Carbohydrate Polymers 101 (2014) 554–564

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Weissella confusa Cab3 dextransucrase: Properties and *in vitro* synthesis of dextran and glucooligosaccharides

Shraddha Shukla[a], Qiao Shi[b], Ndegwa H. Maina[b], Minna Juvonen[b], MaijaTenkanen[b], Arun Goyal[a,*]

[a] Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati 781 039, Assam, India
[b] Department of Food and Environmental Sciences, P.O. Box 27, FI-00014 University of Helsinki, Finland

ARTICLE INFO

Article history:
Received 7 August 2013
Received in revised form
20 September 2013
Accepted 25 September 2013
Available online 3 October 2013

Keywords:
Weissella confusa
Dextransucrase
Dextran
Glucooligosaccharides

ABSTRACT

Food-derived *Weissella* spp. have gained attention during recent years as efficient dextran producers. *Weissella confusa* Cab3 dextransucrase (WcCab3-DSR) was isolated applying PEG fractionation and used for *in vitro* synthesis of dextran and glucooligosaccharides. WcCab3-DSR had a molar mass of 178 kDa and was activated by Co^{2+} and Ca^{2+} ions. Glycerol and Tween 80 enhanced enzyme stability, and its half-life at 30 °C increased from 10 h to 74 h and 59 h, respectively. The ^{1}H and ^{13}C NMR spectral analysis of the produced dextran confirmed the presence of main chain α-(1→6) linkages with only 3.0% of α-(1→3) branching, of which some were elongated. An HPSEC analysis in DMSO revealed a high molecular weight of 1.8×10^{7} g/mol. Glucooligosaccarides produced through the acceptor reaction with maltose, were analyzed with HPAEC-PAD and ESI-MS/MS. They were a homologous series of isomaltooligosaccharides with reducing end maltose units. To the best of our knowledge, this is a first report on native *W. confusa* dextransucrase.

1. Introduction

Various lactic acid bacteria (LAB) among genera *Leuconostoc*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, and *Weissella* produce extracellular α-glucooligosaccharides (GLOS) and polysaccharides catalyzed by glucansucrases (often also called glucosyltransferases, EC 2.4.1.5) (Leemhuis et al., 2013; Patel, Kothari, & Goyal, 2011). The α-glucans differ in their glycosidic linkage composition and organization and are classified into dextrans, mutans, alternans, and reuterans (Leemhuis et al., 2013). The glucansucrases are named accordingly, based on the α-glucans they synthesize, such as dextransucrases (DSR) and alternansucrases (ASR). Because of the hydrocolloid properties and the potential health benefits of α-glucans, they are of high interest in food applications (Hernández, Livings, Aguilera, & Chiralt, 2011; Patel, Majumder, & Goyal, 2012).

Dextrans are α-glucans that contain consecutive α-(1→6) linkages in the main chain and α-(1→2), α-(1→3), or α-(1→4) branch linkages (Bounaix et al., 2009; Naessens, Cerdobbel, Soetaert, & Vandamme, 2005). The type and percentage of branch linkages depend on the dextransucrases elaborated by individual LAB. Dextransucrases catalyze dextran synthesis and are classified as part of the glycoside hydrolase (GH) family 70, which contains solely glucansucrases/glucosyltransferases. The GH70 enzymes are mechanistically, structurally, and evolutionary closely related to GH13 and GH77 enzymes (Stam, Danchin, Rancurel, Coutinho, & Henrissat, 2006). Although glucansucrases are classified as transferases based on their activity, structurally, they are close to glycoside hydrolases. Indeed, the first step in the synthesis is the hydrolysis of sucrose, from which the glucosyl residue is transferred to the growing α-glucan chain. The three-dimensional structure of truncated glucansucrases has recently become available. Based on the findings, it has been proposed that α-glucans are synthesized through the step-wise addition of glucose moieties to the non-reducing end of growing chains (Brison et al., 2012; Ito et al., 2011; Leemhuis et al., 2013; Vujicic-Zagar et al., 2010). When exogenous acceptor molecules are present beside sucrose, glucose moieties can also be transferred to them, and the resulting molecules may in turn act as acceptors, giving rise to acceptor products in addition to α-glucans. The product pattern of glucansucrase for a specific acceptor depends on enzyme specificity. *Leuconostoc mesenteroides* NRRL B-512F dextransucrase predominantly synthesizes α-(1→6) linkages. It produces homologous GLOS in the presence of maltose, with additional glucose moieties all α-(1→6) linked (Robyt & Eklund, 1983). Because of its α-(1→2)-branch synthesizing property, *Lc. mesenteroides* NRRL B-1299 dextransucrase synthesizes three homologous GLOS families with maltose, the major family,

* Corresponding author. Tel.: +91 361 258 2208; fax: +91 361 258 2249.
E-mail address: arungoyl@iitg.ernet.in (A. Goyal).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.09.087

constituting linear α-(1→6) elongated GLOS. The second and third families have α-(1→6)-linked main chains, with a single unit α-(1→2) branch respectively at the terminal and the penultimate unit of the non-reducing end (Dols, Remaud-Simeon, Willemot, Vignon, & Monsan, 1997).

The dextran-producing *Weissella* spp. have received significant attention in the recent years due to their relatively higher dextran production ability, as compared to other lactic acid bacteria, especially in sourdough bread applications (Di Cagno et al., 2006; Galle, Schwab, Arendt, & Gänzle, 2010; Galle et al., 2012; Katina et al., 2009; Schwab, Mastrangelo, Corsetti, & Gänzle, 2008). The genus *Weissella* was proposed in 1993 after reclassification of some *Leuconostoc*-like bacteria (Collins, Samelis, Metaxopoulos, & Wallbanks, 1993). They are rod-shaped heterofermentative LAB from the family *Leuconostocaceae*. Dextran production is a phenotypic criterion for the identification of bacteria under this genus. *Weissella* strains have been isolated from various sources, such as sourdough breads and vegetables (Ahmed, Siddiquia, Armanb, & Ahmeda, 2012; Bounaix et al., 2010; Shukla & Goyal, 2011). Dextrans produced by *Weissella* strains have been reported to possess highly similar structures, consisting of predominantly α-(1→6) linkages and only a few α-(1→3) branch linkages (2.4–4%), with some of the branches elongated (Ahmed et al., 2012; Bejar et al., 2013; Bounaix et al., 2009; Maina, Tenkanen, Maaheimo, Juvonen, & Virkki, 2008; Maina, Virkki, Pynnönen, Maaheimo, & Tenkanen, 2011). They contain less branch linkages than the most commercially used *Lc. mesenteroides* NRRL B-512F dextran. In wheat sourdough, *Weissella confusa* VTT E-90392 synthesized a high yield of dextran, while *Lc. mesenteroides* NRRL B-512F produced almost none under the same conditions. *In situ*-produced *W. confusa* dextran improved the shelf life, volume, and softness of sourdough wheat bread. These positive effects were attributed to the low degree of branching, the high molecular weight of *W. confusa* dextran, and possibly to the GLOS produced concomitantly through acceptor reaction with maltose (Katina et al., 2009). *In situ*-produced exopolysaccharides by LAB are regarded as natural food thickeners from fermentation and could replace other added hydrocolloid additives, leading to clean label foods.

Dextransucrases from *Weissella* spp., especially *W. confusa*, had not been studied until recently. Several dextran-producing *W. cibaria* and *W. confusa* strains exhibited constitutive dextransucrase activity, assigned to a 180 kDa protein (Bounaix et al., 2010). The effect of temperature, pH, and ionic strength on the activity of crude *W. confusa* Cab3 dextransucrase (WcCab3-DSR) has been reported (Shukla & Goyal, 2011). In 2012, the first complete gene sequence encoding dextransucrase from *W. confusa* strain (LBAE K39) became available, followed by the expression of the gene in *E. coli* and the characterization of the recombinant dextransucrase (Amari et al., 2012). The crude dextransucrase from pear-derived *Weissella* sp. TN610 has also been characterized. It induced the solidification of sucrose-supplemented milk and was suggested as a promising, safe food additive (Bejar et al., 2013).

In the present study, the dextransucrase from the highly efficient dextran producer *W. confusa* Cab3, WcCab3-DSR, originating from fermented cabbage, was partially purified using polyethylene glycol partitioning and functionally characterized. The dextran produced by *in vitro* enzyme synthesis was characterized using FT-IR spectroscopy, NMR spectroscopy, and HPSEC. The GLOS produced by acceptor reaction with maltose were purified and analyzed by tandem mass spectrometry. To the best of our knowledge, this was the first study characterizing a native *W. confusa* dextransucrase. Knowing the properties of *W. confusa* dextransucrase and its dextran and GLOS products enhances the usability of this potential dextran producer.

2. Materials and methods

2.1. Microorganism and culture conditions

The bacterial strain *W. confusa* Cab3 (Genbank Accession Number JX649223) isolated from fermented cabbage (Shukla & Goyal, 2011) was used for the production of WcCab3-DSR. The microorganism was maintained in a modified MRS medium (Goyal & Katiyar, 1996) as stabs incubated at 25 °C, stored at 4 °C, and subcultured every two weeks. For the preparation of the inoculum of *W. confusa* Cab3, a loopful of culture from a modified MRS agar stab (Goyal & Katiyar, 1996) was transferred to a 5 ml medium as described by Tsuchiya et al. (1952). *W. confusa* Cab3 was incubated at 25 °C at 180 rpm for 12 h; 1% of the culture was again inoculated in a 2 × 100 ml enzyme production medium in a 250 ml Erlenmeyer flask and incubated at 25 °C for 12 h. The broth was centrifuged at 8000 × *g* at 4 °C for 10 min and the cell-free supernatant containing WcCab3-DSR was subjected to purification.

