Are bacteriophage defence and virulence two sides of the same coin in *Campylobacter jejuni*?

Rogier Louwen*[1] and Peter van Baarlen†

*Department of Medical Microbiology and Infectious Diseases, Erasmus MC, Rotterdam 3015GD, The Netherlands, and †Host–Microbe Interactomics, Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH, The Netherlands

Abstract

The continuous battle for survival in the environment has led to the development or acquisition of sophisticated defence systems in bacteria. These defence systems have contributed to the survival of the bacterial species in the environment for millions of years. Some systems appear to have evolved in a number of pathogenic bacteria towards a role in virulence and host immune evasion. Recently, different bacterial cell envelope components from diverse bacterial species have been linked not only to bacteriophage defence, but also to virulence features. In the present review we focus specifically on the bacterial cell envelope-expressed sialic-acid-containing LOS (lipo-oligosaccharide) structures and Type II CRISPR (clustered regularly interspaced short palindromic repeats)–Cas (CRISPR-associated) genes that both occur in specific Gram-negative pathogens. In *Campylobacter jejuni* circumstantial evidence points at a potential intertwined dual function between sialylated LOS structures and subtype II-C CRISPR–Cas, i.e. in phage defence and virulence. In the present review we discuss whether a dual functionality of sialylated LOS and subtype II-C CRISPR–Cas is exclusive to *C. jejuni* only or could be more widespread within the group of Type II CRISPR–Cas-harbouring bacteria. We conclude from the literature that, at least in *C. jejuni*, circumstantial evidence exists for a complex intertwined dual functionality between sialylated LOS and Type II CRISPR–Cas, and that other bacteria show similar genomic signatures.

Introduction

In the environment bacteria are continuously exposed to biotic and abiotic stressors. Bacteria can fall prey to lytic bacteriophages or can be engulfed and destroyed by amoebae [1]. Bacteria also have to deal with diverse abiotic stress stimuli, including sudden changes in temperature, e.g. during infection of host, exposure to antibiotics, UV light and drought [1]. Bacteria have evolved or acquired usually tightly regulated stress-resistance mechanisms that are important for survival in their environmental niche [1]. Some of these acquired or evolved mechanisms may provide an additional advantage when bacteria infect vertebrates [1]. For example, bacterial survival mechanisms activated upon engulfment by amoebae may also provide efficient protection to the same bacterial pathogens upon invasion of eukaryotic cells or upon phagocytosis by macrophages [2–7].

The bacterial cell envelope is one of the key structures involved in counteracting and responding to biotic [8] and environmental [9] stresses. Interestingly, results from independent studies focussing on *Azotobacter* spp., *Bordetella* spp., *Escherichia coli*, *Pseudomonas* spp., *Salmonella* spp. and *Staphylococcus aureus* appear to suggest that modification of the bacterial cell envelope as a defence mechanism against lytic bacteriophages also contribute to the virulence of these bacteria. For example Protein A, a cell-wall-anchored virulence factor of *S. aureus*, interacts with the Fc region of human IgG. Absence of Protein A was found to enhance bacteriophage binding to *S. aureus*, a strong indication that Protein A blocks a bacteriophage-binding receptor [10,11]. The lipoprotein OmpA of *E. coli* is an important receptor for many T-even-like coliphages, but it is also an important virulence factor [12,13]. When OmpA-bearing *E. coli* strains harbour a plasmid that encodes the outer membrane protein TraT, OmpA is blocked or modified, protecting *E. coli* against infection by the T-even-like coliphages [14,15]. *Pseudomonas* and *Azotobacter* species express EPSs (exopolysaccharides) that provide protection from the surrounding environment. EPSs block access to receptors that can be bound by some bacteriophages which would then lead to infection of bacteria. During this 'arms race', phages have emerged that use these sugars during their infection process, or express enzymes able to dissolve the EPSs, making receptors available again for binding, enabling bacterial infection [16–20]. Also *Salmonella* and *E. coli* species use complex EPS structures on the bacterial cell envelope to avoid binding by bacteriophages during phase variation, and the same EPS decorations can be exploited by some bacteriophages for cellular entry [21–23].

Specific environmental, envelope stress and bacterial membrane physiology have been indirectly linked to a described bacteriophage defence system, CRISPR (clustered regularly interspaced short palindromic repeats)–Cas (CRISPR-associated) [24–28]. In 2002, it was observed that in bacteria

Key words: bacteriophage, clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated (Cas), defence, lipo-oligosaccharide, sialic acid, virulence.

Abbreviations used: Cas, CRISPR-associated; CRISPR, clustered regularly interspaced short palindromic repeats; EPS, exopolysaccharide; GBS, Guillain–Barré syndrome; LOS, lipo-oligosaccharide.

