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Detection of Airborne Lactococcal Bacteriophages in Cheese Plants

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Abstract

The dairy industry adds starter bacterial cultures to heat-treated milk to control the fermentation process during the manufacture of many cheeses. These highly concentrated bacterial populations are susceptible to virulent phages that are ubiquitous in cheese factories. In this study, the dissemination of these phages by the airborne route and their presence on working surfaces were investigated in a cheese factory. Several surfaces were swabbed and five air samplers (polytetrafluoroethylene filter, polycarbonate filter, BioSampler, Coriolis cyclone sampler and NIOSH two-stage cyclone bioaerosol personal sampler) were tested. Samples were then analyzed for the presence of two *Lactococcus lactis* phage groups (936 and c2) and quantification was done by qPCR. Both lactococcal phage groups were found on most swabbed surfaces while airborne phages were detected at concentrations of at least 10^3 genomes/m^3 of air. The NIOSH sampler had the highest rate of air samples with detectable levels of lactococcal phages. This study demonstrates that virulent phages can circulate through the air and that they are ubiquitous in cheese manufacturing facilities.

Introduction

A variety of food products, commodity chemicals, and biotechnology products are manufactured through large-scale bacterial fermentations (13). Because significant amounts of bacterial cells are cultivated in fermentation vats, most of these industries have experienced problems with phage contamination. Phage outbreaks represent an industrial concern because they can slow down the fermentation process and adversely impact product quality, resulting in extra costs and delays (13). The dairy industry has been working to control phage populations for decades (28). Cheesemakers can experience "slow vats" to various levels depending on, among others, the type of fermented milk product, the starter culture system, and the factory design. According to estimations, 0.1 to 10% of all milk fermentations are negatively affected by virulent phages (27).

The dairy industry relies on selected bacterial cultures for the production of an array of fermented milk products. For example, several strains of the low-GC Gram-positive bacterium *Lactococcus lactis* are extensively used for the transformation of milk into cheeses. The lactococcal starter culture population can reach levels of up to 10^9 cfu/ml of milk inside large industrial fermentation vats (24). This ecosystem is also ideal for the multiplication of virulent *L. lactis* phages, which are ubiquitous in dairy environments (3). If phage-sensitive host cells are dominant in the starter culture, a virulent phage inside one of these vats can initiate an infection and its progeny will eventually lyse numerous starter cells and slow down the fermentation process leading to low-quality fermented products (28). Significant progress has been made in the control of phage populations within this food sector as several strategies are now available to keep these bacterial viruses at bay (for review see 13). For example, a typical cheese plant uses

several *L. lactis* strains on a rotation schedule to prevent the build-up of specific phages. Nonetheless, phage infection remains a considerable risk in cheesemaking because phage populations have evolved over time and they can be disseminated through various routes. The source(s) of the phages and their dissemination routes must be identified in order to implement corrective actions to limit their propagation (13).

Virulent phages can originate from a variety of sources. New virulent phages can be introduced into a cheese plant through raw milk, which can be contaminated at low concentration (23). Moreover, some lactococcal phages can withstand pasteurization (1, 21). Novel virulent phages can also emerge within a factory through mutations and recombination events when wild-type phages infect sensitive hosts (18, 35). Reservoirs of phages include the materials and equipments used in the manufacturing process, as well as the fermentation products and by-products (13). Milk with little or no contamination can also be put at risk by the addition of phage-contaminated ingredients such as whey protein concentrates (13, 15). Although rarely documented, phage contamination can also occur through aerosolization. Concentrations of up to 10^8 pfu/m^3 of air have been detected in some areas, mainly downstream of the fermentation process in a German cheese plant (30-32).

Phage aerosolization can occur during air displacements or movements around contaminated surfaces or fluids. It can also occur by liquid splashes, which can aerosolize phages (40). Aerosol contamination represents a major microbiological challenge, especially when the aerosol sources and contents are not known. Studies of the airborne distribution of phages could help identify sources and determine the impact of aerosols on failed milk fermentations.

