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High Stability of Stx2 Phage in Food and under Food-Processing Conditions▽

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Bacteriophages (phages) carrying Shiga toxin genes constitute a major virulence attribute in enterohemorrhagic *Escherichia coli* (EHEC). Several EHEC outbreaks have been linked to food. The survival of such strains in different foods has received much attention, while the fate of the mobile Shiga toxin-converting phages (Stx phages) has been less studied. We have investigated the stability of an Stx phage in several food products and examined how storage, food processing, and disinfection influence the infectivity of phage particles. The study involved a recombinant Stx phage (Δ*stx::cat*) of an *E. coli* O103:H25 strain from a Norwegian outbreak in 2006. Temperature, matrix, and time were factors of major importance for the stability of phage particles. Phages stored at cooling temperatures (4°C) showed a dramatic reduction in stability compared to those stored at room temperature. The importance of the matrix was evident at higher temperatures (60°C). Phages in ground beef were below the detection level when heated to 60°C for more than 10 min, while phages in broth exposed to the same heating conditions showed a 5-log-higher stability. The phages tolerated desiccation poorly but were infective for a substantial period of time in solutions. Under moist conditions, they also had a high ability to tolerate exposure to several disinfectants. In a dry-fermented sausage model, phages were shown to infect *E. coli in situ*. The results show that Stx phage particles can maintain their infectivity in foods and under food-processing conditions.

Shiga toxins (Stx) are a family of related toxins which are involved in human and animal disease by their ability to inhibit protein synthesis. They are produced by either *Shigella dysenteriae* or *Escherichia coli* and constitute a major virulence factor in *E. coli* belonging to the pathogroup Shiga toxin-producing *E. coli* (STEC). The Shiga toxin operon is encoded by lambdoid bacteriophages (phages), which are temperate phages able to integrate into the bacterial chromosome, where they remain stably present as prophages (3). Under stressful conditions, the bacterial SOS response leads to transcription of the prophage, and both Shiga toxin and phage particles are produced and released into the environment (19). Released phage particles can infect new *E. coli* hosts, and the cycle is completed (3).

The main reservoir of STEC is the gastrointestinal tract of ruminants, and the pathogen enters the food chain through fecal contamination of produce or the carcass at slaughter. Sprouts (20), leafy vegetables (6), unpasteurized juices (8), hamburgers (4), and dry-fermented sausages (25, 28) are among foods that have been identified as sources of outbreaks. When STEC causes human disease, it is referred to as enterohemorrhagic *E. coli* (EHEC), and this subgroup of STEC is associated with severe illnesses like hemorrhagic colitis and hemolytic uremic syndrome, which may aggravate to renal failure and even death.

EHEC typically belongs to specific serogroups, with some of the most common being O157, O26, O103, O111, and O145 (13), and it also possesses virulence factors other than the Shiga toxins. These accessory virulence traits are in general stably present in EHEC, as opposed to the Shiga toxin-converting phages (Stx phages), which are mobile and may be lost from the bacterial chromosome during infection (5, 15) and possibly transduced to commensal *E. coli* in the intestinal tract (11). Similar transduction events have also been shown to occur in various food matrices (16).

Because of their mobility, Stx phages play a key role in the evolution of STEC and EHEC (3). It has been suggested that conditions used to inactivate the bacterial hosts may not be sufficient to inactivate the Stx phages (3), and as such, surviving phages may pose an epidemiologic threat, as they maintain the presence of an important virulence gene through harsh conditions. Stx phages have been found free in rivers and sewage systems, likely as a result of fecal contamination (23). The phages are shown to persist longer in aquatic environments than *E. coli* cells and have been reported to be more resistant to decimation procedures like chlorination and pasteurization (23) and to tolerate composting slightly better (17) than the *E. coli* hosts. However, the phages' resistance to environmental stress is not fully known, and the stability of Stx phages in risk products and under conditions related to this has, to our knowledge, not been studied.

The aim of this study was to investigate how storage, food processing, and physical and chemical interventions related to food production influenced the stability of Stx phages. The study involved a recombinant Stx phage with origin from a patient isolate of *E. coli* O103:H25 from an EHEC outbreak in

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2006. We have examined the stability of the recombinant Stx phage during long-term storage at different temperatures, at high and low pH, during desiccation, during freeze-thawing, in heat treatment and fermentation of meat products, in beverages, and during disinfection procedures used in food production facilities.

