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OPTIMIZATION AND CHARACTERIZATION OF AN EXTRACELLULAR POLYSACCHARIDE PRODUCED BY MONILIELLA POLLINIS

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SUMMARY A yeast strain Moniliella pollinis produces an extracellular highly viscous gum-like polysaccharide of glucan type in a simple mineral medium. Optimum conditions for its production and properties are described. The viscosity decreased after lyophilization.

INTRODUCTION

We have found recently that an osmophilic yeast (Moniliella pollinis) can produce mucilaginous, filamentous white gum in good yield. We are not aware of any report in the literature on the production and the optimization of extracellular polysaccharide from Moniliella pollinis, although the production of polyols from this strain has been reported (Hajny et al., 1964; Dooms et al., 1971). The polysaccharide would have a wide range of possible applications. Like other microorganisms, yeast that produce extracellular polysaccharides belong to taxonomically related groups whose members are characteristically mucoid (Slodki, 1980).

MATERIALS AND METHODS

Strain. The strain used originates from the osmophilic unidentified strain isolated by Hajny et al. (1964) from fresh pollen in honey-comb, and received at the Mycothèque de l'Universite Catholiqué de Louvain as MUCL 11525 (=CBS 461.67) in 1968. Subsequently, the strain has been studied for polyol production and described as the type of a new taxon, Moniliella tomentosa var. pollinis (Dooms et al., 1971). Because of its variation in pigmentation and conidiation rate, monoconidial substrains have been selected from the mother strain. All the wild strain and substrains produced highly viscous polysaccharides when cultured in 5% dextrose peptone broth. Their yield varied from 4.77 to 7.49 g/L. However, the highest yield was obtained by the wild strain, while the other MUCL strains of Moniliella tomentosa var. tomentosa and Moniliella acetoabutans produced 0.34 and 3.41 g/L of polysaccharides, respectively. Moniliella tomentosa var. pollinis (type MUCL 11525) (Dooms et al., 1971) has since been taxonomically segregated as Moniliella pollinis (Dooms et al., 1971; de Hoog and Guého, 1984).

The strain (MUCL 11525) was maintained on agar slants prepared with dextrose 20%, yeast extract 1% and urea 1%. After growth at 25°C the strain was transferred and maintained at 4°C except as stated.

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Polysaccharide Production. The polysaccharide was produced either in 500 ml laboratory shaken flasks or in a 2 L capacity of fermentor (Biolafitte). The shaken flasks contained 50 ml of the following culture medium: dextrose 10%, yeast extract 1% and urea 0.1% (Hajny et al., 1964). The culture medium was inoculated with 3 ml of precultured solution (preculture was prepared by inoculating a 3 ml of culture medium with mycelium, shaking at 27°C for 48 h). This was then incubated at 27°C for 5 days at constant shaking (100 rpm). After the specified period of incubation the culture medium was separated from the mycelium by centrifugation (40,000 g). The mycelium was washed twice with warm water. The washings were mixed with the supernatant obtained by first centrifugation. The polysaccharide was precipitated by the addition of 2 volumes of ethanol in the total supernatant. The polysaccharide obtained was dried under vacuum at 40°C, ground and stored under vacuum at ambient temperature.

Assays. Total polysaccharides were estimated in the following samples using the method of Dubois et al. (1956): (a) Precipitated polysaccharide after dissolving it in minimum quantity of distilled water at 60°C. (b) 2 mg each lyophilized and vacuum oven dried powder. Residual glucose in the culture medium was estimated using the method of Nelson and Somogy (Spiro, 1966). Dry weight of mycelium was obtained by drying the well washed mycelium at 105°C until a constant weight was achieved.

Composition. Fifty mg dry powder of polysaccharide was hydrolyzed for 4 h at 100°C in 2 ml of 2 N H_2SO_4. The hydrolyzate was neutralized by $BaCO_3$, filtered on Celite and then passed in an ion exchange column of Zerolite DM-F. The monomers were eluted by double distilled water and freeze-dried. The dry powder (500 µg) was dissolved in 50% ethanol and subjected to two dimensional descending paper chromatography with standard glucose, galactose and other sugars. The solvents used were butanol:acetic acid:water 60:15:25 (Smith, 1960) and ethyl acetate:pyridine:water 50:25:50 (Isherwood, 1951). The spots of reducing sugars were revealed by aniline oxalate at 100°C. The presence of glucose was also estimated using specific enzyme method as described (Finch et al., 1969).

