Effect of Clay Particle Size on Clay–*Escherichia coli*–Bacteriophage Interactions

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Certain clays as well as particulates derived from sediments were found by Roper & Marshall (1974) to inhibit the host–parasite interaction between *Escherichia coli* and a specific bacteriophage. This effect may involve more than one type of sorption phenomenon, since there are functional differences between the sorption of bacteria to particles of bacterial size and larger (Meadows, 1965; Santoro & Stotzky, 1967) and the sorption of colloidal particles to a bacterial surface (Peele, 1936; Lahav, 1962; Marshall, 1968, 1969 *a, b*). Roper & Marshall (1974) suggested that, at low electrolyte concentrations, *E. coli* is protected from bacteriophage attack by an envelope of colloidal particles sorbed to the bacterium, whereas, at high electrolyte concentrations, protection may result both from the presence of a colloidal envelope and from sorption of *E. coli* and bacteriophage to larger particulate material.

The purpose of this investigation was to define more accurately the effects of different particle-size fractions of a montmorillonite on the *E. coli*–bacteriophage interaction at both high and low electrolyte concentrations.

An aqueous suspension of montmorillonite, prepared from Wyoming bentonite (Volclay, American Colloid Co., Skokie, Illinois, U.S.A.), was centrifuged at 12 000 *g* for 20 min and the supernatant was retained. The pellet was resuspended in distilled water, centrifuged at the same speed and the supernatant was combined with the original supernatant. This procedure was repeated until no montmorillonite remained in the supernatant. Similar centrifugations were carried out at separate, decreasing *g* values to give eight particle-size fractions. The effective spherical diameter was then calculated using Stokes' equation (Day, 1965):

$$v = X^2 g(\rho_s - \rho_L) / 18\eta$$

in which *X* is the effective spherical diameter, ρ_s is the density of solid particles (2.60 g cm⁻³), ρ_L is the liquid density (1 g cm⁻³), η_{20} is the viscosity of water at 20 °C (0.010042 poise), and $v = h/t$, in which *h* is the distance of sedimentation (8 cm), and *t* is the time of sedimentation (20 min).

Survival of *E. coli* in the presence of the various montmorillonite fractions was determined in NaCl solutions both at a relatively high specific conductance ($L_s = 13.9$ mS cm⁻¹) and at a low specific conductance ($L_s = 1.39$ mS cm⁻¹). The montmorillonite was dispersed at specific conductance values below 2.2 mS cm⁻¹ (Roper & Marshall, 1974). The treatments employed in the experiments were: (i) *E. coli* (8×10^8 ml⁻¹) plus 1.0 ml spent nutrient broth; (ii) *E. coli* plus 1.0 ml bacteriophage in spent nutrient broth; (iii) *E. coli* plus spent nutrient broth plus montmorillonite fraction (150 µg ml⁻¹, final concentration); and (iv) *E. coli* plus bacteriophage in spent nutrient broth plus montmorillonite fraction. Spent nutrient broth was used in treatments (i) and (iii) to ensure that nutrient additions were the same as in treatments with phage, which were suspended in filtered spent nutrient broth. All treatments were incubated in 250 ml Erlenmeyer flasks on a gyratory shaker for 16 h at 28 °C, after which *E. coli* numbers were determined. In all instances, samples containing montmorillonite were diluted to $L_s = 0.695$ mS cm⁻¹ to desorb the bacteria prior to plating on Mac-

Fig. 1. Effect of particle-size of montmorillonite on the survival of *E. coli* in the presence or absence of bacteriophage. Output/Input = total number of *E. coli* recovered/initial inoculum. Positive values of $\log_{10}$ (Output/Input) represent growth and negative values represent death. At $L_s = 13.9 \text{ mS cm}^{-1}$: $\circ$, no phage; $\bullet$, with phage. At $L_s = 1.39 \text{ mS cm}^{-1}$: $\square$, no phage; $\blacksquare$, with phage.

Conkey agar (Roper & Marshall, 1974); numbers of *E. coli* were adjusted for the volume of diluent employed.

The results (Fig. 1) indicate that almost complete protection of *E. coli* from phage attack was provided by the fine particles. However, particles with an effective spherical diameter greater than $0.6 \mu\text{m}$ did not protect *E. coli* from phage lysis. The electrolyte concentration had little or no effect on the survival of *E. coli* with particles of different sizes. Colloidal particles attach to bacterial surfaces as a result of electrostatic attraction (Marshall, 1971), but colloidal particles exceeding $0.6 \mu\text{m}$ effective spherical diameter may not form a continuous barrier around the bacterium. Thus, phage may make contact with the host surface and cause lysis. This view may be too simplistic because Bystricky, Stotzky & Schiffenbauer (1975) found that although bacteriophage sorbed to clay particles by their tails this did not reduce their ability to infect available hosts (Stotzky, 1977). However, these authors were working with clays in the larger effective spherical diameter range (G. Stotzky, personal communication).

The results presented here indicate that the inhibition of *E. coli*-bacteriophage interaction by colloidal particulates is dependent upon particle size, thereby emphasizing the subtlety of interactions between micro-organisms and certain physical components of their habitat. In addition, the protection of *E. coli* and other faecal bacteria in aquatic systems by particulate matter may have important consequences from a public health viewpoint.

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