[1]To whom correspondence should be addressed (email r.louwen@erasmusmc.nl).

and archaea, a repetitive element called CRISPR was usually accompanied by a set of genes [29]. A few years later it was proposed by three independent groups that the CRISPR–Cas system might be involved in bacteriophage defence, on the basis of observations that some of the repetitive DNA sequences in the CRISPR array displayed 100% sequence identity with bacteriophage protospacers [30–32]. In 2007, it was experimentally established that the Type II CRISPR–Cas system in *Streptococcus thermophilus* functioned as a bacteriophage defence system [33]. Hereafter, it became firmly established that the CRISPR–Cas system functioned in phylogenetically extremely diverse bacteria as a defence system against foreign DNA [24,34–36]. CRISPR–Cas provides defence against bacteriophages by activating a double-stranded nucleic acid degradation response upon detection of bacteriophage RNA or DNA. Recognition of phage DNA is based on identity between bacteriophage sequences and short (approximately 30 bp) DNA fragments represented by CRISPR spacers located in the bacterial genome. Clear examples of the roles CRISPR–Cas may play during a stress response have been established in the bacterial species *Myxococcus xanthus* and *E. coli* [37,38]. In *M. xanthus*, environmental stress was concomitant with the activation of the CRISPR–Cas system and bacterial sporulation [37]. In *E. coli* CRISPR–Cas was activated when *E. coli* bacteria were subjected to cell envelope stresses [38]; downstream activation was under the control of transcriptional stress regulators [39].

The CRISPR–Cas bacteriophage defence systems are divided into three main groups, which are based on the presence of group-specific genes [28]. The Type II CRISPR–Cas system, exemplified by the system originally observed in *Neisseria meningitidis*, is strongly reduced in size, compared with Type I and Type II CRISPR–Cas systems. Type II CRISPR–Cas contains two type-specific *cas* genes, *csn2* or *cas4*, in addition to the conserved *cas* genes *cas9-1-2* [28,40] that allow a further division into three subtypes [41]. Interestingly, the Type II CRISPR–Cas system seems to be overrepresented in pathogenic bacteria that are able to incorporate sialic acid into their cell envelope [40]. In the present review we discuss a potential dual role in viral defence and virulence for two bacteriophage defence systems that are together present in specific Gram-negative pathogens: cell envelope sialylation and Type II CRISPR–Cas.

Sialic-acid-mediated viral defence

The cellular membranes of vertebrates and, to a lesser extent, bacteria, fungi and plants, incorporate sugar structures that harbour the molecule *N*-acetylneuraminic acid, also known as sialic acid [42]. In humans, sialylated sugar structures are determinants of susceptibility to viruses, e.g. mumps virus [43] and influenza virus [44,45]. The interactions between influenza viruses and mammalian sialylated sugar structures have been well studied, and the insights from these studies have led to the development of drugs that are able to inhibit influenza release from infected cells by blocking the viral neuraminidase, an enzyme that cleaves terminal sialic acids enabling the discharge of new influenza viruses [45–48]. Some Gram-negative pathogenic bacteria, including *C. jejuni*, also decorate their LOS (lipo-oligosaccharide) structures with sialic acid residues, with relevance to pathogenicity [49,50].

C. jejuni, a zoonotic human pathogen, is the leading cause of diarrhoea worldwide, with 400–500 million laboratory-confirmed cases each year [51]. *C. jejuni* strains can be divided into two separate groups on the basis of the presence or absence of sialic acid on their outer surface LOS carbohydrate residues [52,53] (Figure 1). Transfer of sialic acids is mediated by the sialyltransferase Cst-II [54–56]. Sialylated *C. jejuni* isolates are highly pathogenic [49,50], cause severe colitis [57] and are associated with reactive arthritis and paralytic diseases such as GBS (Guillain–Barré syndrome) [57,58], a post-infectious life-threatening complication. GBS is suggested to be provoked by molecular mimicry between the sialylated carbohydrate LOS structures of *C. jejuni* and ganglioside epitopes expressed on human peripheral nerves, resulting in autoantibody-induced and complement-mediated nerve destruction [52]. A recent study using 19 different *C. jejuni* isolates and two (complemented) mutants competent or disabled in the ability to express ganglioside-like sialylated LOS structures revealed that the *C. jejuni* ganglioside-like LOS structures provide global protection against most of the lytic bacteriophages that use *C. jejuni* as host [59]. In this study, it was shown that sialic acid did not function as a phage receptor, but rather provided a defence mechanism that protected *C. jejuni* from bacteriophage infection [59], most probably by blocking bacteriophage-produced polysaccharide-degrading enzymes or by blocking compatible bacterial adhesion receptors [8]. Remarkably, ganglioside-like LOS structures expressed on the cell envelope provide serum resistance in humans [60] and play an important role in cellular adhesion on to, invasion into and translocation across intestinal epithelial cells [49,50]. Comparing CRISPR–Cas loci, the presence of sialyltransferase genes and corresponding LOS class and production of sialylated ganglioside-like LOS shows that, in *C. jejuni*, sialic acid decorations are associated with high resistance to bacteriophages and high virulence (Table 1).

