A short note on long-term stability of *Lactococcus lactis* bacteriophages in cheese brine

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1. Introduction

Salting is a commonly practised step during the production of many soft, semi-soft and hard cheeses (1-2). Dry salt can be added to the cheese curd directly (i.e., in Cheddar cheese). However, salting is frequently performed by immersion of formed cheese blocks into brine bathes containing a high concentration of sodium chloride (e.g., 18% to 21% for semi-soft cheeses). Brine bathes are in use continuously for long periods. Hence, there is a permanent leaching of cheese-derived residues (i.e., proteinaceous material, fat, inorganic substances, and microorganisms). A typical salt-tolerant microflora will therefore be present in the brine, and its composition will depend on the "house" microbial population of the cheese factory. For production of smear-ripened cheeses, the brine microflora has a significant effect on the development of the microbial surface layer (3-4). In order to reduce the number of unwanted brine microorganisms, brine bathes are treated periodically by pasteurisation or by filtration through membranes or filter systems (5-7).

In cheese factories, the occurrence of lytic phages infecting dairy starter cultures is still a main cause of faulty or delayed milk fermentation processes (8). Phages can easily spread inside the factory, in particular by phage-contaminated whey or whey aerosols (9). Several routes, how new phages may be introduced into the plant, are known. Raw milk may contain low phage numbers. This has been shown both for *Lactococcus lactis* and *Streptococcus thermophilus* phages (10-12). Temperatures used for standard milk pasteurisation processes have been reported to be ineffective for phage inactivation (13). New lytic phages may also originate from lysogenic starter cultures harbouring prophages in their chromosomal DNAs (8, 14). Induction of temperate phages occurs spontaneously but is also a response to stress conditions of the starter cultures (15-16).

The objective of the present study was to evaluate the abundance of phage in brine tanks of cheese factories and to assess the stability of the brine phage population.

2. Material and Methods

2.1 Sample preparations

Whey and brine samples were centrifuged for 20 min at 17,000xg at 4°C and subsequently filter-sterilized (0.45-µm Minisart filters; Sartorius, Hannover). For determination of phage numbers in cheese, 10 g of the cheese samples were resuspended in 90 ml of 2% tri-sodium citrate (pH 7.5) according to the VDLUFA guidelines (17).

2.2 Bacteria, phages and media

Mesophilic starter cultures were obtained directly from the cheese factories as deep-frozen starter concentrates. After one over-night growth passage in skim milk at 30°C, 26-52 single-colony isolates were obtained from serial dilutions plated on LM17 agar (18). Strains were grown in LM17 broth at 30°C and were frozen in reconstituted skim milk at -76°C for long-term storage. In order to identify phage-sensitive strains, a miniaturized starter activity test in microtiter plates was performed as described before (19).

Phages were propagated on phage-sensitive host strains in LM17 broth fortified with 10 mM $CaCl_2$ as reported before (19). For determination of phage/host spectra, 20 µl of phage lysate were spotted on lawns of different bacterial strains spread in LM17 soft agar on LM17 agar plates. Phage titers were determined by the double-layer plaque assay (20).

2.3 Electron microscopy

Transmission electron microscopy of phage specimen stained negatively with 2% uranyl acetate was done in a Tecnai 10 microscope (FEI Philips, Eindhoven, The Netherlands) at 80 kV as described before (21-22)

