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Bamboo Borer Dust as a Rich Source of Glucose-1-Phosphate

A. RAJOR, RAJESH K. SHARMA,* AND R. M. KOTHARI

School of Biotechnology, Thapar Institute of Engineering and Technology, Patiala-147 001, India

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ABSTRACT

Chemical characterization of bamboo borer dust (BBD) indicated that it contained 2.5 ± 0.5% of an organic entity that was a water-soluble, acid-labile phosphate, nonreducing sugar with a retention period of 5.2 min on a sugar pack column during high pressure liquid chromatography (HPLC). It was subsequently identified and confirmed as glucose-1-phosphate (G-1-P) from its response to phosphoglucomutase and glucose-6-phosphatase treatment. Although the presence of G-1-P in such a large quantity in BBD is inexplicable, it provides a rare and rich source of G-1-P, making it a potential starting material for its isolation in pure state.

Index Entries: *Dendrocalamus strictus; Dinoderus* sp.; G-1-P.

INTRODUCTION

During a visit to several bamboo storage depots of Ballarpur Industries, in Ballarpur (Maharashtra State, India), a large scale infestation of bamboo, *Dendrocalamus strictus*, by borer (*Dinoderus* sp.) was observed. As a result, huge quantities of bamboo borer dust (BBD) was scattered around the premises of the depots. It occurred to us that, while preservation of bamboo was our primary task, salvaging BBD, if possible, also might be looked into, if it could have economic significance or provide a value-added product. During this effort, analyses of BBD collected from several depots revealed that it could be a rich source of glucose-1-phosphate (G-1-P). The present communication summarizes the efforts made in this direction.

*Author to whom all correspondence and reprint requests should be addressed.

MATERIALS AND METHODS

Materials

Bamboo and Borer Dust

Four samples of bamboo (*D. strictus*) dust (BBD) resulting from an infestation by borers, mainly *Dinoderus* sp. (*1*) were collected from Kalmana and Allapalli depots.

Standards

Disodium salt of D-Glucose-6-phosphate (Dihydrate) and D-Glucose-1-phosphate (G-6-P and G-1-P) were procured from Hi-Media, Bombay. Other standards, such as glucose, fructose, cellobiose, xylose, and arabinose, were procured from BDH, London.

Enzymes

Phosphoglucomutase (P-6156, from chicken muscle) and Glucose-6-phosphatase (G-5758, from rabbit liver) were procured from Sigma, St. Louis, MO.

Chemicals

Buffers and laboratory reagents were prepared using analytical reagent grade chemicals (BDH) and demineralized (DM) water.

Enzyme Assays

Phosphoglucomutase and glucose-6-phosphatase were assayed and used as per Najjar (*2*) and Nordlie and Arion (*3*), respectively.

Estimation of BBD Ingredients

Cellulose content was estimated colorimetrically by anthrone method (*4*), hemicellulose by spectrophotometry (*5*), lignin by ethanol-benzene extraction method (*6*), reducing sugars by DNSA method (*7*), and inorganic phosphate (Pi) by Fiske and SubbaRow method (*8*).

HPLC Analysis

Water-soluble extract of BBD, passed through a 0.45-μ membrane and Sepak cartridge, was analyzed for sugars by high pressure liquid chromatography (HPLC), injecting 40-μL samples in a sugar pack column at 90°C, with 50 ppm Ca-EDTA as a mobile phase, at a flow rate of 0.5 mL/min and 1000 psi, like the procedure given in ref. (*9*).

RESULTS AND DISCUSSION

Four samples of BBD from two forest divisions, each collected from 10–15 spottings, were analyzed for cellulose, hemicellulose, reducing sugars, and lignin content. Their average values, expressed in percent on dry wt basis, are given in Table 1.

Table 1
Analyses of BBD

Ingredients	BBD
α-Cellulose	55 ± 2
Hemicellulose	3 ± 1
Reducing sugars	0.4 ± 0.1
Lignin	6 ± 1

Carbohydrate Profiles of BBD on HPLC

These analyses revealed that BBD contained 55 ± 2% α-cellulose, comparable to the amount present in healthy bamboo. The presence of meager (0.4%) reducing sugars indicated that borers do not have a potent cellulase complex to metabolize α-cellulose. Had α-cellulose been metabolized by borers, some of its cellulolytic intermediary metabolites, such as glucose, cellobiose, cello-dextrins, and so on, would have been detected in HPLC profiles. However, on the basis of comparison with the standards or by internal recovery profiles (in which the presence of a particular sugar was suspected, based on its retention time), there was no trace of cellobiose and cellodextrins (intermediary metabolites of α-cellulose) and xylose or arabinose (intermediary metabolites of hemicellulose); the presence of only glucose, fructose, and G-6-P as insignificant peaks in HPLC profiles indicated that these probably are the indigenous ingredients of bamboo and could not be the intermediary metabolites of α-cellulose-degradation. Cumulatively, these three sugars represented about 0.4 ± 0.1% reducing sugars. However, there was a major peak and based on its time of retention, as well as internal recovery profiles, it was tentatively identified as G-1-P.

