A method for detecting and enumerating airborne virulent bacteriophage of dairy starter cultures

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1. Introduction

The permanent threat of severe bacteriophage infections in the dairy environment requires as a main bacteriophage control measurement careful phage monitoring procedures (1). Routinely, this is done by a starter activity test with factory-derived whey samples (2, 3). When internal sources of phage contamination have to be identified, samples taken from successive steps of the production line are also analyzed (e.g., raw milk samples and samples from the bulk starter). During the production of fresh cheese (quarg, cottage cheese) with mesophilic lactic acid streptococci (lactococci), phage can be easily distributed in aerosols, since these production processes demand drainage of whey by means of separators (4). In whey, phage titers can reach up to 10^9-10^{10} phage per ml (5). Therefore, when this phage reservoir is aerosolized, the phage load in the ambient air close to the separator can be tremendous.

In principal, sampling of phage particles from the ambient air may be done by impingement in liquid, impactation on agar surface or by filtration (6). We have used the impingement technique before, to determine the phage dissemination in various ambient air samples from dairy environments (7, 8, 9). However, since sampling is done in gas washing bottles containing a diluted broth as the collecting fluid, this method is not suitable when many samples have to be collected and when the bottles have to be transported to the laboratory. Moreover, only moderate flow rates can be used (up to approximately 10 liters per min), ruling out a rapid screening of large air volumes. As a result, the limit of detection is undesirably high (e.g., $1x10^4$ phage per m^3 ambient air [9]). This prompted us to test whether the filtration technique is suitable for collecting airborne bacteriophages of dairy starter cultures. Following sampling on gelatin filters with a commercially available air sampling apparatus, the viability of the collected phage was assessed under various transportation and storage conditions. Dissimination of airborne phage was documented in a dairy plant in the fresh cheese production area.

2. Material and methods

Bacteria, phages and growth conditions

During a 9-months-period (from May 1994 to January 1995), samples from bulk starter and whey samples were analyzed for the presence of phage. An undefined mixed-strain starter culture of *Lactococcus lactis* (designated as culture B) was used throughout the whole period without rotation of alternative starter cultures. Deep-frozen culture concentrates were inoculated directly into the bulk starter tank. For phage analysis, 52 single-colony isolates were prepared from the culture. Litmus skim milk or M17-broth (10) was used as growth medium (incubation temperature: 30°C). 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.

Two phages which had been isolated from whey samples collected in an earlier investigation in the same factory (9) were included in this study (small isometric-headed phage P463, prolate-headed phage P469). These phages were purified through 3

subsequent single-plaque isolation steps performed with the host strains *Lactococcus lactis* B113 (for phage P463) and *Lactococcus lactis* C22 (for phage P469). High-titer stocks from these phages ($1x10^{11}$ PFU/ml) were obtained in SM-buffer (see Table 1) after cesium chloride step gradient centrifugation.

Determination of starter activity

A miniaturized starter activity test was routinely performed with the 52 bacterial isolates in microplates as described before (9). Acidification capacity of the strains was scored after overnight incubation at 30°C. Phage sensitivity was further verified by monitoring the pH of skim milk during fermentation (30°C) as follows: 50 ml of milk was inoculated with 2 % of individual bacterial strains (overnight cultures) and with 1 % of whey (containing different phage titers).

Sampling

Bulk starter and whey samples were collected from the factory's staff and were prepared for phage analysis as described before (9).

Air sampling was done with an air sampler (model MD8, Sartorius, Göttingen, Germany) on sterile gelatin filter (pore size: 3 µm, diameter: 80 mm). A constant flow rate of 100 liters per min was used. Sampling was routinely limited to 3-6 min, but longer collecting periods up to 12 min were also tested. After sampling, the gelatin filters were transferred immediately into sterile plastic bags. Sample bags specified for a stomacher lab blender (size: 10x15 cm; Seward Laboratory, London, UK) were used. Each filter was immersed in 5 ml of buffer before sealing of the bags. Different buffer systems were tested and are listed in Table 1. It was also tested whether delayed addition of buffer (4 h or 24 h after sampling) would influence viability of phage. Samples were routinely chilled at 4°C. Alternatively, they were left at room temperature or were frozen at -20°C.

Tab. 1: Buffers used for immersion of gelatin filters

Buffer	Composition / references
Sodium phosphate buffer, 0.15 M (pH 7.6)	Prepared from 150 mM $NaH_2PO_4 \times H_2O$ & 150 mM Na_2HPO_4
SM-buffer (pH 7.5)	100 mM NaCl, 8 mM $MgSO_4 \times 7 H_2O$, 50 mM Tris-HCl
TMGS-buffer (pH 7.4)	145 mM NaCl, 10 mM $MgSO_4 \times 7 H_2O$, 10 mM Tris-HCl
1/4-strength Ringer's solution	See reference (13)
1/4-strength Ringer's solution / 10 % (vol/vol) skim milk [a]	See reference (13)
1/4-strength Ringer's solution / 10 % (vol/vol) M17 broth	See references (10 & 13)

[a] 1/4-strength Ringer's solution / 50% (vol/vol) skim milk or pure skim milk were also tested.

Preparation of gelatin filters and determination of phage titers

Immersed filters were liquified for 2 min in a 37°C water bath and were subsequently squeezed by hand using an ink roller for 2 min to assure uniform resuspension and homogenization of the samples. It was also tested whether a 2-min-treatment in a stomacher lab blender apparatus (model 400; Seward Laboratory, London, UK) would allow better resuspension than the manual treatment. For the determination of the phage

titers (plaque-forming units [PFU]) per volume, 0.3-ml-samples were used in the agar double layer technique (plaque assay [11]). A decimal dilution row was used in case of high phage titers. Plaque assays were done in dublicate or triplicate with a single-colony isolate from the starter culture B which had been identified before as phage-sensitive in the miniaturized starter activity test (*Lactococcus lactis*-strain B7). Phage titers were converted to PFU per m^3 ambient air.

3. Results and discussion

Activity tests and phage titers

During the 9-months-period, samples were collected from the bulk starter (n = 162) and from whey (n = 136) on 197 days. Activity of 52 individual isolates obtained from the mesophilic undefined mixed-strain starter culture B was determined in the presence of these samples (Fig. 1). In general, approximately 20 % of the strains tested revealed good growth (acidification) in the presence of whey samples thoughout the 9 months. When the bulk starter samples were tested, acidification was either seen for all strains or for only about 40 % of the individual strains. This response of the culture to continuous phage pressure can be described best as a phage carrier state (also designated as pseudolysogeny [12]): Although phage are permanently present in the dairy environment, the overall phage resistance of the culture is due to a limited number of phage-insensitive strains.