3. Results

3.1 Phage monitoring in brine samples of a large-scale cheese factory

For the phage monitoring in a large cheese factory producing semi-hard cheeses, four different starter cultures were analysed for the presence of strains susceptible to the factory phage population. Culture A was used regularly as the main culture, while cultures B and C were used irregularly as additional cultures. Culture D was not used in the factory but was included in this study as an external control culture containing strains sensitive to the factory phage population. The monitoring was initiated during a phage infection, when high phage numbers were detected in the cheese whey (up to $5x10^9$ plaque-forming units per ml [PFU/ml]). The whey-derived phages were only lytic for strains of starter culture A, but did not infect strains of cultures B, C, and D (Table 1). The same phage patterns were observed in cheese samples analysed before immersion into the brine bath. However, cheese samples collected from cheese blocks after brining did also contain phages active on cultures B, C and D, respectively. A broad spectrum of phages active against all four cultures was also found in the cheese brine, indicating that the cheese blocks became contaminated with phages during the passage in the brine bath (Table 1). Titers of phages specific for culture A were high in the brine ($9x10^7$ PFU/ml), whereas the numbers of phages specific for cultures B ($7x10^2$/ml), C ($5x10^5$/ml), and D ($4x10^5$/ml) were significantly lower (see columns from February 11, Fig. 1). In the brined cheese, phages infecting strains of culture A were enumerated as $6x10^8$ PFU/g.

Brine samples were also collected later during an 8-month interval in the same factory (February 23, July 15, October 8; Fig. 1). Although the phage titers varied in the brine samples, phages active against all four cultures were permanently detected.

3.2 Characterization and diversity of the phage population in brine

Representative brine phages were isolated from single plaques and were propagated on phage-sensitive host strains (i.e., strains A-8, B-10, C-52, and D-49 derived from cultures A-D). For determination of the efficiencies of plating on all culture isolates, lysates was tested with the four host strains from cultures A-D (Table 2). Two phages (P503 and

P502) did only propagate on test strains from the homologous cultures A (P503) and C (P502), while phages P504 (original host: culture B) and P505 (original host: culture D) did also grow on the test strains of culture C (P504) and B (P505). Phages isolated on indicator strains from cultures A, B, and C revealed the same morphology (i.e., small isometric-headed phages belonging to the *L. lactis* 936 phage species), while phage P505 propagated on the test strain of culture D was a member of the prolate-headed phage species c2 (23-24) (data not shown). These results illustrate the diversity of the phage population present in the cheese brine.

Tab. 1: Phage spectra of *L. lactis* strains from starter cultures A-D. 20-µl samples of whey, of resuspended cheese and of brine were spotted onto the lawns of the starter bacteria A-8, B-10, C-52, and D-49 derived from cultures A-D.

Sample:	Phage sensitive isolate from starter culture			
	A (in use)	B	C	D
Whey	+++ [a]	- [c]	-	-
Cheese before brining	+++	-	-	-
Cheese after brining	+++	++ [b]	++	++
Cheese brine	+++	++	++	++

[a] +++ Clear lysis zone in spot test.
[b] ++ Reduced lysis zone with confluent lysis in spot test.
[c] - No lysis zone in spot test (limit of detection: 50 PFU/ml).

