Electronic Journal of Biotechnology ISSN: 0717-3458
Vol.10 No.4, Issue of October 15, 2007

Received November 13, 2006 / Accepted July 9, 2007
DOI: 10.2225/vol10-issue4-fulltext-14
RESEARCH ARTICLE

Phage-resistance of *Salmonella enterica* serovar Enteritidis and pathogenesis in *Caenorhabditis elegans* is mediated by the lipopolysaccharide

Javier Santander
Center for Infectious Diseases and Vaccinology
Biodesign Institute
Arizona State University
1001 South McAllister Av. Tempe
AZ 85287-5401, USA
Tel: 1 480 727 0465
E-mail: jasantanderm@asu.edu

James Robeson*
Laboratorio de Bacteriología
Instituto de Biología
Pontificia Universidad Católica de Valparaíso
Av. Brasil 2950, Valparaíso, Chile
Tel: 56 32 227 3118
E-mail: jrobeson@ucv.cl

Financial support: Vice-Rectoria de Investigación y Estudios Avanzados, Pontificia Universidad Católica de Valparaíso.

Keywords: animal model, bacteriophage prophylaxis, *Caenorhabditis elegans*, phage resistance, *Salmonella*.

Abbreviations: LPS: lipopolysaccharide
O-PS: O-polysaccharide
Pla: virulence plasmid
SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Phage therapy has been used in the past as an alternative therapy against bacterial pathogens. However, phage-resistant bacterial strains can emerge. Some studies show that these phage-resistant strains are avirulent. In this study, we report that phage-resistant strains of *Salmonella enterica* serovar Enteritidis (hereafter *S.* Enteritidis) were avirulent in the *Caenorhabditis elegans* animal model. We isolated phage-resistant strains of S. Enteritidis ATCC 13076 by using three lytic phages (*f2α*SE, *f3α*SE and *f18α*SE). In these mutants, we explored different virulence factors like lipopolysaccharide (LPS), virulence plasmid (Pla), motility and type I fimbriae, all of which may have effects on virulence and could furthermore be related to phage resistance. The phage-resistant strains of *S.* Enteritidis showed loss of O-Polysaccharide (O-PS) and auto-agglutination, present a rough phenotype and consequently they are avirulent in the *C. elegans* animal model. We speculate that the O-PS is necessary for phage attachment to the *S.* Enteritidis cell surface.

Salmonella enterica serovar Enteritidis (hereafter *S.* Enteritidis) causes gastrointestinal disease in humans and is a major public health concern due to its ability to be transmitted via contaminated eggs or egg based and poultry meat products (Dominguez et al. 2002). An approach to the control of *S.* Enteritidis is the use of bacteriophages, which have proven of value in the curtailment of *S.* Enteritidis infection in Cheddar cheese (Modi et al. 2001), vegetables (Leverentz et al. 2001), poultry products (Higgins et al. 2005) and the skin of chickens (Goode et al. 2003). In this latter context, we have recently isolated and described three dsDNA phages that lyse *S.* Enteritidis *in vitro* and additional variants of these phages that lyse *S.* Pullorum (Santander and Robeson, 2002). These phages have a morphology similar to bacteriophage λ of *Escherichia coli*. Furthermore, they form clear plaques in lawns of the *Salmonella* serovars mentioned above (Santander and Robeson, 2002). In addition, we tested these phages in phage prophylaxis assays in *C. elegans* (Santander and Robeson, 2004). All phages tested (*f2α*SE, *f3α*SE and *f18α*SE) protect *C. elegans* from infection and subsequent death by *S.* Enteritidis. We also reported that *S.* Pullorum was able to infect and kill *C. elegans* and that *f3α*SP, a variant of *f3α*SE adapted to *S. enterica* serovar Pullorum, protects *C. elegans* from *S. enterica* serovar Pullorum killing (Santander and Robeson, 2004). Furthermore, recent studies showed that bacteriophage *f3α*SE persists in the avian system (Krüger et al. 2003) and reduces the colonization by *S.* Enteritidis in chicks (Borie et al. 2004) in a similar vein to the report of Fiorentin et al. (2005).

