

The enemys enemy is our friend : phageborne tools for detection and control of foodborne pathogens

Autor(en): **Loessner, Martin. J.**

Objektyp: **Article**

Zeitschrift: **Mitteilungen aus Lebensmitteluntersuchungen und Hygiene = Travaux de chimie alimentaire et d'hygiène**

Band (Jahr): **96 (2005)**

Heft 1

PDF erstellt am: **09.08.2024**

Persistenter Link: <https://doi.org/10.5169/seals-981936>

Nutzungsbedingungen

Die ETH-Bibliothek ist Anbieterin der digitalisierten Zeitschriften. Sie besitzt keine Urheberrechte an den Inhalten der Zeitschriften. Die Rechte liegen in der Regel bei den Herausgebern. Die auf der Plattform e-periodica veröffentlichten Dokumente stehen für nicht-kommerzielle Zwecke in Lehre und Forschung sowie für die private Nutzung frei zur Verfügung. Einzelne Dateien oder Ausdrucke aus diesem Angebot können zusammen mit diesen Nutzungsbedingungen und den korrekten Herkunftsbezeichnungen weitergegeben werden. Das Veröffentlichen von Bildern in Print- und Online-Publikationen ist nur mit vorheriger Genehmigung der Rechteinhaber erlaubt. Die systematische Speicherung von Teilen des elektronischen Angebots auf anderen Servern bedarf ebenfalls des schriftlichen Einverständnisses der Rechteinhaber.

Haftungsausschluss

Alle Angaben erfolgen ohne Gewähr für Vollständigkeit oder Richtigkeit. Es wird keine Haftung übernommen für Schäden durch die Verwendung von Informationen aus diesem Online-Angebot oder durch das Fehlen von Informationen. Dies gilt auch für Inhalte Dritter, die über dieses Angebot zugänglich sind.

The enemys enemy is our friend: phageborne tools for detection and control of foodborne pathogens*

Martin J. Loessner, Food Microbiology Laboratory, Institute of Food Science and Nutrition, Swiss Federal Institute of Technology (ETH) Zürich, Switzerland

Introduction

The foodborne pathogen *Listeria monocytogenes* is the causative agent of epidemic or sporadic listeriosis (11). Many food products are susceptible to contamination with *L. monocytogenes*. Unfortunately, the currently available methods do not achieve full eradication of the organisms from foods or the food production environment, thus there is a need for improved methods to prevent contamination. What is also needed are rapid and sensitive methods for the detection of this opportunistic pathogen.

Bacteriophages – the enemy's enemy

Bacteriophages can, at least in part, be regarded as natural enemies of their host bacteria. They are highly specific for a given group of cells, and have evolved a number of unique principles. The major focus of our work is directed in order to understand the basic principles of interaction between phage and bacterium, and to employ some of the specific biological properties provided by bacteriophages for the purposes mentioned above.

*So, naturalists observe, a flea
Hath smaller fleas that on him prey;
And these have smaller still to bite 'em,
And so proceed ad infinitum...*

*Jonathan Swift
(cited by Joshua Lederberg, 1996)*

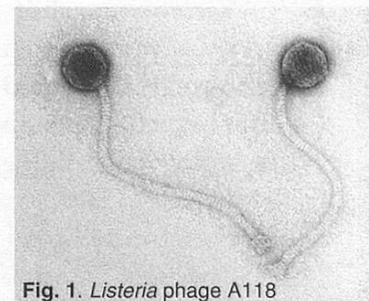


Fig. 1. *Listeria* phage A118

*Lecture presented at the 37th Symposium of the Swiss Society of Food Hygiene, Zurich 29 September 2004

Getting to know each other – nucleotide sequencing provides important information about function, diversity and relationships of phages

