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IMMUNOELECTROPHORETIC CHARACTERIZATION OF LIPOPOLYSACCHARIDES FROM SERRATIA MARCESCENS RESISTANT AND SENSITIVE TO POLYMYXIN B[a]

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Abstract. Lipopolysaccharides (LPS) isolated from Serratia marcescens resistant and sensitive to polymyxin B, were characterized by immunoelectrophoresis. Our results showed that both the free LPS and protein-bound LPS fractions from all strains contained intact LPS present as a neutral component. In addition, most, but not all strains, contained anodic and/or cathodic migrating components. With the exception of those in the polymyxin B resistant strain 08, their exact chemical nature is not known. Although our results do not indicate a correlation between the immunoelectrophoretic characteristics of LPS from S. marcescens with its polymyxin B susceptibility, this does not exclude the possible role of LPS in the in vivo antibiotic resistance mechanism in S. marcescens or other gram-negative bacteria.

Lipopolysaccharides (LPS) are characteristic components of the cell envelope of gram-negative bacteria. They are found as a protein complex, called endotoxin in the outer membrane of the cell envelope. Biologically, preparations of LPS and endotoxins elicit similar responses such as toxicity, pyrogenicity and antigenicity. Structurally, LPS consist of a lipid moiety (lipid A) covalently linked to a core polysaccharide which is attached to several antigenic side-chain polysaccharides.

The role of LPS in antibiotic resistance mechanisms was first observed by Schlecht and Westphal (1970) and later by Tamaki, et al (1971). LPS was suggested to form a barrier to antibiotics and other molecules in the outer

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membrane. Any deletion of the LPS might facilitate penetration of antibiotics, and, hence results in an increased sensitivity of the organism towards the antibiotic molecule.

The effects of polymyxin B on the cell envelope and its components have been studied by several groups. It is known that polymyxin is capable of inactivation of endotoxin (Cooperstock, 1974). Treatment with polymyxin B in Serratia marcescens (Tsang, et al., 1974) and other gram-negative bacteria (Wahn et al., 1968; Koike et al., 1969) causes projections in the outermost layer of the cell envelope and disorganizes the outer membrane. In addition, Lopes and Inniss (1969), by using electron microscopy, observed the breakdown of the ribbon-like structure of LPS from E. coli after treatment with polymyxin B. More recently this antibiotic was demonstrated to bind to the lipid A moiety of the LPS molecule (Teuber and Bader, 1972).

Immunoelectrophoretic characterization of the LPS from a polymyxin B resistant strain of S. marcescens (08) has been reported (Tsang, et al., 1974). The intact LPS molecule was located in the neutral zone, while the protein and the free side-chain polysaccharides migrate towards the anodic compartment and the cathodic compartment, respectively. Similar observations on LPS from other gram-negative bacteria were also reported earlier (Holmgren and Hanson, 1969; Hurvell and Lindberg, 1973). However, the anodic as well as the cathodic migrating components were not characterized.

The purpose of this study is to report the immunoelectrophoretic characteristics of purified LPS fractions isolated from whole cells of S. marcescens resistant and sensitive to polymyxin B.

MATERIALS AND METHODS

Two non-clinical strains (08 and Bizio) were supplied by Dr. P. Alaupovic, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, and four clinical isolates: 6292, 2736 (resistant to polymyxin B) and 13378 and 3910 (sensitive to polymyxin B) were provided by Dr. Marcia Miller of the Peoria School of Medicine, Peoria, Illinois.

All strains were grown in an enriched media with aeration and harvested at the mid-log phase of growth (O.D. = 0.500 at 625 nm) (Tsang, et al., 1974). Cells were harvested and lyophilized. These cells were then treated with hot 45% aqueous phenol, by a slightly modified method of Tsang et al. (1974). The extractable material was then separated into the fractions free-LPS (LPS-f) and protein-bound-LPS (LPS-b) by centrifugation at 12,000 x g.

Antibodies against each strain were prepared by heat killing 10 mg of whole cell bacteria in 10 ml of 0.9% NaCl. Two ml of heat killed bacteria with 1 ml of Complete Freund's Adjuvant were administered intraperitoneally to New Zealand White Rabbits at 5 day intervals for 3 successive injections, with a final booster shot of 3 ml of heat killed bacteria with 1.5 ml of Freund's Adjuvant. Antibodies were then recovered by cardiac puncture.

