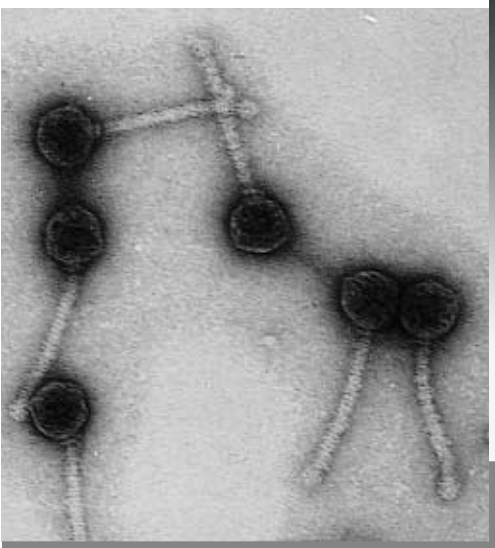
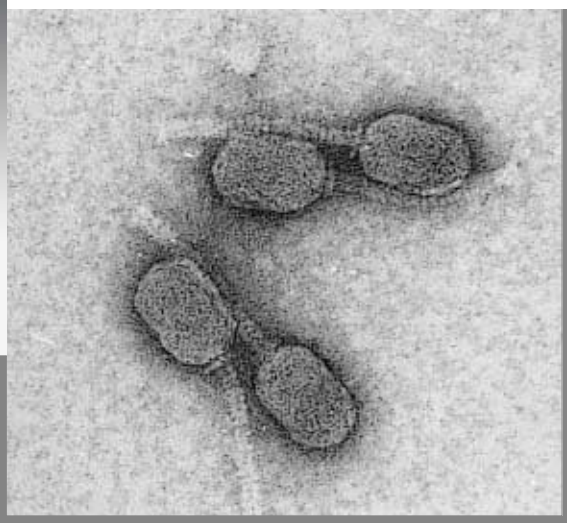
sartorius





Quantitative Detection of Bacteriophage Loads in the Ambient Air of Dairies

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Recommended Step-by-step Procedure Using the Gelatin Membrane Filter Method

Despite worldwide efforts in developing phage-resistant starter cultures, bacteriophages are still the main cause of acid interference and, thus, product spoilage in dairies. Complete and careful phage monitoring is essential to replace phage-sensitive cultures with phage-resistant strains on time and to localize the internal source of infection.

An easy-to-use method will be presented here for quantitative detection of viral bacteriophages in the ambient air of dairies. This method guarantees high stability of the detected bacteriophages under the normal storage and transportation conditions found in practice.

The method was tested using bacteriophages of mesophilic lactic acid streptococci (Lactococcus lactis). The essential criteria for optimizing the routine method were as follows:

- Use of a simple buffer system for the resuspension of the water-soluble Sartorius gelatin filters
- Easy handling properties of the filters after sampling
- High stability of the collected phages – even after the filters had been stored for several days under varying temperature conditions and after repeated determination of the phage titers
- Suitability of the method for the various bacterial phage strains that are commonly found in dairies (see Fig. 1)

1. Collecting the Bacteriophages
The **MD8 Air Sampler**, order no. 16743, which has meanwhile been replaced by the **MD8 airscan**, order no. 16747 (230 V), 16746 (115 V) or 16748 (110 V) (see Fig. 2)

and

Gelatin filters, ∅ 80 mm with a 3 µm pore size, order no. 12602-80-ALK of Sartorius AG, Goettingen, were used.

The following filters are also suitable for bacteriophage sampling:

Gelatin filter disposables, order no. 17528-80-ACD (sterile, single packed), or 17528-80-BZD (sterile, triple packed).

Note: The bacteriophages were sampled at a rate of 100 l per minute during a 3- to 12-minute sampling interval.

2. Transferring the Filters
Transfer these to sterile, disposable polyethylene bags of the appropriate size.

Note: 10 × 15 cm bags, which are available from laboratory equipment dealers, are particularly suited to this purpose.

