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Effect of temperature on production of oligosaccharides and dextran by *Weissella cibaria* 10 M

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ABSTRACT

The formation of HoPS and oligosaccharides in sourdough fermentation improves bread quality but is dependent on the expression of glycansucrases by lactic acid bacteria. Data on the expression of dextransucrases by *Weissella* spp., however, are limited. This study therefore aimed to assess dextansucrase expression in *W. cibaria* 10 M, focusing on the effect of temperature. The effect of temperature on growth, oligosaccharide and dextran synthesis by *W. cibaria* 10 M was determined and the expression and activity of cell-associated dextransucrase from *W. cibaria* 10 M were investigated. The oligosaccharides profiles were measured by thin layer chromatography and high performance anion exchange chromatography coupled to pulsed amperometric detection. Dextran formation was quantified by size exclusion chromatography. *W. cibaria* grew fastest at 30 °C but oligosaccharide formation was highest at 20 °C or less. Dextransucrase expression as measured by reverse transcription quantitative PCR, SDS-PAGE, and activity of cell-associated dextransucrase were maximal at 15 °C. Cold shift incubation, characterized by incubation at 30 °C to obtain biomass, followed by shift to 6 °C to induce dextransucrase expression, supported high dextransucrase activity in laboratory media. Cold shift fermentation of wheat and sorghum sourdoughs supplemented with 15 or 30% sucrose increased the yields of oligosaccharides, and resulted in formation of 16 and 12 g/kg dextran in wheat and sorghum sourdoughs, respectively. Dextran formation was decreased in favour of oligosaccharide formation when doughs were supplemented with maltose. In conclusion, cold shift fermentation of sourdough with *W. cibaria* supports high dextran yields or formation of oligosaccharides without excess acidification.

1. Introduction

The formation of extracellular homopolysaccharides (HoPS) by lactic acid bacteria (LAB) during sourdough fermentation improves texture and storage life of bread (Galle and Arendt, 2014; Tieking and Gänzle, 2005). HoPS formation is accompanied by formation of oligosaccharides, which add nutritional functionality to baked goods through their prebiotic activity (Schwab et al., 2008; Yan et al., 2018). HoPS and oligosaccharide formation by lactic acid bacteria is dependent on the availability of sucrose, and is mediated by extracellular fructansucrases or glucansucrases (Tieking et al., 2003; van Hijum et al., 2006). These enzymes frequently occur in the species *Streptococcus mutans*, in species of the *L. reuteri* group, and in the genera *Leuconostoc* and *Weissella* (Bounaix et al., 2010; Galle et al., 2010; Tieking et al., 2003; van Hijum et al., 2006). Strains of the vertebrate host-adapted *L. reuteri* group are commonly isolated from type II sourdoughs; *Leuconostoc* spp. and *Weissella* spp. occasionally occur in spontaneous sourdoughs or in type I sourdoughs (Gänzle and Ripari, 2016; Gobbetti et al., 2016; Lattanzi et al., 2016). Among HoPS producing lactic acid bacteria, the use of *Weissella* spp. is considered advantageous because *Weissella* ssp. produce high molecular weight dextrans (Chen et al., 2016; Katina et al., 2009). Moreover, *Weissella cibaria* and *W. confusa* generally do not express mannitol dehydrogenase activity and thus accumulate less acetic acid when compared to *Leuconostoc* spp. or lactobacilli (Galle et al., 2010).

Glucansucrases are GH70 enzymes and are categorized based on the linkage types of the glucans formed (van Hijum et al., 2006). Dextran, a α-(1 → 6) linked glucan with α-(1 → 2,6) or (1 → 3,6) branching points, is produced by dextransucrases of *Leuconostoc* spp., *Weissella* spp. and lactobacilli (Bounaix et al., 2010; Naessens et al., 2005; van Hijum et al., 2006). Dextran is one of the first biopolymers produced on an industrial scale and is applied in medicine, separation technology, and as food hydrocolloid (Naessens et al., 2005). Dextransucrases transfer the glycosyl moiety from sucrose to acceptor sugars or to a growing glucan chain to produce α-(1 → 6) linked glucan-oligosaccharides or dextran, respectively (van Hijum et al., 2006). The ratio of