2.2. Enzyme purification

The cell-free supernatant of *W. confusa* Cab3 was fractionated by polyethylene glycol according to the method established by Purama & Goyal (2008). The ice-cold polyethylene glycols of two different molecular weights, i.e., PEG-400 (Qualigens Pvt. Ltd., India) and PEG-1500 (BDH, U.K.), were added to 30 ml of the cell-free supernatant. The PEG-400 concentration varied from 10 to 50% (v/v); 40% (w/v) of the PEG-1500 solution was prepared in distilled water and added to the 30 ml cell-free supernatant to get final concentrations from 5 to 25% (w/v). The mixtures were incubated at 4 °C for 12 h to allow the dextransucrase (WcCab3-DSR) to fractionate, after which they were centrifuged at 12,000 × *g* for 30 min at 4 °C to separate the WcCab3-DSR. The enzyme pellets were dissolved in a 20 mM sodium acetate buffer pH 5.4 and subjected to dialysis against the same buffer using a 14 kDa cut off membrane. The samples were analyzed for dextransucrase activity and protein content.

2.3. Dextransucrase activity assay

The activity assay of WcCab3-DSR was conducted in 1 ml of a reaction mixture containing 20 mM sodium acetate buffer pH 5.4, 5% sucrose, and a 20 μl enzyme sample. The reaction mixture was incubated at 35 °C for 15 min. Aliquots (100 μl) from the reaction mixture were analyzed for reducing sugars by the method described by Nelson (1944) and Somogyi (1945). The absorbance was recorded at 500 nm in a UV–visible spectrometer (Varian, model Cary 100) against a blank. D-Fructose (Hi-Media Pvt. Ltd., India) was used as a standard.

2.4. Protein determination

The protein content of the cell-free extract containing WcCab3-DSR and partially purified WcCab3-DSR were estimated by the method established by Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin as a standard.

2.5. SDS-PAGE analysis

SDS-polyacrylamide gel electrophoresis was performed with a vertical slab mini-gel unit (BioRad) using 1.5 mm thick gel, following the method established by Laemmli (1970); 7.0% (w/v) acrylamide for resolving gel and 4% (w/v) for stacking gel were prepared. For running the enzyme samples (WcCab3-DSR) under non-denaturing conditions, the sample buffer (5×) used did not contain β-mercaptoethanol and the enzyme sample mixed with the sample buffer (62.5 mM Tris–HCl buffer pH 6.8, containing 2.0%,

w/v sodium dodecyl sulfate, 20%, w/v glycerol, and 0.025%, w/v bromophenol blue), was not subjected to heat denaturation. The samples were loaded in duplicate on 7.0% acrylamide gel for non-denaturing SDS-PAGE. The electrophoresis was carried out using a running buffer (200 mM glycine, 0.1% SDS, 50 mM Tris–HCl pH 8.3) with a current of 2 mA per lane. Following the run, the gel was cut into two parts. One part of the gel was subjected to silver staining and the other part of was analyzed for *in situ* activity detection by periodic acid (PA)-Schiff staining protocol (Miller & Robyt, 1986a).

The WcCab3-DSR isolated by 30%, w/v PEG-400 was also run under native condition on a 7.0% gel and subsequently analyzed by periodic acid (PA)-Schiff staining protocol (Miller & Robyt, 1986a). For this the enzyme sample was prepared in 62.5 mM Tris–HCl buffer (pH 6.8), containing 20% (w/v) glycerol, and 0.025% (w/v) bromophenol blue. The electrophoresis was carried out using running buffer (200 mM glycine, 50 mM Tris–HCl pH 8.3) with a current of 2 mA per lane.

2.6. Effects of divalent metals, additives and temperature on WcCab3-DSR

The effects of different divalent cations using $MgCl_2$, $CaCl_2$, $CoCl_2$, $NiSO_4$, $ZnCl_2$, $MnCl_2$ salt solutions (0–10 mM), and EDTA (0–7 mM) on WcCab3-DSR activity was evaluated. The assays were carried out in a 1.0 ml reaction mixture containing salt, WcCab3-DSR preparation (10.5 U/mg, 0.84 mg/ml), and a substrate as described earlier.

The effects of various additives on the stability of WcCab3-DSR at 30 °C were tested. Aqueous solutions of dextran (500 kDa) (Sigma–Aldrich Pvt. Ltd., USA), PEG-8000 (Hi-Media Pvt. Ltd., India), glutaraldehyde (Qualigens Pvt. Ltd., India), glycerol (Qualigens Pvt. Ltd., India) and Tween 80 (Merck, India) were added to 1.0 ml WcCab3-DSR (10.5 U/mg, 0.84 mg/ml) to obtain final concentrations of 2 μg/ml (w/v) dextran (500 kDa), 10 μg/ml (w/v) PEG-8000, 0.1% (v/v) glutaraldehyde, 0.5% (v/v), glycerol and 0.1% (v/v) Tween 80, respectively. Storage stability was also studied by incubating the WcCab3-DSR (10.5 U/mg, 0.84 mg/ml) at various temperatures (0 °C, 4 °C, and −20 °C). The aliquots (20 μl) were taken periodically for residual enzyme activity assays as described earlier. The residual activity and half-life ($t_{1/2}$) of WcCab3-DSR were measured at various temperatures (30 °C, 4 °C, and −20 °C) with respect to time without any additives. The half-life ($t_{1/2}$) of additives-treated WcCab3-DSR was determined only at 30 °C. For determining the thermostability, the WcCab3-DSR preparation (10.5 U/mg, 0.84 mg/ml) was incubated at different temperatures (10–50 °C) for 1 h and the aliquots (10–20 μl) were assayed for residual enzyme activity.

2.7. In vitro synthesis of dextran

For WcCab3-DSR dextran synthesis, 24.0 ml of WcCab3-DSR (10.5 U/mg, 0.84 mg/ml) in 200 ml of 5% (w/v) sucrose in a 20 mM sodium acetate buffer pH 5.4 containing 0.3 mM $CaCl_2$ and 15 mM sodium azide was incubated at 35 °C for 24 h. The viscous solution (200 ml) containing the enzyme-synthesized WcCab3-DSR dextran was treated with three volumes of ethanol (90%, v/v) to precipitate the dextran. The solution was centrifuged at 12,000 × *g* at room temperature for 20 min and the pellet was re-suspended in 500 ml of deionized water. The dextran was precipitated again and the same process was repeated two more times to ensure removal of sucrose and fructose residues. Finally, the precipitated dextran was dissolved in 500 ml of deionized water. The purified dextran solution was analyzed for protein content using bovine serum albumin as the standard (Lowry et al., 1951). The solution was then freeze-dried using a lyophilizer (Christ GmbH, model ALPHA 1-4 LD) at −51 °C at a vacuum pressure of 35×10^{-3} mbar for 24 h. The sample was stored at room temperature for further characterization.

2.8. Production of GLOS by acceptor-reaction

The acceptor products (GLOS) were produced by WcCab3-DSR using maltose as the acceptor molecule following the method established by Dols et al. (1997). The reaction mixture (100 ml) contained 10% (w/v) sucrose, 5% (w/v) maltose, and 12.0 ml of WcCab3-DSR (10.5 U/mg, 0.84 mg/ml) in a 20 mM sodium acetate buffer pH 5.4. The reaction was allowed to proceed for 24 h at 35 °C. The enzyme action was terminated by keeping the reaction mixture in a boiling water bath for 10 min, after which, polymeric dextran was removed by ethanol precipitation with three volumes of pre-chilled ethanol (AltiaEtax A, Finland). After being centrifuged at 12,000 × *g* for 10 min, the supernatant containing fructose, residual sucrose, residual maltose, and GLOS was concentrated to 22 ml with a rotary vacuum evaporator (Heidolph LABOROTA digital 4001) and is referred to as the acceptor-product solution henceforth.

GLOS were purified according to the degree of polymerization (DP) by gel permeation chromatography (GPC) using a Biogel P2 column (5 cm × 95 cm; Biorad, Hercules, USA) with water as the eluent. A 5 ml of 10-fold diluted acceptor-product solution was filtered with 0.45 μm membrane filters (Acrodisc 13, Pall Corporation, Ann Arbor, MI, USA) and injected into the column. The flow rate was first kept at 1.0 ml/min to elute void volume (600 ml) and adjusted to 0.3 ml/min for the collection of 4 ml fractions. The fractions were analyzed for oligosaccharides by HPAEC-PAD (Section 2.10), and the selected fractions of isolated GLOS of varying degrees of polymerization (DP) were subsequently analyzed by tandem mass spectrometry (Section 2.15).

2.9. Estimation of total carbohydrate content

The total carbohydrate content of the purified WcCab3-DSR dextran and acceptor-product solution was determined by the phenol–sulphuric acid method in a micro-titre plate (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Fox & Robyt, 1991). To 25 μl of a sample in a microtitre plate, 25 μl of 5% (v/v) phenol was added and mixed by shaking the plate on a vortex mixer for 30 s. The plate was placed on an ice bath, and 125 μl of concentrated sulphuric acid was added to each well containing the mixture. The plate was again shaken for 30 s to ensure proper mixing of the contents of the wells. The plate was wrapped in a cling film and incubated in a water bath at 80 °C for 30 min. After cooling to room temperature, the absorbance was determined at 490 nm on a multimode microplate reader (Tecan, model Infinite™ 200). Standard graphs using dextran T40 (Sigma–Aldrich, USA) and glucose (Hi-Media Pvt. Ltd., India) in the concentration range 0.1–1.0 mg/ml were plotted for the estimation of the total carbohydrate content of WcCab3-DSR dextran and acceptor-product solution, respectively.

