential for application as a food additive since it catalyzes dextran synthesis in sucrose-supplemented milk, allowing its solidification. A 4257-bp product corresponding to the mature glucansucrase gene was amplified by PCR from TN610. It encoded a polypeptide of 1418 residues having a calculated molecular mass of 156.089 kDa and exhibiting 96% and 95% identity with glucansucrases from *Lactobacillus fermentum* Kg3 and *Weissella cibaria* CMU, respectively.

1. Introduction

Lactic acid bacteria (LAB) produce a wide variety of structurally different exopolysaccharides (EPSs). These include homopolysaccharides that consist of only one monosaccharide, such as glucose (glucans), fructose (fructans) or galactose (galactans), and heteropolysaccharides formed of repeating units of different monosaccharides, including glucose, galactose, fructose, and rhamnose [1,2]. LAB are generally recognized as safe for consumption [3]. EPSs from LAB are commonly used in the food industry to improve the textural properties of a wide variety of food products, including fermented dairy products and baked goods [4,5]. Some of these EPSs have also been reported to be beneficial to human health due to their antitumoral, immunomodulatory, cholesterol-lowering, and biofilm-formation-inhibiting activities [6].

Glucansucrases have been classified as members of the GH70 family of glycoside hydrolases because of similarity to GH13 α-amylases and GH77 amylomaltases [7]. They are relatively large extracellular enzymes mainly produced by LAB strains belonging to *Leuconostoc*, *Streptococcus*, *Lactobacillus*, and *Weissella* species. GS use sucrose to naturally catalyze the synthesis of a variety of α-D-glucans. These include dextrans (mainly produced by GS from *Leuconostoc* and *Weissella* strains) which are characterized by a linear backbone containing α-(1→6) linkages, or predominant α-(1→6) linkages together with α-(1→3), α-(1→2), and/or α-(1→4)-linked branches [8,10–13]. They also include mutans (mainly found in *Streptococcus* strains) that have more than 50% of α-(1→3) linkages in the linear backbone with α-(1→6)-liked branches [14]. The α-D-glucans also comprise alternans (reported only in *Leuconostoc* strains) that contain alternating α-(1→3) and α-(1→6) linkages with α-(1→3)-linked branches [11,15]. Last but not least, they encompass reuterans (produced only by *Lactobacillus reuteri*) with α-(1→4) linkages and some α-(1→6)-linked branches [2,16].

In addition to the synthesis of glucans from sucrose, GS can transfer α-D-glucopyranosyl moiety from the donor (sucrose) onto

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the hydroxyl group of carbohydrate molecules, or onto non-carbohydrate hydroxylated compounds (the acceptor reaction), which leads to the formation of low molecular weight oligosaccharides and glycoconjugates with increased solubility and biological activity, respectively [17,18].

GS genes have been cloned mainly from LAB strains belonging to the *Leuconostoc*, *Lactobacillus*, *Streptococcus*, and *Weissella* genera [10,19–22]. They encode large proteins with approximately 1400–1800 amino acid residues [16]. GS are believed to share a common architecture adopting a permutated $(\beta/\alpha)_8$-barrel [23] containing the catalytic site, and displaying a highly conserved N-terminal domain and a C-terminal glucan-binding domain composed of a series of tandem repeats [24]. The GS from *L. reuteri* 180 is the sole GH70 GS for which a crystal structure has been obtained [25]. It presents a surprising U-shaped 3D-architecture with five distinct structural domains: (i) domain A which is the catalytic domain. It contains a "circularly permuted" $(\beta/\alpha)_8$-barrel; (ii) domain B which is inserted between helix α1 and strand β8 of the $(\beta/\alpha)_8$-barrel and comprising many amino acid residues implied in the substrate donor and acceptor binding sites; (iii) domain C forms the bottom of the "U". Even though most α-amylase superfamily enzymes have this domain its precise function is unclear; (iv) domain IV positioned between domains B and V and with no structural similarity to other proteins; and (v) domain V which contains many sequence repeats of ∼20 amino acid long that have been proposed to be involved in glucan binding. The three conserved catalytic residues [D530, putative catalytic nucleophile; E568, putative acid/base catalyst; and D641, putative transition-state stabilizer (in mature DSRBCB4 of *Leuconostoc mesenteroides* B-1299CB4 numbering)] present in the catalytic core of GS enzymes have been demonstrated to be essential for the enzymatic activity [8]. Due to their similarity to members of the GH13 and GH77 glycoside hydrolase families, GS have been proposed to use an α-retaining double displacement mechanism. In the first step, the α-D-Glc*p*-(1→2)-β-D-Fru*f*-glycosidic bond linking the glucose and fructose moieties of sucrose is cleaved, fructose is released, and a glucosyl-enzyme intermediate wherein the glucosyl unit is covalently attached to the catalytic nucleophile *via* a β-glycosidic linkage is formed. The glucosyl moiety is then transferred to the accepting non-reducing end sugar of a glucan chain under polymerization, with restoration of the α-glycosidic bond [25].