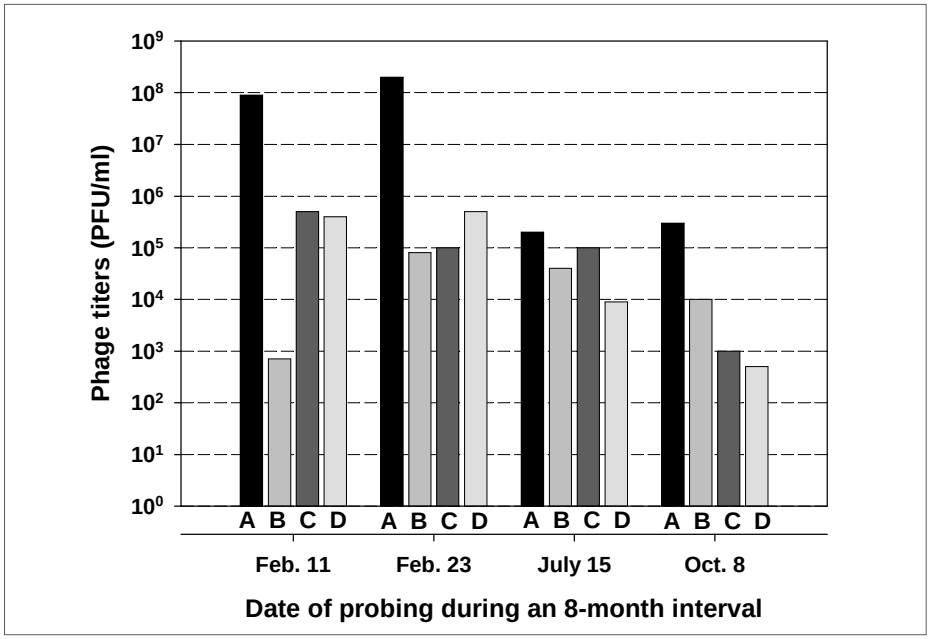

Fig. 1: *L. lactis* phage titers in the brine bath of a large-scale cheese factory. Samples taken from February to October during an 8-month period were analysed for the presence of lytic phages infecting phage-sensitive host strains A-8, B-10, C-52, and D-49 derived from cultures A-D. Culture A was used continuously.

Tab. 2: Efficiencies of plating of *L. lactis* phages isolated from the brine bath of a large-scale cheese factory. High-titer lysates were tested on the strains A-8, B-10, C-52, and D-49 derived from cultures A-D.

Culture isolate *L. lactis*	Phage titer (PFU/ml) of phage isolate			
	P503 (A) [a]	P504 (B) [a]	P502 (C) [a]	P505 (D) [a]
A (in use)	**$6x10^6$**	- [b]	-	-
B	-	**$3x10^{10}$**	-	30
C	-	$1x10^4$	**$2x10^{10}$**	-
D	-	-	-	**$3x10^8$**

[a] The original host cultures used for phage propagation are indicated.

[b] - No plaques (limit of detection: 10 PFU/ml)

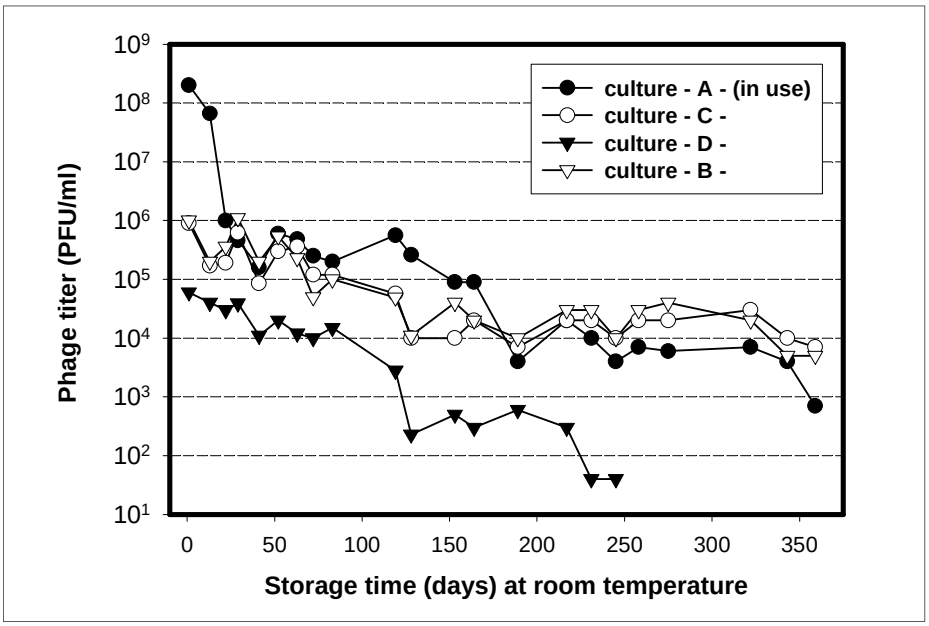

Fig. 2: Stability of *L. lactis* phages in a laboratory reference brine sample taken from a large-scale cheese factory. The sample was stored for 1 year at room temperature. Phage titers were determined regularly on phage-sensitive strains A-8, B-10, C-52, and D-49 derived from cultures A-D. At the time of sampling, culture A was used as the major starter culture.

