J Ind Microbiol Biotechnol (2011) 38:1499–1506
DOI 10.1007/s10295-010-0936-x

ORIGINAL PAPER

A novel dextransucrase is produced by *Leuconostoc citreum* strain B/110-1-2: an isolate used for the industrial production of dextran and dextran derivatives

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Received: 24 August 2010 / Accepted: 27 December 2010 / Published online: 13 January 2011

Abstract The industrial *Leuconostoc* strain B/110-1-2 producing dextran and dextran derivatives was taxonomically identified by 16S rRNA as *L. citreum*. Its dextransucrase enzymes were characterized according to their cellular location and reaction specificity. In the presence of sucrose, the strain B/110-1-2 produced two cell-associated dextransucrases (31.54% of the total glucosyltransferase activity) with molecular weights of 160 and 240 kDa and a soluble dextransucrase (68.46%) at 160–180 kDa. Two open reading frames (ORF) coding for *L. citreum* strain B/110-1-2 dextransucrases were identified. One of them shared a 52% identity with the alternansucrase ASR of *L. citreum* NRRL B-1355 and with a putative annotated alternansucrase sequence found in the genome of *L. citreum* KM20. The structural analysis (HPAEC-PAD, HPSEC, and ^{13}C-NMR) of the polymer and oligodextrans produced by the B/110-1-2 dextransucrases suggest this novel glucansucrase has specificity similar to a dextransucrase but not to an alternansucrase, producing a soluble linear dextran with glucose molecules linked mainly in α-1,6 and α-1,3 with α-1,4 branches. These results enhance the understanding of this industrially significant strain and will aid in distinguishing between physiologically similar *Leuconostoc* spp. strains.

Keywords Dextransucrases · Dextran · Gluco-oligosaccharides · *Leuconostoc*

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Introduction

Dextransucrases (EC 2.4.1.5), which belong to Family 70 of the glycoside hydrolases [16], are cell-associated or soluble extracellular enzymes produced by the soil bacteria belonging to the *Leuconostoc* genus [37]. They catalyze the synthesis of high-molecular-weight glucans (D-glucose polymers) from sucrose. If efficient acceptors like maltose or isomaltose are added to the reaction medium, dextransucrases catalyze the synthesis of low-molecular-weight oligosaccharides and, to a minor extent, high-molecular-weight glucan polymers [19]. Different kinds of glucans with different sizes and structures, depending on the dextransucrase-producing strain, are synthesized [35]. Dextransucrases from *Leuconostoc mesenteroides* have been widely used in the pharmaceutical and alimentary industries since the last century. Dextran produced by *L. mesenteroides* NRRL B-512F (ATCC 10830A) was one of the first biopolymers to be produced on an industrial

scale [15], finding several applications in medicine, separation technology, and biotechnology [14, 40]. *Leuconostoc* spp. strains are applied in fermentative processes to produce polymers of glucose (iron dextran, and glucooligosaccharides) [32]. Oligosaccharides produced by *L. mesenteroides* NRRL B-1299 with one or more D-glucopyranosyl units linked through α(1-2) glucosidic bonds [31] are highly resistant to attack by digestive enzymes [11] and are used as prebiotics in cosmeceutical products and human nutritional applications.

