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Laboratory Characterization of Lipopolysaccharides (LPS)

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There are two types of LPS that can coexist in one strain: smooth and rough. This has been observed in *Pseudomonas aeruginosa* and *Salmonella typhimurium* as well as many other organisms.

Two methods have been used to isolate LPS. Phenol-water extraction has been used for the smooth strains and anhydrous phenol/chloroform/petroleum ether for the rough strains. Both of these techniques are selective in the species of LPS that they isolate. Both provide rather low yields; with some organisms, the yields are impossibly low. Included among these species are *Agrobacterium*, *Bacteroides*, many strains of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. In addition, the methods are selective. For example, with the phenol/chloroform/petroleum ether procedure, the rough core LPS cannot be isolated from a strain of *P. aeruginosa* that contains 80% rough LPS. When the O-polysaccharide is extracted with the phenol-water technique, the O-side chains are enriched.

We have used a technique that is simple. Basically, cells are dissolved in SDS and the LPS precipitated (Darveau and Hancock, J. Bacteriol. 155:831-838, 1983). Yields are about 60%-80% and the LPS is pure by all criteria. Advantages in using the SDS technique are a 50-fold higher yield of some LPSs and the detection of O-side chain-containing LPS in strains previously thought to have only rough LPS.

A purely technical comment concerns the method of running an SDS polyacrylamide gel with LPS in it. A typical artifact that can appear is in the restricted heterogeneity observed. Multiple clusters of O-side chain containing LPS can be caused by hydrophobic interaction between adjacent molecules of LPS or by divalent cation binding of the adjacent LPS molecules. On a purely technical basis, there are three things that can be done to eliminate these problems: addition of EDTA to the gel which removes divalent cations and removal of hydrophobic bonds either by having urea in the gel or by increasing the SDS concentration to 0.5%. Although these are purely technical points, they are important because LPS gels are becoming a major method of documenting the character of LPS in the literature.