

Genome sequence of *Lactobacillus plantarum* phage P2

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Research Article

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Abstract

Lactobacillus plantarum phage P2 was isolated and purified from failed MRS fermentation broth of *Lactobacillus plantarum* IMAU10120. Its morphology indicates this phage belongs to *Siphoviridae* family. Its genome length was 77,937 bp and the G+C content was 39.28 %, including 96 coding sequences (CDS) and 2 tRNA genes. Through genomic and phylogenetic analysis, it revealed that *Lactobacillus plantarum* phage P2 is a novel phage. The predicted proteins were involved in DNA replication and packaging, virus metabolism and host adsorption or lysis were found.

Main Text

Phages were discovered by Herelle and Twort in the early 20th century [1]. Since Whitehead and Cox first discovered harms of phages to the dairy industry, and in order to eradicate or lessen their negative effects, a plethora of research has been conducted [2]. In 1965, *Lactobacillus* phage was first isolated from sewage and then various lactic acid bacteria phages have been reported[3] including *Lactobacillus plantarum* phage Y1, *Leuconostoc mesenteroides* phage Y4, *Leuconostoc sp.* phage Y5, *Leuconostoc sp.* phage Y10, *Leuconostoc sp.* phage Y11, *Leuconostoc sp.* phage Y12, *Lactobacillus sp.* phage Y20, *Leuconostoc sp.* phage R1, *Leuconostoc sp.* phage R2 and *Leuconostoc sp.* phage R3[4], San Francisco *Lactobacillus* phage EV3[5-6], deputy *Lactobacillus casei* phage Φ T25[7], *Lactococcus lactis* virulent phages CHD, QF9, QF12 and QP4[8], *Bifidobacterium* phage LE1, LE2, LE3, LE4, LE5 and LE6[9] and *Lb.paracasei* phage Φ iLp84 and *Lb.paracasei* phage Φ iLp1308[10]. In addition to their negative effects in fermentation industry, bacteriophages have some roles in the identification and differentiation of bacterial species and strains[11]. Phages population are known to be dynamic and genetically diverse. Their genetic material can be integrated into hosts and then replicate. Some specific conditions and/or treatments, such as UV or chemical reagents can induce genome duplication of phages which can result in host bacteria cell lysis[12]. Also, virulent phages can lyse cells directly when the host is infected[13]. In this study, the complete genomic sequence of *Lactobacillus plantarum* phage P2 is presented and compared with those of other *Lactobacillus plantarum* phages. These data will be useful in extending the genomic characteristics of *Lactobacillus* phages and aiding in the identification and control of phage infections.

Lactobacillus plantarum virulent phage P2 was isolated from failed MRS fermentation broth with *L.plantarum* IMAU10120. The phage propagated in MRS broth of *L.plantarum* IMAU10120 and the viable cell was counted on MRS agar combined with a double-layer plaque technique. This was followed by amplification in MRS nutrient broth and subsequently bacteriophages genomic DNA was isolated. Genomic DNA was purified using a phenol-chloroform-isoamyl alcohol method. Whole-genome sequencing was performed on the Illumina Hiseq4000 platform (the Asbios (Tianjin, China) Technology Co., Ltd). High-quality paired-end reads were assembled using SOAPdenovo v2.04 and the assembly results were optimized and corrected using GapCloser v1.12[14]. Gene prediction of *Lactobacillus plantarum* phage P2 was obtained using genemark 3.25[15]. Comparative analysis of *Lactobacillus*

plantarum phage P2 with other known sequences of phages was performed using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Comparative phylogenetic analysis was conducted using the neighbor-joining method in MEGA 5.2^[16].

