APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1982, p. 349–356
0099-2240/82/020349-08$02.00/0

Vol. 43, No. 2

Lysogenic Strains of Lactic Acid Streptococci and Lytic Spectra of Their Temperate Bacteriophages

J. REYROLLE,[1]* M. C. CHOPIN,[2] F. LETELLIER,[1] AND G. NOVEL[1]

Equipe de Génétique Microbienne, U. E. R. des Sciences de l'Alimentation, 14032 Caen Cedex, France[1]; and Laboratoire de Recherches de Technologie Laitière, Institut National de la Recherche Agronomique, 35042 Rennes Cedex, France[2]

Received 1 June 1981/Accepted 29 September 1981

A total of 113 strains of mesophilic strains lactic streptococci of the species *Streptococcus lactis*, *S. lactis* subsp. *diacetilactis*, and *S. cremoris*, chosen from 291 strains that had been previously classified into six groups on the basis of their sensitivity to 132 virulent phages, were subjected to induction with mitomycin C. Among these strains, 43% produced phages capable of forming plaques of lysis on an indicator strain either spontaneously or after induction. There was a close correlation between the lytic spectra of temperate and virulent phages. Among the strains studied, 25% were shown to be indicator strains. These results emphasized the high probability of development of temperate phages in a starter culture containing mesophilic lactic streptococci and therefore their importance as a cause of accidents in cheese making.

Lactic acid streptococci used as starters have been suspected for a long time (3) to be a possible source of lysogenic phages responsible for certain culture failures during cheese making; for a review, see Lawrence et al. (11). Knowledge of the lysogenic and indicator strains that allow multiplication of the phages liberated is therefore important in the choice of starter strains.

The problem of lysogeny in lactic streptococci has been reviewed recently (10). Strains can produce phage particles after induction by UV radiation (6, 7, 9, 13, 14, 17) or by mitomycin C (MC) (7, 18) and sometimes spontaneously (7, 9). The phages were first recognized by means of the electron microscope (6, 7, 13, 14, 16) and then were revealed by the lysis of indicator strains (9), but these latter strains appear to occur only rarely (6, 7, 13).

Lysogeny has been demonstrated in the three species of mesophilic lactic streptococci (*Streptococcus lactis*, *S. lactis* subsp. *diacetilactis*, and *S. cremoris*). Indicator strains are also present in these species (7, 9). Lysogenic strains are not very frequent when lysis of indicator strains is used for their detection (7, 9, 13), whereas a frequency of 50% or more among induced strains may be obtained (7, 16) when the phages are identified only by cellular lysis or with the electron microscope.

In a previous paper, one of us (2) separated 291 strains of lactic streptococci from the three species into six groups by using their sensitivity to 132 virulent phages. In the present investigation, we have induced by MC a sample of strains representative of these six lytic groups, and we have revealed the phage particles by the lysis of at least one indicator strain.

MATERIALS AND METHODS

Origin and cultivation of bacterial strains. Previously Chopin et al. (2) separated 291 strains of lactic streptococci into six groups (G1 to G6) on the basis of their phage sensitivity pattern. Of these strains, 82 were resistant to all of the virulent phages used in that work and were designated group R. For the present study, 113 strains representative of all these bacterial groups were chosen. All of the strains in groups containing a few strains (G1, G2, G4, G5, and G6) were included, whereas of the 121 strains forming group G3, 37 were tested, and of the 82 strains in group R, 21 were represented. Strains belonging to groups G3 and R were chosen on the basis of their phage sensitivity pattern, geographical origin (France or abroad), and the percentage of each species relating to that obtained in the original study (2).

Successive subcultures of Z109 (group R) in M17 broth, has enabled us to obtain a spontaneous variant, Z109B, sensitive to some temperate phages.

All bacterial strains were stored frozen at −40°C in litmus milk. All subcultures were made during the experiment in M17 broth by using a 2% (vol/vol) inoculum and incubation was at 30°C.

Induction of lysogens. After thawing, bacterial strains were subcultured twice in M17 broth. Inocula of 2% (vol/vol) (optical density, 0.05) were then made into 20 ml of M17 broth and divided into two 10-ml samples, which were incubated for 90 min at 30°C (optical density ≈0.1). MC (Sigma Chemical Co.) was added in a final concentration of 1 μg/ml to the assay tube; the other tube served as a control. Incubation was continued for 3 h at 30°C. The tubes were then centrifuged (4,000 × *g*, 10 min), and the supernatants

were filter sterilized through a 0.45-μm pore membrane (Millipore MA).