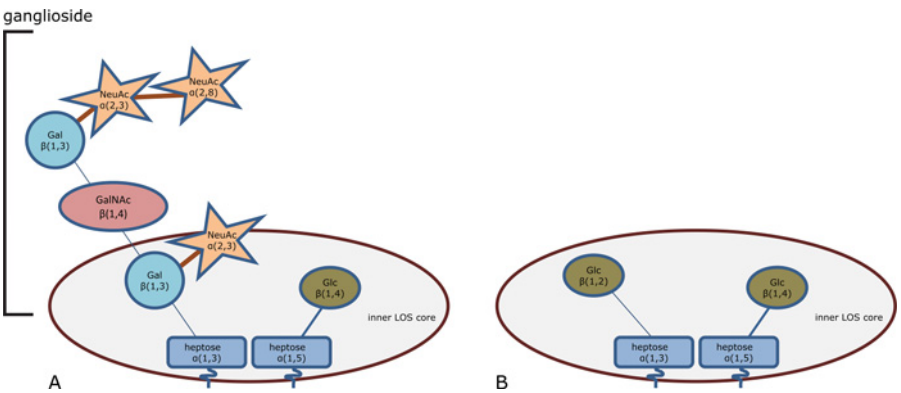
Also in *N. meningitidis*, *Neisseria gonorrhoeae*, *Helicobacter pylori*, *Haemophilus influenzae* and *Pasteurella multocida* ganglioside-like LOS structures contribute to bacterial pathogenicity [61]. As observed for *C. jejuni*, ganglioside-like LOS structures on the bacterial cell envelope of *H. influenzae* appear to play roles in bacteriophage defence and virulence [8,62]. It will be of interest to study whether sialic acid decorations are also involved in bacteriophage defence and virulence in other bacteria belonging to the Type II CRISPR–Cas group as observed for *C. jejuni* (Figure 2).

CRISPR–Cas-mediated bacteriophage defence in *C. jejuni*

Initially, the *C. jejuni* CRISPR element was used for typing purposes, but it was unknown whether or not the *C. jejuni*

Figure 1 | **The presence of sialic acid is determined by the sugar decorations on the heptose backbone**

(**A**) Addition of one glucose molecule to one of the two LOS backbone heptoses makes the addition of Gal and GalNAc residues possible; sialic acids can be added to Gal residues by the *C. jejuni* Cst-II transferase enzyme. The *C. jejuni* Gal-GalNac-NeuAc moieties are also associated with human ganglioside types GM1 or GD1 mimicry [68]; location and numbers of sialic acid residues are the main determinants of mimic type. (**B**) Addition of glucose residues to both heptose sugars appears to prevent addition of Gal residues and thus sialylation and ganglioside-like LOS expression on the *C. jejuni* cell envelope [69].



subtype II-C CRISPR–Cas system was functional as a bacteriophage defence system [63]. In the study of Louwen et al. [59] a first glimpse of a potential role of the *C. jejuni* subtype II-C CRISPR–Cas system in bacteriophage defence was provided. Remarkably, the authors observed, after incubating *cas9*-negative, *cst-II*-positive *C. jejuni* isolates with 29 lytic *C. jejuni*-infecting bacteriophages, that the CRISPR–Cas system only played a minor role in bacteriophage defence in these *C. jejuni* isolates that all expressed ganglioside-like LOS structures GM1 and/or GD1 [59]. Curiously, *cst-II*-positive *C. jejuni* isolates harboured CRISPR–Cas arrays with significantly reduced CRISPR sizes, accompanied by broad alterations in the *cas* genes, or even in one strain by complete absence of a CRISPR–Cas system [59]. The correlation between the presence of the *cst-II* gene and reduced CRISPR sizes and *cas* gene alterations was of interest, since Cst-II-generated ganglioside-like LOS structures on the *C. jejuni* cell envelope provided the strongest resistance to lytic *C. jejuni*-specific bacteriophages [59]. This suggests that in the *cst-II*-positive strains relief from selection on CRISPR–Cas defence functionality might have allowed shrinkage of the CRISPR repeat numbers and CRISPR array size. Intriguingly, loss of *cas9* in *cst-II*-positive *C. jejuni* strains did lead to near-complete loss of virulence, and by using human serum from GBS patients were shown to be more profoundly visible to the human immune system [59]. These results provide a hypothetical scenario where a combination of two different bacteriophage defence mechanisms have resulted in *C. jejuni* isolates that are not only phage-resistant, but also more virulent and linked with post-infectious complications [49,50,53,57,59].

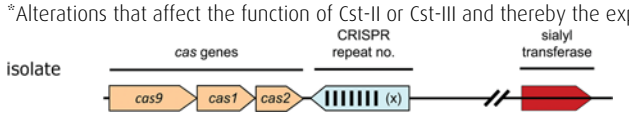
The authors from that study questioned whether the combination of ganglioside-like LOS production and progressively shorter CRISPR repeats was unique for *C. jejuni* only or whether these features could also be found in other pathogenic bacteria. Combinations of ganglioside-like LOS production and subtype II-C CRISPR–Cas have also been observed in other bacterial pathogens including *N. meningitidis* and Pasteurellaceae (*Haemophilus* spp. and *P. multocida*) [61]. Therefore it was investigated *in silico*, using the available complete genome sequences representing these pathogens, whether a link between the presence of sialyltransferases and sequence variation in the subtype II-C CRISPR–Cas loci could be identified [59]. Indeed, correlations between the presence of *cst-II* orthologues and alterations in subtype II-C CRISPR–Cas were detected in *N. meningitidis*, *Haemophilus* spp. and *P. multocida* [59] respectively.

Dual functionalities of bacteriophage defence systems: a blueprint for increased virulence?

