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**Isolation and Characterisation of Bacterial  
Exopolysaccharides Produced by *Lactobacillus*  
*delbrueckii* subsp. *bulgaricus* NCFB 2483 and  
*Sphingomonas elodea* ATCC 31461**



**Massey University**

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Food Technology

at Massey University, Palmerston North,  
New Zealand.

Kelvin Kim Tha Goh

2004



**CERTIFICATE OF REGULATORY COMPLIANCE**

This is to certify that the research carried out in the Doctoral Thesis entitled

Isolation and Characterisation of Bacterial Exopolysaccharides Produced by  
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## ABSTRACT

The aim of this study was to explore the characteristics of a non-gelling exopolysaccharide (EPS) obtained from *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2483 and a gelling EPS obtained from *Sphingomonas elodea* ATCC 31461 (31461). The EPSs were isolated from the two bacterial strains grown in milk permeate-based media. They were purified and then characterised using light scattering and viscometric techniques. A greater emphasis of this research was placed on 2483 EPS since its physical characteristics have not been reported to date. In the case of 31461 EPS, a model for gelation of the sodium gellan was proposed based on rheological and light scattering measurements. The rheological properties of the two EPSs were also compared with several commercial polysaccharides.

Microscopy examination of 2483 EPS was carried out using confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). In CLSM, the lectin SBA (from *Glycine max*) Alexa Fluor 488 conjugate was used to stain the EPS since it has affinity for galactopyranosyl residues present in 2483 EPS. The CLSM micrographs showed a random distribution of EPS aggregates in the culture medium. At high magnification, the SEM micrographs showed web-like EPS structures. These structures were formed during the critical point drying process, when the EPS, which filled the interstices and channels of the protein aggregates, dehydrated. The 2483 EPS aggregates were found to be stable at neutral or low pH (~3.9) but were disrupted at high pH (pH 8-10).

Procedures commonly used to quantify EPS from culture medium were found to be unreliable. In the development of an improved EPS assay, each of the processing steps was examined. Key improvements included the use of Flavourzyme for protein hydrolysis; optimising ethanol concentration to prevent lactose crystallisation yet allowing complete EPS precipitation; and a suitable centrifugation regime to minimise EPS loss. The improved EPS assay gave reproducible results (5% coefficient of variation).

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The isolation of 2483 EPS from milk media proved to be a difficult task because of interference from non-EPS components. An effective and simple approach allowing maximum EPS recovery involved the use of a hydrolysed milk medium which was ultrafiltered (UF) to remove molecular species larger than  $2.5 \times 10^5$  Da. The UF permeate was suitable for the growth of 2483 with an EPS yield of  $\sim 400\text{mg/L}$ . Two EPS fractions (namely a soluble and an insoluble fraction) were isolated by ethanol precipitation and the soluble ‘ropy’ fraction was further purified to achieve  $\sim 98\%$  purity. The elemental analysis of the purified fraction revealed the presence of nitrogen ( $\sim 2.7\%$  w/w). This could be due to the interaction of some peptides (from the growth medium) with the EPS. The polysaccharide composition of the soluble EPS fraction comprised of galactose, glucose, rhamnose and mannose residues (5:1:0.6:0.5). Traces of glucosamine were also found in the fraction.

The purified fraction of 2483 EPS was characterised. Using a capillary viscometer, an intrinsic viscosity of  $\sim 2013\text{mL/g}$  was determined. The flow curves of the 2483 EPS solutions obtained using a rotational viscometer showed shear-thinning behaviour and an exponent value of  $\sim 0.76$  (based on the Cross-type model) is typical of random coil polymers. The concentration dependence of the viscosity plot produced gradients of  $\sim 1.1$  in the dilute domain and  $\sim 3.3$  in the semi-dilute to concentrated domain. The coil overlap parameters at three concentration domains ( $c^*[\eta]$ ,  $c_{cr}[\eta]$  and  $c^{**}[\eta]$ ) were 0.55, 2.86 and 5.67 respectively. The molecular parameters of the 2483 EPS were found via static light scattering measurements to have a weight-average molar mass ( $M_w$ ) of  $\sim 2 \times 10^6$  Da, a z-average root-mean-square radius ( $(r_g^2)_z^{1/2}$ ) of  $\sim 165\text{nm}$  and a low polydispersity index ( $M_w/M_n \sim 1.15$ ). The plot of  $M_w$  versus  $(r_g^2)_z^{1/2}$  gave a gradient of approximately 0.5, which also suggested that the EPS polymer adopted a random coil conformation.

The second part of the research involved gellan gum. Two gellan samples were studied. The first gellan sample was obtained from the fermentation of *Sphingomonas elodea* ATCC 31461 using milk permeate-based medium (31461). The second sample was a commercial high acyl gellan (LT100). Both gellan samples were converted to their

sodium forms (Na-31461 and Na-LT100 gellan) using cation exchange resin and purified. The Na-gellan samples were highly sensitive to changes in  $\text{Na}^+$  concentrations. From oscillatory measurements, it was found that the complex moduli of the two Na-gellan samples superimposed closely at a specific  $\text{Na}^+$  concentration. The model for the conformational changes of Na-gellan molecules from a solution to a gel was proposed based on rheological and light scattering data. At very low  $\text{Na}^+$  concentrations ( $<19\text{mM}$ , in the case of Na-LT100), Na-gellan molecules were single-stranded ( $M_w \sim 2.5 \times 10^5$  Da) and adopted random coil conformation (exponent value based on the Cross-type model of  $\sim 0.76$ ). At a slightly higher  $\text{Na}^+$  concentration ( $\sim 19\text{--}24\text{mM}$ ), Na-gellan molecules formed double-helices which led to a two-fold increase in molecular weight ( $M_w \sim 5.2 \times 10^5$  Da). The double-stranded molecule appeared to be stiffer (exponent value of the Cross-type model  $\sim 0.82$ ) and the mechanical spectra ( $G'$ ,  $G''$ ) demonstrated 'weak gel' characteristics. A further increase in the  $\text{Na}^+$  concentration ( $>24\text{mM}$ ) resulted in the formation of a gel network. The study also found that at low  $\text{Na}^+$  concentration, both single-stranded and double-stranded Na-gellan molecules had a tendency to form aggregates under zero-shear conditions. The interactions involved in these aggregates were considered weak and transient, according to the Cox-Merz plot and light scattering data.



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