Exopolysaccharides from Lactic Acid Bacteria

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Microbial polysaccharides have interesting and attractive characteristics for the food industry, especially when produced by food grade bacteria. Polysaccharides produced by lactic acid bacteria (LAB) during fermentation are extracellular macromolecules of either homo or hetero polysaccharidic nature, and can be classified according to their chemical composition and structure. The most prominent exopolysaccharide (EPS) producing lactic acid bacteria are *Lactobacillus*, *Leuconostoc*, *Weissella*, *Lactococcus*, *Streptococcus*, *Pediococcus* and *Bifidobacterium* sp. The EPS biosynthesis and regulation pathways are under the dependence of numerous factors as producing-species or strain, nutrient availability, and environmental conditions, resulting in varied carbohydrate compositions and beneficial properties.

Keywords: Exopolysaccharides ; Lactic acid bacteria ; Lactic acid fermentation ; fruit ; vegetable ; food

1. Introduction

Lactic acid fermentation (LAF) is a liable method to improve the shelf-life of fruit and vegetables along with nutritional and sensory qualities. Bacterial strains belonging to *Lactobacillus*, *Leuconostoc*, *Weissella* and *Bifidobacterium* genera are commonly involved in food LAF. These lactic acid bacteria can produce lactic acid and other organic acids, carbon dioxide, aromatic compounds, exopolysaccharides (EPS), bacteriocins and enzymes, all involved in the modification of quality of fermented foods. It has also been shown that lactic acid bacteria (LAB) are able to modulate the composition of phenolic compounds and enhance antioxidant activity [1]. Several studies have suggested that lactic acid fermented fruit juices, which exhibit a specific phenolic composition and contain EPS, could constitute a prebiotic food and have an influence on the structure and function of the gut microbiota [2][3]. The ability of LAB to modulate nutritional status of plant-based foods depends on bacterial strains and matrix used for LAF [4]. Among the compounds produced during LAF, EPS have the ability to change rheological properties of food such as viscosity [5], which contributes to the sensory acceptability and stabilization of the products [2]. In the food industry, hydrocolloids gather polysaccharides, which improve textural stability of suspensions, rheological properties of foods, pastry and cake texture and shelf-life by swelling in aqueous medium. It has been shown that EPS produced by *Lactobacillus*, *Leuconostoc* and *Weissella* strains have hydrocolloid properties [6] and would deserve to be tested in food formulation as innovative ingredient.

Depending on the bacterial species, homopolysaccharides (HoPS) and heteropolysaccharides (HePS) can be produced, and at very different levels, typically typically from 50 to 200 mg/L for HePS and up to 10 g/L for HoPS. Up to 70% of the energy of microbial cell can be used to produce EPS. However, this energy consumption is balanced by the protective effect of EPS against stress by the formation of a protecting barrier [7]. The cell is therefore protected from temperature or osmolarity shifts and from toxins and antibiotics. Both quantity and quality of EPS produced depend on the species, the nature and content of available sugars, the presence of micronutrients and fermentation conditions [8].

LAB polysaccharides have interesting and attractive characteristics, including antioxidant activities, and generate an increasing interest for their possible use in the field of food and pharmaceutical industries.

2. EPS Production

2.1. Effect of the Substrate Composition

A method commonly used to demonstrate EPS production by LAB is to grow them on solid MRS medium supplemented with sugars such as sucrose, maltose, fructose, glucose or lactose and to characterize EPS produced according to the appearance of the colonies. Indeed, colonies with HoPS have a viscous appearance whereas those with HePS have a shiny aspect [2]. EPS production can also be determined by evaluating their production in a liquid medium supplemented with various nitrogen and carbon sources and vitamins. Moreover, for EPS production in foods, the influence of sugars already present in the studied matrix must be taken into account.

A specific substrate is required for EPS synthesis and allows the action of specific enzymes on oligosaccharide carbon sources. Sucrose is the most commonly used substrate for HoPS synthesis. EPS production can also be influenced by modifying the nature or/and the amount of nutrients available as well as pH, water activity and oxygen concentration in the culture medium [9]. Indeed, the structure of EPS and their content vary according to carbon, nitrogen, phosphorus or sulphur sources [10]. For instance, a more than five-fold increase in HePS production was achieved by optimization of glucose, yeast extract and ammonium sulphate content in growth media of two *Lactobacillus plantarum* strains [11].

Hence, both pH and nature or amount of sugars should be considered for EPS production in fruit and vegetable products. Sugar content is highly variable in these matrixes, and approximatively ranges from 0.8 in citrus to 19.6 g/100 g in black grape within fruits, and from traces in baby spinach to 6.8 g/100 g in beetroot within vegetables. The main sugars consist mostly in fructose, glucose or sucrose as it was found for litchi and red pepper, black grape and black radish, or pineapple and carrot, respectively. Apart from these major sugars, some products also contain compounds in smaller amounts, such as galactose or maltose in peach [12]. Fruit and vegetable pH values range from around 2.3 in citrus and 3.5 in pineapple to around 6 in cantaloupe melon and carrot. Both pH and main sugars variation could lead to a wide diversity of EPS production from fruit and vegetables.