*Corresponding author

Table 1. *Salmonella* strains and relevant characteristics.

Strains	Relevant characteristics	Reference
S. Enteritidis 13076	Wild-type; Lac-; D_1 ($O_{1,9,12}$); H_m; Mot^-; prototrophic; Pla^+	ATCC
S. Enteritidis *f2α*SE^r	Lac^-; auto-agglutinable; Mot^-; prototrophic; Pla^+; *f2*r *f3*^{r} *f18*^{r}	This study
S. Enteritidis *f3α*SE^r	Lac^-; auto-agglutinable; Mot^-; prototrophic; Pla^+; *f2*r *f3*^{r} *f18*^{r}	This study
S. Enteritidis *f18α*SE^r	Lac^-; auto-agglutinable; Mot^-; prototrophic; Pla^+; *f2*r *f3*^{r} *f18*^{r}	This study
S. Enteritidis *f2f3f18α*SE^r	Lac^-; auto-agglutinable; Mot^-; prototrophic; Pla^+; *f2*r *f3*^{r} *f18*^{r}	This study
S. Typhimurium SL1344	Wild-type; Lac^-; B_1; H_i; Mot^+; Pla^+; His^-	Aballay et al. 2000
S. Enteritidis PT4	Phage type 4; Lac^-	ISP, Chile
E. coli OP50	Sm^r; Pen^r; Neo^r; Lac^+	Aballay et al. 2000
E. coli 39R681	Contains four Plasmids of 98, 42, 23.9 and 4.6 MDa; Lac^+	Mainil et al. 1992

His^-: Histidine auxotroph.
f^r: phage resistant.
Lac^-: Fermentation of lactose negative.
Mot^-: Motility negative.
Neo^r: Neomycin resistant.
Pen^r: Penicillin resistant.
Pla^+: virulence plasmid positive.
Sm^r: Streptomycin resistant.

However, phage resistant strains could emerge and persist. Studies on phage therapy against *Pseudomonas pecoglossida*, a fish pathogen, showed that the phage-resistant bacterial strains lost their virulence (Park et al. 2000). Based on this observation we isolated phage-resistant strains of *S.* Enteritidis and used them to challenge *C. elegans*. We determined that the phage-resistant strains of *S.* Enteritidis were avirulent in the *C. elegans* model and therefore reasoned that phage resistance and virulence towards *C. elegans* should be related. Other bacteriophages of *Salmonella*, as P22, have their attachment site at the lypopolysaccharide (LPS) (Steinbacher et al. 1997). Furthermore, strains of *S.* Enteritidis such as PT7 do not express the long-chain LPS, show auto-agglutination and they are less virulent than *S.* Enteritidis PT4 in Balb/C mice (Chart et al. 1989). Thus, the loss of virulence in our *S.* Enteritidis phage-resistant strains could be due to a change in the LPS molecule. We determined that the strains resistant to *f2α*SE, *f3α*SE and *f18α*SE lost the O-Polysaccharide (O-PS) and show auto-agglutination; consequently, they are unable to kill *C. elegans*. We assume that the O-PS is involved in phage attachment during early infection by these phages in *S.* Enteritidis.

MATERIALS AND METHODS

Salmonella strains, bacteriophages and conditions of culture

Salmonella strains used in this study are listed in Table 1. Strains were routinely cultured at 37ºC in LB medium (Bacto Tryptone, 10 g/liter; Bacto Yeast extract, 5 g/liter; NaCl 5 g/liter) (Sambrook and Russell, 2001) or Nutrient broth (Difco). Media was solidified with 1.5% (wt/vol) agar. When required, the medium was supplemented with streptomycin (Sm; 25 µg/ml), mannose (mann 0.5% wt/vol) or galactose (gal 1.0% wt/vol). Phages used in this study are listed in Table 2. Liquid lysates in LB broth (10^9 - 10^{10} pfu/ml) were propagated in *S.* Enteritidis ATCC 13076 as a host, using pump-aerated cultures at 37ºC (Santander and Robeson, 2002). The final phage suspension was treated with chlorophorm (5 µl/ml), titrated and kept at 4ºC.

