Monitoring and characterization of lactococcal bacteriophages in a dairy plant

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1. Introduction

Bacteriophages attacking mesophilic lactic acid streptococci (lactococci) are known since the 1930's (1). Phage contaminations in dairy plants are still of great economic importance, since phage-derived lysis of the cultures results in slow fermentations or even complete failure to produce lactic acid. Therefore, one overall objective is the control of phage attack on the starter bacteria. Phage infections can be minimized either by traditional approaches or - as has been shown in the last decade - by new genetic tools. In the first case, stringent disinfection programms, rotation of starter cultures and the selection of phage-insensitive strains have been applied (2, 3). For the second approach, phage resistance plasmids have been transferred naturally into phage-sensitive strains by conjugation (4). However, all efforts to control phage infection rely on a profound knowledge and understanding of the phages which may occur in the dairy plant (5).

Three different types of bacteriophages are widespread in dairies throughout the world: small isometric-headed phages of the 936-species, prolate-headed phages of the c2-species, and – to a less extent – large isometric-headed phages of the 949-species [6]. Recently, it has been reported that a new phage type belonging to the P335-species (7) has emerged in dairy environments (8, 9). Therefore, we were interested to investigate for a period of 6 months the current status of phage contamination in a German dairy plant specialized on the production of fresh cheese (quarg). Removal of whey from the curd in a centrifugal separator is a crucial step in quarg production with respect to phage control, since the whey aerosols are known to contain significant numbers of phages which are easily spread in the dairy environment. A long-term phage monitoring has already been performed by us in this factory from 1982 to 1984 (10, 11). The present study is the basis for an evaluation whether a shift in the predominant phage population has occurred or may be expected.

2. Material and methods

Bacteria and growth media

Phage monitoring was performed from May 1992 until October 1992. During this 6-months-period, 5 undefined mixed-strain starter cultures (all were mesophilic lactic acid streptococci [lactococci]) were used successively in culture rotation for the production of quarg. The rotation scheme of the 5 starter cultures is shown in Fig. 1. At the beginning, starter cultures A and B were used alternatively. A severe shortening of cycle intervals occurred from cycle IV to VIII. Later, culture B was replaced by cultures D (cycle VII), C (cycles VIII, X, XII, and XV) and E (cycle XIV). This allowed again a prolongation of the cycle intervals. In general, cycles of culture A were twice as long (25 days in maximum) as the following intermediate cultures. Cultures D and E were only used once during the 6 months.

Fig. 1. Rotation scheme of the 5 undefined mixed-strain starter cultures and lengths of cycles (in days)

Cycle	I	II	III	IV	V–VIII				IX	X	XI	XII	XIII	XIV	XV
Days	(a)	21	10	9	5	3	4	8	19	11	19	10	25	12	(a)
Culture	B	A	B	A	B	A	D	C	A	C	A	C	A	C	C

(a)Total length of cycle not known.

The cultures were inoculated directly as deep-frozen concentrates into the bulk starter vessel. For our investigation, the cultures A, B, C and E were originally obtained as deep-frozen concentrates from the starter culture supplier. Culture D was not available and was omitted in the experiments described here. Upon arrival, 52 single colony isolates were taken from each culture. The strains were grown at 30 °C in litmus skim milk or in M17-broth (12). The bacterial strains were maintained at -72 °C in reconstituted skim milk.

Sampling

The following samples were taken from the factory's staff: (raw) milk from the silo tanks destinated either for the bulk starter vessels or for the coagulation/fermentation vessels, bulk starters, whey separated from the curd, and products (quarg) after packaging. Air samples were drawn from different areas of the factory (on the roof above the fermentation vats and above the packaging facilities, in front of the bulk starter room and in the neighbourhood of the fermentation vats, in the separator room and close to the ultrafiltration equipment). Sampling was routinely done at the beginning and at the end of each cycle of rotation.

Upon arrival in the laboratory, the samples were centrifugated (18,000 x g, 20 min, 4 °C), and the supernatants were filter-sterilized (0.45 µm pore size). In some cases, 10 ml of the samples had to be acidified with 0.3 ml of 10% lactic acid for casein precipitation prior to centrifugation and filtration.

For air sampling, the air was drawn through 100 ml of diluted M17-broth (dilution was 1:14 (v/v) with Ringer solution in order to avoid excessive foaming) in a gas washing bottle using a laboratory vacuum pump with a constant flow rate (approximately 6 ltr/min).

Miniaturized activity test

The activity tests were performed in microplates filled with bromcresol purple (0.01% [w/v]) skim milk supplemented with 0.2 vol. Elliker broth (13). Using a stainless steel multiple inoculator, 52 bacterial culture isolates and the filtrate to be tested were inoculated simultaneously into the microplate wells. After over-night incubation at 30 °C, activity of each isolate with respect to the rate of acid production was determined on basis of the change of the color of the indicator upon acidification.

