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REVIEW ARTICLE

Campylobacter bacteriophages and bacteriophage therapy

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Summary

Members of the genus *Campylobacter* are frequently responsible for human enteric disease with occasionally very serious outcomes. Much of this disease burden is thought to arise from consumption of contaminated poultry products. More than 80% of poultry in the UK harbour *Campylobacter* as a part of their intestinal flora. To address this unacceptably high prevalence, various interventions have been suggested and evaluated. Among these is the novel approach of using *Campylobacter*-specific bacteriophages, which are natural predators of the pathogen. To optimize their use as therapeutic agents, it is important to have a comprehensive understanding of the bacteriophages that infect *Campylobacter*, and how they can affect their host bacteria. This review will focus on many aspects of *Campylobacter*-specific bacteriophages including: their first isolation in the 1960s, their use in bacteriophage typing schemes, their isolation from the different biological sources and genomic characterization. As well as their use as therapeutic agents to reduce *Campylobacter* in poultry their future potential, including their use in bio-sanitization of food, will be explored. The evolutionary consequences of naturally occurring bacteriophage infection that have come to light through investigations of bacteriophages in the poultry ecosystem will also be discussed.

Introduction

Campylobacter the problem

Members of the genus *Campylobacter* are frequently responsible for human enteric disease with occasionally very serious outcomes (Adak *et al.* 2005). The genus comprises 17 species in total (Korczak *et al.* 2006), but it is principally *Campylobacter jejuni* and *Campylobacter coli* that account for the majority of cases of bacterial gastroenteritis in humans, (Allos 2001; Miller and Mandrell 2005). In the United Kingdom in 2009, there were more than 65 000 reported cases, with over 195 000 in the European Union as a whole (EFSA 2011). However, the real number of cases may be much higher, estimated to be around 450 000 in the UK, as a result of substantial under-reporting (Strachan and Forbes 2010). Much of this disease burden is thought to arise from consumption of contaminated poultry products or cross-contamination from them to raw foods (Wingstrand *et al.* 2006; Lindqvist and Lindblad 2008). More than 80% of poultry in the UK harbour these organisms as a part of their intestinal flora and this pattern of high positivity is typical of most other European countries (EFSA 2010). Campylobacters can colonize the chicken intestine in relatively large numbers, often in the region of 7 $\log_{10}$ CFU g^{-1} of the content in the caecum (Rudi *et al.* 2004; Atterbury *et al.* 2005). During processing, release of the intestinal contents inevitably leads to contamination of poultry carcasses destined for human consumption (Kramer *et al.* 2000; Reich *et al.* 2008).

Controlling campylobacters in poultry represents an immense challenge to the agriculture and food industries (Newell *et al.* 2010) as these bacteria are well adapted to avian species such that they may be considered commensal organisms of poultry. Various approaches having been proposed to reduce the numbers of viable campylobacters carried on poultry carcasses that can reach the consumer's kitchen. These approaches target all parts of the poultry meat–processing chain, including those that start at the farm level such as increased biosecurity, competitive exclusion and bacteriophage therapies (Doyle and

Erickson 2006) and proceed through each stage of transport, processing, storage and packing (reviewed by Cox and Pavic 2010). Rigorous application of biosecurity can result in *Campylobacter*-free chickens, but these measures are expensive and difficult to maintain. If one bird becomes infected, the infection spreads rapidly through the entire flock. This is largely because of the susceptibility of chickens to colonization by *Campylobacter* and its ubiquity in the environment (Newell and Fearnley 2003). Moreover, where flocks have been successfully reared to be *Campylobacter*-free through increased biosecurity, the effort may be negated by cross-contamination from *Campylobacter*-positive flocks at the abattoir (Herman *et al.* 2003).

A worrying development is that *Campylobacter* strains isolated from poultry and other farm animals are showing increasing levels of resistance to antibiotics and particularly to the fluoroquinolones (reviewed by Moore *et al.* 2006). This may largely be attributable to the widespread and routine use of antibiotics as growth promoters, a procedure now banned in the EU. While most *Campylobacter* infections are self-limiting, antimicrobial therapy may be indicated for patients with systemic infections or for immunocompromised patients. Antimicrobial resistance among *Campylobacter* isolates therefore has serious implications for the treatment of campylobacterosis in humans.

