RESEARCH LETTER

Characterization of dextran-producing Weissella strains isolated from sourdoughs and evidence of constitutive dextranuscraze expression

Marie-Sophie Bounaix¹, Hervé Robert¹, Valérie Gabriel¹, Sandrine Morel²,³, Magali Remaud-Siméon²,³, Bruno Gabriel¹ & Catherine Fontagné-Faucher¹

¹Laboratoire de Biologie appliquée à l’Agroalimentaire et à l’Environnement (LBAE), Institut Universitaire de Technologie, Université Paul Sabatier, Auch, France; ²Université de Toulouse, INSA, UPS, INP, LISBP, Toulouse, France; and ³CNRS, UMR5504, INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, Toulouse, France

Correspondence: Catherine Fontagné-Faucher, Laboratoire de Biologie appliquée à l’Agroalimentaire et à l’Environnement (LBAE), Institut Universitaire de Technologie, Université Paul Sabatier, 24 rue d’Embaque’s, F-32000 Auch, France. Tel.: +33 562 616 302; fax: +33 562 616 301; e-mail: cathy.faucher@iut-tlse3.fr

Received 28 April 2010; revised 2 July 2010; accepted 6 July 2010.
Final version published online 16 August 2010.
DOI:10.1111/j.1574-6968.2010.02067.x
Editor: André Klier

Keywords
dextranuscraze; homopolysaccharides; exopolysaccharides; Weissella; lactic acid bacteria; sourdough.

Abstract

The study of exopolysaccharide production by heterofermentative sourdough lactic acid bacteria has shown that Weissella strains isolated from sourdoughs produce linear dextrans containing α-(1 → 6) glucose residues with few α-(1 → 3) linkages from sucrose. In this study, several dextran-producing strains, Weissella cibaria and Weissella confusa, isolated from sourdough, were characterized according to carbohydrate fermentation, repetitive element-PCR fingerprinting using (GTG)₅ primers and glucansucrase activity (soluble or cell-associated). This study reports, for the first time, the characterization of dextranuscraze from Weissella strains using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and in situ polymer production (after incubation with sucrose) from enzymatic fractions harvested from both sucrose and glucose culture media. Results demonstrate that dextranuscraze activity was mainly soluble and associated with a constitutive 180-kDa protein. In addition, microsequencing of the active dextranuscraze from W. cibaria LBAE-K39 allowed the design of specific primers that could detect the presence of glucansucrase encoding genes similar to GTFKg3 of Lactobacillus fermentum Kg3 and to DSRWC of W. cibaria CMU. This study hence indicates that sourdough Weissella strains synthesize original dextranuscraze.

Introduction

Oligo- and homopolysaccharides produced from sucrose by lactic acid bacteria (LAB) have received increasing attention mainly because of their potential toward industrial applications such as texturizing agents and prebiotics (Naessens et al., 2005). Polymer production (glucans and fructans) has first been reported for members of the genus Leuconostoc, Streptococcus and Lactobacillus and more recently Weissella (Monsan et al., 2001; Björkroth & Holzapfel, 2006; van Hijum et al., 2006). Glucan synthesis is catalyzed from sucrose by secreted or cell-anchored glucansucrases, which convert the sucrose substrate into high-molecular-weight polymers, with the concomitant release of fructose (Monsan et al., 2001; van Hijum et al., 2006). These reactions occur without any other cofactor; the energy of the osidic bond of sucrose enables the efficient transfer of a glucosyl residue via the formation of a covalent glycosyl-enzyme intermediate allowing the elongation of polymer chains (Moulis et al., 2006; van Hijum et al., 2006). In addition, these enzymes also produce oligosaccharides when acceptor molecules such as maltose are present in the reaction mixture along with sucrose (Monsan et al., 2001; Korakli & Vogel, 2006).

