Effect of Lactose Concentration on the Efficiency of Plating of Bacteriophages on *Streptococcus cremoris*

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The efficiency of plating of phages derived by ultraviolet induction of, or by lytic growth on, certain strains of *Streptococcus cremoris* was found to vary by as much as 10^5 depending on the lactose concentration of the medium in which the indicator bacteria were grown and the length of time the stationary-phase indicator cultures were aged. This effect was noted only when the culture was used as an indicator for phages that had previously grown on an apparently unrelated strain of bacteria. Conditions of culturing and aging had no detectable effect upon the ability of a strain to serve as an indicator for phage that had previously been cultured on the same strain. These observations suggest the presence of some kind of physiologically labile restriction system in strains of *S. cremoris*. The implications of this finding for increasing the sensitivity of the host range test in determining phage susceptibility, whether from induced lysates, whey, or lytic phage stocks, are discussed. It is recommended that, for all such testing, the concentration of lactose in buffered media be increased to such levels as required to obtain a final pH similar to that of a freshly coagulated milk culture, namely, below pH 5.0.

Lytic phages attacking starter cultures of lactic streptococci used in the dairy industry have been recognized as a problem for over 40 years and have been isolated throughout the world (13, 27). These phages have been characterized principally by host range, by which criterion the phages fall into two categories: (i) those with a limited host range (often only one strain) and (ii) those with a multistrain range (6, 7, 10, 17, 25; J. Nyiendo, Ph.D. thesis, Oregon State University, Corvallis, 1975). Recent studies have shown that these two host range groupings correspond roughly to a natural morphological classification of the phages; thus, those phages having limited host ranges also have the same head shape and size (isometric; ca. 60 nm in diameter), whereas phages with broad host ranges have differing head structures, either of different dimensions or of different shape (22).

Many strains of *Streptococcus cremoris* and *Streptococcus lactis* have now been shown to release bacteriophage particles after exposure to the inducing agents ultraviolet light or mitomycin C and are presumably lysogenic (8, 12, 15, 16, 18; B. E. Terzaghi and W. E. Sandine, unpublished data). A variety of morphologically distinguishable temperate phages have been found, all with isometric heads. However, viability (as measured by plaque-forming ability) of

the released particles has either not been demonstrated or, where viability has been shown, the efficiency of plating (EOP) has been low (as judged qualitatively by electron microscopic particle counts) and host range has been limited (8, 12, 15, 16, 18; Terzaghi and Sandine, unpublished data). This raises the question of whether the induced particles are defective in general or whether the conditions for determining viability are inadequate. The inability to find an indicator strain for phages released after induction of *S. lactis* C₂ has led to the former suggestion (11). An indicator strain was found for phages induced from *S. cremoris* R₁, but the precise conditions for successful plaque production were unclear and success was generally attained only with late-stationary-phase or aged cells, as opposed to fresh log-phase cells (15). These findings suggested that, in the testing of induced lysates, indicator conditions have perhaps been deficient rather than that the phage have been defective.

As a result of the need to control phage infection in cheddar cheese manufacture, a variety of methods for selecting and handling the starter cultures have been used (13); the recognition of the existence of widespread lysogeny will, no doubt, see further modification of starter selection techniques. One of the criteria for starter selection is phage sensitivity as determined by a host range test (3, 6, 7, 10, 17, 25); if this test, because of inadequate sensitivity, fails to detect initial low-level lytic responses (which reflect

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potential high-level interactions), then incorrect conclusions regarding the phage sensitivity of strains will be made, and inappropriate starter selection will result.

In this paper, we demonstrate that plaque formation, even with proven indicator strains, is strongly influenced by the pH that the cells had attained during growth (as regulated by the lactose concentration of the medium), the length of time at that pH, and the strain on which the phage was propagated.