2.2. Effect of Bacterial Strain and Incubation Parameters on EPS Production

Apart from medium composition, the species and strain of bacteria influence HoPS production levels. Yu et al. [13] showed that only one strain of *Weissella cibaria* screened from kimchi can achieve a significant EPS production (up to 9.8 g/L) in a dose-dependent way in response to a high sucrose supplementation in the growing media. Similarly, different strains from the same species, *W. cibaria* MG1, MG7 and F33 isolated from cereal environments, showed approximately a 2.8-fold variance in EPS production in a sucrose-MRS broth [14].

Fessard and Remize [15] demonstrated the impact of temperature on EPS production using various LAB isolated from tropically grown fruits and vegetables. *Leuconostoc pseudomesenteroides/mesenteroides*, *Leuconostoc lactis* and *Weissella cibaria/confusa* isolates were able to produce EPS at 30 °C but not at 37 °C. Similarly, dextran production by *L. lactis* AV1n could be observed with growth media containing sucrose (but not glucose, fructose or maltose) and this production occurred at 20 and 30 °C but not at 37 °C [16]. Dextran content was higher at 30 °C than at 20 °C, with 4.15 g/L and 2.96 g/L, respectively. Dextran production decreased to 0.41 g/L when the LAB was grown at 37 °C. These results demonstrated that dextran production by *L. lactis* AV1n was the highest at 30 °C. To manufacture Turkish-type fermented sausages, named Sucuk, *Lb. plantarum* 162 R and *L. mesenteroides* N6 were chosen for their ability to produce EPS [17]. EPS production was higher for *L. mesenteroides* compared to *Lb. plantarum* and the mix of both strains. Moreover, increases in temperature and incubation time led to an increase in hardness, gumminess, and chewiness and to a decrease in adhesiveness. It therefore appears that among LAB species, the capacity and yields of EPS production would be a relevant parameter to consider, in order to select bacterial species and strains that match the desired properties of fermented fruits and vegetable products.

2.3. HoPS Production Pathway

Biosynthesis of HoPS, described in *Weissella*, *Leuconostoc*, *Lactobacillus* and *Pediococcus* genera, is performed by extracellular enzymes, glucansucrases or fructansucrases [6]. Fructansucrase and glucansucrase transfer a monosaccharide from a specific substrate to the growing polysaccharide chains [18]. These enzymes belong to the glycosyltransferase (GTF, E.C. 2.4x.y) group and catalyze the hydrolysis of sugars, the resulting monosaccharide residues being attached to a glycan acceptor chain. These enzymes can be categorized into transglucosidases (E.C. 2.4.1.y; glucan-synthesizing dextran-sucrases, mutansucrases, and reuteransucrases) and transfructosidases (E.C. 2.4.1.y or 2.y; fructan-catalyzing transfructosidases levansucrases and inulosucrases). Glucansucrases are responsible for glucans and fructans synthesis and belong to the alpha-amylase superfamily as part of the glycosides hydrolases (GH), in clan GH-H [2][19].

Most LAB producing HoPS harbor only one glucansucrase gene; however, some LAB genomes have more than one gene encoding the enzyme and are thus able to synthesize different HoPS. Approximately 150 glucansucrase genes have been sequenced and about a third correspond to functional genes [20]. Sequence analysis of glucansucrase encoding genes from *Lactobacillus, Leuconostoc, Weissella* and *Pediococcus* species confirmed that these LAB can be clustered according to the specificity of the EPS glycosidic bonds, rather than the 16S rRNA taxonomy.

3. Conclusions

EPS production by LAB involved in fruit and vegetable fermentation is gaining interest because of both effects on sensory characteristics and potential positive health effects.

The impact of EPS of food texture can be seen as a strategy to limit the use of additives, but requires a production level of several g/L. To that aim, the production of HoPS is more favorable than HePS as several LAB species, such as *L. pseudomesenteroides*, *L. mesenteroides*, *W. cibaria* and *W. confusa* produce these compounds at high levels. Moreover, production level of EPS can be enhanced through adjustment of fermentation medium composition and incubation parameters. However, this optimization should not decrease the general sensory quality of fruit or vegetable fermented foods.

EPS produced during fermentation of fruit or vegetables can exert prebiotic activity, but also antioxidant, anti-inflammatory and cholesterol-lowering activity. Most of previous results were obtained *in vitro*, on cell models or in mice, and will require further investigations to determine if the observed effects can be expected from human diet modifications.

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