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Lactococcus Bacteriophages Isolated from Whey and Their Effects on Commercial Lactic Starters

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ABSTRACT

The incidence of phages of lactic acid bacteria in milk industry and their effects on acidification ability of commercial lactic acid starters were studied. Cheese whey samples (33 samples) were collected from 17 factories. A total of 16 bacteriophages were isolated (12 specific for Lactococcus lactis, 3 for L. diacetylactis and one capable of lysing both species). The results showed that 10% reduction in acidification tests was not good indication of phage in the sample. The majority of samples showed reduction higher than 10%, although only 65% were phage positive. The isolated phages were quite stable and showed no reduction in infectivity even after 20 daily replications. A pool of bacteriophages was prepared from isolates and inoculated in 12 commercial lactic starters. After 8 hours of incubation, only 2 showed reduced acidification. Bacterial strains isolated from commercial starters were tested regarding the phage resistance. Considerable difference in phage sensitivity was observed among different starters (BD, D, O and L. diacetylactis). Five bacteriophages showed no infectivity on any isolates but one was infective for most of isolates.

Key words: Bacteriophage, *Lactococcus*, cheese making, milk, processing

INTRODUCTION

Several factors may affect starter cultures, decreasing their ability to achieve the desired acidity within right time. One of these factors is the infection by bacteriophages. Decrease in the fermentation activity of starters has consequences of technological importance, such as syneresis decrease, development of off flavor and smell and change in texture of final product (Furtado, 1991). Public health hazard may come up due to the possible development of pathogenic bacteria (Gudkov et al., 1984). Bacteriophages are viruses whose infection in bacteria may cause two different consequences: cell lyse, caused by virulent phages, or their permanence in the host cell integrated to its DNA, called prophage or temperate phage, in a state called lysogeny (Adams, 1959). Many *Lactococcus* strains in commercial starters show lysogenic phage which, eventually may release lytic bacteriophages in the milk fermentation process (Reiter and Kirikova, 1976). Nevertheless, according to Jarvis (1984), this is not the main source of phage contamination in processing plants. Milk itself is probably another source of phages, once they can multiply in bacteria naturally found in raw milk (Heap et al., 1978). According to Klaenhammer (1984), bacteriophages are common in cheese processing plants around the world, even under strict hygienic

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conditions, culture rotation and use of phage resistent strains. This work aimed to isolate bacteriophages in cheese whey samples from several milk industries in São Paulo State, Brazil. Their effect on acid-forming ability of commercial milk starters, the stability of their activity in transferences and the sensitivity of isolated bacteria to those bacteriophages were evaluated.

MATERIAL AND METHODS

Whey samples

Whey samples were collected from 17 milk processing plants of different types of cheese along 14 cities in São Paulo State. Thirty three samples were randomly collected regardless of complaints about possible deficiencies in the acid-forming power of starters. Table 1 shows the relation of samples and types of cheese in each plant. Samples were cooled in ice immediately after collection for transportation and then kept at -18ºC until being used.

Cultures and starters

The following strains of *Lactococcus* were used. They were kindly supplied by the Institute of Food Technology (ITAL) in Campinas (SP): *L. lactis* ssp. *cremoris* (NCDO-1999) ITAL 257; *L. lactis* ssp *lactis* (NCDO-2003) ITAL 265; *L. lactis* ssp *lactis* var *diacetylactis* (Wiesby 933) ITAL 343. Twelve types of starters were used for the test of activity of milk starters in the presence of phages and the sensitivity test. Eleven of them had mixed cultures and one had a single culture (*L. diacetylactis)*. These starters are presented in Table 2.

Growth and cultures maintenance methods

The purity of the culture was tested according to the method proposed by Harrigan AND MacCance (1976). They were inoculated in tubes with 10mL of reconstituted sterile skim milk, using 15% inoculum, and then frozen at -18ºC before incubation for cell growth. Reactivation was made incubating at 30ºC until milk coagulation. Transfers were made every 3 months.