2.10. Mono- and oligosaccharide analysis

Monosaccharide analysis of WcCab3-DSR dextran was carried out by high performance anion exchange chromatography (HPAEC); 50 mg of lyophilized WcCab3-DSR dextran sample was hydrolyzed with 1 ml of 50 mM sulfuric acid at 100 °C for 6 h. The hydrolyzed dextran was neutralized by adding a 2 M Na_2CO_3 (~100 μl) solution (filtered with a 0.2 μm membrane). The sample was analyzed by HPAEC-PAD using an ion-chrome system (ICS-3000 system, equipped with the ICS-3000 detector/chromatographic module, Dionex Corporation, USA) with the CarboPac PA-20 analytical column (3 mm × 150 mm) and the CarboPac PA-20 guard column (3 mm × 30 mm). The temperature of the column was adjusted to 30 °C and the injection volume was

25 μl. The eluent used was 250 mM NaOH at 0.2 ml/min. D-Glucose and D-fructose (Hi-Media Pvt. Ltd., India) were used as external standards.

WcCab3-DSR dextran hydrolyzed with a mixture of enzymes (Section 2.14) and acceptor-product solution were analyzed by HPAEC-PAD-applying equipment and the method described by Rantanen et al. (2007). The samples were filtered using 0.45 μm membrane filters (Acrodisc 13, Pall Corporation, Ann Arbor, USA), and the injection volume was 10 μl. D-Glucose (Merck, Germany), D-fructose (Merck), sucrose (Merck), maltose (Sigma–Aldrich), isomaltose (TCI Europe, Belgium), isomaltotriose (TCI Europe), and panose (TCI Europe) were used as external standards.

2.11. Fourier-transform infrared spectrometric analysis

Fourier transform infra-red (FT-IR) spectrum of WcCab3-DSR dextran was recorded in the frequency range of 4000–400 cm^{-1} (Perkin-Elmer Instruments, Spectrum One FT-IR Spectrometer). The dried sample was ground with potassium bromide powder and pressed into pellet for FT-IR measurement.

2.12. Nuclear magnetic resonance spectroscopy analysis

Nuclear magnetic resonance (NMR) spectra of WcCab3-DSR dextran were recorded at 50 °C with a 600 MHz NMR spectrometer (Bruker Avance III NMR spectrometer). The dextran (10 mg/ml) was exchanged three times with D_2O and placed in NMR tubes (Wilmad NMR tubes, 5 mm, ultra-imperial grade, from Aldrich chemical company, Milwaukee, WI, USA). The chemical shifts were referenced to internal acetone (^1H = 2.225 ppm and ^{13}C = 31.55 ppm).

2.13. High performance size exclusion chromatography

For the molar mass analysis, a 2.0 mg/ml solution of the WcCab3-DSR dextran was prepared in DMSO with 10 mM LiBr. The high-performance, size-exclusion chromatography (HPSEC) equipment comprised an integrated autosampler and pump module (GPCmax, Viscotek Corp., Houston, TX, USA), a combined light-scattering and viscometric detector (270 Dual Detector, Viscotek Corp.), and a refractive-index (RI) detector (VE 3580, Viscotek Corp.). The light-scattering detector angles were 7° and 90°. Two LF-804 columns (8 mm × 300 mm, exclusion limit 2×10^6, Showa Denko, Tokyo, Japan) with a LF-G (4.6 mm × 10 mm) guard column were used. Before injection, the sample was filtered using a 0.45 μm membrane filter. The flow rate was 1 ml/min and the injection volume was 100 μl. The HPSEC data were processed with the OmniSEC 4.5 software (Viscotek Corp.) to determine the average molecular weight (M_w) of the WcCab3-DSR dextran. A $d\eta/dc$ value of 0.072 ml/g for dextran in DMSO-based eluent was used (Basedow & Ebert, 1979).

2.14. Enzymatic hydrolysis of dextran and GLOS

The *in vitro*-synthesized WcCab3-DSR dextran was treated by a mixture of *Chaetomium erraticum* dextranase (Sigma–Aldrich, Germany) and *Aspergillus niger* α-glucosidase (E-TRNGL, Megazyme, Ireland) as previously described by Maina et al. (2011). The acceptor-product solution was treated separately with dextranase (3.37 nkat/μl acceptor product solution) and *Bacillus stearothermophilis* α-glucosidase (0.42 nkat/μl acceptor product solution, E-TSAGL, Megazyme, Ireland). After boiling to inactivate the enzymes, the residual oligosaccharides were analysed with HPAEC-PAD (Section 2.10).

2.15. Mass spectrometry analysis

Mass spectrometry was used to determine the molar masses of the GLOS obtained by WcCab3-DSR in the presence of sucrose and maltose. To check whether the GLOS-product mixture was homologous in structure, atmospheric pressure matrix-assisted laser desorption/ionization with ion trap mass spectrometry (AP-MALDI-ITMS) was conducted using the acceptor-product solution filtrated with 0.45 μm membrane filters and desalted with a strongly acidic cation exchanger (Dowex® 50W, Fluka, Sigma–Aldrich, Switzerland). The sample was diluted 500-fold with MilliQ-water before MS analysis, which was performed on an Agilent 6330 ion trap mass spectrometer (Agilent Technologies, Waldbronn, Germany) equipped with an AP-MALDI ion source (Mass Tech Inc. Columbia, MD, USA) in the positive ionization mode (sodium adduct ions $[M+Na]^+$) according to the method described by Chong et al. (2011) with modifications. The capillary voltage was set at 3000 V. The ion optics voltages were set as follows: the capillary exit, 300 V; the skimmer potential, 40 V; and the trap drive value, 70.

Furthermore, the purified fractions containing DP 3–5 were analyzed by electrospray ionization–tandem mass spectrometry (ESI-MS/MS), as previously described (Maina et al., 2013). The analysis was carried out in the negative mode (chloride adduct ions $[M+Cl]^-$) with the Agilent XCT Plus Ion Trap mass spectrometer with an electrospray ion source (Agilent Technology, Palo Alto, CA, USA).

3. Results and discussion

3.1. Isolation of WcCab3-DSR

The cell-free supernatant of *W. confusa* Cab3 (6.0 mg protein/ml) contained 6.0 U/ml crude WcCab3-DSR (Table 1). Because polyethylene glycol fractionation is known as a potential method of isolating dextransucrases (Purama & Goyal, 2008), isolation of WcCab3-DSR applying PEG-400 (20–45%, v/v) and PEG-1500 (5–20%, w/v) were tested (Table 1). The concentration of 30% (v/v) PEG-400 gave the highest specific activity of 10.5 U/mg with 10-fold purification and 25.5% overall yield (Fig. 1S, Table 1); 15% (w/v) concentration of PEG-1500 resulted in a slightly higher specific activity of 10.9 U/mg, with 11-fold purification and 24% overall yield (Fig. 1S, Table 1). WcCab3-DSR (10.5 U/mg, 0.84 mg/ml) isolated by fractionation with 30% (v/v) PEG-400 was selected for further experiments, because PEG-400 is more economical to use than PEG-1500.

WcCab3-DSR obtained after fractionation using 30% PEG-400 and 15% PEG-1500 were analyzed in a single SDS-PAGE gel in duplicate run under a non-denaturing condition (Fig. 1). WcCab3-DSR showed a single band of about 178 kDa molecular mass on the non-denaturing gel stained for proteins (Fig. 1, lanes 1 and 2). The other part of the gel was subjected to activity staining for *in situ* activity detection. The presence of magenta color bands after PAS staining (Fig. 1, lanes 3 and 4) were consistent with the protein staining of the dextransucrase bands. The appearance of some minor faint bands in the activity stained gel (Fig. 1, lanes 3 and 4) might be due to the slight degradation of WcCab3-DSR under the non-denaturing SDS condition applied during their run. Furthermore, to confirm this, WcCab3-DSR isolated by PEG-400 (30%, w/v) was also run under native condition and analysed by PAS staining (Fig. 1, lanes 5). The presence of a single activity band on the native-PAGE, confirmed that the WcCab3-DSR exist as a single enzyme.

Table 1
Isolation of WcCab3-DSR by fractionation with polyethylene glycols.

Sample	Enzyme activity (U/ml)	Vol. (ml)	Total units	Overall activity yield (%)	Protein (mg/ml)	Specific activity (U/mg)	Purification fold
Crude	6.01	30.0	180.4	–	6.0	1.0	1.0
PEG-400 (30%)	8.53	5.4	46.1	25.5	0.84	10.5	10.0
PEG-1500 (15%)	4.90	9.0	44.1	24.4	0.45	10.9	11.0

3.2. Effect of temperature and additives on WcCab3-DSR

WcCab3-DSR exhibited a mesophilic nature and showed stability up to 40 °C, above which it rapidly lost activity during 1 h incubation (Fig. 2A). The optimum temperature range for the assay of WcCab3-DSR was also in the range of 30–35 °C (Shukla & Goyal, 2011). Thus, WcCab3-DSR showed a more thermostable nature as compared with the dextransucrase from *Lc. mesenteroides* NRRL B-640, which rapidly lost its activity above 30 °C after 10 min of incubation (Purama, Agrawal, & Goyal, 2010). Dextranscrase from *W. cibaria* JAG8 was also stable up to 35 °C, but further increase in temperature to 40 °C caused the enzyme to lose 96% of its activity (Tinirikari & Goyal, 2013).