Glucansucrases (EC 2.4.1.5) – also referred as glucosyltransferases (GTF) – are relatively large extracellular enzymes showing an average molecular weight of 170 kDa. They belong to the glycoside hydrolase (GH) family 70 (http://www.cazy.org). Depending on the type of glucosidic linkages as well as the degree and organization of branching, glucansucrases can be classified into different categories (Monsan et al., 2001; van Hijum et al., 2006). Among them are found dextranuscrazes, which produce dextran, a polymer with a linear backbone made of at least 50% α-(1 → 6) glucosidic bonds and α-(1 → 2)-, α-(1 → 3)-
or α-(1 → 4)-linked branches. More than 40 genes encoding GH70 glucansucrases have been isolated and sequence analyzed. Deduced amino acid sequence analysis revealed a signal peptide and a common structural organization with (1) an N-terminal variable domain; (2) a conserved catalytic domain of about 1000 amino acids; and (3) a C-terminal domain of variable length, which is thought to be involved in glucan binding (Korakli & Vogel, 2006; van Hijum et al., 2006).

Weissella genus is phylogenetically related to Leuconostoc and Oenococcus and arose from the reclassification of Leuconostoc paramesenteroides and some related "atypical" heterofermentative lactobacilli (Collins et al., 1993). Weissella cibaria and Weissella confusa are rod-shaped obligate heterofermentative species, which are closely related in the genus (Björkroth et al., 2002; Björkroth & Holzapfel, 2006). These two species have been isolated from a wide variety of fermented products of plant origin (Björkroth et al., 2002; Camu et al., 2007; Kostinek et al., 2007; Chao et al., 2008), in particular from sourdough (De Vuyst et al., 2002; Catzeddu et al., 2006; Valmorri et al., 2006; Iacumin et al., 2009; Robert et al., 2009). They were also occasionally found in dairy products (van der Meulen et al., 2007; Ouadghiri et al., 2009). Additionally, W. cibaria was reported as a member of the human saliva LAB microbial communities (Kang et al., 2006).

Although dextran production is used as one of the phenotypic criteria for the identification of bacteria classified in the genus Weissella (Björkroth & Holzapfel, 2006), an accurate description of the exopolysaccharides from W. cibaria and W. confusa strains was until now only occasional. Several authors reported fructan and/or glucan production by W. confusa and W. cibaria strains (Tieking et al., 2003; Di Cagno et al., 2006; van der Meulen et al., 2007). Based on enzymatic degradation, the presumption of a dextran structure was first suggested by Kang et al. (2006) and Schwab et al. (2008) for W. cibaria strains. Maina et al. (2008) recently reported the production of a linear dextran with > 97% α-(1 → 6) glucosidic linkages by the W. confusa strain DSM 20194 (VTT E-90392).

The aim of the present study is to characterize several Weissella strains that were previously reported as dextran producers (Bounaix et al., 2009). Characterization of polymers by 1H and 13C nuclear magnetic resonance spectroscopy analysis showed that these strains synthesize linear dextran with only a few (2.4–3.3%) α-(1 → 3)-linked branches from sucrose. Here, carbohydrate fermentation patterns, repetitive element (rep)-PCR fingerprinting and dextranucrase activity from six W. cibaria and two W. confusa strains are reported.

**Materials and methods**

**Bacterial strains and growth conditions**

Five strains of W. cibaria (LBAE-C36-1, -D38, -D39, -H25 and -K39) and one strain of W. confusa (LBAE-C39-2) belonging to the culture collection of the Laboratoire de Biologie appliquée à l’Agroalimentaire et à l’Environnement, Université Paul Sabatier (LBAE-UPS, Auch, France) were used in this study. They were initially collected from traditional French sourdoughs (Gabriel et al., 1999). Species affiliation was achieved previously using molecular methods (Robert et al., 2009). Three other LAB strains have been used as reference: W. cibaria DSM 15878T, W. confusa DSM 20196T and Leuconostoc mesenteroides NRRL B-512F. All strains were routinely propagated in De Man, Rogosa and Sharpe (MRS) medium at 30 °C (Biokar).

**Fermentative profiles**

Carbohydrate fermentation patterns of Weissella strains were determined at least in duplicate using API 50CH® strips (API System, BioMérieux, France) according to the manufacturer’s instructions. The results were recorded after 24 and 48 h of incubation at 30 °C.