To reactivate lyophilized commercial milk starters, a fraction of the envelope's content was aseptically transferred to the reconstituted skim milk with sterile spatula and incubated at 20ºC (to prevent changes in any strains proportion in the starters) until coagulation.

Table 1 - Sample List

Plant	Product	Sample code
A	provolone	A_1
	mozarela	A_2
B	mozarela	B_1
	-	B_2
	-*	B_3
C	mozarela	C_1
D	minas frescal	D_1
E	prato	E_1
	mozarela	E_2
F	mozarela	F_1
	parmezian	F_2
	prato *	F_3
G	mozarela	G_1
H	-	H_1
I	minas frescal	I_1
	mozarela	I_2
J	mozarela	J_1
K	-	K_1
	-	K_2
L	mozarela	L_1
	minas frescal	L_2
	ricota	L_3
M	mozarela	M_1
N	mozarela	N_1
O	mozarela	O_1
P	minas frescal	P_1
Q	provolone	Q_1
	edam	Q_2
	cobocó	Q_3
	mini-lanche	Q_4
	provolone	Q_5
	mozarela/ cáccio	
	prato	Q_6
	provolone	Q_7

(-) not specified; (*) analyzed after 6 months

Acid production inhibition test

This test was carried out in reconstituted skim milk at 11% solids, according to Anderson and Meanwell (1942) methods. Cheese whey samples were centrifuged for 10 minutes at 3000g and the supernatant was then filtered through 0.45µm membrane filter. Each tested culture had 2 control tubes and 5 test tubes with 10mL of milk each. Control tubes received 2% of culture (incubated for 18 hours in reconstituted skim milk) and test tubes received culture plus 1mL of filtered cheese whey. All tubes were incubated in water bath at 30ºC for 2 hours and then at 35ºC for 4 hours.

After that the acidity was determined by titration with N/9 NaOH and phenolphtalein indicator.

Test for bacteriophage presence (Hull, 1977)
Amount of 0.05mL of $CaCl_2$ 1 $mol.L^{-1}$ and 0.1mL of the culture to be tested were placed in test tubes for 18 hours at 30ºC in liquid M17 broth (Terzaghi and Sandine, 1975). The mixture was then incubated at room temperature for 15 minutes. Then, each tube received 3mL M17 overlay agar (same M17 agar formulation except that agar is reduced to 7 g L^{-1}), molten and cooled to 50ºC. After slight agitation, the material was overlayed in Petri dishes with a solidified layer of M17 agar. After solidification of the second layer, a drop of whey sample was placed on its surface to test the presence of bacteriophages. Whey samples for this test were obtained from the acid production inhibition test (after centrifugation at 3000g for 10 minutes). This technique allowed enrichment of bacteriophages in the industrial sample by the presence of the specific host bacteria. Plates were incubated at 30ºC for 18 hours where plaques of lysis caused by virus were detected.

Table 2 - Type and composition of commercial starters used in the test of sensitivity to phages

Type	Nominal composition
O_1	2-5% *L. lactis* and 95-98% *L. cremoris*
O_2	2 strains *L. lactis* and 2 *L. cremoris*
O_3	3 strains *L. lactis* and 2 *L. cremoris*
O_4	2 strains *L. lactis* and 2 *L. cremoris*
O_5	*L. lactis* and *L. cremoris*
D_1	80% *L. lactis* and *L. cremoris*, a few *L. diacetylactis*
D_2	80% *L. lactis* and *L. cremoris*, a few *L. diacetylactis*
BD_1	70-80% *L. lactis* and *L. cremoris*, 15-30% *L. diacetylactis*, a few *Leuconostoc*
BD_2	92-99% *L. lactis* and *L. cremoris*, less than 0,5% *L. diacetylactis*, 1-8% *Leuconostoc*
BD_3	92-99% *L. lactis* and *L. cremoris*, less than 0,5% *L. diacetylactis*, 1-8% *Leuconostoc*
BD_4	70-80% *L. lactis* and *L. cremoris*, 15-39% *L. diacetylactis*, a few *Leuconostoc*
S	100% *L. diacetylactis*

