J. Dairy Sci. 89:3791–3799
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Diversity of *Streptococcus thermophilus* Phages in a Large-Production Cheese Factory in Argentina

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ABSTRACT

Phage infections still represent a serious risk to the dairy industry, in which *Streptococcus thermophilus* is used in starter cultures for the manufacture of yogurt and cheese. The goal of the present study was to analyze the biodiversity of the virulent *S. thermophilus* phage population in one Argentinean cheese plant. Ten distinct *S. thermophilus* phages were isolated from cheese whey samples collected in a 2-mo survey. They were then characterized by their morphology, host range, and restriction patterns. These phages were also classified within the 2 main groups of *S. thermophilus* phages (*cos*- and *pac*-type) using a newly adapted multiplex PCR method. Six phages were classified as *cos*-type phages, whereas the 4 others belonged to the *pac*-type group. This study illustrates the phage diversity that can be found in one factory that rotates several cultures of *S. thermophilus*. Limiting the number of starter cultures is likely to reduce phage biodiversity within a fermentation facility.

Key words: Argentinean dairy plant, *Streptococcus thermophilus*, bacteriophage, genetic diversity

INTRODUCTION

It is well recognized that phage infection of the starter cultures can lead to milk fermentation delays, resulting in an unacceptably low production of lactic acid and flavor compounds along with reduced proteolysis and lactose hydrolysis in the fermented products. In extreme cases, phage infection may even lead to the complete loss of the milk product (Neve, 1996). Because of this constant risk, phage control is a primary area of concern when handling lactic acid bacteria (Bruttin et al., 1997; Moineau, 1999). Last year in Argentina, 2.7 billion liters of milk were transformed into cheese (90%) and yogurt (10%). *Streptococcus thermophilus* strains are predominantly found in starter cultures used for Argentinean fermented milks and several varieties of soft and semihard cheeses (Cremoso, Cuartirolo, Port Salut, Holanda, Fontina, Colonia, Edam, and Pategrás; Reinheimer et al., 1997). Thus, it is considered the most technologically important lactic acid bacteria by the dairy industry in Argentina. Unfortunately, several *S. thermophilus* strains used in commercial starters are highly sensitive to autochthonal phages (Suárez et al., 2002).

Over the last 15 yr, several studies from Europe have reported the isolation and detailed characterization of bacteriophages infecting thermophilic starter cultures (Neve et al., 1989; Benbadis et al., 1990; Larbi et al., 1990; Fayard et al., 1993; Brüssow et al., 1994; Bruttin et al., 1997; Le Marrec et al., 1997). Few ecological studies have been carried out to elucidate the origin of *S. thermophilus* phages in dairy environments (Bruttin et al., 1997; Brüssow et al., 1998; Brüssow and Desiere, 2001). Bruttin et al. (1997) identified raw milk as the source of new incoming phages that enter a cheese factory. In this case, the genetic diversity of the *S. thermophilus* phages isolated in the factory was similar outside of the factory. Brüssow et al. (1994) also studied *S. thermophilus* phages collected over 30 yr from batches of yogurt and cheese manufactured in several European countries. The authors characterized 81 lytic phages (40 from yogurt and 41 from cheese-making samples) that showed 46 distinct DNA restriction patterns (11 for phages isolated from yogurts and 35 for phages isolated from cheeses), suggesting more phage diversity in cheese plants. These facts confirm the ubiquitous nature of phages in dairy environments; consequently, considerable research efforts nowadays focus on controlling dairy phages rather than trying to eradicate them (Moineau, 1999; Moineau et al., 2002).

Streptococcus thermophilus phages are currently divided into 2 groups based on the packaging mechanism of their double-stranded DNA (*cos*- and *pac*-type) and

Received November 16, 2005.
Accepted May 3, 2006.
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Table 1. Identification, isolation date, and titers of *Streptococcus thermophilus* bacteriophages isolated from a large-scale-production cheese plant

Phage	Isolation date, mo (2000)	Starter supplier	Starter identification	Sensitive host	Phage titer in the sample, pfu/mL
ALQ1.3	08	A	1	ST1.3	3.5×10^3
ALQ2.2	08	A	2	ST2.2	8.0×10^3
ALQ3.1	08	B	3	ST3.1	8.0×10^2
ALQ3.2	08	B	3	ST3.2	7.5×10^2
ALQ4.2	08	B	4	ST4.2	9.0×10^3
ALQ8.1	09	A	8	ST8.1	2.0×10^3
ALQ8.2	09	A	8	ST8.2	3.0×10^3
ALQ9.1	09	C	9	ST9.1	3.3×10^3
ALQ10.3	09	C	10	ST10.3	3.0×10^5
ALQ13.2	09	D	13	ST13.2	1.7×10^3

the number of major structural proteins (Le Marrec et al., 1997). Seven complete genome sequences of *S. thermophilus* phages are now available; they include the *cos*-type phages DT1, Sfi19, Sfi21, and 7201 as well as the *pac*-type phages O1205, Sfi11, and 2972 (Lévesque et al., 2005). Recently, a PCR method was developed for the rapid detection of *S. thermophilus* phages (Binetti et al., 2005). This detection tool amplifies a variable region within the antireceptor gene of *cos*-type phages (Duplessis and Moineau, 2001), which allows classification of these phages based on their host range.