**Dextranucrase activity measurements**

Dextranucrase activity of the strains was checked as described previously in Bounaix et al. (2009). Briefly, after strain precultivation in MRS broth at 25 °C, a 100 mL culture was prepared (initial OD550 nm = 0.3) in plain MRS (glucose medium) or in MRS containing 4% w/v sucrose instead of 2% w/v glucose (sucrose medium). The pH of the media was initially adjusted to 6.9, and bacteria were grown at 25 °C, 100 r.p.m. The culture was stopped when a pH value of 5.0 was reached. The pH was adjusted at 5.4, an appropriate value for dextranucrase activity, with 5 M sterile NaOH. The culture supernatant containing soluble glucansucrase and the pellet exhibiting cell-associated activity were separated by centrifugation (12 100 g, 20 min, 4 °C). Cells were washed twice with 20 mM sodium acetate buffer pH 5.4 and concentrated twofold within the same buffer. The supernatant and pellet samples were kept at −25 °C until further use. Enzymatic activity was assayed in triplicate using the dinitrosalicylic acid (DNS) method (Sumner & Howell, 1935). One unit of dextranucrase activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol fructose min−1 at 30 °C in 20 mM sodium acetate buffer (pH 5.4) with 292 mM sucrose.

**Electrophoresis and the detection of active dextranucrase (zymogram)**

Supernatant and cell-associated fractions from Weissella cultures were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Each sample (30 μL) was mixed with NuPAGE® LDS sample buffer 4 × (10 μL) (Invitrogen, France) and incubated at 70 °C for 10 min to denature the enzymes reversibly. Electrophoresis
was performed on NuPAGE® 3–8% Tris-acetate gel with the XCell SureLock Minicell system (Invitrogen) at room temperature at constant voltage (150 V). After migration, proteins were stained with the Colloidal Blue Staining kit (Invitrogen). For in situ detection of dextransucrase activity, the gel was first washed three times with sodium acetate buffer (20 mM sodium acetate, pH 5.4, 0.05 g L⁻¹ CaCl₂ and 0.1% v/v Triton X-100) for a total of 60 min to renature dextranase. It was then incubated overnight in the same buffer supplemented with sucrose (10% w/v). Thereafter, dextranase activity was revealed by periodic acid-Schiff staining (Schiff’s reagent, Sigma-Aldrich) of the polymer formed (Miller & Robyt, 1986). The molecular mass was estimated with the Precision Plus Protein Standards all blue purchased from BioRad Laboratories.

**Protein microsequencing**

The supernatant of *W. cibaria* K39 harvested from the glucose medium culture was concentrated up to fivefold with a Centricon (30 kDa cut-off, Millipore) to reach a protein concentration around 1 g L⁻¹, as determined by the Bradford method (Bradford, 1976), and subjected to SDS-PAGE. The proteins were stained with colloidal blue Coomassie and silver staining (ProteoSilver Plus Silver Stain kit, Sigma-Aldrich), which is more sensitive. In addition, zymogram was performed to specifically detect dextranase activity. The unique band detected at 180 kDa was excised from the Coomassie blue-stained gel in sterile conditions and stored in ultrapure water at 4°C. Protein sequencing was conducted by Eurogentec by the ESI-MS-MS analysis (Liege Science Park, Belgium).

**Molecular analyses**

*Weissella* total DNA was prepared according to Robert et al. (2009) or using a DNA extraction kit (DNAeasy Blood and Tissue kit, Qiagen) from overnight cultures grown in MRS medium. PCR amplifications were carried out using a Gradient Master Thermocycler (Eppendorf). Reactions were performed in a total volume of 20 μL containing 1 μL of template DNA (approximately 5–10 ng), 1× reaction buffer, 0.2 mM dNTP, 1.5 mM MgCl₂, appropriate concentration of oligonucleotide primers (Sigma or Eurogentec) and 0.75 U RedGoldstar Taq polymerase (Eurogentec). The PCR involved an initial denaturation step (94°C, 5 min), followed by an appropriate PCR cycling step and a final elongation step (72°C, 10 min).

rep-PCR fingerprinting of *Weissella* strains was performed using the 2 μM (GTG), primer (5’-GTGGTGTTGTTG GTG-3’) (Versalovic et al., 1994). The PCR amplification was achieved using the following conditions adapted from Versalovic et al. (1994): denaturation (94°C, 1 min), annealing (45°C, 1 min) and elongation (72°C, 1 min), for a total of 30 cycles. To limit experimental variations, PCR products from *Weissella* DNA were obtained during a unique PCR experiment and analyzed in the same agarose gel.

