Testing of the virucidal activity of disinfectants with bacteriophages of lactic acid bacteria

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1. Introduction

The impact of bacteriophages infecting bacterial starter cultures during food fermentations is well-known (1). Since many fermentation processes cannot be performed under strict aseptic conditions, phage populations have ample opportunities to attack their bacterial hosts for proliferation. This permanent threat is particularly manifested in the dairy field (2, 3, 4, 5, 6). Due to the constant risk of economic losses, control of phage is an important area of concern in dairies during handling of starter cultures (3, 4, 6, 7).

Disinfection is one major phage control approach in food industry. Disinfectants have to be tested in advance in the laboratory - first in a basic suspension test, but later also under conditions simulating situations found in the industrial area. In order to harmonize test methods in Europe, a CEN committee (TC 216) was established in 1989 (8, 9, 10, 11). We propose here that representative phages of lactic acid bacteria should be used as model viruses for evaluating the virucidal activity of disinfectants used in dairies and other industrial areas. These phages are easy to handle, they yield high-titer lysates, and they have been studied in detail in the last decades (3, 4, 6). Two phages lytic for *Lactococcus lactis* were chosen (phages P008 and P001, respectively) which differ in morphology and which are wide-spread in dairies throughout the world: According to the ICTV International Committee of the Taxonomy of Viruses, phage P008 is accepted as the type-phage of the 936 phage species (with small isometric heads), and phage P001 is a member of the c2 phage species (with prolate heads) (12, 13). Both phages have been deposited in various phage collections (DSM numbers for phages P008 and P001 are DSM 10567 and DSM 4262, respectively). We also included in this study a representative lytic phage of *Streptococcus thermophilus* (i.e., the isometric-headed phage P53) since these bacterial viruses contribute increasingly to improper fermentations during production of yoghurt and *Swiss*- or *Italian*-type cheeses (14, 15).

2. Material and methods

Bacteria, phages and growth conditions

Lactococcus lactis subsp. *lactis* biovar. diacetylactis F7/2 (DSM 4366) was used as the host for the lactococcal phages P008 and P001. Phage P53 was propagated on *Streptococcus thermphilus* 55n. The cultures were stored frozen in litmus skim milk at -72°C for long-time maintenance or were propagated biweekly and stored in between at 4°C. The lactococcal host strain was grown in M17-broth (16) at 30°C, while the thermophilic strain was cultured in modified M17-broth (*th*M17) at 40°C as described earlier (14, 15). Propagation of phage was routinely performed in the presence of 10 mM $CaCl_2$. In order to obtain high-titer lysates, phage were propagated in the bacterial lawn by the agar double layer technique (17) on M17- or *th*M17-bottom plates. Phage (0.1 ml from serial decimal dilutions of a phage stock) and bacteria (0.3 ml from overnight cultures) were seeded in 3 ml of molten soft agar (0.65 % prepared in appropriate growth medium) and were poured on bottom agar plates. Plates revealing confluent lysis were selected,

and phage were harvested from these plates by scraping the soft agar with a bent glass rod and transfering the agar into a test tube. Each plate was subsequently rinsed at room temperature with 5 ml SM-buffer (18) for 15 min, which was also added to the test tube (SM-buffer was prepared without gelatin). After centrifugation in a bench top centrifuge (approx. 4,000 x g, 15 min), the supernatant was passed through a membrane filter (0.45 µm pore size). These test bacteriophage suspensions contained high phage numbers (approx. 1×10^{11} - 8×10^{11} plaque-forming units [PFU] per ml) and were stored at 4°C. For the suspension test trials, the phage stocks were diluted with SM-buffer to obtain the test bacteriophage working suspensions with a titer of approx. 1×10^9 PFU/ml.

Disinfectants and neutralizer

Bacteriophage suspension tests were done with two different types of disinfectants:

(i) Sodium hypochlorite solution (Sigma-Aldrich, Deisenhofen, Germany): The concentration of free available chlorine in the test solution was determined by titration of iodine (liberated from potassium iodide solution) with sodium thiosulfate solutions after acidification (19). 3.3 % - 3.4 % (w/v) available chlorine were determined at the beginning and also at the end of the study.

(ii) A commercially available disinfectant (product A) containing a blend of peracetic acid and hydrogen peroxide: Test kits (Merckoquant, Merck, Darmstadt, Germany) were used for the determination of the concentrations of the active components. Approximately 10 % (w/v) peracetic acid and 30 % (w/v) hydrogen peroxide were determined at the beginning and also at the end of the study.

Water of standardized hardness was used as diluent for the disinfectants (containing per liter double-distilled water: 120 mg $MgCl_2$, 280 mg $CaCl_2$, 280 mg $NaHCO_3$).

For the inactivation of the disinfectants, a neutralizer solution was prepared on basis of M17-broth fortified with 1.5 % (v/v) Tween 80, 0.3 % (w/v) sodium thiosulfate, 0.3 % (w/v) L-cysteine and 0.3 % (w/v) L-histidine (20).

Interfering substances

In order to simulate situations found in dairies, the following interfering substances were chosen:

(i) Acidic whey solution: Acidic whey was prepared from pasteurized low fat milk (1.5 % fat content). 0.3 ml of a 10 % (v/v) lactic acid solution was added to 10 ml of milk. After incubation at room temperature (30 min), milk proteins were sedimented in a bench top centrifuge (approx. 4,000 x g, 15 min). The supernatant was sterilized by membrane filtration (0.45 µm pore size). To obtain a 10 % (v/v) working solution, 1 part of the whey broth was diluted with 9 parts of sterile double-distilled water.

(ii) Skim milk: Reconstituted skim milk was steamed at 100°C on 3 successive days. The milk was diluted 10-fold with double-distilled water to obtain a 10 % (v/v) working solution required for the test.

Both working solutions of interfering substances were stored at 4°C.