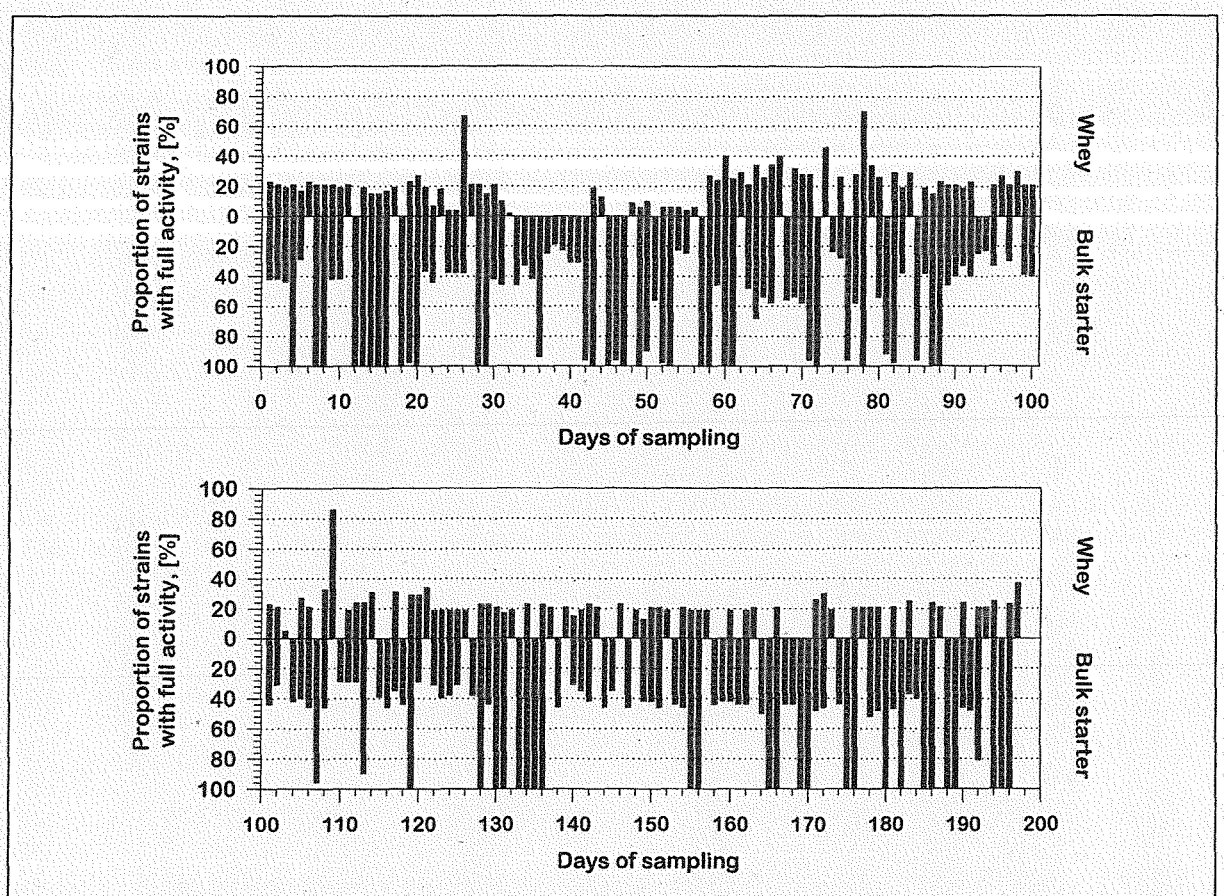

Fig. 1. Distribution of 52 lactococcal single-colony isolates (in %) with respect to their capacity of acidification in the presence of samples from whey (top) and from bulk starters (bottom) collected on 197 days in the dairy factory during a 9-months-period (days without sampling are not indicated in this graph). The bars show the proportion of strains revealing full activity. Strains revealing reduced or no activity are not further specified in this scheme. Missing bars indicate that no corresponding samples had been collected.

In order to assess phage-insensitivity of the 52 individual isolates, their growth response in the presence of 60 phage-containing samples collected in the beginning of the 9-months-period were evaluated (summarized in Figure 2). 10 strains revealed full activity in the presence of at least 80 % of the samples analyzed (e.g., strains B2, B3, B23). However, the majority of the strains tested only showed full activity in the presence of less than 20 % of the samples (e.g., strains B7, B8, B10). According to these results, strain *Lactococcus lactis* B7 was chosen as a suitable indicator strain for the determination of phage titers (in plaque assays) in this study.

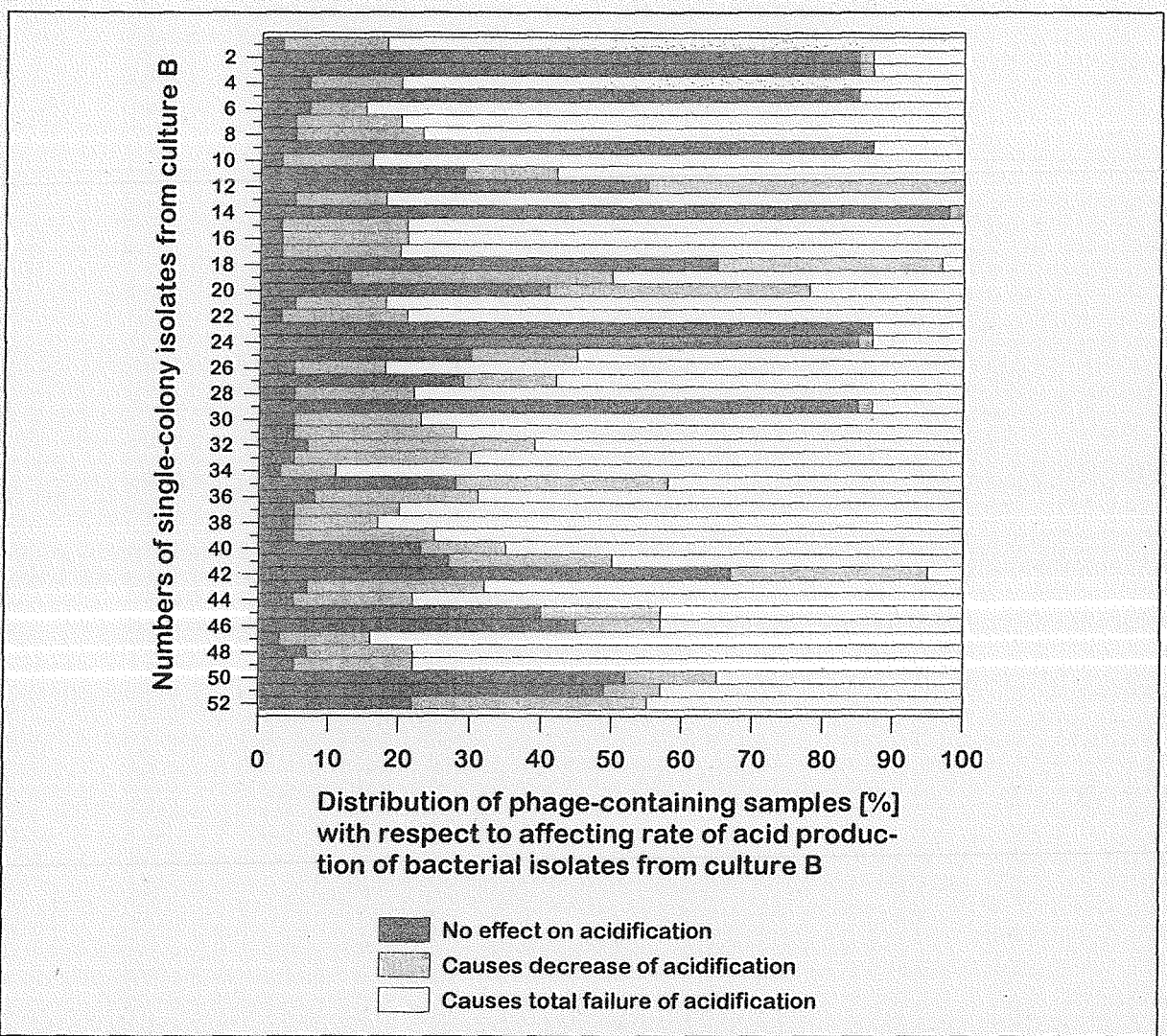

Fig. 2. Distribution of the first 60 phage-containing samples (in %) derived from the factory (either bulk starter or whey samples) with respect to the growth response (ability of acidification) of the 52 lactococcal single-colony isolates.