Amplification of the *Weissella* dextranase encoding gene was carried out using different sets of degenerate or nondegenerate primers (Table 1). Degenerate primers bMAR1F-bMAR2R (Sigma) have been first designed from microsequencing results of the K39 dextranase 180-kDa protein band. From partial sequencing of PCR products, nondegenerate primers dsrK39For-dsrK39Rev were designed (Eurogentec). DNA was amplified as follows: denaturation for 1 min at 94°C, annealing for 1 min at 54°C (bMAR1F-bMAR2R) or 59.8°C (dsrK39For-dsrK39Rev) and elongation for 3 min at 72°C for a total of 38 cycles.

PCR products were subjected to electrophoresis in 1% w/v agarose gel in 0.5× TBE buffer and visualized by staining with ethidium bromide. For amplification products from rep-PCR, separation was conducted in 1.7% agarose gel at 75 V. Smart Ladder™ from Eurogentec were used to estimate the size of the bands. Amplicons from dsrK39 PCR were purified with the MEGASPIN Agarose Gel Extraction kit from Euromedex.

DNA sequencing was conducted by Millegen (Toulouse, France) and the DNA sequence information obtained was analyzed by BLAST. Alignments with known sucrase enzymes downloaded from databases were made using MULTALIN software. The nucleotide and the deduced amino acid sequence of DSRK39 have been submitted to the NCBI nucleotide sequence database under accession number GU237484.2.

**Results and discussion**

**Carbohydrate fermentation**

Phenotypic analysis of the sourdough *Weissella* strains previously assigned to *W. cibaria* and *W. confusa* sp. (Robert et al., 2009) or using a DNA extraction kit (DNAeasy Blood and Tissue kit, Qiagen) from overnight cultures grown in MRS medium. PCR amplifications were carried out using a Gradient Master Thermocycler (Eppendorf). Reactions were performed in a total volume of 20 μL containing 1 μL of template DNA (approximately 5–10 ng), 1× reaction buffer, 0.2 mM dNTP, 1.5 mM MgCl₂, appropriate concentration of oligonucleotide primers (Sigma or Eurogentec) and 0.75 U RedGoldstar Taq polymerase (Eurogentec). The PCR involved an initial denaturation step (94°C, 5 min), followed by an appropriate PCR cycling step and a final elongation step (72°C, 10 min).

rep-PCR fingerprinting of *Weissella* strains was performed using the 2 μM (GTG), primer (5’-GTGGTGTTGTTG GTG-3’) (Versalovic et al., 1994). The PCR amplification was achieved using the following conditions adapted from Versalovic et al. (1994): denaturation (94°C, 1 min), annealing (45°C, 1 min) and elongation (72°C, 1 min), for a total of 30 cycles. To limit experimental variations, PCR products from *Weissella* DNA were obtained during a unique PCR experiment and analyzed in the same agarose gel.

Amplification of the *Weissella* dextranase encoding gene was carried out using different sets of degenerate or nondegenerate primers (Table 1). Degenerate primers bMAR1F-bMAR2R (Sigma) have been first designed from microsequencing results of the K39 dextranase 180-kDa protein band. From partial sequencing of PCR products, nondegenerate primers dsrK39For-dsrK39Rev were designed (Eurogentec). DNA was amplified as follows: denaturation for 1 min at 94°C, annealing for 1 min at 54°C (bMAR1F-bMAR2R) or 59.8°C (dsrK39For-dsrK39Rev) and elongation for 3 min at 72°C for a total of 38 cycles.

PCR products were subjected to electrophoresis in 1% w/v agarose gel in 0.5× TBE buffer and visualized by staining with ethidium bromide. For amplification products from rep-PCR, separation was conducted in 1.7% agarose gel at 75 V. Smart Ladder™ from Eurogentec were used to estimate the size of the bands. Amplicons from dsrK39 PCR were purified with the MEGASPIN Agarose Gel Extraction kit from Euromedex.

DNA sequencing was conducted by Millegen (Toulouse, France) and the DNA sequence information obtained was analyzed by BLAST. Alignments with known sucrase enzymes downloaded from databases were made using MULTALIN software. The nucleotide and the deduced amino acid sequence of DSRK39 have been submitted to the NCBI nucleotide sequence database under accession number GU237484.2.