Presence of G-1-P as Deduced from Chemical Characterization and HPLC

The aqueous extract of BBD, clarified by filtration through Whatman filter paper No. 41, was analyzed by chemical methods. It contained a negligible amount (0.4 ± 0.1%) of reducing sugars, barely detectable (0.13%) inorganic phosphate (Pi), and a large amount (1.4%) of 1.0*N* H_2SO_4-hydrolyzable Pi, with a concomitant release of an organic entity, reducing in nature (Table 2). Cumulatively, these attributes clearly indicated that the BBD filtrate contained an organic phosphate, labile to 1.0*N* H_2SO_4. Upon the application of acid hydrolysate (1.0*N*, 95°C, 30 min) of BBD filtrate on the HPLC column, from the retention time (9.2 min) and its co-elution with standard glucose, it was apparent that the hydrolysate of BBD filtrate contained glucose. Upon estimation of its Pi by the Fiske and SubbaRow method (*8*), as well as reducing sugar by the DNSA method (*7*), it was observed that the amount of reducing sugar (glucose) increased

Table 2
Inorganic Phosphate (Pi) Profiles[a]

Substrate	After hydrolysis with 1.0*N* H_2SO_4	After hydrolysis with phospho-glucomutase followed by 1.0*N* H_2SO_4 hydrolysis	After hydrolysis with Glucose-6 phosphatase
G-1-P	100.7	13.4	NA
G-6-P	0.01	NA	15.81
BBD	13.12	0.04	1.27

[a] All values are expressed as mg/g on a dry basis.
NA, not applicable.

directly proportionate to Pi, indicating that the molecule under identification was probably G-1-P. This was explicable because G-1-P, a nonreducing sugar, is converted into glucose (a reducing sugar) and Pi because of its acid-labile nature; G-6-P, *per se*, is a reducing sugar with acid-nonhydrolyzable phosphate (*2*).

Confirmation of G-1-P from Response to Phosphoglucomutase

In light of the above observations, it was considered worthwhile to confirm the presence of G-1-P from its response to phosphoglucomutase. An aqueous filtrate of BBD and standard G-1-P were individually treated with phosphoglucomutase (pH 7.5, 30°C, 60 min), as described by Najjar (*2*) (Table 2). It was observed that both acid-labile phosphate (G-1-P) and standard G-1-P were converted into acid-resistant phosphate (G-6-P). Concomitantly, the nonreducing sugar (G-1-P) was overwhelmingly converted into a reducing sugar (G-6-P), equilibrium being in favor of G-6-P formation, as earlier shown by Cori et al. (*10*).

$$\underset{(5.5\%)}{\text{G-1-P}} \rightleftharpoons \underset{(94.5\%)}{\text{G-6-P}}$$

This confirmed the presence of G-1-P in BBD.

Presence of G-1-P as Substantiated by Response to Glucose-6-Phosphatase

That the above-identified molecule is indeed G-1-P and not G-6-P was finally substantiated by glucose-6-phosphatase treatment. Upon individual treatment of standard G-1-P and BBD filtrate with glucose-6-phosphatase (pH 6.5, 37°C, 60 min), as described by Nordlie and Arion (*3*), there was no increase in Pi in the BBD filtrate, while the standard G-6-P was hydrolyzed with a stoichiometric release of Pi, which was not possible by acid-hydrolysis (Table 2). This confirmed that the water-soluble,

acid-labile phosphate, nonreducing sugar with a 5.2-min retention time on HPLC in BBD filtrate was G-1-P.

BBD Extract—A Rich Source of G-1-P

It was noted that BBD contained 2.5 ± 0.5% G-1-P. This was reproducibly observed in all four samples collected from different depots. The significance, if any, of the presence of G-1-P in BBD in such a large quantity is not clear at this stage. G-1-P was almost quantitatively extractable in DM water by mere dissolution at ambient temperature. Upon filtration, the clearate contained meager amounts of impurities, thereby making it a rare and rich source of G-1-P.

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REFERENCES

1. Singh, S. (1974), *Indian Pulp Paper* **29,** 11–14.
2. Najjar, V. A. (1955), *Methods Enzymol.* **1,** 294–299.
3. Nordlie, R. C. and Arion, W. J. (1966), *Methods Enzymol.* **9,** 619–622.
4. Updeagraff, D. M. (1969), *Anal. Biochem.* **32,** 420–424.
5. Lise, D. and Yu, E. K. C. (1986), *Appl. Microbiol. Biotechnol.* **24,** 379–385.
6. Browning, B. L. (1967), *Methods of Wood Chemistry,* vol. 2. Interscience, New York, pp. 785–790.
7. Miller, G. L. (1959), *Anal. Chem.* **31,** 426–428.
8. Volkin, E. and Cohn, W. E. (1952), *Methods Biochem. Anal.* **1,** 287–305.
9. Waters HPLC Technical Bulletin for Carbohydrates Analysis (1986), Millipore, Milford.
10. Cori, G. T., Colowick, S. P. and Cori, C. F. (1938), *J. Biol. Chem.* **123,** 375–381.