Isolation and storage of bacteriophages (Terzaghi and Sandine, 1975)
Isolated lysis plaques were removed and transferred to tubes with 6mL of M17 broth, 0.1mL of host culture (18 hours) and 0.05mL of $CaCl_2$ 1$mol.L^{-1}$. Tubes were then incubated at 30ºC for 6 hours when cell lysis was observed. The lysis was detected by the relative clearness of the broth, compared to controls, which are more turbid due to bacteria growth. Tubes where lysis was observed were then filtered through 0.45µm membrane filter and stored at temperatures between 2ºC and 5ºC.

Amplification and titration of phages
A volume of 0.1mL of isolated bacteriophage suspension was transferred to sterile tube with 0.1mL of host bacteria (cultivated at M17 broth at 30ºC for 18 hours) and 0.05mL of $CaCl_2$ 1$mol.L^{-1}$ and kept at room temperature for 15 min. Then, 3mL of M17 overlay agar was added and the resulting material placed in a Petri dish with a solidified layer of M17 agar. The plate was then incubated at 30ºC for 18 hours when there was confluent lysis. Then 5.0mL of M17 broth was added on plate and incubated at 5ºC for 12 hours. The overlay liquid was then collected and stored in sterile recipient between 2 and 5ºC. The successive dilutions of 10^{-1} to 10^{-10} were carried out with Ringer solutions and 0.1mL of each solution was then added to 0.1mL of suspension of the active host bacteria and 0.05mL of $CaCl_2$ $mol.L^{-1}$. The resulting solution was incubated at room temperature for 15 minutes, after which 3mL of M17 overlay agar molten at 50ºC were added. After homogenization, the mixture was placed in Petri dishes with a solid M17 layer. Counting followed dishes incubation at 30ºC for 18 hours. The titre of bacteriophages, expressed in plaque-forming units, was calculated dividing of the numbers of lysis plaques by the corresponding dilution.

Addition of bacteriophages to commercial starters
This test used reconstituted skim milk at 11%. Each starter had 1 control tube and two test tubes for each fermentation hour. Test tubes received 1% of reactivated starter and 0.5% of a mixture of all bacteriophages isolated in the samples. The

control tube received starter only. Tubes were incubated at 30ºC for 8 hours and a sample was taken every hour and acidity was determine by titration with N/9 NaOH.

Phage activity in subcultivated commercial starters

Reactivated starters were subcultivated daily, inoculating 1% of the active culture in milk, and incubated at 20ºC until coagulation. In first day, each culture had 1 control and 3 test tubes. The control received only 1% of starter; test tubes also received 0.5% of the bacteriophages mixture solution besides starter. Then, the same procedures used in the acid production inhibition test were followed. The third test tube, after incubation, was centrifuged at 3000g for 10 minutes. The supernatant was then collected and stored em refrigerator and in the following day, after the second transfer, it was added to the test tubes at 10% proportion. After the tenth day of transference, wheys from each starter were analyzed according to Hull (1977) method to determine the presence of bacteriophages, using the same cultures involved in the bacteriophages isolation (*L. lactis*, *L. cremoris* and *L. diacetylactis*).

Isolation and sensitivity of cultures isolated from commercial starters

Reactivated commercial starters were successively diluted up to 10^{-9} in pepton water 0.1% and 1mL of each solution was plated in APT agar(APT broth agar-Merck). After overlaying with same medium Petri dishs were incubated at 30ºC for 48 hours. Then, isolated colonies were inoculated in milk until coagulation, when they were transferred to M17 broth and incubated at 30ºC for 18 hours. Cultures were then tested with all the isolated bacteriophages to evaluate host sensibility using a lyse plaque test according to Hull (1977).

Electron microscopy of bacteriophages particles

The analysis used a technique with fosfotungstic acid (PTA) 1% pH7.0, according to Herald and Zottolla (1986), on an electronic transmission ZEISS microscope (EM 9S2).