Screening for indicators and lysogens. Each strain was treated as both a potential indicator strain and a lysogen. A pool of filtrates was prepared from groups of filtrates from 10 individual cultures of MC-induced strains by mixing 1 ml of each of 10 filtrates, the pool was tested as a single filtrate by the double agar layer method (20) (see below). When plaques were observed, each filtrate in the pool was tested separately. Any lysis or growth inhibition was further tested for plaque formation by the double agar layer method, and the PFU were counted.

Bacteriophages. (i) Propagation and concentration. Induced bacteriophages were propagated by preparing five plates for each phage by the double agar layer method. Each plate was inoculated with 0.1 ml of the indicator strain and 0.1 ml of a phage dilution to give ca. 10^3 PFU per ml. After incubation at 30°C for 24 h, the upper layer of each plate was collected with 2 ml of 10% M17 broth and centrifuged for 10 min at 4,000 × *g*. The supernatant was then filter sterilized, and the titer was determined. In some cases, the phage was concentrated by ultracentrifugation for 150 min at 30,000 rpm (rotor type 50; Beckman Instruments, Inc.).

(ii) Adsorption. A 0.1-ml sample of an 18-h bacterial culture was added to 0.1 ml of a phage suspension (10^5 PFU/ml) and one drop (0.05 ml) of 1 M $CaCl_2$ and mixed at 30°C for 10 or 30 min and then diluted with 9.8 ml of cold 10% M17 broth. After centrifugation at 4,000 × *g* for 10 min, the supernatant was tested by incubation with an indicator strain for 24 h. Adsorption was expressed as a lowering in phage titer with the control (phage only) as a comparison.

(iii) Nomenclature and storage. Temperate phages were designated by the method of Jarvis (8), using the prefix φ followed by the number of the lysogenic strain and then the number of the indicator strain in brackets. These phages were stored in M17 broth at 4°C during the investigation.

RESULTS

In this study, a strain was considered lysogenic if it produced phages, either spontaneously or after induction, capable of forming plaques on an indicator strain. The immunity of the lysogenic strain toward its own phage was verified.

Percentage of lysogens in each bacterial group. Under these conditions, 49 of the 113 strains tested (43%) were lysogenic (group G1, 75%, 9 of 12 strains; group G2, 57%, 12 of 21 strains; group G3, 57%, 21 of 37 strains; group R, 33%, 7 of 21 strains). Tables 1 and 2 show the distribution of these lysogenic strains with relation to their indicator strains as well as the level of phages liberated after induction by MC. None of the strains belonging to groups G4, G5, and G6 was lysogenic.

Lytic spectrum of temperate phages. (i) In group G1 (Table 1), nine strains were lysogenic. There were two indicator strains (Z119 and Z381) for one of these lysogens, whereas strain Z109B was the only indicator for the others. (ii) In group G2 (Table 1), five strains released phages active against one or more of eight group G2 strains; three of them released phages highly active against strain Z109B. An additional seven of the group G2 strains released phages only active against strain Z109B at a very low titer (10 to 30 PFU/ml). (iii) In group R (Table 1), two strains were lysogenic. One of them (Z253) released a phage active against a group G2 strain (A166) and strain Z109B. The other (Z144) released a phage having strain Z109B as the only indicator. (iv) In group G3 (Table 2), all of the 21 temperate phages released by group G3 strains attacked only group G3 strains. Sixteen of these phages had a narrow lytic spectrum (one to three

TABLE 1. Lytic spectrum of temperate phages released by group G1, G2, and R strains after MC treatment

Group	Indicator strain	Lytic spectrum[a] of phages released by induced strain: G1								
		Z105	Z336	Z341	Z348	Z381	Z351	Z119	1177	A85
G1	Z119	+++								
	Z381	+++								
	Z268									
	Z270									
	A65									
	A119									
G2	Z147									
	Z263									
	Z266									
	Z151									
	A166									
R	Z109B		+	++	++	+	+	+	+	+

[a] +, 10^1 to 10^2 PFU/ml; ++, 10^2 to 10^4 PFU/ml; +++, 10^4 to 10^6 PFU/ml; ++++, >10^6 PFU ml.

strains), whereas the remainder attacked four to six strains. (v) In group R (Table 2), five temperate phages induced from group R strains attacked only group G3 strains. The lytic spectrum of these phages was narrow (one to three strains).