Bacteriophages: natural therapeutic agents

While not a new idea, the dramatic rise in multi-drug resistant bacteria have prompted Western scientists to reassess bacteriophage therapy as an alternative to combat infectious bacteria (reviewed by Sulakvelidze *et al.* 2001; Summers 2001; Monk *et al.* 2010). Bacteriophages are defined as viruses that can infect, multiply and kill susceptible bacteria. They are both ubiquitous and abundant in the environment, with the total number of bacteriophage in the biosphere estimated to be in the region of 10^{31} (Hendrix *et al.* 1999). They can be found in virtually all locations where suitable bacterial hosts proliferate. While bacteriophages can be exploited in many ways, for example their use in bacteriophage-typing schemes or for the rapid identification of bacteria, the recent focus of interest has been on the therapeutic use of bacteriophages (Kutter and Sulakvelidze 2005). Although former Warsaw Pact countries have exploited the use of bacteriophages for therapeutic, prophylactic and disinfection purposes for many years (reviewed by Alisky *et al.* 1998), a few commercial bacteriophage therapy products are now available from various biotechnology companies (reviewed by Monk *et al.* 2010). The application of bacteriophage therapy in the context of food production is attractive, because bacteriophages are already widely present in the foods that we eat and bacteriophage treatment has the potential to be a sustainable measure.

For successful bacteriophage therapy, selection of the bacteriophages to be used is of paramount importance. The bacteriophages selected should be those that have an obligate lytic life cycle and as a consequence will always lyse the bacterial cells they infect and release new bacteriophages. In contrast, those that have a lysogenic life cycle that involves integration of their DNA into the host genome are generally unsuitable for bacteriophage therapy as they may render the host bacterium immune to further infection through the production of a phage-encoded repressor. Furthermore, infection with lysogenic phage may result in the transfer and dissemination of DNA encoding pathogenic traits among their hosts (Cheetham and Katz 1995; Boyd and Brussow 2002).

Bacteriophages, applied as therapeutic agents, offer many advantages over conventional therapies. Firstly, they are already present in the same environments that their hosts inhabit and are easily isolated. They are generally specific so do not damage normal gut flora. They are both self-replicating and self-limiting, multiplying only as long as sensitive bacteria are present.

It is important to understand that bacteriophage replication is a density-dependent process (Levin and Bull 1996; Payne and Jansen 2001; Bull *et al.* 2002) and is critically dependent on the density of host bacteria present. It is proposed that bacteriophages require a host density threshold termed the 'bacteriophage proliferation threshold' (Wiggins and Alexander 1985; Payne and Jansen 2001) to proliferate sufficiently to achieve a crash in the host bacterial population. Various other parameters, including the inoculum size, inoculum timing, bacteriophage absorption rate and burst size are also critical to the success of bacteriophage therapy (Levin and Bull 1996; Payne and Jansen 2001; Weld *et al.* 2004). In addition, the kinetics of bacteriophage absorption to bacteria in the intestinal environment has been proposed to be different from those determined experimentally on laboratory media, because of the viscosity of the mucus layer (Weld *et al.* 2004).

The emergence of resistant bacteria following bacteriophage therapy has always been perceived as a potential obstacle (Barrow 2001) as bacteria constantly mutate to generate diversity. However, while bacteriophage resistance has been reported following experimental bacteriophage treatments (Smith and Huggins 1982; Smith *et al.* 1987a; Sklar and Joerger 2001), it is not necessarily as counterproductive as it appears, as the bacteriophage-resistant types may be less virulent and impaired in their ability to compete with their wild-type counterparts. Development of resistance can be managed by using

bacteriophage 'cocktails' with different combinations of bacteriophages that target different receptors on the host bacteria (O'Flynn *et al.* 2004; Tanji *et al.* 2004). Evidence against the dominance of bacteriophage-resistant populations can also be gained from the examination of natural bacteriophage infections that lead to bacterial succession within the niche rather than the selection and dominance of bacteriophage-resistant bacteria (Connerton *et al.* 2004).

The bacteriophages of *Campylobacter*

First reports of bacteriophages that infect *Campylobacter*

Numerous changes to the taxonomy of *Campylobacter* over the last five decades make it difficult to pinpoint the earliest reports of *Campylobacter* bacteriophages. However, bacteriophages specific to the species we now know as *C. coli* and *C. fetus,* then *Vibrio coli* and *Vibrio fetus*, were isolated from cattle and pigs during the 1960s (Fletcher and Bertschinger 1964; Firehammer and Border 1968; Fletcher 1968). Later temperate bacteriophages were isolated from aborted sheep foetuses in conjunction with their bacterial hosts, which were probably *C. fetus* using the current nomenclature (Bryner *et al.* 1982). *Campylobacter* bacteriophages were also reported to play a role in the auto-agglutination of cells, which interfered with attempts to serotype *Campylobacter* isolates (Ritchie *et al.* 1983).