The genome length of *Lactobacillus plantarum* phage P2 is 77,937 bp and the G+C content was 39.28%, including 96 coding sequences (CDS) and two tRNA genes (Fig. 1), 36 of which encoded for proteins with known functions, including structural protein, and proteins participated in DNA replication and packaging, virus metabolism and host adsorption or lysis (Fig. 1). The predicted proteins encoded by gene LVP2_g018, LVP2_g020, LVP2_g021, LVP2_g022, LVP2_g023, LVP2_g026 and LVP2_g028 were identified as the putative tail protein (cds 18), head-tail joining protein (cds 20), head-tail adaptor (cds 21), tail protein (cds 22), major tail protein (cds 23), distal tail protein (cds 26) and tail fiber protein (cds 28), respectively. Tail protein is a type of the conduit for genome delivery and tail fiber protein has specific adsorption function^[17]. The major tail protein is an important component of the phages tail tube^[18]. Head-tail joining and head-tail adaptor protein is required for assembling phages heads and tails during the last step of morphogenesis^[19]. The predicted proteins encoded by LVP2_g044, LVP2_g059 were identified as the extracellular transglycosylase (cds 44) and ATP-GTP binding protein (cds 59), respectively.

Transglycosylases act on the peptidoglycan of bacterial cell walls and are employed in the adsorption phase; the phages genome is injected in the host cell and release phages progeny in the late stage of the reproduction cycle^[20]. The predicted proteins encoded by LVP2_g012, LVP2_g014, LVP2_g015, LVP2_g035, LVP2_g048, LVP2_g065, LVP2_g069, LVP2_g072, LVP2_g073, LVP2_g074, LVP2_g083, LVP2_g096 were identified as the terminase small subunit (cds 12), terminase large subunit (cds 14), portal protein (cds 15), recombinase/integrase (cds 35), PemK family transcriptional regulator (cds 48), HNH homing endonuclease (cds 65), deoxynucleoside kinase (cds 69), DNA helicase (cds 72), DNA primase (cds 73), single-stranded-DNA-specific exonuclease (cds 74), putative DNA binding protein (cds 83), thymidine kinase (cds 96), respectively. The predicted proteins encoded by LVP2_g037, LVP2_g058 were identified as the DNA polymerase (cds 37 and cds 58) and the predicted proteins encoded by LVP2_g001, LVP2_g003, LVP2_g011, LVP2_g038, LVP2_g041, LVP2_g045, LVP2_g056 were identified as HNH endonuclease. Endonucleases cleave phosphodiester was embedded in polynucleotide chains such as DNA and RNA and HNH endonuclease could cleave phages DNA at multiple sites^[21]. Terminase, consisting of large subunits and small subunits, is a key enzyme initiating DNA packaging^[22]. These genes are involved in phage infection which include the processes of this phage of adsorption, injection, replication, assembly and release.

Genome sequences from 20 other *Lactobacillus* phages obtained from the NCBI with in FASTA format (GenBank accession no. are shown in Table 1) were compared with that of *Lactobacillus plantarum* phage P2. Nucleotide sequences of all 20 phages (including P2) was aligned by ClustalW^[14] and conducted using the test neighbor-joining method with 1000 bootstrap replicates in MEGA5.2 (Fig. 2). The tree showed that *Lactobacillus plantarum* phage P2 is highly homologous to the *Lactobacillus* phage P1, ATCC 8014-B2, PM411, LdI1 (Fig. 2), which indicated that *Lactobacillus plantarum* phage P2 is a novel phage belonging to the *Siphoviridae* family^[23] with phages ATCC 8014-B2, PM411, LDI1, P1. Besides, two

tRNA, tRNA-Pro and tRNA-Gly showed 100% similarity (100% coverage) in phage P1, the sequence of tRNA-Pro showed 94.6% similarity (100% coverage) with the tRNA-Pro of *Lactobacillus* phage Maenad and Satry (GenBank no. NC_047931.1, NC_047931.1), which did not exist in other phages or bacteria. So the LVP2_g028(tail fiber protein), LVP2_g073(DNA primase), LVP2_g001(HNH endonuclease) might play important roles in deciding the lifestyle of phage P2.