Certain metal ions show positive effects on the activity of enzymes. Salts are believed to affect the structure of enzymes, thus affecting their solubility and activity (Forman & Kennedy, 1977). The effects of selected metal ions were studied on WcCab3-DSR activity. The Co^{2+} and Ca^{2+} ions increased the enzyme activity of WcCab3-DSR (Table 2). The enzyme activity was enhanced by 20% and 21% in the presence of 1 mM $CoCl_2$ and $CaCl_2$, respectively (Table 2). Ca^{2+} ion has been reported to be associated with the catalytic sites of dextransucrases (Miller & Robyt, 1986b). $CaCl_2$ (6 mM) increased the activity for *Pediococcus pentosaceus* dextransucrase by 150% and 4 mM $CaCl_2$ by 108% for *Lc. mesenteroides* NRRL B-640 dextransucrase (Patel et al., 2011; Purama et al., 2010). The addition of $ZnCl_2$ (1 mM), $MgCl_2$ (2 mM), and $NiSO_4$ (2 mM) resulted in 9%, 8%, and 7% increase in enzyme activity, respectively (Table 2). On the other hand, $MnCl_2$ affected enzyme activity negatively. At lower 1 mM concentration, $MnCl_2$ decreased the activity by 4%, and at 10 mM $MnCl_2$, the enzyme activity was lost by 42% of its initial value (Table 2). In the presence of 5 mM EDTA, WcCab3-DSR displayed only 2% residual activity (Table 2).

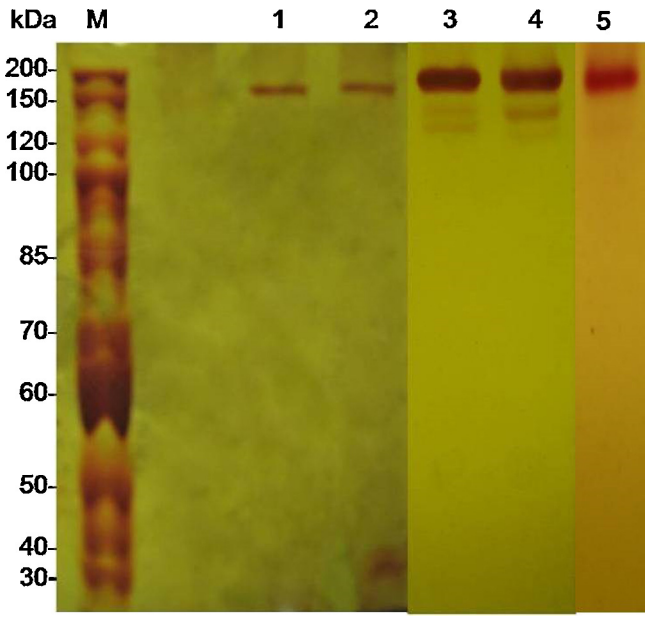

Fig. 1. Non-denaturing SDS-PAGE analyses on 7% acrylamide gel of WcCab3-DSR isolated with PEG-400 (30%, v/v) fractionation (lanes 1 and 3) and PEG-1500 (15%, w/v) fractionation (lanes 2 and 4). Identification of WcCab3-DSR by protein staining (lanes 1 and 2) and staining of dextran formed from sucrose (lanes 3 and 4). PEG-400 (30%, v/v) fraction (lane 5) run on native-PAGE (7% acrylamide gel) and stained with PAS staining using sucrose as substrate. Lane M: protein molecular weight marker (30–200 kDa).

Table 2
Effects of additives on purified WcCab3-DSR.

Effect of metal ions	
Sample	Residual enzyme activity (%)
Control	100
$CoCl_2$ (1 mM)	121
$CaCl_2$ (1 mM)	120
$ZnCl_2$ (1 mM)	109
$MgCl_2$ (2 mM)	108
$NiSO_4$ (2 mM)	107
$MnSO_4$ (10 mM)	58
EDTA (5 mM)	2

Half life ($t_{1/2}$) of WcCab3-DSR treated with various additives and temperatures

Additive	30 °C	4 °C	−20 °C
Control	10.33 h	69.4 d	37.9 d
Glycerol (0.5%)	74.12 h	nd	nd
Tween 80 (1.0%)	59.26 h	nd	nd
PEG-8000 (10 μg/ml)	16.72 h	nd	nd
Dextran T-500 (2 μg/ml)	12.0 h	nd	nd
Glutaraldehyde (0.1%)	9.07 h	nd	nd

nd: not determined.

The effects of different additives on the stability of WcCab3-DSR were studied at 30 °C. The residual activity of WcCab3-DSR after 24 h with glycerol, Tween 80, and PEG 8000 were 80%, 76%, and 37.0%, respectively (Fig. 2B). The dextran (500 kDa) and glutaraldehyde did not stabilize the enzyme, because the residual activity with dextran (500 kDa) and glutaraldehyde after 24 h was only 25% and 16%, respectively (Fig. 2B). The residual activity of WcCab3-DSR with glutaraldehyde at 30 °C after 24 h was even lower than that of WcCab3-DSR without any additive, which was 20%. Thus, glutaraldehyde negatively affected WcCab3-DSR. The storage stability of WcCab3-DSR was also followed at 4 °C and −20 °C. WcCab3-DSR lost only 1.5% and 4% of the activity after 5 d of incubation at 4 °C and −20 °C, respectively (Fig. 2C).

The half-life times ($t_{1/2}$) of WcCab3-DSR and additives-treated WcCab3-DSR were calculated by assuming that the decay followed first-order kinetics (Table 2). Among all the additives used, glycerol and Tween 80 were the most efficient stabilizers, resulting in $t_{1/2}$ of 74.1 h and 59.3 h at 30 °C, respectively (Table 2). A small enhancement in the $t_{1/2}$ (16.7 h) was observed using PEG 8000 at 30 °C as compared with the control (10.3 h). The half-life ($t_{1/2}$) of the enzyme increased from 10.3 h to 69.4 d by decreasing the storage temperature from 30 °C to 4 °C. The WcCab3-DSR was also quite stable at −20 °C with a half-life ($t_{1/2}$) of 37.9 d.

3.3. Characterization of synthesized dextran

The *in vitro*-synthesized WcCab3-DSR dextran was isolated by ethanol precipitation and lyophilized for further structural and functional characterization. The total carbohydrate and protein content of the purified WcCab3-dextran was 98.27% (w/w) and 1.73% (w/w) (7.5 mg/ml and 0.13 mg/ml), respectively. HPAEC-PAD

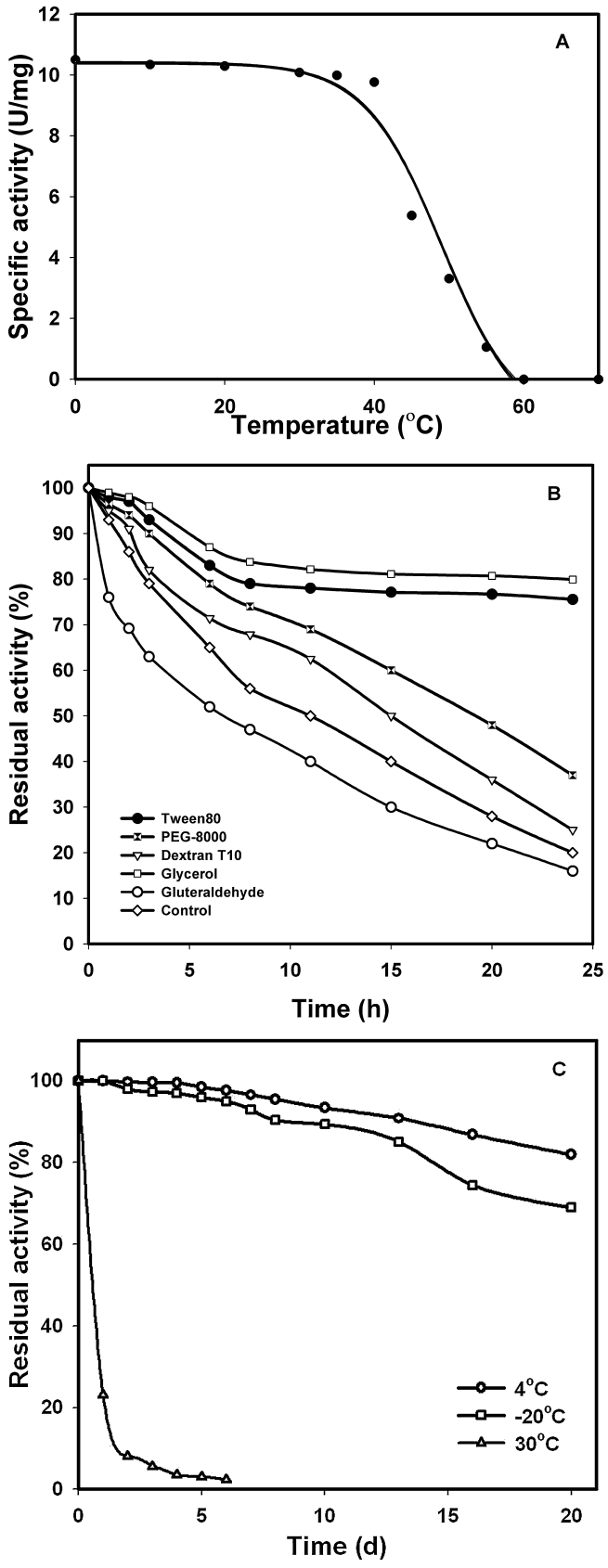

Fig. 2. (A) Effect of temperature on stability of WcCab3-DSR. WcCab3-DSR 10.5 U/mg and 0.84 mg protein/ml) was pre-incubated at different temperatures for 1 h and assayed for residual activity. (B) Effects of various stabilizers on WcCab3-DSR activity. WcCab3-DSR (10.5 U/mg and 0.84 mg protein/ml) in a 20 mM sodium acetate buffer (pH 5.4) was pre-incubated with additives at 30 °C and assayed for activity. (C) Effect of temperatures at 30 °C, 4 °C, and −20 °C on the storage stability of dextransucrase. WcCab3-DSR (10.5 U/mg, 0.84 mg/ml) in a 20 mM sodium acetate buffer (pH 5.4) was pre-incubated at 30 °C, 4 °C, and −20 °C and aliquots were assayed for dextransucrase activity at 35 °C at 1 d intervals.

analysis after acid hydrolysis proved that the purified sample contained no carbohydrate other than glucose.