Determination of phage titers

For the determination of the phage titers (plaque forming units [pfu]), the agar double layer method (14) was used.

Isolation and purification of phages

Phages were purified routinely by 3 single plaque isolation steps. Phage lysates were prepared in M17-broth supplemented with 5 mM $CaCl_2$.

Plasmid analysis

3 ml of cell cultures grown into the late logarithmic phase were used for plasmid-DNA isolation (15). Plasmid-DNA was dissolved in 20 µl TE-buffer (16) and was analyzed by agarose gel (0.65%) electrophoresis in 0.5x TBE-buffer at 7 V/cm (16). Plasmids of known sizes from *Lactococcus lactis* subsp. *cremoris* AC1 were used as size standards in agarose gels (17).

Determination of phage/host spectra

From each starter culture, 3 representative single-colony isolates were chosen which revealed sensitivity to bacteriophages in the starter activity test. Using the agar double-layer technique, multiple samples (whey filtrates and air samples) were spotted with a stainless steel inoculator from a microtiter stock plate onto a lawn of the host bacteria. Lysis of the bacteria was scored after over-night incubation at 30 °C.

Electron microscopy

All whey filtrates were examined in a Philips EM 300 electron microscope using the negative staining technique described earlier (18).

3. Results and discussion

Activity tests and phage titers

From each starter culture, 52 single-colony isolates were analyzed for activity (rate of acid production) in the presence of the sample filtrates or the air samples. To confirm the presence of bacteriophages, the plaquing ability of the samples was tested on a representative starter culture isolate which failed to grow in the activity test.

The milk stock used either for the production of the bulk starters or for the production of quarg in the fermentation vessels was tested in cycles II to XV. In general, no reduction in activity was observed except for the milk samples from cycle III which resulted in a reduced activity of 4 % of the starter isolates (Fig. 2). However, no phage-derived plaques were detected on indicator strains. Therefore, raw milk can be excluded here to be a major source of phage contamination. It is well known from other reports that low titers of virulent phages may be present in raw milk (19, 20).

A severe reduction in starter activity was documented, when the bulk starter samples from the first part of the investigation period were tested, and phage titers up to 1×10^8 pfu/ml were detected. Fig. 3 illustrates that a phage pool was built up from cycle I to VI in the bulk starter. Analysis of samples taken at the beginning and the end of cycle III and IV revealed that the total number of isolates showing reduced or no activity accumulated during each cycle.

Fig. 4 illustrates that high phage titers (up to 3×10^9 pfu/ml in cycle XIII) were detected in all whey samples (with the exception of samples taken at the beginning of cycle XIV). In general, phage titers were equally high at the beginning and at the end of each cycle indicating that a constant high-titer phage pool was present in the fermentation vats. Although no significant phage contaminations were detected during cycles VIII to XV in the bulk starter samples (Fig. 3), phages were still present in these cycles in the whey samples.

Fig. 2. Effect of milk stock samples on the activity (rate of acid production) of the starter cultures and phage titers obtained on one homologous phage sensitive culture isolate. All samples tested were taken at the beginning of each cycle.

Cycle	Sample[a]	Culture used	Distribution of 52 isolates (in %) with respect to rate of acid production[b]	Phage titer (pfu) per ml sample[c]
II	MB	A		–
II	MF	A		–
III	MB	B		n.d.
III	MF	B		n.d.
VIII	MB	C		–
IX	MF	A		–
X	MB	C		n.d.
XIV	MB	E		–
XV	MB	C		n.d.
XV	MF	C		n.d.
			0 % 50 % 100 %	

[a] MB: Milk stock for bulk starter; MF: Milk stock for fermentation vessel
[b] : Full activity; : Reduced activity
[c] – : Not tested; n.d. : Phages not detectable

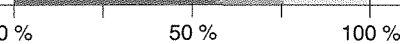
Fig. 3. Effect of bulk starter samples on the activity (rate of acid production) of the starter cultures and phage titers obtained on one homologous phage sensitive culture isolate

Cycle	Sampling time[a]	Culture used	Distribution of 52 isolates (in %) with respect to rate of acid production[b]	Phage titer (pfu) per ml sample[c]
I	e	B		–
II	b	A		–
III	b	B		–
III	e	B		n.d.
IV	b	A		n.d.
IV	e	A		$4x10^2$
V	b	B		n.d.
VI	b	A		$1x10^8$
VIII	b	C		n.d.
VIII	e	C		n.d.
IX	b	A		n.d.
X	b	C		n.d.
XIV	b	E		n.d.
XV	b	C		n.d.
			0 % 50 % 100 %	