The genome sequence was submitted to the GenBank database and is publicly available with the accession number KY381600.1.

Declarations

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Compliance with ethical standards

The authors have no conflict of interest to declare. This article does not contain any studies with human participants or animals performed by any of the authors.

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Tables

Please see the supplementary files section to view the table.

Figures

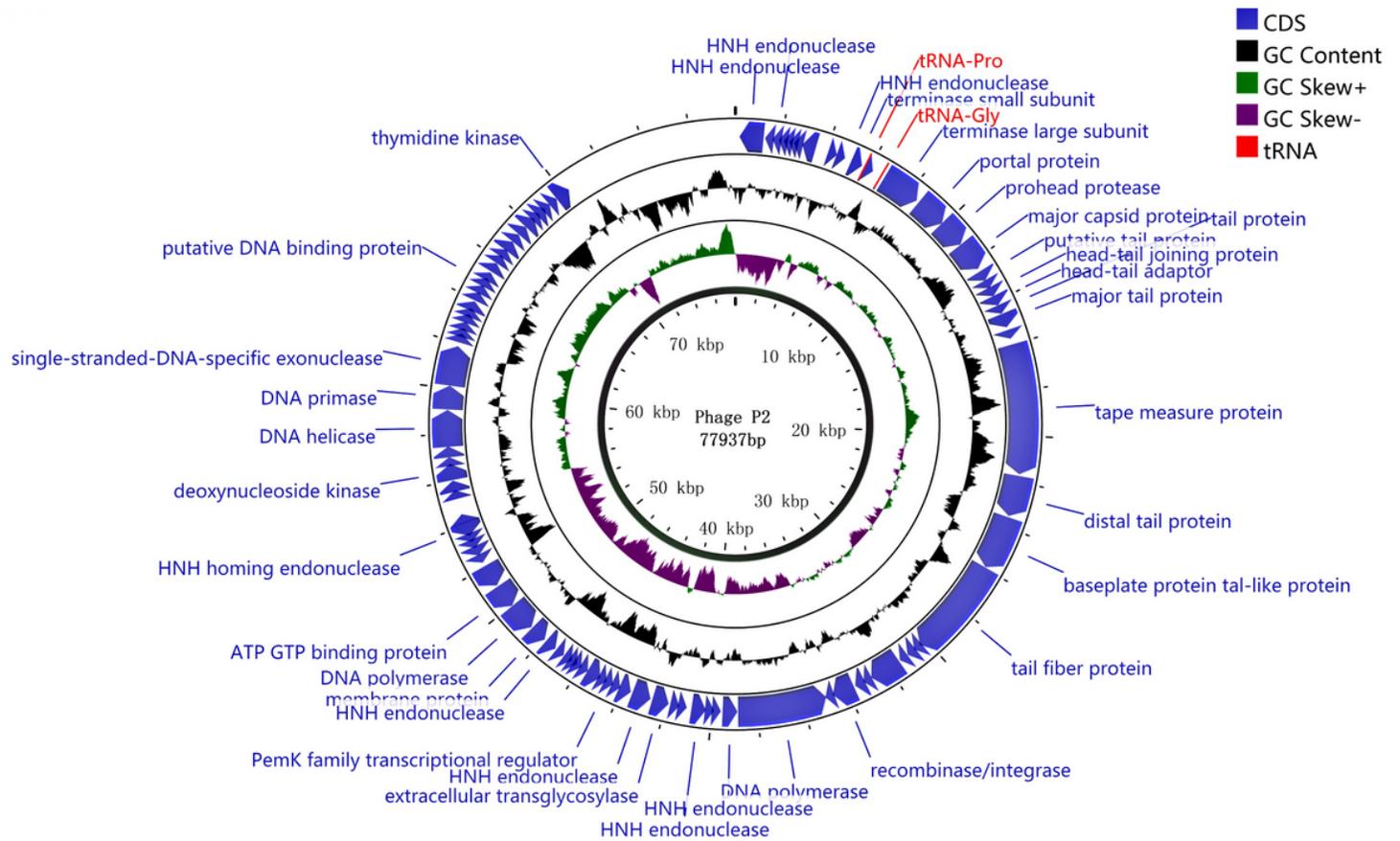


Figure 1