3.3.1. Enzyme-aided structure analysis

An enzyme-aided structure analysis of dextrans is a simple and quick method for determining the structural variation in dextrans produced by different strains or in different conditions (Katina et al., 2009). The method involves specific enzymatic hydrolysis of dextran with a mixture of dextranase and α-glucosidase that converts the dextran to glucose and a set of enzyme-resistant oligosaccharides. The chromatographic profiles of the enzyme-resistant oligosaccharides vary depending on the structural components of dextran, and can therefore be used as structural markers (Maina, 2012). An HPAEC-PAD analysis showed that the enzyme-resistant oligosaccharides from WcCab3-DSR dextran (Fig. 4A) had a similar profile to the oligosaccharides as obtained earlier from *W. confusa* VTT E-90392 and *Lc. mesenteroides* NRRL B-512F dextran (Katina et al., 2009), indicating high similarities in their structural features. Dextrans from these strains have few (<5%) α-(1→3)-linked branches that are either single unit or elongated by two or more α-(1→6)-linked glucosyl units (Maina et al., 2011). Characterization of the resistant oligosaccharides from *W. confusa* VTT E-90392 and *Lc. mesenteroides* NRRL B-512F dextrans showed that they included oligosaccharides with terminal α-(1→3)-linked glucosyl units originating from single-unit branches and α-(1→3)-linked isomaltosyl units that originate from branches that are elongated with α-(1→6) linkages (Maina et al., 2011).

3.3.2. FT-IR analysis

An FT-IR spectrum of the *in vitro* synthesized WcCab3-DSR dextran is presented in Fig. 3A. It shows bands in the 950–1200 cm^{-1} region, which is a typical fingerprinting region for all polysaccharides (Cerna et al., 2003). The bands at 3394 cm^{-1} and 2930 cm^{-1} represent hydroxyl-stretching vibration and C—H stretching vibration of the polysaccharide, respectively. Similar results have been reported earlier (Cao et al., 2006; Shingel, 2002). The transmission peak at 908 cm^{-1} is a characteristic peak for a α-glycosidic bond. The band at 1154 cm^{-1} is assigned to valent vibrations of C—O—C bond and a glycosidic bridge. The peak, at 1022 cm^{-1}, indicates great chain flexibility around the α-(1→6) glycosidic bonds in the dextran. Similar findings were observed by Shingel (2002).

3.3.3. NMR spectroscopy analysis

The ^{1}H spectrum of the WcCab3-DSR dextran (Fig. 3B) showed typical main-chain α-(1→6)-linked glucopyranosyl residues with an anomeric signal centered at 4.97 ppm as also reported earlier (Ahmed et al., 2012; Maina et al., 2008). An additional low intensity anomeric proton signal at 5.32 ppm (Fig. 3B) was also observed in the spectrum, which indicated the presence of α-(1→3)-linked branches (Maina et al., 2008). The remaining five resonances in the ^{1}H spectrum, at 3.97, 3.91, 3.77, 3.73, 3.58, and 3.52 ppm (Fig. 3B), corresponded to H-6b, H-5, H-6a, H-3, H-2, and H-4, respectively of the α-(1→6)-linked glucopyranosyl residues in the main chain (Table 1S).

^{13}C NMR spectroscopy has been used to study the structure of a series of dextrans. Dextrans have typical ^{13}C anomeric signals (C-1), downfield at about 90 ppm, characteristic of anomeric carbon C-1 involved in a glycosidic linkage. The chemical shifts for C-2, C-3, C-4, and C-5 appear in the 70–75 ppm region, and C-6 is normally up-field at about 60 ppm (Seymour, Knapp, & Bishop, 1976). ^{13}C chemical shifts of WcCab3-DSR dextran were

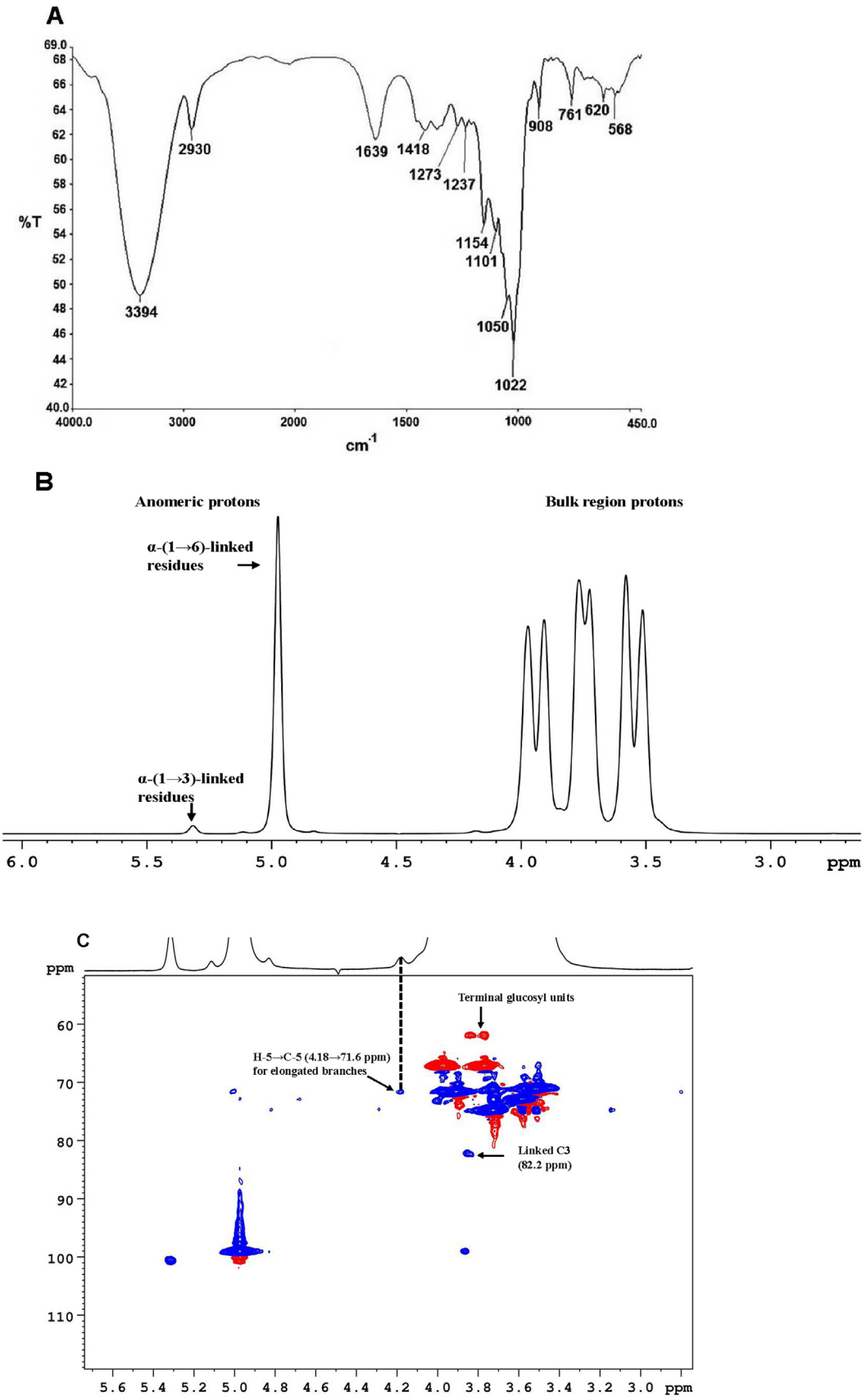

Fig. 3. An FT-IR and NMR spectroscopic analysis of WcCab3-DSR dextran. (A) FT-IR spectrum. (B) The 1D ^{1}H spectrum recorded at 600 MHz in D_2O at 50 °C. (C) The edited HSQC spectrum recorded at 600 MHz in D_2O at 50 °C. Peaks in the NMR spectra are referenced to internal acetone (^{1}H = 2.225 ppm and ^{13}C = 31.55 ppm).