Although lysogeny appears to be widespread among *Listeria* strains, only relatively little information is available on the basic nature of phages from this genus, especially with respect to the possible influence of phages on the host phenotype. We have now sequenced and analyzed the genomes of eight different temperate and virulent bacteriophages infecting *L. monocytogenes* (A118, PSA, A006, A500, P35, A511) and *L. innocua* (B054, B025) (3, 10, 12). Most of the phages feature morphotype B1 and belong to the Siphoviridae family (flexible, non-contractile tail) within the order Caudovirales (tailed phages (1). A511 and B054 are Myoviruses (non-flexible, contractile tail). B054 features an unusual morphology: its tail shaft seems to contract downwards towards the base plate, thereby exposing part of the tail tube proximal to the capsid. The phages have dsDNA genomes between approximately 35 and 50 kb in size. Except for P35, all the Siphoviruses have a quite similar overall similar genome organization: the ORFs are organized into three functional gene clusters in a life-cycle specific manner, which is nicely reflected by the direction of transcription (3). While the DNA molecules of PSA and B025 feature overlapping single-stranded cohesive ends, all other phages carry terminally redundant and circularly permuted genomes. Probable functions could be assigned to a significant number of gene products, such as the lysis genes, terminases, structural components, regulatory functions, and proteins required for DNA recombination, replication, and modification. Bioinformatics suggests that the inter-phage homologies over larger portions of the genomes resulted from horizontal exchange of building blocks and modules, such as morphopoetic genes or DNA recombination/replication genes. P35 infects a large proportion of the serovar 1/2 strains, and appears to be different from the other temperate *Listeria* phages.

Reporter phage – harnessing the biological specificity of a bacterial virus

The idea behind the reporter phage concept is that a virus can transduce genetic information encoding a detectable protein into its specific host cells. Based on its broad host range, the virulent *Listeria* phage A511 was selected for construction of a luciferase reporter bacteriophage (5). A fusion of *luxA* and *luxB* genes from the marine bacterium *Vibrio harveyi* was introduced into the A511 genome, under control of the powerful *cps* promoter. Following infection of *Listeria* cells by A511::*luxAB*, gene expression eventually results in synthesis of the luciferase. Upon addition of the aldehyde substrate, bioluminescence can be quantitatively monitored, even in mixed bacterial floras and in food homogenates or enrichment cultures. Evaluation of the system (6) showed its usefulness as a quick and sensitive method for detection of viable *Listeria* cells in a variety of foods (Fig. 2).

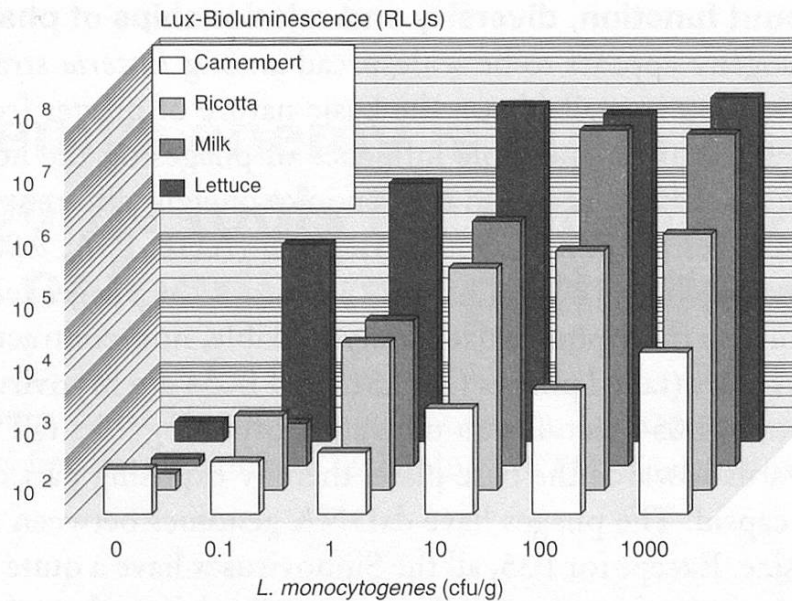


Figure 2 The light emission after infection of *Listeria* cells with A511::luxab in 20 h enrichment cultures allows detection of low numbers of viable *Listeria* cells from different artificially contaminated foods