Isolation of phage-resistant strains of *S.* Enteritidis

An exponential culture of *S.* Enteritidis (2 x 10^8 cfu/ml) was infected with individual strains of phages and a mixture of them. All the infections were made at a multiplicity of infection (MOI) of 1. The mixtures were incubated at 37ºC for 1 hr and plated onto LB agar. The resistant colonies obtained were reisolated. Green indicator plates were used to confirm that the phage resistant strains were phage-free (Provence and Curtiss, 1994). The resistant strains were tested for phage resistance stability and susceptibility to the other phages.

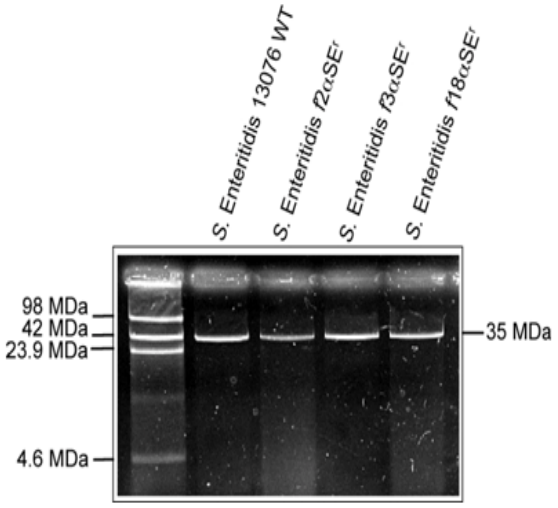

Figure 1. Plasmid profile in agarose (0.5%) gel electrophoresis. 35 MDa correspond to the size of *S.* Enteritidis Pla. *E. coli* 39R681 plasmid profile was used as molecular marker (Mainil et al. 1992).

Table 2. Bacteriophage strains.

Bacteriophages	Relevant characteristics	Reference or source
*f*2*α*SE	Wild type; dsDNA	Santander and Robeson, 2002
*f*3*α*SE	Wild type; dsDNA	Santander and Robeson, 2002
*f*18*α*SE	Wild type; dsDNA	Santander and Robeson, 2002
PT1	Wild type	ISP, Chile
PT2	Wild type	ISP, Chile
PT3	Wild type	ISP, Chile
PT4	Wild type	ISP, Chile
PT5	Wild type	ISP, Chile
PT6	Wild type	ISP, Chile
PT7	Wild type	ISP, Chile
PT8	Wild type	ISP, Chile
PT9	Wild type	ISP, Chile
PT10	Wild type	ISP, Chile

Characterization of the phage-resistant strains of *S.* Enteritidis

The *S.* Enteritidis strains were characterized for type I fimbriae in static broth cultures (Leathart and Gally, 1998) and motility in motility medium (bioMérieux, Marcy I'Etoile, France). LPS presence was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by silver staining (Hitchcock and Brown, 1983). The presence of O-PS was corroborated by Western blot analysis (Harlow and Lane, 1999) of D_1 ($O_{1,9,12}$) using rabbit D_1 polyclonal (1:5000) (Difco) as primary antibody, and alkaline phosphatase-conjugated anti-rabbit IgG (1:10000) (Sigma) as secondary antibody. Plasmid profiles were verified by alkaline lysis and agarose gel (0.5%) electrophoresis (Sambrook and Russell, 2001). Fermentation patterns of various carbohydrates and production of H_2S were determined by using the API 20E system (bioMérieux, Marcy I'Etoile, France). The *S.* Enteritidis strains gave API 20E code number 670455257 (good identification as *Salmonella* spp. 89.0%). Phage typing characterization was done by the macroplaque assay using the ten traditional typing phages (Ward et al. 1987). Agglutination tests were performed on glass microscope slides by mixing 50 µl of antisera against D_1 ($O_{1,9,12}$) and H_m (Difco Laboratories, Detroit, MI) with suspensions of fresh single colonies. Reactions were visualized by phase-contrast microscopy at 10X magnification.