Phage titers were determined in the bulk starter and whey samples derived from the first 44 sampling days (Table 2). In whey, titers varied from $1x10^6$ to $1x10^9$ PFU/ml whey. Phage concentration in bulk starter samples fluctuated from $1x10^1$ to $1x10^{10}$ PFU/ml. No growth failure of individual strains in the microplate assay after overnight incubation was detected, when samples with low phage titers were used ($1x10^1$ to $4x10^2$ PFU/ml; except 1 sample containing $1x10^2$ PFU/ml), but growth was affected in the presence of samples containing higher phage titers. Thus, the lowest limit of detection of starter growth failure in the miniaturized starter activity test is in the range of approximately $1x10^2$ PFU/ml.

Tab. 2: Phage titers in whey and bulk starter samples collected during the first 44 sampling days of this investigation and corresponding results from miniaturized starter activity tests in microplates.

Day of sampling	Phage titers[a] in whey	Detection of growth failure in microplate assays with whey samples[b]	Phage titers[a] in bulk starters	Detection of growth failure in microplate assays with bulk starter samples[b]
1	2×10^8	+	1×10^8	+
2	4×10^6	+	1×10^8	+
3	7×10^6	+	1×10^8	+
4	3×10^7	+	n.d.	-
5	5×10^7	+	7×10^7	+
6	1×10^7	+	./.	./.
7	2×10^7	+	n.d.	-
8	5×10^8	+	n.d.	-
9	5×10^8	+	2×10^8	+
10	9×10^7	+	1×10^8	+
11	1×10^5	+	./.	./.
12	./.	./.	n.d.	-
13	3×10^8	+	4×10^2	-
14	2×10^8	+	n.d.	-
15	9×10^8	+	n.d.	-
16	3×10^8	+	1×10^1	-
17	2×10^8	+	./.	./.
18	./.	./.	n.d.	-
19	1×10^9	+	1×10^3	+
20	4×10^8	+	3×10^2	-
21	5×10^8	+	2×10^8	+
22	8×10^6	+	9×10^7	+
23	1×10^6	+	./.	./.
24	1×10^7	+	1×10^{10}	+
25	1×10^8	+	6×10^9	+
26	4×10^6	+	5×10^8	+
27	1×10^8	+	./.	./.
28	4×10^8	+	n.d.	-
29	5×10^8	+	n.d.	-
30	1×10^8	+	2×10^6	+
31	2×10^7	+	6×10^7	+
32	2×10^6	+	./.	./.
33	./.	./.	4×10^5	+
34	./.	./.	6×10^8	+
35	./.	./.	1×10^8	+
36	./.	./.	n.d.	-
37	./.	./.	6×10^6	+
38	./.	./.	4×10^7	+
39	./.	./.	6×10^7	+
40	./.	./.	2×10^8	+
41	./.	./.	1×10^8	+
42	./.	./.	1×10^2	+
43	3×10^8	+	2×10^1	-
44	./.	./.	n.d.	-

(a) Phage titers are indicated in plaque-forming units (PFU) per ml sample. ./. No sample collected.
n.d. Not detectable (< 5 PFU/ml).

(b) + Growth failure of individual strains (no acidification).
- No growth failure of individual strains detected. ./. No sample collected.

In order to confirm that reduced acidification of milk was due to retarded growth or total growth failure of individual strains in the presence of phage, a representative phage-sensitive (strain B7) and a phage-resistant isolate (strain B23) in addition to the original intact culture B were grown overnight (30°C) in skim milk supplemented with whey samples (1 % vol/vol) containing different phage titers ($3x10^2$ PFU/ml, $3x10^5$ PFU/ml, $3x10^8$ PFU/ml; Figure 3). Phage-resistant strain B23 was not affected by the presence of phage. Acidification of milk by phage-sensitive strain B7 was halted after addition of low phage numbers and was stopped totally in the presence of high phage titers. However, the original culture B revealed good or still significant activity under these conditions. This reflects the good 'phage buffering capacity' of the whole culture due to its adaptation to the phage carrier state.

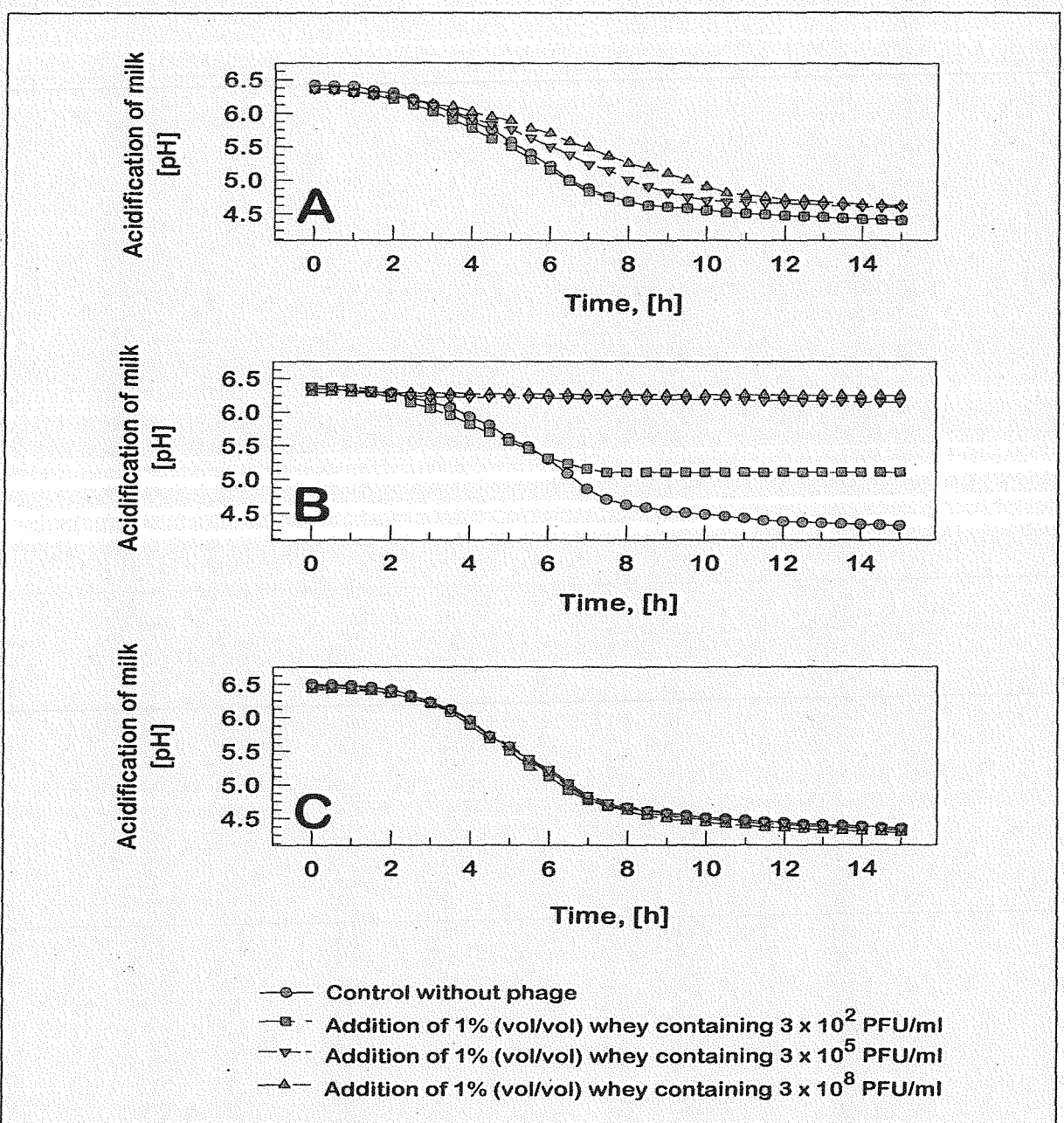

Fig. 3: Acidification of skim milk upon growth of the original undefined mixed-strain starter culture B (A), of the phage-sensitive single-colony isolate B7 (B) and of the phage-resistant isolate B23 (C). The skim milk was inoculated with 2 % of overnight cultures and supplemented with factory-derived whey or bulk starter samples containing different numbers of phage as indicated in the text.