In a mathematical model study on the ecology and evolution of CRISPR–Cas systems, Levin [64] concluded that bacteria with a CRISPR–Cas system might have an evolutionary advantage over bacteria employing other types of resistance, i.e. cell envelope resistance, in case the latter comes with a large enough fitness cost. His model also predicted that cell envelope resistance to phages might have an advantage over CRISPR–Cas, again depending on fitness of individual isolates. The outcomes of the models imply that bacteria with bacteriophage cell envelope resistance might replace CRISPR–Cas-bearing lineages if fitness differences are large enough [64]. Furthermore, it was suggested by Levin [64] that bacteria with a CRISPR–Cas system might acquire bacteriophage cell envelope resistance or vice versa, which could result in a population of bacteria with highest fitness compared with the other variants.

Table 1 | Cst-II-generated ganglioside-like LOS is associated with high resistance to bacteriophages and high virulence

Overview of the CRISPR–Cas locus organization, presence of sialyltransferase genes, LOS class, mimicry to human ganglioside types, capacity to induce GBS and an indication of the virulence and bacteriophage resistance of 20 *C. jejuni* isolates. The Figure shows the generic subtype II-C CRISPR–Cas locus organization *cas9-1-2* (9-1-2); the sialyltransferase genes *cst-II* and *cst-III* which are located at a distance of the CRISPR–Cas locus. The Table shows that specifically isolates belonging to LOS classes A and B harbour increased virulence and phage-resistance capabilities [49,50]. *Alterations that affect the function of Cst-II or Cst-III and thereby the expression of ganglioside-like LOS structures. ND, not determined.



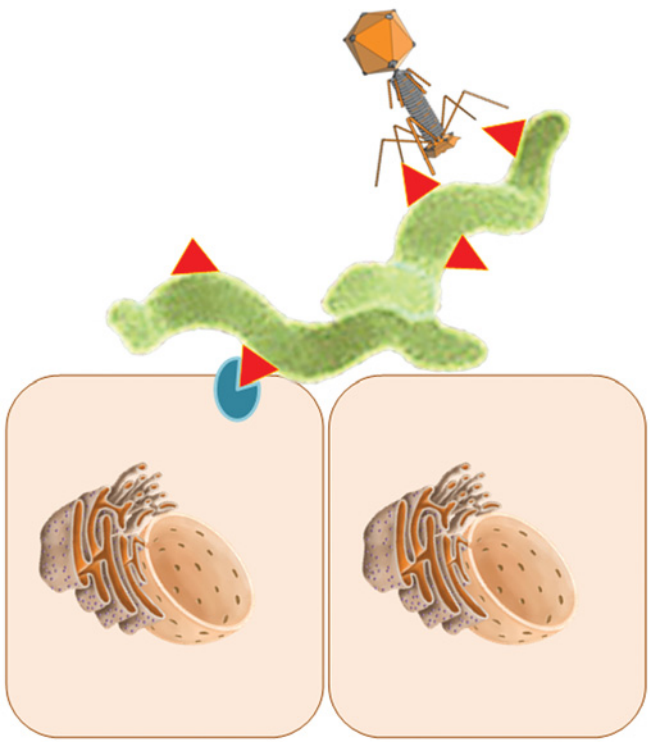
Isolate	Cas genes	Number of repeats	*cst gene*	LOS class	Mimic	GBS induction	Virulence	Phage resistance
GB2	9-1-2	2	*cst-II*	A	GM1/GD1	Yes	High	High
GB3	9-1-2	–	*cst-II*	A	GM1/GD1	Yes	Intermediate	High
GB4	9-1-2	2	–	E	–	No	Low	Intermediate
GB5	9-1-2	–	*cst-II**	B	–	Yes	Low	Low
GB11	9-1-2	–	*cst-II*	A	GM1/GD1	Yes	High	High
GB15	9-1-2	ND	–	D	–	No	Low	Intermediate
GB17	9-1-2	ND	*cst-II**	B	GA1/GM1/GD1	Yes	High	Intermediate
GB19	9-1-2	2	*cst-II*	A	GM1/GD1	Yes	High	High
GB22	9-1-2	3	*cst-II*	A	GM1/GD1	Yes	Intermediate	High
GB23	9-1-2	5	*cst-II*	A	GM2	Yes	High	Low
GB29	9-1-2	4	*cst-III**	C	–	Yes	Low	Low
11168	9-1-2	5	*cst-III*	C	GM1/GM2	No	Low	Intermediate
706	9-1-2	ND	–	D	–	No	Intermediate	Intermediate
R65	9-1-2	4	–	D	–	No	Low	Low
623	9-1-2	ND	–	E	–	No	Low	Intermediate
624	9-1-2	ND	–	E	–	No	Low	Intermediate
9141	9-1-2	ND	–	E	–	No	Intermediate	Low
9146	9-1-2	ND	–	E	–	No	Intermediate	Intermediate
260.94	9-1-2	2	*cst-II*	ND	GM1a	Yes	High	High
81176	–	–	*cst-II*	B	GM2/GM3	No	Intermediate	High

The last two outcomes predicted by the models of Levin [64] have been observed in some of the bacterial species within the subtype II-C CRISPR–Cas group. At least some isolates of *N. meningitidis*, *C. jejuni*, *Haemophilus* spp. and *P. multocida* are characterized by the presence of a CRISPR–Cas system and production of cell envelopes with ganglioside-like LOS structures. It will be of interest to measure whether, within these species, there is a shift at the population level towards an increased proportion of bacteria that harbour both a Type II CRISPR–Cas system and a cell envelope with ganglioside-like LOS structures. Phylogenetic ancestral reconstructions using coalescence theory suggested that *C. jejuni* isolates producing ganglioside-like LOS originated more recently than enteritis isolates that only used CRISPR–Cas for defence [58], suggesting that cell envelope resistance has been acquired more recently. Such analyses, performed for sufficiently large amounts of isolates sampled as unbiasedly as possible, and sampled at least annually, may show eventually whether there is a tendency in *C. jejuni* populations to shift towards increasing proportions of ganglioside-like LOS-producing isolates.