Phage suspension tests

The test phage working suspensions (1×10^9 PFU/ml) and all test solutions were equilibrated to 20°C in a water bath before use. For the actual test, 1 ml of the interfering substance (from the 10 % [v/v] stock solutions), 1 ml of the test phage working solution and 8 ml of the disinfectant test solution were mixed in a test tube placed in a 20°C-water

bath. 0.2-ml-samples were withdrawn in intervals of 5, 15, 30 and 60 min (contact times) and were added to 9.8 ml of ice-cold neutralizer. After a 15-min-inactivation time on ice, decimal dilutions were done in 1/4-strength Ringer's solution (21) fortified with 10 % (v/v) M17-broth and the residual phage titers were determined in dublicate by the soft agar overlay technique in a lawn of the corresponding host bacteria. Suspension tests were also performed in the absence of an interfering substance by replacing the interfering protein solution by 1 ml of hard water. Reference (control) tests were done in parallel, in which the disinfectant test solutions were replaced by 8 ml of hard water.

Virucidal activity was indicated as the $\log_{10}$ reduction in phage titer (i.e., $\log_{10}(N_t/N_o)$, where N_t indicates the number of PFU/ml in the disinfectant-treated suspension after the appropriate contact time and N_o the number of PFU/ml in the untreated suspension.

Validation tests for the suspension test

Validation tests were done in parallel to the actual phage suspension tests: To ensure identical conditions as in the test, the phage working suspensions were diluted 5-fold with SM-buffer to obtain a titer of 2×10^8 PFU/ml. The interfering substances were also used in a 5-fold dilution (in double distilled water) as compared to the actual test. The concentration of the disinfectant solution used for validation was 1.6 times higher than the maximum concentration used in the corresponding suspension test. Four validation tests were done according to the following scheme:

(a) Validation reference control: As the counting reference, 0.1 ml of the interfering substance was mixed in a test tube with 0.1 ml of the phage suspension and with 9.8 ml of M17-broth.

(b) Neutralizer toxicity control: 0.1 ml of the interfering substance was mixed with 0.1 ml of the phage suspension and with 9.8 ml of the neutralizer solution.

(c) Dilution neutralization control: 0.1 ml of the interfering substance was mixed with 0.1 ml of the phage suspension, 9.7 ml of the neutralizer solution and finally with 0.1 ml of the disinfectant solution.

(d) Plaquing ability control: 0.1 ml of interfering substance was mixed with 9.7 ml of the neutralizer solution and finally with 0.1 ml of the disinfectant solution.

All four test tubes were kept on ice for 15 min. After the neutralization period, 0.1 ml of phage suspension were added to the plaquing ability control tube (tube d). Decimal serial dilutions were performed from all 4 controls, and the number of PFU/ml was determined by the soft agar overlay technique as described before.

Electron microscopy

Phage lysates were examined in a Philips EM 300 electron microscope using the negative staining technique described earlier (12, 15).

3. Results and discussion

Selection of phage

Electron micrographs of the lactococcal phages P008 and P001 and of the *S. thermophilus* phage P53 are shown in Fig. 1. All 3 phages are members of the *Siphoviridae* family with non-contractile tails of different lenghts. The phage-derived plaques in the lawn of the corresponding bacterial host strains differ significantly in size, allowing the identification of the lactococcal test phages in the laboratory also in the absence of electron microscopy facilities.