Several *S. thermophilus* phages were previously isolated from various cheese and yogurt samples in Argentina (Suárez et al., 2002). The genetic diversity of *S. thermophilus* phages isolated from these yogurt samples was also previously analyzed (Quiberoni et al., 2003). However, the biodiversity of Argentinean *S. thermophilus* phages isolated from cheese plants has never been investigated. Therefore, the aim of this work was to determine the phage diversity in one cheese plant environment using morphological and genetic parameters. The selected cheese factory transforms 180,000 L of milk per day and uses a rotation of 14 starter cultures containing several *S. thermophilus* strains. A new multiplex PCR was also adapted to rapidly classify *S. thermophilus* phages within one of the 2 groups. Knowledge of the genetic diversity of *S. thermophilus* phages in the factory is essential to ensure the efficacy of the control strategies and to select efficient starter cultures.

MATERIALS AND METHODS

Strains and Cultural Conditions

The cheese plant used 14 different *S. thermophilus* starters (provided by 4 suppliers) in a culture rotation plan (2 starters per week). Ten *S. thermophilus* isolates obtained from the commercial starters were identified as phage sensitive (Table 1). All strains were grown at 42°C in M17 broth (Quélab, Québec, Canada) supplemented with 0.5% (wt/vol) lactose (LM17). They were maintained as frozen stocks (–80°C) in sterile, reconstituted (10%, wt/vol) commercial nonfat dry skim milk at the INLAIN Collection and at the Félix d'Hérelle Reference Center for Bacterial Viruses (www.phage.ulaval.ca).

Molecular Analysis of S. thermophilus Isolates

Pulsed-field gel electrophoresis (**PFGE**) was used to analyze the diversity among the 10 *S. thermophilus* isolates. All strains were inoculated in LM17 broth and incubated overnight at 42°C. Cultures were centrifuged at 12,000 × *g* for 2 min and supernatants were discarded. Cell pellets were washed twice and resuspended in 250 μL of TEE buffer (10 m*M* Tris-HCl pH 9, 100 m*M* EDTA, 10 m*M* EGTA). Solutions were warmed to 37°C for 10 min and mixed with 250 μL of 2% low-melting-point agarose (Bio-Rad Laboratories, Richmond, CA; at 55°C) in TEE buffer. Cell suspensions were placed into the wells of a block maker and placed at 4°C for 15 min. Hardened blocks containing *S. thermophilus* cells were transferred into 2 mL of lysis buffer (10 m*M* Tris-HCl pH 9.0, 100 m*M* EDTA, 10 m*M* EGTA, 30 mg/mL of lysosyme, 0.05% laurylsarcosine), incubated at 37°C for 3 h, transferred into 2 mL of a proteinase-containing solution (10 m*M* Tris-HCl, 100 m*M* EDTA, 10 m*M* EGTA, 1 mg/mL of proteinase K, 1% wt/vol sodium dodecyl sulfate) and incubated overnight at 55°C. The blocks were then washed once at room temperature for 1 h with TE buffer (10 m*M* Tris-HCl pH 8, 1 m*M* EDTA) containing 250 μL of 20 m*M* phenylmethylsulfonyl fluoride in isopropanol, and twice with TE buffer. The genomic DNA trapped within the blocks was restricted with *Sma*I (Roche Diagnostics, Laval, Québec, Canada) at 25°C overnight. The agarose gel (1%) was prepared in 0.5× TBE buffer (45 m*M* Tris-

borate, 1 m*M* EDTA pH 8). The blocks with the restricted DNA were placed into the wells. Pulsed-field gel electrophoresis was performed for 24 h at pulse times from 1 to 90 s, 10 V/cm, using the Gene Navigator system (GE Healthcare Technologies, Waukesha, WI). The gel was stained with ethidium bromide and visualized by UV photography.

Analysis of the Whey Samples for the Presence of Phages

Fourteen cheese whey samples (2 whey samples per week for 7 wk) from an Argentinean industrial plant were analyzed at the INLAIN during August and September 2000. The whey samples were centrifuged (10 min at 5,000 × *g*) and filtered through a 0.45-μm pore size filter (Millipore Indústria e Comércio Ltda., São Paulo, Brazil). The filtrates, with and without a thermal treatment (15 min at 90°C), were used to investigate the presence of phages by means of the spot test, according to the procedure of Svensson and Christiansson (1991). Briefly, the log phase of pure cultures (isolated from commercial starters) was mixed with LM17 soft agar (0.6% wt/vol) and plated as a thin top layer on LM17-Ca (10 m*M* $CaCl_2$) agar (1.2% wt/vol) plates. Aliquots of 50 μL of filtrates suspected of containing phages were spotted on the plates. After incubation at 42°C for 18 h in anaerobic conditions (Gaspak System, Oxoid, Basingstoke, UK), the presence or absence of lysis zones was recorded (Svensson and Christiansson, 1991). The phage turbidity test was also performed by inoculating 5 mL of LM17-Ca broth (10 m*M* $CaCl_2$) with 0.2 mL of an overnight culture of *S. thermophilus* strains and 0.2 mL of whey filtrates. Tubes inoculated only with the strains were used as controls. A total of 3 subcultures were made to increase the concentration of phages. Tubes were incubated at 42°C and the turbidity was compared with the control tubes (Svensson and Christiansson, 1991).