[a] b: Beginning of cycle; e: End of cycle
[b] : Full activity; : Reduced activity; : No activity
[c] – : Not tested; n.d.: Phages not detectable

Fig. 4. Effect of whey samples on the activity (rate of acid production) of the starter cultures and phage titers obtained on one homologous phage sensitive culture isolate

Cycle	Sampling time[a]	Culture used	Distribution of 52 isolates (in %) with respect to rate of acid production[b]	Phage titer (pfu) per ml sample[c]
I	e	B		$1x10^6$
II	b	A		$4x10^8$
II	e	A		$3x10^8$
III	b	B		$1x10^9$
III	e	B		$3x10^8$
IV	b	A		$5x10^8$
IV	e	A		$2x10^8$
V	b	B		$5x10^7$
VI	b	A		$3x10^8$
VI	e	A		$2x10^7$
VIII	b	C		$9x10^6$
VIII	e	C		$2x10^8$
IX	b	A		$5x10^8$
IX	e	A		$5x10^6$
X	b	C		$2x10^6$
XIII	e	A		$3x10^9$
XIV	b	E		n.d.
XIV	e	E		$4x10^7$
XV	b	C		$5x10^6$



[a] b: Beginning of cycle; e: End of cycle
[b] : Full activity; : Reduced activity; : No activity
[c] n.d.: Phages not detectable

The majority of the air samples taken during cycles I and III resulted in reduction of activity of the bacterial isolates, indicating that a phage aerosol was widely distributed in the factory (including air samples taken from the roof above the fermentation vats and above the packaging facilities, air samples taken in front of the bulk starter room, in front of the ultrafiltration equipment, and from the separator room [Fig. 5]). The phage titers were usually in the lower range of detection ($1x10^4$ - $2x10^4$ pfu/m^3 air). However, a high phage titer was documented in the air samples taken in cycle I and III from the separator room (up to $6x10^6$ pfu/m^3 air). A phage aerosol distributed in the factory by the centrifugal separators is likely to account significantly for a constant phage load in the plant. This has already been shown earlier by us (10).

Fig. 5. Effect of air samples on the activity (rate of acid production) of the starter cultures and phage titers obtained on one homologous phage sensitive culture isolate. Samples were taken at the end of each cycle.

Cycle	Sample[a]	Culture used	Distribution of 52 isolates (in %) with respect to rate of acid production[b]	Phage titer (pfu) per ml sample[c]
I	RF	B		n.d.
I	RP	B		n.d.
I	B	B		n.d.
I	F	B		n.d.
I	S	B		$2x10^5$
I	U	B		n.d.
III	RF	B		$2x10^4$
III	RP	B		n.d.
III	B	B		n.d.
III	F	B		–
III	S	B		$6x10^6$
III	U	B		$2x10^4$
XV	RF	C		n.d.
XV	RP	C		n.d.
XV	B	C		n.d.
XV	F	C		n.d.
XV	S	C		n.d.
XV	U	C		n.d.

0 % 50 % 100 %

(a) RF : Air sample taken from the roof above the fermentation vessels
RP : Air sample taken from the roof above the packaging facilities
B : Air sample taken in front of the bulk starter room
F : Air sample taken in front of the fermentation vessels
S : Air sample taken from the separator room
U : Air sample taken in front of the ultrafiltration equipment

(b) : Full activity; : Reduced activity; : No activity

(c) – : Not tested; n.d.: Phages not detectable

Electron microscopy

Whey samples from all cycles were analyzed in the electron microscope for documentation of phage morphologies. All samples taken from those cycles in which cultures A, B, D, or E were used, contained small isometric-headed phages of the 936-species (Fig. 6). However, when culture C was used, whey samples contained prolate-headed phages of the c2-species. This rapid change in the phage population may indicate phage reservoirs in the factory as a source for the rapid build-up of new phage pools. Small isometric-headed phages of the P335-species (6, 7) which have evolved recently in North America (8, 9) were not found during our study. The small isometric-headed phages of the 936-species (6) are widespread in many countries (e.g. Germany [21], France [22], Ireland [23] and New Zealand [24]). Occasionally, prolate-headed phages are reported to predominate (e. g. in Canada [8]). Generally, prolate-headed phages reveal broader host spectra than isometric-headed phages (25).