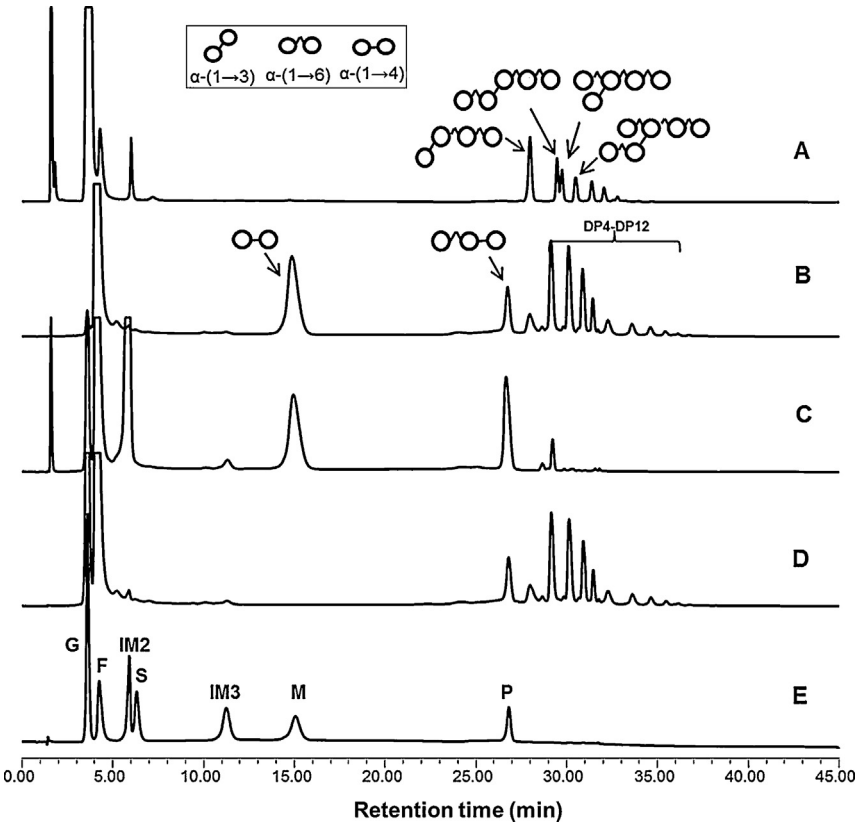

Fig. 4. HPAEC-PAD chromatograms showing (A) resistant oligosaccharides from WcCab3-DSR dextran after dextranase and α-glucosidase treatment, with the structure of the shortest four oligosaccharides illustrated; (B) acceptor-reaction products after WcCab3-DSR incubated with 5% (w/v) maltose and 10% (w/v) sucrose; (C) acceptor products after treatment with dextranase; (D) acceptor products after treatment with *Bacillus stearothermophilis* α-glucosidase; and (E) standards, from left to right G: glucose, F: fructose, IM2: isomaltose, S: sucrose, IM3: isomaltotriose, M: maltose, and P: Panose.

determined by 2D heteronuclear single quantum correlation spectroscopy (HSQC). The edited HSQC spectrum of the WcCab3-DSR dextran (Fig. 3C) showed two anomeric ^{13}C chemical shifts at 99.3 ppm and 100.5 ppm corresponding to the ^{1}H anomeric signals at 4.97 ppm of the α-(1→6)-linked residues, and 5.32 ppm for the α-(1→3)-linked residues, respectively. Apart from the anomeric signals, five ^{13}C NMR resonances at 73.0, 75.0, 71.3, 71.8, and 67.3 ppm were also observed (Fig. 3C), which were assigned to the α-(1→6)-linked residues in the main chains (Table 1S). A typical downfield shift for a linked C-3 of the 3,6-*O*-disubstituted glucopyranosyl units at 82.2 ppm was also observed in the HSQC spectra of the WcCab3-DSR dextran, which further established the presence of α-(1→3)-linked branch residues (Maina et al., 2008).

NMR spectroscopy data also confirmed the presence of elongated branches in WcCab3-DSR dextran. As described previously, the signal at 5.32 ppm represents both α-(1→3)-linked single unit and elongated branches (Maina et al., 2011). The elongated branches can be determined by the occurrence of a signal at ~4.19 ppm that has been assigned to the H-5 signal of the elongated branch point glucopyranosyl unit (→6)-α-D-Glc*p*-(1→3)-) (Maina et al., 2011). In the dextran synthesized by WcCab3-DSR, a peak of 4.18 ppm for the elongated branches was observed in the ^{1}H spectrum (Fig. 3B), and, furthermore, a cross peak (H-5→C-5) of 4.18→71.6 ppm was observed in the HSQC spectrum (Fig. 3C). The terminal units in the main chain, single unit, or elongated branches had a C-6 signal at 61.8 ppm (Fig. 3C).

The relative intensities of the anomeric signals at 4.97 and 5.32 ppm were 97% and 3%, respectively, indicating the relatively low branching degrees in the *in vitro* synthesized WcCab3-DSR dextran. Previous studies have also shown that dextran from *Weissella* strains generally have a lower degree of branching compared with dextran from other strains such as *Lc. mesenteroides* (Bounaix et al., 2009; Maina et al., 2008). This may be a typical feature of *Weissella* strains, which in combination with their high dextran-production efficiency, makes them highly potential for production of commercial dextran with few branch linkages.

3.3.4. HPSEC analysis

An HPSEC analysis of WcCab3-DSR dextran was done in DMSO containing 10 mM LiBr. Although dextrans are commonly analyzed in aqueous solutions, DMSO was preferred in this study, because in aqueous solutions, dextrans are prone to aggregation, which can significantly affect the results (Maina, 2012). Only one major peak for both refractive index (RI) and light scattering at 90° (LS) signals was observed in the HPSEC chromatogram of WcCab3-DSR dextran (results not shown), and the corresponding molar mass was 1.8×10^7 g/mol. Interestingly, when dextran was extracted from the cell mass of *W. confusa* VTT E-90392, HPSEC analysis in DMSO showed a clearly lower molar mass (1.5×10^6 g/mol) (Maina, 2012), compared with WcCab3-DSR dextran obtained *in vitro* in the present study. Irague et al. (2012) recently also reported significantly high molar mass values (10^8 g/mol) for dextran synthesized *in vitro* with *Lc. mesenteroides* NRRL B-512F dextransucrase mutants, as analyzed in an aqueous solution. Based on the characterization, WcCab3-DSR dextran seems well suitable for food applications such as sourdough in which high molar mass dextrans with few branches are desirable (Lacaze, Wick, & Cappelle, 2007).

3.4. Production and characterization of GLOS

The GLOS produced by WcCab3-DSR through an acceptor reaction with maltose were characterized by HPAEC-PAD,

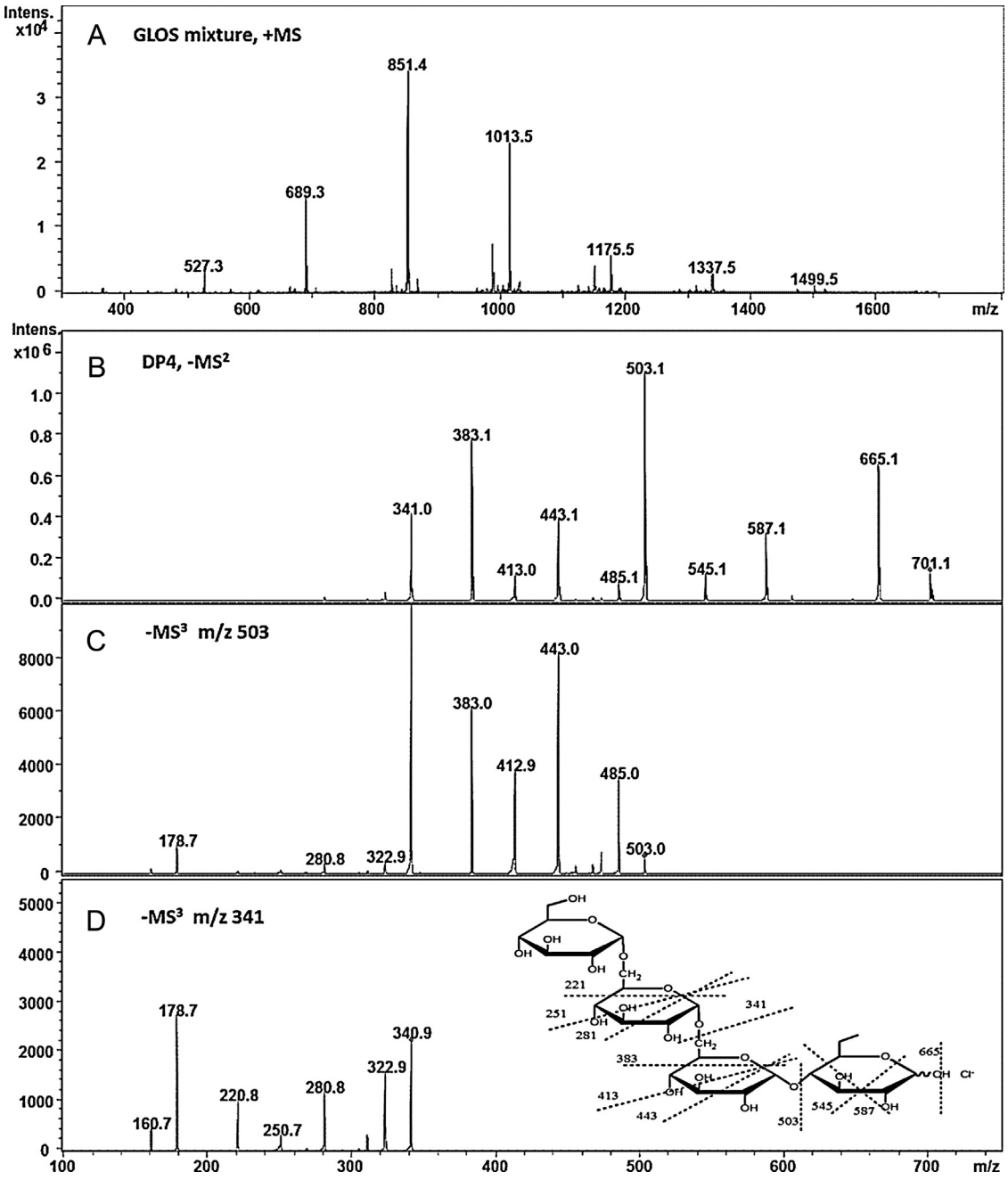

Fig. 5. Mass spectra of WcCab3-DSR maltose acceptor products. (A) The positive mode ($[M+Na]^+$) AP-MALDI-ITMS spectrum of the GLOS-acceptor product mixture. (B) The negative mode ($[M+Cl]^-$) MS^2 and (C and D) MS^3 spectra of the isolated DP4 acceptor product. The MS^3 spectra in C and D are for the fragment ions at *m*/*z* 503 and 341, respectively.

enzyme-aided structural analysis and mass spectrometry. According to HPAEC-PAD analysis, sucrose was depleted during the reaction, while 19% of the added maltose was left unutilized (Fig. 4B). A series of GLOS were produced as shown in the HPAEC-PAD chromatogram (Fig. 4B), with some peaks being predominant, while minor peaks were also present.