3.3 Long-term stability of lactococcal phages in brine

Our experiments have demonstrated the presence of a diverse phage population in brine during several months resulting from leaching of phages from the cheese blocks into the brine. In order to exclude the influence of a permanent input of new phages, phage stability was also tested in a representative brine sample stored at room temperature in the laboratory for 12 months (Fig. 2). Phage titers were determined periodically in this

reference sample on phage-sensitive strains A-8, B-10, C-52, and D-49 representing cultures A - D. The high number of phages specific for culture A decreased rapidly within the first month by 2 orders of magnitude. After this period, the decline of culture A-specific phages was significantly slower and was very similar to the three phage titer curves obtained for cultures B-D. This indicates that approx. 99% of the phage population originating from the cheese blocks was inactivated in the brine within the first month in the reference sample. The surviving phage population was significantly more stable in the salt solution. At the end of the 1-year storage, phages were still detectable with titers of approx. 10^4 PFU per ml (Fig. 2).

3.4 Phage monitoring in brines derived from different cheese factories

In order to evaluate the dissemination of *L. lactis* phages in brine in different cheese factories, brine samples were obtained from seven factories of different geographic locations in Germany, including both large-scale enterprises (factories I and II) and small- to medium-scale factories (factories III to VII). Phage-sensitive *L. lactis* strains derived from the factory-specific starter cultures were used for the determination of phage numbers. High phage titers were recorded for all brine samples analysed (Table 3). In three brine samples (i.e., from factories III, IV, and V, respectively), two morphologically distinct phage types infecting different host strains were present in varying titers. In total, three different *L. lactis* phage morphotypes were found in the different brines (i.e., 936-like, c2-like, and P335-like phages), reflecting the biodiversity of the brine phage population. An unclassified phage revealing an uncommon morphology (isometric-headed, 200-nm long flexible, non-contractile tail) was also documented in the brine from factory IV (data not shown).

Tab. 3: Determination and analysis of *L. lactis* phage population in the brines of two large-scale and five small- to medium-scale enterprises. Phage titers were determined with phage-sensitive strains isolated from the factory-specific starter cultures. In three brines, two morphologically distinct phage types were found in different numbers.

Cheese factory	Factory type	Phage titer(s) (PFU/ml)	Phage species [(d)]
I	LSE [(a)]	$6x10^6$	c2
II	LSE	$8x10^7$	P335
III	SME [(b)]	$3x10^6$ / $2x10^8$ [(c)]	936 / P335
IV	SME	$3x10^5$ / $2x10^6$ [(c)]	P335 / unknown
V	SME	$1x10^6$ / $8x10^5$ [(c)]	936 / c2
VI	SME	$2x10^7$	936
VII	SME	$2x10^7$	936

[(a)] LSE Large scale enterprise

[(b)] SME Small / medium scale enterprise

[(c)] Two titers are indicated for two morphologically distinct phage types isolated with different host strains.

[(d)] Phage species were only assigned on basis of transmission electron microscopy. Phage typing refers to the *L. lactis* phage taxonomy according to (23). c2-phages are prolate-headed while 936- and P335-phages are small isometric-headed phages which differ in tail length. One isometric-headed phage from factory IV with a long (200 nm) non-contractible tail could not be classified.

4. Discussion

To our knowledge, presence of *L. lactis* phages in brine has only been reported once for a Swiss Raclette cheese factory (25), but the phage titers (approx. 10^4 PFU/ml) in the brine were significantly lower than found in our study. Our analysis confirms that brine bathes in cheese factories must be regarded as a large-volume phage reservoir, where high phage titers (> $1x10^8$ PFU per ml brine) can be found.

