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Paper Chromatographic System for the Identification of Glycerol in Bacterial Cell Walls[1]

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ABSTRACT

IKAWA, MIYOSHI (University of New Hampshire, Durham), JAMES W. MORROW, AND SHEILA J. HARNEY. Paper chromatographic system for the identification of glycerol in bacterial cell walls. J. Bacteriol. 92:812–814. 1966.—The solvent system consisting of isopropanol-5% boric acid (7:1, v/v) separates glycerol from the other carbohydrate constituents which are found in hydrolysates of bacterial cell walls. This system is useful for the identification of glycerol even when anhydroribitol and rhamnose are both present, and has been found to be applicable on cell wall hydrolysates as well as on synthetic mixtures.

In the cell walls of the gram-positive bacteria, glucose, galactose, and rhamnose are the most frequently encountered of the non-nitrogenous reducing sugars, whereas mannose, arabinose, fucose, and ribose have been reported less frequently (4, 6–9, 11, 14, 16, 17). In addition to these constituents, the walls of many gram-positive organisms contain glycerol or ribitol, which are specific constituents of a teichoic acid moiety which may constitute part of the cell wall structure (3, 4, 6, 9, 11, 16).

One of the problems in the identification of cell wall components has been to determine the presence or absence of glycerol by paper chromatographic methods when both anhydroribitol (which arises during the acid hydrolysis of ribitol teichoic acids) and rhamnose are present in the hydrolysis mixture. In the commonly used solvent for carbohydrates (*n*-butanol-acetic acid-water, 4:1:1), rhamnose separates from the other cell wall constituents and can be identified, but glycerol and anhydroribitol move ahead of the rhamnose with almost identical R_F values. In a slightly different butanol–acetic acid–water solvent, glycerol and anhydroribitol have been separated by descending paper chromatography by allowing the chromatograms to run for 40 hr (6). Although glycerol and anhydroribitol move identically in most solvent systems, anhydroribitol can be detected in the presence or absence of glycerol by spraying the chromatogram with a modification (see Materials and Methods) of the periodate-Schiff's reagent spray described by Buchanan, Dekker, and Long (5) wherein anhydroribitol gives an intense blue spot after approximately 1 hr, whereas glycerol does not (13). To detect glycerol in the presence of anhydroribitol, the paper can be sprayed with a more concentrated Schiff's reagent (2). Under these conditions, glycerol gives a rapidly appearing color, whereas anhydroribitol gives a slowly developing color (1). We have, however, experienced difficulty in the past in detecting small amounts of glycerol in the presence of anhydroribitol.

By use of boric acid-containing systems, anhydroribitol has been well separated from glycerol by paper electrophoresis (13), paper chromatography (10), and thin-layer chromatography (15). However, in borated systems, rhamnose tends to show migration characteristics similar to glycerol, and the detection of glycerol when rhamnose is present becomes a problem.

In this paper we wish to describe a simple system which will separate glycerol from both rhamnose and anhydroribitol and allow the identification of small amounts of glycerol even when the other substances are both present.

MATERIALS AND METHODS

Chromatograms were developed by the ascending method on sheets of Whatman no. 1 paper. Two to three microliters of the standards in 1% aqueous solution were applied to the paper. Cell wall samples were hydrolyzed in 2 N sulfuric acid in a boiling-water bath for 2 hr, and the hydrolysates were prepared as previously described (13).

For the detection of anhydroribitol, the procedure of Buchanan, Dekker, and Long (5) was modified to

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the following. The dried chromatogram was first sprayed with 2% sodium periodate, and the moist paper was hung in a large covered chromatogram jar. After 5 to 10 min at room temperature, sulfur dioxide gas was passed into the jar until all the liberated iodine was reduced. The paper was then removed from the chamber and was sprayed with 0.1% Schiff's reagent. Anhydroribitol gave a characteristic intense blue spot after about 1 hr.

TABLE 1. *Paper chromatographic behavior of cell wall carbohydrates in isopropanol-5% boric acid (7:1)*

Sugar	Approximate R_F
Glycerol	.56
1,4-Anhydroribitol	.49
L-Rhamnose	.43
Ribitol	.42
D-Ribose	.39
L-Fucose	.33
L-Arabinose	.24
D-Mannose	.24
D-Glucose	.18
D-Galactose	.17
D-Glucosamine	.10

For the detection of reducing sugars and polyols, the Grado and Ballou (10) modification of the periodate-benzidine spray of Viscontini, Hoch, and Karrer (18) was used.