Indicator strains. Of the 113 strains tested, 28 (25%) were lysed by at least one temperate phage. No indicator strain was detected in groups G4, G5, and G6, whereas the percentage of indicator strains was relatively high in the other groups (group G1, 16%, 2 of 12 strains; group G2, 43%, 9 of 21 strains; group G3, 43%, 16 of 37 strains).

The breadth of the spectrum of sensitivity of these strains was very variable (Tables 1 and 2). All of the group G1 and G2 strains and the majority of group G3 indicator strains were sensitive to a small number of phages—15 (one phage), 6 (two phages), and 1 (five phages). There were strains in group G3, however, that had a wide spectrum of sensitivity—five strains (7 to 13 phages). Strain Z109B was an indicator for phages produced by strains of both groups G1 and G2. It was sensitive to 21 temperate phages and showed very low phage titers (1×10^1 to 5×10^2 PFU/ml), but also very high levels (up to 4×10^7 PFU/ml). The spectrum of sensitivity was widened by increasing the titer of the primary lysate up to 10^7 PFU/ml with an ultracentrifuge (φZ105). On the other hand, the same experiment was negative with the primary concentrated lysate (up to 7×10^7 PFU/ml) of φA119 (Table 1).

The titer for a given lysate varied greatly with the indicator strain (Table 3); e.g., from 1×10^1 to 6.8×10^8 PFU/ml could be counted with the lysate of strain A153.

Comparative sensitivity of strains to temperate and virulent phages. Strains that were very sensitive to virulent phages were not necessarily sensitive to temperate phages, and vice versa. Thus, a strain that was sensitive to a large number of virulent phages, e.g., strain A83, (45 phages of 132 tested) was only sensitive to one temperate phage, whereas strains that were sensitive to medium number of virulent phages (e.g., A45 and Z146) (2) were indicators for a large number of temperate phages (14 and 13, respectively, of the 49 observed). On the other hand, strain A57 was sensitive to a large number of virulent and temperate phages (42 and 9, respectively). These temperate phages gave titers higher than 5×10^5 PFU/ml, reaching 6.8×10^8 PFU/ml on strain A57.

Appearance on plaques of lysis. The majority (54%) of the lytic reactions observed appeared as turbid plaques (diameter, 0.2 to 3 mm), whereas 36% showed clear plaques (diameter, 0.2 to 4 mm), sometimes with an indistinct edge or a halo, and 10% showed very small turbid plaques that were visible only when they were numerous and that were impossible to count.

Spontaneous and MC induction. The phage titer after spontaneous induction varied greatly between strains and depended on the indicator strain employed. The percentage distribution of the results of phage titers obtained was as follows: 49%, <10 PFU/ml; 16%, 10 to 10^2 PFU/ml; 23%, 10^2 to 10^4 PFU/ml; 8.6%, 10^4 to 10^6 PFU/ml; 3.8%, $>10^6$ PFU/ml. After induction with MC, the titer of the phages in 90% of cases was multiplied by a factor of 10^1 to 10^3. However, it must be remarked that in 2% of the cases, this factor was 10^6. The highest titer after induction, which was with MC, was 6.8×10^8 PFU/ml

TABLE 1.—*Continued*

				G2								R	
A61	A311	Z151	Z304	Z107	Z147	Z263	Z269	Z272	A140	A24	A119	Z253	Z144
+++	++++	++	++++								+++		
	++++		++++										
			++										
++													
			++										
			+++										
			+++										
+++													
												++	
+++		++++	++++	+	+	+	+	+	+	+		++++	+

TABLE 2. Lytic spectrum of temperate phages released by group G3 and R strains after MC treatment

Indicator strain[a]	Lytic spectrum[b] of phages released by induced strain (group):										
	A18 (R)	A46 (G3)	Z254 (G3)	A88 (G3)	A100 (G3)	Z150 (G3)	Z339 (G3)	Z377 (G3)	Z148 (G3)	Z145 (R)	Z340 (G3)	Z258 (G3)
Z339		+++	++									
Z377		+++	+++									
A14				++	++							
A114	++	+										
Z340		+++	+	++	+				++	++		
A46			++	+++	+++	+++					++	
A45		+++	+++	+	+	+++	++	++	++	++	+++	
Z146		+++	++	+	+	++	+	+	++	++		
A57												
Z125												
Z430							+					
A88								+++				
A69												
A63												
A95											++	++
A83												

[a] All of the indicator strains belong to group G3.
[b] +, 10^1 to 10^2 PFU/ml; ++, 10^2 to 10^4 PFU/ml; +++, 10^4 to 10^6 PFU/ml; ++++, > 10^6 PFU/ml.

with strain A57 and phage A153. Some examples of numerical results obtained after spontaneous and MC inductions are given in Table 4.

