Genome Sequence of Lactobacillus Plantarum Phage P2

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Abstract

Lactobacillus plantarum phage P2 was isolated and purified from failed fermentation broth of *Lactobacillus plantarum* IMAU10120. Its morphology showed this phage belongs to *Siphoviridae*. The genome size of this phage was 77,937 bp in length with an average G+C content of 39.28 %, including 96 coding sequences (CDS) and 2 tRNA genes. Genomic and phylogenetic analysis revealed that phage P2 is a novel phage. Its predicted functional proteins including structural protein and protein involved in DNA replication and packaging, virus metabolism and host adsorption or lysis are presented.

Introduction

Since phage was discovered by Herelle and Twort in the early 20th century[1], more and more researches were published. Since Whitehead and Cox first discovered the problems of phage in the dairy industry, a plethora of research has been conducted in order to eradicate or lessen their effects which prove so costly to the industry[2]. In 1965, the first *Lactobacillus* phage was isolated from sewage; various lactic acid bacteria phages have since been reported[3] including *Lactobacillus acidophilus*[4], *Lactobacillus plantarum*[5], San Francisco *Lactobacillus* EV3[6], deputy *Lactobacilluscasei* Φ T25 and CHD[7] , *Bifidobacterium* LE1, LE2, LE3, LE4, LE5 and LE6[8] and Argentina milk coccus Φ iLp84 and Φ iLp1308[9]. In addition to the problems they cause in the food based fermentation industry, bacteriophages have become useful tools for the identification and differentiation of bacterial species and strains[10]. Phage population are known to be both dynamic and large and genetically diverse. Their genetic material can be integrated into hosts and undergo replication. Some specific conditions and/or treatments, such as UV or chemical reagents can induce gene duplication of phages so that host bacteria are lysed and rendered nonviable. Also, virulent phage can lyse cells directly after the host be infected[11,12]. Therefore, the study of phage genome is of great significance in the prevention of phage contamination and is a useful tool in the study of phage resistant bacterial strains. In this study, the complete genomic sequence of phage P2 is presented and compared with other phages of the *Lactobacillus plantarum* group. These data will be useful in extending the genomic characteristics of *Lactobacillus* phages and aid in the identification and control of phage attacks against *L.plantarum*.

Lactobacillus virulent phage P2 was isolated from a slow starter fermentation employing *L. plantarum* IMAU10120. The phage was propagated using *L. plantarum* IMAU10120 in concert with plating on MRS agar using a double-layer plaque technique. This was followed by amplification in MRS nutrient broth; bacteriophage genomic DNA was subsequently isolated. Genomic DNA was purified using a phenol-chloroform-isoamyl alcohol method. Whole-genome sequencing was performed at the Asbios (Tianjin, China) Technology Co., Ltd. on the Illumina Hiseq4000 platform. High-quality paired-end reads were assembled *de novo* using SOAPdenovo v2.04(http://soap.genomics.org.cn/) and the assembly results were optimized and corrected using GapCloser v1.12.Gene prediction of phage P2 was obtained using genemark(http://topaz.gatech.edu/GeneMark/). Comparative analysis of phage P2 nucleotide and amino acid sequences with other known sequences 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[13].

The phage P2 genome is 77,937 bp in length with a G+C content of 39.28% and 96 coding sequences (CDS). Two tRNA genes were identified (Fig. 1), 36 of which encoded for proteins with known functions, including structural protein, and protein involved in DNA replication and packaging, virus metablism 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, head-tail joining protein, head-tail adaptor, tail protein, major tail protein, distal tail protein and tail fiber protein, respectively. Tail protein is a type of the conduit for genome delivery while tail fiber protein has the function of providing specific adsorption[14]; the major tail protein is an important component of the phage tail tube[15]. Head-tail joining and head-tail adaptor protein is required for joining of phage heads and tails during the last step of morphogenesis[16]. The predicted proteins encoded by LVP2_g044, LVP2_g059 were identified as the extracellular transglycosylase and ATP-GTP binding protein, respectively. Transglycosylases act on the peptidoglycan of bacterial cell walls and are employed in the adsorption phase during the phage genome is injected in the host cell or in the late stage of the reproduction cycle to release phage progeny[17]. 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, terminase large subunit, portal protein, recombinase/integrase, PemK family transcriptional regulator, HNH homing endonuclease, deoxynucleoside kinase, DNA helicase, DNA primase, single-stranded-DNA-specific exonuclease, putative DNA binding protein, thymidine kinase. The predicted proteins encoded by LVP2_g037, LVP2_g058 were identified as the DNA polymerase 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 bonds within polynucleotide chains such as DNA and RNA; HNH endonuclease was demonstrated can cleaves phage DNA at multiple sites[18]. Terminase, consisting of large subunits and small subunits, is a key enzyme initiating DNA packaging[19]. These genes are involved in phage infection and accomplish the process of phage of adsorption, injection, replication, assembly and release. 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. Thus, LVP2_g028 (tail fiber protein), LVP2_g073 (DNA primase), LVP2_g001 (HNH endonuclease) might play important roles in deciding the lifestyle of phage P2.