Circular representation of the *Lactobacillus plantarum* phage P2 genome (Note: The innermost circle indicates the GC skew on the positive and negative strand (green and purple). The second circle indicates the GC content (black). The outer circle indicates predicted CDS located on the positive and negative DNA strand (lavender). Red indicates tRNA coding genes)

Tree scale: 1

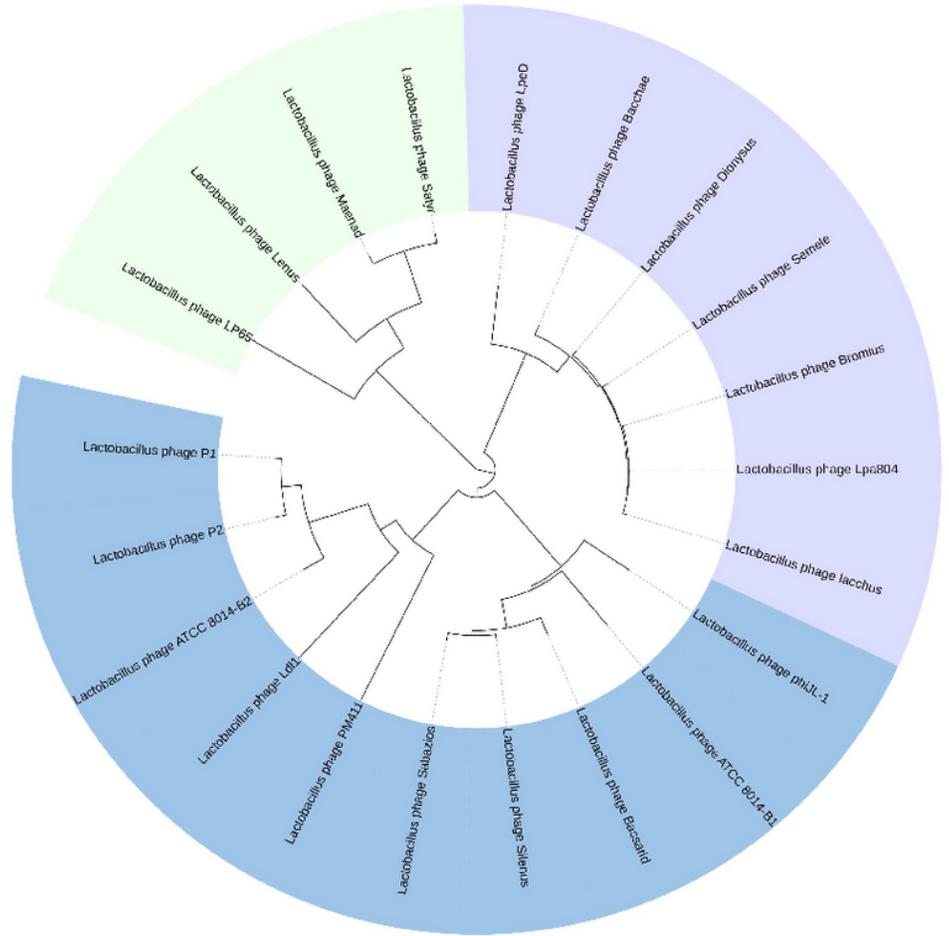


Figure 2

Comparative phylogenetic analysis (Note: Comparative phylogenetic analysis of nucleotide sequences was aligned by ClustalW and performed using the neighbor-joining method in MEGA5.2. Numbers associated with each branch represent bootstrap values)

Supplementary Files

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- [table1.xlsx](#)