Viruses can be found on aerosol particles of various sizes from the sub-micrometer range to tens of micrometers in aerodynamic diameter. Several types of samplers are available for

characterization the viral load in the air (for review see 40). However, no standard procedure exists at present for the sampling of airborne viruses. The efficacy of these samplers is often assessed on the basis of the recovery of infectious viral particles. Many of them have destructive effects on the virus structure, leading to false negative results in viral culture assays (40, 41). Plaque assays are cumbersome because they require the use of several bacterial hosts as the phage host range differs from one isolate to another (12, 25). Plaque assays may not always be feasible because pure culture may not be available if commercial defined-strain or mixed-strain bacterial cultures are used to make the fermented milk products. Consequently, it has been argued that analytical methods that are independent of viral infectivity, such as the detection of phage genomes by quantitative polymerase chain reaction (qPCR), are more suitable for the analysis of dairy samples (3, 9, 10, 12, 17, 33, 42) as well as air samples (41).

The aim of this study was to compare the efficiency of five aerosol samplers in recovering airborne lactococcal bacteriophages in a cheese plant and to assess subsequent detection of the phages by qPCR. The presence of these phages on various working surfaces was also investigated. Although *L. lactis* phages are currently classified into ten genetically-distinct groups (11), only the two most common groups, namely the 936 and the c2, were targeted in this study. Members of these phage groups have been isolated worldwide and belong to the *Siphoviridae* family with a double-stranded DNA genome and a long, non-contractile tail (7, 8, 14, 16, 20-22, 25, 26, 34-36, 38, 39).

Materials and Methods

Sampling site. A factory producing cheddar cheese was chosen for aerosol sampling. This cheese factory uses commercial mesophilic starter cultures containing *L. lactis* strains, which are rotated on a daily basis. A typical aerosol sampling session started at the beginning of cheese production in the morning and lasted for approximately 12 hours. Multiple fermentation vats were filled with pasteurized milk daily. The aerosol sampling was performed in the filling section at the end of the production line near a cheese whey source. Since the air from the initial sections of the cheese production line was moved towards this section, it was likely to contain the largest quantity of airborne lactococcal phages. Due to space restrictions within the factory, the sampling site was conducted in two zones separated by approximately 5 meters.

Air Sampling. Air sampling in the cheese factory was done using five types of samplers (Table 1): 0.4 µm polycarbonate (PC) filters (SKC Inc., Eighty Four, PA), 0.3 µm polytetrafluoroethylene (PTFE) membrane filters (SKC Inc.), BioSamplers (SKC Inc.), a Coriolis cyclone sampler (Bertin Technologies, Montigny-le-Bretonneux, France) and NIOSH two-stage bioaerosol cyclone (BC 251) personal samplers (4).

PC and PTFE filters were mounted on cellulose pads and housed in 37-mm clear styrene three-piece cassettes (SureSeal, SKC Inc.), and were plugged into pumps (GilAir-5, Gilian, Sensidyne, LP, Clearwater, FL) calibrated at a flow rate of 2 L/min. Six filters of each type were evenly distributed in the two sampling areas and used for a period of 710 min. Thus, a total of 1420 liters of air was sampled per filter. The closed-faced filters were positioned with the inlets facing the ground.

BioSamplers were placed in Styrofoam boxes to prevent any broken glass from being scattered in the factory in the event of equipment breakage. The boxes were positioned on tables and the samplers were plugged into pumps (Vac-U-Go Sampling Pump, SKC Inc.). Fifteen ml of sterile water with 0.01% Tween® 20 was used as a sampling liquid in the BioSamplers. Twenty-minute samples were taken with the critical orifices providing a sampling rate of 12.5 L/min. Thus, a total of 250 liters of air was recovered per BioSampler sample.

Ten ml of sterile water with 0.01% Tween® 20 was used in the sampling cones of the Coriolis which was run for a period of 10 minutes at 300 L/min. The evaporated liquid was replaced to provide a final volume of 10 ml after the first sample was taken. The same sampling cone was used three times and the remaining liquid volume was measured after the last sample was taken. Therefore, this sampler concentrated 9000 L of air in each sample in 30 min. The procedure was repeated with five sampling cones at one sampling site.

Four NIOSH samplers were distributed on two sites to provide 350 min sampling periods at 10 L/min using calibrated AirCon-2 pumps (Gilian). Each NIOSH sampler was assembled using a 15 ml (stage 1) plastic tube, a 1.5 ml (stage 2) plastic tube and a 0.4-µm PC capillary pore filter (SKC Inc.). Thus, a total of 3500 liters of air was sampled per assay. The Coriolis sampler and the Airborne Particle Counter (APC, Met One model 3313-3-1, Pacific Scientific Instruments, Grants Pass, OR) were placed 1.3 meters and 2 meters above ground level respectively due to space restrictions. All the other samplers were placed approximately 1 meter above ground level. The APC sampled the air at 28.3 L/min, and airborne particles were analyzed in channels of 0.3, 0.5, 1.0, 5.0, and 10.0 µm.