Lytic sensitivity pattern of strains Z109 and Z109B. To measure the possible heterogeneity of Z109 and Z109B, we had isolated five clones for each strain: Z109.1 to Z109.5 and Z109B.1 to Z109B.5. These clones and the two original strains were typed with four temperate phages (Table 5). Strain Z109B and all its clones (Z109B.1 to Z109B.5) were sensitive to phages. Four of the five substrains of strain Z109 were resistant to the same phages. Plaques of lysis were clearly visible (ca. 10^7 PFU/ml) on plates of the sensitive substrain Z109B. The original strain (Z103), which was heterogeneous, showed turbid plaques that were difficult to count (ca. 50 PFU/ml at the dilution 10^{-5}). At the lowest dilutions, a continuous lawn of bacteria made it impossible to observe lysis, thus explaining the fact that strain Z109 was originally classified in group R (2).

TABLE 3. Effect of the indicator strain on temperate phage count

Phage	Indicator strain	PFU/ml
φA153	A57	6.8×10^8
	Z146	1×10^1
φA69	A57	6×10^8
	Z146	1×10^1
φZ377	A88	3×10^4
	A45	6×10^3
	Z146	1×10^1
φA46	A45	5.6×10^5
	Z146	8×10^5
	A114	6×10^1
	Z340	3.2×10^5

Phage adsorption. The results from adsorption experiments carried out with cells of groups G1, G2, G3, and R and temperate phages of the same groups are given in Table 6. There was a low-percentage adsorption (ca. 30%) in all cases. Temperate phages belonging to groups G1 and G2 adsorbed on all of the strains tested in group G3.

DISCUSSION

The frequency of lysogeny has been determined among a representative sample of strains from the collection of mesophilic lactic acid streptococci previously classed into six lytic groups on the basis of their sensitivity to a group of virulent phages (2). After induction by MC, temperate phages are revealed by examination of plaques of lysis on at least one indicator strain.

In accordance with the method of detection employed, we have not tried to distinguish between lysogeny and pseudolysogeny (1). It has been shown that there was a large proportion of lysogenic strains (43%) among the mesophilic lactic acid streptococci studied. Others authors, using the same criteria, have only found 3 to 11% (7, 9, 13, 15). The fact that there were no lysogenic strains in groups G4, G5, and G6 perhaps only reflects the small size of the sample and the absence of an indicator strain specific to these groups. Group R, defined only by its resistance to all of the lytic phages used (2), contains 33% lysogenic strains, however, and so may be linked to group G3 through five strains and to group G2 through another two strains.

In fact, the actual frequency of lysogeny in these strains should be much higher since (i) the

TABLE 2.—*Continued*

A14 (G3)	A15 (G3)	Z146 (G3)	A50 (G3)	A159 (R)	A69 (G3)	A153 (G3)	Z429 (G3)	571 (G3)	A162 (R)	A91 (R)	A314 (G3)	Z124 (G3)	A63 (G3)
			+	+									
++	++++	++											
++	+++	++	+										
				++	+	+	+						
			++++	++++	++++	++++		++++	++++	++++	++	+++	
												++	++++
			++										
			++										
											++		

conditions of the induction standard adopted are not, a priori, optimum for each strain tested (15), and (ii) numerous phages are revealed, sometimes in a very small number by a single indicator strain.

The proportion of lysogenic strains observed in lactic streptococci examined here is quite comparable to the original proportion of these species inside the groups already defined and in the entire population chosen (for this study). In other words, the presence of lysogeny does not seem to characterize any one species preferentially.

