Bacteriophages as alternative approaches for the biocontrol of foodborne pathogens*

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Listeria monocytogenes – an opportunistic foodborne pathogen

Listeria monocytogenes is a small, Gram-positive rod that is responsible for rare but deadly food-borne infections. The bacterium is widely distributed in nature. It has been found in soil, sewage, decaying plant material and feces of animals and humans (1, 2). *Listeria* is notably well adapted to environmental conditions, predominating also on foods. It is facultative anaerobe, can multiply between 1 and 45 °C, tolerates high salt concentrations (up to 20 %), and can grow in a pH range of 5 to 9.5 (3, 2). *Listeria* has been isolated from a broad variety of foods that include milk, cheese and other dairy products, meat and meat products, poultry, fish and seafood, vegetables and fruits (4, 5). Especially chilled and ready-to-eat foods are of concern. Cases of listeriosis are rather rare, compared to frequent foodborne pathogens such as *Salmonella.* In Switzerland, the BAG statistics list about 50–70 cases of listeriosis per year. However, in the last years there was a significant increase in the number of annual cases (Figure 1).

Listeriosis is a severe disease; very young, old and immunocompromised people and pregnant women are at high risk. Infections of *L. monocytogenes* cause meningitis, encephalitis, sepsis and abortion, with a mortality rate of 15 up to 40 % (6, 7). In conclusion, the ability of *Listeria* to survive and multiply in different foods, its wide distribution, the high mortality rate and the inability for total control in ready-to-eat foods makes the pathogen a major threat for food production and human health. Many conventional conserving methods and procedures are not sufficient to prevent *Listeria* contamination and subsequent infections. Therefore, novel approaches for the control of pathogens are needed and must be evaluated. Such a

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new possibility is offered by the application of specific bacteriophages against *Listeria* (or their lytic enzymes) to high-risk foods.

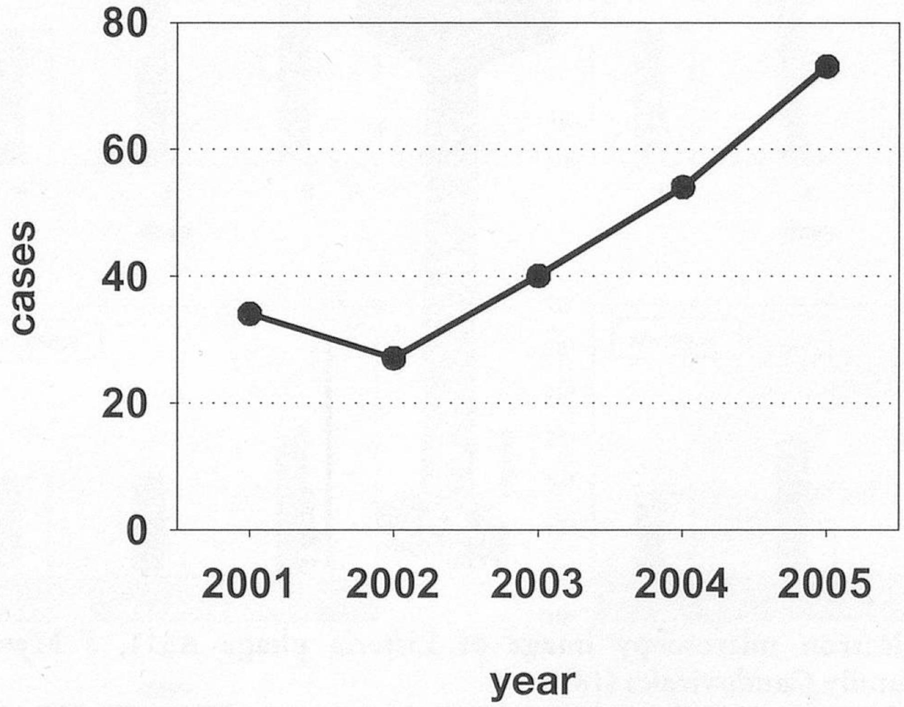

Figure 1 **Annual cases of Listeriosis in Switzerland: (source: BAG, Bern)**

Bacteriophages – the natural enemies as biological preservatives

Bacteriophages are viruses which infect eubacteria – they may be regarded as their natural enemies. Phages are small (20–300 nm) particles, and are composed of nucleic acids and a protein shell. Most phages consist of a capsid and a flexible and sometimes contractile tail structure (Figure 2). They have no own metabolism and depend on the specific host bacterium for multiplication and propagation. In the environment, phages are widely distributed; several estimates suggest that their total number on this planet (all environmental niches together) exceed 10^{31} virus particles (8). They also occur naturally in and on foods (9). Most phages are very specific for one bacterial genus, and therefore do not influence the natural, non-target bacterial flora of a food. Production of phages for the purpose of adding them to foods is straightforward and inexpensive. In fact, phages seem to be quite logical candidates for control of pathogens in foods. Some more recent publications clearly indicate the potential for successful application of phages against pathogens such as *Listeria* or *Salmonella* in foods (10, 11, 12, 13, 14, 15, 16, 17).