Selective enzymatic hydrolysis of the GLOS shed light on their structure. Endo-acting dextranase degraded the GLOS of higher DP into their building blocks, i.e., glucose, isomaltose, isomaltotriose, and panose (based on retention time) (Fig. 4C and E). On the other hand, the α-glucosidase exo-acting on α-(1→4) linkages from the non-reducing end of GLOS, converted only maltose into glucose, leaving the acceptor products intact (Fig. 4D). This indicated that GLOS have consecutive α-(1→6) linked glucopyranosyl chains of varying lengths attached to the non-reducing end of the maltose. Because the GLOS were expected to be homologous to panose, their yield was estimated to be 6.8 g by quantifying GLOS peaks using panose as a standard. This GLOS yield was in accordance with the estimate based on the total carbohydrate content (600 mg/ml as determined by phenol–sulphuric acid method), in the GLOS solution. Because 81% of added maltose (4.1 g) was utilized in the GLOS production, the weight gain of GLOS (2.7 g) was derived from the glucosyl moiety in sucrose (2.7/162 = 16.7 mmol). Because sucrose (10/342 = 29.2 mmol) was depleted, the acceptor reaction toward GLOS consumed 57.2% (16.7/29.2) sucrose, leaving 42.8% sucrose for dextran production.

The AP-MALDI-ITMS analysis of the GLOS-mixture components confirmed that they belong to a homologous hexose series. The

peaks at m/z 527.3, 689.3, 851.4, 1013.5, 1175.5, 1337.5, and 1499.5 corresponded to the $[M+Na]^+$ ions of GLOS from DP3 up to DP9, respectively (Fig. 5A). Nonetheless, oligosaccharides most likely of DP10-DP12 in HPAEC-PAD analysis (Fig. 4B) were not detected in the AP-MALDI-ITMS analysis under the conditions used. Products up to DP10 were isolated by gel filtration with a Biogel P2 column (Fig. 2S). Interestingly, the HPAEC-PAD analysis of the selected fractions showed that minor products particularly co-eluted in the fractions for DP5, DP6, and DP7, possibly representing another homologous series.

Tandem MS in negative mode is useful in the structural analysis of linear mixed-linkage GLOS (Maina et al., 2013). Here, isolated DP3, DP4, and DP5 ($[M+Cl]^-$ ions of m/z at 539.0, 701.1, and 863.2, respectively), were further analyzed by ESI-MS/MS to prove the proposed structures. Cross-ring cleavage fragment ions were observed at m/z 424.8 and 382.8 (loss of 78 and 120 Da, respectively) and at m/z 280.8, 250.6, and 220.6 (loss of 60, 90 and 120 Da, respectively) in the MS^2 spectrum of DP3 (panose) (results not shown). These diagnostic fragment ions (Maina et al., 2013) confirmed the α-(1→4) linkage at its reducing end and the α-(1→6) linkage at the non-reducing end. Similarly, in the MS^2 spectrum of the $[M+Cl]^-$ ion of DP4 (Fig. 5B), fragment ions at m/z 587.1 and 545.1 (loss of 78 Da and 120 Da, respectively) indicated the presence of a α-(1→4) linkage at the reducing end. The ions at m/z 443.1, 413.0, and 383.1 (loss of 60, 90, and 120 Da, respectively) in the MS^3 spectrum, starting with the ion at m/z 503.0, indicated the subsequent α-(1→6) linkage (Fig. 5C). The α-(1→6) linkage at the non-reducing end was confirmed with the MS^3 spectrum of the ion at m/z 340.9 (Fig. 5D), which contained fragment ions at m/z 280.8, 250.7, and 220.8 (loss of 60, 90, and 120 Da, respectively). The MS/MS of DP5 showed a similar trend (Fig. 3S); a α-(1→4) linkage at its reducing end and the remaining linkages being α-(1→6). The findings confirmed that the principal acceptor products (GLOS) were a homologous series of isomaltooligosaccharides that contain maltose at the reducing end. Similar maltose acceptor products have been reported for dextransucrases from *Lc. mesenteroides* NRRL B-512F, and B-1299; however, the latter produced another two series of products bearing a α-(1→2) single unit branch (Brison et al., 2012; Robyt & Eklund, 1983).

The minor acceptor product series has not been previously reported for dextransucrases producing typical dextrans, which are predominantly α-(1→6)-linked α-glucan with a low degree of branching. Cote and Leathers (2005) analyzed the maltose acceptor products of various *Leuconostoc* spp. glucansucrases by thin-layer chromatography, and found that strains producing typical dextrans produced only one series of isomaltooligosaccharides, while strains producing highly branched dextrans produced another series of acceptor products. However, the characterization of the minor acceptor product series synthesized by WcCab3-DSR needs further studies.

4. Conclusions

Recently, *Weissella* spp. has drawn attention for its high dextransucrase and dextran production capacity and the presence of only a few branches in its dextran. Dextransucrase from *W. confusa* Cab3 was isolated in this study by fractionation, using 30% PEG-400, resulting in a specific activity of 10.5 U/mg with 10 fold purification. Activity of WcCab3-DSR was enhanced by Co^{2+} and Ca^{2+} ions (21% and 20%, respectively). Glycerol and Tween 80 significantly enhanced their stability at 30 °C, increasing the half-life ($t_{1/2}$) to 74 h and 59 h, respectively, compared with the same process without any additive (10 h). WcCab3-DSR dextran had a high molar mass of 1.8×10^7 g/mol according to an HPSEC analysis in DMSO. A ^1H and ^{13}C NMR spectral analysis confirmed the presence of main chain α-(1→6) linkages with few (3.0%) α-(1→3) branch linkages. WcCab3-DSR catalyzed acceptor reaction when maltose was present, giving rise to a homologous series of GLOS (DP3–DP12), which carried maltose at the reducing end and additional glucose units all α-(1→6) linked to the non-reducing end.

Acknowledgements

The researchers would like to thank Leena Pitkänen for help in the HPSEC analysis. This study was supported by the Department of Biotechnology; the Ministry of Science and Technology, New Delhi, India (AG); the Academy of Finland (contract number 255755, MT) via the joint WISEDextran project; and the ABS Graduate School in Finland (QS).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.09.087.

References

Ahmed, R. Z., Siddiquia, K., Armanb, M., & Ahmeda, N. (2012). Characterization of high molecular weight dextran produced by *Weissella cibaria* CMGDEX3. *Carbohydrate Polymers*, *90*(1), 441–446.

Amari, M., Arango, L. F. G., Gabriel, V., Robert, H., Morel, S., Moulis, C., et al. (2012). Characterization of a novel dextransucrase from *Weissella confusa* isolated from sourdough. *Applied Microbiology and Biotechnology*, *97*, 5413–5422.

Basedow, A. M., & Ebert, K. H. (1979). Production, characterization and solution properties of dextran fractions of narrow molecular distributions. *Journal of Polymer Sciences: Polymer Symposia*, *66*(1), 101–115.

Bejar, W., Gabriel, V., Amari, M., Morel, S., Mezghani, M., Maguin, E., et al. (2013). Characterization of glucansucrase and dextran from *Weissella* sp. TN610 with potential as safe food additives. *International Journal of Biological Macromolecules*, *52*, 125–132.

Bounaix, M.-S., Gabriel, V., Morel, S., Robert, H., Rabier, P., Remaud-Siméon, M., et al. (2009). Biodiversity of exopolysaccharides produced from sucrose by sourdough lactic acid bacteria. *Journal of Agricultural and Food Chemistry*, *57*, 10889–10897.

Bounaix, M.-S., Robert, H., Gabriel, V., Morel, S., Remaud-Siméon, M., Gabriel, B., et al. (2010). Characterization of dextran-producing *Weissella* strains isolated from sourdoughs and evidence of constitutive dextransucrase expression. *FEMS Microbiology Letters*, *311*(1), 18–26.

Brison, Y., Pijning, T., Malbert, Y., Fabre, E., Mourey, L., Morel, S., et al. (2012). Functional and structural characterization of an α-(1→2) branching sucrase derived from DSR-E glucansucrase. *The Journal of Biological Chemistry*, *287*, 7915–7924.

Cao, W., Li, X.-Q., Liu, L., Yang, T. H., Li, C., Fan, H. T., et al. (2006). Structure of antitumor polysaccharide from *Angelica sinensis* (Oliv.) Diels. *Carbohydrate Polymers*, *66*(2), 149–159.

Cerna, M., Barros, A., Nunes, A., Rocha, S. M., Delgadillo, I., Copikova, J., et al. (2003). Use of FT-IR spectroscopy as a tool for the analysis of polysaccharide food additives. *Carbohydrate Polymers*, *51*(4), 383–389.

Chong, S. L., Nissilä, T., Ketola, R. A., Koutaniemi, S., Derba-Maceluch, M., Mellerowicz, E. J., et al. (2011). Feasibility of using atmospheric pressure matrix-assisted laser desorption/ionization with ion trap mass spectrometry in the analysis of acetylated xylooligosaccharides derived from hardwoods and *Arabidopsis thaliana*. *Analytical and Bioanalytical Chemistry*, *401*, 2995–3009.

