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Communication

Isolation and Characterization of a Pure Mannan from *Oncidium* (cv. *Gower Ramsey*) Current Pseudobulb during Initial Inflorescence Development

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A water-soluble and neutral polysaccharide was extracted from the current pseudobulbs of *Oncidium* "Gower Ramsey" during the early inflorescence stage (flower stalk less than 4 cm) by hot water, precipitated with ethanol, and purified with an anion exchanger. From the data of monosaccharide composition and linkage and anomeric configuration analyses, the polysaccharide was identified as a linear β-1→4 linked mannan.

Key words: current pseudobulbs; *Oncidium*; mannan

Oncidium is a thin-leaf, epiphytic sympodial orchid. The characteristic feature of sympodial orchids is to have sequential identical shoots. As in other epiphytic orchids, such as *Cattleya* and *Dendrobium*, an enlarged bulb-like structure, a pseudobulb, is formed at the base of the stem. The pseudobulb is important in water, mineral, and carbohydrate storage to support both vegetative growth and reproduction.[1–3] The carbohydrate pool in current pseudobulbs varies greatly during inflorescence development,[4,5] and we found a large amount of mucilage in the current pseudobulbs of *Oncidium* "Gower Ramsey" during the early inflorescence stage (flower stalk less than 4 cm).[5]

A mucilaginous substance was extracted from pseudobulbs according to the method of Fedeniuk and Biliaderis with a slight modification.[6] Briefly, pseudobulbs were homogenized and extracted twice with 3 volumes of water at 80 °C for 2 h. The mixed extract was centrifuged at 10,000 *g* for 30 min, and decanted and passed through a 0.45 µm syringe filter to remove any debris. The filtrate was stirred with Celite (30 g/l) for 30 min and centrifuged at 10,000 *g* for 1 h. The supernatant was added with 3 volumes of ethanol at 4 °C and allowed to stand overnight. The precipitate was collected by centrifugation, dissolved in a minimal amount of deionized water, dialyzed extensively against deionized water in a 10-kDa MW cutoff membrane, and lyophilized. Purification of the mucilage was achieved using a DEAE Sepharose fast flow (20 ml, bed height 15 cm)[7] column, by eluting with deionized water and deionized water containing 1 M NaCl in sequence. A small volume of each eluate fraction was spotted on a silica plate (Silica gel 60, Merck, Darmstadt, Germany) and stained with an orcinol reagent (150 ml ethanol, 3.38 ml sulfuric acid, and 3 g 3,5-dihydroxytoluene monohydrate).[8] The majority of carbohydrate was present in the water eluate, implying that the polysaccharide had little if any charges.

The water-eluted polysaccharide was lyophilized and hydrolyzed with 2 M trifluoroacetic acid in a screw-capped tube at 110 °C for 1 h, as described by Redgwell and Hansen.[9] The acid hydrolysate was dried in a Speed-Vac, dissolved in deionized water, and filtered through a Millipore Millex-GX Nylon membrane. The composition of polysaccharide was analyzed with a high pH anion-exchange chromatographic (HPAEC) column (CarboPA1, Dionex, Sunnyvale, CA) using 70 mM NaOH as eluent, and quantified with a pulsed amperometric detector (Dionex).[5] As shown in the Fig. 1 inset, mannose was the almost exclusive monosaccharide component in the polysaccharide. The ratio of mannose to total neutral sugar was estimated to be 96.4%. Other neutral sugars, such as arabinose, galactose, and glucose, were present in miniscule amounts, 1.5%, 0.9%, and 1.2% respectively (Fig. 1, inset).

For the linkage analysis, the mannan was first permethylated using the NaOH/dimethylsulfoxide slurry method.[10] The permethylated derivative was converted into a partially methylated alditol acetate by hydrolysis (2 M trifluoroacetic acid, 121 °C, 2 h), reduction (10 mg/ml $NaBH_4$, 25 °C, 2 h), and acetylation (acetic anhydride, 100 °C, 1 h). The product was

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Abbreviations: GC–MS, gas chromatography–mass spectrometry; HPAEC, high pH anion-exchange chromatography

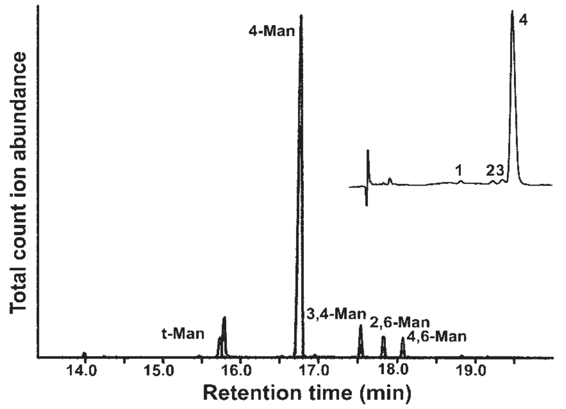

Fig. 1. Composition and Linkage Analysis of the Water-Soluble Polysaccharide Derived from Current Pseudobulbs of *Oncidium* "Gower Ramsey."

Inset: the direct HPAEC profile of the acid hydrolyzates of water-soluble polysaccharide. The HPAEC peaks 1–4 are in the order arabinose, galactose, glucose, and mannose. The major peaks of GC–MS linkage analysis were identified by retention time as compared against the standard and the electron impact-mass spectrum of the partially methylated alditol acetates. t, terminal.