Endolysins – using the weapons of the enemy

Listeria bacteriophages encode several different endolysins, which are cell wall lytic enzymes responsible for release of progeny from infected cells. The *Listeria* phage enzymes represent very specifically acting L-alanine-D-glutamate peptidases or N-acetylmuramoyl-L-alanine amidases (9). The endolysin genes *ply* can be cloned in *E. coli*, and the purified recombinant enzymes enable rapid and highly specific lysis of *Listeria* cells when added exogenously (8). They found a number of interesting applications in biotechnology and molecular biology. Expression and quantitative secretion of functional Ply511 endolysin in the starter culture organism *Lactococcus lactis* was achieved by genetic fusion with a signal peptide sequence of a *Lactobacillus* S-layer protein (2); processing of the propeptide did not influence enzyme activity (Fig. 3). Such genetically engineered starter cultures are potentially useful as a novel biopreservation method, and were shown to suppress the development of a *Listeria* contamination on foods such as camembert cheese (unpublished data).

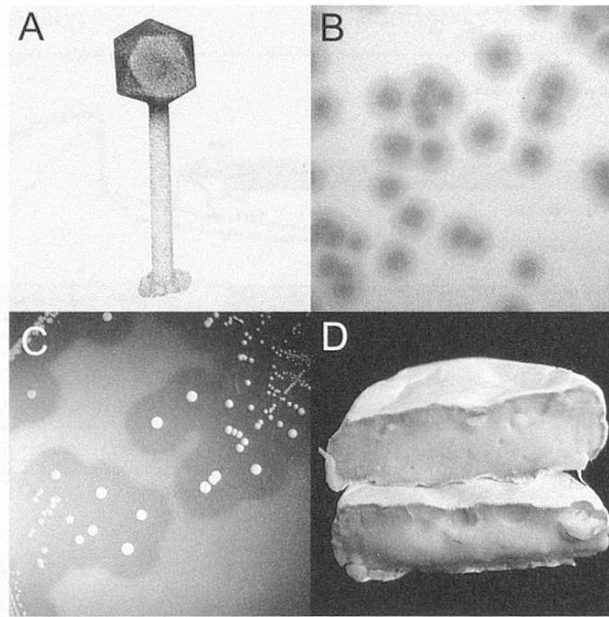


Figure 3 From phage to food: *Listeria* phage A511 (A) encodes an endolysin to destroy its host cells (B). When a modified endolysin gene was cloned into *Lactococcus*, the cells were able to secrete the enzyme (C), which lysed *Listeria* cells on culture media, and could protect Camembert cheese (D) against contamination with the pathogen during production and ripening

CBDs and tail spikes: novel approaches for isolation of bacterial cells

The endolysin enzymes are composed of distinct functional domains: the N-termini generally harbor the catalytic activity, whereas the C-terminal cell wall binding domains (CBD) direct the enzymes to their substrate (4). The CBD domains mediate specific activity of the enzymes towards *Listeria* cell walls, based upon their ability to bind to specific carbohydrate ligands. Genetic fusions between the CBD polypeptides and the green fluorescent protein (GFP) (Fig. 4) demonstrated the highly specific recognition and enabled to quantify the binding properties. Binding kinetics were determined by surface plasmon resonance analysis (Fig. 5), and revealed equilibrium affinities in the nanomolar range, comparable to affinity-matured antibodies.

The CBD-GFP fusions found a number of interesting applications. Besides direct and specific labeling of target cells (Fig. 6), they form the basis for a novel magnetic separation assay (termed CBD-MS), designed for rapid and efficient recovery and detection of *Listeria* cells. The method is based on immobilization of target cells onto the surface of paramagnetic beads coated with recombinant CBD polypeptides. Such CBD-coated beads were shown to be very effective tools for recovery, and provide recovery rates in excess of 90% of *Listeria* cells even from mixed bacterial communities. They also enable sensitive detection of *Listeria* from food homogenates and selective enrichment cultures (13).