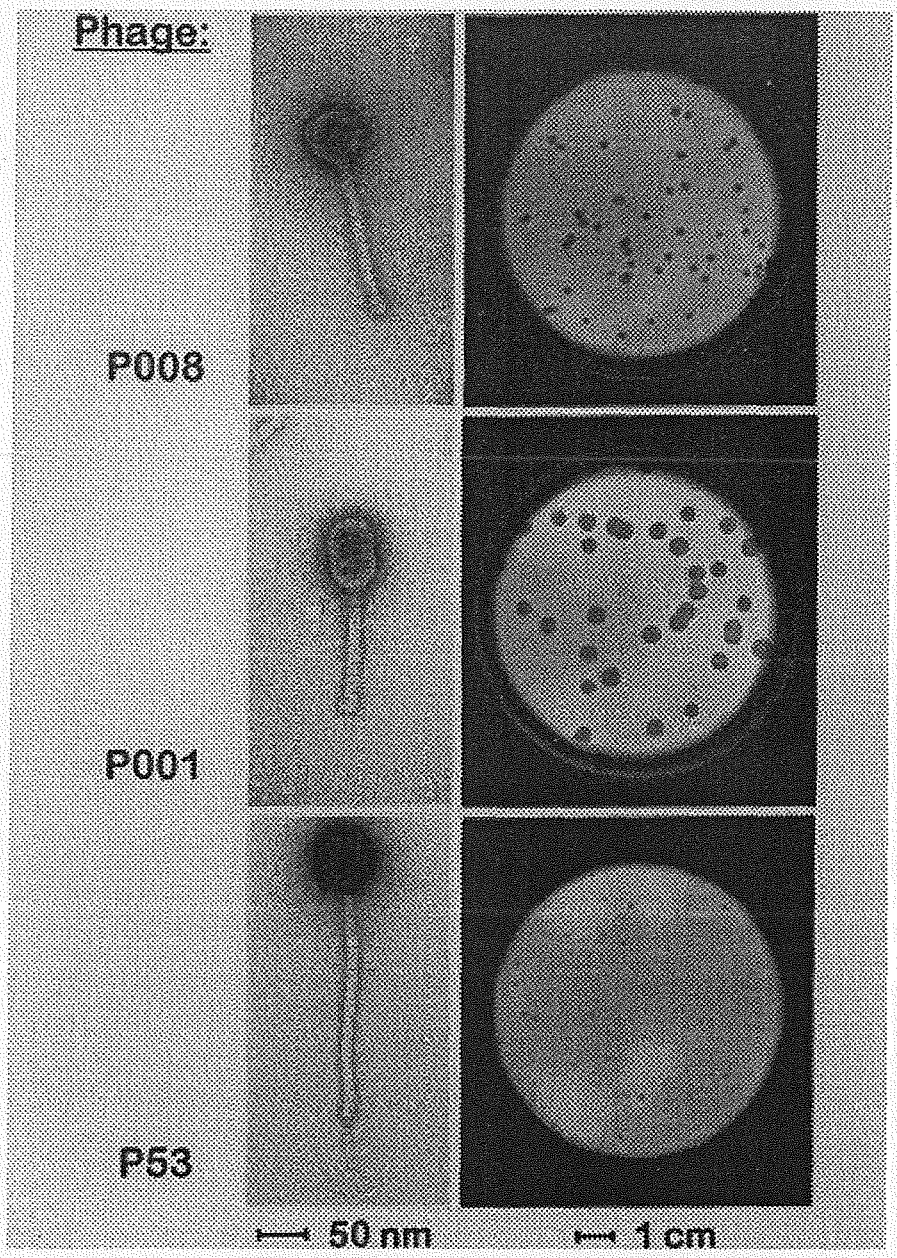

Fig. 1: Morphology of the *Lactococcus lactis* phages P008 and P001 and of the *Streptococcus thermophilus* phage P53. The corresponding lysis zones (plaques) in the lawn of the indicator bacteria are illustrated on the right side. The electron micrographs are shown at the same magnification and the agar plates in reduced size (see bar below the graphs).

Validation tests for the dilution neutralization method

The results of the validation tests are summarized in Fig. 2 for both disinfectant solutions used in this study. The highest product concentrations used in the actual phage suspension tests were considered for the validation methodology and were used in a 1.6 times higher concentration in the validation assays as described before. The tests were first performed in the absence of an interfering substance and then in the presence of acidic whey or skim milk as indicated in Fig. 2. For all three phages tested, phage numbers were not significantly affected by the validation methodology. This indicates that the neutralizer did not reduce the phage titers and was not toxic to the host bacteria. Adequate neutralization of the disinfectant solution prior to the plaque assay is thus ensured.

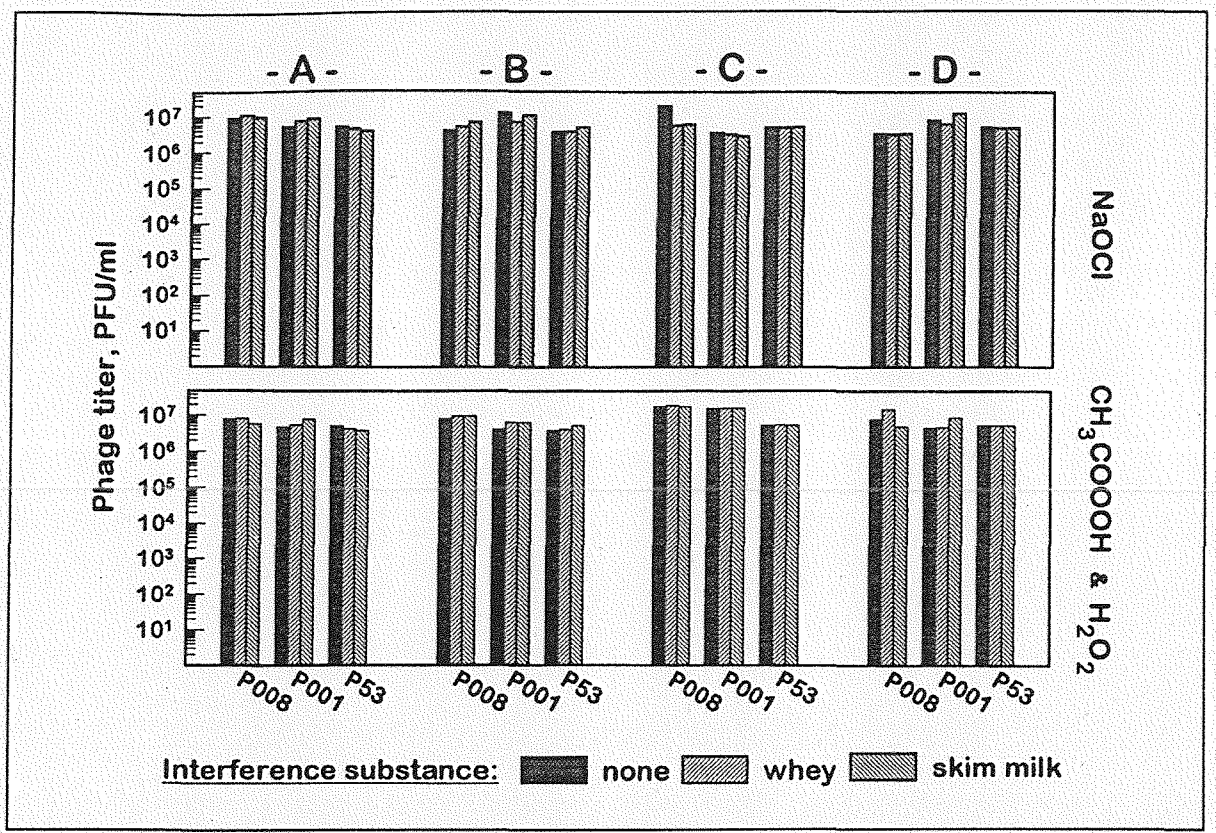

Fig. 2: Phage titers of the test phages P008, P001 and P53 as determined in the validation tests for the disinfectants used in this study (sodium hypochlorite [top] and product A with a blend of peracetic acid and hydrogen peroxide [bottom]). Results are shown for the validation reference control (A), for the neutralizer toxicity control (B), for the dilution neutralization control (C), and for the plaquing ability control (D) as described in the text. The tests were done in the absence and in the presence of interfering substances (whey and skim milk) as indicated in the legend and described in the text.