Phage Counts, Isolation, and Host Range

The double-layer plaque titration method was used to determine the phage titers in the various sample filtrates. One hundred microliters of decimal dilutions of whey sample filtrates (or from the turbidity test) were added to 0.3 mL of log phase indicator cells, mixed with LM17 soft agar, and poured onto the surface of LM17-Ca agar plates supplemented with 100 m*M* glycine (Merck Química Argentina, Buenos Aires, Argentina; Lillehaug, 1997). After incubation at 42°C for 18 h in an anaerobic atmosphere (Gaspak System), the plaques were enumerated (Svensson and Christiansson, 1991). To isolate and purify the phages, well-defined single plaques were picked up and placed in 5 mL of LM17-Ca broth. Tubes were kept for 24 h at 4°C and then inoculated with 0.2 mL of an overnight culture of host strains. Incubation at 42°C was carried out until the total lysis of the culture was reached. This phage purification procedure was carried out 3 consecutive times. The isolated phages were kept at –80°C as high-titer filtrates, with the addition of glycerol (15%, vol/vol), in the INLAIN Collection and at the Félix d'Hérelle Reference Center for Bacterial Viruses. The host range of each phage was investigated by using both the spot test and the turbidity test as described above.

Electron Microscopy

The 10 phages were sedimented at 25,000 × *g* for 60 min using a J2-21 centrifuge (Beckman, Palo Alto, CA) with a JA 18.1 fixed-angle rotor. Phages were washed twice in 0.1 *M* ammonium acetate (pH 7.0), deposited on copper grids provided with carbon-coated Formvar films, stained with 2% potassium phosphotungstate (pH 7.2) or uranyle acetate (pH 4.5), and examined in a Philips EM 300 electron microscope (Philips, Eindhoven, the Netherlands). Magnification was standardized with T4 tails. Isometric capsids were measured between opposite apices.

Phage DNA Analysis

Phage DNA were obtained by phenol-chloroform extractions and concentrated by isopropanol precipitation as described previously (Moineau et al., 1994). DNA pellets were resuspended in 10 m*M* Tris-HCl pH 8. Alternatively, the Maxi Lambda DNA purification kit (Qiagen, Chatsworth, CA) was used for phage DNA purification as specified previously (Labrie and Moineau, 2000). Restriction enzymes (*Eco*RI and *Eco*RV; Roche Diagnostics) were used as recommended by the manufacturer. After restriction, phage DNA samples were heated for 10 min at 70°C to avoid possible cohesive end ligation. DNA was electrophoresed on 0.8% agarose gels in 1× TAE buffer (40 m*M* Tris-acetate, 1 m*M* EDTA) and visualized by UV photography after staining with ethidium bromide (Sambrook et al., 1989). The restriction patterns were captured using the Quantity One electrophoresis analysis system (Bio-Rad Laboratories). The restriction patterns were analyzed with Molecular Analyst Software, Fingerprinting Plus (Version 1.6, Applied Maths 1992–1998; Bio-Rad Laboratories). Calculation of the similarity of restriction profiles was based on the Pearson correlation coefficient (r), and phages were grouped by using the unweighted pair group method with arithmetic averages. The sizes of the phage genomes were estimated by adding the

Table 2. List of primers used for the multiplex PCR[1]

Primer	Sequence, 5′–3′	PCR product length, bp
cos FOR	GGTTCACGTGTTTATGAAAAATGG	170
cos REV	AGCAGAATCAGCAAGCAAGCTGTT	
pac FOR	GAAGCTATGCGTATGCAAGT	427
pac REV	TTAGGGATAAGAGTCAAGTG	

[1]See Figure 4.

size of each visible *Eco*RI and *Eco*RV digested DNA fragment.