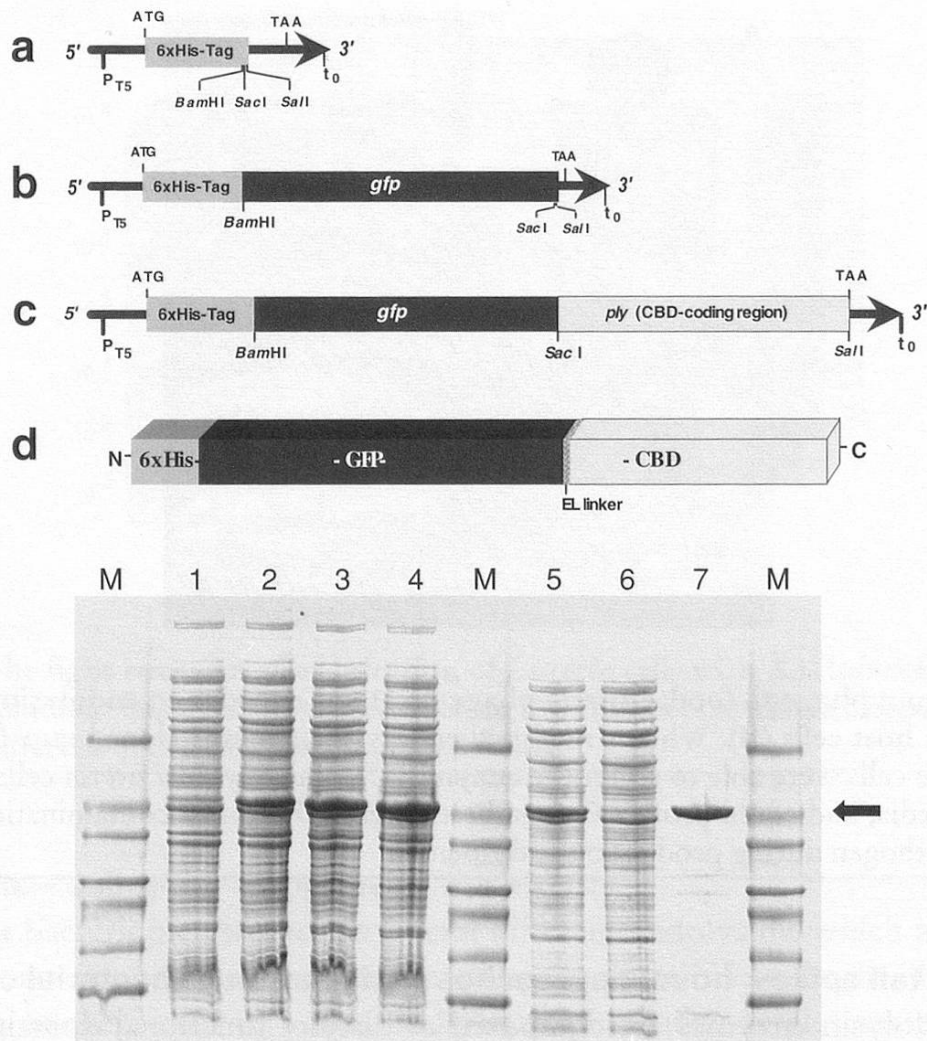


Figure 4 Genetic fusion of the green fluorescent protein gene (GFP) with the cell wall binding domain (CBD) encoding regions of phage *ply* genes. Only relevant regions are shown. Upper panel: vector pQE-30 (a) was basis for construction of pHGFP (b), which then served as backbone for construction of various derivatives carrying fusion genes (c), for the production of hexa-His-tagged chimeras of GFP and CBDs in *E. coli* (d). The lower panel shows overproduction and IMAC purification of HGFP-CBD500 in *E. coli* JM109. Before induction (lane 1) and one hour (lane 2), two hours (lane 3), and 4 hours (lane 4) after induction with IPTG; cleared cell extract (lane 5), flow-through fraction (lane 6), and purified HGFP-CBD500 (47.3 kDa, indicated by black arrow) after elution, buffer exchange, and concentration of the protein (lane 7). Marker protein bands are indicated (lanes M)