The brine phage population is originating from the constant leaching process of cheese blocks. Changes of the surface microstructure and development of the cheese surface barrier layer during brining has been studied by scanning electron microscopy (26). It was concluded that the microstructure of the surface of the cheese blocks of brine-salted cheese was much more compact than the microstructure 1 mm inside the block. It was further shown that the macro-network of water channels was less open near the surface of brined cheeses. These changes apparently do not inhibit the constant release of phage particles from the cheese blocks. Since the outside flux of water from the cheese is approximately twice the inside flux of NaCl from the brine solution (1-2), it can be assumed that a significant number of phages is migrating from the cheese blocks into the brine bathes.

By analysing phage stability in a reference laboratory sample, we demonstrated that approx. 99% of the brine phage population was inactivated within the first month of storage at room temperature. The remaining population, however, appeared to be much more stable, surviving in significant numbers a 1-year storage. Hence brine bathes offer the possibility to analyse the diversity of a phage population in a cheese factory in retrospect, since phages infecting *L. lactis* cultures which were either not in use regularly during the sampling period or not used at all in the factory, could easily be isolated from the brine.

Presence of phages in high-concentrated NaCl environments has been examined for the halophilic host *Halobacterium cutirubrum* capable of growing in saturated NaCl solutions (27). These “halophages” revealed low virulence at high salinity but adopted higher virulence when the salinity was reduced. This response was discussed to allow efficient phage propagation under conditions unfavourable for the host strains, which are likely to die at low salinity. Lactococcal cultures are not active at the high salt conditions of brine bathes, ruling out the possibility of lactococcal phage proliferation in the brine. For *Listeria monocytogenes* it has been shown, that Gram-positive bacteria have the potential to survive for more than 200 days in commercial brine bathes (28).

The preservation of phages in high-molar salt solutions is in fact daily routine in laboratories working with phages. For the purification and centrifugation of phage particles, isopycnic ultracentrifugation steps are performed through CsCl gradients. The phage suspensions are subsequently stored at 4°C in the CsCl solution to assure long-term stability (29).

Brine tanks of large factories may contain large volumes of saline (i.e., >200,000 l). Periodically, the microbial load has to be reduced either by pasteurisation or by filtration through membranes or filter units (5-7). Both strategies, however, do not guarantee a complete inactivation of the brine phage population. Lactococcal phages are known to survive the thermal conditions of pasteurisation (13). Although tested in milk, microfiltration studies have also shown that dairy phages are not completely retained (30-31).

From a practical point of view it must be noted that the personnel of a cheese factory practising cheese brining must be aware of the potential of brine to preserve the lytic activity of dairy phages. Brine bathes are usually areas of high humidity due to condensation

and development of aerosols. These conditions are known to facilitate the distribution of airborne dairy phages (9). Therefore it is strongly recommended to strictly avoid the dispersal of phage-contaminated brine back into the fermentation hall.

Acknowledgement

We would like to thank Inka Lammertz, Angela Back and Bernd Fahrenholz for their technical assistance. The financial support from the board of the dairy company involved in the initiation of the first part of the study is gratefully acknowledged. The second part of this research project was supported by the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn), the AiF and the Ministry of Economics and Technology (AiF-Project No.: 14339 N II). We also thank Prof. Jörg Hinrichs and Dipl.-LM-Ing. Mareile Müller-Merbach (University Hohenheim) for cooperation and discussion within the framework of the AiF project.

5. References

(1) Sutherland, B.J.: Salting of cheese. *In*: Roginski, K., Fuquay, J.W., Fox, P.F. (*eds*) Encyclopedia of dairy sciences. pp. 293-300, Elsevier (2002)

(2) Guinee, T.P., Fox, P.F.: Salt in cheese: Physical, chemical and biological aspects. *In*: Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (*eds*) Cheese: Chemistry, physics and microbiology. 3rd ed., Vol. 1: General aspects. pp. 207-259 Elsevier (2004)

(3) Bockelmann, W.: Smear-ripened cheeses. *In*: Roginski, K., Fuquay, J.W., Fox, P.F. (*eds*) Encyclopedia of dairy sciences. pp. 2391-2401, Elsevier (2002)

