Partial characterization of exopolysaccharides obtained from novel isolates of Weissella Spp.

Ami Patel and JB Prajapati

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Abstract: Current work was aimed to determine the monosaccharide composition and relative molecular weight of the biopolymer obtained from fermented food isolates of W. cibaria using high-performance anion-exchange chromatography pulse amperometric detection (HPAEC) system and size exclusion chromatography (SEC), respectively. Results revealed that both the W. cibaria isolates established production of homo-polysaccharide glucose monomers and had a molecular mass higher than 220 000 Da and suggestive of prospective application as a viscosity improver or stabilizing agent in food formulations.

Keywords: Weissella, homo-polysaccharide, glucan, fermented foods, monosaccharide analysis

Introduction

New technologies that produce healthier foods and utilize minimal or no additives are constantly being explored by the dairy and food industry. With regard to fermented food products, such as yoghurt, butter milk, lassi and cereal based products, the maintenance and enhancement of quality involves addition of food additives such as colorants, flavor enhancing agents and hydrocolloids (Kang et al., 2005). However, these chemical additives are subjected to strict regulation and a current consumer trend is towards healthier and additive-free foods. Exopolysaccharides (EPS) produced by food grade lactic acid bacteria (LAB) carry generally recognized as safe (GRAS) status which make them much more favourable compared to their synthetic counterparts. Microbial EPS own highly desirable rheological changes in the food matrix, such as increased viscosity, improved texture and reduced syneresis, especially in fermented dairy and food products (Patel and Prajapati, 2013).

There is a wide variation in the composition of EPS produced by different LAB. Consequently, it is interesting to characterize EPS produced by different isolates to render their exact structural and chemical composition (Ismail and Nampoothiri, 2010). The monomer composition, the sequence and ring size of the constituting monomeric sugar(s), the location of the glycosidic linkages and the type of non carbohydrate structure constituents determine the primary structure of EPS molecule. In previous work, different strains of LAB were isolated from vegetables and traditional fermented food products of India including idli batter, dhokla batter and dahi to screen for production of EPS (Patel et al., 2012). Based on the biochemical and genetic characterization using 16S rRNA gene sequencing, total six strains were identified either as W. cibaria or W. confusa. These strains shown to possess possible probiotic temperament including antimicrobial activity against clinical pathogens, resistance to bile salts and to low pH (Patel et al., 2012, 2013), resulting in increased efforts to further characterize them. Adequate information is available about the EPS produced by the different strains of genus Lactobacillus and Pediococcus, but not much is known about the genus Weissella.

Considering this fact, EPS was characterized from the two novel strains of W. cibaria (strain 92 and strain 142) involving compositional monosaccharide analysis through HPLC and size exclusion chromatography (SEC) in the current work. These strains were confirmed to produce on an average 570 mg of EPS per litre (dry mass basis) in a semi-defined medium; without any optimization in the growth conditions (Patel et al., 2012).

Materials and Methods

EPS producing bacterial strains, growth conditions and media

Both the strains of W. cibaria 92 and 142 (genbank accession number JN792466 and JN792456) were maintained and stored at -20 °C as stock cultures in de Man- Rogosa-Sharpe (MRS) broth (Merck) containing 10% (v/v) glycerol. Prior to use, frozen cultures were plated onto MRS agar followed by two successive transfers into the respective liquid media.

Precipitation and quantification of EPS
A semi-defined media (SDM) as suggested by Kimmel and Roberts (1998) containing sucrose (5% w/v) was used for the production of EPS. The *Weissella* isolates were inoculated in the liquid medium (2% v/v inoculum) and grown at 37 °C for 24 h followed by removal of bacterial cells by centrifugation (15 000 g, 20 min) and supernatant was collected. The collected cell free supernatant was treated with 2.5 % (v/v) of 80% (w/v) trichloroacetic acid (Merck) to remove proteins and was mixed with three volumes of 95% cold ethanol for overnight at 4 °C to precipitate the EPS. The EPS was recovered by centrifugation, dried at 42 °C, resuspended in distilled water and dialyzed (MW cut off 12-14,000 Da, Spectrum Lab, USA) to remove low molecular weight contaminants. Finally, the samples were freeze dried and weighed. The same procedure was performed on uninoculated media and the weight of the resulting precipitate was subtracted from the amount of EPS produced by the isolates.

Monosaccharide composition of EPS

Preparative SEC was performed on two gel filtration columns in series, Superdex 75 (10/300 GL) and Superdex 200 (10/300 GL) using a FPLC system (GE Healthcare, Stockholm, Sweden). EPS isolates were dissolved in water (2.5 mg/ml) and filtered (0.2 µm PTFE, 13 mm filter, VWR, USA). The column was loaded with 500 µl of the sample and water was used as mobile phase at a flow rate of 0.3 ml/min. The separation was performed at room temperature and detection was achieved with a refractive index detector (Erma Inc., Tokyo, Japan).

**Results and Discussion**

Monosaccharide composition of EPS precipitated from LAB

Preliminary results of the thin layer chromatography (TLC) from the precipitated EPS samples showed a matching spot to glucose standard indicating glucose as one of the chief monomer sugar present in the different samples of EPS.

