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Complete genomic sequence of the temperate bacteriophage ΦAT3 isolated from *Lactobacillus casei* ATCC 393

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Abstract

The complete genomic sequence of a temperate bacteriophage ΦAT3 isolated from *Lactobacillus* (*Lb.*) *casei* ATCC 393 is reported. The phage consists of a linear DNA genome of 39,166 bp, an isometric head of 53 nm in diameter, and a flexible, noncontractile tail of approximately 200 nm in length. The number of potential open reading frames on the phage genome is 53. There are 15 unpaired nucleotides at both 5′ ends of the ΦAT3 genome, indicating that the phage uses a *cos*-site for DNA packaging. The ΦAT3 genome was grouped into five distinct functional clusters: DNA packaging, morphogenesis, lysis, lysogenic/lytic switch, and replication. The amino acid sequences at the NH2-termini of some major proteins were determined. An in vivo integration assay for the ΦAT3 integrase (Int) protein in several lactobacilli was conducted by constructing an integration vector including ΦAT3 *int* and the *att*P (int-*att*P) region. It was found that ΦAT3 integrated at the $tRNA^{Arg}$ gene locus of *Lactobacillus rhamnosus* HN 001, similar to that observed in its native host, *Lb. casei* ATCC 393.

Keywords: Genomic sequence; Bacteriophage; *Lactobacillus casei*; Integration vector

Introduction

Lactic acid bacteria (LAB) are Gram-positive bacteria widely used in the industrial fermentations. A serious problem encountered in the fermentation industry when using LAB as starter cultures is attack by bacteriophages. Cultures of LAB are usually prepared as a mixture of several different microorganisms in the fermentation process. This may lead to intergenomic rearrangements and mutual infections from bacteriophages resulting in genetic polymorphisms and DNA dynamics (Josephsen and Neve, 1998). Consequently, dairy bacteriophages usually share close DNA sequence homology in their genetic modules (Brüssow, 2001). Most of the bacteriophages isolated from lactobacilli thus far are classified as members of the Siphoviridae family, phages with long and noncontractile tails (Ackermann and DuBow, 1987). Differences in their DNA packaging mechanisms allow further division into *pac*-site or *cos*-site phages (Le Marrec et al., 1997). Numerous phages isolated from LAB are now grouped into two newly proposed genera, Sfi11-like or Sfi21-like (Lucchini et al., 1999; Brüssow and Desiere, 2001). While Sfi11-like phages use *pac*-sites, Sfi21-like phages use *cos*-sites. Comparing the genome sequences of eight Sfi21-like Siphoviridae that infect five distinct genera of low GC content Gram-positive bacteria reveals the relatedness of the phages with their host bacteria, suggesting that co-evolution has occurred. With 31–61% amino acid (aa) sequence identity over the DNA packaging, head and tail morphogenesis genes, *Lactobacillus gasseri* phage Φadh ranks third in its relatedness to phage Sfi21 of the reference strain *Streptococcus thermophilus* (Brüssow and Desiere, 2001). Phage A2 of *Lactobacillus casei* (Herrero et al., 1994), which shares the same organization of replication and lysogeny modules with those of *St. thermophilus* phage Sfi21, is recognized as an additional member of the group

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(Proux et al., 2002). The two closest relatives of *St. thermophilus* phage Sfi11 are phig1e and LL-H of *Lactobacillus plantarum* and *Lactobacillus delbrueckii* subsp. *lactis*, respectively, which share up to 58% aa sequence identity over the entire DNA packaging, head and tail modules. Sfi11-like and Sfi21-like phages differ by having two rather than one major head proteins, lacking of proteolytic processing of the major head protein, and the presence of a scaffolding protein (Brüssow and Desiere, 2001). However, genomic similarities over the structural genes of both LAB phage groups have been linked to those of the phages of other branch of Gram-positive bacteria and even to some coliphages (Desiere et al., 1998; Brüssow and Desiere, 2001).