MATERIALS AND METHODS

Growth medium, bacterial strains, and phages. Luria-Bertani (LB) agar (Difco, Detroit) and LB broth supplemented with $CaCl_2$ (10 mM) were used for phage induction and inoculation experiments. LB agar and broth were, when indicated, supplemented with chloramphenicol at a concentration of 25 μg/ml (cm-25).

The Shiga toxin phage in the study originated from a patient isolate of *E. coli* O103:H25 (NIPH-11060424) from a Norwegian outbreak in 2006 (28, 29). Prior to the study, the phage was modified by replacing the *stx* gene with the antibiotic resistance gene chloramphenicol acetyltransferase (*cat*) (Δ*stx::cat*) and transformed to *E. coli* C600 (recombinant phage made by M. Muniesa, University of Barcelona, Spain) (30). This lysogen was used for production of phage particles throughout the study, and for convenience, the recombinant Stx phage is termed Stx phage throughout the article. *Escherichia coli* DH5α was used as the recipient in the transduction experiments. All strains were cultured in LB broth for 16 to 18 h at 37°C and 200 rpm prior to experiments.

Phage induction. Mitomycin C (MitC) was used to induce phage production. An overnight LB broth culture was diluted 1:100 in LB broth, and $CaCl_2$ and chloramphenicol were added. The culture was incubated at 37°C, with shaking at 200 rpm to an optical density at 600 nm (OD_{600}) of 0.45 (~2 h). MitC (0.5 μg/ml) was added, and cultures were incubated for 16 to 18 h in the dark. The induced phage filtrate cultures were centrifuged (3,300 × *g*, 10 min), and the supernatants were sterile filtrated before use (0.22 μm; Nalgene, Rochester, NY).

Enumeration of phages and lysogens (transductants). Infectious phage particles were enumerated by plaque assay. An appropriate dilution series of the phage filtrate was made. The *E. coli* DH5α recipient was grown to mid-exponential growth phase (OD_{600} = 0.4 to 0.6) in LB broth at 37°C and 200 rpm. A volume of 100 μl of diluted phages was added to 900 μl *E. coli* DH5α and 10 mM $CaCl_2$. The phage-recipient mixture was incubated for 30 min at 37°C before it was added to 2.5 ml molten soft agar (0.7% LB broth) and poured onto LB agar plates with $CaCl_2$. The plates were incubated at 37°C overnight, plaques were counted by visual examination, and the phage titer was calculated.

The detection of lysogens followed the same procedure as the phage enumeration, including the incubation for 30 min at 37°C, but thereafter, the phage-recipient mixture was plated on LB agar plates supplemented with chloramphenicol. The agar plates were incubated at 37°C overnight before colonies were counted, subcultured to new LB agar plates, and checked for the presence of the *cat* gene (Cat-F, GGGCGAAGAAGTTGTCCATA; Cat-R, TACACCGTTTT CCATGAGCA) by PCR.

Stability of phages at different temperatures and in ground beef and broth under heat treatment. For long-term stability experiments, phage filtrates of approximately 5×10^9 PFU ml^{-1} in LB broth were used. The suspensions were stored at 24 and 4°C for 60 days. At appropriate time points, infectious phages were enumerated by the plaque assays as described above. Four independent repetitions of the experiments were performed. Aliquots of phage filtrates were also stored at 37°C.

For stability experiments under heat treatment, parallel experiments were performed in meat and in broth at 43, 50, and 60°C. In the meat experiments, 90 g of ground beef was added to 10 ml phage filtrate. Aliquots of 15 g of inoculated ground beef were spread in a thin layer in plastic bags, air was manually removed, and the bags were sealed and allowed to adjust to room temperature prior to heat treatment. For heat treatment experiments in broth, the phage filtrate was diluted 1:10 in LB broth (brought to room temperature) prior to the experiments to ensure the same concentration of phages as that in the ground beef experiments, and aliquots of 1 ml of inoculated broth were transferred to glass tubes. The heat treatment experiments were conducted in water baths to ensure a fast heat transfer, and both the meat and the broth experiments were investigated at the same temperatures (43, 50, and 60°C) and time points (10, 30, and 60 min). After heat treatment, the ground beef samples were diluted 1:10 (wt/vol) in peptone water and homogenized prior to further dilution. The samples were tested for the presence of infectious phage by plaque assays as described above. The experiment was repeated three times.