MATERIALS AND METHODS

Bacteria. *S. cremoris* strains MU0001 and MU0002 were obtained from the New Zealand Dairy Research Institute, where they are labeled R₁ and 368, respectively. The latter strain was previously referred to as the indicator strain AM₁ (15) but is, in fact, a slow milk-coagulating derivative of that strain and has therefore been given a new designation. Strain MU0003 is a derivative of strain MU0001, which has been cured of its ultraviolet-inducible prophages (D. Georghiou, M.S. thesis, Massey University, Palmerston North, N.Z., 1977; D. Georghiou and E. Terzaghi, unpublished data). To avoid hitherto unexplained variability in plating efficiencies, indicator cultures were grown from single-colony isolates of the designated bacterial strains, unless otherwise specified.

Bacteriophages. Phage ϕ MU1, obtained by ultraviolet induction of bacterial strain MU0001 (Georghiou and Terzaghi, unpublished data) was plated on either strain MU0002 or strain MU0003 and then propagated from single plaques on these two hosts as previously described (23). These phage preparations are designated, respectively, ϕ MU1/MU0002 and ϕ MU1/MU0003.

The terms "homologous host" and "heterologous host" will be used to designate, for a particular phage, the relatedness of the bacterial strain on which the phage was previously grown to the strain on which it is being tested; when the same strain is used at both steps, the term "homologous host" will be used. Conversely, the terms "homologous phage" and "heterologous phage" will be used to designate, with regard to a particular host strain, phages that had previously been propagated on that same host strain or some other unrelated host strain, respectively.

Media. Both milk (reconstituted skim milk) and M17 were used as previously described (23). For some experiments, M17 broth with different indicated concentrations of lactose, galactose, or glucose was used instead of the normal 0.5% lactose.

Adsorption. The adsorption of phage to bacteria was measured as follows. Bacteriophages were diluted to 10^5 plaque-forming units/ml, and 0.05 ml was added to an equal volume of the appropriate fresh overnight culture (18 h, 22°C) of bacteria in a centrifuge tube held at 20°C. After 10 min, 10 ml of M17 was added, and the tube was centrifuged at $1,900 \times g$ for 10 min. The supernatant was then immediately assayed for phage, on a sensitive indicator, and the percent adsorption was calculated on the basis of the assay of the control (no bacteria) tube.

Determination of coagulation time and rate of acid production in milk. Coagulation time was determined as described by Limsowtin and Terzaghi (14), and the rate of acid production was measured by the technique of Terzaghi and Sandine (23).

RESULTS

Single-colony isolation. On several occasions strain MU0002, which served as an indicator for phage induced directly from strain MU0001 as well as for those same phage after propagation on strain MU0001 (ϕ MU1/MU0003) (see Materials and Methods for definitions of phage designations, homologous and heterologous), appeared to lose this indicator ability. Similarly, strain MU0003, which normally plated the heterologous phage ϕ MU1/MU0002, would periodically cease serving as host with the customary efficiency. For both strains, the loss in plating efficiency was restricted only to heterologous phages and was not accompanied by any alteration in the capacity to support the growth of homologous phage or by detectable changes in any other demonstrable phenotypic characteristics normally associated with these strains.

Single colonies were isolated from each of the laboratory parental stocks of strains MU0002 and MU0003 and tested (by spotting) (24) for their response to a broad spectrum of lactic streptococcal phages (22). The isolates showed a consistent positive response to their homologous phages, but manifested a moderate degree of variability in their ability to form plaques with heterologous phages. Accordingly, to minimize possible genetic instability or variability, the work reported in this communication was done with single-colony isolates, which had demonstrable indicating ability for heterologous phages. This ability remained stable for at least 30 cell generations in four isolates checked.