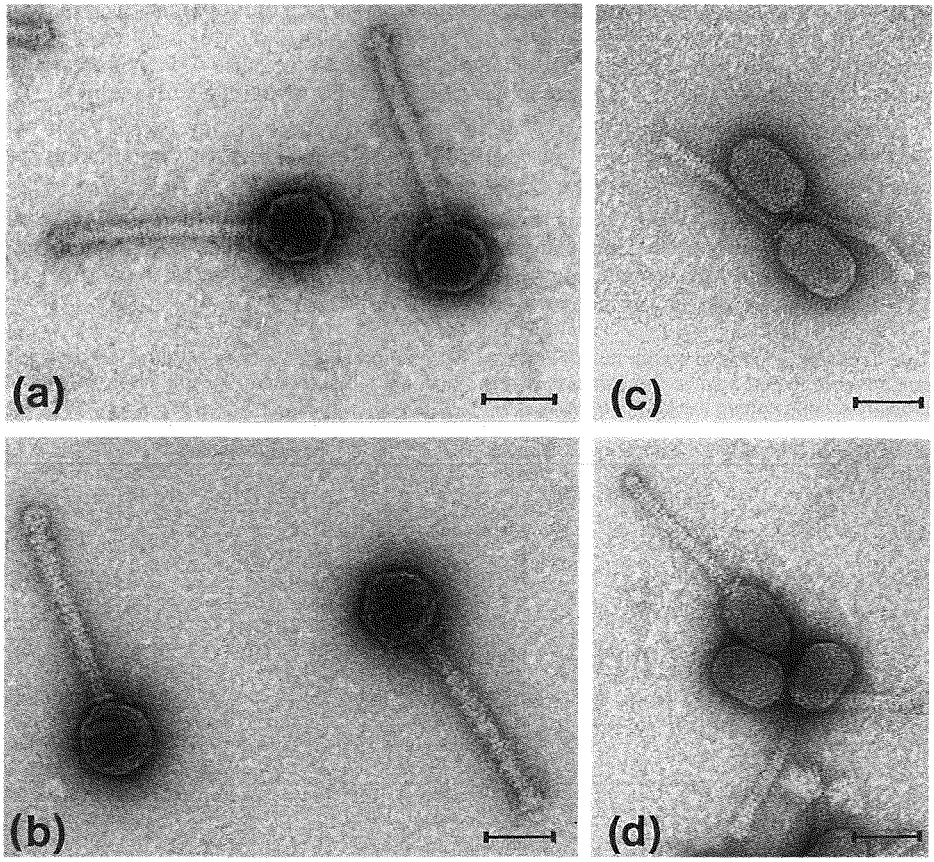


Fig. 6: Electron micrographs of bacteriophages detected in whey samples from cycle I (a), cycle IV (b), cycle VIII (c), and cycle XII (d). The corresponding cultures used were B (in cycle I), A (in cycle IV), and C (in cycles VIII and XII). The bar represents 50 nm.

Phage/host spectra

Since phages were widely distributed in the cheese plant during the 6 months of investigation, it was of considerable interest to determine the phage/host spectra for the detection of cross-reactions of the 5 starter cultures with respect to phage sensitivity. Ideally, phage-unrelated starter cultures should be used in rotation in order to avoid a rapid establishing of a permanent phage pool. From each starter culture, 3 representative isolates revealing high sensitivity to phages were chosen, and sample filtrates were spotted on bacterial lawns of these strains. From the 5 cultures tested, only isolates from culture C failed to reveal cross-reactions with phages derived from other cultures (Table 1). Phages from culture A did also lyse strains from culture E (cycles II, IV, and IX) and vice versa (cycle XIV). Phages from culture A isolated at the end of cycles II, IV, VI, and IX did also lyse strains from culture B: Since culture A was always substituted by culture B in the first part of this study, phages from culture A attacking culture B were already present at the very beginning of the B-cycles. Thus, these phage-related cultures were not suitable for culture rotation. This is also reflected by the cycle intervals in the rotation scheme which became successively shorter during rotation of both cultures (Fig. 1). Phages from

culture A which attacked culture B always occured at the end of the A-cycles. This may indicate that restriction/modification systems present in these cultures could result in the rapid build-up of modified phages. Modified phages attacking culture A did not appear in cycles of culture B which may be due to the significant shorter intervals of culture B.

Starter culture B also revealed a close relationship to culture D (cycle VII; Table 1). This became also evident when the air samples drawn in the separator room during cycles III and VII were analyzed in the cross-reaction test (Table 2).

Phages of culture C which did not reveal any cross-reactions with other cultures, were prolate-headed, while those from cultures A, B, D, and E were isometric-headed. All latter cultures showed cross-reactions with respect to their phage spectra. A summary of the relationship of all cultures is illustrated in Fig. 7.

Tab. 1: Phage spectra of starter culture isolates. Three representative bacterial strains from each culture were tested against the samples (wheys, bulk starters, products) for the determination of cross-reactions among the starter cultures.