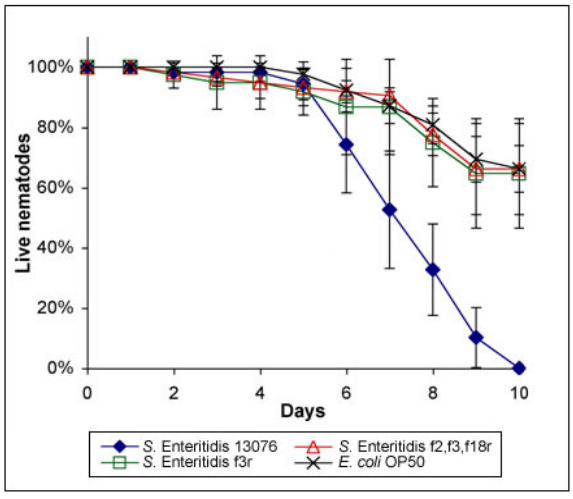

Figure 2. *C. elegans* death assay. Avirulent behavior of *S.* Enteritidis phage resistant mutants in *C. elegans*. Deviations shown correspond to three independent experiments.

C. elegans maintenance

The nematode *C. elegans* Bristol N2 was maintained in modified NG agar (0.35% bacto peptone) and fed with 200 µl of a fresh culture of *Escherichia coli* OP50 per plate (Hope, 1999).

C. elegans death assay

We used the method described by Aballay et al. (2000), without transfers (Santander and Robeson, 2004). Nematodes were incubated at 25ºC for 10 days. Dead nematode counts were performed every 24 hrs eliminating dead specimens from the plate. Thus, we determined the TD_{50} (Time it takes for 50% of the nematodes to die).

RESULTS AND DISCUSSION

Recently we determined that *S.* Enteritidis and *S.* Pullorum kill *C. elegans* (Santander and Robeson, 2004). Furthermore, we used the *C. elegans* model to evaluate protection due to phage prophylaxis against these *Salmonella* strains (Santander and Robeson, 2004), using three lytic phages isolated and characterized before (Santander and Robeson, 2002).

Studies with *P. pecoglossida* had shown that phage resistant strains become avirulent for their fish host (Park et al. 2000), suggesting that the phage attachment site at the bacterial cell surface could be related to pathogenicity. We thought a similar situation could apply to *S.* Enteritidis. To test this hypothesis we isolated *S.* Enteritidis strains

Table 3. Phage typing of *S.* Enteritidis phage-resistant strains.

Strain	Bacteriophages									
	PT1	PT2	PT3	PT4	PT5	PT6	PT7	PT8	PT9	PT10
S. Enteritidis 13076	-	-	Cl	-	Cl	+	++	Cl	-	Cl
S. Enteritidis *f2*^{r}	-	-	-	-	-	-	-	-	-	-
S. Enteritidis *f3*^{r}	-	-	-	-	-	-	-	-	-	-
S. Enteritidis *f18*^{r}	-	-	-	-	-	-	-	-	-	-
S. Enteritidis *f2f3f18*^{r}	-	-	-	-	-	-	-	-	-	-

Cl: lytic halo.
++: 21-80 plaques.
+: 1-20 plaques.
-: no reaction.

