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Quantitative glass-paper chromatography of fungal cell wall acid hydrolysates

During studies of cell wall composition in *Neurospora crassa*, aimed at uncovering possible biochemical defects which might elucidate the nature of morphologic mutants, it became desirable to develop rapid techniques to quantitate amino-, acidic and neutral sugars. Because such cell walls have been reported[1–3] to contain glucose, galactose, mannose, glucosamine, galactosamine, and glucuronic acid, solvent systems were developed for the separation of these components. Glass fiber paper impregnated with silica gel and sodium dihydrogen phosphate was chosen for a variety of reasons. Incorporation of salts has been shown to improve separation of carbohydrates[4–7]. Thin-layer was selected over gas chromatography because previous experience (unpublished) had indicated that the latter method incompletely separated carbohydrates. Glass fiber paper seemed to be advantageous over plates because it is easier to handle and store, there is no breakage problem, small samples of test material can be used, the chromatograms can be saved for later reference, and test strips can be run to test efficiency of separation influenced by conditions. The latter is important in solvent systems in which development time is short; the humidity or temperature of the environment greatly influence separation. The system which was developed fulfilled its initial promise and the results are reported herein.

Materials and methods

Samples. Acid hydrolysates of cell wall fractions in 2–6 *N* HCl were used directly without neutralization.

Chromatography. ITLC SG (Gelman Inst. Co., Ann Arbor, Mich.) glass fiber sheets 20 × 20 cm or glass fiber paper prepared according to HAMILTON *et al.*[8], was impregnated with 1 % NaH_2PO_4 (w/v), allowed to dry at room temperature for 1–3 days and stored in aluminum foil.

The acid hydrolysates and standards were spotted along a line 15 mm from the lower edge of the sheet with a 5 μl pipette, and dried with a stream of warm air from a hair dryer; care was taken not to activate the paper. The paper was developed by the ascending technique at 24°, and air dried. In order to sharpen the separation of the glucose–galactose–mannose complex, the multiple development technique was used. Several solvent systems were examined. The best results were obtained with the following:

(1) Chloroform–methanol–pyridine–water (130:40:1:5);
(2) Ethyl acetate–methanol–acetic acid–water (65:20:7.5:10);
(3) Chloroform–methanol–ammonia–water (130:63:7:4).

Quantitation. The chromatogram was air dried, and the solvent removed by holding the paper horizontally over a hot plate until no solvent odor could be detected. The dried chromatogram was sprayed with reagent grade concentrated sulfuric acid from an atomizer to coat both sides of the paper evenly, and hung vertically in an oven at 230° for 10 min. The charred carbohydrates appeared as brown or dark grey spots and the intensity of these were measured on a spectrophotometric densitometer[9].

Results and discussion

Glass fiber paper impregnated with silica gel and 1% NaH_2PO_4 was most effective, although other concentrations of NaH_2PO_4 and phosphate buffers were tried. The R_F values and time required for development are shown in Table I.

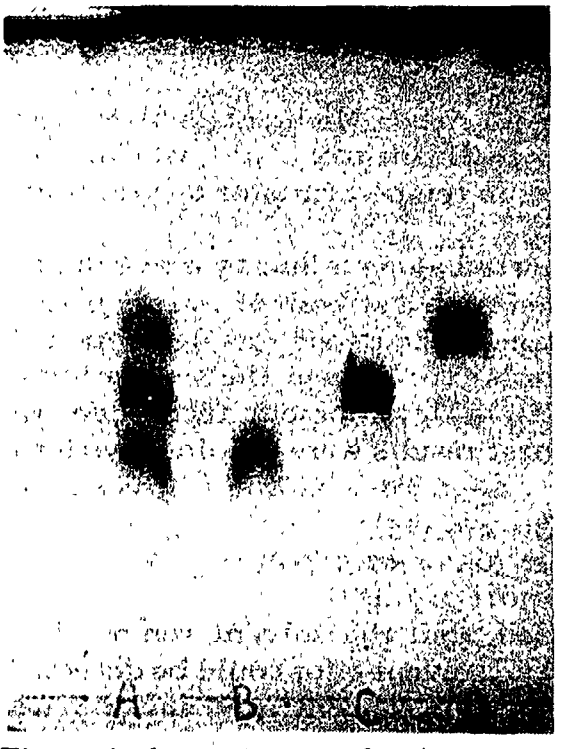
TABLE I

R_F VALUES

	Solvent I	*Solvent II*	*Solvent III*
Galactose	0.36	0.60	0.13[a]
Glucose	0.45	0.70	0.21[a]
Mannose	0.56	0.76	0.29[a]
Galactosamine	0.00	0.00	0.37
Glucosamine	0.00	0.06	0.49
Glucuronic acid	0.00	0.32	0.00
No. of developments	3	1	1
Time for a 15 cm run	15 min	35 min	25 min

[a] Represents major peak which stains with aniline phthalate (see text for discussion).