Air sampling

Results obtained from phage monitoring by the miniaturized (microplate) activity test prompted us to take air samples in the dairy plant in order to elucidate a possible role of airborne infection routes. Samples were analyzed with phage-sensitive strain *Lactococcus lactis* B7. For air sampling, a commercially available air sampler was chosen, which collected airborne phage particles on gelatin filters. Since its suitability for detecting phage of lactic acid bacteria was unknown, a variety of different experiments were performed to obtain an optimized protocol ensuring high viability of the collected phage material on the gelatin filters under conditions imitating transportation and storage conditions. Furthermore, the procedure should ensure easy handling of multiple samples.

Suitability of different buffers for immersion of filters

Use of a buffer ensuring high stability of the collected phage is crucial for monitoring airborne phage. Thus, the different buffer systems listed in Table 1 were tested. For 'worse case' conditions, air samples were taken in close vicinity of whey separators in the dairy plant, since whey is known to be the main phage reservoir in the dairy plant (3, 5). Filters were immediately immersed in chilled buffers and were stored at 4°C until further treatment in the laboratory. After 3 days, all samples were liquefied for 2 min in a 37°C-water bath for determination of phage titers. All samples were subsequently stored in the refrigerator, and determination of phage titers was done again after 7, 9 and 21 days, respectively. Figure 4 shows that phage retrieval was comparable in all 5 buffers tested and high phage concentrations (up to $1x10^8$ PFU/m^3 air) were measured. These values are about 10-fold higher than those determined earlier by the impingement technique in gas washing bottles (9). Storage in the cold and several cycles of repeated liquification of samples for subsequent phage titer determination did not affect viability of phage. In conclusion, Ringer's solution (13) supplemented with 10 % (vol/vol) skim milk was chosen as a convenient buffer system for solubilization of the gelatin filters.

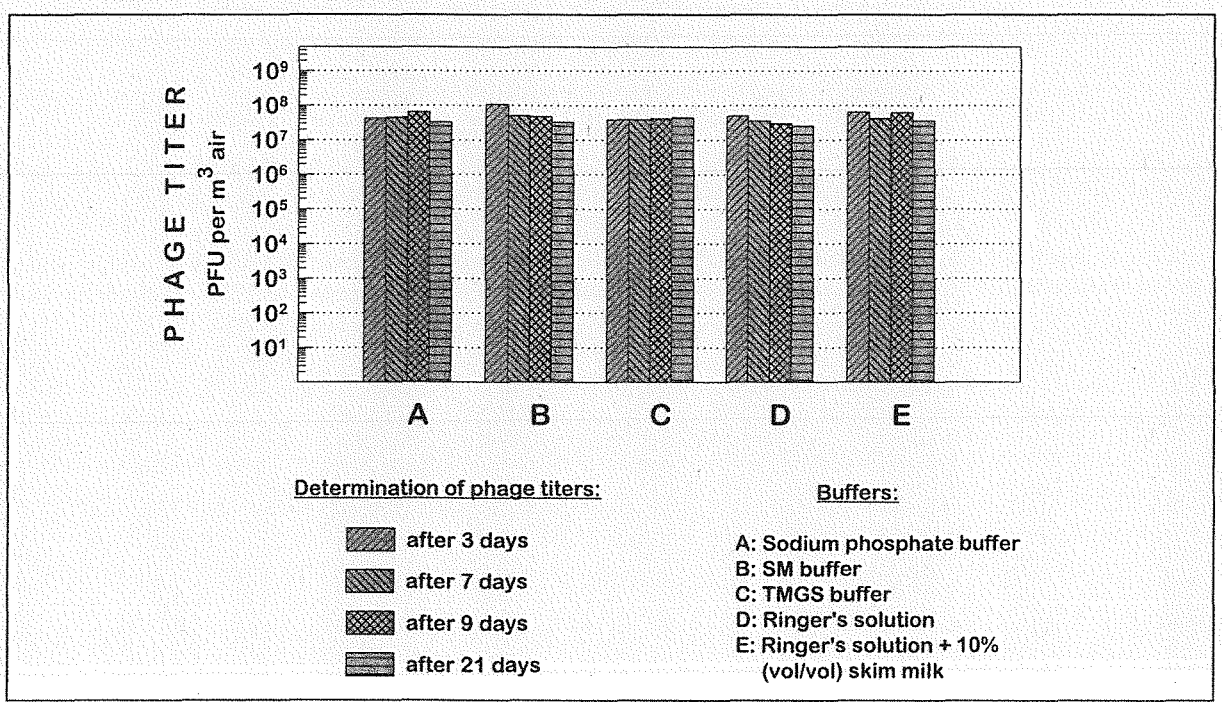

Fig. 4: Titers of undefined phage in air samples collected on gelatin filters. Multiple samples were taken in the vicinity of a whey separator, and the gelatin filters were immersed in different buffers. All samples were stored at 4°C for 3 weeks. During this period, phage titers were determined repeatedly on different days as indicated.

Effect of different storage temperatures on stability of phage on gelatin filters

In order to judge whether lactococcal phage can be stored on the gelatin filters at different temperatures (-20°C, 4°C, room temperature), a factory-derived whey sample containing undefined phage ($2x10^9$ PFU/ml) was added to gelatin filters immersed in 5 ml buffer to yield a final concentration of phage of $1.5x10^7$ PFU/ml buffer. This was done to imitate a natural phage load after air sampling as shown before. For these experiments, Ringer's solution (13) was fortified with different concentrations of skim milk. Samples were frozen for 6 days at -20°C or were kept either at 4°C or at room temperature for 3 days. After determination of phage titers, all samples were stored again for 6 days in the refrigerator at 4°C, and phage titers were repeated. As a control, phage titers were determined immediately after addition of phage to the filters. A minor decrease of phage titers was detected in Ringer's solution upon storage of filters at 4°C (Fig. 5-1) and upon repeated determination of phage concentration (Fig. 5-2). Retrieval of phage was similar in samples resuspended in Ringer's solution fortified with 10 % (vol/vol) or 50 %(vol/vol) skim milk or in pure skim milk. Therefore, fortification of Ringer's solution with a low amount of skim milk is sufficient, in particular with respect to minimizing the risk of growth of microbial contaminations during prolonged storage of filters at room temperature.