Despite their promising properties and attributes, only a few glucansucrase enzymes have so far been cloned and characterized from stains belonging to the genus *Weissella* [10,12]. Accordingly, the present study was undertaken to characterize the extracellular glucansucrase activity from a pear-derived *Weissella* sp. TN610 strain. It aimed to investigate and determine the molecular mass and optimal catalytic conditions of the crude enzyme, as well as the nature of the linkages composing the glucan it produces and its potential application as an additive for the solidification of sucrose-supplemented milk. This paper also reports on the cloning and sequencing of the gene encoding the mature GS from TN610 as well as the comparison of the deduced amino acid sequence with other LAB glucansucrases.

2. Materials and methods

2.1. Screening of LAB isolates for exopolysaccharides production from sucrose

Fifty four lactobacilli, previously isolated from various niches and investigated for antibacterial activities as well as probiotic properties [26,27], were screened in this study for their ability to produce exopolysaccharides (EPSs) from sucrose. The samples were plated on De Man, Rogosa and Sharpe (MRS) agar media (pH 6.5) containing 4% (w/v) sucrose (instead of 2% (w/v) glucose), 10 g/l tryptone, 10 g/l meat extract, 5 g/l yeast extract, 5 g/l sodium acetate, 2 g/l di-sodium phosphate, 2 g/l tri-ammonium citrate, 0.1 g/l $MgSO_4$, and 0.05 g/l $MnSO_4$. After incubation for 24–48 h at 30 °C and under anaerobic conditions, the plates were checked for the presence of colonies displaying a mucoïd phenotype. The isolates containing mucoïd colonies were further screened for extracellular glycosyltransferase activity in liquid MRS sucrose (MRS with 4% (w/v) sucrose instead of 2% (w/v) glucose; pH adjusted to 6.5 with 5 M sterile NaOH) media (MRS-S) as described by Bounaix et al. [15]. In brief, for each strain, a 100 ml culture of MRS-S was inoculated at a final OD_{600} of 0.3 with a 20 h-old preculture grown on MRS broth at 30 °C without shaking, and then incubated on a rotary shaker (100 rpm) at 25 °C until pH 5. The pH was then adjusted to 5.4 using 5 M sterile NaOH. Finally, the culture was centrifuged at 4 °C (10,000 rpm, 20 min), and the supernatant fraction was assayed in triplicate for glycosyltransferase activity using the dinitrosalicylic acid (DNS) method. One glycosyltransferase unit (U) was defined as the amount of enzyme capable of releasing 1 μmol fructose and/or glucose/min from 292 mM sucrose at 30 °C in 20 mM sodium acetate buffer (pH 5.4).

2.2. DNA isolation and manipulation

The total DNA from the *Weissella* sp. TN610 strain was prepared according to Serror et al. [28]. General molecular biology techniques were performed as described by Sambrook et al. [29]. PCR amplifications were performed using primers either from PROLIGO (Paris, France) or Eurogentec (Serain, Belgium). They were carried out using Phusion high-fidelity DNA polymerase from New England Biolabs or DAp Goldstar® DNA polymerase from Eurogentec. The amplified fragments were purified with the Wizard SV Gel and PCR Clean-Up system (Promega) and then submitted to nucleotide sequencing using an automated 3100 Genetic Analyzer (Applied Biosystems). The data from DNA sequencing was then analyzed using the Basic Local Alignment Search Tool BlastN.

2.3. Taxonomic identification of the selected strain

PCR amplification was performed on the 16S rRNA gene from the TN610 selected strain using primers specific for *Weissella* genus: weifor (5′-CGTGGGAAACCTACCTCTTA-3′) and weirev (5′-CCCTCAAACATCTAGCAC-3′) [30]. PCR conditions were an initial denaturation step (5 min at 94 °C) followed by 30 cycles of 30 s at 94 °C, 30 s at 61 °C, 1 min at 72 °C, and a final additional extension step of 7 min at 72 °C. Amplification was carried out using DAp Goldstar® DNA polymerase from Eurogentec. PCR amplifications conducted on the 16S rRNA gene from *Weissella cibaria* LBAE-K39, *Lactobacillus plantarum* DSM 20174, and *Leuconostoc citreum* NRRL B-1355 with the same primers and under the same PCR conditions were used as controls.

2.4. In situ detection of active glycosyltransferase from Weissella sp. TN610

The supernatant fraction from the *Weissella* sp. TN610 culture was submitted to SDS-PAGE analysis. *In situ* detection of active glycosyltransferase was performed as described in Bounaix et al. [12]. In brief, 30 μl of the sample (approximately 30 mU of glycosyltransferase as measured by the DNS assay) were mixed with 10 μl of NuPAGE® LDS Sample Buffer 4× (Invitrogen, France) and incubated at 70 °C for 10 min to reversibly denature the enzyme. Electrophoresis was carried out on NuPAGE® 3–8% Tris–acetate mini gel (Invitrogen, France) at room temperature and a constant voltage of 150 V for 1 h. After migration, the resolved proteins were stained with a Colloidal Blue Staining kit (Invitrogen, France). To