The limit of detection for each sampler (Table 3) was determined by taking into consideration the volume of air sampled, the dilution factor of the liquid suspension, and the limit of detection of the qPCR reaction.

Surface sampling. Several surfaces in the cheese factory were swabbed with polyester fiber tipped plastic applicators (Fisher Scientific Company, Ottawa, Ontario, Canada). The tips were first wet in sterile water with 0.01% Tween® 20, cut after swabbing, and kept in 1 ml of the same liquid. Whenever possible, the areas swabbed were approximately 100 cm^2. However, the exact size of the areas swabbed is not known in some cases owing to the limited or uneven surfaces. Therefore, these results are considered qualitative.

Sample treatment and analysis. All samples were kept on ice after on site sampling and then stored at 4˚C until analyses, which were performed within 48 hours. PTFE and PC filters were eluted by placing 5 ml of sterile water with 0.01% Tween® 20 directly into the three-piece cassettes and by shaking the filters on a vortexer for 20 min. The volume of liquid in the BioSamplers and the Coriolis samplers was measured and the liquid was stored at 4˚C. For the NIOSH samplers, 250 µl of sterile water with 0.01% Tween® 20 was placed in each tube. The 1.5 ml tubes were pulse-shaken on a vortexer until the pellet was no longer visible. The 15-ml tubes were processed in the same way; however, the tubes were also shaken upside-down to clean the upper part of the tube. No DNA extraction was necessary for qPCR analysis. Every sample was analyzed by qPCR in triplicate as a minimum.

Quantitative polymerase chain reaction primer and standard curve design. Primers for quantitative polymerase chain reactions (qPCR) were designed using the Beacon Designer 4.0 program (PREMIER Biosoft International, Palo Alto, CA) for SYBR Green protocols. The primer pair used for the detection of the lactococcal 936 phage group has already been published (41). The targeted region was within the *orf6* of phage P008, a gene coding for a highly conserved structural protein within the 936 group (22, 35). Reverse and forward primers were 5'-CCAGCAGTAGGGCGAACAAAG-3' (positions 5187 to 5167) and (5'-TGAGGGAGACGGAACAAACGG-3' (positions 5035 to 5055), respectively. For the c2 group, we targeted the gene coding for the highly conserved major capsid protein (20). Reverse and forward primers were 5'-GCATTAAAGCCAACTGATAGC-3' (positions 9642 to 9662) and 5'-AGTAAGAGGGATAGCGAACC-3' (positions 9432 to 9451) respectively.

The qPCR experiments were performed on a DNA Engine Opticon 2 apparatus (Bio-Rad Laboratories, Hercules, CA) in 96-well plates. The qPCR reaction mixture consisted of 0.5 µM forward primer, 0.5 µM reverse primer and a 1X final concentration of iQ SYBR Green Supermix (Bio-Rad Laboratories) in a final volume of 25 µl, 5 µl of which was the template DNA or sample. The standard curve was built using 10^0 to $5x10^6$ plasmid copies (see below) per reaction tube and was repeated in duplicate on each plate. The results from three plates were pooled to determine the equations of the slope used to calculate phage concentration. The protocol used for qPCR was 94°C for 3 min (hot start) followed by 35 cycles of 1) 94°C for 20 s; 2) 60°C or 53°C for 30 s for 936 and c2, respectively; 3) plate reading; and 4) 72°C for 25 s. A melting curve was calculated with readings every 0.2°C from 50°C to 95°C holding the temperature for 1 s. The cycle threshold or C(t) was set at a fluorescence intensity of 0.011 for all plates.

For both phage groups, plasmids containing the amplicon obtained with the primers were used as template for the construction of standard curves. Plasmids were obtained using the TOPO cloning reaction (Invitrogen, Carlsbad, CA) and purified using the QIAprep Spin Miniprep Kit (Qiagen Inc., Mississauga, ON, Canada). The optical density of the purified plasmid at 260 nm was used to determine its concentration and aliquots of known concentrations were prepared.

