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The size and shape of three water-soluble, non-ionic polysaccharides produced by lactic acid bacteria: A comparative study

Marianne Øksnes Dalheim[a], Nina Bjørk Arnfinnsdottir[b], Göran Widmalm[c], Bjørn E. Christensen[a,*]

[a] NOBIPOL, Department of Biotechnology, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway
[b] Department of Physics, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway
[c] Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

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ABSTRACT

Three water-soluble, non-ionic extracellular polysaccharides (EPS) obtained from lactic acid bacteria (*S. thermophilus* THS, *L. helveticus* K16 and *S. thermophilus* ST1) were subjected to a comparative study by means of multidetector size-exclusion chromatography, providing distributions and averages of molar masses, radii of gyration and intrinsic viscosities. All polysaccharides displayed random coil character. Further analysis of the data reveals differences in chain stiffness and extension that could be well correlated to structural features. The calculated persistence lengths ranged from 5 to 10 nm and fall within the range typical for many single-stranded bacterial or plant polysaccharides. The ST1 polysaccharide had the highest molar mass but the lowest persistence length, which is attributed to the presence of the flexible (1 → 6)-linkage in the main chain.

1. Introduction

Lactic acid bacteria (LAB) are used as probiotics, i.e., live microorganisms that may confer a health benefit to the host, and in production of fermented foods (Divya, Varsha, Nampoothiri, Ismail, & Pandey, 2012; Patrick, 2012; Popova et al., 2012; Quigley, 2010). These gram-positive bacteria are generally regarded as safe (GRAS) and an important application is as dairy starters in production of yogurt and cheese (Patel & Prajapati, 2013). In yogurt production a number of positive effects result as a consequence of using these types of bacteria, e.g. mild flavouring, improved texture, ropiness and sensory characteristics (Purwandari, Shah, & Vasiljevic, 2007). In Mozzarella cheese-production increased moisture content and improved melting properties were regarded as positive outcomes from using a LAB strain (Broadbent, McMahon, Welker, Oberg, & Moineau, 2003). It is the *in situ* production of beneficial components during fermentation that makes these probiotic LAB particularly interesting.

Microbial exopolysaccharides can be found in the cellular external environment either as capsules associated with the bacterial cell or loosely attached as slime, which may be released into the surrounding environment of the bacteria (Cescutti, 2009; Nwodo, Green, & Okoh, 2012). The former type of polymeric material is referred to as a capsular polysaccharide (CPS) (Widmalm, 2013) and the latter material is in contrast often simply denoted as an exopolysaccharide (EPS) (Ruas-Madiedo, 2014). These biopolymers play a pivotal role for the bacteria in the ecological niches they populate. In particular as a physical barrier acting as a protective shield, being part of cell-cell recognition processes and colonization through biofilm formation (Ruas-Madiedo, 2014).

Two groups of exopolysaccharides can be identified in LAB strains, namely homopolysaccharides consisting of a single type of sugar residue and heteropolysaccharides having different sugar residues within the polymer (De Vuyst & Degeest, 1999; Fontana, Li, Yang, & Widmalm, 2015; Nwodo et al., 2012). The bioactivity of the EPS is due to their action as antagonists to toxins from other bacteria; they act as a physical hindrance to the toxin targeting eukaryotic cells thereby avoiding the interaction either by blocking the toxin *per se* or by acting as a toxin-scavenger (Ruas-Madiedo, 2014; Ruas-Madiedo et al., 2010).

Lactobacillus helveticus and *Streptococcus thermophilus* are both of the genera belonging to LAB. *S. thermophilus* THS, *L. helveticus* K16 and *S. thermophilus* ST1 produce heteropolysaccharides having

* Corresponding author. Tel.: +47 73 59 33 27; fax: +47 73 59 12 83.
E-mail addresses: marianne.dalheim@ntnu.no (M.Ø. Dalheim), nina.arnfinnsdottir@ntnu.no (N.B. Arnfinnsdottir), gw@organ.su.se (G. Widmalm), bjorn.e.christensen@ntnu.no (B.E. Christensen).

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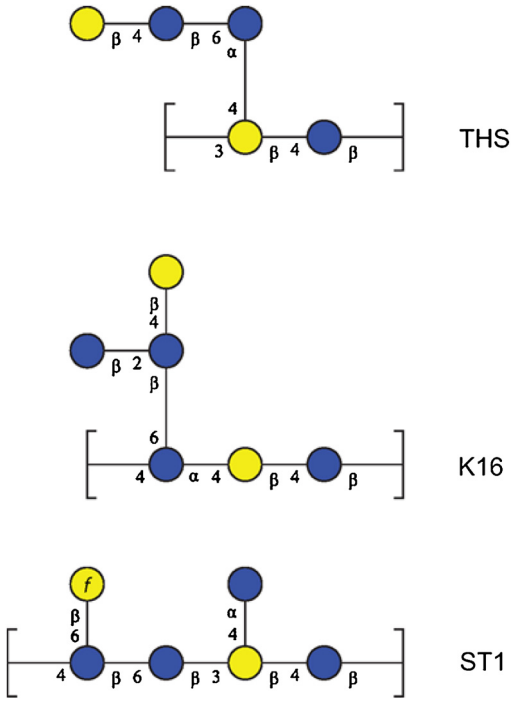

Fig. 1. Schematic representation in CFG-format of the repeating units from the EPS produced by *S. thermophilus* THS, *L. helveticus* K16 and *S. thermophilus* ST1. Note the presence of lactose as a structural element in all of the backbones of the polysaccharides as well as in the THS and K16 side-chains.