Virucidal activity of disinfectants in the phage suspension test

Figures 3 and 4 summarize the kinetics of inactivation of the three phages tested by the sodium hypochlorite solution and by the product A (containing peracetic acid and hydrogen peroxide) at 20°C. In all experiments, the initial concentration of the phages in the suspension test was similar (approx. 1 x 10^8 PFU/ml). For each disinfectant, inactivation curves are shown for 3 concentrations. The disinfectants were considered to be effective against the phages when they produced a 4-$\log_{10}$ reduction of the phage titer during the contact times chosen (i.e., ideally within 15 min, but at least after 60 min). The lactococcal phage P008 revealed the highest sensitivity to sodium hypochlorite in the absence of interfering substances: Reduction of phage titer greater than 4 $\log_{10}$ was achieved within a contact time of 60 min (at room temperature) by 0.18 % (v/v) of the disinfectant. Lactococcal phage P001 revealed the highest persistance to the disinfectant in the absence of interfering proteins, since 1.2 % (v/v) was required for adequate inactivation, while a minimum of 0.4 % (v/v) of the disinfectant was needed to inactivate the *S. thermophilus* phage P53 within 60 min. These concentrations for inactivation had to be increased significantly, when interfering proteins were added to the suspension tests. In particular, inactivation of phage P008 was affected by the addition of whey or skim milk. Under these conditions, 1 % - 1.2 % (v/v) (in presence of whey) and 2.4 % - 2.8 % (v/v) (in presence of skim milk) of sodium hypochlorite solution were required for appropriate inactivation. During the disinfection trials performed in 1 % (v/v) whey, phage

P001 again revealed the highest resistance to sodium hypochlorite. However, in the suspension test fortified with 1 % (v/v) skim milk, the highest concentration of the disinfectant (2.4 %-2.8 % [v/v] of NaOCl solution) was required for inactivation of phage P53.

Disinfection experiments performed with the product A (i.e., a blend of peracetic acid and hydrogen peroxide) also showed differences in the inactivation kinetics of the three test phages. Without interfering protein, inactivation of phage P008 and phage P53 required similar concentrations of the product (14 ppm). As shown before, phage P001 was the most persistent phage and the required 4-$\log_{10}$ reduction was achieved by 20 ppm of the disinfectant. Upon addition of whey or skim milk, it was also necessary to increase the concentration of the product to a maximum of 150 ppm (for inactivation of phage P001 in diluted skim milk) as shown in Fig. 4.

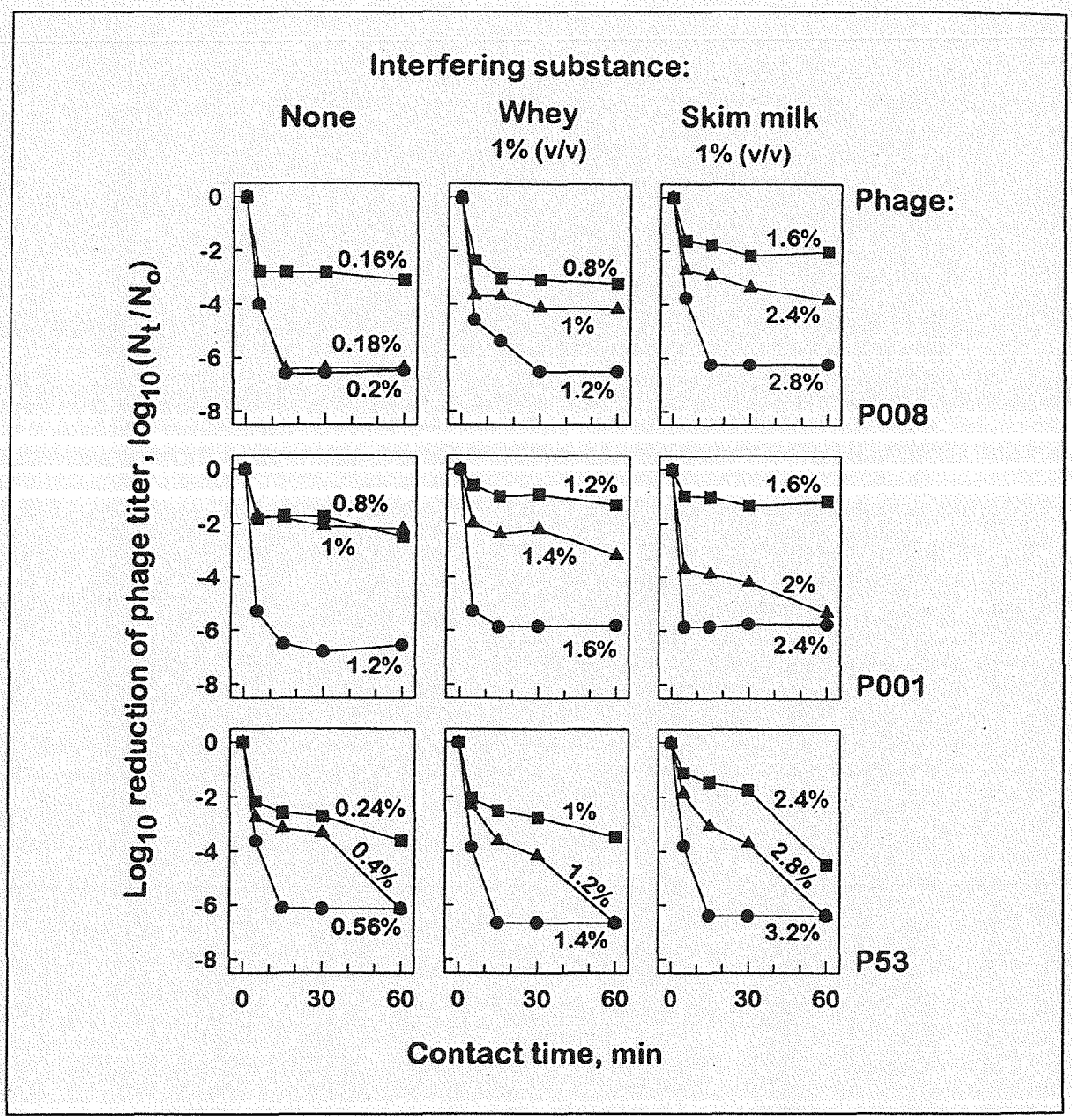

Fig. 3: Inactivation of the *L. lactis* phages P008 (top) and P001 (middle) and of the *S. thermophilus* phage P53 (bottom) by sodium hypochlorite in a phage suspension test. The concentrations used in the tests are indicated in the graphs. The tests were performed in the absence of interfering substances (left) or in the presence of 1 % (v/v) acidic whey (middle) or 1 % (v/v) reconstituted skim milk (right).