For all aerosol samplers, viral extraction efficiency for DNA quantification was verified by inoculating the samplers with a known concentration of 936-like phages. The phage suspension was inoculated into the liquid sampling media for the Coriolis and BioSampler and was extracted after sampling HEPA filtered air. The phage suspension was allowed to dry in the NIOSH and filters prior to air sampling. Viral extractions were done as described for the environmental samples.

Specificity of the primers. The primer pairs were tested on 23 different lactococcal phages of the 936 group and 7 different phages of the c2 group (Table 2) as well as one phage of the P335 group used as a negative control. All these phages had previously been isolated from cheese factories and kept at the Felix d'Hérelle Reference Center for Bacterial Viruses (www.phage.ulaval.ca). Lysates were diluted in sterile water and used directly as a template for qPCR. Both primer pairs were used on all lysates to verify the specificity of the primers towards different isolates of the same phage groups. Results were confirmed both by the melting curves and by migration on 2% agarose gels for 30 min at 100 V. Furthermore, the PCR-amplicons obtained from 28 randomly selected positive air and surface samples were sequenced to ensure primer specificity.

Statistical analysis. For the air samples, data were expressed as median and interquartile range. Statistical analyses were performed using the mean values. One-way ANOVA was used to analyze experimental factors associated with the comparison of samplers. For qPCR results, values were log transformed to stabilize the variances. Reported P values are based on these transformations. The univariate normality assumptions were verified with the Shapiro-Wilk tests. Brown and Forsythe's variation of Levene's test was used to verify the homogeneity of variances. The Tukey multiple comparison technique was applied *posthoc* to the ANOVA. The results were considered significant with P values ≤ 0.05. All analyses were conducted using the SAS statistical package, version 9.2 (SAS Institute Inc.).

Results

Testing of primer pairs for specificity in qPCR assays. To confirm that the selected primers were able to detect a diverse set of phages belonging to either the 936-like or the c2-like phages, we tested them in qPCR assays using the phages listed in Table 2. Both pairs of 21-nucleotide primers were specific to either the 936- or the c2-like phages (Table 2). No false negative or false positive results were obtained with the primers. According to the standard curves obtained, the limit of detection varied from 1 to 50 genome copies per reaction tube for the 936 qPCR protocol and from 5 to 50 genome copies per reaction tube for the c2 qPCR protocol. Moreover, all the sequenced qPCR amplicons were in accordance with the anticipated phage groups as identified by the qPCR protocol.

Size distribution of airborne particles. The size distribution of airborne particles in the cheese factory was monitored throughout the sampling period by collecting data with the APC for 5 min at a time followed by 15 min waiting times. Mean counts of particles per cubic meter of air for each of the six channels of the APC are shown in Figure 1. Airborne particle concentration and size distribution was stable throughout the study.

Comparison of air samplers for the recovery of lactococcal phages. Samples from samplers spiked with known concentrations of 936-like phages were analyzed by qPCR. Concentrations detected were within the same order of magnitude as the theoretical concentrations (data not shown). Viral extractions from the samplers were satisfactory for proper comparison of sampler

efficiencies. Results obtained from the qPCR analysis of the aerosol samples are summarized in Table 3. Interestingly, lactococcal phages belonging to both groups were detected with all air samplers, indicating that a significant number of phages were suspended in the air within the cheese plant. However, the sampler efficiency varied considerably.

The first stage of the NIOSH sampler was more efficient at detecting both 936-like- and c2-like phages (Table 3). When the results for the detection of both phage groups are combined, 22 out of 24 samples (92%) were positive in the first stage and 16 out of 24 (67%) were positive in the second stage. The differences in the concentration of airborne phages found in the first and second stages of the NIOSH sampler were very significant, with 16-fold more phages being detected in the first stage than in the second stage ($p<0.0001$) for 936-like phages. No difference was observed for c2-like phages. It should be noted that the NIOSH sampler had the lowest limit of detection.

A total of 75 out of 104 (72%) samples from the Coriolis were positive for lactococcal phages when the results of the two phage groups are combined (Table 3). The concentrations of airborne c2-like phages detected were significantly higher with the Coriolis sampler than with the stages 1 (23-fold, $p<0.0001$) and 2 (17-fold, $p=0.002$) of the NIOSH sampler. By contrast, detection of 936-like phages was significantly lower than with stage 1 (5-fold, $p<0.0001$) and significantly higher than with stage 2 (3-fold, $p=0.002$) of the NIOSH.