RESULTS AND DISCUSSION

Effect of Different Concentrations of Carbon Source on the Production of Polysaccharide. It is well known that microbial polysaccharides are normally secondary metabolites, provided an excess of carbon source is present. Since Moniliella pollinis is an osmophilic yeast it was interesting to examine the effect of various concentrations (10%, 20% and 40%) of dextrose on the production of polysaccharide. Table 1 shows that by increasing the dextrose concentrations from 10% to 20% almost 1.5 fold polysaccharide production increased, but further increase to 40% had no effect. However, mycelial dry weight increased steadily with increasing dextrose concentration; thus, production of polysaccharide was not related directly to growth. This result is similar to our earlier observations with Glomerella cingulata (Sarkar et al., 1985).

Table 1. Influence of different concentration of carbon source on the production of polysaccharide.

Concentration of dextrose (%)	Polysaccharide (g^{-1})	Mycelium dry wt. (g^{-1})	Conversion of glucose into polysaccharide (%)
10	3.95	6.85	3.95
20	5.73	11.40	2.86
40	5.45	19.80	1.362

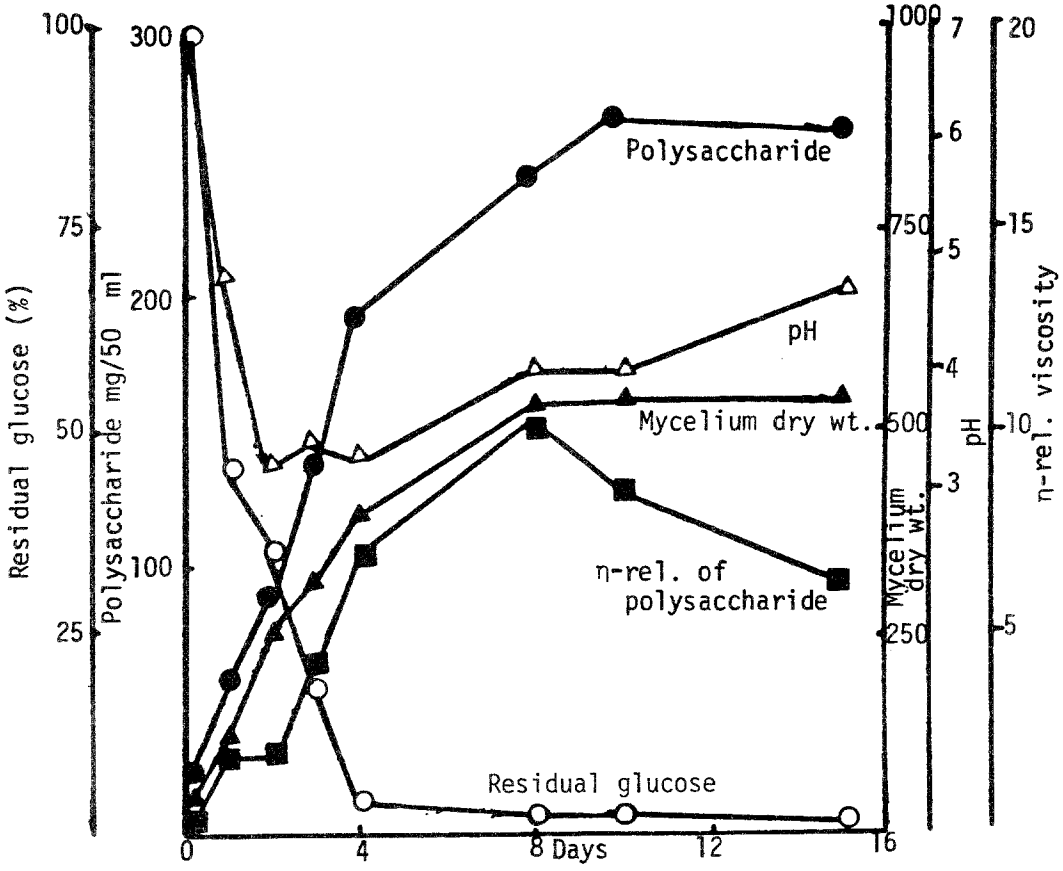

Figure 1. Effect of inoculation time on the production of polysaccharide from Moniliella pollinis.

