

Analysis has shown the purified polysaccharide to be a polysaccharide having a repeating unit containing the following components:

7 D-gluco units comprising 1 unit of 6-substituted glucose, 2 units of 4-substituted glucose, 2 units of 3-substituted glucose, and 2 units of 4,6-disubstituted glucose; 1 D-galacto unit comprising 3-substituted galactose; 1 acetate unit; and 1 pyruvate unit; the above components including 1 4,6-disubstituted glucose branch point and 1 side chain terminated by a 4,6-O-(1-carboxyethylidene)-D-glucose unit.

The purified polysaccharide has an optical rotation $[\alpha]_{22} = -15^\circ$ (C 0.68_{H₂O}) which indicates that all the sugars are linked in the β -D configuration.

The viscosity and flow properties of the polysaccharide of the present invention may be described in terms of the consistency index k and the flow behaviour index n , as suggested by Krumel and Sarkar, "Flow Properties of Gums useful in the Food Industry", in Food Technology, April 1975 pp 36-44, Vol. 29(4).

The apparent viscosity (η) in centipoises was measured using a cone and plate viscometer at various rates of shear (D) in sec^{-1} . A plot of $\log \eta$ against $\log D$ for a 1% by weight solution of the polysaccharide according to the present invention at 25° C. gave a straight line graph which gave a k value (η extrapolated to a shear rate of 1 sec^{-1}) of 4600 cps and an n value (the slope of the graph plus 1) of 0.22. A commercial sample of food grade xanthan gum sold under the Trade Mark Keltrol by Kelco of San Diego, California gave under the same conditions values of k and n of 5,000 and 0.23 respectively.

The following Examples illustrate the invention:

EXAMPLE 1

10 Liter Batch Fermentation

Exopolysaccharide production by *Pseudomonas* sp NCIB 11264 was followed in a 10 liter batch fermentation, without pH control, for 50 hours at 30° with volume/volume aeration and an impeller speed of 350 rpm. The doubling time of the organism under these conditions was 140 minutes.

Logarithmic growth continued for some 18 hours at which point (E_{520} 6.0) all nutrients were apparently in excess, although the oxygen tensions were not determined. It is known, however, that exopolysaccharide synthesis is at a maximum when the oxygen tension is non-limiting. At this stage, polysaccharide could be detected by isopropanol precipitation, although exopolymer had been detectable in increasing amounts in culture supernatants from 12-13 hours after inoculation utilising a more sensitive viscometric assay. Thus, although exopolysaccharide production apparently commenced during the late exponential phase of growth, the formation was maintained maximally for another 20 hours during the stationary growth phase, before the rate of production eventually began to decrease. This fermentation pattern is typical of a secondary metabolite.

Of the glucose utilised, only 30% was converted into exopolysaccharide the other 70% being metabolised to establish and maintain the culture.

EXAMPLE 2

Steady State Exopolysaccharide Production

Exopolysaccharide-producing cultures of *Pseudomonas* sp NCIB 11264 were maintained in a steady state for

up to 500 hours. The defined medium based on that described by Gray et al (1966) supplemented with glucose (10 mg/ml), was used in all the previously described continuous culture studies.

As a result of these investigations, the initial concentrations of some of the components were reduced, and continuous polymer production followed under conditions of imposed nitrogen limitation. Conditions were optimised at pH 7.0 ± 0.1 with a growth temperature of $30^\circ \pm 1^\circ$ and an aeration rate of 500 ml/min.

After inoculation, the culture was grown as a batch and allowed to establish for 24 hours before the flow rate was adjusted to 44 ml/hour. The course of the fermentation, run at a dilution rate of 0.08 hr^{-1} , was followed for 500 hours. An impeller speed of 900 ± 10 rpm was maintained throughout. Steady state values for total cell density, polysaccharide level and glucose conversion remained constant—after 100 hrs these values were respectively 0.26 ($E_{520} \times 10^{-1}$), 1.6 mg/ml and 40%, while after 500 hours they were 0.26 ($E_{520} \times 10^{-1}$), 1.6 mg/ml and 45%. There was no evidence to indicate cultural deterioration or development of mutant strains.

Polysaccharide samples analysed were of a constant composition and solutions of the polymer (0.1 mg/ml) had a similar relative viscosity (1.7 ± 0.05) when measured at 25° with a modified Zimm-Crothers rotating cylinder type viscometer (55 mA), indicating that there had been no change in the molecular weight of the exopolymer produced over the period of the fermentation.

We claim:

1. A process for the preparation of a polysaccharide which comprises cultivating *Pseudomonas* sp NCIB 11264 (ATCC 31260) to yield an exocellular polysaccharide.

2. The process of claim 1 wherein cultivation is effected in a continuous manner.

3. The process of claim 2 wherein the cultivation in a continuous manner is conducted under nitrogen-limited conditions.

4. The process of claim 2 wherein the cultivation is effected in a cultivation medium comprising NH_4Cl and KH_2PO_4 supplemented with a supplementary carbon source.

5. The process of claim 4 wherein the cultivation medium is supplemented with glucose or sucrose.

6. The process of claim 1 wherein the cultivation is effected at 25° C. to 35° C.

7. The process according to claim 1 wherein the pH of the cultivation medium is maintained at above 6.

8. The process according to claim 7 wherein said pH is maintained at 6.5 to 8.

9. The process of claim 1 wherein the exocellular polysaccharide is isolated by precipitation with an organic water-miscible solvent and is then deionised.

10. The process of claim 9, wherein the isolated polysaccharide is freeze-dried.

11. The process of claim 1 wherein said exocellular polysaccharide is recovered.

12. A process for the preparation of an exocellular polysaccharide which process consists essentially of the steps of:

(a) inoculating a nutrient medium with *Pseudomonas* sp NCIB 11264 (ATCC 31260);