Stability of phages in the dry-fermented sausage model. Sausage batter was fermented *in vitro* using sterile centrifuge tubes, as previously described by Heir et al. (14). Phage filtrates were added to meat batter (final concentration of approximately 10^7 PFU ml^{-1}) containing the following ingredients (concentration in the water phase of the meat batter): 0.9% dextrose, 3.8% NaCl, and 100 ppm nitrite. Starter culture (LS-25, *Lactobacillus sakei* and *Staphylococcus carnosus*; Gewürzmüller, GmbH, Germany) was added to the batter to a final cell concentration of approximately 10^8 CFU ml^{-1}. *E. coli* DH5α to a final concentration of approximately 10^8 CFU ml^{-1} was added, where appropriate, to the batter. Aliquots of mixed batter (30 to 35 g) were stuffed into 50-ml sterile centrifuge tubes (here named tube-sausages), and centrifuged (600 × *g*, 2 min). The tubes were stored for up to 32 days at 24°C. Four different series of tube-sausages were made with (i) LB broth (control), (ii) phages, (iii) phages and *E. coli* DH5α, and (iv) phages (diluted 1:10) and *E. coli* DH5α, with the latter having a 10-fold reduced amount of phages compared with those in series ii and iii. At different time points during the storage period, samples were withdrawn, and phage titer was measured. Samples were diluted 1:10 (wt/vol) in peptone water and homogenized prior to further dilution. The presence of infectious phages and transductants (lysogens) was detected by plaque assays and selective plates, respectively, as described above. Two biological and three technical replicates were included.

TABLE 1. Disinfectants used in suspension testing of the studied Stx phage

Disinfectant	Active compound(s)[a]	% user solution[b]	pH(s)[c]
Ethanol	Ethanol	70	
Virkon S	Peroxide agents and acid	1.0	2.6
Oxy Des	Peroxide agents and acid	0.5	
Oxysan ZS	Peroxide agents	0.08	2.2–2.8
Sekumatic FD	Glutaraldehyde and ethanol	1.0	10
Aco Hygiene Ultra Des	Cationic surfactants (QAC) and acid	1.0	7
TP99	Cationic surfactants and acid	1.0	8.5–9.1
Aco Hygiene Des GA	QAC and glutaraldehyde	0.5	7

[a] Specific information on contents presented in the study by Møretrø et al. (22). QAC, quaternary ammonium compound.
[b] User solution in this experiment. All concentrations used are within the user range recommended by the producer.
[c] pH in user solution according to the producer.

Stability of phages in beverages and at low and high pH. Phage filtrate (approximately 10^8 PFU ml^{-1}) was diluted 1:10 in tap water (pH 7.4), semifat milk (pH 6.7), 100% pure orange juice (pH 3.9) and apple juice (pH 3.7), and LB broths adjusted to pH 3, 4, 5, 8, 9, and 10. The beverages were all purchased from local retailers and allowed to adjust to room temperature prior to inoculation. All trials were kept at 20°C throughout the experiment, and samples for plaque assays were withdrawn after 10 min, 30 min, 120 min, and 24 h.

Stability of phages on stainless steel. Aliquots of 100 μl of phage filtrate (approximately 10^9 PFU ml^{-1}) were transferred to coupons of stainless steel (AISI 304, 2B; Norsk Stål AS, Nesbru, Norway), air dried for 1 h at 20°C in a safety hood, and subsequently stored at 24°C for 24 h in a sealed plastic box at 98% relative humidity (RH) (21). To detach the phage particles, the coupons were treated in one of two ways, as follows: (i) coupons were placed in individual tubes with 5 ml peptone water and subjected to sonication for 2, 5, or 10 min in a water bath (30°C, 45 kHz/100 W) (Bransonic 3510; Bransonic Ultrasonic B.V., Soest, Netherlands), or (ii) each coupon was thoroughly swabbed with a moist cotton swab. The phage-peptone solution and the swabs were incubated with *E. coli* DH5α and applied in a plaque assay.