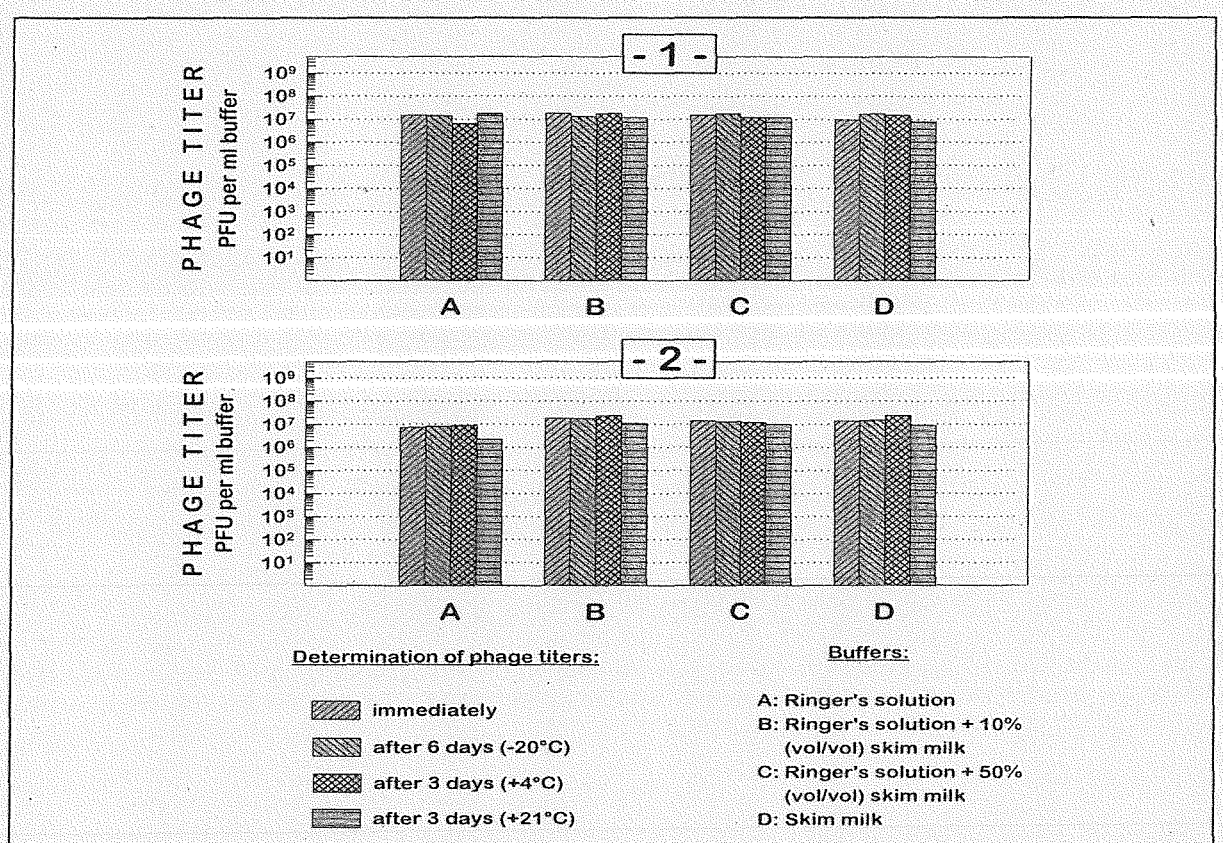

Fig. 5: Viability of undefined phage on gelatin filters immersed in milk-based buffer (Ringer's solution) or in pure milk. Phage obtained from a factory-derived whey sample were added in a final concentration of $1.5x10^7$ PFU/ml buffer to each sample. Samples were stored at different temperatures. After phage enumeration (1), all samples were stored at 4°C for redetermination of phage numbers (2).

Influence of the mode of mechanical resuspension of gelatin filters

The scope of the following experiment was to assess, whether manual homogenization of the gelatin filters was sufficient or whether phage retrieval could be improved by mechanical treatment. Gelatin filters were again loaded with 5 ml of different buffers and

were supplemented with the factory-derived phage from whey (final concentration on filters: 1.5×10^7 PFU/ml). Homogenization and resuspension was done after heating in the 37°C water bath for 2 min manually as described before or by placing the sealed plastic bags for 2 min in a stomacher lab blender apparatus. Data shown in Figure 6-1 show that the mode of mechanical treatment was not a critical step for obtaining high and uniform phage titers. When phage titer determinations were repeated after storing the filters for 2 days in the refrigerator, equivalent data were obtained. Thus, manual homogenization of the filters is a convenient technique after air sampling.

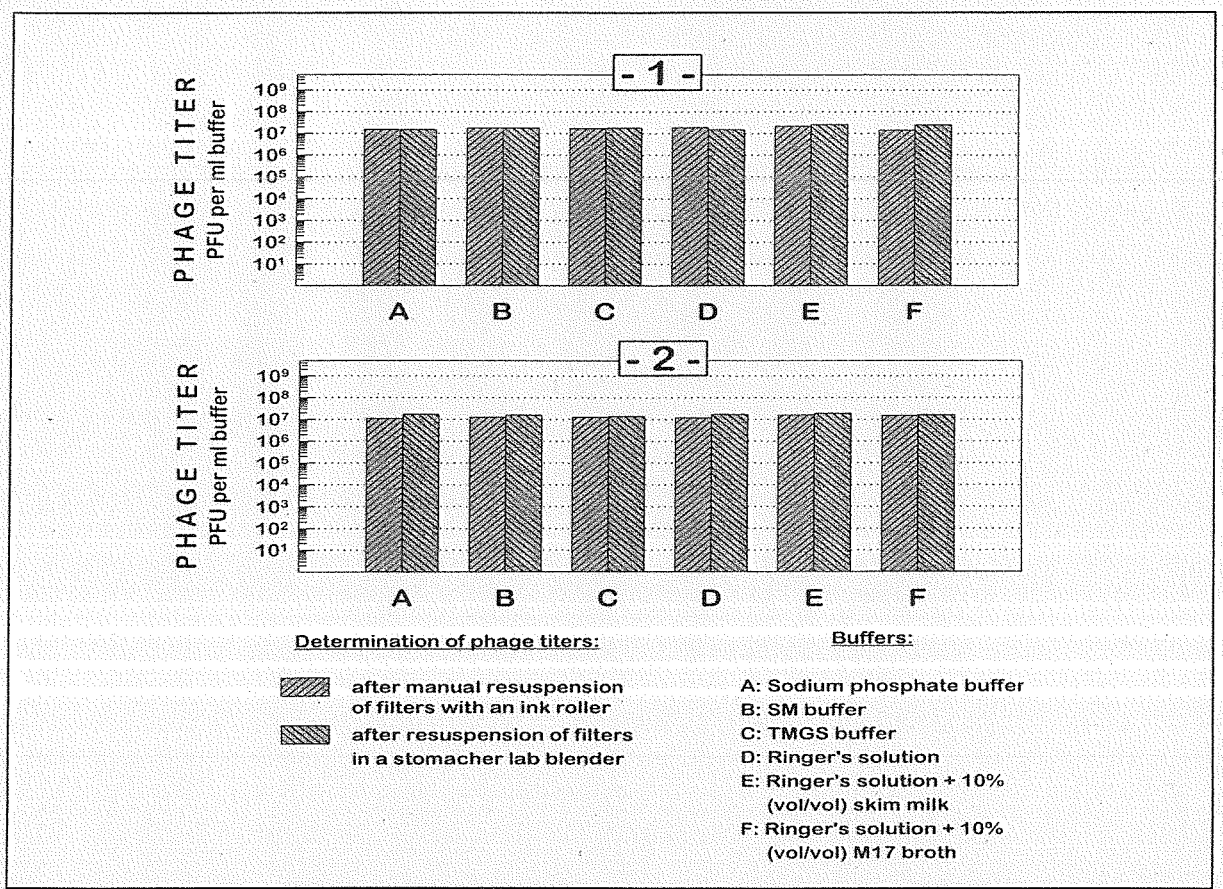

Fig. 6: Viability of undefined phage on gelatin filters immersed in different buffers and effect of two different types of resuspension (manually by means of an ink roller or using a stomacher lab blender). Phage obtained from a factory-derived whey sample were added in a final concentration of 1.5×10^7 PFU/ml buffer to each sample. Phage enumeration was done immediately (1) and was repeated after 2-days-storage of samples at 4°C (2).