The PC and PTFE filters showed equivalent phage recovery giving almost the same number of positive samples. No significant difference was found between the two filter types in the concentration of airborne viruses recovered. However, statistical analyses based on mean values showed that PC filters allowed the detection of 3-fold more airborne 936 phages than the

265 Coriolis. Overall, the two filters were still less efficient than the NIOSH and Coriolis samplers
266 (Table 3). They were particularly deficient in the recovery of c2-like phages.

267 The BioSampler showed a very high detection limit in terms of viral genomes per m^3 of
268 air (Table 3). Every phage genome detected in qPCR analysis of the BioSampler samples
269 represented 10^4 phages genomes/m^3 of air. Only 1 sample out of 18 allowed the detection of
270 airborne 936-like phages, whereas 3 out of 18 qPCR reactions revealed c2-like phages. However,
271 for positive samples, the BioSampler detected the highest concentrations of airborne viruses. This
272 sampler allowed the detection of more than 100 fold higher concentrations of airborne viruses
273 than stages 1 and 2 of the NIOSH and more than 10 fold higher concentrations than the two
274 filters and the Coriolis sampler.

275

276 **Surfaces.** Various surfaces were swabbed in the factory to check for the presence of c2-like and
277 936-like phages. Using the swabbing method, the limit of detection for the 936-like phages was
278 1.4 phage genomes per cm^2 and was five-fold higher for the c2 group. Both phage groups were
279 found on most of the surfaces swabbed (100 cm^2) as evidenced by qPCR assays (Table 4).
280 Concentrations of 936-like phages ranged from 3 genome copies/cm^2 on the top of a closed
281 fermentation tank to 9.1×10^3 genome copies/cm^2 on the handle of a door leading to the office area
282 of the cheese plant, whereas c2 concentrations reached 3.1×10^3 genome copies/cm^2 on the surface
283 of cleaning material.

Discussion

The source(s) of phages as well as their dissemination routes should be identified in a cheese plant in order to implement long-term corrective actions to limit phage propagation and improve overall product quality (13). Contamination sources and dissemination routes are not easy to identify as several control points need to be checked. Because virulent dairy phages have a narrow host range (12, 25), it is not practicable to use culture assays to detect them in various dairy environments including air samples. Additionally, phages can deteriorate and lose their infectivity during sampling and sample processing (40). Molecular biology methods independent of phage infectivity and of bacterial hosts can facilitate the analysis of the viral content of environmental samples (40). In this study, quantitative PCR using SYBR Green fluorescence and primers specific to conserved regions was successfully performed to detect the two main lactococcal phage groups in various samples from a cheese plant, including air samples.

Only a few studies to date have shown that airborne phages can be detected in industrial cheese plant settings (30-32). We also had some anecdotal evidences that ventilation breakdowns lead to increased phage contamination. Our analyses of air samples confirmed that lactococcal phages can be disseminated through the airborne route since up to $2.7x10^4$ and $6.6x10^4$ lactococcal phage genomes were detected per m^3 of air using the BioSampler (Table 3). Considering that it takes only a few infectious phages to infect phage-sensitive *L. lactis* cells and start the phage lytic cycle, cheese factories should possess adequate ventilation and control the airflow to minimize phage dissemination as much as possible.

Our study also clearly demonstrates that phage genomes can be found on various surfaces including floors, walls, cleaning materials, pipes, door handles, and office tables. While it is not known if these viral nucleic acids are still part of infectious phages, it is safe to assume that they were at some point. These findings underscore the need to train workers regarding the importance of surfaces as sources of phage contaminations. They also suggest that the use of appropriate cleaning procedures and effective sanitizers needs to be carefully evaluated to reduce the risks of phage problems.

Although we successfully detected phages in aerosols using different air samplers, not all samplers showed the same level of efficiency. The air sampling devices used in earlier studies on airborne phages in cheese plants (30, 32), were based on a gelatin membrane filtration and on impaction on agar. Although the devices were able to detect phages, they have practical drawbacks. For example, gelatin membrane filtration can dry and break during prolonged sampling or it can dissolve if liquid droplets are sampled. By contrast, impaction on agar relies on plaque assays, which is difficult to apply when a variety of bacterial strains are employed daily. Besides the concern about drying during prolonged sampling with this latter system, only large aerosol particles can be sampled. In fact, there is no standard and approved protocol to detect viral aerosols, let alone phages.