Campylobacter bacteriophage characteristics

The most frequently encountered *Campylobacter* bacteriophages are the double-stranded DNA, tailed bacteriophages, with icosahedral heads, belonging to the family *Myoviridae* (Table 1). Reports from the Russian Federation have also described bacteriophages belonging to the *Siphoviridae* and *Podoviridae* families, but few details are available regarding the characteristics of these bacteriophages (Connerton *et al.* 2008).

Sixteen bacteriophages that make up the most widely used bacteriophage typing system (Frost *et al.* 1999; see next section) were characterized by Sails *et al.* (1998). These could be subdivided into three groups according to their genome size and head diameter. Two bacteriophages with head diameters of 140·6 and 143·8 nm and large genome sizes of 320 kb were classified as group I. Five bacteriophages were classified into Group II and had average head diameters of 99 nm and average genome sizes of 184 kb. Group III contained nine bacteriophages with average head sizes of 100 nm and average genome sizes of 138 kb. The sixteen bacteriophages could also be categorized into four groups based on their patterns of lysis against spontaneous, transposon-insertion and defined mutants of *C. jejuni* (Coward *et al.* 2006). While the bacteriophage genomic DNA is often resistant to digestion with any of the standard restriction endonucleases, *Hha*I has proven to be useful to discriminate some group III bacteriophages (Sails *et al.* 1998). This enzyme was also used to classify and subdivide a group of bacteriophages isolated by Hansen *et al.* (2007).

Lysogenic, or temperate, bacteriophages were first described in *C. fetus* (see previous section). Evidence of their presence in *C. jejuni* was not definitively demonstrated until relatively recently when genome sequence data for several strains of *C. jejuni* became available. It then became apparent that prophages were in fact present in some, but not all, strains examined. Specifically,

Table 1 Members of *Myoviridae* family bacteriophages specific to *Campylobacter*

Life cycle	Approx. genome size (kb)	Average head diameter (nm)	Group*	Comment†	Use in bacteriophage therapy
Lytic	320	143	I	Rare with only two known isolates	Unlikely due to instability‡
Lytic	180	83–99	II	Uncommon	Proven applications§
Lytic	140	100–130	III	Most common type isolated from poultry sources	Proven applications¶
Lysogenic	40**	90	NA††	Sequence identified in many poultry strains‡‡	Not suitable

*Classification proposed by Sails *et al.* (1998).

†The relative frequency estimated from the following studies: Atterbury *et al.* (2003a); El-Shibiny *et al.* (2007); Hansen *et al.* (2007); Hwang *et al.* (2009); Loc Carrillo *et al.* (2005, 2007); Sails *et al.* (1998).

‡Frost *et al.* (1999).

§Carvalho *et al.* (2010b) El-Shibiny *et al.* (2009); group II classification surmised from reported genome size of constituents of bacteriophage cocktail).

¶Loc Carrillo *et al.* (2005); Scott *et al.* (2007b); Wagenaar *et al.* (2005).

**Estimated from genome sequence RM1221.

††Not applicable.

‡‡Barton *et al.* (2007); Clark and Ng (2008); Parker *et al.* (2006); Scott *et al.* (2007a).

Mu-like bacteriophage sequences were identified in *C. jejuni* RM1221 (Fouts *et al.* 2005), and similar sequences were also found in many other *C. jejuni* strains (Parker *et al.* 2006; Barton *et al.* 2007; Scott *et al.* 2007a; Clark and Ng 2008) but are notably absent in the prototype genomic sequence of *C. jejuni* NCTC 11168 (Parkhill *et al.* 2000). Genomic rearrangements, identified in a *C. jejuni* isolate used for bacteriophage therapy trials in poultry, were found to be associated with intra-genomic inversions between Mu-like prophage DNA sequences (Scott *et al.* 2007a). Unlike the parental strain, these strains were resistant to infection by virulent bacteriophages, inefficient at colonization of the broiler chicken intestine and spontaneously produced bacteriophage CampMu virions, which could be visualized by electron microscopy (Fig. 1).