Stability of different lactococcal phage types on the gelatin filters

The experiments done so far were performed with undefined phage from the factory - either by air sampling or by adding directly undefined phage from a factory-derived whey sample to the filters. Since 2 types of lactococcal phage are the most abundant in dairy environments throughout the world (small isometric-headed phage of the 946-phage species and prolate-headed phage of the c2-phage species [14, 15]), we analyzed their stability on the gelatin filters in the presence of different buffers. The small isometric-headed phage P463 and the prolate-headed phage P469 were used, which had been isolated and purified before from wheys obtained from the same factory (9). Final concentration of these phages on the filters was 1.5×10^7 PFU/ml buffer (phage P463) and 5×10^6 PFU/ml (phage P469). After the first enumeration of phage titers, the samples were

stored for 2 days either in the cold at 4°C or were kept frozen for one day at -20°C, and phage titers were determined again. The data reveal that viability of the two phages was not significantly affected by storage conditions (selection of buffer, temperature).

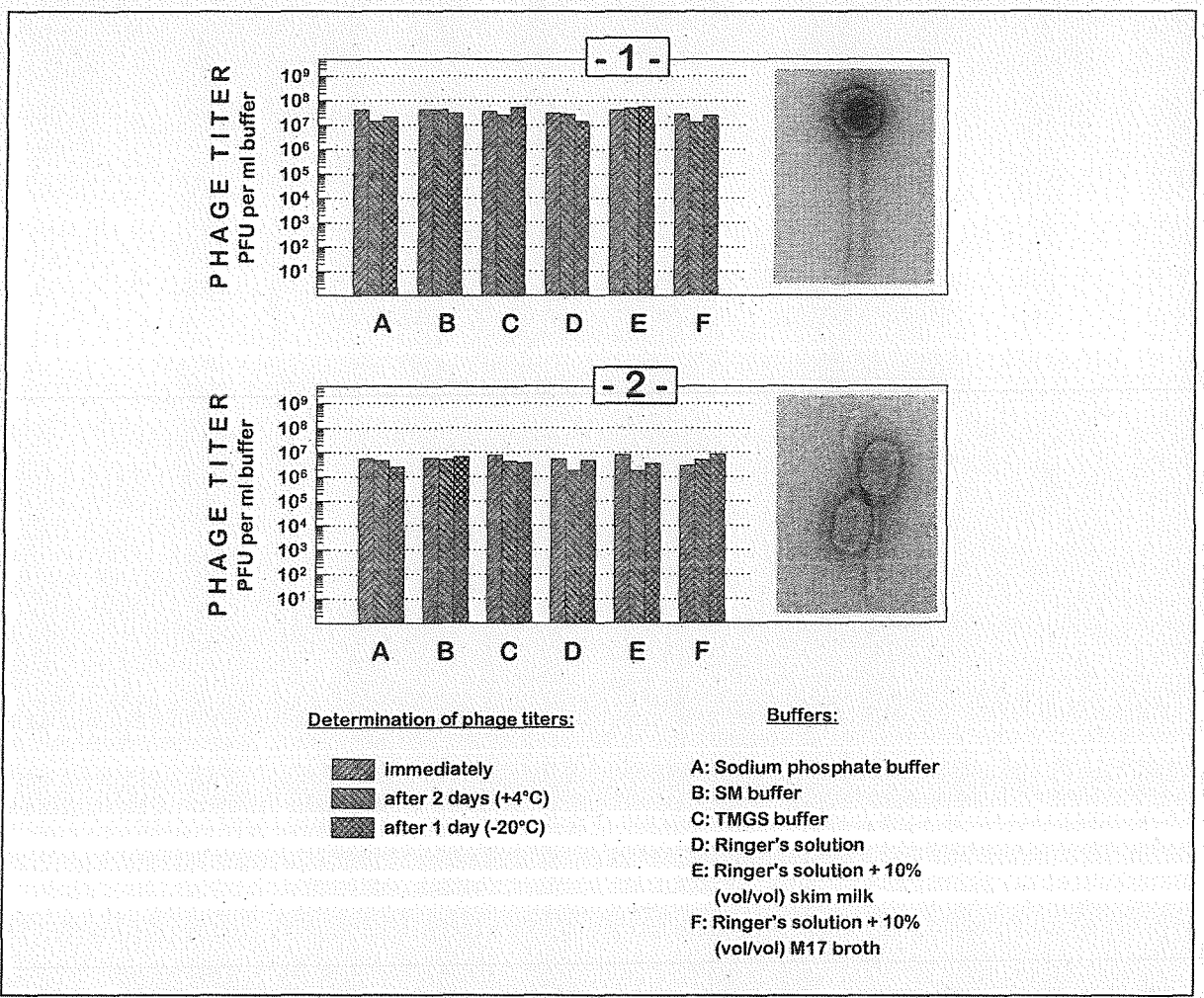

Fig. 7: Viability of two purified defined phages (small isometric-headed phage P463 [1], prolate-headed phage P469 [2]) on gelatin filters resuspended in different buffers. These phages have been obtained from factory-derived whey samples collected in a previous investigation (9) and were added in a final concentration of 1.5×10^7 PFU/ml (phage P463) and 5×10^6 PFU/ml (phage P469). Samples were stored at different temperatures as indicated in the text. Morphology of the two phages is shown in the graphs at a magnification of 80.000 x.

Effect of delayed addition of buffer to filters after air sampling

In order to answer the question whether a delay in adding buffer to the filters after air sampling will influence phage viability, various air samples were taken in parallel. To obtain high phage numbers, the air sampler was placed close to the whey separators in the dairy plant. Filters were immersed in Ringer's solution (plus 10 % [vol/vol] skim milk) after 4 h or after 24 h. As a control, buffer was added immediately after sampling. The filters were kept at room temperature, at 4°C or frozen at -20°C. Determination of phage titers was done with parallel samples after 4 h or after 24 h, respectively. Subsequently, all filters were stored for 4 weeks at 4°C in the refrigerator, and determination of phage titers was repeated after this time for all samples. Figure 8-1 illustrates that filters may be stored dry after sampling without loss of phage activity (enumerated phage load in all samples was approximately 1×10^8 PFU/m³ ambient air). Even after long-time storage of filters, high

values were obtained again (Fig. 8-2). These data are in accordance with earlier results that lactococcal phage are highly resistant to environmental stress and survive spray-drying (16).