Variation in concentration of lactose. A previous publication on the use of strain MU0002 as an indicator stated that stationary-phase cultures were most effective in producing plaques of the induced MU0001 lysate (15). That work was done using an unbuffered medium (M16) in which the pH dropped from approximately 7 to approximately 4.3. Since no evidence for β -glycerophosphate incorporation into lactic streptococci has yet been found (J. Thompson, personal communication), the principal difference between M16 and (buffered) M17 medium is that in M17 the bacteria attain a final pH of approximately 5.8 upon reaching stationary phase (and ca. 5.2 upon aging). The possibility of restoring indicator ability by growing bacteria in M17 to a lower pH was therefore investigated, and this was accomplished by increasing the

concentration of lactose to 1%. After growth in this medium, the ability of strain MU0002 to produce plaques of phage ϕ MU1 directly after ultraviolet induction of MU0001 was tested and found to be greatly improved.

To ascertain how closely this restoration was related to the pH of the medium, a comparison of the EOP of the phages ϕ MU1/MU0003 and ϕ MU1/MU0002 was made on strains MU0002 and MU0003, respectively, grown for 18 h at 22°C in different concentrations of lactose. The results of one set of platings are given in Fig. 1A for strain MU0002 and in Fig. 1B for strain MU0003. Qualitatively similar results were found in other experiments but with up to a 100-fold variation in the EOP. From these data the following observations can be made.

(i) The final pH of the culture falls linearly as the concentration of lactose increases up to a concentration of approximately 0.8%, at which point it stabilizes at around pH 5.0. This correlates with the response of the culture turbidity, which would imply that at lower lactose concentrations this nutrient is limiting.

(ii) No increase in culture turbidity occurs once pH 5 has been attained, although acid production apparently continues (in accord with previous reports [26]) (see also Fig. 2).

(iii) The EOP on a given strain for a heterologous phage increases as the lactose concentration increases, or as the pH falls.

(iv) Although variation is observed in the concentration of lactose at which an increase in the EOP begins, for both strains, a maximal EOP is reached at a lactose concentration of 0.8% or greater and a pH of approximately 5.0.

(v) The EOP on a given strain for a phage propagated on its homologous host does not vary substantially with the concentration of lactose.

To determine whether, once restored, the ability to produce visible plaques was now independent of the culture conditions, a comparison of EOP was done with these same isolates and phages, but the lactose concentration of the medium was reversed upon subculturing. Table 1 shows that, in agreement with the results of Fig. 1, irrespective of the lactose concentration the EOP of phages on their homologous hosts does not change, but for phages on heterologous hosts, a 10- to 40-fold higher EOP is achieved with 1% lactose. However, a possible influence of previous culture conditions is noted with heterologous hosts in that the maximal EOP, attained from a culture maintained at 1% lactose, is three- to sixfold greater than that of a culture transferred from 0.5 to 1% lactose. It should be noted that here also there is great variability from isolate to isolate and culture to culture in the absolute values of the EOP and in the mag-

FIG. 1. Growth, final pH, and heterologous phage EOP of *S. cremoris* strains grown in M17 containing various concentrations of lactose. (A) EOP of ϕ MU1/MU0003 on strain MU0002 when the phage titer is 1.6×10^{10} plaque-forming units/ml on strain MU0003, irrespective of the lactose concentration. (B) EOP of ϕ MU1/MU0002 on strain MU0003 when the phage titer is 3.6×10^8 plaque-forming units/ml on strain MU0002, irrespective of lactose concentration.

nitude of the ratios of the EOP values of cells grown with the various lactose regimes. However, this does not detract from the fact that the relative EOP values are consistent, and that the 1% lactose cultures yield much higher values than the 0.5% lactose cultures. This point is also illustrated in Tables 2 and 3.