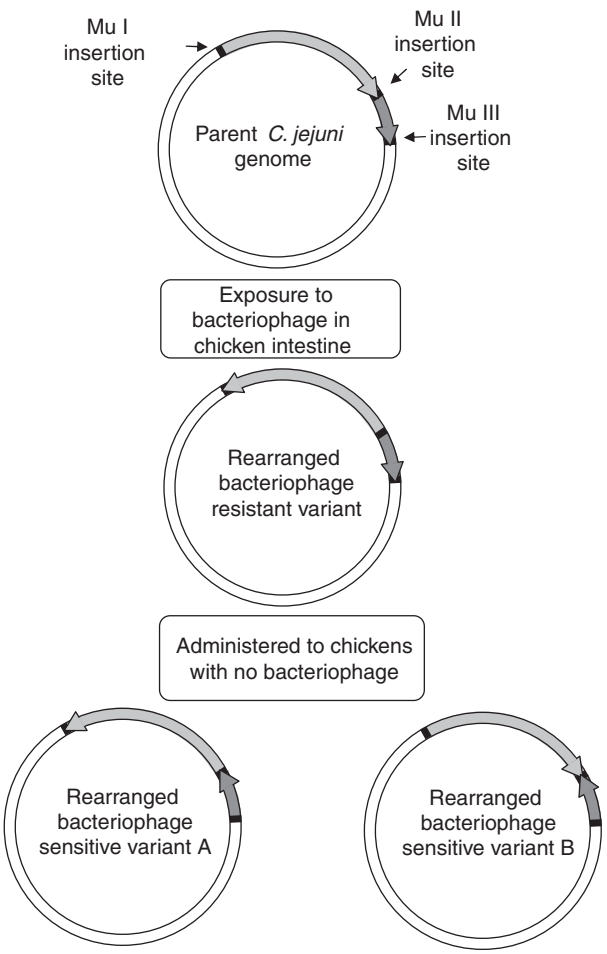

Figure 1 An example of the progression of rearrangements of the *Campylobacter jejuni* HPC5 genome following bacteriophage exposure in chickens, involving the Mu-like prophage sequence as described by Scott *et al.* (2007a).

Bacteriophage typing

Until relatively recently when molecular techniques became available, subtyping of campylobacters was historically challenging with many different systems being proposed but no one system being universally accepted. Bacteriophage typing systems that had been used very successfully for *Salmonella* were developed for *Campylobacter* spp. (Grajewski *et al.* 1985; Salama *et al.* 1990; Khakhria and Lior 1992; Sails *et al.* 1998; Frost *et al.* 1999) and these were subsequently compared with other classification schemes of the time (Gibson *et al.* 1995; Hopkins *et al.* 2004). Although the majority of *C. jejuni* isolates could be bacteriophage-typed inevitably some could not, even so there are many examples in the literature where bacteriophage typing was utilized to differentiate *Campylobacter* spp. and the technique proved potentially more discriminatory than serotyping or biotyping (Salama *et al.* 1990).

Isolation of *Campylobacter* bacteriophages

Campylobacter bacteriophages have been isolated wherever their hosts are present such as the faeces of pigs, cattle and sheep (Firehammer and Border 1968; Bryner *et al.* 1970, 1973); abattoir effluent, sewage, manure and the excreta of both broiler and layer chickens (Grajewski *et al.* 1985; Salama *et al.* 1989; Khakhria and Lior 1992; Sails *et al.* 1998; Connerton *et al.* 2004; Atterbury *et al.* 2005; El-Shibiny *et al.* 2005; Loc Carrillo *et al.* 2007) and even from poultry meat (Atterbury *et al.* 2003a; Tsuei *et al.* 2007). Most isolations of bacteriophages have been through application of filtered suspensions to *Campylobacter* host strains in a soft agar lawn, where plaques can be visualized following incubation. Where bacteriophage numbers are low and could otherwise be missed, an enrichment method was adopted whereby *Campylobacter* cells are incubated with the sample for 18 h prior to detection to increase the overall numbers of bacteriophages to be detected (Carvalho *et al.* 2010a).