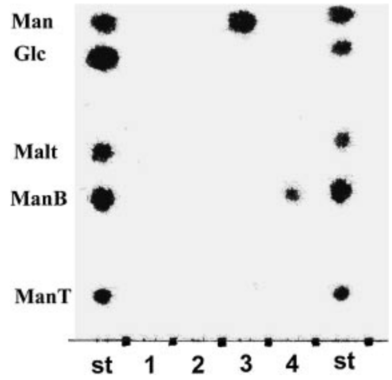

Fig. 2. TLC Chromatogram of the Hydrolyzates of the *Oncidium* Mannan by Different Enzymatic Treatments.

(1) α-amylase, (2) α-mannosidase, (3) β-mannosidase, (4) β-mannanase. Man, mannose; Glc, glucose; Malt, maltose; ManB, mannobiose; ManT, mannotriose.

dissolved in an appropriate amount of hexane for GC–MS analysis. GC–MS was carried out using a Hewlett-Packard Gas Chromatograph 6890 connected to an HP 5973 Mass Selective Detector and fitted with an HP-5MS fused silica capillary column (30 m $\times$ 0.25 mm ID). The sample was injected onto the column in the splitless mode. The column head pressure was maintained at about 8.2 psi to give a constant flow rate of 1 ml/min using helium as the carrier gas. The oven temperature was programmed to 60 °C for 1 min, increased to 90 °C after 1 min, and then to 290 °C for 25 min.[10] As Fig. 1 shows, the 1,4-linkage predominated. Since each of the less methylated residues was more abundant than the terminal residue, they cannot be branching points, and probably arose from incomplete methylation. These results indicate that the backbone of the mannan is a linear 1,4 chain.

In order to distinguish the C-1 anomeric configuration, mannan (80 µg) was incubated with α- or β-glycosidases (16.7 nkat), such as α-mannosidase (EC 3.2.1.24, Sigma M-7257), α-amylase (EC 3.2.1.1, Sigma A-2771), β-mannosidase (EC 3.2.1.25, Sigma M-9400), and β-mannanase (EC 3.2.1.78, Megazyme E-BMANN) at 37 °C for 24 h. The hydrolyzates were desalted by passage through a cation exchanger (Dowex-50W, H^+ form, Sigma) and then an anion exchanger (Dowex-1, Cl^- form, Sigma).[11] The neutral hydrolysate was dried by Speed-Vac and analyzed on a thin-layer plate (Silica gel 60, Merck) by two consecutive runs with ethyl acetate, pyridine, and H_2O (20:7:5, by volume).[11] The products were visualized by spraying with a mixture of aniline/diphenylamine/phosphoric acid (2:2:43 w/v in 97:3 v/v acetone–water), and heated to 100 °C.[12] As shown in Fig. 2 β-type hydrolytic enzymes, such as β-mannosidase and β-mannanase, cleaved the polysaccharide to yield mannose and mannobiose respectively, but α-type glycosidases, such as α-mannosidase and α-amylase, were inactive.

These results taken together indicate that the *Oncidium* mucilage is a β-1,4-linked linear mannan.

Thus far, mannans have been categorized into pure mannans, glucomannans, and galactomannans, based on their compositions and structures.[13] The mannan we have described here is in excellent agreement with the definition of a "pure" linear mannan, having more than 95% mannose content and a high degree of uniformity in the structure.

As is well known in many monocotyledon families, such as Amaryllidaceae, Araceae, Iridaceae, Liliaceae, and Orchidaceae, the mannans in bulbs and tubers are glucomannans.[14–17] For example, the ratios of glucose/mannose of the polysaccharides in the bulb of *Lillum testaceum*[17] and the young tuber of *Orchis morio*[14] were 3/7 and 1/3.3 respectively. However, the *Oncidium* pseudobulb mannan had monosaccharide constituents other than mannose at a very low level (Fig. 1, inset). In addition, the uronic acid level of *Oncidium* mannan was determined according to the method of Blumenkrantz and Asboe-Hason.[18] The extremely low level of uronic acid content, estimated to be 1.12% (w/w) of total carbohydrate, agrees with the insignificant charged nature as revealed by the result of DEAE Sepharose column purification. Therefore, it is reasonable to suggest that *Oncidium* mannan is a neutral polysaccharide.

To our knowledge, this is the first report describing a pure mannan that exists in the pseudobulbs of *Oncidium*. Moreover, it is so rich in the current pseudobulb during the early inflorescence stage, estimated at above 6.0% on a dry mass basis, that the physiological importance as a storage material it might play in the flowering-related organ cannot be overlooked.

In higher plants, pure mannans are also widespread in non-leguminous seeds, such as Palmae, coffee beans, and some *Umbelliferae* species.[19–21] Recently, Petkowicz *et al.*[22] also isolated a pure mannan from the seed of *Schizolobium amazonicum*, a Leguminosae species. The characteristics of pure mannans from seeds are mostly insoluble in water, and thus might contribute to the hardness of seeds to resist mechanical damage.[13,20] But the pure mannans isolated from the *Oncidium* pseudobulb and yam tuber[23] are water soluble. It is reasonable to speculate that these two types of pure mannan play different physiological roles.

In general, mucilage is a heterogeneous and complex polysaccharide. In linseed, for example, the mucilage consists of neutral and acidic polysaccharides.[6] Moreover, the polysaccharide exhibits a synergistic viscosity phenomenon when mixed with other polysaccharides. Pavlova and Grigorova[24] demonstrated that the viscosity of a mannan isolated from yeast is very low, but the mixture of mannan–xanthan (20:80) was 900% and 70% higher than the mannan and xanthan solutions alone, respectively. Hence it is conceivable that the mucilage nature of polysaccharides in *Oncidium* pseudobulb might not only be attributable to mannan. Further studies on other kinds of polysaccharides in the organ should be done.

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