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oligosaccharides to polysaccharides depends on the concentration of suitable acceptor carbohydrates (Hu et al., 2017; Robyt and Eklund, 1983). Maltose, an abundant sugar in wheat sourdough but not in sorghum sourdoughs, is a preferred glucosyl acceptor for dextransucrases (Galle et al., 2010; Hu et al., 2017). Oligosaccharide formation in sourdough does not impact bread texture, however, α-(1 → 6) linked gluco-oligosaccharides with a DP higher than 3 increase the fibre content of bread (Hu et al., 2017; Schwab et al., 2008; Yan et al., 2018).

The formation of HoPS and oligosaccharides in sourdough fermentation is also dependent on the expression of glucansucrases by lactic acid bacteria. Reuteransucrases in *L. reuteri* are expressed constitutively (Schwab and Gänzle, 2006) while dextransucrase expression in *Leuconostoc mesenteroides* is induced by sucrose (Quirasco et al., 1999). Data on the expression of dextransucrases by *Weissella* spp., however, are limited (Bounaix et al., 2010). This study therefore aimed to assess dextansucrase expression in *W. cibaria* 10 M, focusing on the effect of temperature, a major parameter for fermentation control in industrial and artisanal fermentations (Gänzle et al., 1998). Oligosaccharide formation was quantified by high performance anion exchange chromatography with pulse amperometric detection (HPAEC-PAD) and thin layer chromatography (TLC) was used as a fast analysis method; dextran was quantified by size exclusion chromatography and its size was assessed by asymmetric flow field flow fractionation coupled to multi-angle laser scattering. Dextran- and oligosaccharide formation during growth in laboratory media was compared to product formation in wheat and sorghum sourdoughs as substrates differing in the content of maltose as glucosyl acceptor.

2. Materials and methods

2.1. Strains and growth conditions

Weissella cibaria 10 M expressing the dextransucrase DsrM (Chen et al., 2016) was routinely cultivated on modified de Man Rogosa Sharpe (mMRS) agar (Schwab et al., 2008) from glycerol stock stored at −80 °C. For preparation of working cultures, colonies were picked from the agar plate, cultured at 30 °C in mMRS broth containing 24 mM maltose, 22 mM glucose and 22 mM fructose for 16 h, and subcultured in MRS containing 85 mM sucrose (sucMRS) as sole carbon source for 16 h. Media were prepared by adding filter sterilized sugar solutions to autoclaved basal media.

2.2. Growth curve of W. cibaria 10 M

W. cibaria 10 M was subcultured with a 1% inoculum in mMRS broth and incubated at 6, 20, and 30 °C. Samples were taken periodically for determination of the pH and the optical density (OD). The optical density was determined with 200 µL samples at 600 nm in a micotitreplate reader.

2.3. Effect of temperature on dextran synthesis by W. cibaria 10 M in the presence of 0.5 M sucrose

W. cibaria 10 M was subcultured at 1% inoculum in MRS broth with 0.5 M sucrose, incubated at 30 °C to the exponential phase of growth, corresponding to an optical density (OD) at 600 nm of 0.2–0.4, and further incubated at 6, 15, 20, 25, or 30 °C for 24 h. Cells were removed by centrifugation for 20 min at 4 °C and polysaccharides in the supernatant were precipitated by addition of two volumes of cool ethanol, followed by incubation at 4 °C overnight. Polysaccharides were re-dissolved in water, insoluble material was removed by centrifugation, and polysaccharides in the supernatant were precipitated again with ethanol, collected by centrifugation, and freeze dried. The size distribution of polysaccharides was analysed by size exclusion chromatography using a Superdex 200 column (GE Healthcare Life Sciences, Mississauga, ON, Canada) eluted with water at a flow rate of 0.3 mL/min. Polysaccharides were quantified with a refractive index (RI) detector using dextran preparations (Sigma-Aldrich, Oakville, ON, Canada) with a molecular weight ranging from 5×10^6 to 4×10^7 as standard. Reactions were carried out in duplicate or triplicate.

