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Characterization of glucan-producing *Leuconostoc* strains isolated from sourdough

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ABSTRACT

Sourdough was previously demonstrated to be a fruitful biotope for isolation of lactic acid bacteria producing exopolysaccharides and more accurately diverse glycan polymers which have interesting applications as texturing agents or prebiotics. Characterization of polymers by ^{1}H and ^{13}C NMR spectroscopy analysis demonstrated that these strains could synthesize glucans of high structural variety and containing different amounts of α-(1→2), α-(1→3) and α-(1→6) linkages. In this study, fifteen glucan-producing *Leuconostoc mesenteroides* and *L. citreum* strains from sourdoughs were characterized according to carbohydrate fermentation, rep-PCR fingerprinting using $(GTG)_5$ primers and glycansucrase activity (soluble or cell-associated). Enzyme characterization using SDS-PAGE and *in situ* polymer production after incubation with sucrose correlated with synthesis of classical or α-(1→2) branched dextrans, alternan and levan. In addition, the presence of genes coding for alternansucrase was detected by PCR and partially characterized by sequence analysis. We thus provide new information on the biodiversity of glucan production by sourdough *Leuconostoc* strains.

1. Introduction

Extracellular polysaccharides or exopolysaccharides (EPS) from wild-type lactic acid bacteria (LAB) have potential for development and exploitation as natural, safe food additives or functional food ingredients with both health and economic benefits (Naessens et al., 2005, Tieking and Gänzle, 2005, Lacaze et al., 2007). Based on their composition, EPS are divided in two classes: heteropolysaccharides composed of different monosaccharides, such as glucose, galactose and rhamnose, and homopolysaccharides containing only one type of monosaccharide, either glucose (glucans) or fructose (fructans) (De Vuyst et al., 2001; Monsan et al., 2001).

Glucans and fructans are synthesized from sucrose by *Leuconostoc*, *Streptococcus* and some *Lactobacillus* LAB strains (Monsan et al., 2001; van Hijum et al., 2006). For instance, dextran synthesized by *Leuconostoc mesenteroides* was one of the first biopolymers produced at industrial scale and has numerous applications in pharmaceutical, food and biotechnology industries (Naessens et al., 2005). Glucan and fructan synthesis is catalyzed by secreted or cell-anchored extracellular glucansucrases and fructansucrases, respectively, with the concomitant release of either fructose or glucose (Monsan et al., 2001; van Hijum et al., 2006). These reactions are carried out without cofactors, as the energy required for the reaction is provided by the hydrolysis of the sucrose glycosidic linkage (Moulis et al., 2006; van Hijum et al., 2006). In addition, glycansucrases can also produce oligosaccharides by a transglucosylation reaction from the sucrose donor to exogenous acceptor molecules (Monsan et al., 2001; Korakli and Vogel, 2006).

Glucansucrases (GS, E.C. 2.4.1.5; also referred as glucosyltransferase (GTF)) are glycosyltransferases which belong to the glycoside hydrolase family 70 (Coutinho and Henrissat, 1999; http://www.cazy.org) and primary structures of several glucansucrases are available. Depending on the enzyme specificity, a wide range of glucans can be produced. According to the glucosidic linkages present in the polymer, these enzymes are classified as: i) dextransucrase when the resulting polymer is characterized by a linear backbone of α-(1→6)-linked glucosyl residues along with some α-(1→2), α-(1→3) or α-(1→4) branching; or ii) mutansucrase when the polymer contains more than 50% α-(1→3) glucosidic linkages in the linear backbone, or iii) alternansucrase when the polymer contains alternating α-(1→6) and α-(1→3) glucosidic linkages, or iv) reuteransucrase when the glucan is composed of α-(1→4) glucosidic bonds with α-(1→6) branching (Monsan et al., 2001; van Hijum et al., 2006). Fructansucrases (FS) are fructosyltransferases (FTF) that belong to the glycoside hydrolase family 68 with levansucrase (LS, E.C. 2.4.1.10) and inulosucrase (IS, E.C. 2.4.1.9) that synthesize fructose polymers linked in their main chain by β-(2→6)

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and β-(2→1) osidic bonds respectively (Velazquez-Hernandez et al., 2009). Although glucan- and fructansucrase catalyze analogous reactions, they have different reaction mechanisms and differ strongly in protein structure and size (van Hijum et al., 2006, Korakli and Vogel, 2006).

Different sourdough lactobacilli (*Lb. sanfranciscensis*, *Lb. fermentum*, *Lb. pontis*, *Lb. panis*, *Lb. reuteri*, *Lb. acidophilus*), *L. mesenteroides* and *Weissella* strains were shown to produce various EPS such as fructans (levan or inulin) and/or glucans (dextran, reuteran or mutan) as recently reviewed (Tieking and Gänzle, 2005). Suitability of EPS produced by sourdough LAB to replace or reduce plant hydrocolloids used in the breadmaking process has been suggested in order to improve dough rheological parameters and bread quality (Tieking and Gänzle, 2005; Di Cagno et al., 2006; Lacaze et al., 2007). Additionally, polymers and oligosaccharides produced by some of these LAB strains exhibit prebiotic properties (Korakli et al., 2002; Korakli and Vogel, 2006).