The NIOSH sampler gave the most reliable results in this study. It had the highest proportion of positive samples, low between-samples variability, and the lowest detection limit. The two stages of the NIOSH sampler were analyzed separately to take advantage of the aerodynamic size separation that took place with this sampler. At a sampling rate of 10 L/min, the 50% cut-off is 2.1 µm for the first stage and 0.41 µm for the second stage while the remainder of the aerosol is captured by the third stage. The second stage of this sampler detected the lowest

328 airborne concentrations of 936-like phages while the first stage detected the highest
329 concentration. This suggests that most airborne 936-like phages where bound to larger particles.
330 However for the c2-like phages, there was no difference in the concentrations detected in the two
331 stages of the sampler, indicating that these phages were present at similar concentrations on the
332 smaller and the larger particles. It is not clear at present why this difference was observed. Both
333 phage groups belong to the *Siphoviridae* family but have somewhat different morphology.
334 Phages belonging to the 936 group have an isometric capsid approximately 60 nm in diameter
335 and a long non-contractile tail ranging from 140 nm to 200 nm (morphotype B1) whereas c2-like
336 phages have a prolate capsid (60 nm x 40 nm) and a 100-nm-long non-contractile tail
337 (morphotype B2) (26).

338 Of all the samplers tested, the BioSampler allowed the highest recovery of airborne phage
339 concentrations. However, given its very high detection limit and the few positive samples
340 collected, this sampler was less suitable for the determination of the airborne phage
341 concentrations.

342 The Coriolis sampler has the advantage of collecting a large volume of air in a very short
343 time period. However, with its very high flow rate (300 L/min), this sampler can draw in particles
344 with greater inertia (greater aerodynamic size) than can the filters (2 L/min), the NIOSH
345 (10 L/min) or the BioSampler (12.5 L/min). Considering that the mass of a particle is
346 proportional to the cubic value of the radius, a few large particles can drastically raise the
347 concentration of airborne viruses detected. The presence of these larger aggregated particles in
348 the liquid sample can also cause large differences in viral concentrations between aliquots (see
349 c2-like phages in Table 3). Our attempts to reduce these variations consisted of purifying the viral
350 DNA with commercial kits and exposing the samples to sonication (data not shown). However,

viral DNA purification lowered the concentrations of DNA and led to the underestimation of the viral load in the air sample while sonication had no effect on the sample variability (data not shown). This inherent variability may be due to the variation of the concentrations of airborne viruses over the course of a day reflected by the short sampling period of the Coriolis. A sampler that slowly collects its sample, like the NIOSH sampler or PC and PTFE filters, likely provides a more representative evaluation of the airborne viral concentration over the course of a day.

In conclusion, various types of samplers were successfully used to collect airborne viruses, but the NIOSH sampler was the most efficient. Most samplers detected concentrations of at least 10^3 genomes/m^3 of air for both the lactococcal 936-and the c2-phage groups. The NIOSH sampler results indicate that a significant portion of the airborne phages was bound to small particles (< 2.1 µm). Since these smaller particles can remain airborne for longer periods of time and are influenced by air movements, it is likely that they can be carried far away from their aerosolization source. Although the dynamics of airborne viral transmission are poorly understood (29), appropriate ventilation practices should reduce airborne dissemination. Finally, a qPCR protocol was effectively adapted to detect lactococcal phages. However, it is not known whether these phages were active or inactive, or whether they were inactivated by the sampling/elution procedure. The detection level and the limit of detection are the most important characteristics to consider when choosing a sampler for field studies.

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Table 1: Sampler descriptions

Sampler	Principle	Sampling rate used (L/min)	Documented physical collection efficiency	Reference
Polycarbonate (PC) filters (0.4 µm pore size)	Filtration	2	78 to 99.99% for particles 0.035 to 1 µm. (Data obtained with similar filter from different manufacturer)	(19)
Polytetrafluoroethylene (PTFE) membrane filters (0.3 µm pore size)	Filtration	2	More than 96% efficiency for particles 10 to 900 nm	(6)
BioSampler	Liquid impaction	12.5	>1.0 µm; close to 100% 0.5 µm; 90%	(37)
Coriolis cyclone sampler	Liquid impaction	300	0.8 µm; 62% 1.6 µm; 70% 2.4 µm; 80% 4.4 µm; 100% 16 µm; 109%	(2)
NIOSH two-stage bioaerosol cyclone (BC 251) personal samplers	Dry surface impaction (stages 1 and 2) and filtration (stage 3)	10	First stage 50% cut-off: 2.1 µm Second stage 50% cut-off: 0.41 µm	Product data sheet

Table 2: Primer specificity for c2 and 936 detection and quantification.