Incidence of *Campylobacter* bacteriophages in poultry

The incidence of *Campylobacter* bacteriophages in UK poultry was determined to be approximately 20%, of 205 chickens sampled in the UK in 2002 (Atterbury *et al.* 2005). The presence of bacteriophages in these birds resulted in a statistically significant 1·8 $\log_{10}$ CFU g^{-1} difference ($P < 0{\cdot}001$) in *Campylobacter* counts between those birds that harboured bacteriophages and those that did not. In a 2004 study from Denmark, the isolation rate of *Campylobacter* bacteriophages from broiler intestines and abattoir samples was approximately 3% of 312

sampled, but interestingly the rate was approximately 50% from ten duck samples (Hansen *et al.* 2007). In organic flocks which are generally close to 100% colonized by *Campylobacter* (Heuer *et al.* 2001), the incidence of bacteriophages in *Campylobacter*-positive organic birds from a UK flock was found to be 51% positive from 37 birds sampled (El-Shibiny *et al.* 2005) probably due to organic birds having greater exposure to the environment and therefore to a greater range of *Campylobacter* types and their associated bacteriophages. A study of a naturally bacteriophage-infected broiler chicken barn indicated that both bacteriophages and its host *Campylobacter* could be carried over from one flock to the next (Connerton *et al.* 2004).

There is little data from other countries regarding the incidence of *Campylobacter* bacteriophages. In Korea, 20% of 30 chicken intestinal samples yielded *Campylobacter* bacteriophages, while sewage and abattoir effluent were negative (Hwang *et al.* 2009). In New Zealand, 28% of 39 pooled whole-chicken rinses were positive for *Campylobacter* bacteriophages, while vegetable and retail poultry samples were negative (Tsuei *et al.* 2007). No *Campylobacter* bacteriophages were isolated from 53 surface water samples from New Zealand (Bigwood and Hudson 2009).

Survival of *Campylobacter* bacteriophages

In general, bacteriophages have evolved to survive the same potentially hostile environments that their hosts endure and *Campylobacter* bacteriophages are no exception. While there is little published research on the specific survival characteristics of *Campylobacter* bacteriophages, they have been shown to be able to survive their journey from the chicken intestine to the surface of poultry meat along with their hosts (Atterbury *et al.* 2003a). This is an important point as it is therefore clear that man has almost certainly been continuously exposed to surviving bacteriophages associated with poultry meat since poultry first became an important food source. The general robustness of bacteriophages is an advantage for therapeutic agents as they can, for example, be simply added to drinking water or to feed (Carvalho *et al.* 2010b), provided that the intended targets are intestinal pathogens. However, as the bacteriophage capsid is essentially protein, it is perhaps not surprising that some bacteriophages are sensitive to the low pH encountered in the stomach or proventriculus (Leverentz *et al.* 2001). To enhance their use in general intestinal bacteriophage therapy, combination of bacteriophages with an antacid (Smith *et al.* 1987b; Koo *et al.* 2001) or selection of appropriate low-pH-tolerant bacteriophages can improve their effectiveness. The former technique has been demonstrated to be effective in *Campylobacter* bacteriophage therapy where bacteriophages were mixed with $CaCO_3$ (Loc Carrillo *et al.* 2005) prior to administration to chickens.

Genome sequences

Despite great interest in the bacteriophages that infect *Campylobacter* and ever improving technologies capable of sequencing whole genomes of bacteria and other organisms, the genomic characterization of these bacteriophages has been particularly slow. In common with the prototype bacteriophage T4 of *Escherichia coli*, *Campylobacter* bacteriophages have DNA modifications that make them difficult to clone and sequence. Molecular characterization of *Campylobacter*-specific bacteriophages is vital for continued development of their therapeutic applications to avoid the inadvertent transfer or mobilization of harmful genes (Connerton *et al.* 2008), enhanced selection procedures for appropriate bacteriophages and the quality control of bacteriophage therapy products. An important breakthrough was the publication of the sequences and genomic analysis of two *Campylobacter* group II bacteriophages with broad lytic activity against both *C. jejuni* and *C. coli* isolates (Timms *et al.* 2010). The genomes of the two bacteriophages studied were extremely similar at the nucleotide level despite the fact that they were isolated from different places and the isolations were separated by fourteen years. Both bacteriophages contained numerous copies of radical S-adenosylmethionine genes, and these were suggested to be involved in enhancing bacterial metabolism during infection. Other bacteriophage genes identified appeared to have been acquired from a wide range of bacterial species. The sequencing of members of the Group III bacteriophages, which are the most commonly encountered *Campylobacter*-specific bacteriophages, is keenly awaited.

