

PROCESS FOR THE PREPARATION OF POLYSACCHARIDE 9

This invention relates to a polysaccharide possessing useful flow and gel-forming properties, and to a process for its preparation.

Polysaccharides from microbiological sources are becoming increasingly important in many different industrial applications where materials with particular flow properties are required. Microbial exopolysaccharides can possess unique properties and furthermore can be more easily produced to a uniform specification than plant or algal polysaccharides.

Polysaccharides, such as locust bean gum and alginates, are widely used in industry, as emulsifiers, stabilizers and thickeners. In the food industry, they are used as emulsion stabilizers for ice-cream, as gelling agents for milk puddings, as thickeners for sauces and as foam stabilizers for beer. They are also used in the manufacture of paper and textiles and for thickening drilling muds in oil drilling. Xanthan gums are also increasingly widely used in a range of applications.

We have discovered a polysaccharide possessing in aqueous systems pseudoplastic flow and shear thinning properties which are remarkably similar to those of xanthans and alginates, suggesting similar commercial applications. This polysaccharide can be prepared by cultivating a polysaccharide-producing strain of *Pseudomonas* sp deposited at the National Collection of Industrial Bacteria, Torry Research Station, 135 Abbey Road, Aberdeen under the Number NCIB 11264, in a nutrient medium therefor. This polysaccharide-producing strain has also been deposited at the American Type Culture Collection in Rockville, Md. under the number ATCC 31260.

Pseudomonas sp NCIB 11264 (ATCC 31260) was isolated from a carbohydrate-rich industrial effluent. Its morphology and physiology may be summarised as follows (all temperatures in degrees centigrade):

Morphology

Oxid CM3 Nutrient Agar 25°

Gram-negative, small-medium parallel-sided rods, becoming short rods and sometimes coccobacilli.

Motile. Flagella position: single, polar (electron micrographs).

Colonies (6 days): 2 mm, whitish, opaque (translucent confluent growth), circular, entire, low convex, smooth, soft, easily dispersed, no variation.

Physiology 30°

Catalase	+
Kovacs' oxidase	+
Growth at 37°	+ (colony growth rate at 37° approximately equal to rate at 25°)
Growth at temperatures in excess of 40°	very poor
Anaerobic growth, glucose agar	—(slight)
Hugh & Leifson Glucose	oxidative
Peptone water	} no acid
sugars, Andrades' indicator	
Glucose, Lactose, Fructose	
Sucrose, Maltose, Mannitol	
Glycerol, Starch	
Kosers' citrate	+
Starch hydrolysis	—
King et al A & B	—

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Arginine, Møllers	—
Gelatin hydrolysis	—
Casein hydrolysis	—
NH ₃ from tryptone	—
NO ₃ ' to NO ₂ ' or N ₂	—
Christensen's urease	alkaline \leq 1½ days
DNAse	—
Egg yolk plate reaction	—
Voges - Proskauer test	—
Methyl red	—
Indole	—
Polypectate degradation	—

The micro-organism can be cultivated, under aerobic conditions, in any convenient medium in which it will grow and produce exocellular polysaccharide. Typical media include complex broths, e.g. a 1% nutrient broth, or a chemically defined medium such as that described by Gray et al. (*Biochimica et Biophysica Acta*, 117, 22 32, 1966), with a supplementary carbon source of, for example, glucose or sucrose. A supplement of about 2% w/v in the medium is desirable.

A particularly preferred defined medium (the glucose-supplemented Gray et al medium) for use in the cultivation of *Pseudomonas* sp NCIB 11264 has the following composition:

Glucose	20 g/liter
NH ₄ Cl	2.66 g/liter
KH ₂ PO ₄	5.44 g/liter
NaOH to pH 7	approx. 1.5 g/liter
solution of trace elements*	6 ml/liter
*a solution containing	MgSO ₄ · 7H ₂ O 10 g
	MnCl ₂ · 4H ₂ O 1 g
	FeSO ₄ · 7H ₂ O 0.4 g
	CaCl ₂ · 2H ₂ O 0.1 g
	Distilled water to 1 liter

The culture may be effected batch-wise or in a continuous manner, according to conventional practice. Continuous cultures are preferably conducted under nitrogen-limiting conditions, e.g. about 0.5 g NH₄Cl/liter. With a glucose-supplemented medium, the glucose conversion is about 30% in batch cultures, but up to 75% in continuous cultures.

A temperature of from 25° C. to 35° C. is satisfactory, a temperature of about 30° C. being optimal.

In general, polysaccharide production is found to be enhanced when an excess of carbon source is present, under nitrogen-limited conditions. Preferably the pH of the medium should not fall below 6 and may conveniently be from 6.5 to 8.0.

The exocellular polysaccharide may be isolated from the culture supernatant (free from cells) by precipitation with an organic water-miscible solvent such as isopropanol and deionized, e.g. by a conventional desalting using dialysis. Conveniently, unwanted cellular matter can be removed by trypsin digestion, e.g. by digesting an aqueous solution of the polysaccharide buffered to pH 7-8, e.g. using 0.2 M HEPES buffer, at about 30° C. in the presence of a bacteriostat such as mercuric chloride. For example, 3.6 liters of solution, buffered to pH 7-8 with 0.2 M HEPES, are treated with 20 mg of the enzyme and mercuric chloride (1.5 ml of a saturated alcoholic solution) for 5 days at 30° C.

After dialysis, the isolated material can be freeze-dried to give the purified dry exopolysaccharide.