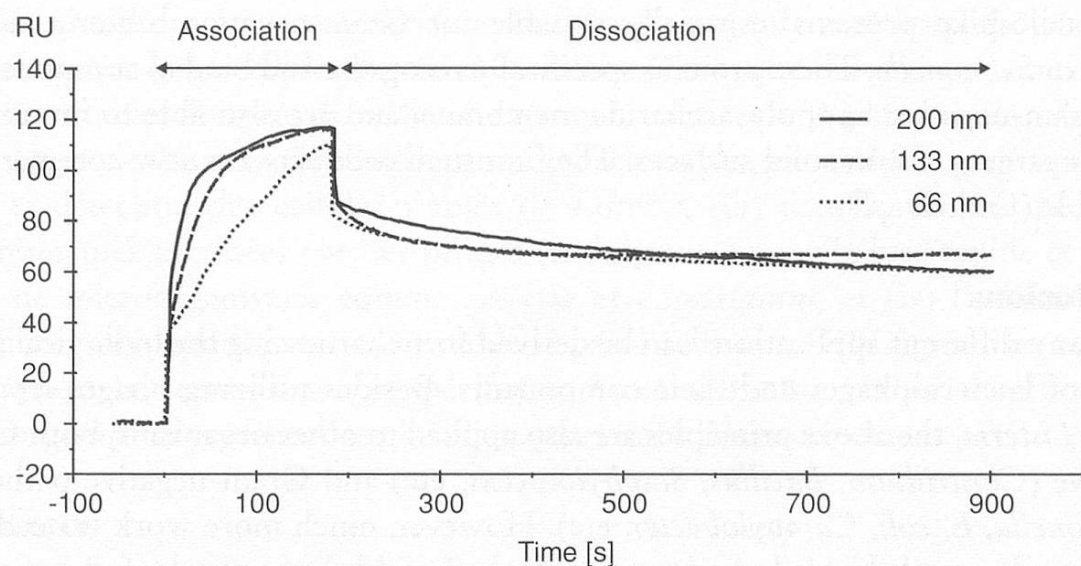


Figure 5 Sensorgram of binding of CBD500 to the surface of *L. monocytogenes* cells immobilized on the modified surface a Pioneer C1 biochip. Association kinetics and dissociation kinetics were determined using a BIAcore 2000 surface plasmon resonance analyzer, at the indicated analyte concentrations and times. Kinetic data (association rates, dissociation rates, equilibrium association constants) for the molecular binding affinity of the CBD polypeptides to the cell wall ligands could be calculated directly from the curves