The *Leuconostoc* sp. strain B/110-1-2 was isolated from sugarcane juice and has been used for dextran production from sugarcane molasses in a large-scale plant, obtaining up to 1 ton per day of technical-grade dextran. There are basically three stages in dextran production: first, the propagation of the *Leuconostoc* sp. from a pure strain in the laboratory and its further propagation in the plant, until sufficient cells have been obtained for a high concentration of the generated dextransucrase enzyme. The culture supernatant free of cells or the whole culture (optional) is used for the enzymatic synthesis of dextran in a sucrose solution that takes place in the second stage. The solution also contains yeast extract, a source of phosphate as a buffer, traces of salts of some metals such as Mg, Mn, Fe, and sodium hydroxide to neutralize the excess acid formed during fermentation. The third stage consists of the dextran recovery through precipitation with ethyl alcohol, washing, reprecipitation, dissolving, drying, grinding, and packaging. [4]. Recently, the technical-grade dextran polymer produced by the above-mentioned procedure using the dextransucrase present in the culture supernatant of the strain B/110-1-2 has been submitted to a physical and chemical characterization using infrared spectroscopy (IR), ^{1}H and ^{13}C NMR spectroscopy and compared with that of a commercial native dextran 512F obtained from Sigma-Aldrich Corp. The spectra obtained for both native dextrans were very similar. This result suggests both native dextrans have a similar structure [7, 8]. Strain B/110-1-2 has also been applied in the production of iron-dextran at the pilot-plant scale. A new process has been developed at ICIDCA that substitutes traditional synthesis by previous formation of β-FeOOH. This intermediate is then purified through liquid–solid separation in a decanting centrifuge. Production of 5,000-Da dextran is a three-step process: synthesis and purification of β-FeOOH followed by production of injectable iron dextran [21]. The dextransucrase present in the culture supernatant of the strain B/110-1-2 has also been used for the development of controlled-release solid dosage forms (soluble drugs) [7]. In the present study, the B/110-1-2 strain was taxonomically identified by 16S rRNA analysis. Activity, cellular localization, molecular weight, and reaction specificity of its dextransucrase enzymes were determined. The detection of a novel dextran polymer and dextransucrase ORF is discussed.

Materials and methods

Bacterial strains and culture media

The *Leuconostoc* sp. strain B/110-1-2 was obtained from the Cuban Research Institute on Sugarcane by-Products (ICIDCA) culture collection. It was isolated from sugarcane juice (A. Martínez pers. comm.). Stock cultures were maintained at −80°C in 15% (v/v) glycerol. Erlenmeyer flask cultures were grown in a rotary shaker at 30°C, 175 cycles min^{-1} in standard medium (20 g/l sucrose, 20 g/l potassium hydrogen phosphate, 20 g/l yeast extract, 0.2 g/l $MgSO_4$ $7H_2O$, 0.01 g/l $MnSO_4$ H_2O, 0.01 g/l NaCl, 0.02 g/l $CaCl_2$, 0.01 g/l $FeSO_4$ $7H_2O$) with an adjusted pH of 6.9. Cultures were grown until the pH dropped to around 5.0–4.8. Glucose instead of sucrose was added in the standard medium for chromosomal DNA purification purposes in order to eliminate the interference in the purification procedure of the dextrans produced by the strain B/110-1-2 in the presence of sucrose.

Preparation of fractions containing dextransucrase enzymes: enzyme activity assays

The supernatant and cellular fractions of the B/110-1-2 cultures contain soluble and insoluble dextransucrase enzymes, respectively. They were separated by centrifugation at 10,000 × *g* and 4°C for 15 min. They were collected from cultures grown in 50 ml of standard medium supplemented with 40 g/l sucrose. The cellular fractions were suspended in 100 ml of 20 mM (pH 5.4) sodium acetate, 2 mM $CaCl_2$, and $NaNO_3$ 0.1% (W/V). Both fractions were kept at −20°C until they were analyzed.

Enzyme reactions were assayed under standard conditions at 30°C, in 20 mM sodium acetate buffer (pH 5.4), 0.05 g/l $CaCl_2$, 1 g/l $NaNO_3$, and 100 g/l sucrose. The dextransucrase activity was determined by measuring the release of reducing sugars with the dinitrosalicylic acid method [41]. One U is defined as the amount of enzyme that catalyzes the formation of 1 μmol/min fructose under the assay conditions.

Malto-oligosaccharides and dextran production

Acceptor reactions were carried out at 30°C for 24 h, in 20 mM sodium acetate buffer (pH 5.4), 0.05 g/l $CaCl_2$, 1 g/l $NaNO_3$, 200 g/l sucrose, 50 g/l maltose, and 0.5 U/ml dextransucrase. The acceptor reaction products synthesized by the dextransucrase DSR-S purified from *L. mesenteroides*

NRRL-B-512F (ATCC 10830A) supernatant were used as controls for comparative purposes. The synthesis of dextran polymer was carried out under the same conditions as above except that maltose was not added to the reaction medium.