Disinfection tests. A panel of eight different disinfectants commonly used in the food industry were tested for their effects on phages (Table 1), including ethanol (Kemetyl Norge AS, Vestby, Norway), Virkon S (Antec Inc., Sudbury, United Kingdom), Oxy Des (ACO Norge AL, Tønsberg, Norway), Oxysan ZS (Ecolab), Sekumatic FD (Ecolab), Aco Hygiene Ultra Des (ACO Norge AL), TP99 (Ecolab), and Alco Hygiene Des GA (ACO Norge AL). A full description of the agents is presented in the study by Møretrø et al. (22).

A modified version of the Council of Europe suspension test EN 1276 (9) was used to test the effect if disinfectants toward phages. Phage filtrates containing approximately up to 10^9 PFU ml^{-1} were used in the experiments. The phage suspension in LB was exposed to the different disinfectants at recommended user concentrations for 5 and 30 min following a 1:10 dilution (10 ml) in Dey/Engley

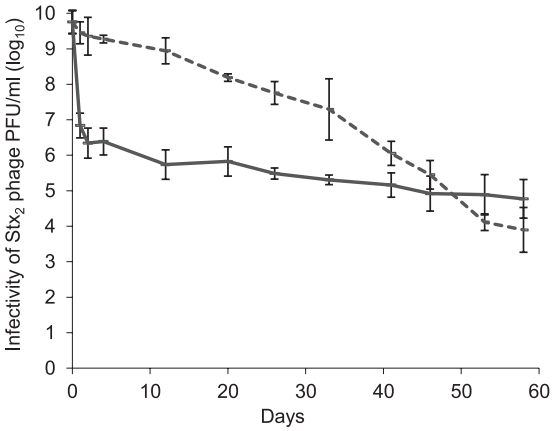

FIG. 1. Infectivity of the studied Stx phage stored in LB broth at 4°C (solid line) and 24°C (dashed line). The results are the means from four independent experiments.

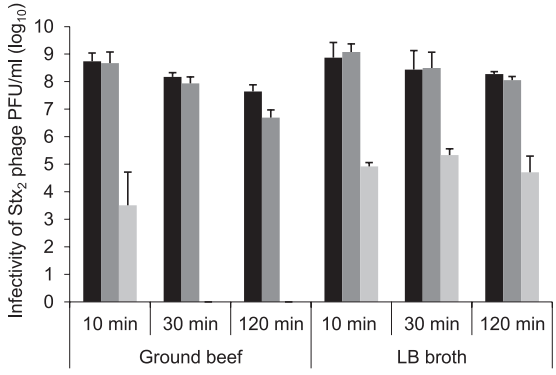

FIG. 2. Infectivity of the studied Stx phage in ground beef and LB broth after incubation at 43°C (black bars), 50°C (dark gray bars), and 60°C (light gray bars) for 10, 30, and 120 min. The results are the means from three independent experiments.

neutralizing broth (Difco Laboratories, Detroit, MI). Subsequently, the solution was diluted in peptone water and applied in a plaque assay. The tests were performed at 20°C. The efficacy of the disinfectants was calculated as the difference between the log-transformed numbers of phages exposed to distilled water (dH_2O; control) and disinfectant. The tests were performed in duplicate with three biological replicates on different days and with freshly prepared solutions.

RESULTS

Stability at different temperatures. The stability of phage particles in LB broth at two temperatures (4 and 24°C) was investigated over a 60-day time span. At day zero, the phage titer was 5×10^9 PFU ml^{-1}. After 1 day, there was a drop in phage titer of almost 3 log for the phages stored at 4°C (Fig. 1). After the initial drop in titer, the infectivity of phages at 4°C remained relatively stable for the next 60 days. In contrast, a steady and continuous decrease in phage titer was observed at 24°C throughout the study period. For storage exceeding 50 days, 4°C showed a higher stability of phages than 24°C. At 37°C, a 4-log reduction was recorded after 7 days, and no phage activity was observed after 28 days (not shown). Freezing resulted in a >5-log reduction after 2 days (not shown).