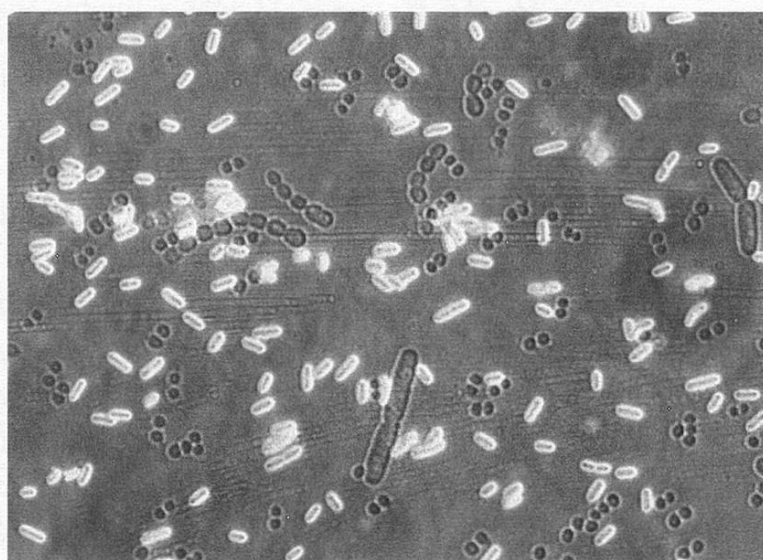


Figure 6 The GFP-CBD500 fusion protein selectively labels *Listeria* cells in a mixed bacterial culture (including various Bacilli, Streptococci, and Staphylococci)

An alternative approach to using CBD from endolysins, is the use of phage short tail spike proteins, especially suitable for Gram-negative bacteria such as *E. coli* or *Salmonella*. These proteins specifically recognize and bind to components of the Gram-negative lipopolysaccharide membrane, and are also able to immobilize LPS or target cells on solid surfaces. The first such products are now commercially available (EndoTrap®).

Conclusions

Many different applications can be derived from harnessing the biological specificity of bacteriophages and their components. Besides utilizing phages from the genus *Listeria*, the above principles are also applied to other organisms, both Gram-positive (*Clostridium*, *Bacillus*, *Staphylococcus*, etc) and Gram-negative pathogens (*Salmonella*, *E. coli*, *Campylobacter*, etc). However, much more work is needed to better understand the underlying molecular principles and to make full use of it – a major topic of the current research in our laboratory.

Summary

We are studying the interactions of bacteriophages infecting foodborne pathogens with their host bacterial cells. In this short review, we are briefly summarizing some recent research with respect to (i) a description of *Listeria* bacteriophages, (ii) the use of genetically modified reporter bacteriophages for the detection of viable *Listeria* cells, (iii) the application of recombinant phage-encoded proteins (endolysins) for the rapid and specific lysis of microorganisms such as *Listeria* and *Clostridium*, and (iv) the use of the cell wall binding polypeptide domains (CBD) from endolysins for the specific recognition, immobilization, and detection of microorganisms from foods and the environment.

Zusammenfassung

Wir untersuchen die Interaktionen von Bakteriophagen mit ihren Wirtszellen, Lebensmittel-assoziierten Krankheitserregern. In dieser kurzen Übersicht werden neuere Forschungsergebnisse kurz zusammengefasst, im Hinblick auf (i) eine Beschreibung von *Listeria* Bakteriophagen, (ii) die Anwendung von genetisch modifizierten Reporter-Bakteriophagen für den Nachweis von lebenden *Listeria* Zellen, (iii) die Anwendung von rekombinanten phagenkodierte Proteinen (Endolysine) für die schnelle und spezifische Lyse von Mikroorganismen wie *Listeria* oder *Clostridium*, und (iv) die Nutzung der zellwandbindenden Polypeptid-Domänen (CBD) der Endolysine für die spezifische Erkennung, Immobilisierung und den Nachweis von Mikroorganismen aus Lebensmitteln und der Umwelt.

Résumé

Nous étudions les interactions des bactériophages infectant les pathogènes alimentaires avec leurs cellules bactériennes hôtes. Dans cette brève revue, nous avons résumé certaines données de recherche récentes relatives à (i) la description des bactériophages de *Listeria*, (ii) l'utilisation de bactériophages modifiés génétiquement pour la détection des cellules viables de *Listeria*, (iii) l'application de protéines recombinantes encodées par des phages (endolysines) pour la lyse rapide et spécifique de microorganismes comme *Listeria* et *Clostridium*, et (iv) l'utilisation de domaines polypeptidiques de liaison de la paroi cellulaire (CBD) pour les endolysines pour la reconnaissance spécifique, l'immobilisation et la détection des microorganismes présents dans les aliments et dans l'environnement.