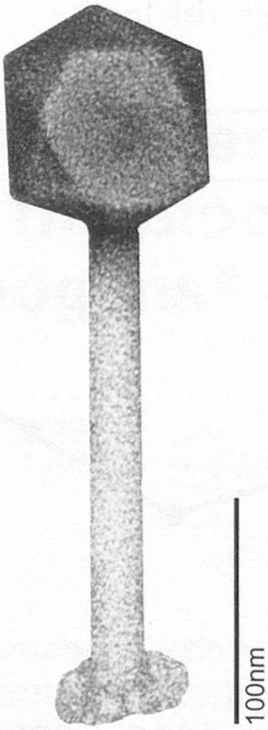

Figure 2 **Electron microscopy image of Listeria phage A511, a Myovirus of the Family Caudovirales** (18)

We investigated the usefulness of the *Listeria* phages A511 against *Listeria monocytogenes* strain Scott A on different foods, during storage at refrigeration temperature (6 °C). Phage A511 is a virulent, broad host range virus, and can kill approx. 95 % of all strains of *L. monocytogenes* serovars ½ and 4 (18).

Several different food items (mozzarella cheese, chocolate milk, hot dogs and shrimps) were purchased at local stores, and artificially contaminated with approx. 10^3 cfu/g. After 1 h storage at 6 °C, *Listeria* phage A511 was added (approx. 3×10^8 pfu/g). The foods were further stored at 6 °C for six days. At defined time points *Listeria* cell counts and phage titer were determined. Figure 3 shows the results for growth of *L. monocytogenes* with and without the addition of phage. In mozzarella brine and chocolate milk, no *Listeria* cells could be detected in the phage treated samples, over the entire time period of the experiment. Compared to the control, this corresponds to a reduction of more than 4 log units in mozzarella brine and units in chocolate milk. On hot dogs, *Listeria* could be detected on day 6 only (detection limit 10 cfu/g), indicating a reduction of more than 2 log units (99 %). On shrimps, reduction of *L. monocytogenes* by phage was not possible below the detection limit. However, compared to the control, reduction was still more than 2 log units (99 %).