Solvent system 1 permitted the rapid separation of glucose, galactose and mannose. The spots were well defined; however, multiple development with the same solvent system was applied to improve separation. The best results were obtained by three 15-min developments during which time the solvent moved about 15 cm up the paper. (Fig. 1 shows such a chromatogram.)



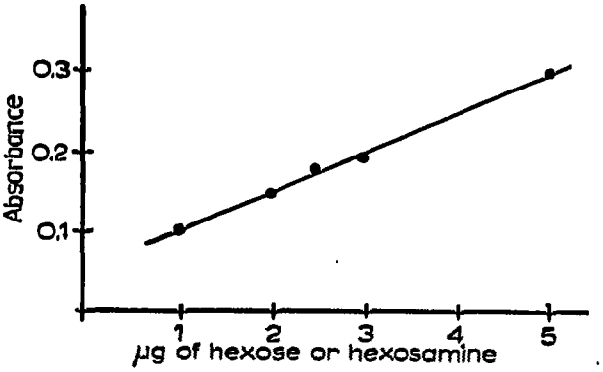


Fig. 1. A chromatogram showing separation of a mixture of glucose, galactose and mannose (A), galactose (B), glucose (C) and mannose (D).

Fig. 2. Densitometer readings of charred spots on glass fiber paper chromatograms, demonstrating linearity among varying quantities of standards.

In solvent system 2, glucuronic acid formed a compact spot and was clearly separated from the neutral and amino-sugars. If the polarity of this system was reduced, glucose, galactose and mannose could be separated by multiple developments.

Solvent system 3 was employed for the separation of amino-sugars. It was observed that neutral sugars form two spots in this solvent system; one stained with aniline phthalate and the other with ninhydrin. However, the amino-sugars appeared unaffected and formed single, well-defined, compact spots. This has also been observed by RANDERATH[7] and WEICKER AND BROSSMER[10]. Apparently the silica gel exerts a catalytic effect on the amination of hexoses and pentoses for this effect has not been observed when separations are carried out on Kieselguhr G nor are amino-sugars formed if ammoniacal solvents are used in the absence of silica gel.

Quantitation of 1–5 μg samples by the H_2SO_4-char technique proved to be linear within this range, giving absorbancy readings between 0.1 and 0.3. (Fig. 2 shows the standard curve, all sugars charred similarly.)

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1 P. R. MAHADEVAN AND E. L. TATUM, *J. Bacteriol.*, 90 (1965) 1073.
2 J. R. TREVITHICK AND R. L. METZENBERG, *J. Bacteriol.*, 92 (1966) 1016.
3 E. BARBOSA, *Ph. D. Thesis*, Tulane University, 1966.
4 E. STAHL AND W. KALTENBACH, in E. STAHL (Editor), *Thin-Layer Chromatography. A Laboratory Handbook*, Academic Press, New York, 1965, p. 235.
5 Y. S. OVODOV, E. V. EVTUSHENKO, V. E. VASKOVSKY, R. G. OVODOVA AND T. F. SOLOV'EVA, *J. Chromatog.*, 26 (1967) 111.
6 J. NĚMEC, K. KEFURT AND J. JARÝ, *J. Chromatog.*, 26 (1967) 116.
7 K. RANDERATH, *Thin Layer Chromatography*, Academic Press, New York, 1966, p. 235.
8 J. G. HAMILTON, J. R. SWARTWOUT, O. N. MILLER AND J. E. MULDREY, *Biochem. Biophys. Res. Commun.*, 5 (1960) 226.
9 J. R. SWARTWOUT, J. W. DIECKERT, O. N. MILLER AND J. G. HAMILTON, *J. Lipid Res.*, 1 (1960) 281.
10 H. WEICKER AND R. BROSSMER, *Klin. Wochschr.*, 39 (1961) 1265.

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