Samples for immunoelectrophoresis were prepared by dissolving 5 mg of LPS in 0.5 ml of 0.9% NaCl and 0.5 ml of 0.5% SDS in tris buffer (0.05M, pH 7.6). Immunoelectrophoresis was performed in 1% agar plates made in 0.05M

Veronal (S/P) buffer pH 8.6, with a current of 10ma/frame for 45 min in a Gelman Deluxe Electrophoresis Chamber. Antibodies were then added and double gel diffusion (Ouchterlony, 1962) and precipitation was allowed to occur for 24 hr in a humidity chamber at 37°C. Slides were then washed in 0.9% NaCl for twenty-four hours to remove any free proteins, then dried and stained with Amido Black.

RESULTS AND DISCUSSION

It was previously reported that intact LPS from a polymyxin B resistant strain of S. marcescens (08) was located in the neutral zone after immunoelectrophoresis of the purified LPS fraction (Tsang, et al., 1974). The position of the precipitin arc suggests that there is a lack of charged groups in the intact LPS of this resistant strain. In this study, this behavior will be compared with that of LPS isolated from the other resistant and sensitive strains of S. marcescens.

Table 1 presents the relationship of the minimal inhibitory concentrations of polymyxin B of the six strains with the corresponding yields of their lipopolysaccharide fractions. In general, the yields of total LPS (free LPS + protein-bound-LPS) from the resistant strains are considerably higher than those of the sensitive ones.

The immunoelectrophoretic results showed that LPS preparations from all strains were antigenic to their corresponding antibodies. All fractions showed the presence of a precipitin arc in the neutral compartment. These results are consistent with those reported for S. marcescens (Tsang, et al., 1974) as well as for other gram negative bacteria (Holmgren, 1969; Hurvell and Lindberg, 1973). However, the identification of the anodic and cathodic migrating components was slightly more difficult. With the exception of those from the resistant strain 08 of S. marcescens, which were identified as the protein component of the endotoxin and the side-chain polysaccharide, respectively (Tsang, et al., 1974), the anodic and cathodic migrating precipitin arcs cannot be generally categorized as either the protein or the free side-chain polysaccharides of the LPS molecules (Orskov, 1970; Hornstein, 1972; Larsson, 1973; Orskov, 1973; Bokhout, 1974).

Figures 1-6 show the immunoelectrophoretic patterns of the free-LPS (LPS-f) and protein-bound-LPS (LPS-b) of the resistant strains (Fig. 1-3) and sensitive strains (Fig. 4-6). Figure 1a shows two precipitin components, one neutral, and one cathodic migrating, of the-free-LPS (LPS-f) of strain 08, while Figure 1b shows three components from the protein-bound-LPS preparation of the same strain: a neutral, a cathodic, and an anodic migrating component. These results agree with previously reported observations (Tsang, et al., 1974), and will serve as a reference for comparison with the LPS fractions extracted from the other five strains.

The free-LPS fraction (LPS-f) from 6292 was shown to contain a single homogenous neutral component (Fig. 2a). Although the protein-bound-LPS fraction (LPS-b) of the same strain, contained an identical component, its intensity was much greater. In contrast to those of strain 08 and 6292, the free-LPS of 2736 contained a single neutral component which was much closer to the antigen well (Fig. 3a). Similarly, the protein-bound-LPS was shown to contain an extremely intense neutral component and a weak anodic migrating one (Fig. 3b). With the

exception of S. marcescens 08 where all precipitin components have been analyzed and identified, no attempt was made to further characterize the chemical nature of the anodic and cathodic migrating components in the other two resistant strains. It remains uncertain as to which is the protein component of the endotoxin or the free side-chain polysaccharide of the LPS molecule.

Figures 4-6 show the results of the immunoelectrophoresis of the LPS preparations from the sensitive strains. With the exception of those from 3910, it is quite apparent that the free-LPS fractions from the sensitive strains were much less antigenic with their antibodies and were present as small, faint precipitin lines in the neutral compartments (Figures 4a and 4b; 6a and 6b). In addition to the neutral component, protein-bound-LPS of strain Bizio contained anodic and cathodic migrating components (Fig. 4b). Both the free-LPS and protein-bound-LPS fractions of 3910, similar to those from the resistant strain 2736, contained only one neutral component close to the antigenic wells (Fig. 5a-5b).

