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# Lipid Composition of Gram-Negative Bacteria, Sensitive and Resistant to Streptomycin

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Analyses of streptomycin-sensitive and -resistant gram-negative bacteria show that, contrary to previous reports, the development of antibiotic resistance is not accompanied by changes in membrane lipid or fatty acid composition.

The appearance of bacteria resistant to the commonly used therapeutic antibiotics has prompted many studies on the mechanism of resistance. Differences in total lipid or fatty acid composition, or both, have been associated with an increased antibiotic resistance in various bacteria, and it has been suggested that the lipid composition may be of importance in preventing the entrance or binding of the antibiotic to the cell (2, 9, 11, 13-15). However, the divergence of results obtained does not allow any general conclusions to be drawn with regard to the role of lipids in the development of resistance. Much of the work was carried out by using hospital isolates in which the sensitive and resistant strains were not necessarily closely related. It is known that the lipid composition of the bacterial cell can vary with the age of the culture and growth conditions (6, 12). Streptomycin has been shown to affect membrane permeability (1, 7, 8) and bacterial lipid composition (3), but the lipids of streptomycin-sensitive and -resistant cells have not been examined. We have therefore compared three gram-negative organisms with their derived streptomycin-resistant mutants, grown under identical conditions.

Mutants resistant to 400 μg of streptomycin per ml were derived from *Escherichia coli* K-12, *Pseudomonas fluorescens* N1, and *Serratia marcescens* (NCTC 1377) (10).

The parent strains, *E. coli* K-12 and *S. marcescens*, are sensitive to 5 μg of streptomycin per ml, and *P. fluorescens* is sensitive to 30 μg of streptomycin per ml. *E. coli* B, which is naturally resistant to 100 μg of streptomycin per ml, was also analyzed. Lipids were extracted from lyophilized cells, purified, and analyzed as previously described (3). Fatty acid methyl esters were prepared with boron trifluoride in methanol by using a temperature of 60 C to prevent degradation of cyclopropane fatty acids. Gas-liquid chromatography was carried out on a Packard model 7508 gas chromatograph by using a column (1.8 m by 4 mm) containing 25% diethyleneglycol succinate-2% phosphoric acid on 100 to 120 mesh Gas-Chrom Q.

All strains were analyzed at six hourly intervals during growth to measure the variation in content of cyclopropane fatty acids, which is known to occur with culture age (12). Typical results obtained for *E. coli* K-12 after 6 h of growth (approximately mid-log phase) and 24 h of growth (stationary phase) are shown in Table 1. The growth pattern, phospholipid content, and fatty acid composition of the derived streptomycin-resistant mutant closely followed that of the parent strain. The proportion of the individual phospholipids was unaltered in the mutant, and fatty acid analyses of the individual phospholipids failed to detect any differences between the sensitive and resistant cells. The analysis of the lipids of another strain of *E. coli*, B, which is moderately resistant to streptomycin, revealed a very different fatty acid composition from that of *E. coli* K-12 (Table 1). Since the streptomycin-resistant mutant of K-12 has the same lipid composition as the parent strain, it seems unlikely that the resistance to streptomycin of *E. coli* B can be related to the lipid composition, as might have been concluded if only *E. coli* B and K-12 had been compared. The differences in the affinity for dihydrostreptomycin of 70*S* ribosomes from several *E. coli* strains observed by Chang and Flaks (5) support this interpretation of the results. Although different types of resistance to streptomycin in *E. coli* have recently been described, it was thought that if the lipid composition of the cell membrane were of importance in resistance, differences should have been apparent in the strains studied. Analyses of the derived streptomycin-resistant mutants of both *S. marcescens* and *P. fluorescens* also failed to detect any alteration

TABLE 1. *Analysis of growth pattern and fatty acid composition of E. coli K-12, the derived streptomycin-resistant mutant (SmR), and E. coli B*[a]

| Strain | Time of harvesting (h) | Optical density at 700 nm | Dry wt (mg/ml of medium) | % Lipid (mg/100 mg of cells) | Lipid P (%) | Phospholipids (%) | | | Fatty acids (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Phosphatidyl ethanolamine | Phosphatidyl glycerol | Diphosphatidyl glycerol | C14:0 | C15:0 | C16:0 | C16:1 | C17:0Δ[b] | C18:0 | C18:1 | C19:0Δ[b] |
| K-12 | 6 | 1.32 | 0.51 | 6.5 | 4.0 | | | | 3.5 | | 39.3 | 30.2 | 8.0 | 0.4 | 18.8 | |
| | 24 | 2.9 | 1.0 | 6.0 | 4.3 | 77 | 9 | 14 | 4.9 | | 43.4 | 1.7 | 35.5 | 0.4 | 3.6 | 10.4 |
| K-12 (SmR) | 6 | 1.25 | 0.52 | 7.0 | 3.9 | | | | 4.1 | | 42.0 | 33.0 | 6.7 | 0.3 | 14.1 | |
| | 24 | 2.7 | 1.02 | 5.2 | 3.0 | 78 | 8 | 14 | 5.3 | | 46.0 | 1.4 | 37.6 | 0.3 | 1.3 | 8.1 |
| B | 6 | 1.7 | 0.81 | 5.2 | 4.0 | | | | 3.5 | 0.5 | 31.3 | 15.2 | 19.2 | 1.0 | 26.4 | 3.3 |
| | 24 | 2.4 | 1.04 | 5.2 | 4.0 | | | | 5.3 | 1.5 | 34.5 | 1.2 | 31.8 | 0.8 | 4.3 | 22.0 |

[a] Replicate 5-ml samples from an overnight culture of cells in Buntings medium (4) were inoculated into a series of 1-liter conical flasks containing 200 ml of medium. Mutants were grown in the presence of 400 μg of streptomycin per ml. Cells were harvested by centrifugation at 20,000 × *g*, washed once with water, and lyophilized.

[b] Δ, Cyclopropane fatty acid.

in lipid or fatty acid composition from that of the parent strains. Our experiments indicate that the reported differences in lipid composition of antibiotic-resistant and -sensitive cells (2, 9, 11, 13–15) possibly reflect strain variations rather than an alteration in the permeability of the cell membrane to antibiotics.


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