High-performance size exclusion chromatography (HPSEC)

Glucan molecular weight distributions were determined by HPSEC. For dextran analyses, two Shodex OH-Pack SB-805 and SB-802.5 columns were maintained in series, using an eluent containing 0.45 M of $NaNO_3$ and 1% of ethylene glycol at a flow rate of 0.3 ml/min. Columns and guard columns were maintained at 70°C, and samples were filtered through a 0.45-µm-pore-size filter (Sartorius) before injection [25]. The reaction was stopped after 24 h by heating 5 min at 95°C in a boiling water bath. Calibration standards of commercial dextrans of 2,000, 530, 70, and 10 kDa were used (Sigma-Aldrich).

^{13}C-NMR analyses of dextran polymers

All NMR spectra were recorded on an Advance 500-MHz spectrometer (Bruker) using a 5-mm z-gradient TBI probe. The data were acquired and processed using XWINNMR 3.5 software. The temperature was 343 K. Quantitative ^{13}C-NMR were recorded as previously described [12, 13] using an inverse gated sequence taken from the Bruker pulse sequence library, and using a 90° pulse, 25,000-Hz sweep width, 2.5-s relaxation delay, and 0.63-s acquisition time. A total of 30,000 scans were recorded. Proton spectra were acquired by using a 30° pulse; 12,500-Hz sweep width, 2.5-s relaxation delay, and 2.0-s acquisition time. A total of four or 16 scans were recorded. Signal assignments were made by the comparison of spectra from products and acceptors of the branching reaction.

SDS-PAGE and zymograms

Protein electrophoresis under denaturing conditions (SDS-PAGE) was performed with the XCell *SureLock*™ Mini-Cell system, with NOVEX Tris–Acetate gels of 1.5-mm thickness, NuPAGE® anti-oxidant and NuPAGE® Tris–Acetate SDS Running Buffer (for Tris–Acetate gels) from Invitrogen. The NuPAGE® Sample Reducing Agent (3 µl) and NuPAGE®LDS Sample Buffer (4×) sample buffer (7.5 µl) were mixed with 20 µl of sample and heated at 70°C for 10 min prior to being loaded onto gels. Samples containing suspended cells were centrifuged at 10,000 × *g* in a micro-centrifuge before being applied to the gels. Approximately 2 mU of enzyme, as measured by the DNS assay, was loaded onto the gels and electrophoresis was carried out for 1 h at 150 V. The gels were then stained for glucansucrase activity in situ by the procedure of Miller and Robyt [22]. Bio-Rad protein standards (Precision Plus Protein™ All Blue Standard, Bio-Rad, Inc.) were included in all electrophoresis runs.

DNA extraction and purification

Chromosomal DNA of B/110-1-2 strain was purified with the Blood and Cell Culture DNA Maxi kit (Qiagen). Agarose gel electrophoresis was performed by standard procedures [34]. PCR amplification products and gel extraction were performed using QIAquick kit (Qiagen).

PCR amplification of 16S rDNA operon

Primers designed to amplify the complete sequence of the 16S rDNA operon were designated as **R16S-1** (5′-AGAG TTGACATGCCTCTCAG-3′) and **R16S-2** (5′-TACGGTT ACCTTGTTACGACTT-3′). Amplicons were synthesized with a Perkin Elmer Gene Amp PCR System 2400 thermocycler. The reaction was carried out in a total volume of 20 µl containing 2.5 U *Taq* Polymerase (New England Biolabs), 50 ng chromosomal DNA of the strain B/110-1-2, 10 µM of each primer, 200 µM of each dNTP, and 2 µl of *Taq* Polymerase Buffer 10× supplemented with 15 mM $MgCl_2$. The following program was applied: 30 cycles at 94°C for 30 s, 50°C for 1 min and 30 s, 72°C for 2 min, and a final additional extension step of 72°C for 10 min. The amplicon was cloned in the T-vector pUCmT (TaKaRa Biotechnology).