resistant to *f2α*SE, *f3α*SE and *f18α*SE and to a mixture of all three phages. We found that all *S.* Enteritidis phage-resistant strains that we isolated were phage-free. These strains were stably resistant to the corresponding phage and were also resistant to the other phages. These results suggested that the attachment site at the surface of *S.* Enteritidis is the same for the three phages assayed. In addition, the phage resistant strains were phage-typed with the ten traditional phages used for phage-typing *S.* Enteritidis (Ward et al. 1987) and were shown to be resistant to all the phages utilized in the phage-typing test (Table 3), suggesting a common attachment mechanism for all these typing phages as well. Furthermore, *S.* Enteritidis PT7, a derivative of S. Enteritidis PT4 which is unable to express the long-chain LPS (Chart et al. 1989), is resistant to *f2α*SE, *f3α*SE and *f*18*α*SE (Santander and Robeson, 2002). However, *S.* Enteritidis PT7 has a different phage-type profile in comparison with our *S.* Enteritidis 13076 phage-resistant mutants (Table 3), perhaps because some of the traditional typing phages do not interact with the O-PS as *f2α*SE, *f3α*SE and *f18α*SE apparently do during phage infection.

In the context of phenotypic variation in *Salmonella* we were aware that conversions in phenotype are associated to acquisition or loss of either a temperate phage or a plasmid in *S.* Enteritidis (Baggesen et al. 1997; Gregorova et al. 2002). Therefore, we thought that phage-resistance in our *S.* Enteritidis 13076 isolates could be connected to such phenomena. However, our phage resistant strains preserve their plasmid content (Figure 1) and were tested to be phage-free using green agar plates. These results indicate that our phage-resistant derivatives are mutants obtained by phage-mediated selective pressure against the wild type strain with a complete O-PS.

Infection experiments with *C. elegans* showed that the *S.* Enteritidis phage-resistant strains were unable to kill the worm in contrast to the wild-type strain (Figure 2). Both a *f3α*SE resistant strain and a strain isolated as a triple phage-resistant mutant, were avirulent in the *C. elegans* assay (Figure 2). Since our data indicated that phage resistance and loss of virulence were related, we characterized the main virulence factors of phage-resistant strains, which were likely to change. All the phage resistant strains presented phenotypic markers similar to the wild-type except that the phage-resistant strains showed auto-agglutination, which made impossible the detection of somatic and flagellar antigens (Table 1). However, these strains were non-motile and have the virulence plasmid (Pla), an important virulence factor in *Salmonella* (Rychlik et al. 2006; Figure 1). The auto-agglutination test indicated that the LPS had changed. The LPS profiles showed a notable difference between the phage-resistant strains and the wild-type, as expected (Figure 3a). Therefore, the phage-resistant strains of *S.* Enteritidis 13076 presented a rough phenotype. Western blot analysis also showed that the *S.* Enteritidis phage resistant strains had lost the O-PS (Figure 3b). These results indicated that motility may not be required by *S.* Enteritidis to kill the worm and that the O-PS is an important virulence factor for the infection and subsequent killing of *C. elegans*.

It is known that *S.* Typhimurium requires an intact O-PS to trigger programmed cell death in *C. elegans* and consequently kill the worm (Aballay et al. 2001; Aballay et al. 2003). This last study is in agreement with our observations that the *S.* Enteritidis phage-resistant mutants with an incomplete O-PS (Figure 3) fail to kill *C. elegans* (Figure 2).

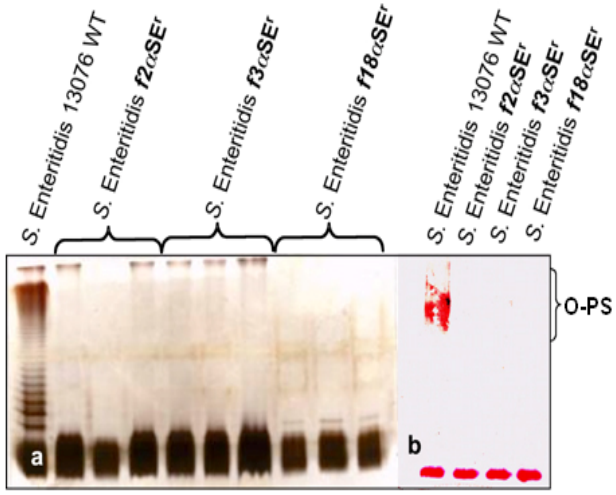

Figure 3. (a) LPS profile in SDS-PAGE (10%). *S.* Enteritidis ATCC 13076 WT and phage resistant variants, which show a rough phenotype; (b) Western blot analysis for detection of O-PS ($O_{1,9,12}$; 1:5000) in *S.* Enteritidis phage-resistant strains.

