AN EVALUATION OF TWO BACTERIOPHAGES AS SEWAGE TRACERS

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Abstract. Two bacteriophages – phage 80 of *Staphylococcus aureus* and a P2-like phage (φMWD 1) of *Escherichia coli* (H2S +) – were evaluated as sewage tracers. Background plaque concentrations on the *S. aureus* host were < 1 100 mL−1 in seven (raw and treated) effluents tested but, on *E. coli* (H2S +), they ranged from < 1 100 mL−1 in oxidation pond effluents to 1.1 × 10³ 100 mL−1 in primary treated meatworks effluent. Thus, phage 80 appears to be a suitable tracer for both raw and treated sewage but φMWD 1 may only be suitable for use in secondary treated effluent. In frozen samples, concentrations of both tracer phages were reduced by 90% within 2 days, but decreased more slowly over the following 68 days to around 5% of the original (unfrozen) titre. In a field test, the two phages were used simultaneously to trace the movement of oxidation pond effluents down a river system. Timing of assay of frozen samples and the advantages and limitations of bacteriophages as sewage tracers are discussed.

1. Introduction

Laboratory-cultured bacteriophages of *Escherichia coli* (coliphages) are the most commonly used viral tracers of water movement. They have been used to monitor river flow rates (Niemelä and Kinnunen, 1968; Wimpenny et al., 1972; Kinnunen, 1978), predict estuarine mixing patterns (Statham, 1974) and trace groundwater movement (Kinnunen and Jokinen, 1972; Martin and Thomas, 1974; Fletcher and Myers, 1974; Schaub and Sorber, 1977; Noonan and McNabb, 1979). They have also been used to follow effluent movement through a sedimentation basin (Kawata and Olivieri, 1974), through soils (Duboise et al., 1974; Schaub and Sorber, 1977) and along overland flow treatment plots (Schaub et al., 1980).

The principal advantages of using bacteriophages as water tracers are: (i) their greater safety when compared to bacterial tracers, and (ii) their potential for simulation of enteric virus behavior in aquatic environments. The possibility of freezing samples for later assay has also been noted by some authors (e.g., Keswick et al., 1982), but this procedure does not appear to have been critically evaluated.

The principal disadvantage of most coliphage tracers is encountered in studies of sewage or sewage-polluted waters. These environments are likely to contain significant concentrations of phages to which the host bacterium is susceptible and which will interfere with tracer detection. Schaub and Sorber (1977) overcame this problem by autoclaving effluent used in through-soil phage transport studies. However, most investigators have simply ensured that tracer coliphage concentrations greatly exceeded background levels, which ranged from < 10 pfu (plaque forming units) mL⁻¹ in river water (Wimpenny et al., 1972) to 10 000 pfu 1 mL⁻¹ in effluent (Kawata and Olivieri, 1974).
The high tracer : background ratio approach is most successful where pollution from a single source is followed into otherwise uncontaminated water, and least successful where additional sewage discharges occur immediately upstream of remote sampling sites. Seely and Primrose (1982) noted that tracer phage concentration procedures will also exacerbate background interference problems. They emphasized the importance of selecting appropriate phage-host systems for water tracing, rejected phages of *E. coli* and *Pseudomonas aeruginosa* because of likely background interference, and suggested a rare phage of *Salmonella typhimurium* as a potential tracer. Recently, Purdy *et al.* (1986) used a phage of *Bacillus* sp. to trace sewage movement from a coastal outfall. Other possibilities include phages of *Serratia marcescens* and *Staphylococcus aureus*, both of which have been used to index human enteric virus survival in sewage, the former by Carstens *et al.* (1965) and the latter by Weber-Schutt (1966). Drury and Wheeler (1982) later evaluated the *S. marcescens* phage as a sewage tracer but phages of *S. aureus* do not appear to have been investigated further for this purpose.

The present study was designed to investigate the suitability as sewage tracers of an *S. aureus* phage and a coliphage, in terms of background concentrations in a range of effluents and survival in frozen samples. These two elements were then combined in a field evaluation of the two tracers.

2. Materials and Methods

2.1. Laboratory Studies

A range of effluent types was assayed for phages on 17 hosts – *Staphylococcus aureus* (Heatley Oxford strain; ATCC 9144) and 16 reference culture *E. coli* strains. Coliform and faecal coliform bacteria concentrations were also determined (APHA, 1985). The two hosts giving the lowest phage titres – *S. aureus* and an *E. coli* (H₂S +) strain were selected as potential tracer hosts.

Phage 80 of *S. aureus*, to which the Heatley Oxford strain is susceptible, was selected for evaluation as a tracer. The enumeration and propagation techniques recommended by Blair and Williams (1961) were followed, except that culture in tryptic soy broth was used to obtain a high phage titre for field use.

Six known coliphages (T2, T4, MS2, φX174, φII, and R17) either failed to lyse, or gave poor lysis on the *E. coli* (H₂S +) strain. Therefore, a large, clearly defined plaque from a raw sewage sample was selected and repeatedly subcultured to provide a pure tracer phage stock. Electron microscopy showed this phage to have an icosahedral head with a straight tail. It was tentatively identified as a P2-like phage and was designated tracer coliphage φMWD 1 (Lane, 1986).

To test the feasibility of delayed assay of frozen samples, the survival of both tracer phages in 100 mL samples, stored in screw-capped plastic bottles at −18 °C, was examined over a 70-day period. Coliphage φMWD 1 was stored in river water, and phage 80 in river water and raw sewage. Randomly selected samples (4 × 100 mL replicates) were thawed for assay and then discarded.