**Results and discussion**

**Carbohydrate fermentation**

Phenotypic analysis of the sourdough *Weissella* strains previously assigned to *W. cibaria* and *W. confusa* sp. (Robert et al., 2009) or using a DNA extraction kit (DNAeasy Blood and Tissue kit, Qiagen) from overnight cultures grown in MRS medium. PCR amplifications were carried out using a Gradient Master Thermocycler (Eppendorf). Reactions were performed in a total volume of 20 μL containing 1 μL of template DNA (approximately 5–10 ng), 1× reaction buffer, 0.2 mM dNTP, 1.5 mM MgCl₂, appropriate concentration of oligonucleotide primers (Sigma or Eurogentec) and 0.75 U RedGoldstar Taq polymerase (Eurogentec). The PCR involved an initial denaturation step (94°C, 5 min), followed by an appropriate PCR cycling step and a final elongation step (72°C, 10 min).

rep-PCR fingerprinting of *Weissella* strains was performed using the 2 μM (GTG), primer (5’-GTGGTGTTGTTG GTG-3’) (Versalovic et al., 1994). The PCR amplification was achieved using the following conditions adapted from Versalovic et al. (1994): denaturation (94°C, 1 min), annealing (45°C, 1 min) and elongation (72°C, 1 min), for a total of 30 cycles. To limit experimental variations, PCR products from *Weissella* DNA were obtained during a unique PCR experiment and analyzed in the same agarose gel.

Amplification of the *Weissella* dextranase encoding gene was carried out using different sets of degenerate or nondegenerate primers (Table 1). Degenerate primers bMAR1F-bMAR2R (Sigma) have been first designed from microsequencing results of the K39 dextranase 180-kDa protein band. From partial sequencing of PCR products, nondegenerate primers dsrK39For-dsrK39Rev were designed (Eurogentec). DNA was amplified as follows: denaturation for 1 min at 94°C, annealing for 1 min at 54°C (bMAR1F-bMAR2R) or 59.8°C (dsrK39For-dsrK39Rev) and elongation for 3 min at 72°C for a total of 38 cycles.

PCR products were subjected to electrophoresis in 1% w/v agarose gel in 0.5× TBE buffer and visualized by staining with ethidium bromide. For amplification products from rep-PCR, separation was conducted in 1.7% agarose gel at 75 V. Smart Ladder™ from Eurogentec were used to estimate the size of the bands. Amplicons from dsrK39 PCR were purified with the MEGASPIN Agarose Gel Extraction kit from Euromedex.

DNA sequencing was conducted by Millegen (Toulouse, France) and the DNA sequence information obtained was analyzed by BLAST. Alignments with known sucrase enzymes downloaded from databases were made using MULTALIN software. The nucleotide and the deduced amino acid sequence of DSRK39 have been submitted to the NCBI nucleotide sequence database under accession number GU237484.2.
and (3) type strain DSM 15878T, with some variations in the differentiations: (1) D39, D38 and K39, (2) C36-1 and H25 identified within *W. cibaria* and *W. confusa* the tested conditions. Two strains (D38 and K39) isolated from different sourdough samples showed the same carbohydrate fermentation profile. On the other hand, *W. cibaria* D38 and D39, originating from the same sourdough sample, exhibited different patterns and differed by lactose, melibiose, raffinose, rhamnose, ribose, tagatose and trehalose fermentation. Sourdough strain C36-1 was the only strain able to produce acid from inulin. These results thus indicate the natural biodiversity of exopolysaccharide-producing *Weissella* strains from sourdough. Notably, the reference strain DSM 20196T could not produce acid from sucrose in the tested conditions.

**rep-PCR genomic fingerprinting**

Analysis of PCR products obtained using (GTG)\_5 primers allowed further characterization of the *Weissella* strains. Profiles from *W. confusa* strains were clearly discriminated from *W. cibaria* ones (Fig. 1). Different fingerprints were identified within *W. cibaria* strains that allowed three group differentiations: (1) D39, D38 and K39, (2) C36-1 and H25 and (3) type strain DSM 15878T, with some variations in the band pattern (Fig. 1). The sourdough strain *W. confusa* C39-2 displayed a different pattern from the type strain DSM 20196T. These results show that (GTG)\_5-PCR fingerprinting can be used for a rapid species affiliation to *W. confusa* or *W. cibaria*.