From this study, it can be demonstrated (a) although there was a slight difference in the yields of LPS from resistant and sensitive strains of S. marcescens the isolation of LPS was independent of their antibiotic susceptibility, (b) the isolated LPS fractions were antigenic against their corresponding antibodies for each strain, (c) the neutral components were identified as intact LPS, (d) there was no difference in the net charge of the intact LPS of the resistant and sensitive strains, and (e) additional charged components were present in some strains, but not all.

Even though there seems to be a slightly higher yield of LPS from the resistant strains, it is premature to attempt to correlate the immunoelectrophoretic characteristics of the isolated LPS fractions with polymyxin B susceptibility in S. marcescens. However, this does not exclude the role of LPS in the in vivo antibiotic resistance mechanism towards various antibiotics. It would be of interest to study the immunoelectrophoretic behavior of the LPS isolated from S. marcescens after polymyxin B treatment.

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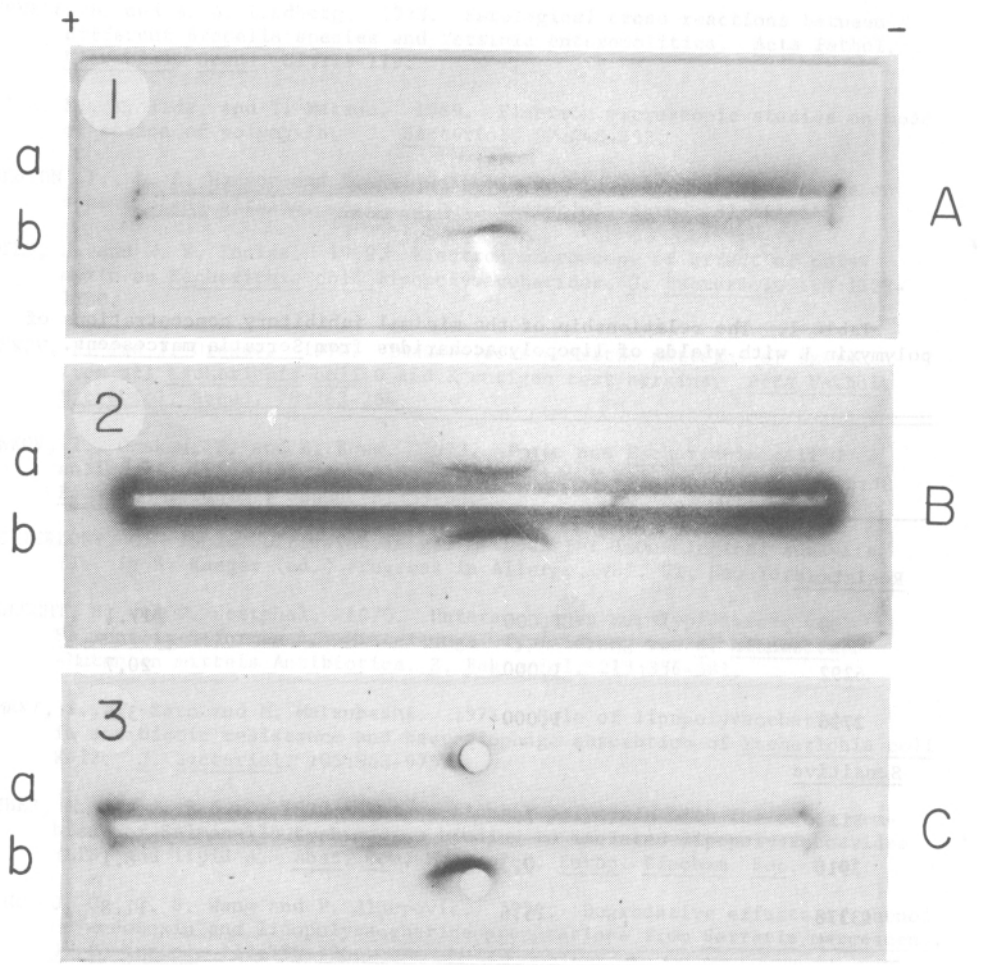
Table 1. The relationship of the minimal inhibitory concentrations of polymyxin B with yields of lipopolysaccharides from Serratia marcescens.