Cycle	Sample[a]	Sampling time[b]	Culture used	Culture isolate[c]											
				A 9	A 5	A 25	B 37	B 2	B 9	C 15	C 2	C 50	E 4	E 1	E 7
I	W	e	B	–	–	–	⊕	⊕	⊕	–	–	–	–	–	–
II	W	b	A	⊕	⊕	⊕	–	–	–	–	–	–	⊕	–	–
II	W	e	A	⊕	⊕	⊕	⊕	–	–	–	–	–	⊕	–	–
III	W	b	B	–	–	–	⊕	⊕	⊕	–	–	–	–	–	–
III	W	e	B	–	–	–	⊕	⊕	⊕	–	–	–	–	–	–
IV	W	b	A	⊕	⊕	⊕	–	–	–	–	–	–	⊕	–	–
IV	W	e	A	⊕	⊕	⊕	⊕	–	–	–	–	–	–	–	–
V	W	b	B	–	–	–	⊕	⊕	⊕	–	–	–	–	–	–
VI	W	b	A	⊕	⊕	⊕	–	–	–	–	–	–	–	–	–
VI	B	b	A	⊕	⊕	–	–	–	–	–	–	–	–	–	–
VI	W	e	A	⊕	⊕	⊕	⊕	–	–	–	–	–	–	–	–
VII	W	b	D	–	–	–	⊕	⊕	⊕	–	–	–	–	–	–
VII	W	e	D	–	–	–	⊕	⊕	⊕	–	–	–	–	–	–
VIII	W	b	C	–	–	–	–	–	–	–	–	⊕	–	–	–
VIII	W	e	C	–	–	–	–	–	–	–	⊕	⊕	–	–	–
IX	W	b	A	⊕	⊕	⊕	–	–	–	–	–	–	–	–	–
IX	W	e	A	⊕	⊕	⊕	⊕	–	–	–	–	–	–	⊕	⊕
X	W	b	C	–	–	–	–	–	–	–	⊕	⊕	–	–	–
XII	P	e	C	–	–	–	–	–	–	⊕	⊕	⊕	–	–	–
XIII	W	e	A	⊕	⊕	⊕	–	–	–	–	–	–	–	–	–
XIV	W	e	E	–	⊕	–	–	–	–	–	–	–	–	⊕	⊕
XV	W	b	C	–	–	–	–	–	–	–	⊕	⊕	–	–	–

(a) W : Whey
B : Bulk starter
P : Product

(b) b : Beginning of cycle
e : End of cycle

(c) – : No lysis of culture isolate
⊕ : Lysis of culture isolate

Plasmid analysis

In order to evaluate the strain diversity in the undefined mixed-strain starter cultures, plasmid profiles were determined from all 52 isolates obtained from each culture. Thirteen representative plasmid patterns from each culture are shown in Fig. 8.

Plasmid profiles from cultures A, B and E were homogeneous and revealed one predominating pattern. Plasmid profiles from the isolates of culture C were highly heterogeneous and basically different from those of the former cultures. Therefore, culture C represents best an undefined mixed-strain starter culture comprising of many different strains.

Tab. 2: Phage spectra of starter culture isolates. Three representative bacterial isolates from each culture were tested against the air samples for the determination of cross-reactions among the starter cultures.

Cycle	Sample[a]	Culture used	Culture isolate[b]											
			A 9	A 5	A 25	B 37	B 2	B 9	C 15	C 2	C 50	E 4	E 1	E 7
III	RF	B	–	–	–	–	–	–	–	–	–	–	–	–
III	RP	B	–	–	–	–	–	–	–	–	–	–	–	–
III	B	B	–	–	–	–	–	–	–	–	–	–	–	–
III	F	B	–	–	–	–	–	–	–	–	–	–	–	–
III	S	B	–	–	–	⊕	–	–	–	–	–	–	–	–
III	U	B	–	–	–	–	–	–	–	–	–	–	–	–
VII	RF	D	–	–	–	–	–	–	–	–	–	–	–	–
VII	RP	D	–	–	–	–	–	–	–	–	–	–	–	–
VII	B	D	–	–	–	–	–	–	–	–	–	–	–	–
VII	F	D	–	–	–	–	–	–	–	–	–	–	–	–
VII	S	D	–	–	–	⊕	⊕	–	–	–	–	–	–	–
VII	U	D	–	–	–	–	–	–	–	–	–	–	–	–

[a] RF : Air sample taken from the roof above the fermentation vessels
RP : Air sample taken from the roof above the packaging facilities
B : Air sample taken in front of the bulk starter room
F : Air sample taken in front of the fermentation vessels
S : Air sample taken from the separator room
U : Air sample taken in front of the ultrafiltration equipment

