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Polysaccharide production from callus cultures of *Cereus peruvianus* Mill. (Cactaceae)

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ABSTRACT *- The stems of adult plants and calli of* Cereus peruvianus *were used for polysaccharide extraction and characterization. The carbohydrate contents of polysaccharide extracted from stems and calli tissues were estimated to be 58.08% and 35.21%, respectively. The ratio carbohydrate contents of the plant stems and callus cells was approximately 1.65:1. The glycosyl composition of the stems and callus polysaccharides consisted mostly in galactose, arabinose, and rhamnose. This shows that calli induced from hypocotyl are capable of producing significant amounts of the same polysaccharides present in stems of adult plants.*

Key words: callus culture, polysaccharides, columnar-cactus.

INTRODUCTION

Plants of *Cereus peruvianus* Mill., a cactus species, provide various compounds of economic, pharmacological, and industrial interest. Among the products that may be directly obtained from this cactus species are viscous gum with potential industrial applications, such as the flocculation of impurities in drinking water (Nozaki et al. 1993), pollutant abatement from effluents of paper and pulp industries (Barros and Nozaki 2002), and cosmetics manufacturing (Alvarez et al. 1992) and a pectic compound (pectic acid) that can be used to prepare jams, jellies, gums, yogurts, and other products (Alvarez et al. 1995). The major gum fraction of *C. peruvianus* is an *O*-acetylated and uronic acid-containing rhamnoarabinogalactan, whose intrinsic viscosity may exceed 1000 mL g^{-1}, and almost protein-free polysaccharide forms viscous solution upon redissolution (Alvarez et al. 1992).

C. peruvianus plants can be easily propagated by seeds and through vegetative propagation; however, variations in plant growth have been detected and were attributed to the diverse environmental conditions (Weiss et al. 1993). Climatic, soil, and water conditions affect the plant growth and metabolism. The different environmental conditions may also induce variations in the production of compounds of pharmacological and industrial interests.

Plant cell culture can be considered an alternative via for the synthesis of natural products at an industrial scale (Ellis 1988). It is a means to obtain various plant components that may be employed to produce any product of interest.

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Recent studies have shown that *C. peruvianus* calli have been explored for *in vitro* selection as an important source of alkaloids (Oliveira and Machado 2003). A comparison of the alkaloid production in the stems and calli of adult plants indicates higher alkaloid amounts in the latter. The callus culture in *C. peruvianus* may also be a potential polysaccharide source. Thus, in the present study, we investigated the production of polysaccharides and the sugar composition in callus cultures of *C. peruvianus*.

MATERIAL AND METHODS

Callus culture

Stems of adult plants and calli of *C. peruvianus* were used for polysaccharide extraction and characterization. The stems were collected from plants maintained on the campus of the State University of Maringá (Maringá, PR., Brazil) for 15 years. The long-term callus tissue cultures were obtained from hypocotyl in MS medium containing B5 vitamins, 0.8% agar (Select Agar-Invitrogen Life Technologies), 3% sucrose, 4.0 mg L^{-1} of 2.4- dichlorophenoxyacetic acid and 4.0 mg L^{-1} of N-(2-furanylmethyl)-1H-purine-6 amine (Oliveira et al. 1995), at 32 °C during a 16-hour photoperiod, and under 15 μ mol m^{-2} s^{-1} irradiance (by fluorescent tubes 20 W). Calli were generated from pieces of hypocotyl stalks of six seedlings that grew from seeds collected from a single plant (Oliveira et al. 1995). The calli (6 years old) were subcultured in fresh medium in intervals of 30-60 days.

Polysaccharide extraction

The freeze-dried stems (30 g) and calli (30 g), following addition of 1 volume of tap water, were separately mixed in the blender; after filtration the viscous solution was precipitated with 2-3 volumes of ethanol. Precipitate was further washed with solvent to provide a pigment-free powdery gum.

Acid hydrolysis of the gum (0.5 g) was performed with 1 M sulfuric acid at 100 ºC during 6 h. After the hydrolysis procedure, the solution was neutralized with 4 M NaOH to pH 7.0. The hydrolysis product was lyophilized separately.