![Fig. 1. (GTG)\_5-PCR fingerprints of Weissella cibaria and Weissella confusa strains.](https://academic.oup.com/femsle/article-abstract/311/1/18/600887)

**Table 2. Differential phenotypic characteristics of glucan-producing Weissella strains**

<table>
<thead>
<tr>
<th>Acid from</th>
<th><em>W. cibaria</em></th>
<th><em>W. confusa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DSM 15878T</td>
<td>C36-1</td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arbutin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Glycogen</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Inulin</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lactose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Melibiose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Raffinose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ribose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tagatose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Trehalose</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

+, Positive; w, weakly positive; –, negative after 48-h incubation at 30 °C; T, type strain. In addition to the carbohydrates listed in the table, all of the strains tested could ferment amygdalin, cellobiose, fructose, gentiobiase, glucose, maltose, mannone, potassium gluconate, salicin, xylose, N-acetylglucosamine and could hydrolyze esculin.

**Dextranucrase activity**

The dextranucrase production level of the different *Weissella* strains cultivated with sucrose or glucose as the carbon source was determined and compared with those obtained from the well-characterized dextran-producing strain *L. mesenteroides* NRRL B-512F (Fig. 2). The values determined for the *Weissella* strains grown in a sucrose medium ranged from 0.02 to 0.27 U mL\(^{-1}\) (Fig. 2a). Most strains exhibited only soluble detectable activity. Only D39, DSM 20196T and the reference NRRL B-512F strains displayed a cell-associated activity (Fig. 2a). Interestingly, all *Weissella* strains showed only soluble dextranucrase activity when glucose was used as the carbon source instead of sucrose (Fig. 2b). In these conditions, no activity was detected for the reference NRRL B-512F strain, which is known to synthesize a sucrose-inducible dextranucrase (Monsan *et al*., 2001; van Hijum *et al*., 2006). To our knowledge, dextranucrase activity without sucrose induction has never been reported for *Weissella* strains. Future studies could reveal whether it is
a general feature of dextranucrase from Weissella genus. So far, constitutive wild-type glucansucrases have only been described for Streptococcus sp. and some Lactobacillus strains, notably Lactobacillus reuteri (van Geel-Schutten et al., 1999; Monsan et al., 2001; Kralj et al., 2004; Schwab & Gänzle, 2006; Arsköld et al., 2007). Furthermore, soluble dextranucrase activities obtained with glucose as the carbon source were always higher than those produced with sucrose (Fig. 2b). Indeed, depending on the studied strains, a 1.4–5.5-fold increase of activity level was observed when glucose was used instead of sucrose. Cell growth determined in both culture conditions was quite similar, with a maximum of 1.5-fold increase in the specific growth rate (data not shown), except for W. confusa DSM 20196 that grew poorly in a sucrose medium in view of the carbohydrate fermentation profile. This increase in the dextranucrase activity level can be assigned to an enhanced enzyme production with glucose as carbon source. Such results suggested that a possible repression by fructose could occur when sucrose is used as carbon source.

**Dextranucrase band pattern**

The protein content in the soluble and cell-associated fractions was analyzed by electrophoretic analyses and zymograms (Fig. 3). All sourdough Weissella strains revealed a dextranucrase at 180 kDa, which is similar to the dextranucrase visualized from the reference NRRL B-512F strain. A similar band pattern was obtained for both conditions of culture i.e. with sucrose or glucose as the carbon source (Fig. 3a and b, respectively). Conversely, no active band was detected with the reference strain NRRL B-512F when cultivated in glucose growth conditions (Fig. 3b); thus confirming the well-known sucrose induction of dextranucrase. The most intense bands were observed for soluble fractions that previously exhibited higher enzyme activity in DNS assays. This confirms that W. cibaria and W. confusa 180 kDa dextranucrase is mainly soluble and is produced either with sucrose or with glucose as the carbon source. Besides, an additional faint band was detected at 300 kDa for the W. confusa DSM 20196T strain and only in sucrose growth conditions (Fig. 3a, not visible in the supernatant), suggesting the presence of an additional sucrose-inducible dextranucrase.