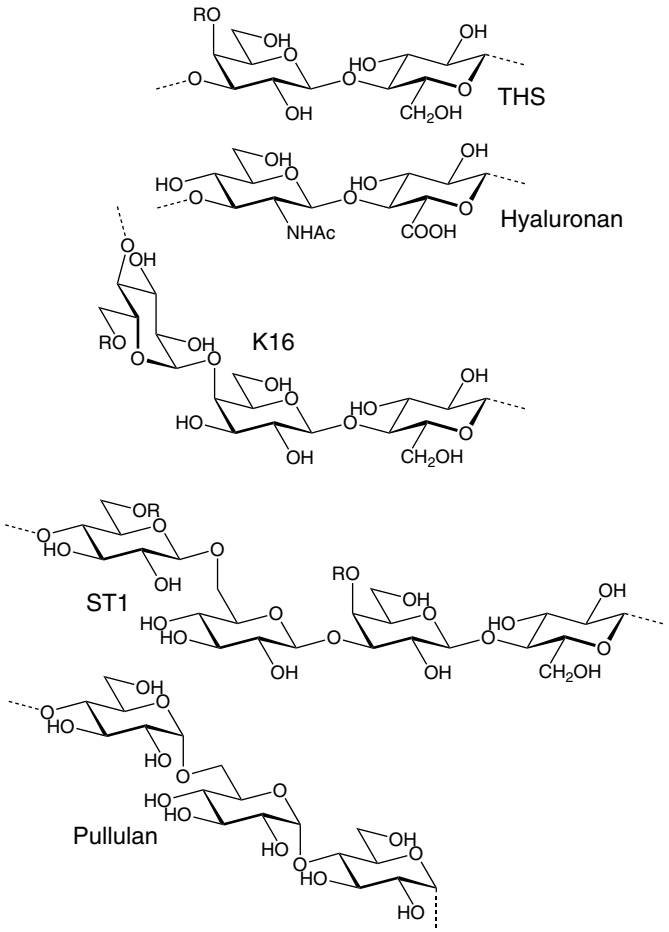

Fig. 2. Chemical structures of THS, hyaluronan, K16, ST1 and pullulan polysaccharides. R denotes different side-chains.

glucose and galactose as constituents of the branched pentasaccharide repeating units (RUs) for the THS strain and hexasaccharide RUs for the K16 and ST1 strains (Fig. 1) (Nordmark, Yang, Huttunen, & Widmalm, 2005; Säwén, Huttunen, Zhang, Yang, & Widmalm, 2010; Yang, Staaf, Huttunen, & Widmalm, 2000). The EPS preparations used for structural determination employing in particular NMR spectroscopy gave highly viscous solutions. We herein extend the characterization of these EPS by analysing them by size exclusion chromatography with multi angle laser light scattering and viscometry (SEC-MALLS-VISC), providing information about chain extension and stiffness. It further allows a comparative analysis between the three polysaccharides in solution (under identical conditions and with the same methodology). To our knowledge few, if any, comparisons of this type have been published for bacterial EPS.

The three polysaccharides are neutral but water-soluble. The absence of charged groups should render them largely insensitive to variations in pH and ionic strength. The physical properties are therefore expected to depend only on the geometry of the sugar residues and the glycosidic linkages, and the chain length distributions. To gain further insight into the structure-function relationships we find it useful to compare these EPS to structurally related water-soluble polysaccharides which have been thoroughly described in the literature.

The backbone of THS has structural similarities with hyaluronan (HA) by having a repeating disaccharide structure with alternating β-(1 → 3)- and β-(1 → 4)-linkages (Cowman & Matsuoka, 2005) (Fig. 2). The differences lie in the substitution patterns: HA has a carboxylic group (intrinsic pK_a = 2.9, Cleland, Wang, & Detweiler, 1982) due to the D-Glc*p*A residue and an *N*-acetyl group at position 2 of the D-Glc*p*NAc residue, whereas THS has in addition to an axial hydroxyl on C4 of the second unit a bulky trisaccharide attached to it. HA behaves in aqueous solution as a single semi-flexible random coil with an intrinsic persistence length (at infinite ionic strength) of about 7 nm (Mendichi, Soltes, & Schieroni, 2003).

The ST1 EPS has a more complex structure, where the backbone consists of a tetrasaccharide repeating unit, in addition to single residue side-chains on two of the residues. We did not find analogues to this particular structure, but since it contains an α-(1 → 6)-linkage, which is known to be particularly flexible compared to (1 → 3)- and (1 → 4)-linkages (Pendrill, Säwén, & Widmalm, 2013), we found it useful to compare it to pullulan (Liu, Brant, Kitamura, Kajiwara, & Mimura, 1999), which consists of maltotriose repeating units joined by α-(1 → 6)-linkages (Fig. 2).

K16 has a backbone without (1 → 6)-linkages, but the diaxial α-(1 → 4)-linkage is expected to provide chain flexibility over the corresponding diequatorial equivalent, such as cellulose or a β-mannan backbone, for example. K16 has further a branched trisaccharide side-chain, and bears a slight structural relationship to xanthan.

In the present study we simultaneously analyse under identical experimental conditions the dilute solution properties of three EPS from lactic acid bacteria, using multi-detector SEC analysis as previously conducted for alginates (Vold, Kristiansen, & Christensen, 2006), chitosans (Christensen, Vold, & Vårum, 2008), barley mixed linkage glucans (Christensen et al., 2001), and hyaluronan (Kristiansen, Dalheim, & Christensen, 2013). Direct comparison to other polysaccharides with related chemical features, particularly the geometries of the backbones, was further performed.