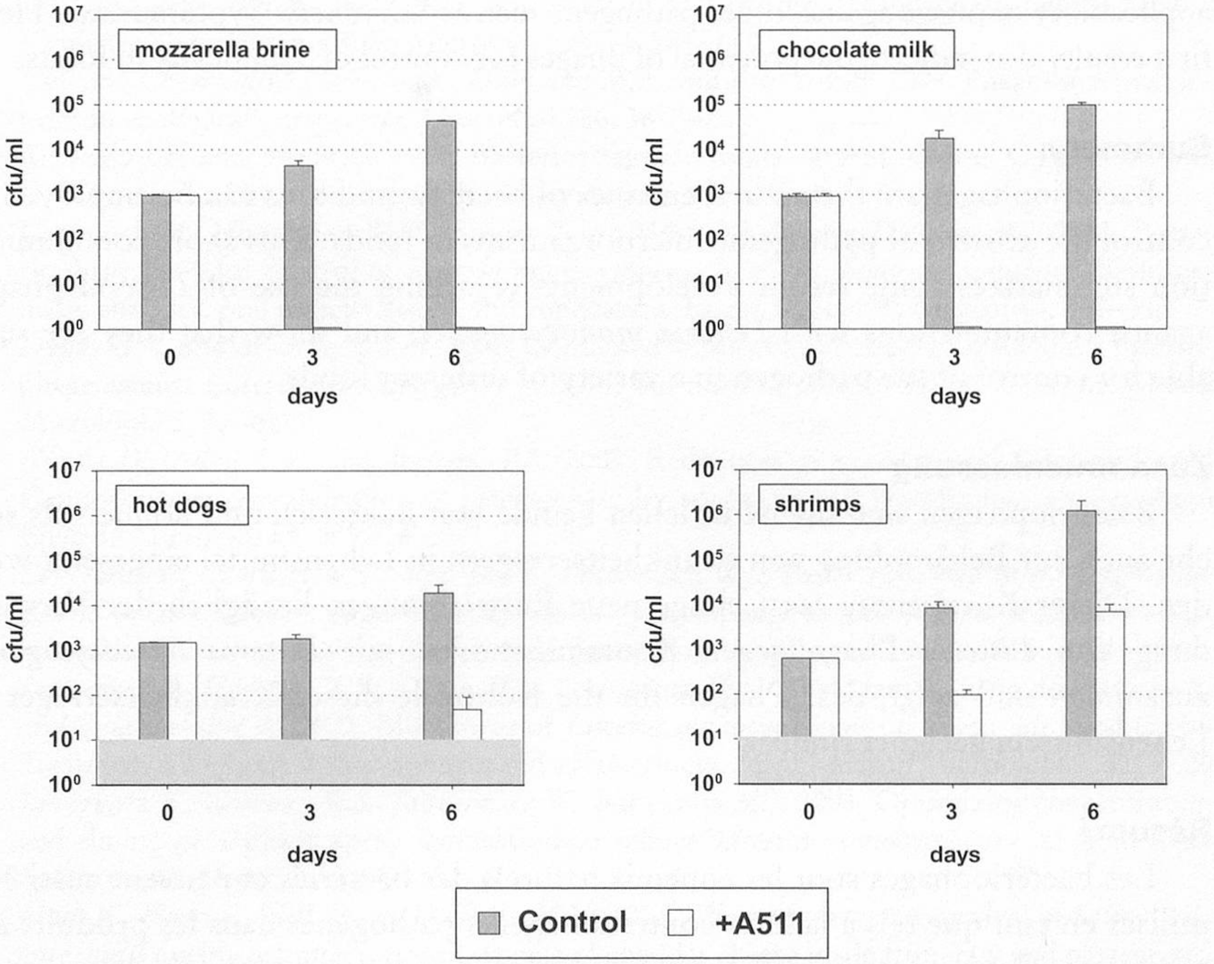

Figure 3 **Growth of *L. monocytogenes* Scott A (SV 4b) without (control) and with addition of phage (+A511) on different foods**

The concentration of phage A511 remained constant in all foods, reflecting the stability of the phage particle in the different environments. The effect of other *Listeria* phages (P100 or P35) was also evaluated, and our results indicate an effectiveness on *L. monocytogenes* similar to that of A511 (data not shown).

For the broad host range *Listeria* Phage P100, who is very similar to A511, the complete genome sequence was recently published (1). Bioinformatic analyses revealed no unwanted genes or potentially allergenic proteins; high dose oral feeding study in rats showed no adverse effects; GRAS approval was granted by the FDA, and the phage is already commercially available.

Conclusions

Current results clearly show the promising and largely overlooked potential of bacteriophages as biological control agents and preservatives. However, the effectiveness depends on the type of food (ingredients, matrix), and the initial phage concentration. Further research is required with respect to optimization of phage application, especially with respect to the individual foods. We are also currently investigating the applicability of phage against other pathogens such as *Salmonella* Typhimurium. Here, first results also indicate the potential of phages for control of *Salmonella* in foods.

Summary

Bacteriophages are the natural enemies of bacteria, and thus can be employed to control the growth of pathogenic microorganisms in foods. This short communication summarizes some recent developments regarding the use of *Listeria* phages against contaminations with *Listeria monocytogenes,* and show that they are suitable for control of the pathogen in a variety of different foods.

Zusammenfassung

Bakteriophagen sind die natürlichen Feinde von Bakterien und können als solche auch zur Bekämpfung von Krankheitserregern in Lebensmittel eingesetzt werden. Dieser Kurzbeitrag fasst einige neue Entwicklungen bezüglich der Verwendung von *Listeria* Phage gegen Kontaminationen mit *Listeria monocytogenes* zusammen und zeigt, dass Phagen für die Kontrolle dieser Krankheitserreger in Lebensmitteln geeignet sind.

Résumé

Les bactériophages sont les ennemis naturels des bactéries et peuvent aussi être utilisés en tant que tels à la lutte contre les agents pathogènes dans les produits alimentaires. Cette contribution brève résume quelques nouveaux développements concernant l'utilisation des bactériophages de *Listeria* contre des contaminations avec des *Listeria monocytogenes* et montre que des bactériophages sont appropriés pour le contrôle de ces agents pathogènes dans les produits alimentaires.

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