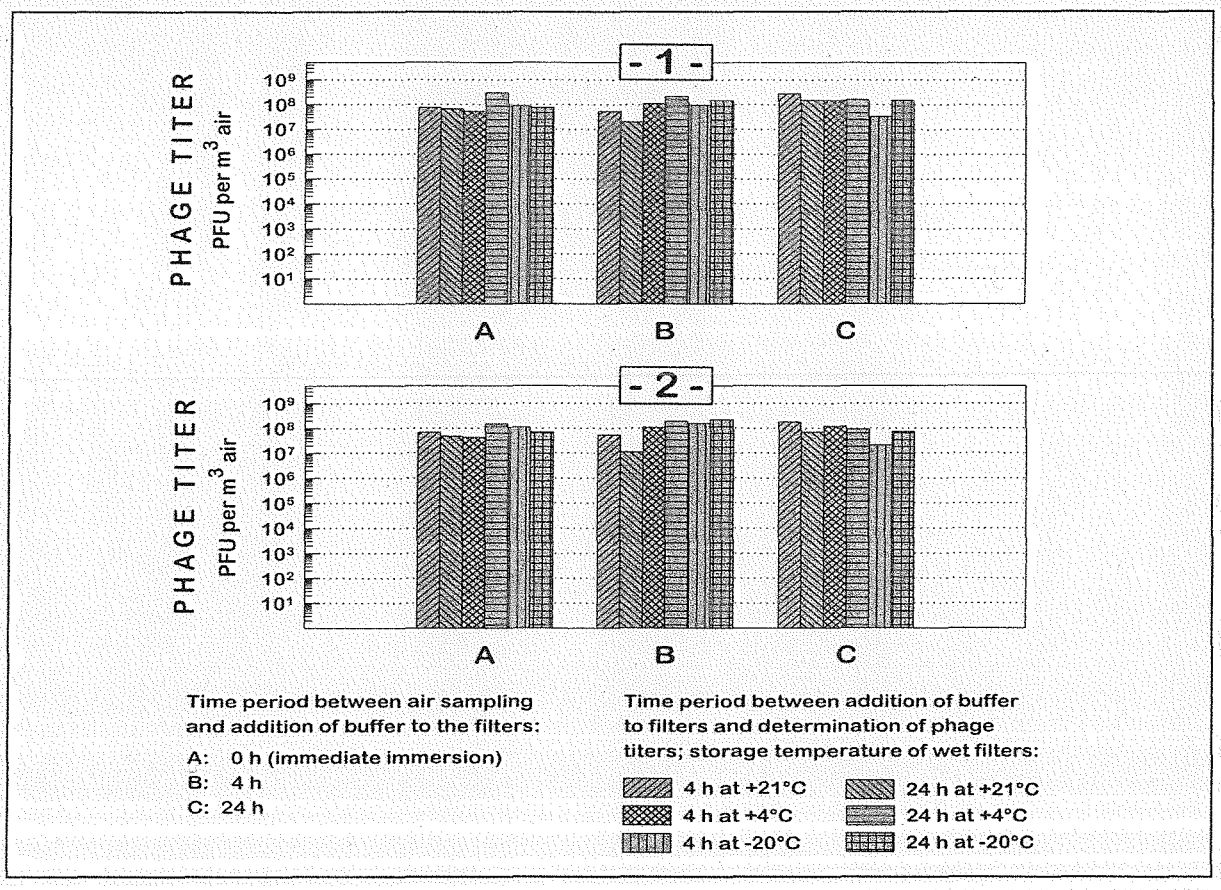

Fig. 8: Effect of delayed addition of buffer to gelatin filters after air sampling on enumeration of phage numbers. Multiple air samples were collected in the vicinity of a whey separator. Buffer (Ringer's solution supplemented with 10 % (vol/vol) skim milk) was added at different times after sampling. Determination of phage numbers was done after different storage conditions of samples with respect to storage time and storage temperature (1). Determination of phage titers was repeated for all samples stored for 4 weeks at 4°C (2).

Monitoring of airborne phage in the dairy environment

Taking air samples from different locations of the dairy plant, the applicability of the air sampling method proposed in this communication was shown. A concentration gradient from as high as $2x10^8$ PFU/m^3 air in the vicinity of the whey separators to as low as $2x10^2$ PFU/m^3 air close to the fermentation vessels was determined with our standard method (Figure 9). In critical areas (e.g. the bulk starter room), no phage particles were detected in air samples. The limit of detection was determined to be below 5 PFU/m^3 air at the conditions used here (flow rate of 100 liters per min; maximum sampling time of 12 min; resuspension of filter in 5 ml buffer, determination of phage titer by plating a 1-ml - sample in the plaque assay). The data did also confirm the high efficiency of the air filtration systems used in the plant: Although a significant phage load ($2x10^2$-$1x10^4$ PFU/m^3 air) was detected in the ambient air next to the sterile filter equipment for the fermentation vessels, the filtered air was either free of phage, or phage were found at the limit of detection only.

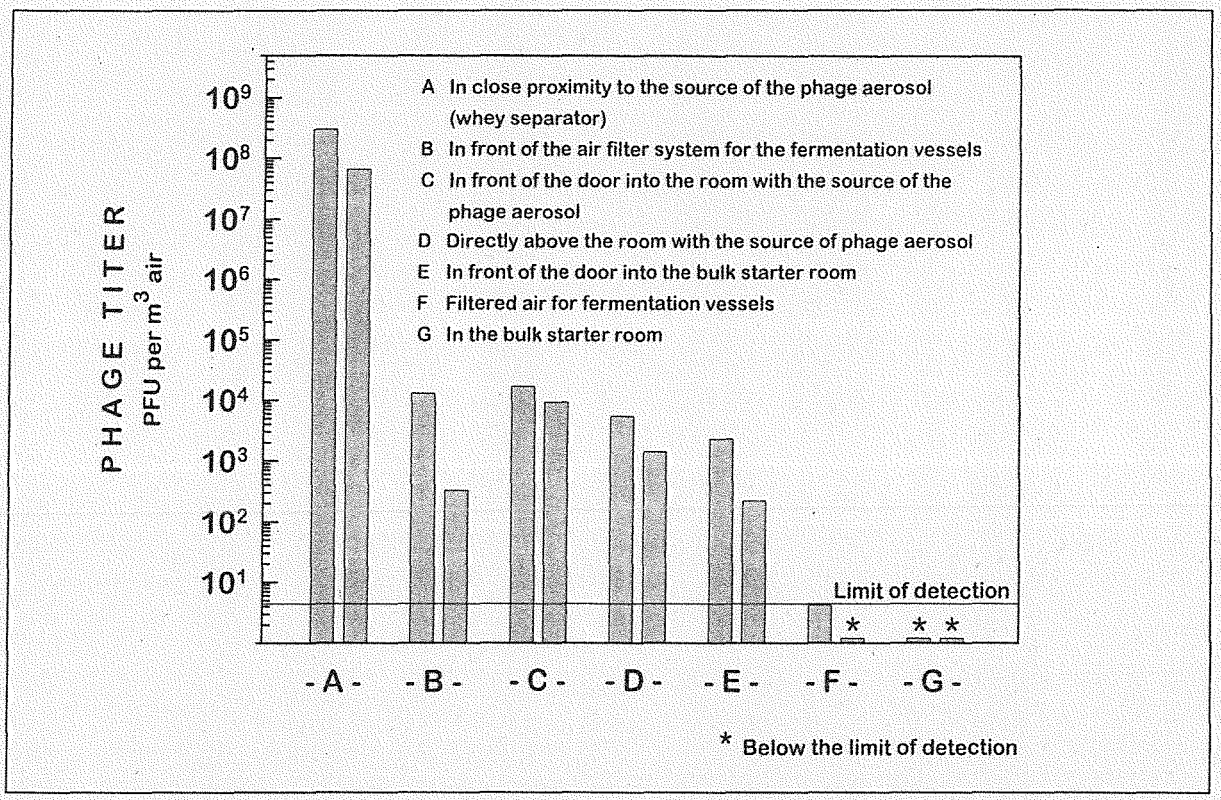

Fig. 9: Numbers of phage in various air samples collected in one dairy factory. Two results obtained either on different days (A, B, F) or on one day but at two close locations (in a distance of approximately 1-5 m [C, D, E, G]) are shown for each area. The limit of detection is indicated in the graph.