Knockout mutagenesis of the *C. jejuni* subtype II-C CRISPR–Cas marker gene *cas9* suggested that there might be a mechanism, at least partially depending on Cas9 function, that is (co-)regulating the production of ganglioside-like LOS structures in clinical *C. jejuni* isolates obtained from GBS patients [59]. A recent study showed that Cas9, together with two small non-coding RNA species, was involved in degradation of the mRNA encoding an immunogenic lipoprotein that was located at the cell envelope of *Francisella novicida* [65]. It is intriguing that at least one protein belonging to the Type II CRISPR–Cas bacteriophage defence system is involved in regulation of endogenous protein production or, as suggested by *cas9*-knockout mutagenesis experiments in *C. jejuni*, LOS production at the bacterial cell envelope. More evidence is gradually appearing that CRISPR spacers and small non-coding RNA molecules can display high sequence identity with genes encoding proteins involved in bacterial cell envelope synthesis, virulence or stress [31,41,65–67], allowing for several intriguing research questions to be posed for Type II CRISPR–Cas harbouring ganglioside-like LOS-expressing bacteria: (i) is production of ganglioside-like LOS as a means of phage defence becoming more widespread in bacteria, for instance in the genera *Neisseria*, *Campylobacter*, *Haemophilus* and *Pasteurella*?; (ii) does production of ganglioside-like LOS confer an evolutionary advantage on bacteria?; (iii) will the proportions of bacteria that harbour CRISPR–Cas and have the additional

Figure 2 | Ganglioside-like LOS is linked with increased virulence and bacteriophage resistance in *C. jejuni*

Cst-II-generated ganglioside-like LOS, depicted by a red triangle, has been shown to enhance *C. jejuni* (green) adherence on to the intestinal epithelial cell line Caco-2 (light pink). This increased adherence went together with increased numbers of *C. jejuni* bacteria that invaded into, and translocated across, a (polarized) Caco-2 cell monolayer [49,50]. Cst-II-generated ganglioside-like LOS structures (GM1 and GD1) also strongly contribute to resistance of *C. jejuni* to infection by bacteriophage (orange and brown), probably by interfering with binding of bacteriophage to the *C. jejuni* cell envelope [59].



capacity to produce ganglioside-like LOS increase at the population level?; (iv) is there further proof for co-regulated dual functionality between the two bacteriophage defence systems CRISPR–Cas and ganglioside-like LOS with respect to phage defence and virulence?; (v) is an interaction between the subtype II-C CRISPR–Cas and ganglioside-like LOS bacteriophage defence systems active during host infection including defence against host-immune response?; and (vi) are the features of LOS sialylation described also operational in LPS (lipopolysaccharide)-expressing bacteria?

Conclusion

In *C. jejuni*, production of ganglioside-like LOS and alterations in its subtype II-C CRISPR–Cas bacteriophage defence system appear to be correlated in more invasive highly pathogenic isolates. At least in *C. jejuni*, ganglioside-like LOS and subtype II-C CRISPR–Cas are involved in bacteriophage defence and virulence, suggestive of dual functionality and co-regulation of these two important bacterial processes. Evolutionary models propose that isolates combining both CRISPR–Cas and cell envelope resistance to bacteriophages might experience higher competitive fitness and proportionally increase at the population level. Additional research is required to further elucidate the regulation of production of ganglioside-like LOS at the bacterial cell envelope and the Type II CRISPR–Cas system that may well have evolved different functionalities in bacteria harbouring this system.

References

1 Erken, M., Lutz, C. and McDougald, D. (2013) The rise of pathogens: predation as a factor driving the evolution of human pathogens in the environment. Microb. Ecol. **65**, 860–868

2 Matz, C., Nouri, B., McCarter, L. and Martinez-Urtaza, J. (2011) Acquired type III secretion system determines environmental fitness of epidemic *Vibrio parahaemolyticus* in the interaction with bacterivorous protists. PLoS ONE **6**, e20275

3 Hagele, S., Kohler, R., Merkert, H., Schleicher, M., Hacker, J. and Steinert, M. (2000) *Dictyostelium discoideum*: a new host model system for intracellular pathogens of the genus *Legionella*. Cell. Microbiol. **2**, 165–171

4 Matz, C., Moreno, A.M., Alhede, M., Manefield, M., Hauser, A.R., Givskov, M. and Kjelleberg, S. (2008) *Pseudomonas aeruginosa* uses Type III secretion system to kill biofilm-associated amoebae. ISME J. **2**, 843–852