RESULTS AND DISCUSSION

Sample analysis

Table 3 shows the difference of acidity in percentage of Dornic degrees (%ºD) of the milk fermented by lactic bacteria (*L. lactis*, *L. cremoris* and *L. diacetylactis*) inoculated with 33 samples of whey samples collected in cheese plants. Most samples had an acid production reduction above 10%, which, according to many authors, would indicate the presence of phages (Anderson and Meanwell, 1942). A few samples had a reduction of acidity production below 10%. Nevertheless, only 13 samples had phage particles that lysed *L. lactis* and 4 samples lysed *L. diacetylactis*. No sample had phages that lysed *L. cremoris*. These data suggested that at least under the conditions of this experiment, the hypothesis that a decrease of 10% or more in the acidification was indicative of the presence of bacteriophages was not consistent. Although most of the samples with phages particles had an acidity difference above 10%, one sample (sample K_1) had zero decrease, and *L. lactis* phages particles had been detected. Anyway, a widespread presence of phage particles were real: 12 samples had *L. lactis* phages, 3 had *L. diacetylactis* phages and 1 had both *L. lactis* and *L. diacetylactis* phages. That mean 65% of all samples had some type of phage, even considering only one strains of each *Lactococcus* species. It was reasonable to expect that if higher number of bacteria strains were tested they would have lead to a higher number of positive results, since phages were strain and species specifics.

Table 4 shows the number of phages particles calculated by Halvorson and Ziegler (1932) method and the acidity difference of fermented milk with and without whey addition. That relation has not been detected in this work. On the other hand, Figure 1 showed that there was a positive correlation between acidification reduction between samples inoculated with *L. lactis* and *L. cremoris* (r=0.73) and between *L. diacetylactis* and *L. cremoris* (r=0.75) (correlation datum from Table III). This suggested that different samples had some kind of milk fermentation inhibitor that acted similarly to bacteria.

Table 3 - Difference of acidity in % of ºD of milk fermented by (A) *Lactococcus cremoris;* (B) *L. lactis* and (C) *L. diacetylactis*, with and without addition of cheese whey from plants.

		Acidity difference (% ºD)		
Samples	**Control acidity**	**A**	**B**	**C**
A_1	54	38,4	28,3	33,3
A_2	56	36,5	36,0	32,1
B_1	58	25,0	0,0	13,5
B_2	42	25,0	23,8	11,6
C_1	55	40,3	41,1(*)	16,1
D_1	55	9,8	13,2	15,0
E_1	53	29,6	28,0	28,5
E_2	44	30,5	18,1(*)	21,7
F_1	46	30,6	22,7(*)	28,0
F_2	50	13,6	30,7	27,2
G_1	53	24,0	28,0	35,6
H_1	47	14,2	20,8(*)	9,1
I_1	47	36,9	37,7	30,6
I_2	45	0,0	17,4	11,7
J_1	42	31,9	21,2(*)	30,4
K_1	47	(23,2)	0,0(*)	15,2
K_2	43	15,0	27,6	11,6
F_3	48	4,2	15,2	(1,9)
B_3	51	28,0	33,3	27,3
L_1	50	31,9	36,1(*)	43,8
L_2	58	(7,1)	12,5(*)	8,1
L_3	45	37,5	25,5(*)	11,1
M_1	51	24,0	19,1	32,1(*)
N_1	56	40,7	38,1	45,7
O_1	50	41,6	40,0(*)	26,9
P_1	54	32,1	39,6	32,1(*)
Q_1	61	33,9	31,6(*)	32,8(*)
Q_2	60	35,0	37,9	31,1(*)
Q_3	48	20,4	19,6	16,2
Q_4	63	31,7	35,0	33,8
Q_5	61	31,6	41,9	30,6
Q_6	63	35,0	48,3(*)	34,8
Q_7	66	37,6	35,9(*)	58,5

(*) bacteriophages presence

The nature of this inhibitor was not known, but one could suspect of an antimicrobial agent that was used for treatment of animals health or a cleaning agent used in production or processing plants. Other possible explanation could be the presence of similar receptors sites in the strains.

