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Sequence analysis of the *Lactobacillus plantarum* bacteriophage ΦJL-1☆

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Abstract

The complete genomic sequence of a *Lactobacillus plantarum* virulent phage ΦJL-1 was determined. The phage possesses a linear, double-stranded, DNA genome consisting of 36,677 bp with a G+C content of 39.36%. A total of 52 possible open reading frames (ORFs) were identified. According to N-terminal amino acid sequencing and bioinformatic analyses, proven or putative functions were assigned to 21 ORFs (41%), including 5 structural protein genes. The ΦJL-1 genome shows functionally related genes clustered together in a genome structure composed of modules for DNA replication, DNA packaging, head and tail morphogenesis, and lysis. This type of modular genomic organization was similar to several other phages infecting lactic acid bacteria. The structural gene maps revealed that the order of the head and tail genes is highly conserved among the genomes of several *Siphoviridae* phages, allowing the assignment of probable functions to certain uncharacterized ORFs from phage ΦJL-1 and other *Siphoviridae* phages.

Keywords: Bacteriophage; ΦJL-1; Sequence analysis; Structural proteins; *Lactobacillus plantarum*

1. Introduction

Lactic acid bacteria (LAB) are used as starter cultures in the production of various fermented foods. Bacteriophage (phage) infection of LAB has been a major problem in the dairy industry, causing slow fermentation or complete starter failure and, thus, economic losses. Due to their economical importance, dairy phages (mainly *Lactococcus lactis* or *Streptococcus thermophilus* phages isolated from dairy industry or products) became the most thoroughly studied phage group in the database (Brüssow, 2001). *Lactobacillus* phages received much less attention, probably because they are less of a practical problem in the fermentation industries (Altermann et al., 1999).

Currently, over 20 LAB phage genome sequences are available in databases. Most of these sequences are from lactococcal or streptococcal phages. A few are from *Lactobacillus* phages, including *Lactobacillus plantarum*

Abbreviations: LAB, lactic acid bacteria; MRS, DeMan Rogosa Sharpe; ORF, open reading frame; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; DNA, deoxyribonucleic acid; RBS, ribosome binding site; NTP, nucleotide triphosphate; gp, gene product; pI, isolectric point; aa, amino acid.

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phage ϕgle (Kodaira et al., 1997), *Lactobacillus gasseri* phage ϕadh (Altermann et al., 1999), *Lactobacillus delbrueckii* ssp. *lactis* phage LL-H (Mikkonen et al., 1994), and *Lactobacillus casei* phage A2 (Proux et al., 2002). These available phage sequences have provided significant information on the biology of the individual phages. Many new insights have been revealed from the detailed and comparative analysis of these sequences in areas of phage evolution, genetic diversity, horizontal/vertical gene transfer, module similarity, and lytic/lysogenic cycles (Brøndsted et al., 2001; Brüssow and Desiere, 2001; Desiere et al., 1999; Desiere et al., 2000; Lucchini et al., 1999; Mahanivong et al., 2001). Compared with other organisms, the total number of phage sequences in the database is small. More phage genome sequences from a diverse array of phages and comparative sequence analysis are needed to elaborate upon a sequence-based theory and to improve our understanding of these viruses and their interaction with their hosts.

In the United States, commercial vegetable fermentations are usually natural fermentations without addition of starter cultures (Fleming et al., 1995). With the increasing interest in reducing waste brine disposal, low-salt fermentation is currently being developed. This will require greater control of the non-lactic flora and is likely to involve the use of starter cultures. *L. plantarum* BI7 and its derivative, MU45 (deficient in malolactate fermenting ability), have been evaluated as starter cultures for controlled cucumber fermentations and as biocontrol microorganisms for minimally processed vegetable products in the USDA-ARS Food Fermentation Laboratory. Since vegetable fermentation systems are not sterile, the starter cultures may be susceptible to infection by phages naturally present in these environments. A virulent bacteriophage, ΦJL-1 (active against both *L. plantarum* BI7 and MU45), was recently isolated from a commercial cucumber fermentation. Some of its biological properties were described previously (Lu et al., 2003). The phage has an isometric head, a long non-contractile tail, and belongs to morphotype B1 within the *Siphoviridae* family. Tail fibers were not observed. Phage ΦJL-1 has a linear, double-stranded, DNA genome of 36.7 kb. SDS–PAGE revealed the presence of six structural proteins. Using *L. plantarum* MU45 as a host, the phage ΦJL-1 had an average burst size of 22 and a latent period of 35 min. Little is known about the genetic content, organization, or functions of genes in ΦJL-1. A better understanding of the genetics and biological properties of the *Lactobacillus* phage ΦJL-1 is fundamental to the understanding of phage–host interactions and possibly to the development of phage-control strategies for controlled vegetable fermentations and biocontrol systems using *L. plantarum* BI7 or MU45.

The objectives of this study were to determine and analyze the complete genome sequence of the *L. plantarum* phage ΦJL-1, to identify the structural genes (including the major head and tail protein genes), and to explore the genomic organization of the phage.

2. Materials and methods

2.1. Bacterial strain, phage, and media

L. plantarum MU45 was grown in MRS broth (Difco Laboratories, Detroit, MI) at 30 °C. Phage ΦJL-1 was propagated on *L. plantarum* MU45 in MRS medium supplemented with 10 mM $CaCl_2$ at 30 °C (Lu et al., 2003).

2.2. Purification of ΦJL-1 and isolation of phage DNA

Phage ΦJL-1 particles were concentrated from 1 L of phage lysate by PEG precipitation and then resuspended in 6 mL of 10 mM Tris–HCl buffer (pH 7.4). The phage suspension was intentionally vortexed (Daigger Vortex-Genie 2, A. Daigger and Company, Inc., Vernon Hills, IL) at the highest speed for 2 min in an attempt to generate defected phages. The mixture of intact and defected phage particles was separated and purified by CsCl density gradient centrifugation at 600,000×*g* for 6 h at 15 °C (Sorvall micro-ultra-centrifuge with rotor S100AT6, RC-M150 GX, Sorvall, Newtown, CT). Two visible bands, consisting of intact and defective phages, respectively, were collected separately, and dialyzed against 3 L of 10 mM Tris–HCl buffer (pH 7.4). Phage DNA was isolated as described by Lu et al. (2003).

2.3. Electron microscopy

CsCl-purified phage samples were negatively stained with 2% (w/v) aqueous uranyl acetate (pH 4.0) on a carbon-coated grid and examined by transmission electron microscopy (JEOL JEM-100S, Japan Electronics and Optics Laboratory, Tokyo, Japan) at an accelerating voltage of 80 kV. Electron micrographs were taken at a magnification of 50,000× and printed at 85,000× (V. Knowlton, Center for Electron Microscopy, NC State University, Raleigh, NC).

2.4. Sequence and analysis of ΦJL-1 DNA

DNA sequencing was carried out at the Department of Energy Joint Genome Institute (JGI) sequencing facility (Walnut Creek, CA) and Davis Sequencing (Davis, CA) using shotgun cloning and primer walking sequencing strategies. Sequence annotation was performed using the Global Annotation of Multiplexed On-site Blasted DNA Sequences software package (Altermann and Klaenhammer, 2003). Briefly, five ORFs were manually determined based on several criteria (see Results and discussion). Based on these ORFs, a training model was built using build-icm provided in the glimmer package to identify the remaining