Phage isolates from cheese factories	Group	936		c2	
		qPCR	Gel	qPCR	Gel
DM1	936	+	+	-	-
DM2	936	+	+	-	-
DM3	936	+	+	-	-
DM4	936	+	+	-	-
DM5	936	+	+	-	-
DM6	936	+	+	-	-
DM7	936	+	+	-	-
DM8	936	+	+	-	-
DM9	936	+	+	-	-
DM10	936	+	+	-	-
DM11	936	+	+	-	-
DM12	936	+	+	-	-
DM13	936	+	+	-	-
JG4	936	+	+	-	-
JG9	936	+	+	-	-
JG10	936	+	+	-	-
GL1	936	+	+	-	-
GL3	936	+	+	-	-
GL7	936	+	+	-	-
GL8	936	+	+	-	-
GL9	936	+	+	-	-

GL10	936	+	+	-	-
GL11	936	+	+	-	-
LS13	c2	-	-	+	+
CB17	c2	-	-	+	+
CB27	c2	-	-	+	+
GR3	c2	-	-	+	+
GR4	c2	-	-	+	+
GR6	c2	-	-	+	+
bIL67	c2	-	-	+	+
GR2	P335	-	-	-	-

Table 3: Airborne concentrations of 936 and c2 phages in a cheese factory.

Sampler	**936**			**c2**		
	Positive/ total assays[a]	Median concentration (1st quartile; 3rd quartile)[b]	LOD[c]	Positive/ total assays[a]	Median concentration (1st quartile; 3rd quartile)[b]	LOD[c]
NIOSH (stage 1)	12/12 (100%)	5346 (2494; 6010)	10	10/12 (83%)	162 (124; 208)	50
NIOSH (stage 2)	11/12 (92%)	280 (159; 449)	10	5/12 (42%)	223 (139; 465)	50
Coriolis	43/52 (83%)	580 (249; 2905)	62	32/52 (62%)	1848 (792; 31077)	318
PC filter	15/18 (83%)	2399 (1372; 3483)	492	5/18 (28%)	5377 (3562; 5595)	2523
PTFE filter	14/18 (78%)	1538 (1271; 2093)	492	5/18 (28%)	3378 (3232; 3917)	2523
BioSampler	1/18 (6%)	27903	6986	3/18 (17%)	51750 (44905; 66148)	35821

[a] Number of positive qPCR assays versus the total number of assays (% positive samples).

[b] Median concentration of genomes/m^3 of air taking only positive samples into account.

[c] Limit of detection of viral genomes/m^3 of air by qPCR.

Table 4: Estimated concentrations of 936-like and c2-like genomes per cm^2 of a swabbed surface.

Surface description	**936**		**c2**	
	Genomes/cm^2	**Positive/total assays** [a]	**Genomes/cm^2**	**Positive/total assays** [a]
Door handle	9106 ± 789	3/3	869 ± 69	3/3
Rough floor	6115 ± 785	3/3	1113 ± 120	3/3
Top of paper towel dispenser	6079 ± 800	3/3	458 ± 109	3/3
Cleaning material	1854 ± 271	3/3	3138 ± 449	3/3
Smooth floor	448 ± 145	15/15	564 ± 403	9/10
Wall	407 ± 169	3/3	945 ± 66	3/3
Push for hand sanitizer	266 ± 126	3/3	0	0/3
Office table	60 ± 28	3/3	83 ± 41	3/3
Top of electrical panel	53	1/4	0	0/5
Top of stainless pipe	30 ± 7	3/3	260 ± 59	3/3
Stair ramp	11 ± 10	4/6	67 ± 44	3/6
Top of closed tank	3 ± 1	3/3	12 ± 2	3/3
Vertical panel	2	1/3	9	1/3
Vertical surface (electrical)	0	0/3	43 ± 5	3/3

[a] Number of positive qPCR assays versus the total number of assays.

Figure 1: Mean size distribution of airborne particles in a cheese factory shown according to the frequency of particle count in each channel of the APC. Error bars represent standard deviations.