Bacteriophage isolation

Bacteriophages produced round lyse plaques with clear borders and interior free of bacteria growth, which were host specific. Phage suspension around 10^8 PFU/mL (plaque forming unit) was prepared for bacteriophages storage. Storage for 4 months caused no change in activity. Figure 2 showed photomicrograph of an *L. lactis* bacteriophage isolated from one industry. Given the presence of bacteriophages in the cheese industry of São Paulo, it would be essential do adopt preventive measures, especially regarding the sanitation of the processing plant, adequate handling of whey in the industry and rotation of inoculation cultures to reduce contamination levels.

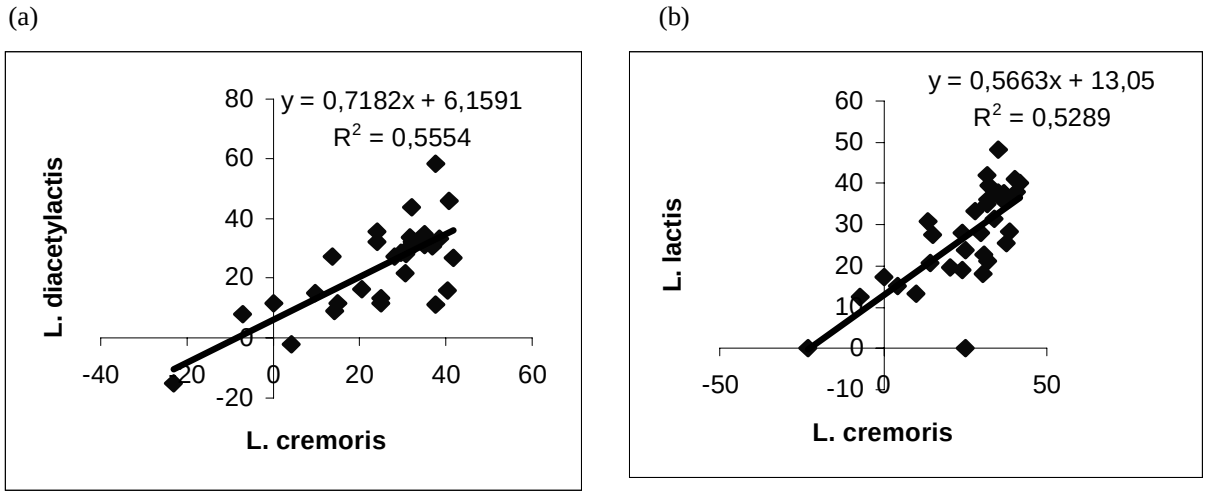

Figure 1 - Correlation between acidification reduction data among samples inoculated with *L. diacetylactis* and *L. cremoris* (r=0.75) (a) and between *L. lactis and L. cremoris* (r=0.73) (b).

Table 4. Acidity difference (%ºD) and number of phage particles found in whey samples.

Samples	Difference (%ºD)	particles/mL
C_1	41.1	>1.6*
E_1	18.1	>1.6*
F_1	22.7	>1.6*
H_1	20.8	0.51*
J_1	21.2	>1.6*
K_1	0.0	>1.6*
L_1	36.1	1.6*
L_2	12.5	0.22*
L_3	25.5	1.6*
O_1	40.0	>1.6*
Q_1	31.6	>1.6*
Q_6	48.3	0.22*
Q_7	35.9	0.92*
M_1	32.1	>1.6**
P_1	32.1	>1.6**
Q_1	32.8	>1.6**
Q_2	31.1	0.92**

(*) -*L. lactis* ssp *lactis* bacteriophages*;*
(**) - *L. lactis* ssp *diacetylactis* bacteriophages

