Repeated sequences and the sites of genome rearrangements in bacteriophages of *Lactobacillus delbrueckii* subsp. *lactis*

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**Summary.** We have sequenced the KIS-element, a 1.5 kb insertion segment present in the genome of *Lactobacillus delbrueckii* subsp. *lactis* phage LL-K, but absent from its close relative, phage LL-H. The KIS-element showed some sequence features of a transposable element: it was flanked by direct repeats of a 20 nt long sequence which was in the genome of LL-H as a target sequence. The KIS-element contained two putative ORFs. The C-terminal part of ORF333 consisted of clusters of direct repeats, capable of coding Lys/Arg-Gly-Asp motifs, which are known to be able to bind to glycoproteins. A homologous counterpart of the KIS-element was also found in the genome of prolate-headed *L. delbrueckii* subsp. *lactis* phage JCL1032, even though the phage JCL1032 is not a close relative of phage LL-K. The nucleotide sequence comparison between KIS-element and its homologous counterpart in JCL1032 showed that there have occurred several genome rearrangements at the repeat clusters.

**Introduction**

Lactic acid bacteria are subject of increasing research interest because of their importance as industrial and probiotic microorganisms [1]. In recent years also bacteriophage-host interactions have been the subject of intense scrutiny, most notable in lactococci, but increasingly also in *Streptococcus, Lactobacillus* and *Leuconostoc* species [2]. A large number of new phages have been isolated. In many of the cases they are characterized by only morphology and host range, but an increasing number of them have been classified also into phage families on the basis of genetic studies [3, 4]. Because of the large number of characterized phages that are related to each other to various degrees, the phages of lactic acid bacteria offer good tools to analyze virus evolution.

It has been shown that modular evolution is important for the evolution of prokaryotes. The modular evolution of bacteriophages is based on families of interchangeable genetic elements (modules) each of which carries out a particular biological function [5]. The exchange of the modules occurs by
recombination among a population of different bacteriophages. The evolution of lambdoid-phages is a good example of modular evolution [6]. But almost nothing is known about possible modular evolution in Lactobacillus phages.

In the present study we used Lactobacillus delbrueckii subsp. lactis phages LL-H and LL-K, which are almost identical when compared by Southern hybridization, heteroduplex analysis or restriction mapping of their DNAs [7]. There is however a size difference between the genomes of these phages [7]: in LL-K there is an insertion (or deletion in LL-H), about 1.5 kb in size, and accordingly we call it here KIS-element (LL-K Insertion Sequence).

Phage JCL1032, also used in this study, is a prolate-headed phage [8]. Surprisingly, it has short DNA regions (about 1–4 kb in size) homologous with isometric-headed phages. This was the first report on the DNA homology between isometric- and prolate-headed phages of lactic acid bacteria. Interestingly, one of those homologous regions is homologous with the KIS-element [8].

In this study we have characterized the KIS-element, and its homologous counterpart in phage JCL1032 at the level of nucleotide sequences.

**Materials and methods**

**Phages and bacteria**

*Lactobacillus delbrueckii* subsp. *lactis* phages LL-H and LL-K were isolated from cheese processing plants in Finland, and were obtained from Valio, Cooperative Dairies' Association in Helsinki, Finland (for details see [7]). Phage JCL1032 was isolated in Switzerland, and was kindly provided by Dr. Jimeno Federal Dairy Research Institute, Bern Switzerland [8]. The indicator strain of LL-H and LL-K phages, *L. delbrueckii* subsp. *lactis* LKT, and the indicator strain of JCL1032, *L. delbrueckii* subsp. *lactis* ATCC 15808, originate from the French Collection of Lactic Acid Bacteria (INRA, Jouys-en-Josas, France). The *L. delbrueckii* subsp. *lactis* strains LL23 and LL78, used in Southern hybridization experiment of this study, were obtained from Valio. All lactobacilli strains were grown in MRS broth (Difco, Detroit, U.S.A.) at 37 °C. For phage propagation MRS broth was supplemented with 20 mM CaCl₂.

**Phage DNA isolation**

For phage DNA isolation, phage lysates (30 ml) were centrifuged at 10 000 × g for 15 min to remove the cell debris. Phages were pelleted from the lysate by centrifugation at 100 000 × g for 2 h 30 min. The phage pellet was suspended into 500 µl of 10 mM Tris-HCl pH 8.0, 10 mM MgCl₂. Phage proteins were removed by extracting with equal volume of phenol-chloroform-isooamylalcohol (25:24:1), and DNA was precipitated from the aqueous phase with two volumes ethanol.

**Isolation of total Lactobacillus DNA**

Lactobacilli were grown overnight in MRS-broth. The cells were pelleted by centrifugation at 10 000 × g for 10 min. The cells were washed two times with 10 mM Tris-HCl pH 7.0, and resuspended in 10 mM Tris-HCl pH 7.0, 12% polyethyleneglycol (PEG 6000), lysozyme 10 mg/ml. The suspension was incubated at 37 °C for 30 min. Spheroplasts were collected.