Carbohydrate contents of the lyophilized hydrolyzed product (0.5 g) were estimated by a colorimetric assay based on reaction with phenol (5% w v^{-1} in water) and concentrated sulfuric acid. Absorbance was determined at 450 nm and 490 nm (Sturgeon 1990).

Gas chromatographys

Monosaccharides were analyzed and the sugars identified by gas chromatographys. The monosaccharide was derived according to Sweeley et al. (1963). TMSI derivatives were then injected into a Finnigan gas chromatographer equipped with a flame ionation detector and an OV-1 gas capillary column (50 m long and 0.25 mm diameter). Hydrogen was the carrier gas and the following temperature program was used: 150 ºC (30 s^{1}, 5 ºC min^{-1}) until 250 ºC; the temperature was maintained at 250 ºC for another 10 min.

RESULTS AND DISCUSSION

The carbohydrate contents of extracted polysaccharide from shoot and callus of *C. peruvianus* were estimated at 58.08% and 35.21%, respectively. Nevertheless, a large number of plant cultures failed to produce the expected natural products or produced only small amounts of the compounds of interest (Lindsey and Yeoman 1986). Modifications in inorganic nutrients and temperature and variations in carbon source and concentrations are important parameters that must be optimized to obtain successful callus establishment, maintenance, and development. They may even stimulate a productivity increase of the desired compounds in cultured cell plants. A relationship between culture medium containing tyrosine and total alkaloid production was reported in *C. peruvianus* callus cultures (Oliveira and Machado 2003). Actually, callus increased the capacity for alkaloid synthesis by 11%.

Data of the glycosyl composition for each isolated polysaccharide indicate that the callus culture of *C. peruvianus* makes up 4.0% of the dry weight in mucilage, while that of higher plants makes up 7.4%.

The structural features of the gummy polysaccharide of *C. peruvianus* columnar-cactus have been studied: galactose, arabinose, and rhamnose appear to be the common monosaccharide constituents (Alvarez et al. 1992). In the present study, the glycosyl composition for polysaccharide from stem and callus tissue also consisted mostly in galactose, arabinose, and rhamnose in the proportions 5.6:2:1 and 24:1:2.6, respectively. However, other monosaccharides, such as fructose, were also detected on the calli (Figure 1).

The differential glycosyl proportion for the polysaccharide from stem and callus culture may be explained by differential mechanisms such as a shift in the metabolic flow from the sucrose in the callus culture medium. Polysaccharide production in callus cultures has been closely related to the differentiation response induced by the relatively high (10^{-5} M) concentration of 2.4-D (Honda et al. 1996). Addition of 2.4-D was found to be essential in culture of tuberose callus for a high yield of extracellular polysaccharides. Honda et al. (1996) showed that the