3. Adding Buffer to the Sterile Polyethylene Bag
Add 5-10 ml of buffer to the bag. The following simple buffer system is recommended:
¼ Ringer solution with the addition of 10% skim milk.

Note: Ringer solution is used in the preparation of sample dilutions of milk and milk products and is available in tablets from laboratory equipment dealers. ("Milk and milk products – Preparation of samples and dilutions for microbial examination. IDF Standard 122B, 1992"). Skim milk should be prepared from powdered skim milk. The Ringer solution, after autoclaving (20 min., 121 °C), is mixed with the skim milk (which had been previously autoclaved for 15 min. at 115 °C).

4. Sealing the Filter Bags
Seal the bags to ensure trouble-free storage or transportation to the research laboratory.

Note: It is highly recommended to store the filters in a cool place and to reprocess them as soon as possible. The buffer can be added at a later time (for testing, the buffer was subsequently added after 4 and 24 hours, respectively). Thus, high flexibility is guaranteed in the choice of when to reprocess the filters (Fig. 3).

5. Prior to Determining the Phage Titers
Before determination of the phage titers, the filters should be warmed for 2 minutes in 37 °C water first, then be manually resuspended | homogenized in buffer (likewise for 2 minutes).

6. Determining the Phage Titers
Use the agar overlay method with phage-sensitive, multi-strain culture isolates (Adams 1950).

Note: It is necessary to determine the titers using defined, individual strains. If a dairy employs an individual culture strain or a defined, multi-strain culture, then these strains must also be used as the indicator strains. However, if undefined multi-strained cultures have been used, then the phage-sensitive culture isolates must be isolated prior to test begin. Generally, it is sufficient to prepare cultures of individual colony isolates first, because their acidification response can then be monitored in the presence of a sterile-filtered whey product.

7. Storing the Remaining Sample Quantities
Storing these quantities as documented samples in a refrigerator for several weeks, in case the phage titer determination must be repeated, is no problem. There is no reduction in the titers when the samples are rewarmed and homogenized.

8. Monitoring
It is recommended that dairies use their own whey products when monitoring air for phage titer testing.

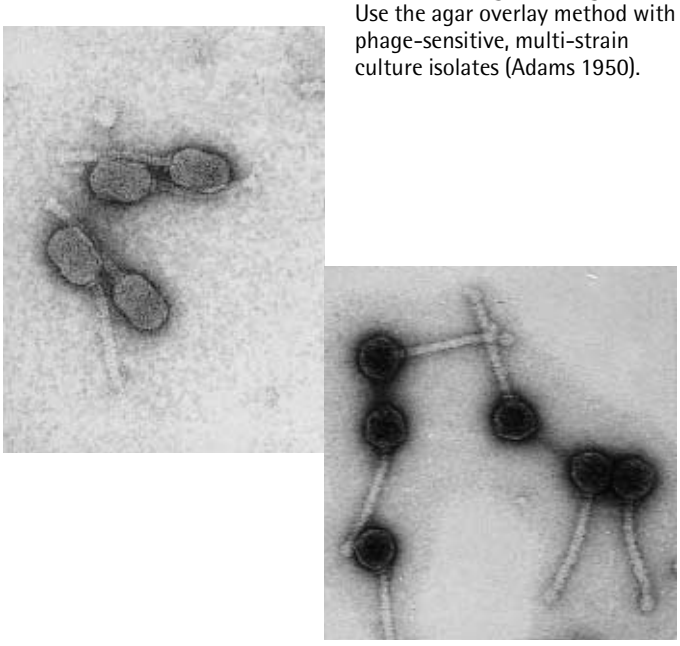


Figure 1:
Scanning electron micrographs of 2 bacteriophage strains of mesophilic lactic acid streptococci (Lactococcus lactis) with round (isodiametric) or longish (prolated) heads, which are commonly found in dairies. The bar is scaled to 50 nm.