[b] – : No lysis of culture isolate
⊕ : Lysis of culture isolate

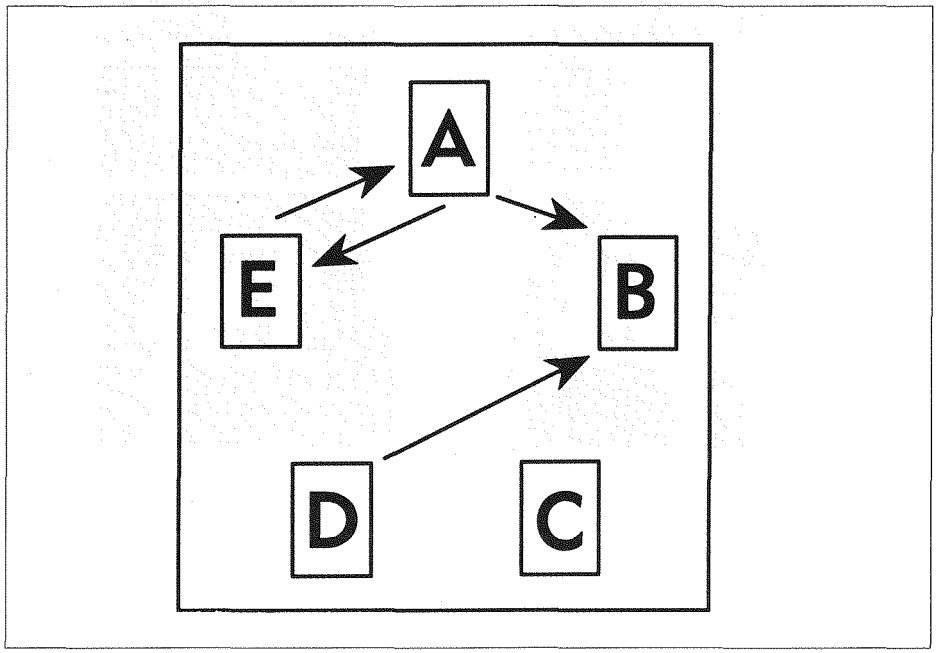


Fig. 7: Cross-reactions of the 5 starter cultures with respect to phage sensitivity. The arrows point to those cultures which contain strains lysed by phages obtained from other cultures.

