Bacteriophage Control of Meat Spoilage by *Brochothrix thermosphacta*

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Background

Bacteriophage (phage)-host systems in refrigerated foods have been described (Whitman *et al.*, 1971) and phage are naturally present in meat, fish, poultry and milk. Virulent, psychrotrophic phage for *Pseudomonas* spp. (Greer, 1982) and *Brochothrix thermosphacta* (Greer, 1983) have been isolated and purified from spoiled retail beef. In subsequent studies with artificially inoculated beef, homologous phage could inhibit *Pseudomonas* growth and extend the retail case life of beef steaks (Greer, 1986). This suggested the potential for a unique biocontrol method but a restricted spectrum of activity limited practical value (Greer and Dilts, 1990).

Although *B. thermosphacta* phage had been characterized (Greer, 1983; Ackermann *et al.*, 1988) their interactions with bacterial hosts in chilled meats was not investigated. The present study was undertaken to determine if phage could inhibit the growth of *B. thermosphacta* on pork fat and delay the onset of undesirable off-odours.

Objectives

1. To determine the effects of homologous phage on the growth of *B. thermosphacta* in aqueous muscle extracts and on pork fat.

2. To determine the effects of homologous phage on the spoilage of pork fat during simulated retail display.

Methods

Bacteria and Phage

A wild type strain *B. thermosphacta* B2 and the homologous phage A3 were isolated and purified from spoiled, beef rib steaks (Greer, 1983). Bacteria and phage were cultivated in tryptic soy broth or agar (Difco) containing 1 mM $CaCl_2$. High titer phage lysates were prepared from confluently lysed plates following the agar overlay technique. Both phage and bacteria were diluted in 0.1% (w/v) peptone water to obtain the necessary concentrations for inoculation.

Inoculation

The effect of phage on bacterial growth in aqueous extracts of beef muscle and at the surface of pork fat was examined following inoculation and storage under simulated retail display conditions at 7°C.

Sterile, aqueous extracts of homogenized beef *longissimus thoracis* muscle were prepared as described previously (Greer, 1986) and flasks containing 25 ml of extract were inoculated to give an initial concentration of *B. thermosphacta* of about 10^7 CFU/ml. Phage were co-inoculated at 10^7 or 10^6 PFU/ml and bacterial growth was estimated by absorbance at 600 nm.

Sterile pork fat discs (10 cm^2) were prepared from *longissimus thoracis* muscle (Greer *et al.*, 1995) and inoculated to give an initial concentration of *B. thermosphacta* of 10^5 CFU/ml. Phage were coinoculated at a concentration of 10^7 PFU/ml. Inoculated tissues were placed in petri plates, overwrapped in an oxygen permeable film and growth was determined by plate counts on tryptic soy agar. At each storage time, 5 flasks and 5 tissue discs were sampled and data were presented as means.

Sensory

At daily intervals for up to 9 days of retail display, fat tissue was evaluated for the intensity of off-odours using a 5-member sensory panel and a 4-point subjective scale (1 = no off-odour; 4 = prevalent off-odour). To quantify case life, samples were also rated on an acceptance scale (1 = unacceptable; 5 = acceptable).

Results and Discussion

The data in Fig. 1 show a phage concentration-dependent inhibition of the growth of *B. thermosphacta* in a beef extract medium. No bacterial growth was observed when initial phage concentrations were 10^7 PFU/ml (multiplicity of infection = 1.0). Phage were also determined to restrict *B. thermosphacta* growth on fat during simulated retail display (Fig. 2) and inhibit the development of intense

off-odours (Fig. 3). On the basis of odour acceptance data the case life of phage treated samples was extended beyond the duration of the storage interval (>9 d) while the acceptability of fat inoculated with bacteria alone, deteriorated within 1.6 d.

The present data complement previously published studies with *Pseudomonas* spp. where phage were found to reduce bacterial growth and double the storage life of inoculated beef steaks (Greer, 1986). Others have shown that phage can inhibit the growth of spoilage pseudomonads in refrigerated milk (Ellis *et al.*, 1973).

Conclusions

The current study provides the first known evidence that homologous phage can interact with a *B. thermosphacta* host at an inoculated fat surface, reduce bacterial populations and extend storage life by more than one week. If phage are to be advocated as "natural", biopreservatives efficacy must be confirmed in more complex ecosystems where the indigenous flora is comprised of a diversity of *B. thermosphacta* strains acquired under the more practical circumstances in commercial environments.

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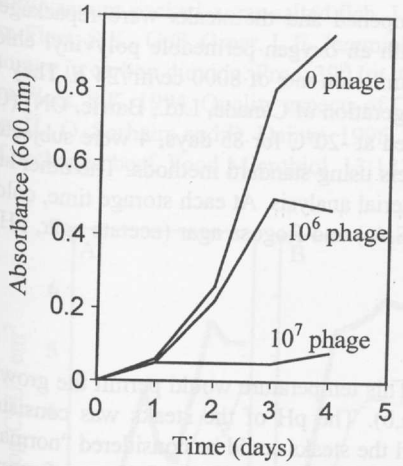

Figure 1. Effect of bacteriophage on growth of *B. thermosphacta* in an aqueous beef extract at 7°C

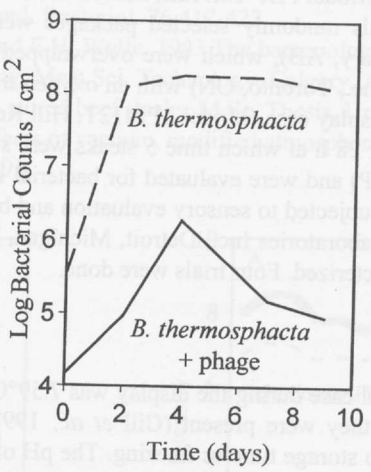

Figure 2. The effect of bacteriophage on the growth of *B. thermosphacta* on pork fat at 7°C

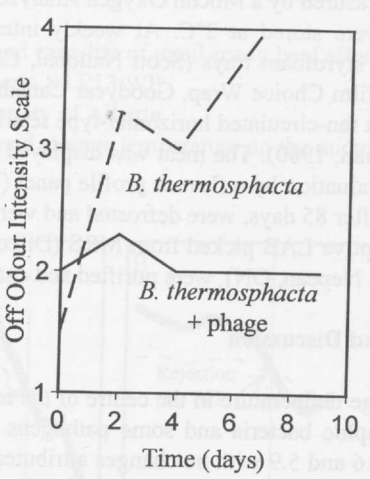

Figure 3. The effect of bacteriophage on the intensity of off-odours during *B. thermosphacta* growth on pork fat at 7°C. 1 = no off odour; 4 = prevalent off-odour.