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Analysis of two theta-replicating plasmids of *Streptococcus thermophilus*

Stéphanie L. Girard [a,b], Sylvain Moineau [a,b,c,*]

[a] *Département de biochimie et de microbiologie, Faculté des sciences et de génie, Université Laval, Quebec City, Que., Canada G1K 7P4*
[b] *Groupe de recherche en écologie buccale, Faculté de médecine dentaire, Université Laval, Quebec City, Que., Canada G1K 7P4*
[c] *Félix d'Hérelle Reference Center for Bacterial Viruses, Université Laval, Quebec City, Que., Canada G1K 7P4*

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Abstract

We report the characterization of two new theta-replicating plasmids of *Streptococcus thermophilus* (pSMQ-312b and pSMQ-316) as well as the further analysis of pSMQ-308. The nucleotide sequences of pSMQ-312b and pSMQ-316 were determined and both contained 6710 bp. In fact, the two sequences were identical, despite that the plasmids were isolated from two different *S. thermophilus* strains as demonstrated by pulsed-field gel electrophoresis. Comparative analyses indicated that the two plasmids were highly related to the previously characterized *S. thermophilus* plasmid pSMQ-308 (8144 bp). Plasmid stability tests showed that pSMQ-312b/316 was more stable in LM17 medium while pSMQ-308 was the most stable in milk. The presence of the plasmids did not modify the acidification profile of the *S. thermophilus* strains during growth in milk and under time–temperature conditions mimicking an industrial process. These theta-replicating plasmids are unique genetic material for the construction of stable cloning vectors for industrially relevant strains of *S. thermophilus*.

Keywords: *Streptococcus*; Theta replication; Plasmid; Lactic acid bacteria; Plasmid stability; Milk; Milk acidification; Food grade; Fermentation; Cheese; Yogurt

1. Introduction

Streptococcus thermophilus is a low G + C Gram-positive bacterium commonly used for the manufacture of a wide array of fermented dairy products. In the past decade, the increased industrial use of this generally recognized as safe (GRAS) organism has led to numerous fundamental studies. The overall goal of these studies was to improve general knowledge of this non-pathogenic streptococcal species with the purpose of selecting better strains or improving specialized strains through genetic modification. The latter can be achieved using cloning tools developed through the molecular characterization of resident plasmids.

* Corresponding author. Address: Groupe de recherche en écologie buccale, Faculté de médecine dentaire, Université Laval, Quebec City, Que., Canada G1K 7P4. Fax: +1 418 656 2861.
E-mail address: Sylvain.Moineau@bcm.ulaval.ca (S. Moineau).

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Plasmids have been reported to be present in only 20–30% of *S. thermophilus* strains (Geis et al., 2003; Girard et al., 1987; Herman and McKay, 1985; Janzen et al., 1992; Somkuti and Steinberg, 1986; Su et al., 2002; Turgeon and Moineau, 2001). However, a recent study showed a much higher prevalence of plasmids (59%) in *S. thermophilus* strains isolated from cultured dairy products made by traditional processes using the natural microbiota of milk (Turgeon et al., 2004). These non-commercial strains might be an interesting reservoir of unique genetic material. Most *S. thermophilus* plasmids are cryptic and almost all employ a rolling-circle (RC) mechanism of replication (Geis et al., 2003; Petrova et al., 2003; Petrova and Gouliamova, 2006; Shareck et al., 2004; Turgeon et al., 2004). *Streptococcus thermophilus* plasmids have recently been classified into six groups based on their replication machinery, four of which use a RC mechanism (Turgeon et al., 2004). One *S. thermophilus* plasmid (pSMQ-308) in the fifth group was experimentally shown to replicate using a theta mechanism, while the mode of replication of the sixth group is currently unknown (Turgeon et al., 2004).

Several cloning vectors have been constructed for the genetic analysis of *S. thermophilus* (reviewed by Shareck et al., 2004). To date, all these molecular tools contain a RC replicon derived from *S. thermophilus* cryptic plasmids. One technical advantage of the RC replicon is its expanded host range, which allows preliminary cloning in an alternative host such as *Escherichia coli*. Replacement hosts are particularly valuable, as *S. thermophilus* is notoriously difficult to transform. However, promiscuity may also lead to undesirable plasmid transfers in foods or the environment. Moreover, RC vectors are often unstable, particularly when they contain a large DNA insert. This instability is presumably linked to the formation of single-stranded DNA during replication (Shareck et al., 2004; Kiewiet et al., 1993). Lastly, RC plasmids have a high degree of incompatibility when two plasmids belonging to the same replication family are present in the same cell (Turgeon et al., 2004). Since genetic studies often require compatible cloning vectors, this is a significant drawback.

Theta-replicating plasmids are more stable than RC plasmids and have a limited host range, at least in some bacterial species such as *Lactococcus lactis* (Ehrlich et al., 1991; Émond et al., 2001; Janniere et al., 1990; Kiewiet et al., 1993), making them good candidates for the construction of cloning vectors. We previously identified the first theta-replicating plasmid in *S. thermophilus*, namely pSMQ-308 (8144 bp) (Turgeon et al., 2004). In a related study, we also showed that this plasmid belonged to a DNA homology group containing two other *S. thermophilus* plasmids, namely pSMQ312b and pSMQ-316 (Turgeon and Moineau, 2001). All three plasmids were found in *S. thermophilus* strains isolated from artisanal cheeses.

The aim of this study was to characterize these two other *S. thermophilus* plasmids, which are presumably highly related to pSMQ-308.

2. Material and methods

2.1. Bacterial strains, plasmids, and media

The bacterial strains and plasmids used in this study are listed in Table 1. The *S. thermophilus* strains were grown at 42 °C in M17 broth (Quelab) supplemented with 0.5% (w/v) lactose (LM17), unless otherwise specified. Plasmid DNA from *S. thermophilus* was isolated according to a previously described method (O'Sullivan and Klaenhammer, 1993) with the following modification: QG buffer (Qiagen Inc.) was added before adding the silica used to clean the DNA. Once the plasmid DNA had been isolated, it was further purified using a continuous CsCl gradient as reported elsewhere (Turgeon et al., 2004).

2.2. DNA sequencing and analyses

The *S. thermophilus* plasmids pSMQ-308, pSMQ-312b, and pSMQ-316 belong to the same DNA homology group (Turgeon et al., 2004). The primers used to sequence pSMQ-308 were also used to start the sequencing of pSMQ-312b and pSMQ-316, with isolated plasmid DNA as a template. Additional primers were designed from the new nucleotide sequence, and the sequencing was completed by primer walking on the two strands of each plasmid. DNA sequencing was performed with an ABI Prism 3700 apparatus at the genomic platform of the Centre Hospitalier de l'Université Laval. DNA was analysed using the GCG Wisconsin Package version 10.3 (Genetics Computer Group, Madison, WI, USA) and the BioEdit sequence alignment editor (Hall, 1999). The open-reading frames (ORFs) were compared with databases using ORF finder and Blast version 2.2.10 (Altschul et al., 1997).

2.3. Pulsed field gel electrophoresis analysis

High molecular weight fragments of *S. thermophilus* chromosomal DNA were prepared for pulsed field gel electrophoresis (PFGE) using the protocol of Le Bourgeois et al. (1989). Genomic DNA trapped in agarose