Variation in concentration of galactose and glucose. To test whether the effect of sugar

FIG. 2. Strains MU0002 and MU0003 were grown for 18 h at 22°C in both 0.4 and 0.8% lactose-containing broth and then were allowed to age at 4°C. After the indicated periods of time, the culture pH and the EOP of the heterologous phage were determined. (A) Strain MU0002 and phage ϕ MU1/MU0003; (B) strain MU0003 and phage ϕ MU1/MU0002. EOP was calculated on the basis of the homologous host titrations performed at the same time. Symbols: 0.4% lactose, pH (○); 0.4% lactose, EOP (□); 0.8% lactose, pH (●); 0.8% lactose, EOP (■).

concentration upon heterologous phage indicator ability is a function of the specific sugar metabolized (as opposed, for example, to being simply a function of the final fermentatively attained pH), cultures were grown in 0.5 and 1.0% lactose, glucose, and galactose. After aging for several days at 4°C, these cells were tested for their ability to serve as both homologous and heterologous hosts. Whereas cells served as homologous hosts equally well when grown on any

of the three sugars, their efficiency as heterologous hosts when grown on glucose or galactose was only 1 to 10% the efficiency of cells grown on lactose (Table 2). It should be noted that although at the lower sugar concentration, lactose metabolism yields a lower pH than does metabolism of the other sugars, at the higher concentration all three sugars yield similar final pH values.

In spite of the fact that lactose-grown cells were better heterologous hosts at all sugar concentrations, cells grown on glucose and galactose did, nevertheless, manifest improved heterologous indicator capacity with increased sugar levels.

Bacteriophage heterogeneity. It is conceivable that the apparent effect of lactose concentration on the EOP of phages on their heterologous hosts is attributable to heterogeneity in the phage population such that one class might propagate on cells grown at low lactose and the other, less frequent class might propagate on the cells grown at higher lactose concentrations. To test this, stocks were made from the phages that grew on heterologous hosts at 0.5 and 1.0% lactose, respectively, and their EOP was determined on their original homologous hosts (now heterologous hosts). It can be seen (Table 3) that irrespective of the lactose concentration, pairs of stocks derived from the same heterologous host behave identically (cf. lines 1 and 2 or lines 3 and 4). In fact, the two critical factors determining the plating behavior of a phage on its heterologous host appear to be the host upon which it was propagated and the lactose concentration in which the current (heterologous) host was grown, as illustrated by comparison of lines 1 and 2 with line 5, or lines 3 and 4 with 6.

Adsorption. To determine whether the differences in EOP resulted from an inability to adsorb the phage, possibly through blockage or loss of receptor sites, or instead reflected the action of some postadsorption step in the phage growth cycle, the adsorption of the phage to bacteria grown in 0.5 and 1% lactose, glucose, and galactose was tested. Metabolism of varying concentrations of lactose, glucose, and galactose did not significantly alter the ability of the bacteria to adsorb the heterologous phage (Table 4).

Aging of culture. The preceding data indicated that the increased EOP resulted from some intracellular mechanism that was sensitive to either the lower pH or prolonged lactose metabolism. In an effort to distinguish between them, the heterologous host EOP was measured at regular intervals for 14 days on both strains

TABLE 1. *EOP of phages on homologous and heterologous hosts subcultured at 0.5 and 1.0% lactose^a*

Phage	Lactose concn (%)		MU0003		MU0002	
	Subculture 1	Subculture 2	Titer	EOP	Titer	EOP
ϕ MU1/MU0003	0.5	0.5	8.6×10^9	1.0	8.5×10^5	1.0×10^{-4}
	0.5	1.0	1.0×10^{10}	1.0	8.4×10^6	8.4×10^{-4}
	1.0	1.0	9.1×10^9	1.0	2.3×10^7	2.5×10^{-3}
	1.0	0.5	9.1×10^9	1.0	2.6×10^6	2.9×10^{-4}
ϕ MU1/MU0002	0.5	0.5	1.2×10^4	1.6×10^{-5}	7.5×10^8	1.0
	0.5	1.0	1.2×10^5	1.1×10^{-4}	1.1×10^9	1.0
	1.0	1.0	4.7×10^5	6.3×10^{-4}	7.5×10^8	1.0
	1.0	0.5	1.1×10^4	1.2×10^{-5}	9.1×10^8	1.0

^a Subculture 1 (after 18 h at 22°C) was diluted (1%) into fresh medium (subculture 2), which was then incubated for 18 h at 22°C and 24 h at 4°C.