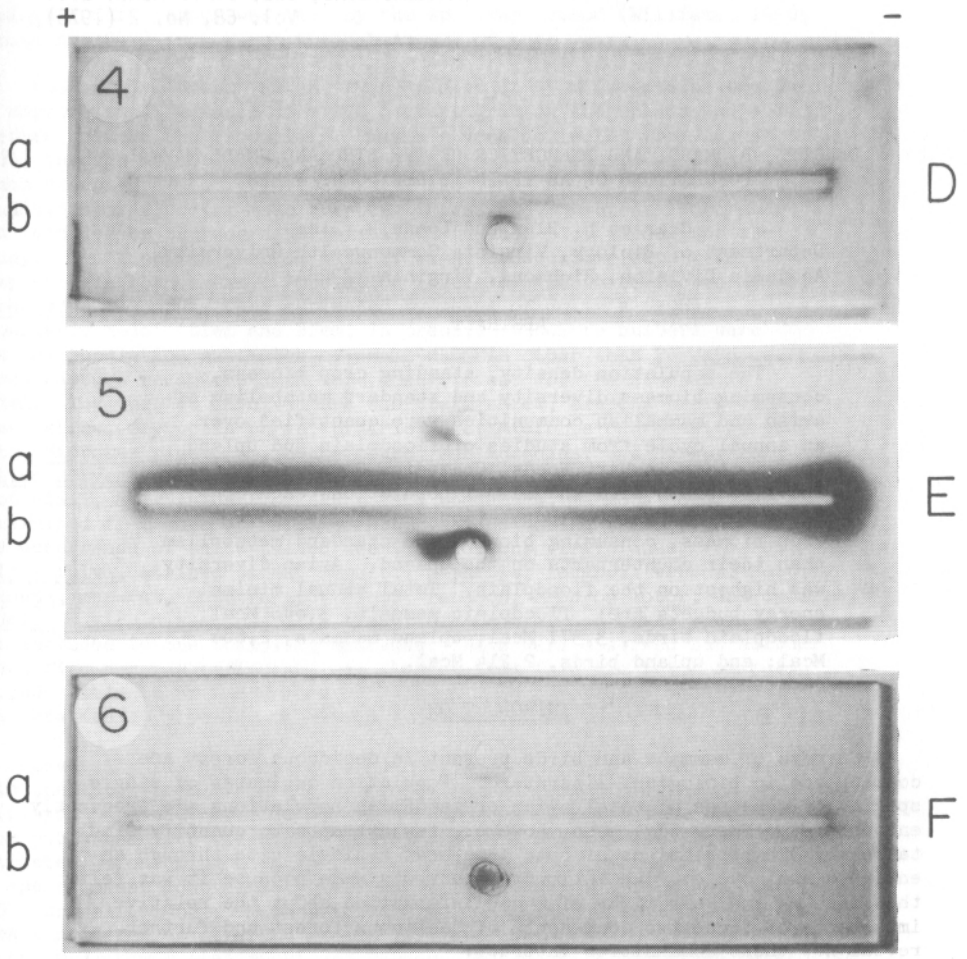
Strains	MIC[a] μg/ml	Yield[b]
Resistant		
08	1,000	17.1
6292	1,000	20.7
2736	1,000	3.6
Sensitive		
Bizio	7.8	8.1
3910	0.39	8.1
13378	15.6	11.8

[a]MIC = minimal inhibitory concentration

[b]Yield of LPS was expressed in mg/g of dry whole cells



Figures 1, 2, and 3. Immunoelectrophoretic patterns of Serratia marcescens resistant to polymyxin B. Antibodies in the three central troughs: A: against whole cell 08; B: against whole cell 6292; C: against whole cell 2736. Antigens applied: 1a: 08 LPS-f; 1b: 08 LPS-b; 2a: 6292 LPS-f; 2b: 6292 LPS-b; 3a: 2736 LPS-f; 3b: 2736 LPS-b.



Figures 4, 5, and 6. Immunoelectrophoretic patterns of Serratia marcescens sensitive to polymyxin B. Antibodies in the three central troughs: D: against whole cell Bizio; E: against whole cell 3910; F: against whole cell 13378. Antigens applied: 4a: Bizio LPS-f; 4b: Bizio LPS-b; 5a: 3910 LPS-f; 5b: 3910 LPS-b; 6a: 13378 LPS-f; 6b: 13378 LPS-b.