Multiplex PCR

As indicated above, *S. thermophilus* phages are classified into 2 groups based on their structural protein profiles and their mode of DNA packaging (Le Marrec et al., 1997). Comparative genome analyses have also indicated that genes coding for the structural proteins are conserved among each group of phages (Lévesque et al., 2005). To rapidly classify a newly isolated *S. thermophilus* phage within one of these 2 groups, a multiplex PCR was developed. First, 2 pairs of primers (one per phage group) were designed from the conserved regions of the gene coding for the major capsid protein in the phages for which the complete genome is available (4 *cos*-type and 3 *pac*-type; Table 2). The PCR reactions were performed in a total volume of 50 μL containing 125 μ*M* deoxynucleoside triphosphate (Pharmacia Biotech, Baie d'Urfé, Québec, Canada), 5 μ*M* concentrations of the 4 primers, 2.5 U of *Taq* DNA polymerase (Roche Diagnostics), *Taq* buffer (20 m*M* Tris-HCl pH 8.4, 1.5 m*M* magnesium chloride, 50 m*M* potassium chloride), and 1 μL of the phage lysate. Phages DT1 (Tremblay and Moineau, 1999) and 2972 (Lévesque et al., 2005) were used as *cos*- and *pac*-type positive controls, respectively. Phage DT1 was propagated on *S. thermophilus* SMQ-301, whereas phage 2972 was propagated on *S. thermophilus* RD534. A negative control (without the template) was included for all PCR assays to eliminate the possibility of contamination. Polymerase chain reaction amplifications were performed on a Robocycler gradient apparatus (Stratagene, La Jolla, CA), and conditions were set as follows: 5 min at 94°C, followed by 35 cycles (45 s at 94°C, 45 s at 53°C, 1 min at 73°C), and a final step of 5 min at 73°C. The PCR products were separated on a 2% agarose gel in TAE buffer (40 m*M* Tris-acetate, 1 m*M* EDTA), stained with ethidium bromide, and visualized under UV light (Sambrook et al., 1989).

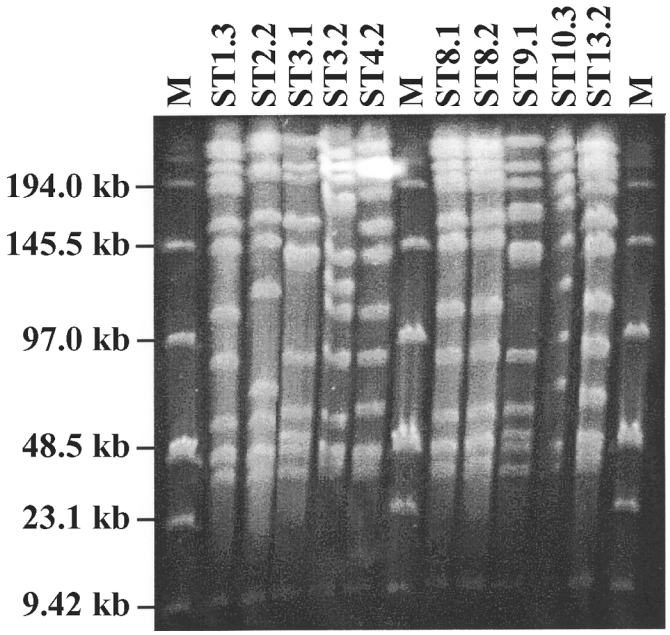

Figure 1. Pulsed-field gel (PFG) electrophoresis patterns of phage-sensitive *Streptococcus thermophilus* strains. M = low-range PFG markers (New England BioLabs, Ipswich, MA).

RESULTS

Molecular Analysis of S. thermophilus Commercial Strains

As indicated in the preceding section, 10 *S. thermophilus* strains were obtained from the commercial starters and found to be phage sensitive. Overall, the *Sma*I restriction patterns were relatively similar among the 10 phage-sensitive *S. thermophilus* isolates. Nonetheless, 7 different PFGE patterns were distinguished among the 10 *S. thermophilus* phage-sensitive isolates (Figure 1). The isolates identified as ST1.3, ST8.1, and ST8.2 showed identical patterns, as did ST4.2 and ST13.2. It should be noted that these 5 strains were also highly related to each other (Figure 1). Interestingly, ST1.3, ST8.1, and ST8.2 were isolated from 2 different starter cultures (starters 1 and 8) provided by the same supplier (Table 1). On the other hand, ST4.2 and ST13.2 were isolated from 2 starters from different suppliers, indicating that highly related *S. thermophilus* strains are used by different starter culture suppliers. Finally, isolates ST3.1 and ST9.1 displayed very similar profiles, whereas the *Sma*I patterns of strains ST2.2, ST3.2, and ST10.3 were unique. In conclusion, 7 different phage-sensitive *S. thermophilus* strains were isolated from the 14 commercial starter cultures.