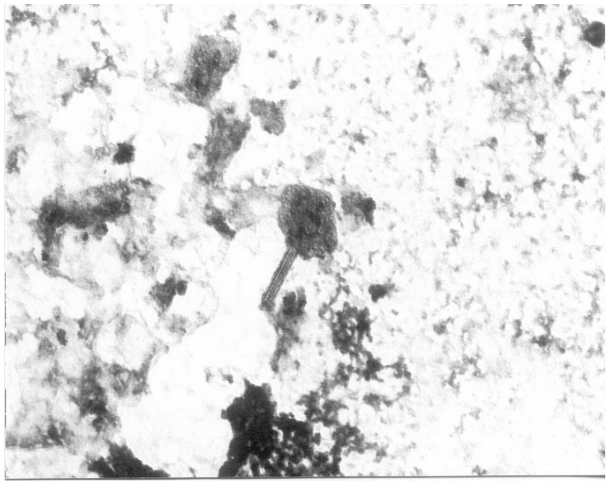

Figure 2 - Bacteriophage of *L. lactis* isolated from industrial whey (transmission electronic microscope - 57,000 times)

Influence of the bacteriophages mixture on commercial milk starters

Table 5 shows milk acidity and the degree of inhibition caused by presence of the bacteriophage mixture isolated in commercial cultures of milk bacteria (commercial starters). Acidification after 8 hours of incubation with commercial starters ranged from 56 to 87 ºD, which was expected given their different strain composition (Table 2).

Table 5 - Acidity (ºD) of milk fermented by different commercial starters and inhibition percentage (%ºD) by addition of bacteriophages mixture at 4, 6 and 8 hours of incubation.

Commercial starter	Inhibition percentage			
	Acidity (ºD)	4hs	6hs	8hs
BD_1	14	0,0	0,0	0,0
BD_2	75	0,0	0,0	0,0
BD_3	87	0,0	0,0	0,0
BD_4	82	0,0	0,0	0,0
0_1	64	0,0	0,0	0,0
0_2	57	4,4	0,0	0,0
0_3	56	0,0	6,1	9,2
0_4	72	4,0	14,0	28,0
O_5	83	0,0	0,0	0,0
D_1	87	0,0	0,0	0,0
S	62	0,0	0,0	0,0

Most commercial starters were not sensitive to inoculated phages, except O_3 and O_4 starters. Although Stadhouders and Leeders (1984) have suggested that incubation for shorter periods (6 hours) was more adequate to evaluate starters behavior in the presence of bacteriophages, results after 8 hours were qualitatively identical to those after 6 hours in our case. Quantitative difference was higher after 8 hours of incubation, indicating that this period generated more sensitive results than 6 hours. Since subculturing of starters has been a common practice in industry, a test with daily transference was carried out on the following commercial starters: BD_1; D_2; O_1 and S (*L. diacetylactis*). Results of the tests after 20 transferences are shown on Table 6.

There was a variation in the inhibition of acidity caused by addition of bacteriophages and the different numbers of transferences. Variation was smaller in simple cultures (*L. diacetylactis*), which was explainable once a sensitive behavior change could only be due to a genetic mutation. In the case of starters BD_1 and D_2, phages and variation in the acidity inhibition occurred after the 10^{th} transference. Limsowtin et al. (1978) attributed such change to the variation in initial composition of starters. This phenomenon could be attributed to differences in the lag period and growth rates, in the tolerance to final metabolic products, in nutritional requirements or variations in the physiological state of cells (Collins, 1962).

Table 6 - Variation of acidity percentage (ºD) of milk fermented by commercial starter (BD_1; D_2; O_1 and *L. diacetylactis*) daily transferences with and without bacteriophages addition.