TABLE 2. *EOP of phages on homologous and heterologous hosts cultured on 0.5 and 1.0% lactose, glucose, and galactose^a*

Host	Sugar concn (%)	pH	Phage (titer)	Titer	EOP
MU0003	Lactose, 0.5	5.3	ϕ MU1/MU0002 (4.4×10^8)	7.5×10^5	1.7×10^{-3}
	Glucose, 0.5	6.0		8.0×10^3	1.8×10^{-5}
	Galactose, 0.5	5.6		7.0×10^3	1.6×10^{-5}
	Lactose, 1.0	4.9		3.8×10^6	8.6×10^{-3}
	Glucose, 1.0	4.7		3.7×10^5	8.4×10^{-4}
	Galactose, 1.0	5.0		5.0×10^5	1.1×10^{-3}
MU0002	Lactose, 0.5	5.7	ϕ MU1/MU0003 (7.7×10^9)	3.0×10^5	3.9×10^{-5}
	Glucose, 0.5	6.0		2.3×10^4	3.0×10^{-6}
	Galactose, 0.5	5.9		6.3×10^4	8.2×10^{-6}
	Lactose, 1.0	5.1		3.2×10^7	4.2×10^{-3}
	Glucose, 1.0	4.9		4.1×10^6	5.3×10^{-4}
	Galactose, 1.0	5.0		8.0×10^5	1.0×10^{-4}

^a Each culture was grown in the indicated medium from 1% inocula of a 0.5% lactose overnight culture of the appropriate strain of bacterium. The cultures were incubated for 18 h at 22°C and then stored for 5 days at 4°C. The titer of the two phage stocks was determined on the 0.5% lactose homologous host culture.

TABLE 3. *EOP of phages of heterologous origins^a*

Phage/old host/new host				Lactose concn (%)	MU0003		MU0002	
					Titer	EOP	Titer	EOP
(1) ϕ MU1/MU0002/MU0003 (tose)	(1.0% lac-		0.5	1.5×10^9	1.0	6.5×10^2	4.3×10^{-7}	
			1.0	ND ^b		8.0×10^5	5.3×10^{-4}	
(2) ϕ MU1/MU0002/MU0003 (tose)	(0.5% lac-		0.5	6.0×10^8	1.0	5.5×10^2	9.2×10^{-7}	
			1.0	ND		4.0×10^5	6.7×10^{-4}	
(3) ϕ MU1/MU0003/MU0002 (tose)	(1.0% lac-		0.5	$<10^1$	$<10^{-7}$	6.3×10^8	1.0	
			1.0	2.4×10^6	3.8×10^{-3}	ND		
(4) ϕ MU1/MU0003/MU0002 (tose)	(0.5% lac-		0.5	$<10^1$	$<10^{-7}$	5.2×10^8	1.0	
			1.0	3.1×10^6	6.0×10^{-3}	ND		
Controls (original stocks)								
(5) ϕ MU1/MU0003			0.5	1.1×10^9	1.0	8.2×10^2	7.5×10^{-7}	
			1.0	ND		5.0×10^5	4.5×10^{-4}	
(6) ϕ MU1/MU0002			0.5	$<10^1$	$<10^{-7}$	9.3×10^8	1.0	
			1.0	2.0×10^6	2.1×10^{-3}	ND		

^a Phages plating on their heterologous hosts at both 0.5 and 1.0% lactose were expanded into stocks by growth on the heterologous host (new homologous host) in M17 broth containing 0.5% lactose. Heterologous plating hosts were prepared as in Table 1.

^b ND, Not determined.