PCR amplification of putative dextransucrase encoding genes

Primers were designed based on the sequence of *asr*, *dsrS*, and *dsrE* genes from GenBank™ (accession numbers AJ250173, I09598 and AJ430204, respectively). DNA fragments were generated by LA PCR (Long and Accurate PCR) using the Expand High Fidelity PCR System (Roche Applied Science) with a Perkin Elmer Gene Amp PCR System 2400 thermocycler and 50 ng of genomic DNA, 10 µM of forward and reverse primers (Table 1), 200 µM of each dNTP, and 5 µl of Expand High Fidelity buffer, 10× conc. with 15 mM $MgCl_2$ in a total reaction volume of 50 µl. The thermal cycling was 1 cycle at 94°C for 2 min, ten cycles of 94°C for 15 s, 50°C for 30 s, 68°C for 10 min, 20 cycles of 94°C for 15 s, 50°C for 30 s, 68°C for 10 min plus 5 s for each cycle, and 1 cycle 68°C for 15 min. The amplicons were cloned in the vector pGEM3Zf(+) (Promega).

Table 1 Oligoprimers used for LA PCR amplification

Designation	Description	5′–3′ Sequence[a]
asr-dir-PS	nt 195-220	***C****ATG****G***AACAACAAGAAACAGTTACCCG
asr-inv-PS	nt 6335-6365	AGCTTGCAAAGCACGCTTATC*AAT*CCATAGC
dsrS-dir-PS	nt 230-263	*ATG*CCATTTACAGAAAAAGTAATGCGGAAAAAGC
dsrS-inv-PS	nt 4809-4780	GTATGCTGACACAGCATTTCCATTATTATCA*AAT*TGG
dsrE-dir-PS	nt 567-593	*ATG*AGAGACATGAGGGTAATTTGTGAC
dsrE-inv-PS	nt 9070-9045	AATTTGAGGT*AAT*GTTGATTTATCACC

ATG and *AAT* in italic type represent, respectively, the start and stop codons. Nucleotides in *bold type* and *underlined* represent mismatches with the sequence bearing *asr* in order to transform the start codon in a *Nco*I cleavage site

nt nucleotides

[a] Primer sequences are designed from the non-coding strand (-dir-PS) and from the complementary strand (-inv-PS)

DNA sequencing and sequence analysis

Amplicons were sequenced in both directions by Genome Express, France. Nucleotide sequence analysis was performed with Vector NTI 10.0 software (Invitrogen). Sequence alignment and phylogeny estimation were done using the program MAFFT, version 6.717b (available at http://align.bmr.kyushu-u.ac.jp/mafft/software). The sequence alignment was performed by using the L-INS-I method and the default parameters of the program. The tree was constructed using the neighbor-joining method with bootstrap values for 500 replicates.

ClustalW2 [20] and BLAST [2] Internet programs were used to perform the sequence alignments of the dextransucrase open reading frame (ORF). ClustalW2 is available at http://www.ebi.ac.uk/Tools/clustalw2/index.html.

Results and discussion

Taxonomic classification of *L. citreum* strain B/110-1-2

The strain B/110-1-2 is a microaerophilic, nonmotile, nonspore-forming Gram-positive bacterium. Cells are ovoid and arranged in chains of 3–4 cocci. Colonies on sucrose solid media are viscous. This strain was primarily identified as *L. mesenteroides* on the basis of its morphological properties and by the API 50-CH test (bioMérieux, Marcy l'Etoile, France). However, sequence analysis of the 16S rDNA operon (GenBank accession number FJ716698) showed that strain B/110-1-2 shares a 99% identity with the *L. citreum* strains included in the analysis (Fig. 1). It appears that taxonomy based only on phenotypic or biochemical tests is not enough to detect differences between *L. mesenteroides* and *L. citreum* because several former *L. mesenteroides* strains [CW28, NRRL B-1355, NRRL B-742 (ATCC 13145)] have been reclassified as *L. citreum* based on the sequence analysis of 16S rDNA operon [10, 28, 29].