Transference	BD_1	D_2	O_1	*L. diacetylactis*
1	3,7	0,0	2,0	7,0
2	3,7	0,0	2,0	5,0
3	7,1	2,0	0,0	0,0
4	5,9	0,0	9,8	6,0
5	3,9	2,0	0,0	4,1
6	7,8	0,0	2,2	4,4
7	8,2	0,0	14,0	8,1
8	0,0	0,0	6,1	0,0
9	0,0	0,0	12,2	4,1
10	0,0*	0,0*	12,2	0,0
11	11,0*	15,7*	0,0	0,0
12	10,0*	7,8*	0,0	4,1
13	0,0*	0,0*	0,0	0,0
14	13,2*	3,9*	4,1	0,0
15	0,0*	0,0*	0,0	0,0
16	11,0*	5,9*	10,9	0,0
17	7,8*	0,0*	7,8	0,0
18	19,0*	2,0*	4,1	0,0
19	20,7*	3,9*	17,6	0,0
20	15,8*	1,9*	2,0	0,0

(*)bacteriophages presence

Bacteriophages isolated in cultures inoculated and incubated with BD_1 and D_2 starters after 20 transferences were able to attack the *Lactococcus* strains used in their isolation, hence it was very likely that those bacteriophages were the same ones inoculated in the beginning of the fermentation. In the case of starter D_2, bacteriophages were maintained after the 10^{th} transferences but there was no significant alteration in their capacity of acidification. This could be caused by the presence of *L. lactis* and *L. cremoris* not sensitive to a bacteriophage specific to *L. diacetylactis*, which would allow normal levels of acid production, as related by Sandine et al. (1960). Nevertheless, this kind of infection certainly leads to variations in the ability to form diacetyl and other secondary components, and then interfere with the flavour and quality of the final product. Besides, the presence of such cryptic bacteriophage in a processing plant could have disastrous consequences if acid-producing cultures that were sensitive to such phage were introduced in the process. In the case of starter BD_1, besides the emergence and permanence of the bacteriophage, there was a significant inhibition in milk acidification, showing that it had a bacterium sensitive to the phage, which was responsible for the acid formation.

Table 7 - Sensitivity to phages of bacteria isolated in commercial starters

starter/ culture	Bacteriophages														
	c_1	**e_2**	**f_1**	**j_1**	**k_1**	**l_1**	**l_2**	**l_3**	**m_3**	**o_1**	**p_1**	**q_1**	**q_2**	**q_6**	**q_7**
BD_1-1	-	-	-	-	-	-	-	-	-	-	S	S	-	-	-
2	-	-	-	-	-	-	-	S	-	S	S	S	-	-	-
3	-	-	-	-	-	-	-	S	-	-	S	S	-	-	-
4	-	-	-	-	-	-	-	S	-	-	S	S	-	-	-
5	-	-	-	-	-	-	-	-	-	-	S	S	-	-	-
6	-	-	-	-	-	-	-	-	-	-	S	S	-	-	-
7	-	-	-	-	-	-	-	-	-	S	S	S	-	-	-
8	-	-	-	-	-	S	-	-	-	-	S	S	-	-	-
9	-	-	-	S	S	-	-	S	-	S	S	S	-	-	-
10	-	-	-	-	-	-	-	S	-	-	S	-	-	-	-
11	-	-	-	-	-	-	-	S	-	-	S	-	-	-	-
12	-	-	-	-	-	-	-	S	-	-	S	-	-	-	-
13	-	-	-	-	-	-	-	S	-	-	S	S		-	-
D_2- 1	-	-	-	-	-	-	-	-	-	-	S	S	S	-	-
2	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-
6	-	-	-	-	-	-	-	-	S	S	S	S	-	-	-
7	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-
9	-	-	-	-	-	-	-	-	-	S	S	-	-	-	-
10	-	-	-	-	-	-	-	-	-	S	S	-	S	-	-
11	-	-	-	-	-	-	-	-	-	S	S	-	-	-	-
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14	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-
15	-	-	-	-	-	-	-	-	-	S	S	-	-	-	-
16	-	-	-	-	-	-	-	-	-	S	S	-	-	-	-
17	-	-	-	-	-	-	-	-	S	-	-	S	S	-	-
O_3- 1	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-
O_4- 1	-	S	-	-	S	S	-	S	S	-	S	-	-	-	-
2	-	-	-	-	-	-	-	-	S	-	S	S	-	-	-

Sensitivity: S; Resistance: -

Starter O_1 did not keep bacteriophages after successive transference, but its acid-production ability varied greatly. This variation was probably due to inherent characteristics of the culture, and not to the presence of phages. Phages were washed out by successive dilutions due to the lack of hosts.