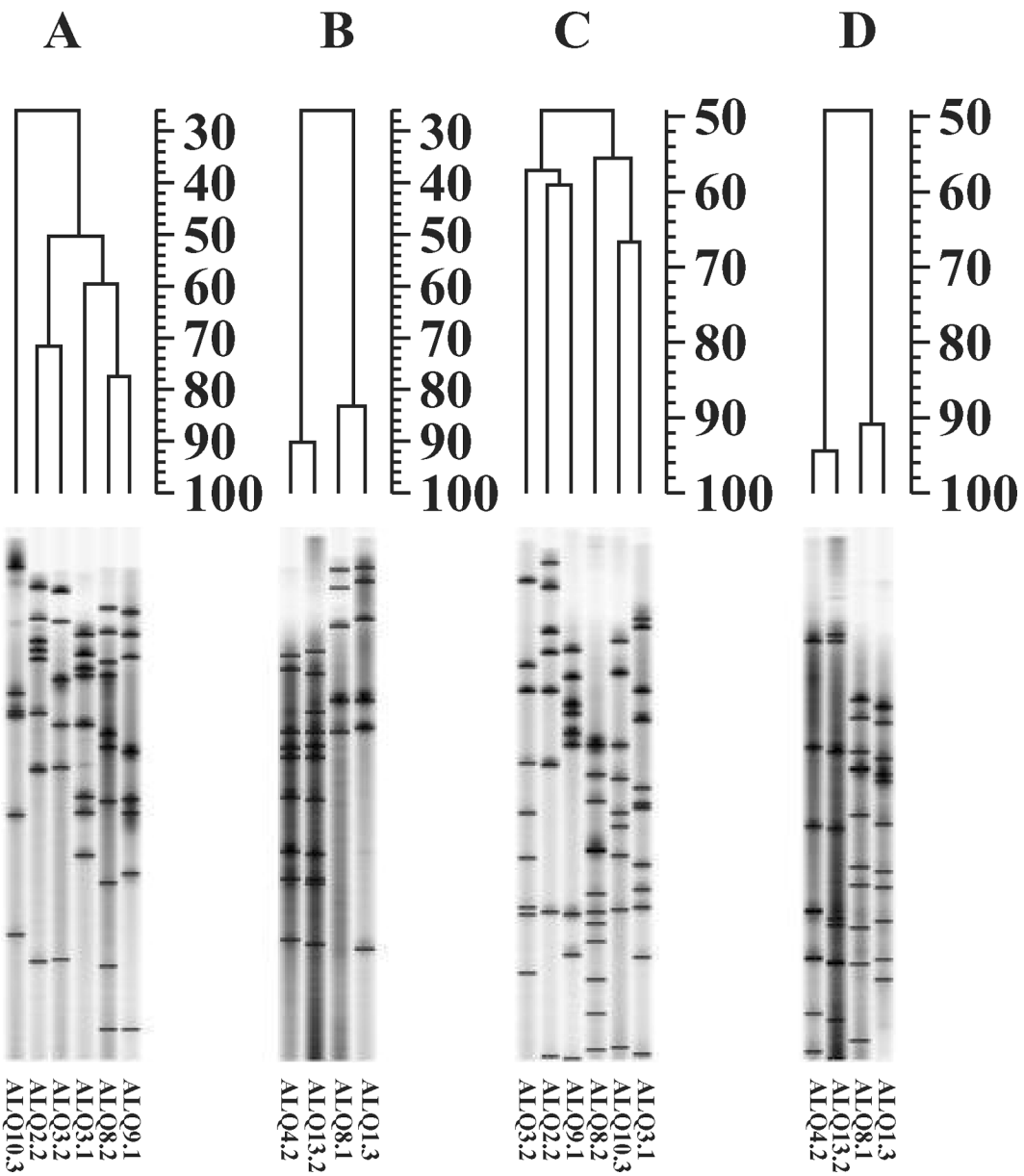

Figure 2. Restriction profiles of the 10 *Streptococcus thermophilus* phages. *Eco*RI restriction profiles of the *cos*-type (A) and *pac*-type (B) phages; *Eco*RV restriction profiles of the *cos*-type (C) and *pac*-type (D) phages.

Phage Isolation

Nine cheese whey samples (samples 1 to 4, 8 to 10, 13, and 14) from a total of 14 were found to contain phages infecting the host strains analyzed. Based on the phage DNA restriction profiles and host range data (see below), a total of 10 distinct *S. thermophilus* bacteriophages were isolated from these 9 samples. Two whey samples contained 2 different *S. thermophilus* phages (ALQ3.1 and ALQ3.2, ALQ8.1 and ALQ8.2). The same phage (ALQ13.2) was also isolated in 2 whey samples (samples 13 and 14). Table 1 indicates the isolation date of each phage, their titers in the whey cheese samples, and the respective *S. thermophilus* strains used for their isolation. The number of viral particles in the whey samples ranged from 7.5×10^2 to 3.0×10^5 pfu/mL.

Restriction Patterns

Based on the phage DNA restriction profiles obtained with *Eco*RI and *Eco*RV, 10 different patterns were detected (Figure 2). In a few cases, some phages displayed related restriction patterns. For example, phages ALQ4.2 and ALQ13.2, which can propagate on the same

Table 3. Host range of the *Streptococcus thermophilus* bacteriophages isolated from whey cheese samples[1]

	Phage									
Host	ALQ2.2 (*cos*)	ALQ3.1 (*cos*)	ALQ3.2 (*cos*)	ALQ8.2 (*cos*)	ALQ9.1 (*cos*)	ALQ10.3 (*cos*)	ALQ1.3 (*pac*)	ALQ4.2 (*pac*)	ALQ8.1 (*pac*)	ALQ13.2 (*pac*)
ST1.3				+			+		+	
ST2.2	+									
ST3.1		+								
ST3.2			+							
ST4.2			+					+		+
ST9.1			+		+					
ST10.3						+				

[1]A blank indicates the phage insensitivity of the strain.

host (Figure 1 and Table 3), showed very similar *Eco*RV and *Eco*RI patterns (Figure 2). Similarly, phages ALQ1.3 and ALQ8.1 replicating on the same strain (Table 3), exhibited related restriction profiles (Figure 2). The genome sizes of these phages were in the same range (data not shown) as those previously determined by genome sequencing studies (Lévesque et al., 2005).