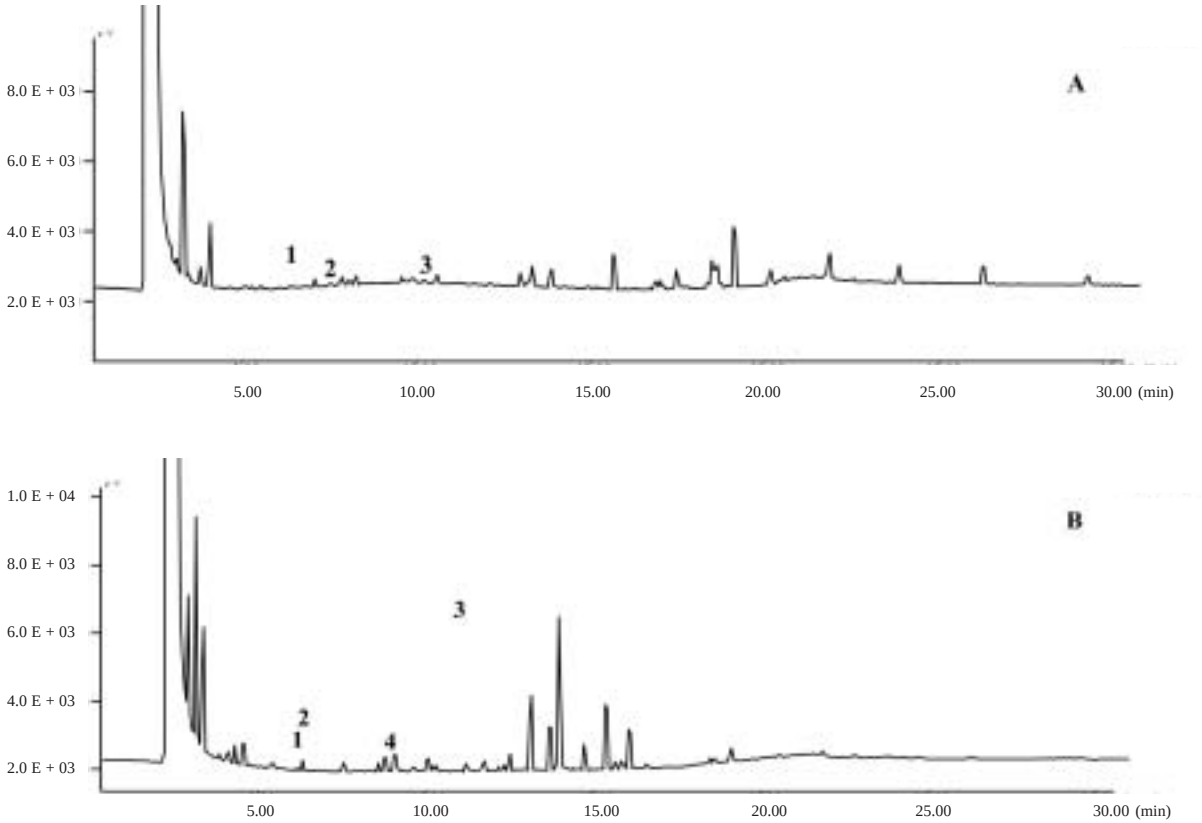

Figure 1. Gas chromatogram of polysaccharide hydrolyzed with flame ionization detection. Hydrolyzed from stem (A) and calli (B) of *Cereus peruvianus*. Peaks: 1. TMS-arabinose, 2. TMS-rhamnose, 3. TMS-galactose, and 4. TMS-fructose

biosynthesis of extracellular polysaccharide components was controlled by plant growth regulators supplemented in the medium. The *C. peruvinaus* calli showed differential gene expression (Machado et al. 1993, Torquato et al. 1995, Mangolin et al. 1999) that could result in an altered sugar proportion.

In *C. peruvianus* we found that hypocotyls-induced calli are capable of producing significant amounts of the same polysaccharides present in adult plant stems. While the plant growth of *C. peruvianus* is different under the diverse environmental conditions, the calli are clonal tissues that show rapid propagation. A 5-7 fold growth rate was observed over a period of 30 days (Oliveira-Collet et al. 1996). Furthermore, the polysaccharide synthesis could be induced after medium optimization with special attention to the growth regulator balance that controls dedifferentiation mechanisms, the highest 2.4-D proportion with the addition of exo-glycosil as additional supplement on culture medium, and with the establishment of suspension callus cultures. *C. peruvianus* plants do require appropriate and large areas for culture where the calli may be subcultured *in vitro*, indefinitely propagated on the optimized medium and used as an alternative polysaccharide source, substituting the use of adult plants.

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Produção de polissacarídeos em cultura de calos de *Cereus peruvianus* Mill. (Cactaceae)

RESUMO - *Tecidos de caules de plantas adultas e de calos foram usados para a extração e caracterização de polissacarídeos. O conteúdo de carboidratos extraído dos polissacarídeos de caules e de calos foi estimado em 58,08% e 35,21%, respectivamente. A relação do conteúdo de carboidratos entre os caules das plantas e as células de calos foi aproximadamente 1,65:1. A composição glicosídica dos polissacarídeos de caules e de calos foi principalmente galactose, arabinose, e ramnose, mostrando assim, que os tecidos de calos induzidos a partir de hipocótilos são capazes de produzir quantidades significantes dos mesmos polissacarídeos presente nos caules de plantas adultas.*

Palavras-chaves: cultura de calos, polissacarídeos, mandacaru.

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