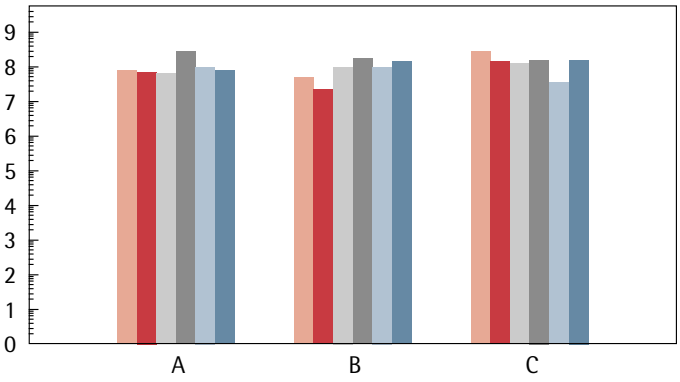
Conclusion

With the method presented here, virulent bacteriophages can be detected over a wide concentration gradient (Fig.4). In the immediate vicinity of a phage aerosol source, it is possible to detect phage loads of up to 3×10^8 plaque-forming units per m^3 of ambient dairy air.

The lower limit of detection is less than 5 phages per m^3 ambient air (Fig.4). In the case of mesophilic lactic acid streptococci, there are essentially two different phage strains that cause acid interference (phages with round, isodiametric heads and those with longish, prolated heads; see the scanning electron micrographs, Fig.1). The method presented is equally suited to both phage strains.

Figure 2
MD8 airscan air sampler with Gelatin filter disposables

Figure 3:
Influence of different storage conditions on the phage titers (plaque-forming units per m^3 of air). Air samples were taken in parallel at a dairy near a phage aerosol source, and the phage titers were determined by employing a phage-sensitive Lactococcus lactis strain. The data reveals that the different handling of the filters in view of (I) the time of adding the buffer after sampling, (II) the time of the filter reprocessing after having added the buffer and the chosen storage temperature, is not critical to phage titer properties. After the phage titers were determined, the samples were kept in a refrigerator for 4 weeks and the titers were determined once again: a significant reduction in the phage titers had not resulted even after this time had elapsed (data not shown).



I. Time between sampling and adding buffer to the filters:

A 0 h (immediate resuspension)

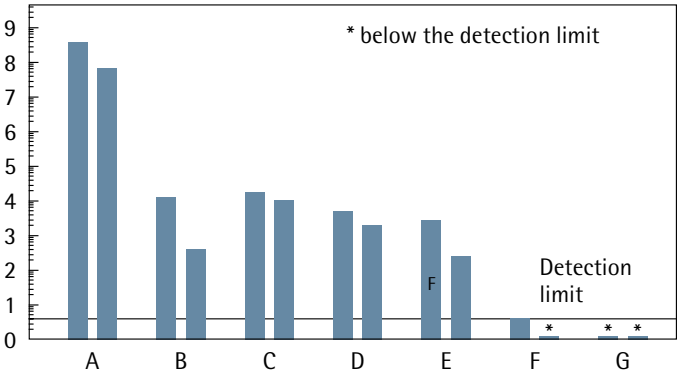
B 4 hrs.

C 24 hrs.

II. Time between adding buffer to the filters and determining phage titers; storage temperatures of wet filters:

- 4 hrs. (storage temperature: 21 °C)
- 24 hrs. (storage temperature: 21 °C)
- 4 hrs. (storage temperature: 4 °C)
- 24 hrs. (storage temperature: 4 °C)
- 4 hrs. (storage temperature: -20 °C)
- 24 hrs. (storage temperature: -20 °C)

Figure 4
Bar graph summarizing the phage titers of the ambient air in a dairy. The samples were taken at various locations within the dairy. In each case, 2 measured values are shown, which were either recorded on different days or at different places within the respective locations.



A In the immediate vicinty of the source of phage aerosols
B Upstream of the fermentation tank's sterile filter system
C In front of the door leading to the room with the source of the phage aerosols
D Above the room with the source of the phage aerosols
E In front of the door leading to the starter culture room
F Filtered incoming air for the fermentation tank
G Within the starter culture room

Literature:

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Meth. Med. Res., Chicago 3 (1950) Sect. pp. 1-73.

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

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