**Studies of dextranucrase encoding gene**

The sourdough strain K39, which produced the highest soluble enzyme activity, was selected to perform protein sequencing in order to design specific primers. Indeed, a first attempt to detect dextranucrase encoding genes from W. cibaria and W. confusa strains (Bounaix et al., 2009) was unsuccessful using degenerate primers DegFor-DegRev (Kralj et al., 2003), targeting conserved regions within the
catalytic domains of LAB glucansucrases (Fig. 4), as also reported by several authors (Tieking et al., 2003; Di Cagno et al., 2006; van der Meulen et al., 2007; Schwab et al., 2008).

As shown in Fig. 4a, six peptides were generated during microsequencing of the K39 soluble 180 kDa dextransucrase, and the peptide sequences showed similarity with the glucansucrase GTFKg3 of Lactobacillus fermentum Kg3 (Kralj et al., 2004). A first set of degenerate primers bMAR1F-bMAR2R was designed (Fig. 4b and Table 1). PCR amplification yielded a 2500-bp amplicon from

Fig. 3. Sucrase activity patterns of cell-associated (C) and soluble (S) fractions produced from Weissella cibaria and Weissella confuza strains and from Leuconostoc mesenteroides NRRL B-512F used as a reference, grown on sucrose (a) or glucose (b) -containing media. Strain designations are listed above each corresponding gel lane. Cell-associated fractions were all concentrated twofold. M, molecular weight marker (250, 150, 100 and 75 kDa from top to bottom).

Fig. 4. (a) Schematic representation of glucansucrase GTFKg3 from Lactobacillus fermentum Kg3. The four different domains shown are as follows: (i) N-terminal signal sequence; (ii) variable region; (iii) catalytic domain; and (iv) C-terminal glucan-binding domain. Positions and amino acid sequences of the peptides generated during microsequencing of the 180 kDa dextransucrase from Weissella cibaria K39 are indicated with arrows. (b) Zoom in on the catalytic domain and position of the different peptides used for primer design; the three catalytic residues are represented above the arrows.
Partial sequencing of this fragment allowed to design nondegenerate oligonucleotide primers dsrK39For and dsrK39Rev (Fig. 4b and Table 1). Using these primers, a 1950-bp fragment was obtained from *W. cibaria* strains DNA, but no fragment was amplified from the two *W. confusa* strains DNA. Partial sequencing of the PCR products from strain K39 confirmed the similarity to dextransucrase (Fig. 5). The corresponding predicted amino acid sequence, named DSRK39, showed 98% identity to GTFKg3 of *L. fermentum* Kg3 (Kralj et al., 2004) and DSRWC from *W. cibaria* CMU (Kang et al., 2009) as well as 64.4% identity to DSR-S from *L. mesenteroides* NRRL B-512F, which is in agreement with the dextransucrase activity.

Notably, the regions in the vicinity of the catalytic triad (D, E, D) are relatively conserved in these enzymes. This is usually found in enzymes that produce a linear dextran with few α-(1→3) linkages as it is the case for *W. cibaria* K39 dextransucrase, which produces a dextran with 2.8% α-(1→3)-linked branches.

In conclusion, the present study explored the production and characterization of dextransucrase from several dextran-producing *W. cibaria* and *W. confusa* strains. Our results demonstrate that dextransucrase activity expressed by *Weissella* is constitutive and is mainly assigned to a 180-kDa soluble protein. Dextran-producing *Weissella* strains have promising applications in several sectors. Some studies have already pointed out that exopolysaccharides from *W. cibaria* improve the textural properties of bread (Di Cagno et al., 2006; Schwab et al., 2008; Katina et al., 2009). Schwab et al. (2008) demonstrated the production of isomaltooligosaccharides as reliable prebiotics during the breadmaking process with *W. cibaria* 10M. In addition, a dextran-producing *W. cibaria* strain has been suggested as a probiotic for applications in oral health, as it offers the ability to inhibit *Streptococcus mutans* biofilm formation, both in vitro and in vivo (Kang et al., 2006). Future studies on *Weissella* sucrase enzymes will expand our knowledge on the diversity of these dextransucrases, which is useful for various applications.

Acknowledgements

Part of the work was financially supported by the Region of Midi-Pyrénées, France. The authors wish to thank Eliane Auret, Laurent Labadie and Philippe Rabier for their technical contribution.

References