Recently we first reported promising production of diverse glucans from sucrose by *Leuconostoc* and *Weissella* strains isolated from sourdoughs (Bounaix et al., 2009). They were identified by NMR analysis as usual dextrans, putative alternans and dextrans with high content of α-(1→2) linkages. In the present study, we provide further characterization of these *L. mesenteroides* and *L. citreum* sourdough strains in terms of carbohydrate fermentation, repetitive element-PCR fingerprinting and description of the polymer-synthesizing enzymes.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Nine *Leuconostoc citreum* (LBAE-A7, -B2, -B7, -B13, -C10, -C11, -C12, -E16, and -H6) and six *Leuconostoc mesenteroides* (LBAE-A9, -G15, -G28, -K24, -K29, and -K30) EPS-producing strains belonging to the Culture Collection of the Laboratoire de Biologie appliquée à l'Agroalimentaire et l'Environnement-Université Paul Sabatier (LBAE-UPS, Auch, France) were used in this study. They were originally collected from different traditional French sourdoughs (Gabriel et al., 1999) as indicated by the first letter of the strain denomination. Strains were previously assigned to *L. mesenteroides* and *L. citreum* species by molecular methods (Robert et al., 2009). Five other *L. mesenteroides* and *L. citreum* strains were used as reference strains: ATCC 8293 (equivalent to NRRL B-1118), NRRL B-512F, B-742, B-1299 and B-1355. All strains were routinely propagated in De Man, Rogosa and Sharpe (MRS) medium at 30 °C (Biokar, Beauvais, France).

2.2. Fermentative profiles

Carbohydrate fermentation patterns of *Leuconostoc* strains were determined at least in duplicate using API 50CH® system (API system, BioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. The results were recorded after 24 and 48 h of incubation at 30 °C.

2.3. Glycansucrase recovery and assay

Each strain was first cultivated in MRS broth at 25 °C for 20 h, then a 100 mL culture was prepared (initial $OD_{600}=0.3$) in MRS-sucrose (MRS containing sucrose 4% instead of glucose 2%), with pH adjusted to 6.9 with 5 M sterile NaOH. The incubation was performed at 25 °C, 100 rpm until pH 5.0. Finally, pH was adjusted to 5.4 with 5 M sterile NaOH in order to optimize glycansucrase activity (Naessens et al., 2005). The culture was centrifuged at 4 °C (12,100×*g*, 20 min) to separate culture supernatant containing soluble glycansucrase and pellet with cell-associated activity. Cells were washed twice with 20 mM sodium acetate buffer pH 5.4 and

Table 1
Fermentation profiles of glucan-producing *Leuconostoc* strains.

	Ln. mesenteroides								*Ln. citreum*									
Strains	A9	G15	G28	K24	K29	K30	ATCC 8293	NRRL B-512F	NRRL B-1299	NRRL B-1355	NRRL B-742	A7 B7	B2	B13	C10	C11	C12	E16	H6
Acid from																			
Amygladin	+	+	+	+	w	−	+	−	+	w	w	−	−	w	w	w	+	−	w
Arabinose	+	+	+	+	+	−	+	+	+	w	+	+	+	−	+	+	+	+	+
Arbutin	+	+	+	+	+	−	w	w	+	w	+	+	+	+	+	+	+	w	+
Cellobiose	+	+	+	+	+	w	+	−	−	−	w	−	+	w	w	w	+	w	w
Galactose	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−	−
Gentobiose	w	+	+	+	+	−	+	−	−	−	w	−	−	−	−	−	+	w	w
Lactose	w	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−	−
Maltose	+	+	+	+	+	+	+	+	w	−	+	+	+	+	+	+	+	+	+
Mannitol	w	w	−	w	w	−	w	+	w	−	−	w	w	w	w	w	−	w	w
Mannose	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+
Melibiose	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	−	−
MethylDglucoside	+	+	+	+	+	+	+	+	+	+	+	+	−	w	w	w	+	+	w
Potassium gluconate	w	+	−	−	−	w	w	w	+	w	w	w	w	w	w	w	w	w	w
Raffinose	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	−	−
Rhamnose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	w	−	−
Ribose	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	−	−
Starch	−	−	−	w	w	w	−	−	−	−	−	−	−	−	−	−	−	−	−
Tagatose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Turanose	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Xylose	+	+	w	+	+	+	+	+	+	+	−	+	+	+	+	+	w	+	−
2-keto-gluconate	−	−	−	−	w	w	−	−	w	−	w	w	w	w	w	w	w	−	w
5-keto-gluconate	w	w	−	−	−	w	−	w	−	−	−	−	−	−	−	−	−	−	−
Polymer group	I	II	I	I	III	III	II	I	III	IV	V*1	III	IV	IV	III	IV	III	IV	I

+, positive; w, weakly positive; −, negative after 48 h of incubation at 30 °C. In addition to the carbohydrates listed in the table, all of the strains tested could ferment glucose, fructose, salicin, sucrose, trehalose and N-acetylglucosamine and could hydrolyse esculin.
Polymer group was previously reported for sourdough and reference strains (Bounaix et al., 2009; *1 Seymour et al., 1979): I, classical dextran with about 95% α-(1→6) and 5% α-(1→3) linkages; II, mixture of classical dextran and levan; III, dextran with high content of α-(1→2) linkages; IV, glucan with high content of α-(1→3) linkages, putative alternan; V, mixture of dextran containing α-(1→4) branch linkages and dextran with high degree of single α-(1→3) branched glucose residues (comb-like structure).