4. Conclusions

Collecting airborne lactococcal phage on gelatin filters is a convenient method for phage monitoring in a dairy environment, and lactococcal phage are resistant to the stress conditions during sampling. In principal, the choice of buffer is not a critical step, however we recommend Ringer's solution fortified with 10 % (vol/vol) skim milk to ensure high stability of these bacterial viruses. Filters should be immersed in a minimum of 5 ml buffer and sealed in sterile plastic bags of convenient size (e.g., 10x15 cm). Preferentially, samples should be chilled until determination of phage titers, but handling at room temperature or freezing is also acceptable. Therefore, this technique is also suitable for mailing various samples to the laboratory after sampling, when direct analysis cannot be done in the factory.

Mesophilic dairy starter cultures (*Lactococcus lactis*) may either be composed of many undefined strains in mixed-strain cultures or contain 2 or more individual strains (defined starters) (3, 5). In the latter case, the individual strains can be taken directly as indicator strains for phage titer determination. However, when undefined cultures are used, identification and selection of phage-sensitive strains is a prerequisite for monitoring airborne phage by means of a standard plaque assay (2). Phage of lactococcal starters may also be detected in whey samples by DNA probes specific for the phage present in the dairy, either by dot blot hybridization or by PCR methodology (17, 18). The suitability of a DNA/DNA hybridization technique has been shown recently for airborne genetically modified bacteria collected on gelatin filters (19). These methods may also be adopted for the detection of airborne lactococcal phage and would avoid the necessity to isolate indicator strains. However, the sensitivities of these methods are much lower than determination of plaque-forming units in the plaque assay.

Acknowledgement

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6. Summary

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

26 Lactococcal bacteriophages (starter cultures, fresh cheese)

For the assessment of the bacteriophage load in the ambient air of dairies producing fresh cheese, air samples were taken with an air sampling apparatus, and a simple method was elaborated for transportation and preparation of samples and subsequent determination of phage titers. Sampling was done on gelatin filters which were immersed in a simple buffer system (quarter-strength Ringer's solution supplemented with 10 % (vol/vol) skim milk is recommended). The filters were stored in sterile plastic bags. Determination of phage titers (plaque-forming units (PFU) per m^3 air) was done with phage-sensitive isolates of the starter culture in use (*Lactococcus lactis*). The collected phage material revealed high stability on the gelatin filters under routine storage and transportation conditions - also after prolonged storage for several days at different temperatures and after repeated determination of phage titers. The method is suitable for different phage types known to be widespread in dairies (in particular: small isometric- and

prolate-headed phages of the 936 and c2 phage species, respectively). The limit of detection was found to be below 5 PFU per m^3 of ambient air, when sampling was done for 12 min with a flow rate of 100 liters per min. A phage load of up to $2x10^8$ PFU per m^3 ambient air was determined in close proximity of the whey separators which are known to be the main source of phage aerosols. Within the production area, a concentration gradient down to as low as $2x10^2$ PFU per m^3 ambient air (close to the fermentation vessels) was documented.

Zusammenfassung

Neve, H., Berger, A., Heller, K.J.: **Eine Methode zur quantitativen Erfassung der virulenten Bakteriophagen von Starterkulturen in der Raumluft.** Kieler Milchwirtschaftliche Forschungsberichte **47** (3) 193-207 (1995)

26 Laktokokken-Bakteriophagen (Starterkultur, Frischkäse)

Zur Abschätzung der Phagenbelastung in der Betriebsraumluft Frischkäse-produzierender Betriebe wurden Luftproben mit einem Luftkeimsammelgerät genommen und eine einfach zu handhabende Routinemethode zum Transport und zur Aufarbeitung der Proben mit nachfolgender Phagentiterbestimmung erarbeitet. Die Probennahme erfolgte auf Gelatinefilter, die in einem einfachen Puffersystem gelöst und zum Probentransport in sterile Plastikbeutel verschweißt wurden (gut geeignet ist 1/4-starke Ringerlösung mit 10% Magermilchzusatz). Die Phagentiterbestimmung (Plaque-bildende Einheiten (PbE) pro m^3 Raumluft) erfolgte mit phagensensitiven Isolaten der jeweiligen eingesetzten Starterkultur (*Lactococcus lactis*). Das gesammelte Phagenmaterial zeigte auf den Gelatinefiltern unter praxisüblichen Transport- und Lagerbedingungen eine ausreichend hohe Stabilität - auch nach längerer mehrtägiger Lagerung bei unterschiedlichen Temperaturen und nach wiederholter Phagentiterbestimmung. Die Methode ist für unterschiedliche Phagentypen, die in den milchverarbeitenden Betrieben weit verbreitet sind, gleichermaßen gut geeignet (insbesondere Phagen mit isometrischen und Phagen mit prolaten Köpfen der Phagenspecies 936 und c2). Die untere Nachweisgrenze der Methode lag niedriger als 5 PbE pro m^3 Raumluft bei 12-minütiger Probennahme und einer Saugleistung von 100 Liter pro min. In Nähe der Separatoren, die eine wesentliche Phagenaerosol-Quelle darstellen, wurde eine Phagenbelastung von bis zu $2x10^8$ PbE pro m^3 Raumluft gemessen. Innerhalb des Produktionsbereiches wurde ein Konzentrationsgradient bis hinunter auf $2x10^2$ PbE pro m^3 Raumluft in Nähe der Produktionstanks dokumentiert.

Résumé

Neve, H., Berger, A., Heller, K.J.: **Une méthode pour la détermination quantitative des bactériophages virulents de levains présents dans l'air ambiant.** Kieler Milchwirtschaftliche Forschungsberichte **47** (3) 193-207 (1995)

26 Bactériophages (levains, fromage frais)

Pour évaluer la teneur en bactériophages de l'air ambiant des usines fabriquant des fromages frais, on a prélevé des échantillons d'air à l'aide d'un appareil de prise d'échantillon d'air et développé une méthode de routine simple pour transporter et

préparer les échantillons et pour déterminer ensuite les titres phagiques. L'échantillonnage était effectué sur des filtres de gélatine qui étaient dissous dans un simple système tampon et soudés dans des sacs en plastique stériles pour transporter les échantillons (une solution de Ringer (1/4) à laquelle était additionné 10 % (vol/vol) du lait écrémé s'est avérée appropriée). On a déterminé les titres des phages (unités formant des plaques (UFP) par m^3 d'air ambiant) à l'aide des isolats sensibles aux phages du levain utilisé (*Lactococcus lactis*). Sur les filtres de gélatine les phages collectés ont montré, dans des conditions de stockage et de transport normales, une stabilité tout à fait satisfaisante - même après un stockage prolongé pendant plusieurs jours à des températures différentes et une valeur répétable des titres phagiques. La méthode s'est avérée appropriée pour les différents types de phages qui sont très répandus dans les usines transformatrices du lait (en particulièrement les phages à tête isométrique et à tête allongée (prolate) des espèces 936 et c2). La limite inférieure de détection de la méthode était inférieure à 5 UFP/ m^3 d'air ambiant (échantillonnage de 12 min, volume aspiré: 100 litres/min). A proximité des séparateurs qui se sont avérés une source essentielle d'aérosols de phages, on a mesuré une teneur allant jusqu'à $2x10^8$ UFP/m^3 d'air ambiant. Dans la zone de production, on a établi un gradient de concentration descendant jusqu'à $2x10^2$ UFP par m^3 d'air ambiant à proximité des tanks de fermentation.