Phage Morphology

All phages were members of the *Siphoviridae* family and of the B1 morphotype (Ackermann, 2001; Figure 3). They showed isometric capsids of 61 nm in diameter and long, more or less rigid tails of 298 × 8 nm with conspicuous cross-striations and a base plate of 12 × 3 nm (Figure 3). Phages tended to adsorb to bacterial debris and to form large rosettes of several phage particles.

Multiplex PCR Phage Classification

A multiplex PCR method was previously developed to classify *Lactococcus lactis* phages into one of the 3 predominant groups (Labrie and Moineau, 2000). Three sets of primers, one for each group, were designed based on conserved regions of their genomes and were optimized to give PCR products of different sizes. Two of the 3 sets of primers targeted the gene coding for the major capsid protein. Using the same approach, a multiplex PCR method was developed to catalog the 10 isolated phages into the 2 *S. thermophilus* phage DNA packaging groups (*cos*- and *pac*-type; Figure 4). Two sets of primers were designed, one for the *cos*-type and another for the *pac*-type group, based on conserved regions in the gene coding for the major structural proteins of phages for which the complete genomic sequence is known (Lévesque et al., 2005; Table 2, Figures 4A and B). The virulent phages DT1 (*cos*-type) and 2972 (*pac*-type) were used as controls. By using the multiplex PCR method and phage lysates, 6 phages (ALQ2.2, ALQ3.1, ALQ3.2, ALQ8.2, ALQ9.1, and ALQ10.3) gave a PCR product of 170 bp, indicating that they belonged to the *cos*-type group, whereas the remaining 4 phages (ALQ1.3, ALQ4.2, ALQ8.1, and ALQ13.2) yielded a PCR fragment of 427 bp, distinctive of the *pac*-type phage group (Table 2, Figure 4C).

Host Range

The 10 phages were tested against the 7 distinct *S. thermophilus* strains identified above; their host range

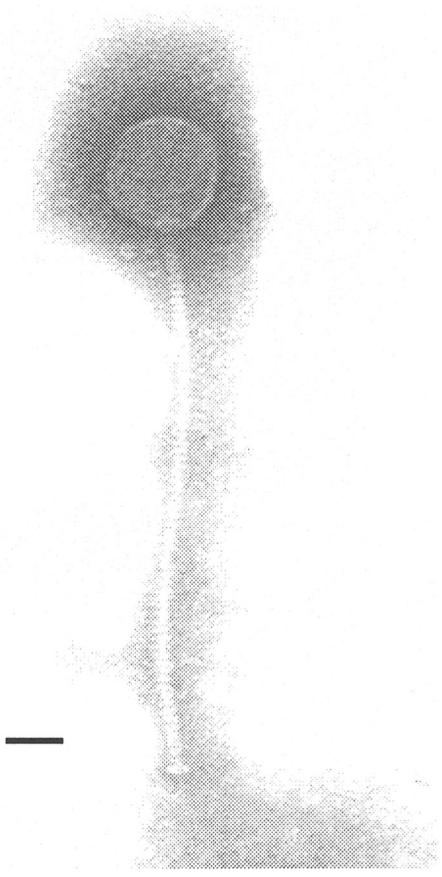

Figure 3. Electron micrograph of *Streptococcus thermophilus* phage ALQ1.3 isolated from whey cheese samples. Bar represents 30 nm.