The major monosaccharide resulting from acid hydrolysis of the EPS obtained from the *W. cibaria* strain 92 and strain 142 was glucose (Figure 2). For both EPS samples, along with glucose peak, other two small peaks of galactose and mannose were observed in the HPLC chromatogram. However, they were also

<table>
<thead>
<tr>
<th>Sample/strain</th>
<th>Sugar composition percentage dry weight</th>
<th>Total sugar</th>
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<tbody>
<tr>
<td></td>
<td>Fucose</td>
<td>Arabinose</td>
</tr>
<tr>
<td>EPS strain 92 – broth media</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>EPS strain 92 – agar plates</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>EPS strain 142 – broth media</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>EPS strain 142 – agar plates</td>
<td>0.0</td>
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</table>
Fig. 2: Chromatogram showing monosaccharide composition of the EPS polymer (a) precipitated from agar plates of *W. cibaria* strain 92, (b) precipitated from broth of *W. cibaria* strain 92, (c) precipitated from agar plates of *W. cibaria* strain 142, and (d) precipitated from broth of *W. cibaria* strain 142, against standard monosaccharide sugars during HPAEC analysis.
Fig. 3: Chromatogram showing relative molecular weight of the EPS polymer (a) precipitated from agar plates of *W. cibaria* strain 92, (b) precipitated from broth media of *W. cibaria* strain 92, (c) precipitated from agar plates of *W. cibaria* strain 142, and (d) precipitated from broth media of *W. cibaria* strain 142 with dextran standards after running size exclusion chromatography.
present in control sample (only MRS broth) which indicated that it might be belonging to some media component and co-precipitated with biopolymer fraction when treated with ethanol. The EPS obtained from agar plates contained less amount of mannose as compared to the EPS collected from broth medium (Fig.1). Therefore, they were considered as contaminants from the media, specifically from yeast extract. Moreover, galactose can come as background contaminant from the agar agar powder used during the preparation of media.

As shown in Table-1, the biopolymers obtained from *W. cibaria* strain 92 and strain 142 were composed of approximate 61% of glucose in their structure when obtained from agar medium. These results are in harmony with other reported studies (Katina et al., 2009). Galle et al. (2010) also reported presence of glucose as a main constituent of EPS obtained from *W. cibaria* strains. Apart from this, when EPS was precipitated from broth media, it was found to comprise about 48% and 40% glucose with more percentages of mannose as assumed contaminant from the media. Fucose, arabinose, rhamnose, galactose, glucose, xylose and mannose were run as standard monomers to compare the retention type of the unknown sugar moieties from the EPS samples. The retention times of these standard monosaccharides were 5.86, 13.27, 14.88, 16.66, 20.58, 24.81 and 28.80 min for fucose, arabinose, rhamnose, galactose, glucose, xylose and mannose, respectively. In Fig. 2 chromatogram illustrates the monosaccharide analysis of EPS from *W. cibaria* strain 92 and strain 142 in parallel to standard sugars. For the biopolymer of *W. cibaria* 92, the monosaccharide sugar (glucose) had retention time of 20.48 and 20.57 min belonging from solid media and broth, respectively. Identical retention times were obtained for EPS belonging to *W. cibaria* 142 viz., 20.49 and 20.53 min for solid media and broth samples, respectively. Results indicate that the EPS from both *W. cibaria* isolates were found to be a homo-poly-saccharide composed of glucose monomers.

In a study reported by Bouaix et al. (2010), all the *Leuconostoc* and *Weissella* (including mainly *W. cibaria*) strains produced glucans assimilating sucrose from the growth medium. Similarly, Shukla and Goyal (2011) reported glucan formation from *W. confusa* when grown in sucrose-supplemented culture medium, while production of polymeric dextran in sourdoughs with concomitant formation of shorter iso-malto-oligosaccharides was found to the relationship between molecular mass and the retention time of standard dextran in a size exclusion chromatography (Fig. 3). Di Cagno et al. (2006) reported that EPS from sourdough isolate *W. cibaria* had an apparent molecular mass of approximately \(10^4\) Da. In that, the synthesis of EPS was found from sucrose only. While in an experiment conducted by Bouaix et al. (2010), the *W. cibaria* EPS have a size greater than \(10^5\) Da. Kim et al. (2008) isolated *Weissella hellenica* SKkimchi 3 from kimchi and purified EPS from sucrose containing medium. The resulting EPS reported to be a glucan type having 203,000 Da relative molecular mass.

In general, EPSs synthesized by LAB appear to have high molecular weight ranging from \(4.0 \times 10^4\) to \(6.0 \times 10^4\) Da. This data suggested that EPS produced by the two *W. cibaria* strain 92 and strain 142 is not having very high molecular mass, but its mass is large enough for its application as a viscosifying, gelling, or stabilizing agent in food formulations (Kang et al., 2005; Kim et al., 2008).

**Conclusions**

Partial characterization revealed that EPS obtained from the two *W. cibaria* isolates 92 and 142 comprises glucose monomers in the structure suggesting production of glucan kind of biopolymer and these homo-polysaccharides is having a molecular mass higher than 220 000 Da as determined from size exclusion chromatography. In this context, the location of the glycosidic linkages and structural analysis through NMR spectroscopy is further aimed in order to characterize the biopolymer completely and to find suitable application at industrial level.

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