TABLE 4. Adsorption of phage to homologous and heterologous hosts grown on different concentrations of lactose, glucose, and galactose

Phage	Adsorption to strain:	Sugar concn (%)	% Adsorption
ϕ MU1/MU0002	MU0002	Lactose, 0.5	90
	MU0003	Lactose, 0.5	86
		Glucose, 0.5	80
		Galactose, 0.5	72
		Lactose, 1.0	68
		Glucose, 1.0	64
		Galactose, 1.0	54
ϕ MU1/MU0003	MU0003	Lactose, 0.5	87
	MU0002	Lactose, 0.5	96
		Glucose, 0.5	75
		Galactose, 0.5	95
		Lactose, 1.0	73
		Glucose, 1.0	50
		Galactose, 1.0	85

MU0003 and MU0002 grown for 18 h at 22°C in different concentrations of lactose ranging from 0.2 to 2% and then held at 4°C. Both strains showed two kinds of response, reflecting again the lactose concentration. Examples are given in Fig. 2 for lactose concentrations of 0.4 and 0.8%. For both strains, the EOP when measured on the heterologous host grown at an elevated lactose concentration (0.8%; Fig. 2A and B) increased with time up to 10^3 -fold, reaching a maximum of 10% of the titer measured on the homologous host (which number remained approximately constant). After 14 days, the bacteria began to die, making backgrounds too faint to detect plaques accurately. Although the pH of the 0.8% lactose cultures declined slightly during the course of aging, this was not sufficient to explain the very marked increase in EOP. In contrast, the lactose-limited (<0.6%) cultures showed little or no effect of aging upon either the pH of the culture or upon the ability of the culture to serve as a heterologous host (0.4%; Fig. 2A and B).

Acid production by isolates grown in milk. The possibility that selection might have occurred for slow milk-coagulating cells (9) in cultures grown at a higher lactose concentration, and that these might serve as better indicators, was tested by determining the milk-coagulating ability of strains after growth in different lactose concentrations. The time required for milk coagulation and the rate of acid production as a result of prior incubation in either 0.5 or 1% lactose were not appreciably different for a given strain, although characteristic strain differences between MU0002 and MU0003 were observed.

DISCUSSION

We have observed that a phage (ϕ MU1/MU0002) grows with greatly reduced

efficiency on a heterologous host (MU0003); however, if propagated on that host (to generate the phage ϕ MU1/MU0003), it now grows with high efficiency on that host, but with low efficiency on the original host. This observation formally suggests the operation of a host-controlled modification/restriction system (2). Previous evidence (4, 14, 19) relating to the existence of such systems in lactic streptococci, all of which is circumstantial, has recently been reviewed (13). As yet, enzyme purification, which would be required for the demonstration of a restriction system similar to that found in well-studied bacteria, has not been reported. In this communication, we have presented data that, although not bearing directly on the question of the molecular basis of the putative modification and restriction, do further define how this restriction system might be operating in lactic streptococci and, perhaps more importantly, point out the practical significance of the phenomenon.

In the course of studies of this apparent modification/restriction system, it was noted that growth of the cells at elevated lactose levels led to a partial breakdown in the restriction activity (Fig. 1). Further investigation revealed a number of interesting features of this phenomenon.

(i) The effect is transient in that the cells behave largely according to the lactose level of the medium in which they are grown, irrespective of the prior history of the inoculum (Table 1); (ii) aging of the cells grown at elevated lactose concentrations further reduces the ability to restrict growth of heterologous phage (Fig. 2); (iii) whereas the effect is most readily attributable to the low pH attained by the high-lactose cultures, the observation that growth of cells on similar levels of the component monosaccharides glucose and galactose, which yield comparable final pH values, are not as efficient heterologous hosts indicates that pH alone is not the critical element (Table 2); (iv) whereas growth on lactose is most effective in improving heterologous EOP, comparison of cultures grown at low and high levels of glucose and galactose shows that these sugars are also effective, and thus lactose metabolism alone is not the critical element either (Table 2).