As we had expected, there was a narrow specificity in the lytic reaction between the temperate phage and the bacterium. All of the temperate phages arising from group G3 strains were able to lyse group G3 strains and only these strains. This specificity also characterizes certain phages arising from group G1 and G2 strains. However, the indicator strain, Z109B, is sensitive to both group G1 and G2 temperate phages. Strain Z109, classified in group R, has been shown to be heterogeneous with regard to its sensitivity to phages. This variability of lactic acid streptococci has been studied thoroughly by Limsowtin et al. (12), in particular in strains growing on medium M17. Strain Z109 has been shown to be sensitive to a high concentration of virulent phage 160 (2; M. C. Chopin, unpublished data), confirming that this strain belongs to group G2 and that the strains of groups G1 and G2 are related through their lytic spectra. The existence of an intermediate strain between two lytic groups has already been shown for group G3 and G6 strains by means of virulent phages (2).

It may be remarked that the variation of the original strain Z109 toward strain Z109B consists of a loss of resistance to virulent phages with the simultaneous acquisition of sensitivity to temperate phages. Comparable examples ex-

TABLE 4. Examples of spontaneous and MC inductions in some selected strains

Phage		Indicator strain		Spontaneous induction (PFU/ml)	MC induction (PFU/ml)	Induction factor[a]
No.	Group	No.	Group			
φZ258	G3	A95	G3	2×10^3	0.8×10^3	~1
φA61	G2	Z109B	R	4.6×10^4	2.0×10^5	4
φA91	G3	A57	G3	2.0×10^6	5.5×10^7	2.8×10^1
φZ105	G1	Z119	G1	1.0×10^4	4.9×10^5	4.9×10^1
φA153	G3	A57	G3	4.2×10^6	6.8×10^8	1.6×10^2
φ571	G3	A57	G3	1.8×10^5	1.3×10^8	7.2×10^2
φZ254	G3	Z146	G3	<10	3.0×10^3	$>3.0 \times 10^2$
φA15	G3	A46	G3	1.1×10^4	2.9×10^7	2.6×10^3
φA46	G3	A45	G3	9.0×10^1	5.6×10^5	6.2×10^3
φZ253	G2	Z109B		6.0×10^2	2.0×10^7	3.0×10^4
φA63	G3	Z125	G3	<10	2.0×10^6	$>2.0 \times 10^5$

[a] (MC-induced PFU per milliliter)/(spontaneously induced PFU per milliliter).

TABLE 5. Sensitivity pattern of strains Z109, Z109B, and their substrains to four temperate phages[a]

Phage	Sensitivity pattern of strains[b]											
	Z109	Z109.1	Z109.2	Z109.3	Z109.4	Z109.5	Z109B	Z109B.1	Z109B.2	Z109B.3	Z109B.4	Z109B.5
φZ107 (Z109)	±	−	−	+	−	−	+	+	+	+	+	+
φA311 (Z109)	±	−	−	+	−	−	++	++	++	++	++	++
φZ304 (Z109)	±	−	−	+	−	−	+	+	+	+	+	+
φA61 (Z109)	±	−	−	+	−	−	+	+	+	+	+	+

[a] Phages issued from group G2 strains.
[b] ±, Very turbid plaques difficult to count; +, turbid plaques; ++, clear plaques with turbid edges.

ist in lytic phages. An *S. cremoris* strain sensitive to certain lytic phages produced several variants resistant to certain phages when it was exposed to these phages, and it gained sensitivity to other phages to which the original strain was insensitive (4). Very recently, Sinha (18) described a mutant of *S. cremoris* which showed a lytic spectrum that was the inverse of that of the original strain. In the case of strain Z109B, it was obtained without previous exposure to any other phage and without the aid of any mutagen, only cultivation and conservation at 4°C on medium M17. Like the other strains mentioned, Z109 is an *S. cremoris* strain—it forms the link between groups G2 and G1; this does not change the composition of the latter group, since this is entirely composed of *S. cremoris* strains (2).

The inability of a temperate phage to multiply on certain strains of its own or a different lytic group is sometimes due to the resistance of these strains to the adsorption of the phage. However, strains insensitive to lysis were sensitive to adsorption in a manner similar to that shown by sensitive strains. The blockage occurred during a later stage of the development of the phage. Therefore, distribution into lytic groups only partially depends on the specificity of the adsorption. On the other hand, the speed of adsorption of the temperate phages studied generally appears to be slower than that of the virulent phages. However, virulent phages may sometimes be adsorbed slowly (M. C. Chopin, unpublished data).

