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# Production of Insoluble Exopolysaccharide of *Agrobacterium* sp. (ATCC 31749 and IFO 13140)

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## Abstract

*Agrobacterium* isolated from soil samples produced two extracellular polysaccharides: succinoglycan, an acidic soluble polymer, and curdlan gum, a neutral, insoluble polymer. Maize glucose, cassava glucose, and maize maltose were used in fermentation medium to produce insoluble polysaccharide. Two *Agrobacterium* sp. strains which were used (ATCC 31749 and IFO 13140) in the production of insoluble exopolysaccharide presented equal or superior yields compared to the literature. The strain ATCC 31749 yielded better production when using maize maltose, whose yield was 85%, whereas strain IFO 13140 produced more when fed maize glucose, producing a yield of 50% (on reducing sugars).

**Index Entries:** Microbial exopolysaccharides; *Agrobacterium* sp.; microbial gums; curdlan; fermentation.

## Introduction

An insoluble and extracellular microbial polysaccharide composed completely of D-glucose residues linked by β-D-1,3, was isolated and identified through Gram-negative bacteria culture, *Alcaligenes faecalis*, *myxogenes* variety, mutant 10C3K, currently classified as ATCC of *Agrobacterium* sp. The exopolysaccharide (EPS) was tested and found to be soluble and form a gel in a warmed aqueous suspension and was named "curdlan" (*1–3*). This gum was approved by the Food and Drug Administration (FDA) in 1996, being used in food industry owing to its ability of producing an excellent, hard, and resistant gel.

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The production of curdlan can be done through continuous and batch systems, using a chemically defined medium, having a carbohydrate as the main source of carbon. The glucose is the most commonly used carbohydrate in different concentrations: varying from 4% to 8% *(4–6)*, producing about 50% gum on a mass basis. Lee et al. *(7)* obtained excellent results using 10% of maltose (yield of 48%) and sucrose (yield of 47%) as carbon sources in the production medium. Using the same concentration of glucose in the evaluated conditions, those researchers reached a yield of 40%. They used fructose, galactose, lactose, and raffinose too, but with lower yield. According to Sutherland *(8)*, the hydrolyzed starch and glucose are on a large scale accepted by industry for microbial transformation, although they are found in different levels of purity. They are available substrates worldwide and in general, have lower prices. This fact is valid for sugarcane molasses, which need a clarification process before use. So more, research to find a more economical carbon source is essential for industrial production of the polysaccharide.

The gum is easily separated from the fermentation medium owing to its solubility in the alkaline medium *(9,10)*, eliminating the use of solvents very common in the separation of others polysaccharides. The carbon source used in fermentation is the most expensive part of the process.

## Materials and Methods

### *Microorganism*

It was acquired two lyophilized strains of bacterium *Agrobacterium* sp. from the American Type Culture Collection (ATCC 31749, United States) and from the Institute for Fermentation of Osaka (IFO 13140, Japan).

### *Carbon Sources*

Cassava's glucose syrup: Commercial food product, furnished by Indemil Indústria e Comércio de Milho Ltda, Paranavaí, Pr, Brazil (commercial label: Manicandy® 4084). The composition declared by producer is presented in the Table 1. Maize's glucose syrup: commercial food product, furnished by Corn Products Brasil Ingredientes Industriais, Balsa Nova, Pr, Brazil (commercial label: Excell® 1040). The composition declared by producer is presented in the Table 1. Maize's high maltose syrup: commercial food product, furnished by Corn Products Brasil Ingredientes Industriais, Balsa Nova, Pr, Brazil (commercial label: Mor-sweet® 1557). The composition declared by producer is presented in the Table 1.

### *Production of EPS*

The production of the insoluble EPS was carried out in flasks Schott. Cultivation at 30°C for 15 d at 120 rpm. A chemically defined medium was

Table 1
Characteristics of the Glucose and Maltose Syrups of Commercial Origin

| Characteristic | Cassava's glucose syrup Manicandy® | Maize's glucose syrup Excell® | Maize's high-maltose syrup Mor-sweet® |
|---|---|---|---|
| Substance dries (%) | 83–84.5 | 81–83 | 85 |
| Equivalent dextrose (%) | 37–41 | 38–40 | n.c. |
| pH | 4.5 | 4.5–5.5 | 4.5–5.5 |
| $SO_2$ (ppm) | 150 | 150 | n.c. |
| Dextrose (%) | 16.9 | 15 | 12 |
| Maltose (%) | 13.2 | 12 | 42 |
| Other sugars (%) | 69.9 | 73 | 46 |

n.c., not certain. The symbol ® stands for commercial products trademark.

used, described by Nakanishi et al. *(4)* (g/100 mL): Glucose, 4; $(NH_4)_2HPO_4$, 0.15; $KH_2PO_4$, 0.10; $MgSO_4$, 0.05; $Fe_2(SO4)_3{\cdot}7H_2O$, 0.005; $MnSO_4{\cdot}nH_2O$, 0.002; $CoCl_2{\cdot}6H_2O$, 0.001; $ZnCl_2$, 0.001; $CaCO_3$, 0.30. The pH was adjusted to 7.0. Commercial products of this study substituted the glucose. The quantity syrups used was calculated based on total reducing sugar (TRS) concentration. The samples were collected at predetermined periods to determine TRSs.

### *Total Reducing Sugars*

The technique described by Nelson *(11)* was used for TRS determination.

### *Polysaccharide Recovery*

The production medium containing insoluble EPS was centrifuged and the sediment was solubilized in a solution of 1 *N* NaOH. After centrifugation the sediment (cells and insoluble solids) was discarded and the supernatant (solubilized EPS) was precipitated by neutralization, using a solution of 1 *N* HCl. The insoluble material was water washed and dried by lyophilization. The percent yield was calculated based on the EPS weight (g) obtained compared with the TRS weight (g) consumed in the process.