(4) Mounier, J., Gelsomino, R., Goerges, S., Vancanneyt, M., Vandemeulebroecke, K., Hoste, B., Scherer, S., Swings, J., Fitzgerald, G.F., Cogan, T.M.: Surface microflora of four smear-ripened cheeses. Applied Environmental Microbiology **71** 6489-6500 (2005)

(5) Bruch, R.: Membranfiltration in Molkereien. Deutsche Molkereizeitung **10** 494-505 (1998)

(6) Kammerlehner, J.: Salzlakeregeneration. Deutsche Molkereizeitung **5** 210-212 (1999)

(7) Laackmann, E., Laackmann, H.-P.: Salzbadpflege in einer Käserei. Deutsche Milchwirtschaft **12** 498-501 (2003)

(8) Josephsen, J., Neve, H.: Bacteriophage and antiphage mechanisms of lactic acid bacteria. *In*: Salminen, S., von Wright, A., Ouwehand, A. (*eds*), Lactic acid bacteria – Microbiology and functional aspects, 3rd ed., pp. 295-350, Marcel Dekker (2004)

(9) Neve, H., Berger, A., Heller, K.J.: A method for detecting and enumerating airborne virulent bacteriophages of dairy starter cultures. Kieler Milchwirtschaftliche Forschungsberichte **47** (3) 193-207 (1995)

(10) McIntyre, K., Heap, H.A., Davey, G.P., Limsowtin, G.K.Y.: The distribution of lactococcal bacteriophage in the environment of a cheese manufacturing plant. International Dairy Journal 1: 183-197 (1991)

(11) Bruttin A., Desiere, F., D'Amico, N., Guerin, J.-P., Sidoti, J., Huni, B., Lucchini, S., Brüssow, H.: Molecular ecology of *Streptococcus thermophilus* bacteriophage infections in a cheese factory. Applied and Environmental Microbiology **63** 3144-3150 (1997)

(12) Madera, C., Monjardin, C., Suarez, J.E.: Milk contamination and resistance to processing conditions determine the fate of *Lactococcus lactis* bacteriophages in dairies. Applied and Environmental Microbiology **70** 7365-7371 (2004)

(13) Chopin, M.C.: Resistance of 17 mesophilic lactic *Streptococcus* bacteriophages to pasteurization and spray-drying. Journal of Dairy Research **47** 131-139 (1980)

(14) Moineau, S., Pandian, S., Klaenhammer, T. R.: Evolution of a lytic bacteriophage via DNA acquisition from the *Lactococcus lactis* chromosome. Applied and Environmental Microbiology **60** 1832-1841 (1994)

(15) Chopin, A., Bolotin, A., Sorokin, A., Ehrlich, S. D., Chopin, M.C.: Analysis of six prophages in *Lactococcus lactis* IL1403: different genetic structure of temperate and virulent phage populations. Nucleic Acids Research **29** 644-651 (2001)

(16) Lunde, M., Aastveit A.H, Blatny J.M., Nes, I.F.: Effects of diverse environmental conditions on phiLC3 prophage stability in *Lactococcus lactis*. Applied and Environmental Microbiology **71** 721-727 (2005)

(17) Anonymous: VDLUFA Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik (Methodenbuch) Band VI, p. M5.2, 6. Ergänzung (2003) VDLUFA Verlag Darmstadt

(18) Terzaghi, B.E., Sandine, W.E.: Improved medium for lactic streptococci and their bacteriophages. Applied Microbiology **29** 807-813 (1975)

(19) Neve, H, Kemper, U., Geis, A., Heller, K.J.: Monitoring and characterization of lactococcal bacteriophages in a dairy plant. Kieler Milchwirtschaftliche Forschungsberichte **46** (2) 167-178 (1994)

(20) Adams, M.H.: Bacteriophages. Interscience Publishers, Inc., New York (1959)

(21) Neve, H., Back, A., Heller, K.J.: Prophage screening in a *Streptococcus thermophilus* starter culture collection. Kieler Milchwirtschaftliche Forschungsberichte **56** (4) 265-275 (2004)