We then sought to investigate further the nature of the genetic defect that led to the rough phenotype of the *S.* Enteritidis phage-resistant mutants. It is known that *manE* (or *pmi*) *Salmonella* mutants have a defect in phospho-mannose isomerase which cannot convert, mannose into mannose-6P and consequently, in the absence of mannose their O-PS is incomplete (Collins et al. 1991). Also, there are mutants in the *galE* gene encoding UDP-galactose-4-epimerase which converts UDP-glucose in UDP-galactose, leading to the synthesis of the LPS O-antigen side chain and core. Therefore, in the absence of galactose, *galE* mutants become rough. However, addition of small amounts of galactose to the growth medium of these mutants results in production of sufficient UDP galactose to restore LPS production; but if *galE* mutants are fed excess galactose they accumulate UDP galactose, which is toxic to the cell (Adhya, 1996). To test whether any of these phenotypic changes had occurred in the *S.* Enteritidis phage-resistant mutants, we grew these strains in nutrient broth, which in contrast to LB broth does not have galactose or mannose (data not shown). We found that the strains grew in the presence and absence of both mannose and galactose and that these sugars did not have any effect on the LPS profile (data no shown). These facts indicate that defects in the *galE* (or the *galK* or *galU* related genes) and the *manE* gene are not the cause of the incomplete O-LPS chain.

These results collectively imply that *S.* Enteritidis avirulence in *C. elegans* and phage-resistance are related. Consequently, in connection with phage therapy or prophylaxis, use of the bacterial viruses referred to in this study should not be hampered by the eventual emergence of phage-resistant mutants, since they become avirulent. However, should they remain a sanitary problem it is always possible to search for additional phages to which they may serve as hosts and wherein such phages may presumably target outer membrane proteins.

ACKNOWLEDGMENTS

We are particularly grateful to Dr. Roy Curtiss III (Arizona State University, Biodesign Institute) for providing his lab facilities to perform some of our experiments and Dr. Melha Mellata (Arizona State University, Biodesign Institute) for her criticism and assistance in determining large plasmid profiles.

REFERENCES

ABALLAY, Alejandro; YORGEY, Peter and AUSUBEL, Frederick M. *Salmonella typhimurium* proliferates and establishes a persistent infection in the intestine of *Caenorhabditis elegans*. *Current Biology*, November 2000, vol. 10, no. 23, p. 1539-1542.

ABALLAY, Alejandro and AUSUBEL, Frederick M. Programmed cell death mediated by *ced-3* and *ced-4* protects *Caenorhabditis elegans* from *Salmonella typhimurium*-mediated killing. *Proceeding of the National Academy of Sciences of the United States of America*, February 2001, vol. 98, no. 5, p. 2735-2739.

ABALLAY, Alejandro; DRENKARD, Eliane; HILBUN, Layla R. and AUSUBEL, Frederick M. *Caenorhabditis elegans* innate immune response triggered by *Salmonella enterica* requires intact LPS and is mediated by a MAPK signaling pathway. *Current Biology*, January 2003, vol. 13, no. 1, p. 47-52.

ADHYA, Sankar. The galactose operon. In: NEIDHARDT, Frederick C.; INGRAHAM, John L.; LOW, K. Brooks; MAGASANIK, Boris; SCHAECHTER, Moselio and UMBARGER, H. Edwin eds. *Escherichia coli and Salmonella typhimurium: cellular and molecular biology*. 2nd ed, American Society for Microbiology, Washington, D.C. 1996, vol. 2, p. 1503-1512.