The spontaneous liberation of temperate phages by the cells of mesophilic lactic acid streptococci has already been established for a strain of *S. lactis* and for three strains of *S. cremoris* (9), but other authors were unable to demonstrate this in 50 *S. lactis* strains isolated from raw milk (6), probably in the absence of indicator strains. The maximum production reported is 2×10^4 phages per ml on a commercial medium (PRM, phage-resistant medium) (7).

Spontaneous production of phages appears to be very frequent in the selection of strains employed in this study (25.6% of the strains tested). This frequency is probably still underestimated because many indicator strains are little sensitive, and results should be verified by electron microscopical examination of the lysates.

TABLE 6. Adsorption of some temperate phages on selected bacterial strains

Phage		Adsorption strain		Lytic activity of phage	% Adsorption[a]
No.	Group	No.	Group		
φZ304	G2	Z109B	R	+	32[b]
		Z119	G1	−	36[b]
		A45	G3	−	36[b]
		Z430	G3	−	20[b]
φA119	G2	Z268	G2	+	36[b]
		Z109	R	−	29[b]
φZ105	G1	Z119	G1	+	32[c]
		Z109	R	+	41[c]
		Z430	G3	−	54[c]
φZ254	G3	A45	G3	+	**36**[d]
		Z430	G3	−	**0**[d]
		Z119	G1	−	**0**[d]

[a] The results express the means of 2 to 4 independent measurements.
[b] Phage enumerated on strain Z268.
[c] Phage enumerated on strain Z119.
[d] Phage enumerated on strain A45. The results in boldface type were obtained after 30 min of adsorption. Other results were obtained after 10 min of adsorption.

Spontaneous liberation of phages was often very intense and could be greater than 10^6 phage particles per ml.

Phage production induced by MC, under the standard conditions chosen, rarely exceeded 6×10^8 phage particles per ml (Table 4). The level of induction, defined as the ratio between the efficiency of plating after induction and the efficiency of spontaneous phage production, depends on (i) the level of spontaneous phage production, i.e., strains giving a high level of spontaneous lysis show a lower level of induction than strains giving a weak spontaneous lysis, and (ii) the sensitivity of the indicator strain, e.g., the lysate of strain Z377, where the efficiency of plating varies between three different indicator strains. Certain strains (e.g., Z258) do not appear to be susceptible to induction under the standard conditions (Table 4).

The absence of indicator strains has complicated the study of the temperate phages of lactic acid streptococci for a long time (11, 14, 16). Kozak et al. (9) demonstrated the presence of at least 21% indicator strains in a sample of 87 strains belonging to the three species at the maximum dilution of the lysates (10^{-3}). New Zealand authors observed a much smaller frequency, in the region of 10% (3 of 29 strains) (13) and 8% (4 of 50 strains) (6).

The results of the present study are more nearly comparable to those of Kozak et al. (9), since a frequency of 25% was observed over a total of 113 strains.

The indicator strains recognized here, show a certain number of characteristics; (i) the spectrum of their sensitivity is generally narrow (1 to 2 phages), although sometimes it is wide (8 to 14 phages), (ii) the efficiency of phage production (efficiency of plating) can vary considerably for a given lysate action on different indicator strains, (iii) the majority of indicator strains are lysogenic (group G3, 9 of 16 strains; group G2, 4 of 9 strains; group G1, 2 of 2 strains).

The presence in a strain of one (or perhaps several) temperate phage (5) does not interfere (or interferes only slightly) with the lytic production of another phage of the same group. Three of the four group G3 indicator strains having a wide spectrum were lysogenic (Tables 2 and 4). However, it is not impossible to imagine that such strains are the best indicators when their prophage(s) have been eliminated, and the distribution of indicator strains between the 3 species appear to be almost identical to that occurring within groups G1 to G3.

To conclude, it has been shown that the majority of the collection of 113 strains of mesophilic lactic streptococci classed on the basis of their sensitivity to virulent phages (2) are lysogenic, and that the temperate phages produced spontaneously or after induction by MC belong to the same sensitivity groups as the virulent phages characterizing the groups of Chopin et al. (2). This correlation between temperate and virulent phages through their lytic spectrum confirms the hypothesis of Lawrence et al. (11) and corroborates the morphological similarities established between these phage types (7, 19). It may therefore be said that temperate phages really appear to form a reservoir or a source of virulent phages, capable of lysing sensitive strains after mutation to gain virulence. It is even possible, given the intensity of spontaneous production of certain temperate phages and the possible multiplication of these phages on other strains in a starter, that temperate phages may be a cause of accidents in cheese making.