Campylobacter bacteriophage therapy

Therapeutic application of *Campylobacter* bacteriophages to reduce *Campylobacter* numbers in poultry

Bacteriophage treatment of chickens was first reported in 2005 by Wagenaar *et al.* (2005) and Loc Carrillo *et al.* (2005). These initial experiments involved group III bacteriophages (Table 1). Wagenaar *et al.* (2005) compared the effects of both therapeutic and preventative treatment of broiler chickens, using two bacteriophages used in the bacteriophage typing scheme of Frost *et al.* (1999). A 3 $\log_{10}$ CFU g^{-1} decline in caecal counts of *C. jejuni* was observed within 48 h of bacteriophage treatment of infected chickens when compared with

non-bacteriophage-treated controls. Preventative bacteriophage treatment prior to infection with *Campylobacter* delayed but did not prevent the onset of colonization in young birds compared with controls. No adverse effects of bacteriophage treatment on the treated chickens were observed in either application.

Loc Carrillo *et al.* (2005) selected broad lytic spectrum bacteriophages from broiler chickens and administered these to birds infected with typical broiler *C. jejuni* isolates. The efficacy of different doses of the bacteriophages, administered in antacid suspension, was determined to establish the optimum dose. All the experimental bacteriophage treatments of *C. jejuni*-colonized birds resulted in the bacteriophages persisting and replicating in the chicken intestinal tract. The optimum dose for bacteriophage therapy was reported to be 7 $\log_{10}$ PFU, with the higher (9 $\log_{10}$ PFU) and lower doses (5 $\log_{10}$ PFU) of bacteriophage being generally less effective (Loc Carrillo *et al.* 2005). A possible reason for the highest dose being less effective has been postulated to be because of bacteriophage aggregation and nonspecific association with digesta or non-host bacteria (Rabinovitch *et al.* 2003). The reductions observed in the *Campylobacter* levels of the colonized birds following bacteriophage administration were between 1·5 $\log_{10}$ and 5 $\log_{10}$ CFU g^{-1} of intestinal contents compared with controls. Similar reductions in *Campylobacter* numbers were observed by Scott *et al.* (2007a) using a different group III bacteriophage.

The group II bacteriophages (Table 1) were used as bacteriophage therapy treatments by El-Shibiny *et al.* (2009) and Carvalho *et al.* (2010b). Similar results to those obtained from the use of the group III bacteriophages were obtained in terms of net reductions in *Campylobacter* numbers. However, some members of this group appear to have a broad ability to infect *Campylobacter* strains that include representatives of the *C. coli* and *C. jejuni* species. The cross-species lytic spectrum of the group II bacteriophage members is in contrast to the more commonly isolated and therefore more readily available group III bacteriophages. In practice, bacteriophage cocktails containing members of both groups probably represent the most appropriate scenario for bacteriophage therapy treatment. Future trials involving treatment of commercial birds that are naturally infected with *Campylobacter* would be the next logical step forward for this technology.

Bio-sanitisation

In addition to therapy applications, where bacteriophages are administered to the live animals, bacteriophages may be applied directly to foods such as poultry meat or onto environmental surfaces in processing facilities to reduce numbers of food-borne pathogens in foods (Sulakvelidze and Barrow 2005). As temperature and atmospheric conditions in these circumstances would prevent growth of *Campylobacter* and replication of bacteriophages, the numbers of campylobacters are reduced through passive inundation or 'lysis from without' alone. Where a large number of bacteriophages adsorb to the host bacterium, the cell wall is compromised causing the bacterium to swell and burst (Delbruck 1940). Two studies demonstrated the potential use of bacteriophages to reduce *Campylobacter* numbers on the surface of experimentally contaminated chicken skin in this way (Atterbury *et al.* 2003b; Goode *et al.* 2003). Although the reductions were relatively small 1–2 $\log_{10}$ CFU cm^{-2}, a greater reduction was achieved when the action of the bacteriophages was combined with freezing (Atterbury *et al.* 2003b).