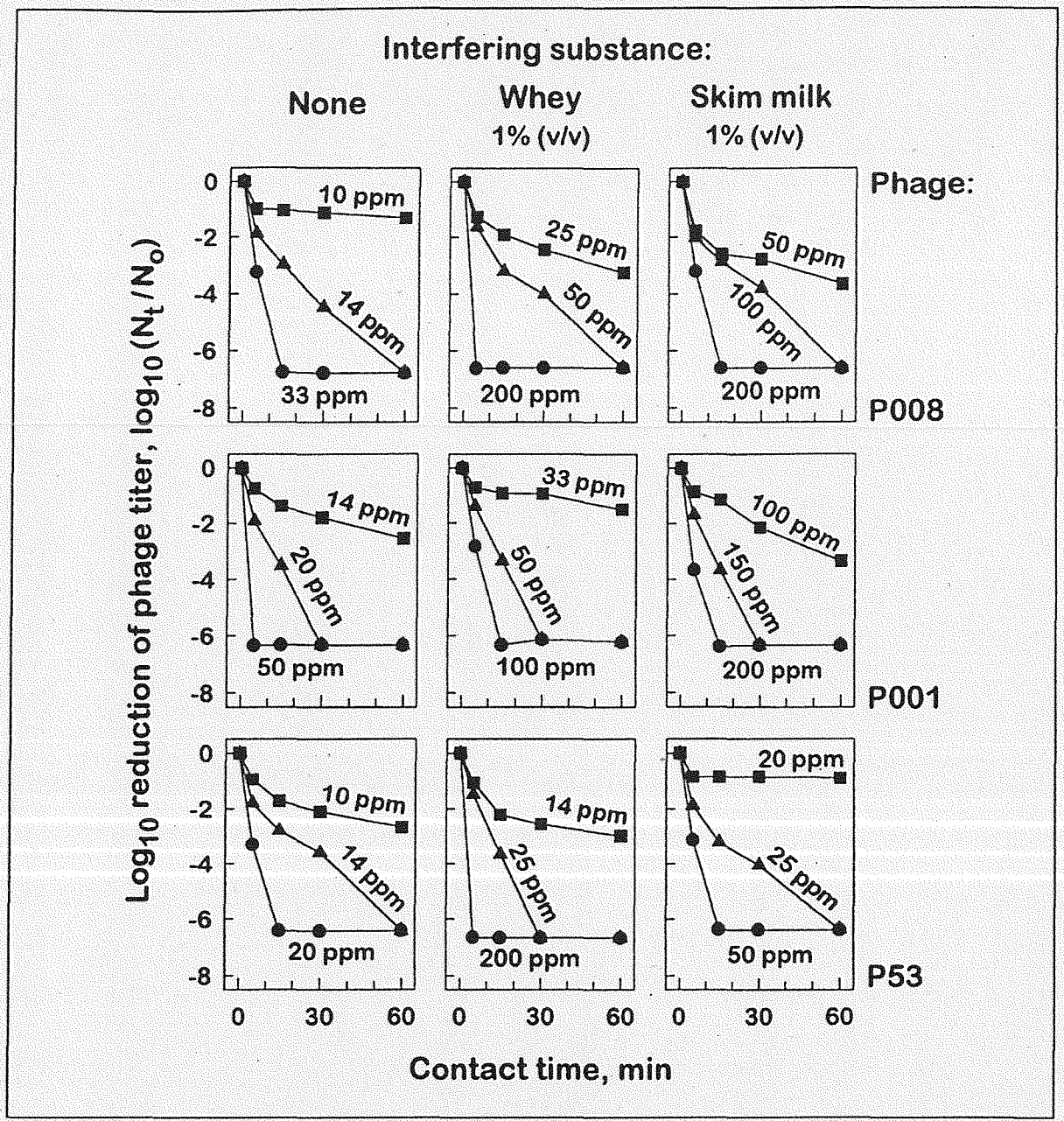

Fig. 4: Inactivation of the *L. lactis* phages P008 (top) and P001 (middle) and of the *S. thermophilus* phage P53 (bottom) by product A (consisting of a blend of peracetic acid and hydrogen peroxide) in a phage suspension test. The concentrations used in the tests are indicated in the graphs. The tests were performed in the absence of interfering substances (left) or in the presence of 1 % (v/v) acidic whey (middle) or 1 % (v/v) reconstituted skim milk (right).

Stability of the phage suspensions

The test phage suspensions in SM-buffer were routinely stored at 4°C. In order to assess, whether phage remained active during storage, phage titers were determined successively during an approximately 4-month period either for the undiluted test suspensions or for a 10-fold dilution (in SM-buffer). The results shown in Fig. 5 reveal that phage titers remained stable during storage for the whole period (115 days), indicating that these lysates could be used for the disinfection experiments also after prolonged storage without the risk of losses in phage titers.