Sensitivity of commercial starters isolated cultures

Since culture rotation is one of the methods used in industrial processes to control phage particles, it is important to determine the sensitivity level of starters bacteria to the bacteriophages found in the industry. This work was carried out with isolating 108 isolates from commercial starters BD_1, D_2, O_3 and O_4. Thirteen of 56 strains isolated in starter BD_1, or 23%, were sensitive to at least one of the phages. Seventeen of 43 strains (40%) from starter D_2 were sensitive. One of the 5 strains that formed starter O_3 and 2 of the 4 O_4 strains were sensitive. There was a wide variation in sensitivity among isolated strains (Table 7). No starter isolate was sensitive to phages c_1, f_1, j_1, l_2, q_6 e q_7. On the other hand, almost every isolate (except for isolate 17, isolated from starter D_2) was sensitive to phage p_1. The absence of infective phages in certain isolates might be attributed to the non utilization of such culture in the plant where its isolation took place (Henning et al., 1968). Also, if the tested starters were only recently used in the plant there was no sufficient time for the multiplication of bacteriophages to be experimentally detected.

CONCLUSIONS

There is a wide presence of phage particles in the milk industry of São Paulo State. 65% of the analyzed samples were infected with some kind of phage, in tests with a single strain of each *Lactococcus* species. If more strains species of bacteria was included, probably more samples would have been contaminated, once phages are specific to species and often even to strains.

Therefore, it is essential to adopt preventive measures, especially regarding sanitation of the processing plant, adequate whey handling and rotation of starter inoculation cultures to decrease contamination levels. The hypothesis that a decrease of 10% or more in the acidification capacity was indicative of the presence of bacteriophages did not hold, possibly due to the presence of another inhibitor of fermentation in milk, such as microbial agents that were used to control animal health or compound used in the cleaning and sanitation of the production or processing plant, which acted similarly to phages in different bacteria species. Although commercial starters have good acidification behavior in the presence of bacteriophages processing plants, the possibility of maintenance in dormant stage of phage in industry that may cause occasional damages should be considered.

RESUMO

Para ampliar conhecimentos sobre a incidência de bacteriófagos de bactérias lácticas na indústria de leite do Estado de São Paulo e a sua influência sobre a capacidade acidificante de fermentos lácticos disponíveis em nosso mercado, o presente trabalho foi conduzido com o intuito de esclarecer a real situação dos laticínios no Estado. Foram coletadas 33 amostras de soro de queijo em 17 laticínios. Foram isolados 16 bacteriófagos, 12 específicos para *Lactococcus lactis*, 3 para *L. diacetylactis* e um capaz de lisar ambos os microrganismos. Os experimentos mostraram que, uma diminuição de 10% na acidez em presença de soro suspeito, ao contrário do estabelecido na literatura, não reflete a veracidade da presença de bacteriófagos na amostra, uma vez que a maioria apresentou redução acima desse valor embora atividade fágica estivesse presente em apenas 65% das indústrias avaliadas. Utilizando uma mistura de bacteriófagos isolados, procedeu-se testes com 12 fermentos lácticos comerciais, e, em 8 horas, somente 2 tipos mostraram diferença na capacidade de acidificação. Verificou-se também que os fermentos, quando submetidos a repicagem diária, mantiveram os bacteriófagos após 20 subcultivos. A partir dos fermentos comerciais foram obtidas as bactérias componentes, que foram testadas individualmente quanto à resistência aos bacteriófagos, mostrando que o nível de sensibilidade varia consideravelmente entre os diferentes tipos de fermentos (BD, D, O e um de *L. diacetylactis*). Cinco bacteriófagos isolados não se mostraram infectivos a nenhum dos microrganismos isolados, enquanto 1 deles, mostrou-se infectivo para a quase totalidade dos isolados.

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