5 Wagner, M. and Horn, M. (2006) The Planctomycetes, Verrucomicrobia, Chlamydiae and sister phyla comprise a superphylum with biotechnological and medical relevance. Curr. Opin. Biotechnol. **17**, 241–249
6 Stolfa, G. and Koudelka, G.B. (2012) Entry and killing of *Tetrahymena thermophila* by bacterially produced Shiga toxin. mBio **4**, e00416–00412
7 Lainhart, W., Stolfa, G. and Koudelka, G.B. (2009) Shiga toxin as a bacterial defense against a eukaryotic predator, *Tetrahymena thermophila*. J. Bacteriol. **191**, 5116–5122
8 Labrie, S.J., Samson, J.E. and Moineau, S. (2010) Bacteriophage resistance mechanisms. Nat. Rev. Microbiol. **8**, 317–327
9 Jordan, S., Hutchings, M.I. and Mascher, T. (2008) Cell envelope stress response in Gram-positive bacteria. FEMS Microbiol. Rev. **32**, 107–146
10 Foster, T.J. (2005) Immune evasion by staphylococci. Nat. Rev. Microbiol. **3**, 948–958
11 Nordstrom, K. and Forsgren, A. (1974) Effect of Protein A on adsorption of bacteriophages to *Staphylococcus aureus*. J. Virol. **14**, 198–202
12 Nicholson, T.F., Watts, K.M. and Hunstad, D.A. (2009) OmpA of uropathogenic *Escherichia coli* promotes postinvasion pathogenesis of cystitis. Infect. Immun. **77**, 5245–5251
13 Prasadarao, N.V., Wass, C.A., Weiser, J.N., Stins, M.F., Huang, S.H. and Kim, K.S. (1996) Outer membrane protein A of *Escherichia coli* contributes to invasion of brain microvascular endothelial cells. Infect. Immun. **64**, 146–153
14 Pedruzzi, I., Rosenbusch, J.P. and Locher, K.P. (1998) Inactivation *in vitro* of the *Escherichia coli* outer membrane protein FhuA by a phage T5-encoded lipoprotein. FEMS Microbiol. Lett. **168**, 119–125
15 Riede, I. and Eschbach, M.L. (1986) Evidence that TraT interacts with OmpA of *Escherichia coli*. FEBS Lett. **205**, 241–245
16 Sutherland, I.W. (1995) Polysaccharide lyases. FEMS Microbiol. Rev. **16**, 323–347
17 Hanlon, G.W., Denyer, S.P., Olliff, C.J. and Ibrahim, L.J. (2001) Reduction in exopolysaccharide viscosity as an aid to bacteriophage penetration through *Pseudomonas aeruginosa* biofilms. Appl. Environ. Microbiol. **67**, 2746–2753
18 Castillo, F.J. and Bartell, P.F. (1974) Studies on the bacteriophage 2 receptors of *Pseudomonas aeruginosa*. J. Virol. **14**, 904–909
19 Temple, G.S., Ayling, P.D. and Wilkinson, S.G. (1986) Isolation and characterization of a lipopolysaccharide-specific bacteriophage of *Pseudomonas aeruginosa*. Microbios **45**, 81–91
20 Moll, D.M. and Vestal, J.R. (1992) Survival of microorganisms in smectite clays: implications for Martian exobiology. Icarus **98**, 233–239
21 Stirm, S. (1968) *Escherichia coli* K bacteriophages. I. Isolation and introductory characterization of five *Escherichia coli* K bacteriophages. J. Virol. **2**, 1107–1114
22 Steinbacher, S., Miller, S., Baxa, U., Weintraub, A. and Seckler, R. (1997) Interaction of *Salmonella* phage P22 with its O-antigen receptor studied by X-ray crystallography. Biol. Chem. **378**, 337–343
23 Perry, L.L., SanMiguel, P., Minocha, U., Terekhov, A.I., Shroyer, M.L., Farris, L.A., Bright, N., Reuhs, B.L. and Applegate, B.M. (2009) Sequence analysis of *Escherichia coli* O157:H7 bacteriophage PhiV10 and identification of a phage-encoded immunity protein that modifies the O157 antigen. FEMS Microbiol. Lett. **292**, 182–186
24 Barrangou, R. (2013) CRISPR-Cas systems and RNA-guided interference. Wiley Interdiscip. Rev.: RNA **4**, 267–278
25 Barrangou, R. and Horvath, P. (2012) CRISPR: new horizons in phage resistance and strain identification. Annu. Rev. Food Sci. Technol. **3**, 143–162
26 Koonin, E.V. and Makarova, K.S. (2009) CRISPR-Cas: an adaptive immunity system in prokaryotes. F1000 Biol. Rep. **1**, 95
27 Sorek, R., Kunin, V. and Hugenholtz, P. (2008) CRISPR: a widespread system that provides acquired resistance against phages in bacteria and archaea. Nat. Rev. Microbiol. **6**, 181–186
28 Makarova, K.S., Haft, D.H., Barrangou, R., Brouns, S.J., Charpentier, E., Horvath, P., Moineau, S., Mojica, F.J., Wolf, Y.I., Yakunin, A.F. et al. (2011) Evolution and classification of the CRISPR-Cas systems. Nat. Rev. Microbiol. **9**, 467–477
29 Jansen, R., Embden, J.D., Gaastra, W. and Schouls, L.M. (2002) Identification of genes that are associated with DNA repeats in prokaryotes. Mol. Microbiol. **43**, 1565–1575
30 Bolotin, A., Quinquis, B., Sorokin, A. and Ehrlich, S.D. (2005) Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology **151**, 2551–2561