Stability of phages in ground beef and broth under heat treatment. There were no large differences in the stability of phages in ground beef compared to that in broth at either 43 or 50°C (Fig. 2). However, at 60°C, no infective phages were present after 30 min, whereas in LB broth, infective phages were still present after 2 h, the latter representing a nearly 4-log reduction compared with the initial phage concentration.

Stability of phages in the dry-fermented sausage model. As EHEC has the ability to survive in dry-fermented sausages over time, the stability of Stx phages in an *in vitro* dry-fermented sausage model was investigated. Two model experiments, both with and without a starter culture (LS-25), were used. After 4 days, there was a drop in phage titer of 3.5 logs when LS-25 was added to the batter, while there was a 2-log reduction when no starter culture was used. After 11 days, no differences between the two models were observed, and there was almost a 5-log reduction. The fermentation and storing process of 21 days gave a reduction of phage titer of more than 5 logs for the phages in the dry-fermented sausage model. Transductants were isolated from the sausages for up to 11 days after the start of the experiments. A higher level of transductants was found in the sausages containing an initial smaller amount of phages compared with those in the experiments containing higher phage levels, but the numbers of transductants in both cases were low (50 CFU g^{-1} and 5 CFU g^{-1}, respectively). However, given the low infectious dose of STEC, the amount of transductants was significant.

Stability of phages in beverages and at low and high pH. Adjustment of the pH of the phage filtrate resulted in a large impact on the stability of phages (20°C). No infectious phages were present after 10 min at pH 3. At pH 4 and 10, a 3- to 4-log reduction was recorded after 24 h, while pH 5, 8, and 9 gave a 0.5- to 1-log reduction for the same period of time. In the pH range of 4 to 10, the infectivity of the phages after storage at 120 min was affected only to a minor degree. The decrease in infective phages was less than 1 log in these cases.

Several beverages are neutral or acidic. The stability of phages in milk and water was high after 24 h, with only a 0.5-log reduction. The stability in the juices was considerably lower, with a 5-log reduction after 2 h in apple and orange juices and no infective phages after 24 h.

Stability of phages on stainless steel. After 24 h at 98% RH, there was a 7-log reduction in phage titer when desiccated on coupons of stainless steel (20°C).

Disinfection tests. The eight different disinfectants tested showed highly variable efficiencies in deactivating phages in phage filtrates at 20°C (Fig. 3). Most disinfectants had user concentrations of 0.5 to 1%. The 0.1% solutions had almost no effect on the stability (data not shown), while the 1% solutions gave a marked difference in reduction after 5 min. The oxidizing agents (Virkon S, Oxy Des, and Oxysan ZS) reduced the amount of infective phages by more than 5 logs. Exposure to disinfectants for 30 min did not give a higher reduction for 10% ethanol, Ultra Des, TP99, and Des GA, while after 30 min of exposure to 70% ethanol, no phages survived. Lower titers of phages, 10^6 and 10^4 PFU ml^{-1}, showed the same reduction pattern as that of titers of 10^8 PFU ml^{-1} (not shown).

The inactivation of Stx phages under exposure to selected food related conditions is summarized in Table 2.

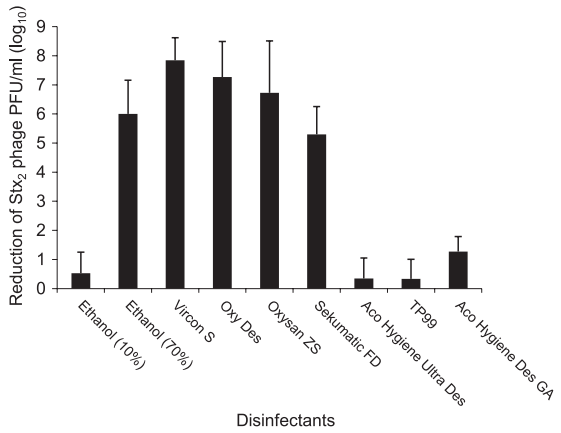

FIG. 3. Decimation of the studied Stx phage exposed to eight different disinfection solutions for 5 min. User concentrations were 1% of all disinfectants, except for ethanol (10 and 70% user solutions).