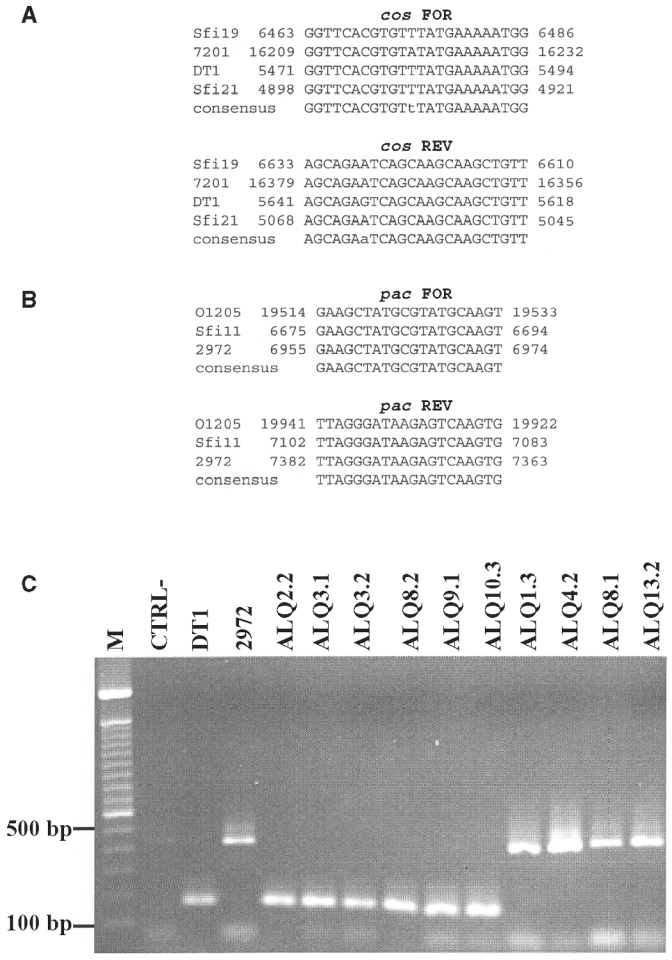

Figure 4. Multiplex PCR for the detection of the 2 groups of *Streptococcus thermophilus* phages. Nucleotide sequence alignments of the conserved regions used to design the primers for the *cos*-type (A) and the *pac*-type (B) phages. The genes coding for the major capsid protein were analyzed in 4 *cos*-type phages: phage Sfi19 (*orf39*, GenBank accession number AF115102), phage 7201 (*mpl-7201*, AF145054), phage DT1 (*orf8*, AF085222), and phage Sfi21 (*orf9*, AF115103). Similarly, the genes coding for the major capsid protein were analyzed in 3 *pac*-type phages: phage O1205 (*orf31*, U88974), phage Sfi11 (*orf348*, AF145054), and phage 2972 (*orf9*, AY699705). The corresponding coordinates are indicated on the left and right sides. (C) Classification of the 10 isolated *S. thermophilus* phages by the multiplex PCR. M = 100-bp DNA ladder (Invitrogen Life Technologies, Carlsbad, CA); CTRL = control.

is shown in Table 3. In agreement with the PFGE data, the 7 *S. thermophilus* strains exhibited different phage-sensitivity patterns. Some strains were also phage related (i.e., sensitive to the same phages). For example, *S. thermophilus* ST3.2, ST4.2, and ST9.1 were sensitive to phage ALQ3.2, indicating that these strains should not be used in the same starter culture and should be used sparingly in a starter rotation system. *Streptococcus thermophilus* ST1.3 and ST4.2 were sensitive to 3 phages, whereas ST9.1 was sensitive to 2 phages. The other 4 strains were sensitive to only one distinct phage (Table 3). Interestingly, *S. thermophilus* ST1.3 and ST4.2 were sensitive to *cos*- and *pac*-type phages, whereas the other 5 strains were sensitive to only *cos*-type phages. Phage ALQ3.2 showed the broader host range because it could propagate on 3 of the 7 distinct strains, whereas the 9 other phages could infect only one of these strains.

DISCUSSION

Knowledge of the phage types present in dairy plants and an understanding of the mechanisms creating this phage diversity are needed for efficient management of a defined culture rotation system (Casey et al., 1993; Moineau and Lévesque, 2005). Despite the technological relevance of *S. thermophilus* and the marked increase in the number of incidents related to streptococcal phage infections in the last 15 yr (Suárez et al., 2002), few data are available on the indigenous phages in Argentina (Quiberoni et al., 2003). Nevertheless, some European studies have reported the isolation and detailed characterization of bacteriophages from thermophilic dairy manufacture (Neve et al., 1989; Benbadis et al., 1990; Larbi et al., 1990; Fayard et al., 1993; Brüssow et al., 1994; Bruttin et al., 1997). Very few of these *S. thermophilus* phages were isolated from large-scale-production cheese plants.

The factory environment is an important reservoir of phages and it is mainly responsible for phage permanence in dairy plants. In the present study, 10 distinct *S. thermophilus* phages were isolated during a relatively short period of 2 mo from only 14 whey samples derived from an Argentinean cheese plant that produces 80 tons of cheese daily. A great diversity in the phage population was observed during our monitoring, although phage titers were relatively low (from 7.5×10^2 to 3.0×10^5 pfu/mL) in whey samples. However, a high phage titer could be obtained when these phages were amplified in the laboratory, indicating that they can be highly virulent under the appropriate environmental conditions. The intensive starter rotation system used by the cheese plant managers to control phages is likely responsible for the phage biodiversity in this industrial environment. At least 7 commercial *S. thermophilus* strains could serve as a host for these phages.