31 Mojica, F.J., Diez-Villasenor, C., Garcia-Martinez, J. and Soria, E. (2005) Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J. Mol. Evol. **60**, 174–182
32 Pourcel, C., Salvignol, G. and Vergnaud, G. (2005) CRISPR elements in *Yersinia pestis* acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. Microbiology **151**, 653–663
33 Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D.A. and Horvath, P. (2007) CRISPR provides acquired resistance against viruses in prokaryotes. Science **315**, 1709–1712
34 Barrangou, R. and van der Oost, J. (2013) CRISPR-Cas Systems: RNA-Mediated Adaptive Immunity in Bacteria and Archaea, Springer, Berlin
35 Horvath, P. and Barrangou, R. (2010) CRISPR-Cas, the immune system of bacteria and archaea. Science **327**, 167–170
36 Karginov, F.V. and Hannon, G.J. (2010) The CRISPR system: small RNA-guided defense in bacteria and archaea. Mol. Cell **37**, 7–19
37 Viswanathan, P., Murphy, K., Julien, B., Garza, A.G. and Kroos, L. (2007) Regulation of *dev*, an operon that includes genes essential for *Myxococcus xanthus* development and CRISPR-associated genes and repeats. J. Bacteriol. **189**, 3738–3750
38 Perez-Rodriguez, R., Haitjema, C., Huang, Q., Nam, K.H., Bernardis, S., Ke, A. and DeLisa, M.P. (2011) Envelope stress is a trigger of CRISPR RNA-mediated DNA silencing in *Escherichia coli*. Mol. Microbiol. **79**, 584–599
39 Westra, E.R., Pul, U., Heidrich, N., Jore, M.M., Lundgren, M., Stratmann, T., Wurm, R., Raine, A., Mescher, M., Van Heereveld, L. et al. (2010) H-NS-mediated repression of CRISPR-based immunity in *Escherichia coli* K12 can be relieved by the transcription activator LeuO. Mol. Microbiol. **77**, 1380–1393
40 Haft, D.H., Selengut, J., Mongodin, E.F. and Nelson, K.E. (2005) A guild of 45 CRISPR-associated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes. PLoS Comput. Biol. **1**, e60
41 Chylinski, K., Le Rhun, A. and Charpentier, E. (2013) The tracrRNA and Cas9 families of type II CRISPR–Cas immunity systems. RNA Biol. **10**, 726–737
42 Angata, T. and Varki, A. (2002) Chemical diversity in the sialic acids and related α-keto acids: an evolutionary perspective. Chem. Rev. **102**, 439–469
43 Villar, E. and Barroso, I.M. (2006) Role of sialic acid-containing molecules in paramyxovirus entry into the host cell: a minireview. Glycoconjugate J. **23**, 5–17
44 Bukrinskaya, A.G., Petrova, I.G., Podchernyaeva, R., Prokazova, N.V., Zvezdina, N.D. and Bergelson, L.D. (1989) Gangliosides influence experimental influenza virus infection in mice. J. Gen. Virol. **70**, 2433–2437
45 Weis, W., Brown, J.H., Cusack, S., Paulson, J.C., Skehel, J.J. and Wiley, D.C. (1988) Structure of the influenza virus haemagglutinin complexed with its receptor, sialic acid. Nature **333**, 426–431
46 Nobusawa, E., Ishihara, H., Morishita, T., Sato, K. and Nakajima, K. (2000) Change in receptor-binding specificity of recent human influenza A viruses (H3N2): a single amino acid change in hemagglutinin altered its recognition of sialyloligosaccharides. Virology **278**, 587–596
47 Thompson, C.I., Barclay, W.S. and Zambon, M.C. (2004) Changes in *in vitro* susceptibility of influenza A H3N2 viruses to a neuraminidase inhibitor drug during evolution in the human host. J. Antimicrob. Chemother. **53**, 759–765
48 Viswanathan, K., Koh, X., Chandrasekaran, A., Pappas, C., Raman, R., Srinivasan, A., Shriver, Z., Tumpey, T.M. and Sasisekharan, R. (2010) Determinants of glycan receptor specificity of H2N2 influenza A virus hemagglutinin. PLoS ONE **5**, e13768
49 Louwen, R., Heikema, A., van Belkum, A., Ott, A., Gilbert, M., Ang, W., Endtz, H.P., Bergman, M.P. and Nieuwenhuis, E.E. (2008) The sialylated lipooligosaccharide outer core in *Campylobacter jejuni* is an important determinant for epithelial cell invasion. Infect. Immun. **76**, 4431–4438
50 Louwen, R., Nieuwenhuis, E.E., van Marrewijk, L., Horst-Kreft, D., de Ruiter, L., Heikema, A.P., van Wamel, W.J., Wagenaar, J.A., Endtz, H.P., Samsom, J. et al. (2012) *Campylobacter jejuni* translocation across intestinal epithelial cells is facilitated by ganglioside-like lipooligosaccharide structures. Infect. Immun. **80**, 3307–3318
51 Ruiz-Palacios, G.M. (2007) The health burden of *Campylobacter* infection and the impact of antimicrobial resistance: playing chicken. Clin. Infect. Dis. **44**, 701–703