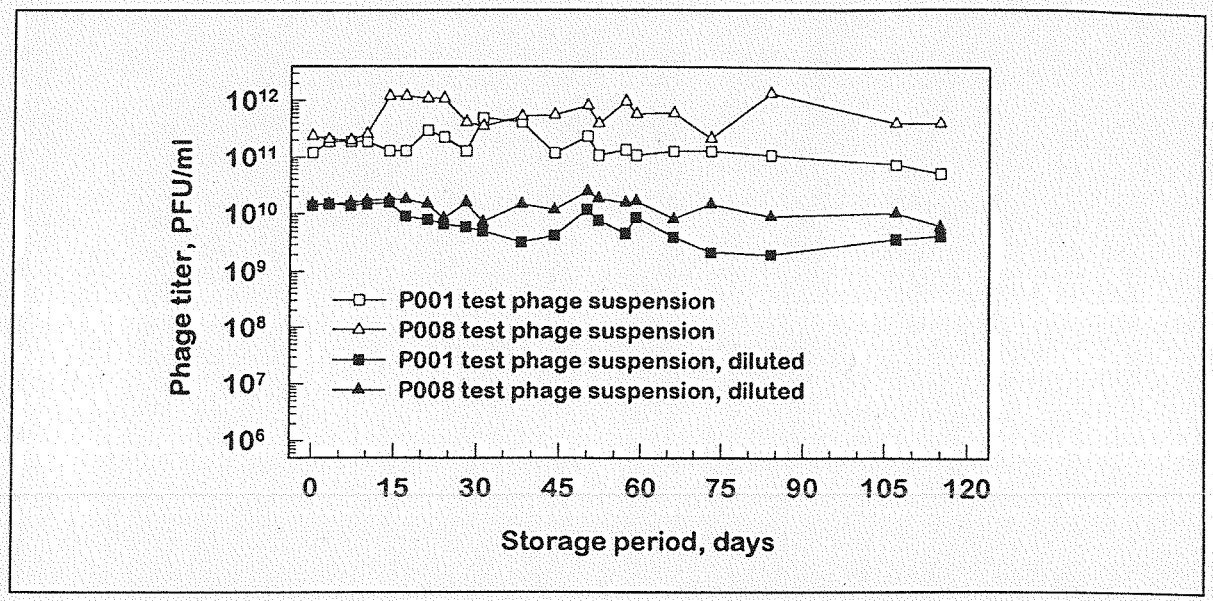

Fig. 5: Stability test of phage suspensions of *L. lactis* phages P008 and P001 in SM-buffer and of corresponding 10-fold dilutions (also in SM-buffer). After preparation, the phage suspensions were stored at 4°C and phage titers were determined repeatedly during a 115-d-period.

Effect of age of phage suspensions on the inactivation kinetics

For the lactococcal phage P008 and P001, disinfection experiments with sodium hypochlorite were done in parallel with fresh and with 4-months old phage suspensions under test conditions indicated in Fig. 6 (i.e., without and in the presence of interfering proteins). The response of the phages to the disinfectant was not significantly affected by the age of the phage suspensions.

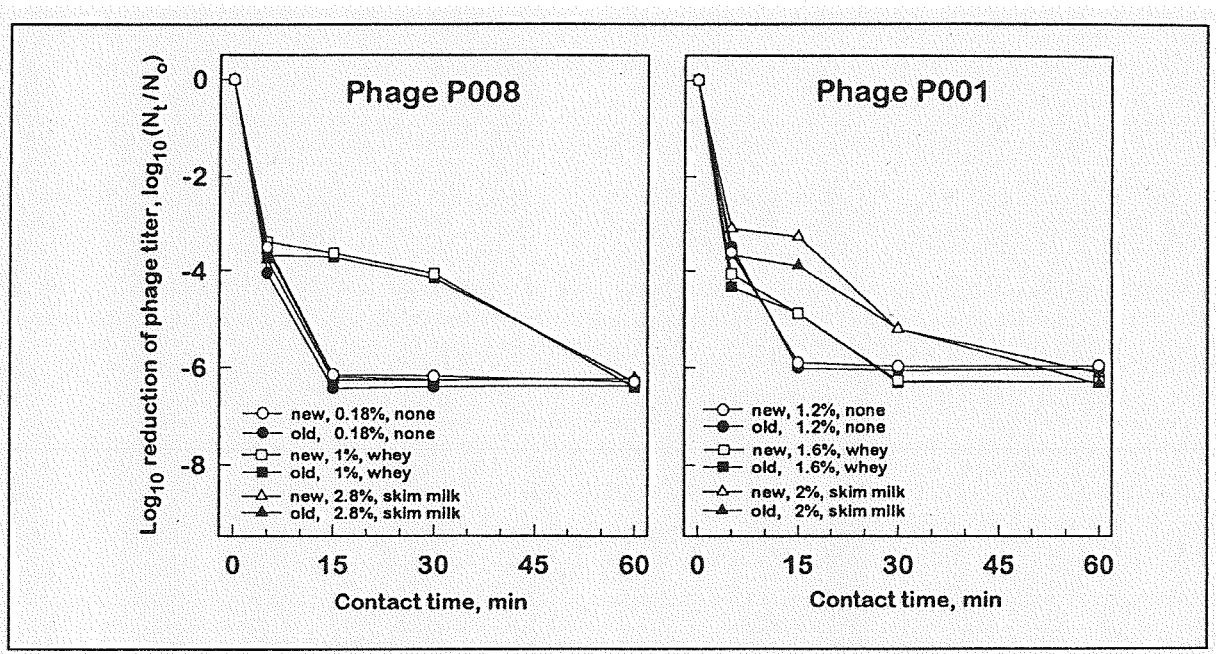

Fig. 6: Inactivation of the *L. lactis* phages P008 (left) and P001 (right) by sodium hypochlorite in a phage suspension test. The concentrations used in the tests are indicated in the graphs. The tests were performed in the absence of interfering substances or in the presence of 1 % (v/v) acidic whey or 1 % (v/v) reconstituted skim milk as indicated in the graphs. Disinfection trials were performed with freshly prepared phage lysates and with old lysates which have been stored for 4 months at 4°C as shown in the legends. The actual concentrations of the disinfectants are also shown in the legends.

4. Conclusions

The disinfection experiments reported in this study reveal that phages of lactic acid bacteria are suitable as model viruses for testing the virucidal activity of disinfectants. Small isometric-headed and prolate-headed lactococcal phages have also been used as test viruses in other studies (20, 22). All investigations have shown that these phages reveal different responses to disinfectants, justifying to consider both phage types as test viruses in a standard suspension test.

These phages are predominant in the dairy field (12, 13, 23). High-titer lysates which are stable during storage, can be obtained with ease as shown in this study. We have also shown that a phage lytic for *S. thermophilus* may be used as a test virus, since *S. thermophilus* phages are also a major cause for growth failure of thermophilic starter cultures (14, 15). With respect to the control of water quality, bacteriophages of other bacterial species have already been suggested earlier as model viruses for testing disinfectants, including phage of *Escherichia coli* (24, 25, 26, 27), phage of *Bacteroides fragilis* (26, 28, 29) and phage of *Pseudomonas aeruginosa* (30, 31). For one phage of the latter species, the morphological effects of disinfectants have also been studied in detail by electron microscopy (31).

Disinfectants become less effective in the presence of interfering substances (i.e. whey or skim milk). Thus testing the virucidal activity of disinfectants under conditions simulating their practical use is crucial. Since disinfection is usually done after cleaning (32), it was decided for the present study to use low concentrations of interfering proteinaceous substances in the test (i.e., 1 % [v/v] whey or skim milk solution, respectively).