Collins, M. D., Samelis, J., Metaxopoulos, J., & Wallbanks, S. (1993). Taxonomic studies on some *Leuconostoc*-like organisms from fermented sausages: Description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. *Journal of Applied Microbiology*, *75*(6), 595–603.

Cote, G. L., & Leathers, T. D. (2005). A method for surveying and classifying *Leuconostoc* spp. glucansucrases according to strain-dependent acceptor product patterns. *Journal of Industrial Microbiology and Biotechnology*, *32*(2), 53–60.

Di Cagno, R., De Angelis, M., Limitone, A., Minervini, F., Carnevali, P., Corsetti, A., et al. (2006). Glucan and fructan production by sourdough *Weissella cibaria* and *Lactobacillus plantarum*. *Journal of Agricultural and Food Chemistry*, *54*, 9873–9881.

Dols, M., Remaud-Simeon, M., Willemot, R. M., Vignon, M. R., & Monsan, P. F. (1997). Structural characterization of the maltose acceptor-products synthesized by *Leuconostoc mesenteroides* NRRL B-1299 dextransucrase. *Carbohydrate Research*, *305*(3/4), 549–559.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350–356.

Forman, H. J., & Kennedy, J. (1977). Effects of chaotropic agents versus detergents on dihydroorotatedehydrogenase. *The Journal of Biological Chemistry*, *25*, 3379–3381.

Fox, J. D., & Robyt, J. F. (1991). Miniaturization of three carbohydrate analyses using a micro-sample plate reader. *Analytical Chemistry*, *195*(1), 93–96.

Galle, S., Schwab, C., Arendt, E., & Gänzle, M. (2010). Exopolysaccharide-forming *Weissella* strains as starter cultures for sorghum and wheat sourdoughs. *Journal of Agricultural and Food Chemistry*, *58*, 5834–5841.

Galle, S., Schwab, C., Dal Bello, F., Coffey, A., Gänzle, M. G., & Arendt, E. K. (2012). Influence of *in situ* synthesized exopolysaccharides on the quality of gluten-free sorghum sourdough bread. *International Journal of Food Microbiology*, *155*(3), 105–112.

Goyal, A., & Katiyar, S. S. (1996). Regulation of dextransucrase productivity of *Leuconostoc mesenteroides* B-512F by the maintenance media. *The Journal of General and Applied Microbiology*, *42*, 81–85.

Hernández, H. G., Livings, S., Aguilera, J. M., & Chiralt, A. (2011). Phase transitions of dairy proteins, dextrans and their mixtures as a function of water interactions. *Food Hydrocolloids*, *25*(5), 1311–1318.

Irague, R., Rolland-Sabaté, A., Tarquis, L., Doublier, J. L., Moulis, C., Monsan, P., et al. (2012). Structure and property engineering of α-D-glucans synthesized by dextransucrase mutants. *Biomacromolecules*, *13*(1), 187–195.

Ito, K., Ito, S., Shimamura, T., Weyand, S., Kawarasaki, Y., Misaka, T., et al. (2011). Crystal structure of glucansucrase from the dental caries pathogen *Streptococcus mutans*. *Journal of Molecular Biology*, *408*(2), 177–186.

Katina, K., Maina, N. H., Juvonen, R., Flander, L., Johansson, L., Virkki, L., et al. (2009). In situ production and analysis of *Weissella confusa* dextran in wheat sourdough. *Food Microbiology*, *26*(7), 734–743.

Lacaze, G., Wick, M., & Cappelle, S. (2007). Emerging fermentation technologies: Development of novel sourdoughs. *Food Microbiology*, *24*(2), 155–160.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680–685.

Leemhuis, H., Pijning, T., Dobruchowska, J. M., van Leeuwen, S. S., Kralj, S., Dijkstra, B. W., et al. (2013). Glucansucrases: Three-dimensional structures, reactions, mechanism, α-glucan analysis and their implications in biotechnology and food applications. *Journal of Biotechnology*, *163*(2), 250–272.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*, *193*(1), 265–275.

Maina, N. H. (2012). *Structure and macromolecular properties of Weissella confusa and Leuconostoc citreum dextrans with a potential application in sourdough. EKT-series 1553*. Finland: University of Helsinki (Doctoral Thesis)

Maina, N. H., Juvonen, M., Domingues, R. M., Virkki, L., Jokela, J., & Tenkanen, M. (2013). Structural analysis of linear mixed-linkage glucooligosaccharides by tandem mass spectrometry. *Food Chemistry*, *136*, 1496–1507.

Maina, N. H., Tenkanen, M., Maaheimo, H., Juvonen, R., & Virkki, L. (2008). NMR spectroscopic analyses of exopolysaccharides produced by *Leuconostoc citreum* and *Weissella confusa*. *Carbohydrate Research*, *343*, 1446–1455.

Maina, N. H., Virkki, L., Pynnönen, H., Maaheimo, H., & Tenkanen, M. (2011). Structural analysis of enzyme-resistant isomaltooligosaccharides reveals the elongation of α-(1→3)-linked branches in *Weissella confusa* dextran. *Biomacromolecules*, *12*(2), 409–418.

Miller, A. W., & Robyt, J. (1986). Detection of dextransucrase and levansucrase on polyacrylamide gels by the periodic acid-Schiff stain: Staining artifacts and their prevention. *Analytical Biochemistry*, *156*, 357–363.

Miller, A. W., & Robyt, J. F. (1986). Activation and inhibition of dextransucrase by calcium. *Biochimica et Biophysica Acta*, *880*(1), 32–39.

Naessens, M., Cerdobbel, A., Soetaert, W., & Vandamme, E. J. (2005). Leuconostoc dextransucrase and dextran: Production properties and applications. *Journal of Chemical Technology and Biotechnology*, *80*, 845–860.

Nelson, N. A. (1944). photometric adaptation of the Somogyi method for the determination of glucose. *The Journal of Biological Chemistry*, *153*(2), 375–380.

Patel, S., Kothari, D., & Goyal, A. (2011). Purification and characterization of an extracellular dextransucrase from *Pediococcus pentosaceus* isolated from the soil of north east India. *Food Technology and Biotechnology*, *49*(3), 297–303.

Patel, S., Majumder, A., & Goyal, A. (2012). Potentials of exopolysaccharides from lactic acid bacteria. *Indian Journal of Microbiology*, *52*(1), 3–12.

Purama, R. K., & Goyal, A. (2008). Identification effective purification and functional characterization of dextransucrase from *Leuconostoc mesenteroides* NRRL B-640. *Bioresource Technology*, *99*, 3635–3642.

Purama, R. K., Agrawal, M., & Goyal, A. (2010). Stabilization of dextransucrase from *Leuconostoc mesenteroides* NRRL B-640. *Indian Journal of Microbiology*, *50*(1), S57–S61.

Rantanen, H., Virkki, L., Tuomainen, P., Kabel, M. A., Schols, H. A., & Tenkanen, M. (2007). Preparation of arabinoxylobiose from rye xylan using family 10 *Aspergillus aculeatus* endo-1,4-ß-D-xylanase. *Carbohydrate Polymers*, *68*(2), 350–359.

Robyt, J. F., & Eklund, S. H. (1983). Relative, quantitative effects of acceptors in the reaction of *Leuconostoc mesenteroides* B-512F dextransucrase. *Carbohydrate Research*, *121*, 279–286.

Schwab, C., Mastrangelo, M., Corsetti, A., & Gänzle, M. (2008). Formation of oligosaccharides and polysaccharides by *Lactobacillus reuteri* LTH5448 and *Weissella cibaria* 10 M in sorghum sourdoughs. *Cereal Chemistry*, *85*(5), 679–684.

Seymour, F. R., Knapp, R. D., & Bishop, S. H. (1976). Determination of the structure of dextran by ^{13}C-nuclear magnetic resonance spectroscopy. *Carbohydrate Research*, *51*(2), 179–194.

Shingel, K. I. (2002). Determination of structural peculiarities of dextran, pullulan and c-irradiated pullulan by Fourier-transform IR spectroscopy. *Carbohydrate Research*, *337*, 1445–1451.

Shukla, S., & Goyal, A. (2011). 16S rRNA based identification of a glucan hyper-producing *Weissella confusa*. *Enzyme Research*, http://dx.doi.org/10.4061/2011/250842

Somogyi, M. (1945). A new reagent for determination of sugars. *The Journal of Biological Chemistry*, *160*(1), 61–68.

Stam, M. R., Danchin, E. G. J., Rancurel, C., Coutinho, P. M., & Henrissat, B. (2006). Dividing the large glycoside hydrolase family 13 into subfamilies: Towards improved functional annotations of α-amylase-related proteins. *Protein Engineering Design and Selection*, *19*, 555–562.

Tinirikari, J. M. R., & Goyal, A. (2013). Purification, optimization of assay, and stability studies of dextransucrase isolated from *Weissella cibaria* JAG8. *Preparative Biochemistry and Biotechnology*, *43*(4), 329–341.

Tsuchiya, H. M., Koepsell, H. J., Corman, J., Bryant, M. O., Feger, V. H., & Jackson, R. W. (1952). The effect of certain cultural factors on production of dextransucrase by *Leuconostoc mesenteroides*. *Journal of Bacteriology*, *64*(4), 521–526.

Vujicic-Zagar, A., Pijning, T., Kralj, S., López, C. A., Eeuwema, W., Dijkhuizen, L., et al. (2010). Crystal structure of a 117 kDa glucansucrase fragment provides insight into evolution and product specificity of GH70 enzymes. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 21406–21411.