52 Godschalk, P.C., Heikema, A.P., Gilbert, M., Komagamine, T., Ang, C.W., Glerum, J., Brochu, D., Li, J., Yuki, N., Jacobs, B.C. et al. (2004) The crucial role of *Campylobacter jejuni* genes in anti-ganglioside antibody induction in Guillain–Barré syndrome. J. Clin. Invest. **114**, 1659–1665
53 van Belkum, A., van den Braak, N., Godschalk, P., Ang, W., Jacobs, B., Gilbert, M., Wakarchuk, W., Verbrugh, H. and Endtz, H. (2001) A *Campylobacter jejuni* gene associated with immune-mediated neuropathy. Nat. Med. **7**, 752–753
54 Cheng, J., Yu, H., Lau, K., Huang, S., Chokhawala, H.A., Li, Y., Tiwari, V.K. and Chen, X. (2008) Multifunctionality of *Campylobacter jejuni* sialyltransferase Cst-II: characterization of GD3/GT3 oligosaccharide synthase, GD3 oligosaccharide sialidase, and trans-sialidase activities. Glycobiology **18**, 686–697
55 Gilbert, M., Brisson, J.R., Karwaski, M.F., Michniewicz, J., Cunningham, A.M., Wu, Y., Young, N.M. and Wakarchuk, W.W. (2000) Biosynthesis of ganglioside mimics in *Campylobacter jejuni* OH4384. Identification of the glycosyltransferase genes, enzymatic synthesis of model compounds, and characterization of nanomole amounts by 600-MHz ^{1}H and ^{13}C NMR analysis. J. Biol. Chem. **275**, 3896–3906
56 Godschalk, P.C., Kuijf, M.L., Li, J., St Michael, F., Ang, C.W., Jacobs, B.C., Karwaski, M.F., Brochu, D., Moterassed, A., Endtz, H.P. et al. (2007) Structural characterization of *Campylobacter jejuni* lipooligosaccharide outer cores associated with Guillain–Barré and Miller Fisher syndromes. Infect. Immun. **75**, 1245–1254
57 Mortensen, N.P., Kuijf, M.L., Ang, C.W., Schiellerup, P., Krogfelt, K.A., Jacobs, B.C., van Belkum, A., Endtz, H.P. and Bergman, M.P. (2009) Sialylation of *Campylobacter jejuni* lipooligosaccharides is associated with severe gastro-enteritis and reactive arthritis. Microbes Infect. **11**, 988–994
58 van Doorn, P.A., Ruts, L. and Jacobs, B.C. (2008) Clinical features, pathogenesis, and treatment of Guillain–Barré syndrome. Lancet Neurol. **7**, 939–950
59 Louwen, R., Horst-Kreft, D., de Boer, A.G., van der Graaf, L., de Knegt, G., Hamersma, M., Heikema, A.P., Timms, A.R., Jacobs, B.C. et al. (2013) A novel link between *Campylobacter jejuni* bacteriophage defence, virulence and Guillain–Barré syndrome. Eur. J. Clin. Microbiol. Infect. Dis. **32**, 207–226
60 Louwen, R., van Baarlen, P., van Vliet, A.H.M, van Belkum, A., Hays, J.P. and Endtz, H.P. (2012) *Campylobacter* bacteremia: a rare and under-reported event? Eur. J. Microbiol. Immunol. **2**, 76–87
61 Severi, E., Hood, D.W. and Thomas, G.H. (2007) Sialic acid utilization by bacterial pathogens. Microbiology **153**, 2817–2822
62 Zaleski, P., Wojciechowski, M. and Piekarowicz, A. (2005) The role of Dam methylation in phase variation of *Haemophilus influenzae* genes involved in defence against phage infection. Microbiology **151**, 3361–3369
63 Schouls, L.M., Reulen, S., Duim, B., Wagenaar, J.A., Willems, R.J., Dingle, K.E., Colles, F.M. and Van Embden, J.D. (2003) Comparative genotyping of *Campylobacter jejuni* by amplified fragment length polymorphism, multilocus sequence typing, and short repeat sequencing: strain diversity, host range, and recombination. J. Clin. Microbiol. **41**, 15–26
64 Levin, B.R. (2010) Nasty viruses, costly plasmids, population dynamics, and the conditions for establishing and maintaining CRISPR-mediated adaptive immunity in bacteria. PLoS Genet. **6**, e1001171
65 Sampson, T.R., Saroj, S.D., Llewellyn, A.C., Tzeng, Y.L. and Weiss, D.S. (2013) A CRISPR-Cas system mediates bacterial innate immune evasion and virulence. Nature **497**, 254–257
66 Zhang, Q., Rho, M., Tang, H., Doak, T.G. and Ye, Y. (2013) CRISPR-Cas systems target a diverse collection of invasive mobile genetic elements in human microbiomes. Genome Biol. **14**, R40
67 Hoe, C.H., Raabe, C.A., Rozhdestvensky, T.S. and Tang, T.H. (2013) Bacterial sRNAs: regulation in stress. Int. J. Med. Microbiol. **303**, 217–229
68 Ang, C.W., Jacobs, B.C. and Laman, J.D. (2004) The Guillain–Barré syndrome: a true case of molecular mimicry. Trends Immunol. **25**, 61–66
69 Gilbert, M., Parker, C.T. and Moran, A.P. (2008) *Campylobacter jejuni* lipooligosaccharides: structures and biosynthesis. In Campylobacter (Nachamkin, I., Szymanski, C.M. and Blaser, M.J., eds), pp. 493–504, ASM Press, Washington

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