2.4. Effect of temperature on oligosaccharide synthesis by W. cibaria 10 M in the presence of 0.5 M sucrose and 0.25 M maltose

W. cibaria 10 M was subcultured at 1% inoculum in MRS broth with 0.5 M sucrose and 0.25 M maltose and incubated at 20, 25, 30 °C for 24 h. Cells were removed by centrifugation and the supernatant was heated to 90 °C for 10 min to inactivate extracellular dextransucrase. Samples were diluted 100-fold with water, filtered by 0.22 µm filter, and oligosaccharides were analysed by a by high performance anion exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD) on a Carbopac PA20 column (Dionex, Oakville, Canada) as described (Schwab et al., 2008). Other microbial metabolites were quantified with an RI detector after separation on an Aminex 87H column as described (Galle et al., 2010).

2.5. Expression and activity of cell-associated dextransucrase from W. cibaria 10 M

W. cibaria was subcultured at 1% inoculum in MRS broth with 0.5 M sucrose and incubated at 30 °C to the exponential phase of growth, corresponding to an OD_{600nm} of ~0.4. Cultures were then transferred to 6, 15, 20, 25, or 30 °C and further incubated for 24 h. Cells were harvested by centrifugation, resuspended in 1 mL phosphate buffered saline (pH 7.4) and the OD_{600nm} was adjusted 10. Cells were lysed by a bead beater. Expression of cell-associated DsrM was assessed by SDS-polyacrylamide gel electrophoresis using a 4–16% Mini-PROTEAN® TGX™ Precast Gel (BIO-RAD, Mississauga, Canada) and Coomassie staining. DrsM activity was determined with 50 µL of cell lysate added to 0.45 mL 20 mM Na Acetate (pH 5.2) containing 100 mM sucrose. Reactions were incubated at 4, 6, 15, 20, 25 and 30 °C for 1–6 h, stopped by heating to 95 °C for 10 mins, and DsrM activity was determined by enzymatic quantification of fructose (Fructose Assay Kit, Sigma-Aldrich). DsrM activity was normalized by using the protein concentration of the cell lysate as determined by the absorption at 280 nm. The specific hydrolysis and total activity of DsrM (1 U) was defined as release of 1 µmol/min of free fructose or glucose, respectively, released per 1 mg cellular protein.

2.6. Quantification of dsrM mRNA in cultures of W. cibaria 10 M

The expression of *dsrM* from *W. cibaria* 10 M was quantified by reverse-transcriptase-quantitative PCR (RT-qPCR). Bacteria were grown in the MRS broth containing sucrose at different temperature (6, 15, 20, 25, 30 °C) to an OD_{600nm} of 0.4. RNA was extracted using RNAprotect Bacteria Reagent and a RNeasy Minikit (Qiagen, USA) and was subjected to DNAase treatment with RQ1 RNase-Free DNase Kit (Promega,Madison, USA) to eliminate genomic DNA. The treated RNA was reverse transcribed to cDNA using QuantiTect Reverse Transcription Kit (Qiagen, USA). qPCR was performed on 7500 Fast Real-Time PCR System (Applied Biosystems, Life Technologies, Burlington ON) with QuantiFast SYBR Green (Qiagen). DNase-treated RNA samples served as negative controls. Relative gene expression was calculated according to Pfaffl (2001) as follows:

$$relative\ gene\ expression = \frac{2^{\Delta Cp_{target}(control-sample)}}{2^{\Delta Cp_{reference}(control-sample)}};$$

where ΔCP is the difference in CP values for control and sample conditions. The gene coding for 16S rRNA was used as reference gene; gene expression was normalized to expression at 30 °C. The primers used for the quantification of gene expression of *dsrM* are: DSR-F′: AGACTGGT GAACGCTTGTATC and DSR-R′: CTGCCTGAACTTGTGGATTTG. Results