DISCUSSION

The present study shows that Stx phages can tolerate conditions relevant for the storage and handling of foods. We found that the stability of the Stx phage was highly influenced by physical factors and the matrix embedding the phage particles.

At storage temperatures of 4 and 24°C, viable phages were detected throughout the test period of 60 days, but the decline of stability showed a different pattern at room temperature compared to that at refrigeration temperature (Fig. 1). The finding that room temperature was more conserving in the short run was rather surprising, as cooling temperatures are expected to be more conserving for viral particles than room temperature. In a study comparing the long-term survival of phages, the stability of these microorganisms was higher at 4°C than at 26°C (7). The sudden drop in the measured phage titer at refrigeration followed by a stationary phase could suggest a cold agglutination of the phage particles, resulting in an underestimation of phages, but nevertheless, the results clearly demonstrated that Stx phages are able to persist and maintain infectivity during storage for more than 2 months. At 37°C, the phage titer was significantly lower than those at the storage temperatures, and no active phages were detected halfway through the test period of 60 days. The results indicate that the optimal temperature for Stx phage stability is around 24°C, in contrast to the optimal growth temperature of the bacterial host, which is about 37°C. This may, however, be beneficial to the phage population, as it enables them to persist in the environment until a susceptible bacterial host reappears. From a food safety perspective, our results indicate that refrigerated storage will reduce the infectivity of Stx phages present in food and thus is a well-suited temperature for inhibition of the phage as well as the bacterial host.

Thermal inactivation is regarded as a central postprocessing method for reducing the level of STEC in food, and several food-borne outbreaks are due to undercooked beef or ground beef (12, 26). As expected, heating also reduced the infectivity of the phages, and the reduction was faster at the highest temperature (Fig. 2). In preliminary experiments, we found that no phages tolerated 80°C for 10 min (data not shown), while at 60°C, which is a recommended holding temperature of hot foods, a time-dependent stability was shown (Fig. 2). It is recommended that hamburgers reach an internal temperature of 71.1°C (26), but undercooked hamburgers are a frequent cause of EHEC infections (18). We found that at 60°C, the matrix seemed to have an effect on the inactivity. A higher reduction of phages was observed in heat-treated meat than in heat-treated broth. This difference between the matrices was not seen at 43 or 50°C nor was it seen in control samples of phages in meat at 20°C for 2 h (data not shown), eliminating the possible underestimation of infective phages because they adsorb to structures in the meat. The difference in heat transfer between the two matrices might be important. The pH in the ground beef samples (pH 5.7) was lower than that in the LB broth (pH 7.1). The mildly acidic conditions in combination with heating might explain the smaller amount of infective phages in the ground beef sample. Nevertheless, it is worth noticing that the phages remained infective for more than 2 h at 60°C in broth.

The high stability of phages stored in milk and water confirmed the stability of phages in neutral medium at room temperature. For acidic beverages like apple and orange juices, the stability was considerably lower, and a similar low stability was seen in acidified LB broth, confirming the great effect constituted by the pH itself. This corresponds with a study by Imamovic et al. (16), who reported that transduction in food matrices was prevented by low pH and at low temperatures.

Dry-fermented sausages have traditionally not been considered a risk product due to the combination of low pH, low water activity, and high salt content. However, several outbreaks have been related to the survival of STEC in dry-fermented sausages (10, 25, 27, 28, 33) and raised the question of whether the phages can also withstand these conditions. Our *in vitro* model showed a substantial reduction of phages during fermentation and maturation, and a higher initial drop in phage titer was observed in sausages made with starter culture than in those made without it. The starter culture ensured a pH of 4.6 within the first days of fermentation, and it is likely that the phage reduction was a direct effect of the decreased pH, which corresponds with the results from the acidic beverages and acidified broth. Eventually, the indigenous background flora of the meat batter also lowered the pH in the sausages

TABLE 2. Summary of loss of infectivity of the studied Stx phage exposed to conditions relevant for food and food processing