Key words

bacteriophage, *Listeria*, endolysin, lysis, detection, bacterial luciferase, affinity

References

- 1 Ackermann H.-W.: Tailed bacteriophages: the order Caudovirales. *Adv Virus Res* **51**, 135–201 (1999)
- 2 Gaeng S., Scherer S., Neve H. and Loessner M.J.: Gene cloning and expression and secretion of *Listeria monocytogenes* bacteriophage-lytic enzymes in *Lactococcus lactis*. *Appl. Environ. Microbiol.* **66**, 2951–2958 (2000)
- 3 Loessner M.J., Inman R.B., Lauer P. and Calendar R.: Complete nucleotide sequence, molecular analysis and genome structure of bacteriophage A118 of *Listeria monocytogenes*: Implications for phage evolution. *Mol. Microbiol.* **35**, 324–340 (2000)
- 4 Loessner M.J., Kramer K., Ebel F. and Scherer S.: C-terminal domains of *Listeria* bacteriophage murein hydrolases determine specific recognition and high affinity binding to the bacterial cell wall carbohydrates. *Mol. Microbiol.* **44**, 335–349 (2002)
- 5 Loessner M.J., Rees C.E.D., Stewart G.S.A.B. and Scherer S.: Construction of luciferase reporter bacteriophage A511::*luxAB* for rapid and sensitive detection of viable *Listeria* cells. *Appl. Environ. Microbiol.* **62**, 1133–1140 (1996)
- 6 Loessner M.J., Rudolf M. and Scherer S.: Evaluation of luciferase reporter bacteriophage A511::*luxAB* for detection of *Listeria monocytogenes* in contaminated foods. *Appl. Environ. Microbiol.* **63**, 2961–2965 (1997)
- 7 Loessner M.J. and Scherer S.: Organization and transcriptional analysis of the *Listeria* phage A511 late gene region comprising the major capsid and tail sheath genes *cps* and *tsb*. *J. Bacteriol.* **177**, 6601–6609 (1995)
- 8 Loessner M.J., Schneider A. and Scherer S.: A new procedure for efficient recovery of DNA, RNA, and proteins from *Listeria* cells by rapid lysis with a recombinant bacteriophage endolysin. *Appl. Environ. Microbiol.* **61**, 1150–1152 (1995)
- 9 Loessner M.J., Wendlinger G. and Scherer S.: Heterogeneous endolysins in *Listeria monocytogenes* bacteriophages: a new class of enzymes and evidence for conserved holin genes within the siphoviral lysis cassettes. *Mol. Microbiol.* **16**, 1231–1241 (1995)
- 10 Zimmer M., Sattelberger E., Inman R., Calendar R. and Loessner M.J.: Genome and proteome of *Listeria monocytogenes* phage PSA: an unusual case for programmed +1 translational frameshifting in structural protein synthesis. *Mol. Microbiol.* **50**, 303–317 (2003)

- 11 Vazquez-Boland J.A., Kuhn M., Berche P., Chakraborty T., Dominguez-Bernal G., Goebel W., Gonzalez-Zorn B., Wehland J. and Kreft J.: *Listeria* pathogenesis and molecular virulence determinants. Clin. Microbiol. Rev. 14, 584–640 (2001)
- 12 Dorscht J., Zimmer M., Schmelcher M., Calendar R. and Loessner M.J.: Comparative Genomics of *Listeria* phages (manuscript in preparation)
- 13 Kretzer J.W., Lehmann R., Korn C. and Loessner M.J.: High-affinity cell wall-binding domains (CBD) for specific recognition, immobilization, and magnetic separation of *Listeria* cells. (submitted for publication)

Correspondence address: Martin J. Loessner, Institute of Food Science and Nutrition, Swiss Federal Institute of Technology Zurich, ETH Center, Schmelzbergstrasse 7, CH-8092 Zürich, Switzerland, Phone +41 44 632 3335, E-mail: martin.loessner@ilw.agrl.ethz.ch, <http://www.food-microbiology.net>