Genome sequences from 19 other *Lactobacillus* group phages were obtained from the NCBI in FASTA format (Genebank accession no. are shown in Table 1). Nucleotide sequences of all 20 phages (including P2) was aligned by ClustalW and conducted using the test neighbor-joining method with 1000 bootstrap replicates in MEGA5.2 (Fig. 2). BLASTn comparison against the NCBI non-redundant nucleotide database revealed that Phage P2 has the closest relative with *Lactobacillus* phage Maenad and *Lactobacillus* phage Satyr, query coverage and identity are (%):77, 93, 80 and 92, respectively. The tree showed that

phage P2 is highly homologous to the *Lactobacillus* phage ATCC 8014-B2, PM411, LdI1 (Fig. 2). These results indicate that phage P2 is a novel phage of the family *Siphoviridae* with phage ATCC 8014-B2, PM411, LDI1.

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

Declarations

Acknowledgements

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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.

References

1. Briggiler M M, Garneau J E, Tremblay D, et al (2012) Characterization of two virulent phages of *Lactobacillus plantarum*. Appl Environ Microb 78(24): 8719-8734. https://doi.org/10.1128/AEM.02565-12
2. De Klerk H C, Coetzee J N, Fourie J T (1965) The fine structure of *Lactobacillus* bacteriophages. J Gen Microbiol 38(1):35-38. https://doi.org/10.1099/00221287-38-1-35
3. Séchaud L, Rousseau M, Fayard B, et al (1992) Comparative study of 35 bacteriophages of *Lactobacillus helveticus*: morphology and host range. Appl Environ Microb 58(8): 1011-1018. https://doi.org/10.1128/AEM.58.3.1011-1018.1992
4. Yoon S S, Barrangou-Poueys R, Breidt F, et al (2002) Isolation and characterization of bacteriophages from fermenting sauerkraut. Appl Environ Microb 68(2): 973-976. https://doi.org/10.1128/AEM.68.2.973-976.2002
5. Foschino R, Venturelli E, Picozzi C (2005) Isolation and characterization of a virulent *Lactobacillus sanfranciscensis* bacteriophage and its impact on microbial population in sourdough. Curr Microbiol 51(6): 413-418. https://doi.org/10.1007/s00284-005-0122-y
6. Mercanti D J, Ackermann H W, Quiberoni A (2015) Characterization of two temperate *Lactobacillus paracasei* bacteriophages: morphology, kinetics and adsorption. Intervirology 58(1): 49-56. https://doi.org/10.1159/000369207
7. SunthornthummasS , Doi K , Rangsiruji A , et al (2016) Isolation and characterization of *Lactobacillus paracasei* LPC and phage ΦT25 from fermented milk. Food Control 73: 1353-1361. https://doi.org/10.1016/j.foodcont.2016.10.052

8. Suárez V, Moineau S, Reinheimer J, et al (2010) Argentinean *Lactococcus lactis* bacteriophages: genetic characterization and adsorption studies. J Appl Microbiol 104(2): 371-379. https://doi.org/10.1111/j.1365-2672.2007.03556.x
9. Mercanti D J, Carminati D, Reinheimer J A, et al (2011) Widely distributed lysogeny in probiotic *Lactobacilli* represents a potentially high risk for the fermentative dairy industry. Int J Food Microbiol 144(3): 503-510. https://doi.org/10.1016/j.ijfoodmicro.2010.11.009
10. Gillis A, Mahillon J (2014) Phages preying on *Bacillus anthracis*, *Bacillus cereus*, and *Bacillusthuringiensis*: past, present and future. Viruses 6(7): 2623-2672. https://doi.org/10.3390/v6072623
11. Ofir G, Sorek R (2018) Contemporary phage biology: from classic models to new insights. Cell 172(6): 1260-1270. https://doi.org/10.1016/j.cell.2017.10.045
12. Hurwitz B L, Ponsero A, Thornton J, et al (2018) Phage hunters: Computational strategies for finding phages in large-scale 'omics datasets. Virus Res 244: 110-115. https://doi.org/10.1016/j.virusres.2017.10.019
13. Tamura K, Peterson D, Peterson N, et al (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28(10): 2731–2739. https://doi.org/10.1093/molbev/msr121
14. Yoichi M , Abe M , Miyanaga K , et al (2005) Alteration of tail fiber protein gp38 enables T2 phage to infect *Escherichia coli* O157:H7. J Biotechnol 115(1): 101-107. https://doi.org/10.1016/j.jbiotec.2004.08.003
15. Lisa G P, Voula K, Logan W D, et al (2009) The phage A major tail protein structure reveals a common evolution for long-tailed phages and the type VI bacterial secretion system. P Natl Acad Sci USA 106(11): 4160-4165. https://doi.org/10.1073/pnas.0900044106
16. Maxwell K L, Yee A A, Arrowsmith C H, et al (2002) The solution structure of the bacteriophage lambda head-tail joining protein, gpFII. J Mol Biol 318(5): 1395-1404. https://doi.org/10.1016/S0022-2836(02)00276-0
17. Fokine A, Miroshnikov K A, Shneider M M, et al (2008) Structure of the bacteriophage φKZ lytic transglycosylase gp144. J Biol Chem 283(11): 7242-7250. https://doi.org/10.1074/jbc.M709398200
18. Fan XT, Jiang YL, Li K, et al (2018) F-CphI represents a new homing endonuclease family using the Endo VII catalytic motif. Mobile DNA-UK 9(1): 1-11. https://doi.org/10.1186/s13100-018-0132-5
19. Yang L, Yang Q, Wang M, et al (2019) Terminase large subunit provides a new drug target for herpesvirus treatment. Viruses 11(3): 219. https://doi.org/10.3390/v11030219

Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

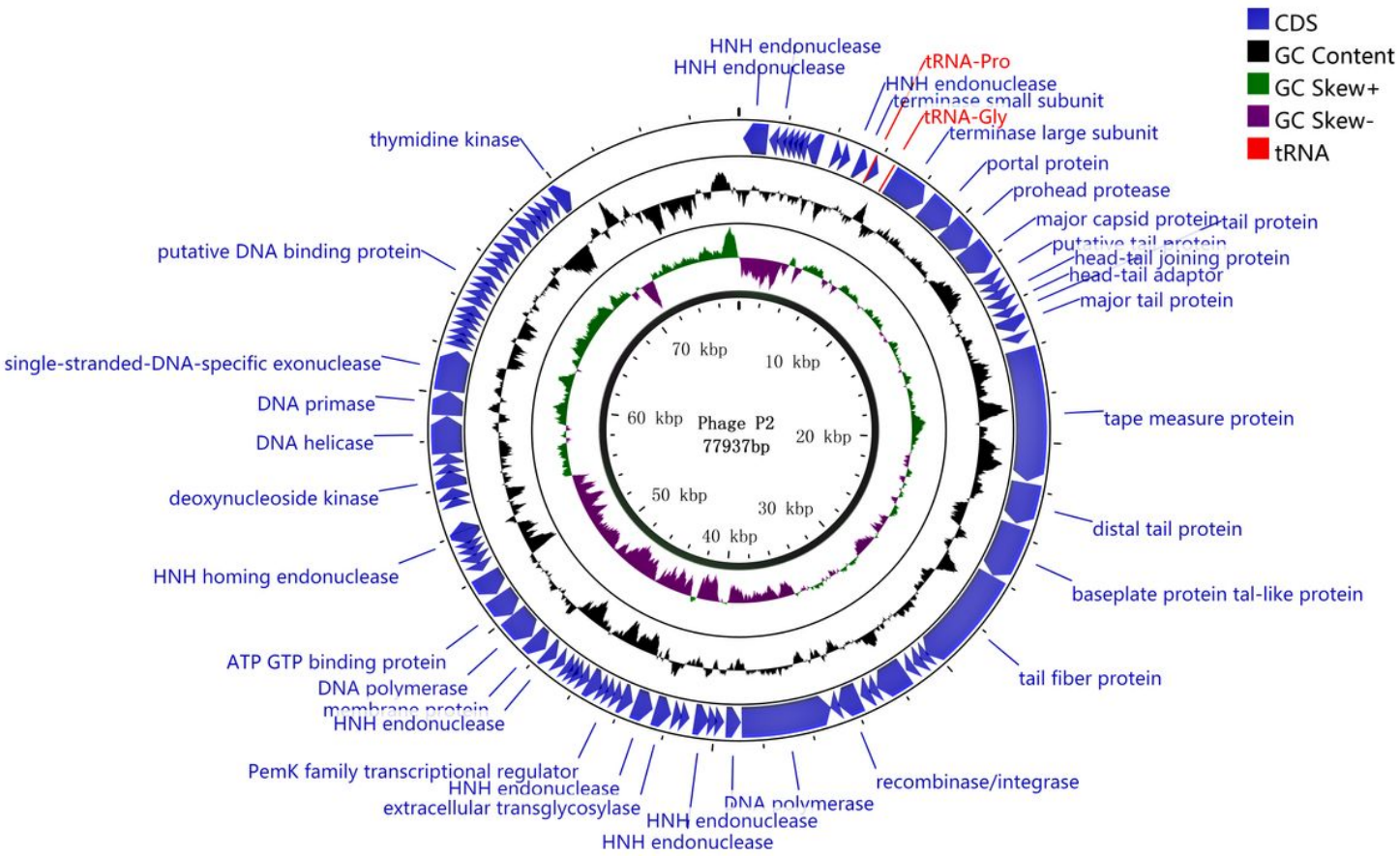

Figure 1

Circular representation of the 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)