Condition	Duration	Temp/pH	Loss of infectivity[a]
Storage	1 mo	4°C	++
		24°C	+
		37°C	+++
Freezing	48 h	−18°C	+++
Heating in ground beef	10 min	43°C	+
		50°C	+
		60°C	++
pH (at room temp)	24 h	pH 3	+++
		pH 4, 10	++
		pH 5, 8, 9	+
Desiccation	24 h	24°C	+++

[a] +++, ≥5-log reduction in the number of PFU ml^{-1}; ++, 2- to 4-log reduction; +, ≤1-log reduction.

without a starter culture, and the phage titer was then equal in both types of sausage. However, after 20 days, which is around the time needed to complete the maturation of dry-fermented sausages, no infective phages were detected in either of the sausages. Transductants were observed only until halfway through the maturation process, but it is worth noticing that a higher level of transductants was found when a reduced amount of phage was added. This unexpected result could be due to residual MitC in the phage filtrate, and despite the dilution effect in the meat batter, MitC still might have constituted an effect. The starting point in phage titer in these experiments was much higher than what would be expected in commercial foods, but the high titers allowed us to detect the decline pattern over time. As transduction is a rare event, the high titers also enabled us to detect transductants in the sausage model. These experiments show that phages keep their infectivity for extended periods in the sausage during the production process and that transduction in food does occur, albeit at a low rate. Considering the vast amounts of foods being produced, even rare events may not be negligible. Based on these results, it is not possible to state that phages cannot survive a dry-fermented sausage process.

Phages are sensitive to desiccation, with a general loss of 90 to 95% of the original titer soon after drying. On the other hand, the remaining part of the titer can be maintained for months (1). The high immediate loss corresponds well with our observed decline of phages after desiccation on stainless steel, but we did not measure the stability over time. Stainless steel is a frequently used material in the food industry, and in practice, the challenge is to keep the environment dry enough to prevent bacterial growth. Several microniches in food production facilities would hold a moist environment, and the humidity would influence both the rate of surface drying and the survival of hosts. STEC has been shown to survive for more than 19 days on stainless steel in 70% relative humidity (21). Temperature is another crucial factor in the safe production of food, and it is desirable to keep the temperature as low as possible in most types of food production facilities (21). Studies have shown that the survival of STEC is higher at 4 to 15°C than at 20 to 35°C, under dry conditions, but with the highest survival under moist conditions (34, 35). Both high humidity and low temperature will have a positive influence on the stability of phages over time, but as reported earlier in this study, Stx phages do have a greater ability to maintain infectivity at room temperature in a shorter time (<40-day) perspective. Hence, a low temperature (≤4°C) should be regarded as safer with respect to the Stx phages.

Cleaning and disinfection are important in reducing the amount of bacteria, including STEC. Several studies have examined the efficacy of disinfectants against STEC (21, 24, 32), but little focus has been on the efficiency of disinfectants toward phages within a food safety perspective. The eight disinfectants tested in our study are all effective against bacteria in the *Enterobacteriaceae* family, and no surviving *Salmonella* (>5-log reduction) was observed after exposure in a disinfection suspension test (22). However, the disinfection test showed high variability in reducing the amount of infective phages. The five efficient disinfectants had either a low or a high pH, while the least effective disinfectants all had a more neutral or mild alkali pH. In a disinfection study of a contact surface (31), Virkon S, a bactericidal and virucidal disinfectant, was highly effective in killing phages, while the efficiency of quaternary ammonium compound (QAC)-containing agents showed a minor effect on ϕX174, a phage with a DNA-based genome (31). This is hand in hand with our results, where the two QAC-containing disinfectants (Aco Hygiene Ultra Des and Aco Hygiene Des GA) reduced the stability of the Stx phages only to a minor degree.

Several EHEC outbreaks have been linked to fermented sausages (2, 10, 25, 27), and also, the Stx phage in this study originates from an outbreak where such a product was the culprit (28, 29). The *E. coli* cells can survive these harsh environments with low water activity, low pH, and high salt content, but no investigation has reported the fate of phages under these conditions. Although further research on the implications of Stx phages in different food is needed, our study shows that phages can remain infective for a substantial period of time in the environment, are able to persist in several food products, and are also resistant to several disinfectants.

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