Dextransucrase activity in cellular and supernatant fractions of *L. citreum* strain B/110-1-2

The strain B/110-1-2 produces both cell-associated and soluble dextransucrase enzymes. Cell-associated (insoluble) dextransucrases represent 31.54% of the total glucosyltransferase activity (Fig. 2IIa). Higher levels (68.46%) of soluble dextransucrase activity detected in the culture supernatant are in agreement with the extracellular localization of most dextransucrases produced by lactic-acid bacteria belonging to the genus *Leuconostoc* [24, 37]. In *L. mesenteroides* NRRL B-1299 (ATCC 11449) an insoluble form of dextransucrase is responsible for the 60–95% of the whole glucosyltransferase (dextransucrase) activity produced [18, 33, 39]. The *L. citreum* NRRL B-1355 strain produces an alternansucrase (EC 2.4.140), that is predominantly cell-associated and it represents less than 10% of the whole glucosyltransferase activity in the supernatant fraction of active young cultures [45]. All of this is supported by the fact that the N-terminal domain from almost all sequenced dextransucrase encoding genes is preceded by a well-conserved signal peptide, leading to secretion of the enzymes to the extracellular medium [42].

The dextransucrase zymograms of cell and supernatant fractions of B/110-1-2 show this strain produces four dextransucrases: three cell-associated and a soluble enzyme. The soluble form and one of the cell-associated forms share an approximate molecular weight of 160–180 kDa (Fig. 2II-b, II-b′). Enzymes with similar sizes have been found in several *L. mesenteroides* strains, for example: in *L. mesenteroides* NRRL B-512F the principal dextransucrase produced is a protein of 170 kDa (DSR-S) that corresponds to the product of the gene (*dsr*S) [43], DSR-B in *L. mesenteroides* NRRL B-1299 [23],

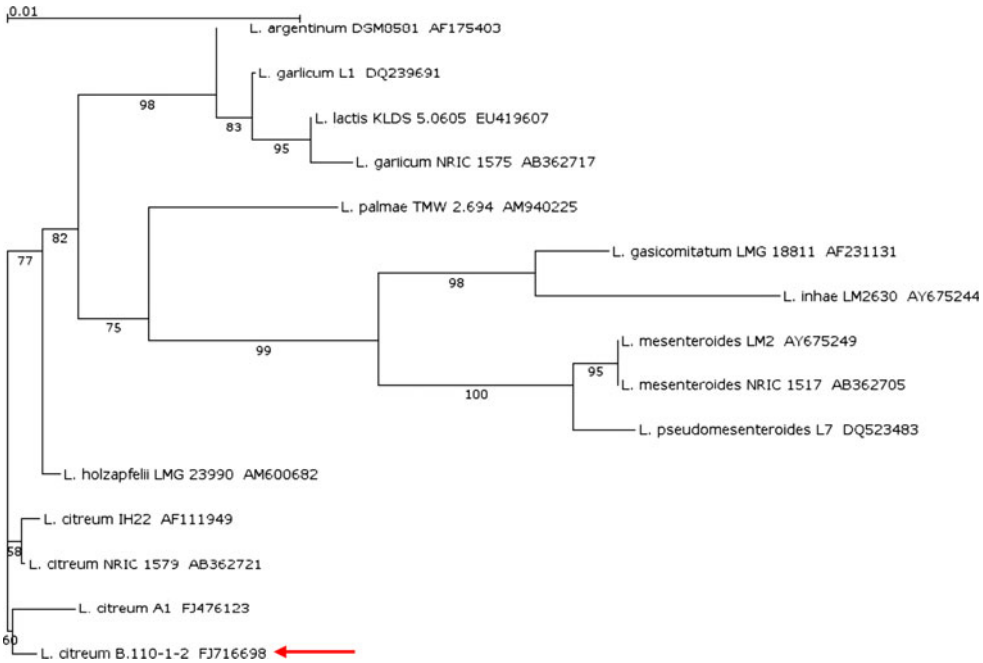

Fig. 1 Phylogenetic position of strain B/110-1-2 within the genus *Leuconostoc*. Accession numbers of the sequences in the GenBank database are shown. The strain B/110-1-2 is indicated by an *arrow*