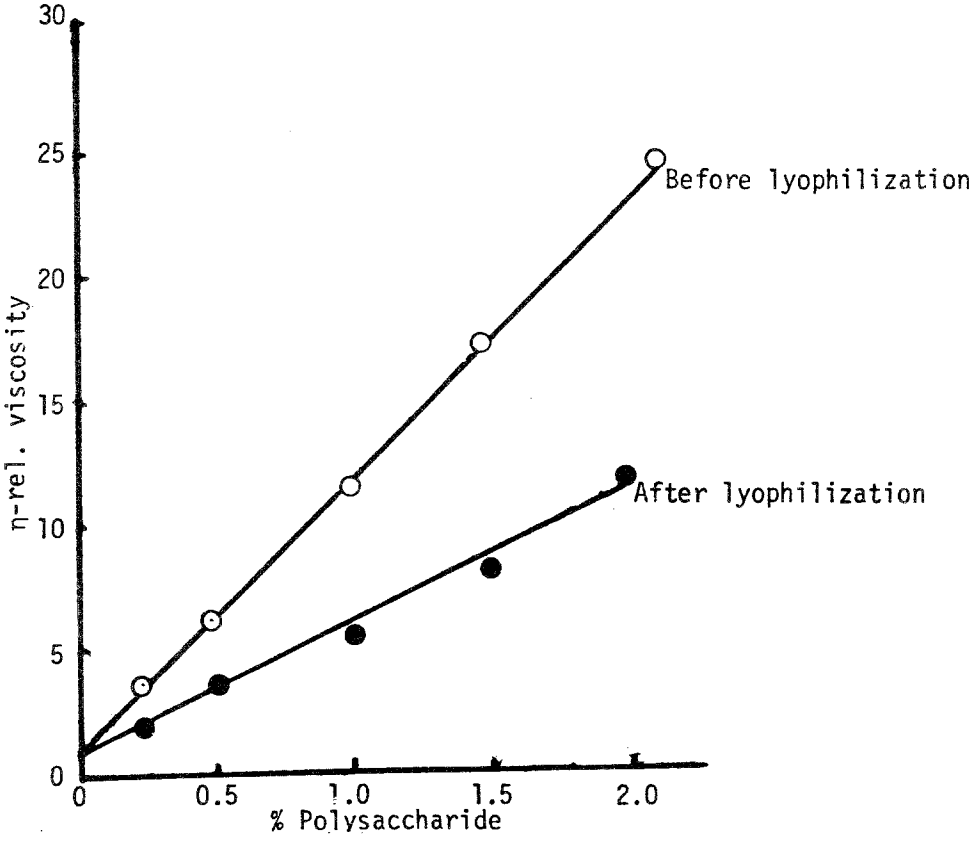

Figure 2. Effect of various concentrations of polysaccharide on the viscosity, before and after lyophilization.

Effect of Inoculation Time on the Production of Polysaccharide. Fig. 1 shows the production of polysaccharide in shaken flasks. The polysaccharide concentration increased rapidly during four days of incubation and more slowly after this period. The pH of the culture medium decreased drastically within two days of incubation and thereafter increased. The maximum quantity of polysaccharide was produced when the pH of the culture medium reached pH 4.0. The viscosity of the culture medium initially increased in parallel with polysaccharide production but after eight days while the concentration of polysaccharide remained high the viscosity decreased. However, the polysaccharide content remained quite stable even after fifteen days of incubation, suggesting that there was no major depolymerase activity in the culture medium, unlike that observed in Glomerella cingulata (Sarkar et al., 1985).

Characterization. The polysaccharide produced was white, gum-like, and highly soluble in cold and hot water. When the viscous polysaccharide solution was lyophilized and the resultant white product solubilized in water a less viscous solution was obtained (Fig. 2). Lyophilization might have altered the reticulary system of the polysaccharide. Drying under vacuum in an oven at 40°C gave a white granular product which retained the viscosity and other rheological properties.

Elementary analysis of polysaccharide revealed that it contained carbon (39%), nitrogen (0.21%), hydrogen (5.8%) and oxygen (53%). The polysaccharide consisted of mostly glucose and traces of uronic acid, confirmed by paper and gas liquid chromatographies. The composition is glucose (90%), uronic acid (8.7%) and mannose (1%), in some agreement with the results of Weijman (1979) who analyzed the sugar composition of the intact cells of Moniliella suaveolens by gas chromatography and found α- and β-glucose as predominant sugar and α, β mannose and α,β,-galactose in small amounts.

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