(22) Vegge, C.S., Brondstedt, L., Neve, H., Mc Grath, S., van Sinderen, D., Vogensen F.K.: Structural characterization and assembly of the distal tail structure of the temperate lactococcal bacteriophage TP901-1. Journal of Bacteriology **187** 4187-4197 (2005)

(23) Jarvis, A.W., Fitzgerald, G.F., Mata, M., Mercenier, A., Neve, H., Powell, I.B., Ronda, C., Saxelin, M., Teuber, M.: Species and type phages of lactococcal bacteriophages. Intervirology **32** 2-9 (1991)

(24) Neve, H., Laborius, A., Heller, K.J.: Renaissance alter Phagentypen? Deutsche Molkerei Zeitung **15** 25-29 (2004)

(25) Schmidlin, J., Spillmann, H.: Phagenkontaminationslage in schweizerischen Käsereien: III. Stufenkontrolle in einer Raclette-Käserei. Schweizerische Milchwirtschaftliche Forschung **8** 65-70 (1979)

(26) Melilli, C., Carco, D., Barbano, D.M., Tumino, G., Carpino, S., Licitra, G.: Composition, microstructure, and surface barrier layer development during brine salting. Journal of Dairy Science **88** 2329-2340 (2005)

(27) Daniels, L.L., Wais, A.C.: Virulence in phage populations infecting *Halobacterium cutirubrum*. FEMS Microbiology Ecology **25** 129-134 (1998)

(28) Larson, A.E., Johnson, E.A., Nelson, J.H.: Survival of *Listeria monocytogenes* in commercial cheese brines. Journal of Dairy Science **82** 1860-1868 (1999)

(29) Sambrook, J., Russel, D.W.: Molecular cloning: A laboratory manual. 3rd ed. pp. 2.47-2.51, Cold Spring Harbor Laboratory Press (2001)

(30) Mistry, V.V., Kosikowski, F.V.: Influence of milk ultrafiltration on bacteriophages of lactic acid bacteria. Journal of Dairy Science **69** 2577-2582 (1986)

(31) Gautier, M., Rouault, A., Mejean, S., Fauquant, J., Maubois, J.L.: Partition of *Lactococcus lactis* bacteriophage during the concentration of micellar casein by tangential 0.1 µm pore size microfiltration. Lait **74** 419-423 (1994)

6. Summary

Neve, H., Dietrich, J., Heller, K.J.: **A short note on long-term stability of *Lactococcus lactis* bacteriophages in cheese brine**. Kieler Milchwirtschaftliche Forschungsberichte **57** (3) 191-200 (2005)

26 Microbiology (*Lactococcus lactis,* starter cultures, bacteriophages, brine, phage stability)

Brine samples from a large-scale cheese factory were analysed during an 8-month period for the presence of *Lactococcus lactis* bacteriophages. Phages infecting the main starter culture were found in high numbers in the brine throughout the whole sampling time (up to $2x10^8$ plaque-forming units [PFU] per ml). Lower phage titers were also documented in the brine samples continuously with strains from two additional cultures that were used irregularly as additional cultures. The phages belonged to the small isometric-headed 936 phage species. Control strains of a fourth culture (not used in the cheese factory) allowed the isolation and propagation of another distinct prolate-headed c2-like phage type from the same brine samples. A reference brine sample was stored in the laboratory for a 1-year period at room temperature, and phage titers were determined regularly with test strains from all four lactococcal cultures. Although 99% of the initial phage population was inactivated within the first month of storage, the residual phage population revealed a notably long-term high stability. At the end of the 1-year storage, phages were still detectable with titers of approx. 10^4 PFU per ml. High phage numbers were also recorded in brine samples from seven other cheese factories. Hence brine bathes must be regarded as a large-volume phage reservoir with high numbers of infective phages.