In conclusion, harvesting lactococcal phage from agar plates revealing confluent lysis is a simple and convenient approach to obtain high-titer lysates. The phage lysates are stable for several months and the age of the lysate does not affect the results of the disinfection trials. It is suggested to adopt the methodology of this study for establishing a European standard suspension test for the assessment of the virucidal activity of disinfectants in the food area and related fields of the biotechnological industry.

Acknowledgement:

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5. References

1. Sanders, M.E.: In: Phage ecology (Goyal, S.M., Gerba, C.P., Bitton, G., eds.), New York: John Wiley & Sons, pp. 211-244 (1987)
2. Cogan, T.M, Accolas, J.P.: In: Dairy Microbiology, Vol. I., The microbiology of milk, 2nd. ed. (R.K. Robinson, ed.), London: Elsevier, pp. 77-114 (1990)
3. Hill, C.: FEMS Microbiology Reviews **12** 87-108 (1993)
4. Klaenhammer, T.R., Fitzgerald, G.F.: In: Genetics and biotechnology of lactic acid bacteria (Gasson, M.J., de Vos, W.M., eds.), London: Blackie A & P, pp. 106-168 (1994)
5. Neve, H.: In: Dairy starter cultures (Cogan, T.M., Accolas, J.-P., eds.), Weinheim, New York, Basel, Cambridge, Tokyo: VCH-Verlag, pp. 157-189 (1996)
6. Daly, C., Fitzgerald, G.F., Davis, R.: In: Lactic acid bacteria: Genetics, metabolism and applications (Venema, G., Huis in't Veld, J.H.J., Hugenholtz, J., eds.), Dordrecht: Kluwer Academic Publishers, pp. 3-14 (1996)
7. Everson, T.C.: Bulletin of the IDF **263** 24-28 (1991)
8. v. Klingeren, B., Leussink, A.B., Wijngaarden, L.J.: Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abt. B **164** 521-548 (1977)
9. Bloomfield, S.F., Arthur, M., Looney, E., Begun, K., Patel, H.: Letters in Applied Microbiology **13** 233-237 (1991)

10. Bloomfield, S.F., Looney, E.: Journal of Applied Bacteriology **73** 87-93 (1992)
11. Bloomfeld, S.F., Arthur, M., Begun, K., Patel, H.: Letters in Applied Microbiology **17** 119-125 (1993)
12. Braun, V., Hertwig, S., Neve, H., Geis, A., Teuber, M.: Journal of General Microbiology **135** 2551-1560 (1989)
13. Jarvis, A., Fitzgerald, G.F., Mata, M., Mercenier, A., Neve, H., Powell, I.B., Ronda, C., Saxelin, M., Teuber, M.: Intervirology **32** 2-9 (1991)
14. Krusch, U., Neve, H., Luschei, B., Teuber, M.: Kieler Milchwirtschaftliche Forschungsberichte **39** 155-167 (1987)
15. Neve, H., Krusch, U., Teuber, M.: Applied Microbiology and Biotechnology **30** 624-629 (1989)
16. Terzaghi, B.E., Sandine, W.E.: Applied Microbiology **29** 807-813 (1975)
17. Adams, M.H. (ed.): In: Bacteriophages, New York: Interscience Publishers, Inc., (1959)
18. Sambrook, J., Fritsch, E.F., Maniatis, T. (eds.): Molecular Cloning, Cold Spring Harbor Laboratory Press (1989)
19. Richardson, G.H. (ed.): In: Standard methods for the examination of dairy products. 15th ed., Washington: American Public Health Association, pp. 344-446 (1985)
20. Lembke, J., Teuber, M.: dmz Deutsche Molkerei-Zeitung **18** 2-6 (1981)
21. Anonymus: International standard FIL-IDF 73, Milchwissenschaft **31** 606-611 (1976)
22. v. Rheinbaben, F., Bansemir, K.-P., Heinzel, M.: Zentralblatt für Hygiene und Umweltmedizin **192** 419-431 (1992)
23. Neve, H., Kemper, U., Geis, A., Heller, K.J.: Kieler Milchwirtschaftliche Forschungsberichte **46** 167-178 (1994)
24. Harakeh, M.S.: FEMS Microbiology Letters **23** 27-30 (1984)
25. Maillard, J.-Y., Beggs, T.S., Day, M.J., Hudson, R.A., Russel, A.D.: Applied and Environmental Microbiology **60** 2205-2206 (1994)
26. Jofre, J., Ollé, E., Ribas, F., Vidal, A., Lucena, F.: Applied and Environmental Microbiology **61** 3227-3231 (1995)
27. Tyrrell, S.A., Rippey, S.R., Watkins, W.D.: Water Research **29** 2483-2490 (1995)
28. Tartera, C., Bosch, A., Jofre, J.: FEMS Microbiology Letters **56** 313-316 (1988)
29. Pintó, R.M., Abad, F.X., Roca, R.M., Riera, J.M., Bosch, A.: FEMS Micobiology Letters **82** 61-66 (1991)
30. Maillard, J.-Y., Beggs, T.S., Day, M.J., Hudson, R.A., Russel, A.D.: Letters in Applied Microbiology **17** 167-170 (1993)
31. Maillard, J.-Y., Hann, A.C., Beggs, T.S., Day, M.J., Hudson, R.A., Russel, A.D.: Journal of Medical Micobiology **42** 415-420 (1995)
32. Mrozek, H.: In: Reinigung und Desinfektion in der Lebensmittelindustrie (Wildbrett, G., ed.), Hamburg: Behr's Verlag, pp. 189-202 (1996)

6. Summary

Neve, H., von Maurich, A., Heller, K.J.: **Testing of the virucidal activity of disinfectants with bacteriophages of lactic acid bacteria**. Kieler Milchwirtschaftliche Forschungsberichte **48** (4) 359-370 (1996)