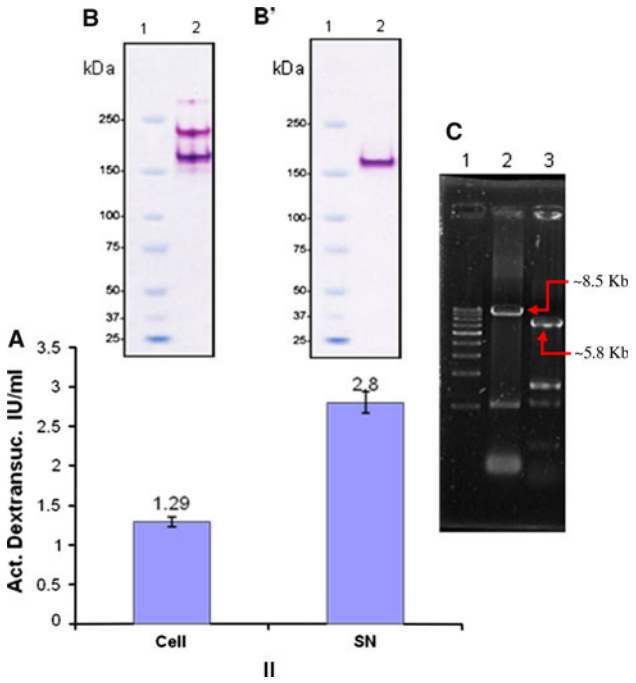

Fig. 2 Dextransucrase production and detection of dextransucrase amplicons in *L. citreum* strain B/110-1-2. Quantification of dextransucrase activity (**a**) and zymograms (**b**) of cellular (Cell) and supernatant (SN) fraction, 1b: protein marker (Bio-Rad). **c** DNA electrophoresis of 8.5 kb (2c) and 5.8 kb (3c) amplicons encoding for dextransucrase enzymes amplified by LA PCR with primers dsrE-dir-PS, dsrE-inv-PS and asr-dir-PS, asr-inv-PS, respectively. 1c: 1-kb DNA Step Ladder (Promega)

DSR-D in *L. mesenteroides* Lcc4 [27], DSR-P in *L. mesenteroides* IBT-PQ [9, 30], DsrX in *L. mesenteroides* CGMCC [44], and dexYG in *L. mesenteroides* 0323 [46]. There are also some examples of *L. citreum* strains having dextransucrase enzymes in the range of 160–180 kDa, like DSR-C in *L. citreum* NRRL B-1355 (GenBank accession number: CAB76565.1) [Arguello-Morales unpublished result], DSR-b742 in *L. citreum* NRRL B-742 (ATCC 13146) (GenBank accession number: AAG38021.1) [17]. Recently it has been reported that the gene encoding dextransucrase (LcDS) from *L. citreum* HJ-P4 is also around 170 kDa [1].

Another cell-associated dextransucrase produced by the strain B/110-1-2 has an approximate molecular weight of 220–240 kDa (Fig. 2II-b). A similar molecular weight has been found in the *L. citreum* NRRL B-1355 (ASR alternansucrase) [3]. Kim and Robyt [17] reported an activity band of 240 kDa in the constitutive mutant *L. citreum* B-742CB. Nevertheless, Chellapandian et al. [9] suggested that the 240 kDa protein may represent a multimer of dextransucrase subunits. It is possible that the 220–240 kDa cell-associated dextransucrase from *L. citreum* B/110-1-2 described in this study is a similar multimer since no alternansucrase activity was detected in this strain.

Amplification of DNA fragments encoding for dextransucrase enzymes

Two amplicons could be detected from chromosomal DNA of *L. citreum* B/110-1-2 (Fig. 2II-c, lines 2 and 3). Analysis of the partial sequence of the 8.5 kb amplicon, amplified with the primers dsrE-dir-PS and dsrE-inv-PS (Fig. 2II-c, line 2) revealed that it shares 98% identity with the sequence of dextransucrase DSR-E from the strain *L. mesenteroides* NRRL B-1299 [5] and with a putative

dextransucrase encoding sequence found in the genome of *L. citreum* KM20. A dextransucrase of molecular mass of approximately 313 kDa (like DSR-E) was detected with a very low expression level in zymograms (cellular fraction) of the strain *L. citreum* B/110-1-2 (Fig. 2II-b, line 2), although it could not be detected by nuclear magnetic resonance (^{13}C-NMR) spectroscopy dextrans with the linkage pattern like the ones produced by DSR-E enzyme (Fig. 4).