In this report, we present the complete nucleotide sequence of a temperate bacteriophage ΦAT3, which was induced from *Lb. casei* ATCC 393 with 0.2 μg/ml mitomycin-C. ΦAT3 has a linear genome of 39,166 bp, an isometric head, and a noncontractile tail of 200 nm in length. Fifty-three open reading frames (ORFs) were identified on the phage genome. We compared the nucleotide sequences of *att*B, *att*L, *att*R, and *att*P regions and identified a 15-bp core sequence; the attachment site *att*B is located in a putative tRNA^{Arg} gene. We compare sequences of the phage major structural proteins, immunity system, integration protein, and the putative origin of replication with those of known phages. The activity of ΦAT3 Int is also assayed using an integration vector that is based on the sequences of ΦAT3 *int*, *att*P region, and a potential *int* promoter (P_{IN}).

Results and discussion

The complete genomic sequence of ΦAT3 has been determined. The functions of most predicted ORFs are unknown although they share sequence homology with comparable ORFs of *Lb. casei* phage A2 within the replication and downstream regions. The morphology of temperate bacteriophage ΦAT3, isolated from *Lb. casei* ATCC 393, is very similar to that of other Sfi21-like phages. ΦAT3 belongs to the Siphoviridae family (Canchaya et al., 2003); the electron micrograph of the phage reveals an isometric head of 53 nm in diameter and a noncontractile tail of approximately 200 nm in length. A tail fiber from the baseplate can also be seen (Fig. 1A). Some extremely long tails, polytails, were also observed (Fig. 1B), these are not

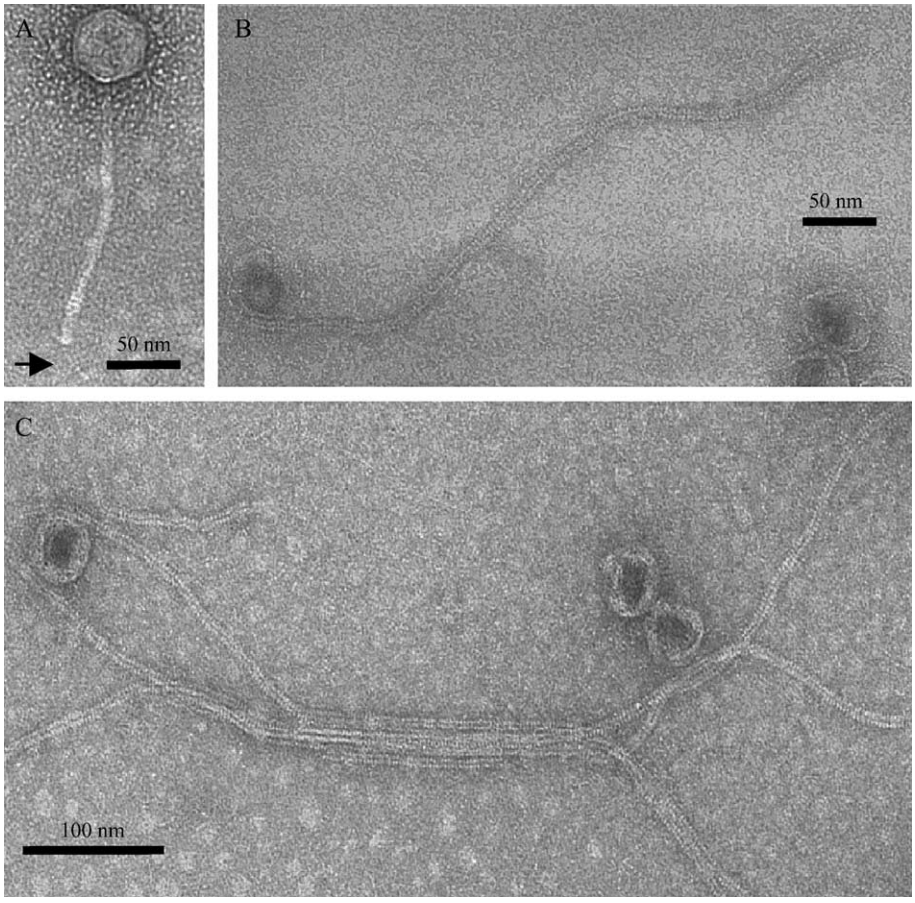

Fig. 1. (A) Electron micrograph of ΦAT3. The phage has an isometric head of 53 nm diameter and a noncontractile tail of approximately 200 nm. A tail fiber extruded from the baseplate is highlighted with an arrow. (B and C) The two electron micrographs, also showing some extra long tails, are associated with empty or defective heads.