BAGGESEN, D.L.; WEGENER, H.C. and MADSEN, M. Correlation of conversion of *Salmonella enterica* serovar *enteritidis* phage type 1, 4, or 6 to phage type 7 with loss of lipopolysaccharide. *Journal of Clinical Microbiology*, January 1997, vol. 35, no. 1, p. 330-333.

BORIE, C.; ZURITA, P.; SanTander, J.; KRÜEGER, E.; SÁNCHEZ, M.L.; RAMÍREZ, S. and ROBESON, J. Effect of the bacteriophage *f3α*SE in *S. eneteritidis* colonization in avian model. In: *XIX Panamerican congress of Veterinary Sciences* (24th - 28th October 2004, Buenos Aires, Argentina). Abstracts 2004, p. 23.

CHART, Henrik; ROW, Bernard; THRELFALL, E. John and WARD, Linda R. Conversion of *Salmonella enteritidis* phage type 4 to phage type 7 involves loss of lipopolysaccharide with concomitant loss of virulence. *FEMS Microbiology Letters*, July 1989, vol. 60, no.1, p. 37-40.

COLLINS, L.V.; ATTRIDGE, S. and HACKETT, J. Mutations at *rfc* or *pmi* attenuate *Salmonella typhimurium* virulence for mice. *Infection and Immunology*, March 1991, vol. 59, no. 3, p. 1079-1085.

DOMINGUEZ, C.; GOMEZ, I. and ZUMALACARREGUI, J. Prevalence of *Salmonella* and *Campylobacter* in retail chicken meat in Spain. *International Journal of Food Microbiology*, January 2002, vol. 72, no. 1-2, p. 165-168.

FIORENTIN, Laurimar; VIEIRA, Nilson D. and BARIONI, Waldomiro Jr. Oral treatment with bacteriophages reduces the concentration of *Salmonella* Enteritidis PT4 in caecal contents of broilers. *Avian Pathology*, June 2005, vol. 34, no. 3, p. 258-263.

GOODE, R.D.; ALLEN, V.M. and BARROW, P.A. Reduction of experimental *Salmonella* and *Campylobacter* contamination of chicken skin by application of lytic

bacteriophages. *Applied and Environmental Microbiology*, August 2003, vol. 69, no. 8, p. 5032-5036.

GREGOROVA, Daniela; PRAVCOVA, Martina; KARPISKOVA, Renata and RYCHLIK, Ivan. Plasmid pC present in *Salmonella enterica* serovar Enteritidis PT14b strains encodes a restriction modification system. *FEMS Microbiology Letters*, September 2002, vol. 214, no. 2, p. 195-198.

HARLOW, E. and LANE, D. *Using Antibodies: A Lab Manual*. Cold Spring Harbor Laboratory, 1999. 495 p. ISBN 0-87-969544-7.

HIGGINS, J.P.; HIGGINS, S.E.; GUENTHER, K.L.; HUFF, W.; DONOGHUE, A.M.; DONOGHUE, D.J. and HARGIS, B.M. Use of a specific bacteriophage treatment to reduce *Salmonella* in poultry products. *Poultry Science*, July 2005, vol. 84, no. 7, p. 1141-1145.

HITCHCOCK, P.J. and BROWN, T.M. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. *Journal of Bacteriology*, April 1983, vol. 154, no. 1, p. 269-277.

HOPE, Ian A. *C. elegans: A practical approach*. Oxford University Press, 1999. 274 p. ISBN 0-19-963739-5.

KRÜGER, E.; SANTANDER, J.; ZURITA, P.; BORIE, C. and ROBESON, J. Persistence in chicken of *f3αSE*, a biocontroling bacteriophage of *Salmonella enteritidis*. In: *XXVChilean Congress of Microbiology, Chilean Society for Microbiology* (22nd - 25th October, 2003, Universidad de Antofagasta, Chile) Abstracts 2003, p. 35.

