

## COMPARATIVE STUDY OF ERWINIOPHAGES 49 AND 59 BASED ON RESTRICTION ANALYSIS OF THEIR GENOMES

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Bacteriophages as vectors for obtaining recombinant DNA molecules have been widely used in genetic engineering and biotechnology. Also, it was found possible to use separate virion components for protection, delivery and introduction of medicines. Discovery of these application opportunities requires reconsideration of well known phages and isolation and investigation of new viruses of bacteria.

In 1984 F. Tovkach et. al found two closely related temperate phages 59 and 49 in *Pectobacterium carotovorum* 288. Physical and genetic organization of phage 59 was actively investigated, while phage 49 was described only partially.

The lysate of the strain *Erwinia horticola* 450 was centrifuged in order to obtain concentrated suspension of phage particles. Virion purification was performed by using step gradient and isopycnic centrifugation with CsCl. Genomic DNA was obtained as described by Maniatis et. al (1984). Restriction analysis was arranged according to Tovkach et. al (1988) with the next endonucleases: ClaI, DraI, EcoRI, HpaI, KpnI, PvuI, PvuII, Sall ta XhoI.

The genome size of the phage 49 DNA determined by summing up DraI, EcoRI, PvuI, PvuII and HpaI restricts was 61,0; 47,7; 44,0; 39,0 and 50,5 kbs respectively. The average size of phage DNA is 48,4 kbs. The standard error calculation is 12,4%, which is too large for restriction analysis.

Endonucleases Sall and EcoRI helped to define the exact size of the phage 49 DNA. The average size obtained based on restriction with Sall was 47,8 kbs. The molecular mass determined by summing up 11 EcoRI-fragments was 47,7 kbs. In this way the genome size of phages 49 and 59 (47.7 kb) can be considered identical within the experimental error.

The highly active endonucleases made it possible to reveal the restriction fragment patterns of phage 49 DNA, which is typical for permuted genomes. In HpaI, DraI ta XhoI restriction patterns some submolar fragments were detected. The HpaI-cleaved mixture contained submolar fragment with size about 5 kbs. Similar particle was identified in the Sall-cleaved mixture of phage 59, which has permuted genome. In addition, a submolar fragment was found in the XhoI-cleaved mixture of phage 49 DNA. We were unable to detect heterogeneous in size terminal fragments similar to the ones in the phage 59 DNA, which describes accidentally permuted genomes. In some case phage 49 has discrete terminal fragments leading to the overvalued size of phage DNA in comparison with its real one.

Obtained data allow to conclude that genome of phage 49 is cyclically permuted as the genome of phage 59 is. However, this permutation has discrete nature.