26 Bacteriophages of lactic acid bacteria (disinfectants, virucidal activity, suspension test)

To test the virucidal activity of disinfectants used in the area of food processing, virulent bacteriophages of lactic acid bacteria were chosen as model viruses for the preparation of a European CEN-standard, since these phages are wide-spread in dairies. For this quantitative suspension test, the well-characterized *Lactococcus lactis* phages P001 (prolate-headed phage type) and P008 (isometric-headed phage type) were used, which have already been deposited in national and international reference culture collections. The suitability of the method was also demonstrated for a virulent (isometric-headed) phage of *Streptococcus thermophilus* (phage P53), since phages of this bacterial species

have recently also been reported to cause significant decreases of lactic acid production in the factories. High-titer stock lysates were obtained, when phage-derived plaques were harvested from agar plates revealing confluent lysis (titer ranging from 1×10^{11} - 8×10^{11} plaque forming units [PFU] per ml). The stock phage lysates were diluted to obtain virus working suspensions revealing a titer of 1×10^{9} PFU/ml. To consider interfering proteinaceous substances, virucidal activity was tested in the presence of 1 % (v/v) acidic whey or - optionally - 1 % (v/v) skim milk. After incubation at room temperature (20°C), the test samples were diluted 50-fold in a validated neutralization solution. The phages tested revealed different levels of resistance. The titers of the stock lysates remained stable for several months. The phage lysates could be used for the test method during a storing period of several months at 4°C without a significant effect on reproducibility, indicating that sensitivity to disinfectants was not affected by the age of the lysates.

Zusammenfassung

Neve, H., von Maurich, A., Heller, K.J.: **Prüfung der viruziden Wirkung von Desinfektionsmitteln mit Bakteriophagen von Milchsäurebakterien.** Kieler Milchwirtschaftliche Forschungsberichte **48** (4) 359-370 (1996)

26 Bakteriophagen der Milchsäurebakterien (Desinfektionsmittel, Viruzidie, Suspensionstest)

Zur Viruzidieprüfung von Desinfektionsmitteln im Lebensmittelbereich wurden virulente Bakteriophagen von Milchsäurebakterien als Modellviren für die Erarbeitung eines europäischen CEN-Normenentwurf ausgewählt, da diese Phagen in den milchverarbeitenden Betrieben weit verbreitet sind. Für diesen quantitativen Suspensionstest wurden die gut charakterisierten *Lactococcus lactis* Phagen P001 (prolater Phagentyp) und P008 (isodiametrischer Phagentyp) verwendet, die bereits in nationalen und internationalen Referenz-Stammsammlungen hinterlegt wurden. Die Eignung des Verfahrens wurde auch für einen virulenten Bakteriophagen von *Streptococcus thermophilus* (Phage P53) gezeigt, da Phagen dieser Bakterienspecies auch in zunehmendem Maße Säuerungsstörungen in den Betrieben verursachen. Von den Phagen ließen sich hochtitrige Ausgangslysate durch Abernten der Plaques von Agarplatten mit konfluenter Lyse erzielen (Titer von 1×10^{11} - 8×10^{11} Plaque-bildende Einheiten pro ml [PbE/ml]). Die Ausgangslysate wurden für die Versuche auf einen Arbeitstiter von 1×10^{9} PbE/ml eingestellt. Zur Berücksichtigung eines Eiweißfaktors wurde die viruzide Wirkung in Gegenwart einer Proteinbelastung von 1 % Sauermolke oder - optional - 1 % Magermilch geprüft. Nach Inkubation bei Raumtemperatur (20°C) wurden die Versuchsansätze zur Inaktivierung des Desinfektionsmittels in einer validierten Neutralisationslösung 1:50 verdünnt. Die Phagen zeigten ein unterschiedliches Resistenzverhalten. Die Titer der Ausgangslysate blieben über mehrere Monate stabil. Die Phagenlysate konnten während einer mehrmonatigen Lagerung bei 4°C ohne nachweisbare Effekte auf die Reproduzierbarkeit in das Prüfverfahren eingebracht werden, so daß die Empfindlichkeit gegen die geprüften Desinfektionsmitel nicht vom Alter der Lysate beeinflußt wurde.

Résumé

Neve, H., von Maurich, A., Heller, K.J.: **Utilisation de bactériophages des bactéries lactiques pour l'étude de l'effet virucide des désinfectants.** Kieler Milchwirtschaftliche Forschungsberichte **48** (4) 359-370 (1996)

26 Bactériophages des ferments lactiques (désinfectants, activité virucide, test de suspension)

Pour tester l'activité virucide des désinfectants utilisés dans le domaine de la transformation des aliments, nous avons choisi des bactériophages virulents de bactéries lactiques comme virus modèles pour élaborer un standard CEN européen car ces phages sont très répandus dans les industries laitières. Pour ce test de suspension quantitatif nous avons utilisé les phages bien caractérisés P001 de *Lactococcus lactis* (phages à tête allongée, prolate) et P008 (à tête isodiamétrique) qui ont déjà été déposés dans les collections de cultures nationales et internationales. On a pu démontrer que la méthode est aussi apte à être utilisée pour un bactériophage virulent de *Streptococcus thermophilus* (phage P53) compte tenu que les phages de cette espèce bactérienne comptent parmi ceux qui sont responsables des difficultés d'acidification dans les industries laitières. Des stocks de phages à titre élevé étaient obtenus en prélevant sur boîte les plages de lyse confluantes (titres entre 1×10^{11} - 8×10^{11} unités formant des plages (ufp/ml)). Les stocks ont été dilués pour obtenir des suspensions de travail ayant un titre de 1×10^{9} ufp/ml. Pour tenir compte des substances protéiques interférentes, l'effet virucide a été testé en présence de sérum acide à 1 % (v/v) ou bien de lait écrémé à 1 % (v/v). Après incubation à température ambiante (20°C) les échantillons ont été dilués (1:50) dans une solution de neutralisation validée pour inactiver le désinfectant. Les phages étudiés présentaitent différent niveaux de résistance. Les titres des stocks étaient stables pendant plusieurs mois. Pour la méthode il était possible d'utiliser les stocks pendant plusieurs mois à 4°C sans observer d'effets significatifs sur la reproductibilité, indiquant gue la sensibilité aux désinfectants n'était pas influencée par l'âge des stocks de phages.