Future applications

Bacteria in their natural environments frequently form biofilms comprised of single or multiple bacterial species attached to a surface and embedded in an extra-cellular polymeric matrix. These matrices may help bacteria to overcome environmental stresses such as aerobic conditions, desiccation, heating, disinfectants and acidic conditions and thereby increase their potential to survive. The application of bacteriophages to reduce biofilms of several different bacterial species has been demonstrated (Hibma *et al.* 1997; Hughes *et al.* 1998), and reduction of *Campylobacter* biofilms using bacteriophages has been demonstrated by Siringan and Connerton (2010). Moreover, engineered bacteriophage enzymes have been employed to disperse biofilms by breaking down components of the extra-cellular polymeric matrix (Lu and Collins 2007). Bacteriophages may therefore play an important role in the control of attachment and formation of biofilms by *Campylobacter* in situations where such biofilms occur in nature, and they have the potential for application in industrial situations leading to improvements in food safety.

Evolutionary consequences of natural bacteriophage infection

Bacteriophages influence the strains of *Campylobacter* that populate chickens

The ubiquity of campylobacters in the environment represents a paradox considering their fastidious nature, sensitivity to atmospheric oxygen and their lack of identifiable global stress response mechanisms (Murphy *et al.* 2006), limited genome size, and on top of all this, they must cope with attack by bacteriophages. To counteract

such environmental challenge, they appear to generate diversity within their populations at a genomic level. Recombination of genetic material between *C. jejuni* genotypes *in vivo* can be demonstrated to be a frequent event that gives rise to heterogenic populations (Schouls *et al.* 2003; Avrain *et al.* 2004; Fearnhead *et al.* 2005). It is this very heterogeneity that made subtyping challenging before the advent of molecular techniques. The effect of such heterogeneous populations is that some strains are better colonizers and persist in chickens for much longer periods of time than others (Gaynor *et al.* 2004; Jones *et al.* 2004; McCrea *et al.* 2006). When chickens are exposed to multiple genotypes of *Campylobacter*, one strain tends to dominate, although this dominant type may change several times particularly if the birds are exposed to multiple *Campylobacter* types over the rearing cycle (El-Shibiny *et al.* 2005, 2007). The selection of the dominant type may be influenced by the presence of bacteriophages (Connerton *et al.* 2004). This was elegantly demonstrated by Scott *et al.* (2007b) who showed that in the absence of bacteriophages, a bacteriophage-sensitive strain out competed a bacteriophage-insensitive strain to become the dominant strain. However, when bacteriophages were administered to birds co-infected with both strains, the situation was reversed, with the insensitive strain becoming dominant and the sensitive strain being reduced to a minority population. Individually the strains were equally able to colonize birds, but it was clear that the strain insensitive to the bacteriophages was associated with a competitive fitness disadvantage in the absence of bacteriophages but not in their presence. These findings have implications regarding the types of strains isolated from different sources, as the presence of bacteriophages may bias the isolation rates of different strains colonizing the same intestinal environment.

Genomic rearrangement of *Campylobacter jejuni* in response to bacteriophage predation

Examination of *Campylobacter* strains that had been subjected to experimental bacteriophage predation in chickens revealed that the selective pressure exerted by bacteriophage predation influenced the evolution of the *Campylobacter* genome. Large segments of the genome, up to 590 kb in length, were found to be inverted in some strains that had acquired the bacteriophage-insensitive phenotype, where the recombination breakpoints were associated with the presence of Mu-like prophage sequences (Scott *et al.* 2007a; see section 3·7). When these bacteriophage-insensitive strains were reintroduced into chickens without bacteriophage predation, they exhibited an increase in dose dependence, indicative of a deficiency in colonization ability. However, once colonization had been established, the campylobacters recovered from these chickens had reverted to a bacteriophage-sensitive phenotype. On examination, these isolates were found to have undergone a further round of genome rearrangement also involving the Mu-like prophage elements. These secondary reversion strains were not only bacteriophage sensitive but had also regained the ability to efficiently colonize chickens. An example of the progression of rearrangements involving the Mu-like prophage sequence is shown in Fig. 1. Such rearrangements observed in response to bacteriophage predation in the chicken intestine were not evident in cultures propagated in the laboratory.

Conclusions

It is perhaps unsurprising that in our attempts to harness the power of virulent *Campylobacter* bacteriophages, firstly as typing reagents and more recently for therapeutic reduction and bio-sanitization of poultry meat, we have learnt a great deal about the general ecology and population biology of *Campylobacter*. Bacteriophages hold not only the potential to become a powerful tool for the reduction of campylobacters in poultry but are of key importance to understanding the fundamental dynamics of *Campylobacter* populations in avian species.

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