Brüssow et al. (1994) reported a more diverse *S. thermophilus* phage population within cheese plants than within yogurt factories. Isolation of the same *S. thermophilus* phages within a yogurt plant has also been reported (Quiberoni et al., 2003). During this 2-mo survey, 10 distinct phages were isolated and only one was isolated twice (ALQ13.2). This diverse phage popula-

tion is likely due to the rotation of multiple strains of *S. thermophilus* in the cheese starters, as compared with yogurt starters. In fact, a limited number of commercial *S. thermophilus* strains possess the relevant industrial phenotypes for yogurt manufacture, for example, exopolysaccharide production (Broadbent et al., 2003). Moreover, the yogurt and cheese factories are, to some extent, 2 distinct ecological conditions. Milk for yogurt production undergoes a heat treatment at 90°C, which kills most phages (Quiberoni et al., 1999, 2004), whereas raw or pasteurized milk is used in cheese fermentation, and many phages will resist pasteurization (Chopin, 1980). Yogurt production is also a relatively aseptic process in which the fermented product has minimal exposure to the factory environment. In contrast, cheese factories experience phage contamination from various sources (Moineau and Lévesque, 2005).

The *S. thermophilus* phages isolated in this study showed the same basic characteristics as those previously isolated in other countries (Neve et al., 1989; Benbadis et al., 1990; Larbi et al., 1990; Fayard et al., 1993; Brüssow et al., 1994; Bruttin et al., 1997; Le Marrec et al. 1997; Tremblay and Moineau, 1999). However, the length of their noncontractile tail was slightly longer (approximately 300 nm) than previously reported (230 to 260 nm; Brüssow et al., 1998). Argentinean *S. thermophilus* phages have previously shown other differences, such as larger burst sizes and longer tails, as compared with the reference phages (Suárez et al., 2002). Nonetheless, they could still be classified within the 2 known groups of *S. thermophilus* phages. Some of these phages were highly related, as defined by specific restriction patterns, which indicate that they probably experienced genome rearrangements by deletion, duplication, or acquisition of novel phage DNA. This observation has already been inferred from previous comparative genome analyses (Desiere et al., 1998; Lévesque et al., 2005).

The DNA packaging mechanism of the phage genome was previously proposed as the basis for dividing *S. thermophilus* bacteriophages into 2 distinct groups (Le Marrec et al., 1997). The authors also demonstrated that *cos*-containing phages possessed 2 major structural proteins (32 and 26 kDa), in contrast to the *pac*-containing phages, which possessed 3 major structural proteins (41, 25, and 13 kDa). Phages belonging to both groups were isolated in our study. No obvious correlation was detected between the grouping based on packaging mechanism and the host range data obtained with the 7 industrial *S. thermophilus* strains. However, 5 of the *S. thermophilus* strains were sensitive only to *cos*-type phages, whereas the 2 others (ST1.3 and ST4.2) were sensitive to both groups of phages. The latter finding was of interest because it was previously suggested that phage-sensitive *S. thermophilus* strains were rarely infected by members of both phage groups (Lévesque et al., 2005). From a practical point of view, it would be appropriate to remove these 2 strains from the starter cultures because they are sensitive to the highest number of phages as well as to members of both *S. thermophilus* phage groups. Such strain replacement would reduce the risk of creating new virulent phages, for example, by recombination between incoming phage genomes.

One significant novelty of this study was the development of a PCR method to rapidly classify *S. thermophilus* phages within one of the 2 known groups. Two other PCR methods were previously developed for the detection of *S. thermophilus* phages and not for classification purposes. The first PCR-based phage detection method was developed for cheese whey from a factory that produced Mozzarella cheese with complex undefined starter cultures (Brüssow et al., 1994). This detection tool was based on the sequence information from a phage DNA element conserved in many, but not all, *S. thermophilus* phages. A second PCR method was developed based on the amplification of a variable region (V2) of the antireceptor gene of *cos*-type *S. thermophilus* phages (Binetti et al., 2005). The strength of this latter PCR method was due to the correlation between the typing profiles and the host ranges, which could lead to a rational starter rotation system.

As additional *S. thermophilus* phages are tested, it is possible that our 2 sets of primers may not classify all of them because of sequence variations. Reducing the hybridization temperature may still enable the classification of some phages, but modifying the primer or primers (and target) could constitute another strategy (Labrie and Moineau, 2000).

In conclusion, the results of this study reflect the phage diversity found in a single cheese plant with a considerable rotation of commercial cultures of *S. thermophilus*. Consequently, limiting the number of starter cultures is likely to reduce the phage biodiversity within a fermentation facility. Our findings that Argentinean *S. thermophilus* phages could be classified within the 2 known phage groups complement the previous phage epidemiology data from European countries (Brüssow et al., 1998). A detailed knowledge of the genetic diversity of *S. thermophilus* phages and regular monitoring of the phage population within an industrial environment are essential for the successful application of phage control measures and for the development of adapted antiphage strategies.

ACKNOWLEDGMENTS

This work was supported by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, Proyecto

PICT 09-8200; Argentina), Universidad Nacional del Litoral (Project CAI+D 2002, no. 22-155; Argentina) and by a grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada (Ottawa, ON, Canada) to S. M.

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