Resistance to Coliphage Infection Induced in 

*Escherichia coli* by Growth in the Presence of a Surfactant

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Heavy slime exudates surrounding *Escherichia coli* cells grown in media containing 1% sodium dodecyl benzene sulfonate apparently block adsorption of coliphages to susceptible strains.

In a comparison of the effect of various synthetic detergents on bacterial growth, Anderson (2) observed that, when strains of coliforms were grown in media containing sodium dodecyl benzene sulfonate (NaDDBS), cell proliferation initially lagged, but cells exuded large amounts of slime. However, after several hours, a period of logarithmic growth ensued paralleling that of the control and indicating no marked loss in viability. Electron micrographs (2) revealed an amorphous slime layer immediately surrounding the cell, suggesting that this slime layer might mechanically block phage receptor sites and thus prevent phage adsorption.

Such a phenomenon was observed by Danz and Schultz (3) with a highly "gelatinous" mutant strain of *Psuedomonas aeruginosa*. Although the parent strain was highly susceptible to a particular strain of phage, the mucoid strain was resistant to the majority of specific phages tested. Maxted (4) found that the presence of a hyaluronic acid capsule conferred phage resistance on a group A streptococcus strain. Removal of the capsule with hyaluronidase rendered the bacterium susceptible to phages.

As a preliminary test of the phage-blocking hypothesis, susceptible strains of *Escherichia coli* were inoculated as streaks on nutrient agar plates containing 1.0% NaDDBS and seeded with droplets of representative T-even and T-odd phage strains. No zones of clearing developed on the NaDDBS-containing agar. However, normal droplet plaques formed on the control plate and on plates containing 1.0% dodecyl benzene (Nalkylene 500) or 1.0% of sodium benzene sulfonate, each representing a molecular portion of NaDDBS (Fig. 1). Plates containing 1.0% Triton X-100 likewise showed no inhibition of plaque formation.

Phages held in 1.0% NaDDBS-nutrient broth for 60 min still retained their ability to produce normal lysis of susceptible cells. On the nutrient agar-NaDDBS plates, viable phages were recovered from the droplet inoculation areas after 24 h of incubation.

A viscosity increase concomitant with the increase in slime was shown by Ostwald viscometer measurements.

![Fig. 1. Streaks of E. coli grown on nutrient agar, and nutrient agar plus surfactants. Seeded with droplets of suspension of coliphages T1, T6, and T6. Upper left, Nutrient agar control; upper right, nutrient agar plus dodecyl benzene; lower left, nutrient agar plus NaDDBS; lower right, nutrient agar plus sodium benzene sulfonate.](http://aem.asm.org/)

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Fig. 2. One-step growth curves of coliphage T-5 showing the effect of growth in the presence of surfactant on phage adsorption and replication. O, Control; □, cells grown in the presence of 1% NaDDBS; Δ, cells grown in the presence of NaDDBS and centrifuged, and deoxyribonuclease added to pellet before one-step growth experiment was begun. Multiplicity of infection is 0.1.

Rate of adsorption of phage T-5 to E. coli was compared by using cells grown in the presence of NaDDBS, Triton X-100 (nonslime inducing), and nutrient broth only. The presence of NaDDBS-induced slime surrounding the cells reduced the rate of adsorption.

One-step growth experiments, from Adams (1), were used to measure the extent of inhibition of adsorption. In the presence of slime, no phage replication occurred (Fig. 2).

With a similar E. coli surfactant system, Pollack and Anderson (5) concluded that the high viscosity induced by the slime was apparently due to a high deoxyribonucleic acid content (10%), suggesting cell membrane leakage. Accordingly, deoxyribonuclease was added to a 24-h NaDDBS culture seeded with phage, in an attempt to remove the slime layer. After this procedure, a one-step growth experiment revealed that adsorption resulting in phage replication had occurred in a portion of the treated cells (Fig. 2).

The results obtained suggest that the surfactant-induced slime may mechanically block phage receptor sites, inhibiting phage adsorption. The major blocking agent may well be deoxyribonucleic acid, as indicated by the high viscosity and induction of phage adsorption and replication after treatment with deoxyribonuclease.

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