RESULTS

Table 1 lists the approximate R_F values of the major non-nitrogenous cell wall carbohydrates and glucosamine for the solvent system consisting of seven volumes of isopropanol and one volume of 5% (w/v) boric acid. By trial and error, these proportions were found to give the best resolution of glycerol from both anhydroribitol and rhamnose. Decreasing the proportion of 5% boric acid improved the separation of rhamnose from glycerol, but not of anhydroribitol from glycerol. With no boric acid present, isopropanol-water (9:1) achieved some separation of glycerol from anhydroribitol. Increasing the proportion of 5% boric acid decreased the separation of rhamnose from glycerol and did not improve the separation of anhydroribitol from glycerol.

The applicability of this method to mixtures of sugars is shown in Fig. 1, and, to cell wall hydrolysates, in Fig. 2. The absence of glycerol in

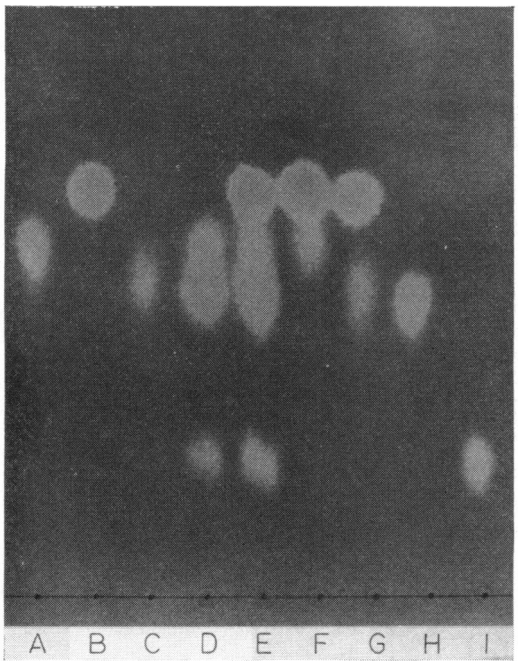

FIG. 1. *Paper chromatography of carbohydrate mixtures in isopropanol—5% boric acid (7:1). (A) Anhydroribitol; (B) glycerol; (C) rhamnose; (D) anhydroribitol, ribitol, rhamnose, glucose; (E) glycerol, anhydroribitol, ribitol, rhamnose, glucose; (F) glycerol, anhydroribitol; (G) glycerol, rhamnose: (H) ribitol; (I) glucose.*

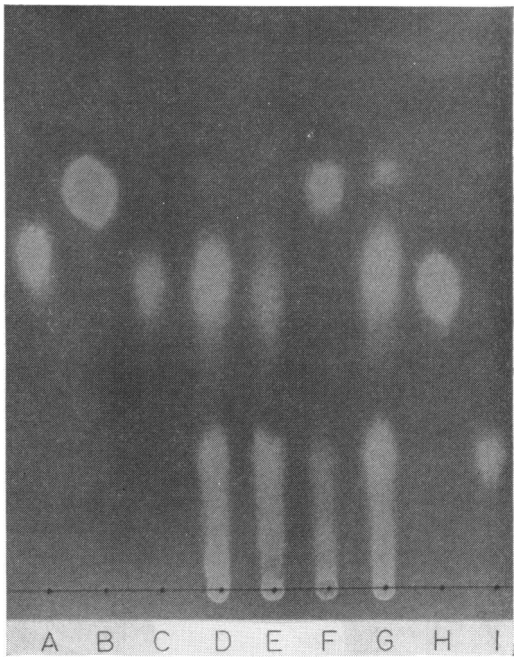

FIG. 2. *Paper chromatography of cell wall hydrolysates in isopropanol—5% boric acid (7:1). (A) Anhydroribitol; (B) glycerol; (C) rhamnose; (D) Lactobacillus casei cell wall; (E) L. plantarum cell wall; (F) L. delbrueckii cell wall; (G) Streptococcus faecalis cell wall; (H) ribitol; (I) glucose.*

the cell walls of *Lactobacillus casei* (ATCC 7469) and *L. plantarum* 17-5 (ATCC 8014) and its presence in the cell walls of *L. delbrueckii* LD-2 (Rogosa) and *Streptococcus faecalis* R (ATCC 8043) agree with previous results (11, 12).

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