In the absence of direct evidence to the contrary, it would be possible to propose that in fact ϕ MU1/MU0002 is not structurally identical to ϕ MU1/MU0003 and that the specific phage receptor sites on the bacterial surface are correlated with the specific sugar being metabolized by the bacteria. The experiments reported in Tables 3 and 4 were designed to test this general class of proposal. The former demonstrates the identity, in respect to restriction, of ϕ MU1/MU0002 and ϕ MU1/MU0003/MU0002

and the identity of ϕ MU1/MU0003 and ϕ MU1/MU0002/MU0003. The latter shows that although there are some interesting consistent minor variations in phage adsorption to bacteria grown under different sugar regimes, both phage adsorb satisfactorily to their heterologous hosts, irrespective of sugar regime.

Thus, we may conclude that in respect to this restriction ability, whatever the underlying molecular mechanism, the bacteria are sensitive to metabolism of sugars beyond a certain very low limiting concentration, and that among the sugars tested, lactose was by far the most effective. Recent preliminary experiments (E. Terzaghi, unpublished data) suggest that an extracellular diffusible element, which accumulates with time, is actively required in restriction breakdown at the time of phage infection. Taken altogether, the evidence suggests that some new, hitherto unobserved mechanism of modification/restriction is operating in this system. This does not preclude the simultaneous existence and operation of other restriction systems in lactic streptococci that are perhaps more similar to those already described.

In other bacterial systems, the efficiency of restriction has been affected by growth in minimal medium or by heat treatment of bacteria (2). A thermolabile restriction endonuclease has been purified from *Thermus aquaticus* (21), but no pH-sensitive endonucleases have yet been reported. This may not be too surprising since the well-studied bacteria do not normally undergo as drastic changes in pH as that of industrial lactic streptococcal strains. Inasmuch as these starter strains can also be subjected to wide temperature variations (22 to 39°C), the possibility of a temperature effect on plaque production with these heterologous phages was investigated by growth at 22, 30, and 34°C, but no increase in plaque production was noted; indeed the contrary was observed.

It may be of practical concern that the aging effect is most striking with cells grown in lactose excess, the situation that occurs in milk. In the present experiments (Fig. 1), cell growth as measured by absorbance does not increase substantially with increasing lactose beyond a concentration of 0.8%. It is at this concentration that the aging effect becomes striking, in respect to both the slow drop in culture pH and the dramatic rise in heterologous phage EOP.

Decisions on starter strains to be used in dairy factories are influenced strongly by the detection and lytic action of phages as determined in the host range test (13). On the basis of this phage typing, classifications have been made of both lactic streptococcal phage (3, 25) and strains of lactic bacteria (7, 21). However, host range is not generally considered an acceptable method

of classifying phages (1), and the lack of success in controlling phage infection in the dairy industry might be partially attributable to too much faith in the specificity of this test. As these results show, phages could be present and potentially lytic in the vats, but the demonstration of this lytic ability may fail because of the limitations imposed by the testing conditions.

Because bacteria grown in milk are difficult to use, synthetic media are preferred for laboratory work. However, it is during lactic streptococcal growth in milk that phage infection becomes an industrial concern, and synthetic media should therefore duplicate as much as possible the conditions in milk. Calcium has often been cited as one additive affecting plaque production (13), and calcium is now added routinely to synthetic media. Milk buffering capacity can also be duplicated, in the presence of calcium, by use of glycerophosphate (5, 23). In light of the data presented here, it is recommended that for studying lactic streptococcal phages in buffered media such as M17, the lactose level of the medium used to grow the bacterial indicator be increased to a level sufficient to give a final pH below 5. This change is particularly recommended for use under conditions of possible heterologous host interactions, as in testing for phage presence in whey, determinations of host range, or detection of induced temperate phages. Although very little testing has been done on the advantages of any addition of lactose to plating agar, preliminary data suggest that no improvement occurs.

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