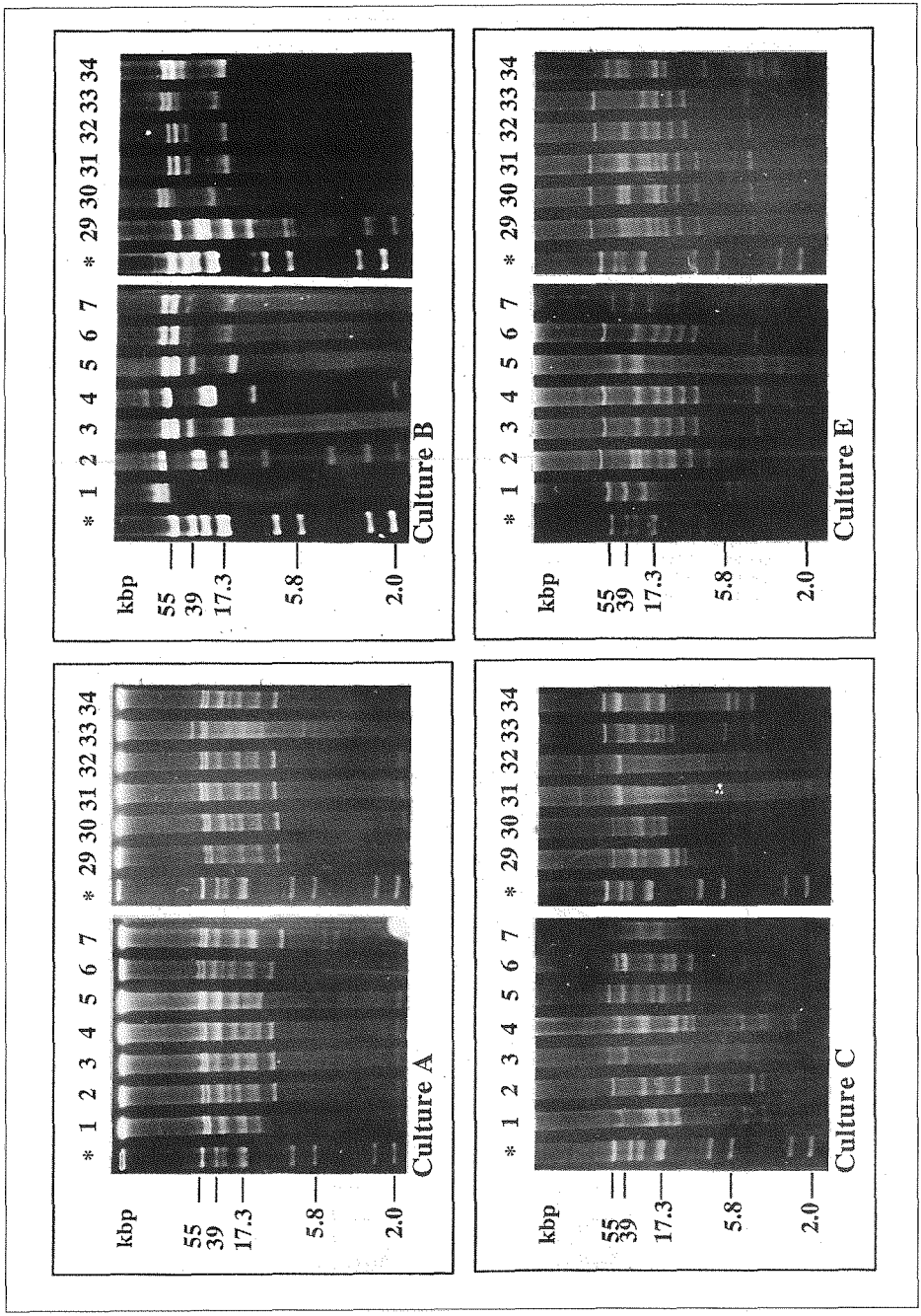


Fig. 8: Plasmid profiles of 13 representative single colony isolates (strains 1-7 and 29-34) from the starter cultures A, B, C, and E, respectively. In the lanes indicated by the asterisks, the plasmids of known sizes from *Lactococcus lactis* subsp. *cremoris* AC1 are shown (sizes from 2.0 - 55 kilobase pairs [kbp]).

4. Conclusions

Starter culturtes A, B, D, and E used in rotation for production of quarg, were phage-related, and small isometric-headed phages proliferated on these strains. In cultures A, B, and E, one strain was predominating according to the plasmid analysis. With respect to phage sensitivity, starter culture C did not reveal cross-reactions with the former cultures, and phages attacking culture C were prolate-headed. Furthermore, strain composition of culture C was heterogeneous and different from the other cultures. Therefore, culture C could be used in rotation with culture A. However, to ensure long intervals of cycles in rotation, phage reservoirs present in the factory have to be eliminated. This is crucial for phage protection of the bulk starters and the cultures in the fermentation vats. From our study, no evidence has been found that a new phage type (of the P335-species) has evolved in the factory so far.

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5. References

(1) Whitehead, H.R., Cox, G. A.: New Zealand Journal of Science and Technology **16** 319-320 (1935)
(2) Cogan, T.M., Peitersen, M., Sellars, R.L.: Bulletin of the IDF **263** 16-23 (1991)
(3) Everson, T.C.: Bulletin of the IDF **263** 24-28 (1991)
(4) Hill, C.: FEMS Microbiology Reviews **12** 87-108 (1993)
(5) Neve, H., Teuber, M.: Bulletin of the IDF **263** 3-15 (1991)
(6) Jarvis, A., Fitzgerald, G.F., Mata, M., Mercenier, M., Neve, H., Powell, I.B., Ronda, C., Saxelin, M., Teuber, M.: Intervirology **32** 2-9 (1991)
(7) Braun, V., Hertwig, S., Neve, H., Geis, A., Teuber, M.: Journal of General Microbiology **135** 2551-2560 (1989)
(8) Moineau, S., Fortier, J., Ackermann, H.-W., Pandian S.: Canadian Journal of Microbiology **38** 875-882 (1993)
(9) Moineau, S., Pandian, S., Klaenhammer, T.D.: Applied and Environmental Microbiology **59** 197-202 (1993)
(10) Budde-Niekiel, A., Möller, V., Teuber, M.: Milchwissenschaft **40** 477-481 (1985)
(11) Möller, V., Teuber, M.: Milchwissenschaft **43** 482-486 (1988)
(12) Terzaghi, B.E., Sandine, W.E.: Applied Microbiology **29** 807-813 (1975)
(13) Elliker, P.R., Anderson, A., Hanneson, G.: Journal of Dairy Science **39** 1611-1612 (1956)
(14) Adams, M.H.: Interscience Publishers, Inc., New York (1959)
(15) Anderson, D.G., McKay, L.L.: Applied Environmental Microbiology **46** 549-552 (1983)
(16) Sambrook, J., Fritsch, E.F., Maniatis, T.: Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)
(17) Neve, H., Geis, A., Teuber, M.: Journal of Bacteriology **157** 833-838 (1984)
(18) Lembke, J., Krusch, U., Lompe, A., Teuber, M.: Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene - I. Abt. Originale **C1** 79-91 (1980)
(19) Heap, H.A., Limsowtin, G.K.Y., Lawrence, R.C.: New Zealand Journal of Dairy Science and Technology **13** 16-22 (1978)
(20) Jarvis, A.W.: New Zealand Journal of Dairy Science and Technology **22** 93-103 (1987)
(21) Loof, M., Lembke, J., Teuber, M.: Systematic and Applied Microbiology **4** 413-423 (1983)
(22) Prevots, F., Mata, M., Ritzenthaler, P.: Applied and Environmental Microbiology **56** 2180-2185 (1990)
(23) Coveney, J.A., Fitzgerald, G.F., Daly, C.: Applied and Environmental Microbiology **53** 1439-1447 (1987)
(24) Jarvis, A.W., Heap, H.A.: New Zealand Journal of Dairy Science and Technology **19** 125-128 (1984)
(25) Heap, H.A., Jarvis, A.W.: New Zealand Journal of Dairy Science and Technology **15** 75-81 (1980)