The sequence analysis of the approximately 5.8-kb amplicon from chromosomal DNA of B/110-1-2, obtained with the primers asr-dir-PS and asr-inv-PS (Fig. 2II-c, line 3) permitted the identification of a novel dextransucrase open reading frame (ORF) (GenBank accession number: FJ844434), sharing a 52% identity with the ASR of *L. citreum* NRRL B-1355 and with a putative alternansucrase sequence in the genome of *L. citreum* KM20.

Analysis of oligosaccharides and dextran produced by *L. citreum* B/110-1-2 dextransucrases

The acceptor reaction products formed by supernatant and cell-associated dextransucrases of *L. citreum* B/110-1-2 were compared with those produced by dextransucrase DSR-S, from *L. mesenteroides* NRRL-B-512F. In the presence of maltose as the acceptor molecule, the B/110-1-2 soluble dextransucrase synthesizes a series of isomaltodextrin analogs very similar to the ones produced by the DSR-S. These products range from the trisaccharide panose to homologous saccharides harboring 12 units of glucose [10]. The specificity of the soluble dextransucrase of B/110-1-2 strain (similar to one of DSR-S) was confirmed by ^{13}C-NMR analysis of the high-molecular-weight (HMW) dextran produced by the supernatant fraction, which was identical to the dextran produced by the DSR-S enzyme (data not shown).

The use of high-performance anionic exchange chromatography (HPAEC-PAD) enabled the detection of the B/110-1-2 cell-associated dextransucrases which synthesized malto-oligosaccharides with retention times very similar to the ones produced by the DRS-S as well, except that two additional peaks clearly appeared immediately after the products with degrees of polymerization (DP) of seven and eight, respectively (data not shown). High-performance size-exclusion chromatography profiles (HPSEC) of the products from the polymerization reaction carried out by the cell-associated dextransucrases of the *L. citreum* strain B/110-1-2 showed that part of the oligosaccharides were not elongated and were accumulated in the reaction medium (DP < 8) whereas the others were elongated until formation of a HMW dextran polymer (2×10^6 Da) occurred (Fig. 3). This is a very similar pattern to that described for the alternansucrase ASR from the *L. citreum* strain NRRL-B-1355 [26]. ^{13}C-NMR analysis (Fig. 4) of this dextran (produced by the cell-associated dextransucrases of the *L. citreum* strain B/110-1-2) showed a case of intermediate branching, represented by three residues of D-glucosyl joined by α-D-(1,6) links, and one 1,4,6-tri-*O*-substituted by