Zusammenfassung

Neve, H., Dietrich, J., Heller, K.J.: **Eine Kurzmitteilung über die Langzeitstabilität von *Lactococcus lactis* Bakteriophagen in Käsesalzlake**. Kieler Milchwirtschaftliche Forschungsberichte **57** (3)191-200 (2005)

26 Mikrobiologie (*Lactococcus lactis,* Starterkulturen, Bakteriophagen, Salzlake, Phagenstabilität)

Salzbadproben einer großen Käserei wurden über einen 8-monatigen Zeitraum auf die Anwesenheit von *Lactococcus lactis* Bakteriophagen untersucht. Über den gesamten Untersuchungszeitraum wurden Phagen in hoher Zahl (bis zu $2x10^8$ Plaque-bildende Einheiten [PbE] pro ml) nachgewiesen, die Stämme der Betriebsstarterkultur infizieren konnten. Die Salzbadproben enthielten auch in niedriger Konzentration Phagen für zwei weitere Kulturen, die unregelmäßig als Zusatzkulturen verwendet wurden. Die nachgewiesenen Phagen wurden der 936-Phagenspecies mit isodiametrischen Köpfen zugeordnet. Mit einem Kontrollstamm aus einer Kultur, die nicht im Betrieb verwendet wurde, gelang die Isolierung und Anzucht eines anderen Phagentyps (c2-Phagengruppe mit prolaten Köpfen). Mit Teststämmen aller vier Laktokokken-Kulturen wurden die Phagentiter regelmäßig in einer Salzbad-Rückstellprobe, die für ein Jahr im Labor bei Raumtemperatur gelagert wurde, bestimmt. 99% der ursprünglichen Phagenpopulation wurden zwar bereits im ersten Monat der Lagerung inaktiviert, jedoch wiesen die überlebenden Phagen eine bemerkenswerte Langzeitstabilität auf. Somit waren nach einem Jahr noch Phagen in einer Konzentration von nahezu 10^4 PbE pro ml nachweisbar. Hohe

Phagentiter wurden auch in Salzbadproben aus sieben weiteren Käsereien nachgewiesen. Daher müssen Salzbäder als großvolumiges Phagenreservoir mit hohen Konzentrationen an infektiösen Phagen betrachtet werden.

Résumé

Neve, H., Dietrich, J., Heller, K.J.: **Flash sur la stabilité longue durée des bactériophages *Lactococcus lactis* en saumure fromagère.** Kieler Milchwirtschaftliche Forschungsberichte **57** (3) 191-200 (2005)

26 Microbiologie (*Lactococcus lactis*, cultures de levain, bactériophages, saumure, stabilité des phages)

Pour une période de 8 mois, des échantillons de saumure d'une grande fromagerie étaient analysés sur la présence de bactériophages *Lactococcus lactis*. Tout au long de la période d'examen, un nombre élevé de phages étaient détectés (jusqu'à $2x10^8$ unités formant plaque (pfu) par ml) qui pouvaient infecter les souches des cultures de levain principales. Les échantillons de saumure contenaient également des phages à faible concentration pour deux autres cultures régulièrement utilisées comme culture complémentaire. Les phages détectés étaient attribués au groupe de phages de l'espèce 936 à tête isométrique. Grâce à une souche de contrôle d'une culture n'étant pas employée dans la fromagerie, on a réussi à isoler et à propager un autre type de phage (groupe de phage c2 à tête prolongée). Avec des souches d'essai des quatre cultures *Lactococci,* les titres de phage étaient régulièrement déterminés dans un échantillon de référence de saumure, conservé à température ambiante au laboratoire pour un an. 99 % de la population originale étaient déjà inactivés pendant le premier mois de stockage, néanmoins les phages survivants faisaient preuve d'une stabilité longue durée remarquable. Ainsi, même après un an, des phages étaient encore détectables dans une concentration d'environ 10^4 unités formant plaque (pfu) par ml. Des titres de phage élevés étaient également détectés dans des échantillons de saumure provenant de sept autres fromageries. C'est la raison pour laquelle les bains de saumure doivent être considérés comme grand réservoir de phage à forte concentration de phages infectieux.