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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

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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-

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