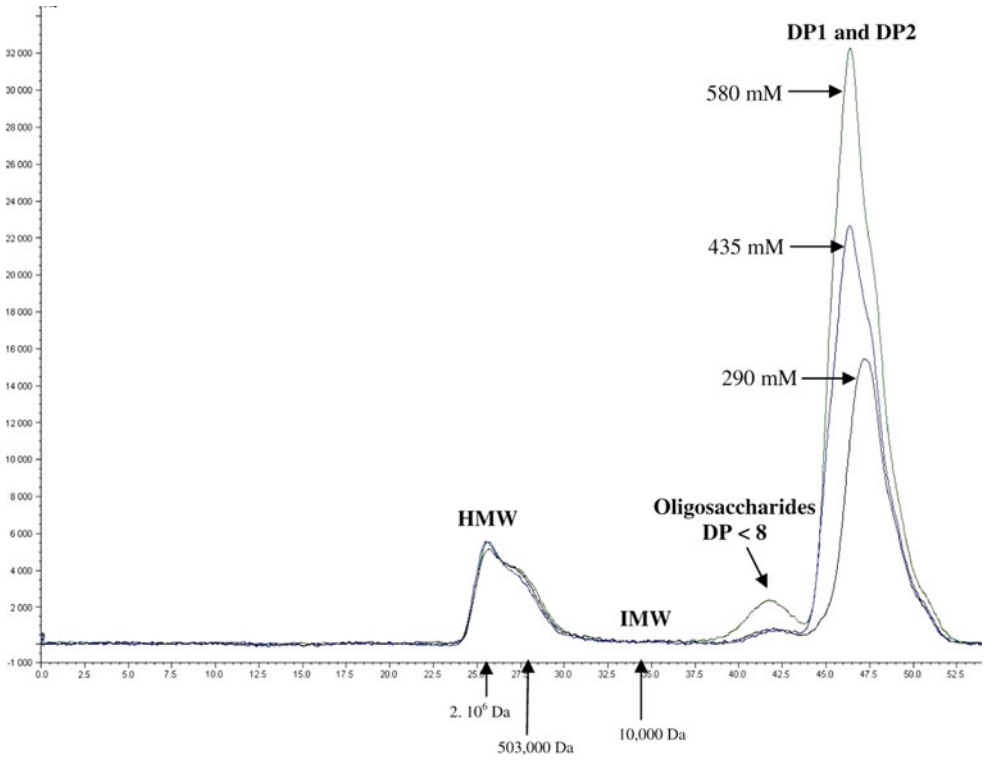

Fig. 3 HPSEC profiles of the products synthesized by *L. citreum* B/110-1-2 cell-associated dextransucrases during the polymerization reaction from sucrose 290, 435, and 580 mM, respectively. *HMW* high-molecular-weight polymers, *IMW* products of intermediate molecular weight, *DP2* sucrose, *DP1* monosaccharides

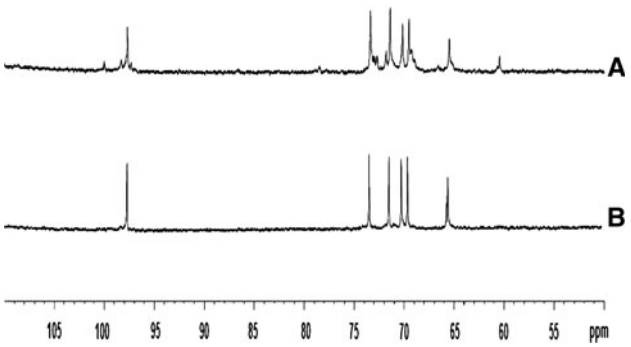

Fig. 4 ^{13}C NMR spectra of glucan polymers produced by *L. citreum* B/110-1-2 cell-associated dextransucrases (*A*). Dextran T-70 produced by *L. mesenteroides* NRRL B-512F DSR-S dextransucrase (*B*)

a non-reducing D-glucosyl group. The two additional picks, minor picks which appear in the region of 60–70 ppm, are due to the sugars in the branching and the non-reducing ends. The anomeric region also shows two minor picks related to the sugars of the branching and the non-reducing end terminal. The pick shown in the region 75–85 ppm (79.54) is assumed to be the C-4 atom involved in the branching. A similar ^{13}C-NMR profile was previously found in the dextran present in fraction L of *Streptobacterium dextranicum* NRRL B-1254 [36], and in the fraction L of *L. citreum* NRRL-B-742 (ATCC 13146) [38]. On the other hand, Brossard [6] working on the structural determination of the dextran produced by the whole culture (supernatant and cell fraction together) of the strain B/110-1-2, found this dextran formed by α(1–6) (93%), α(1–3) (6%) links and α(1–4) (1%) branches. According to the results of the present study, it is concluded that this type of dextran polymer is produced by the cell-associated dextransucrases of the *L. citreum* strain B/110-1-2, or at least by one of them. Further characterization of the novel dextransucrase open reading frame (ORF) identified in this study will be crucial for the proper identification of the dextransucrase responsible for the synthesis of the above-mentioned polymer.

The data obtained in this study enhances the understanding of this industrially significant strain and will aid in distinguishing between physiologically similar *Leuconostoc* strains. As the cell-associated dextransucrases from *L. citreum* B/110-1-2 are able to produce branched oligosaccharides it may be easily incorporated as an immobilized biocatalyst for linear α-1,6 and α-1,4 branched isomaltooligosaccharide production. Such oligosaccharides have potential applications as prebiotics, i.e., non-digestible carbohydrates, which are specifically metabolized by beneficial strains of the human or animal intestinal microbiota.

Acknowledgments R. Fraga holds a contract as Research Associated from INRA, Toulouse. We express our gratitude to Elisse Champion and Richard Guyton by their technical support and advice for the HPAEC-PAD and HPSEC analysis, respectively. We also thank Dr. Joan Combie for the style correction of the manuscript.

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