6. Summary

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)

26 Lactococcal bacteriophages

During a 6-months period, phage monitoring was performed in the quarg production section of a dairy factory practising rotation of 5 undefined mixed-strain starter cultures.

A wide distribution of virulent phages was shown in samples from the bulk starters, from wheys and those drawn from the air. Phage load was up to $3x10^9$ plaque forming units (pfu) per ml whey and up to $6x10^6$ pfu per m^3 air from the separator room. A significant shift in phage population occurred during the course of this study: While small isometric-headed phages were found in the beginning, prolate-headed phages appeared later upon introduction of culture C into the rotation scheme. This culture was not related to the other 4 cultures with respect to phage sensitivity. The latter were all phage-related and only partly suitable for culture rotation.

Zusammenfassung

Neve, H., Kemper, U., Geis, A., Heller, K.J.: **Monitoring und Charakterisierung von Laktokokken-Bakteriophagen in einer Molkerei**. Kieler Milchwirtschaftliche Forschungsberichte **46** (2) 167–178 (1994)

26 Laktokokken-Bakteriophagen

Über einen 6-monatigen Untersuchungszeitraum wurde in der Quarkproduktion einer Molkerei, in der die Rotation von 5 nicht-definierten Vielstamm-Kulturen praktiziert wurde, ein Bakteriophagen-Monitoring durchgeführt. Die Untersuchung zeigte eine weite Verbreitung von virulenten Bakteriophagen im Betrieb in den Betriebsstarter-, Molken- und Luftproben. Die Phagenbelastung betrug bis zu $3x10^9$ Plaque-bildende Einheiten (PbE) pro ml Molke und bis zu $6x10^6$ PbE pro m^3 Luft aus dem Separatorenraum. Im Verlauf der Untersuchung wurde ein ausgeprägter Wechsel in der Phagenpopulation dokumentiert: Während am Anfang Phagen mit isodiametrischen Köpfen auftraten, wurden später Phagen mit prolaten Köpfen nachgewiesen, nachdem die Starterkultur C in die Rotation aufgenommen wurde. Diese Kultur war hinsichtlich ihrer Phagenempfindlichkeit nicht mit den anderer 4 Kulturen verwandt. Die letzteren Kulturen zeigten eine Phagenverwandtschaft und waren somit für eine Kulturenrotation nur bedingt geeignet.

Résumé

Neve, H., Kemper, U., Geis, A., Heller, K.J.: **Monitoring et caractérisation de bactériophages de Lactococcus dans une laiterie**. Kieler Milchwirtschaftliche Forschungsberichte **46** (2) 167–178 (1994)

26 Bactériophages de Lactococcus

Pendant 6 mois on a surveillé des bactériophages dans le département d'une laiterie fabriquant de la caillebotte qui emploie le système de rotation de 5 cultures mixtes pas définies. On a démontré une large distribution de bactériophages virulents dans l'usine dans les échantillons du levain industriel, du sérum et de l'air. La contamination a maximalement atteint $3x10^9$ pfu (unités capables de former des plages)/ml de sérum et maximalement $6x10^6$ pfu/m^3 de l'air dans la chambre d'écrémeuse. Au cours de l'essai on a observé un changement prononcé dans la population des phages: on a observé, au début, des phages à tête isométrique et plus tard des phages à tête „prolate", après qu'on a introduit la culture C dans la rotation. Cette culture n'était pas parent des autres 4 cultures en ce qui concerne la sensibilité phagique. Les dernières cultures ont montré une parenté phagique et étaient, par conséquent, utilisables seulement sous réserve pour une rotation de culture.