ACKNOWLEDGMENTS

This work was partly supported by a grant from the Mission de la Recherche, Ministère des Universités, France.

We thank Annette Rouault for her technical assistance and Anne B. Dickinson for her translation.

LITERATURE CITED

1. **Barksdale, L., and S. B. Arden.** 1974. Persisting bacteriophages infections, lysogeny, and phage conversion. Annu. Rev. Microbiol. **28**:265–299.
2. **Chopin, M. C., A. Chopin, and C. Roux.** 1976. Definition of bacteriophage groups according to their lytic action on mesophilic lactic streptococci. Appl. Environ. Microbiol. **32**:741–746.
3. **Czulak, J., and J. Naylor.** 1956. Host-phage relationship of cheese starter organisms. III. Significance in selection and maintenance of starter cultures in commercial use. J. Dairy Res. **23**:131–133.
4. **Frölich, G., I. Degle, and M. Busse.** 1978. Variation of phage resistance of *Streptococcus cremoris* upon lysis with virulent phage. Milchwissenschaft **33**:428–431.
5. **Heap, H. A., and R. C. Lawrence.** 1977. The contribution of starter strains to the level of phage infection in a commercial cheese factory, N. Z. J. Dairy Sci. Technol. **12**:213–218.
6. **Heap, H. A., and G. K. Y. Limsowtin.** 1978. Induction of *Streptococcus lactis* strains found in raw milk. N. Z. J. Dairy Sci. Technol. **13**:16–22.
7. **Huggins, A. R., and W. E. Sandine.** 1977. Incidence and properties of temperate bacteriophages induced from lactic streptococci. Appl. Environ. Microbiol. **33**:184–191.
8. **Jarvis, A. W.** 1978. Serological studies of a host range mutant of lactic streptococcal bacteriophage. Appl. Environ. Microbiol. **36**:785–789.
9. **Kozak, W., M. Rajchert-Trzpil, J. Zadjel, and W. T. Dobrzanski.** 1973. Lysogeny in lactic streptococci producing and not producing nisin. Appl. Microbiol. **25**:305–308.
10. **Lawrence, R. C.** 1978. Action of bacteriophage on lactic acid bacteria: consequence and protection. N. Z. J. Dairy Sci. Technol. **13**:129–136.
11. **Lawrence, R. C., T. D. Thomas, and B. E. Terzaghi.** 1976. Reviews of the progress of dairy science: cheese starters. J. Dairy Res. **43**:141–193.
12. **Limsowtin, G. K. Y., H. A. Heap, and R. C. Lawrence.** 1978. Heterogeneity among strains of lactic streptococci. N. Z. J. Dairy Sci. Technol. **13**:1–8.
13. **Lowrie, R. J.** 1974. Lysogenic strains of group N lactic streptococci. Appl. Microbiol. **27**:210–217.
14. **McKay, L. L., and K. A. Baldwin.** 1973. Induction of prophage in *Streptococcus lactis* C2 by ultraviolet irradiation. Appl. Microbiol. **25**:682–684.

15. **Meister, K. A., and R. A. Ledford.** 1979. Optimum cultural conditions for induction of temperate bacteriophages in lactic streptococci. J. Food Protect. **42**:396–400.
16. **Park, C., and L. L. McKay.** 1975. Induction of prophage in lactic streptococci isolated from commercial dairy starter cultures. J. Milk Food Technol. **38**:594–597.
17. **Reiter, B., and M. Kirikova.** 1976. The isolation of a lysogenic strain from a multiple strain starter culture. J. Soc. Dairy Technol. **29**:221–225.
18. **Sinha, R. P.** 1980. Alteration of host specificity to lytic bacteriophages in *Streptococcus cremoris*. Appl. Environ. Microbiol. **40**:326–332.
19. **Terzaghi, B. E.** 1976. Morphologies and host sensitivities of lactic streptococci and their bacteriophages. N. Z. J. Dairy Sci. Technol. **II**:155–163.
20. **Terzaghi, B. E., and W. E. Sandine.** 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Environ. Microbiol. **29**:807–813.