## Results and Discussion

The two strains studied responded differently during production with commercial sources of carbon. Figures 1 and 2 show the TRS consumption of each commercial product and the laboratory glucose.

When using maize glucose ("Excell") as carbon source, the two strains showed a satisfactory production of EPS, considering yield of 55% for strain ATCC 31749 and 50% for strain IFO 13140 based on TRS. The strain IFO 13140 showed the best result for the three alternative sources researched.

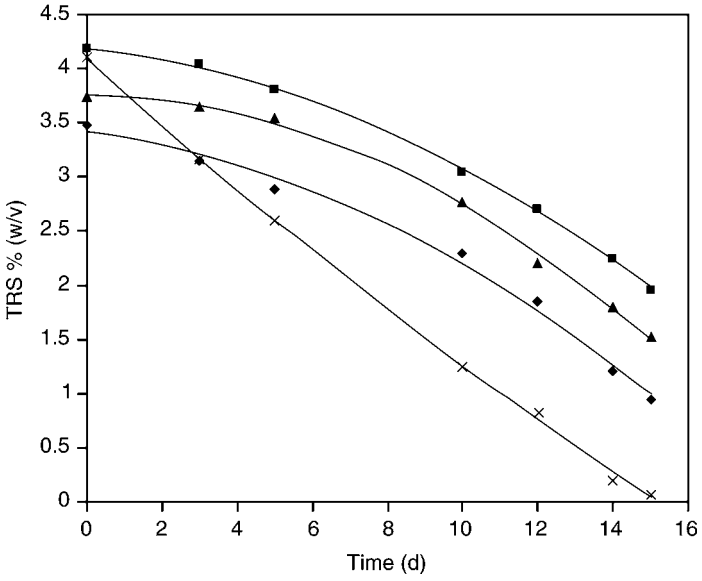


**Fig. 1.** Observation of the consumption of total reducing sugars (TRSs) during insoluble polysaccharide production by strain ATCC 31749 of *Agrobacterium* sp. in a medium made with different carbon sources. ◆, cassava glucose; ■, maize maltose; ▲, maize glucose; ×, laboratory glucose—control.

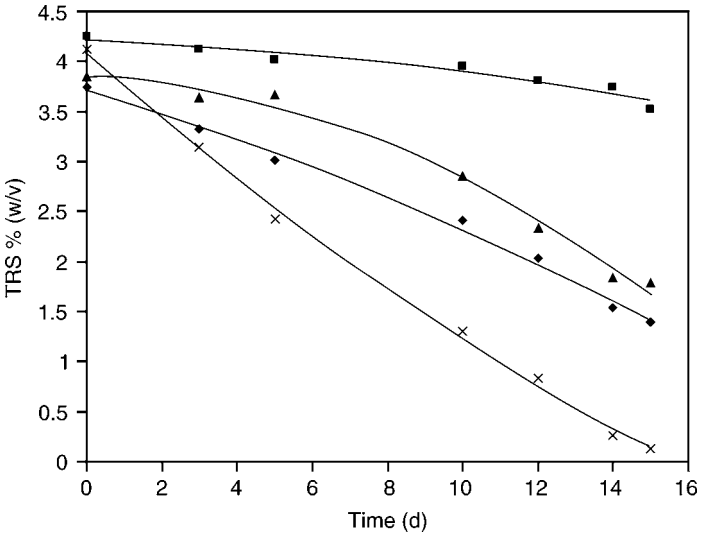


**Fig. 2.** Observation of the consumption of total reducing sugars (TRSs) during insoluble polysaccharide production by IFO 13140 de *Agrobacterium* sp. in a medium made with different carbon sources. ◆, cassava glucose; ■, maize maltose; ▲, maize glucose; ×, laboratory glucose—control.

The two strains were able to use the carbohydrates presented in the commercial product "Mor-sweet" (maize maltose). Strain ATCC 31749 was more efficient than the other ones, showing a yield of 85%, whereas the

strain IFO presented a yield of 48% of insoluble EPS on the consumed TRS during fermentation.

The use of cassava glucose ("Manicandy") produced insoluble EPS with the two strains employed too, but it was indeed the commercial product which had lower yield results, as follows of 44% on the consumed TRS for strain ATCC 31749 and 45% for strain IFO 13140.

## Conclusion

Although the commercial carbohydrates used did not have a highly defined chemical composition, they were useful in the production of insoluble EPS from *Agrobacterium* sp. However, the two strains of *Agrobacterium* sp. did not consume completely the present sugars in the commercial products. Lee et al. *(2)* cite monosaccharides (glucose, fructose, and galactose), disaccharides (maltose and sucrose), and a trisaccharide (raffinose) as usual carbon sources used by *Agrobacterium* sp. in the curdlan production. In the composition of the studied commercial products, some carbohydrates are not reported in literature as producers of the polysaccharide (*see* Table 1; "other sugars" are trisaccharide, oligosaccharides, and dextrins). These sugars were not metabolized in the medium and, therefore, they were not converted into polysaccharides. It is also possible that certain carbohydrates of the medium can control the curdlan biosynthesis (rarely explored in literature), which explains the low sugar consumption in the medium of production.

The commercial products used show relatively lower cost than the commercial glucose cost—50% Excell, 20% Mor-sweet, and 60% Manicandy—cheaper than the glucose. However, the amount used for producing culture medium resulted in a higher final cost of polysaccharide compared with the cost of glucose used as carbon source. The latter was completely metabolized by the strains used in this study and the commercial carbohydrates were not consumed completely by microorganism, which does not justify its use in the commercial processes.

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