MOLECULAR SIZE AND SUB-UNIT STRUCTURE OF A NOVEL EXOPOLYSACCHARIDE FROM *LACTOBACILLUS DELBRUECKII* SUBSP. *BULGARICUS* NCFB 2074

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Introduction. Exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) are widely used as food thickening and gelling agents. The subunit structures of many of these have been elucidated over the last 5 years or so. Moreover there is information on the EPS gene clusters involved, including the genes that are predicted to regulate chain length during the polymerisation. Many strains synthesise EPS of high molecular mass, although the actual size and shape is often not determined (1). This is a consequence of the analytical techniques such as Size Exclusion Chromatography (SEC) that are commonly available.
We have used High Performance Size Exclusion Chromatography (HPSEC) and Multi-Angle Laser Light Scattering (MALLS) to determine the size of a novel EPS from *Lb delbrueckii* subsp *bulgaricus* NCFB 2074.

Methods. Freeze-dried samples of exopolysaccharides were prepared using the method of Marshall (2) from strains of *Lb. delbrueckii* susp. *bulgaricus*. Samples were then dissolved in distilled water at 50°C and then passed through a cell disrupter (Constant Cell Disruption Systems) at different pressures (10, 20 and 45 kpsi). The same sample was also passed through the orifice up to 6 times. Following disruption, samples were recovered and their molecular size determined by SEC, HPSEC and by MALLS.

Results and discussion. Monomer analysis and 1D- and 2D-NMR have shown that this novel EPS is a highly branched hepta-saccharide composed by repeat units of galactose and glucose in a molar ratio of 4:3. (3) (Fig. 1) SEC has found, as with most EPS from the dairy LAB, the molecular mass is in excess of 1.8 million kDa. The value is larger than that of the available standards used to calibrate SEC columns. A similar molecular mass (~2 million kDa) was also found using HPSEC and MALLS. The EPS from this strain is susceptible to chain shortening when subjected to the pressure exerted in a cell-disrupter; however there appears to be a "limiting" size of around 500 kDa which is maintained and is not further reduced on subsequent passage through the disrupter.

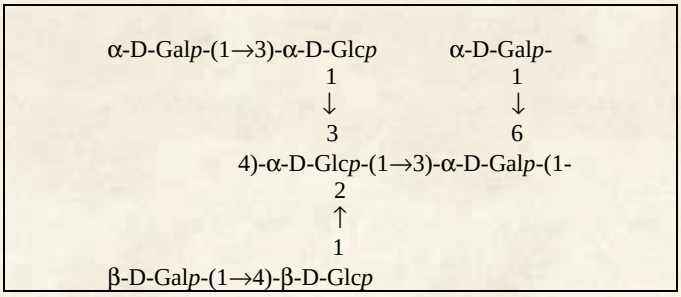

Fig. 1. Structure of the EPS produced by Lb. delbrueckii subsp. bulgaricus NCFB 2074.

Conclusions. Results suggest that *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2074 posses a novel EPS with a large molecular mass which it is susceptible to physical disruption. Under these conditions its size can be reduced if it is subjecting to pressure. The structure however remains unchanged. This therefore has particular significance to scale up and commercial exploitation of these dairy strains for use as texture-enhancing starter cultures for yoghurt and related dairy products as degradation may result in different rheology in the final retail product.

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