LEATHART, J.B.S. and GALLY, D.N. Regulation of type 1 fimbrial expression in uropathogenic *Escherichia coli*: heterogeneity of expression through sequence changes in the *fim* switch region. *Molecular Microbiology*, April 1998, vol. 28, no. 2, p. 371-381.

LEVERENTZ, Britta; CONWAY, William S.; ALAVIDZE, Zemphira; JANISIEWICZ, Wojciech J.; FUCHS, Yoram; CAMP, Mary J.; CHIGHLADZE, Ekaterine and SULAKVELIDZE, Alexander. Examination of bacteriophages as a biocontrol method for *Salmonella* on fresh-cut fruit: a model study. *Journal of Food Protection*, August 2001, vol. 64, no. 8, p. 1116-1121.

MAINIL, J.G.; BEX, F.; DREZE, P.; KAECKENBEECK, A. and COUTURIER, M. Replicon typing of virulence plasmids of enterotoxigenic *Escherichia coli* isolates from cattle. *Infection and Immunity*, August 1992, vol. 60, no. 8, p. 3376-3380.

MODI, R.; HIRVI, Y.; HILL, A. and GRIFFITHS, M.W. Effect of phage on survival of *Salmonella enteritidis* during manufacture and storage of cheddar cheese made from raw and pasteurized milk. *Journal of Food Protection*, July 2001, vol. 64, no. 7, p. 927-933.

PARK, Se Chang; SHIMAMURA, Ichiro; FUKUNAGA, Minoru; MORI, Koh-Ichiro and NAKAI, Toshihiro. Isolation of bacteriophages specific to a fish pathogen, *Pseudomonas plecoglossicida*, as a candidate for disease control. *Applied and Environmental Microbiology*, April 2000, vol. 66, no. 4, p. 1416-1422.

PROVENCE, D.L. and CURTISS, R. III.Isolation and characterization of a gene involved in hemagglutination by an avian pathogenic *Escherichia coli* strain. *Infection and Immunity*, April 1994, vol. 6, no. 4, p. 1369-1380.

RYCHLIK, I.; GREGOROVA, D. and HRADECKA, H. Distribution and function of plasmids in *Salmonella enterica*. *Veterinary Microbiology*, January 2006, vol. 112, no. 1, p. 1-10.

SAMBROOK, Joseph and RUSSELL, David W.*Molecular Cloning: A Laboratory Manual*. 3rd ed. Cold Spring Harbor Press, 2001. 2344 p. ISBN 0-87-969577-3.

SANTANDER, Javier and ROBESON, James. Isolation and characterization of bacteriophages active against *Salmonella enteritidis* and their assay on *Salmonella pullorum*. *Acta Microbiológica*, November 2002, vol. 8, no. 1, p. 17-22.

SANTANDER, Javier and ROBESON, James. Bacteriophage prophylaxis against *Salmonella enteritidis* and *Salmonella pullorum* using *Caenorhabditis elegans* as an assay system. *Electronic Journal of Biotechnology* [online]. 15 August 2004, vol. 7, no. 2. Available from Internet: http://www.ejbiotechnology.info/content/vol7/issue2/full/1/index.html. ISSN 0717-3458.

STEINBACHER, Stefan; MILLER, Stefan; BAXA, Ulrich; BUDISA, Nediljko; WEINTRAUB, Andrej; SECKLER, Robert and HUBER, Robert. Phage P22 tailspike protein: crystal structure of the head-binding domain at 2.3 Å, fully refined structure of the endorhamnosidase at 1.56 Å resolution, and the molecular basis of O-antigen recognition and cleavage. *Journal of Molecular Biology*, April 1997, vol. 267, no. 4, p. 865-880.

WARD, L.R.; DE SA, J.D. and ROWE, B. A phage-typing scheme for *Salmonella enteritidis*. *Epidemiology and Infection*, October 1987, vol. 99, no. 2, p. 291-294.