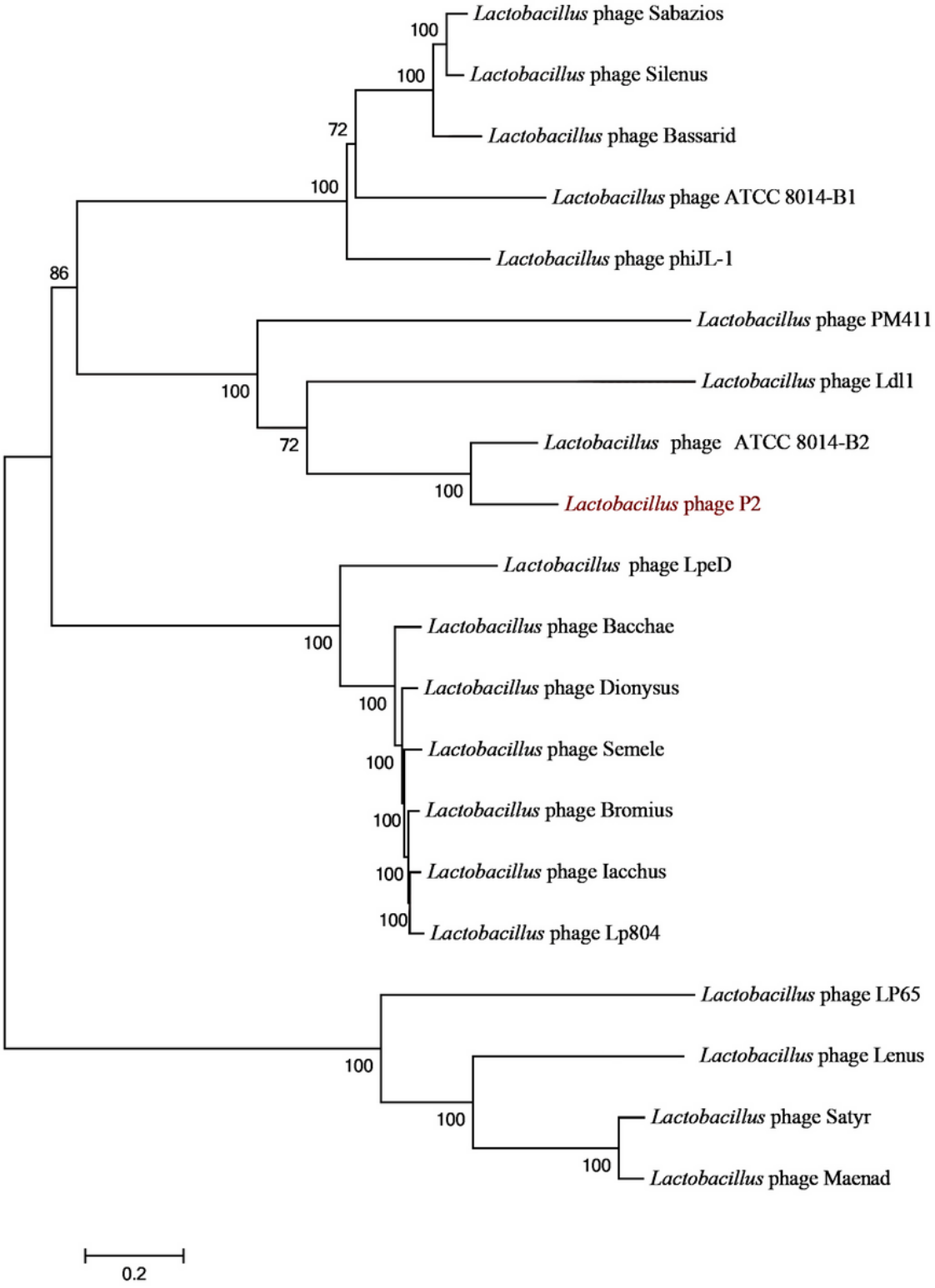

Figure 2

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

This is a list of supplementary files associated with this preprint. Click to download.

- table1.xlsx