

Abstract

The KPP2, KPP3, KPP4, KPP5, KPP6, KPP7, KPP10, KPP11, KPP30, KPP42, KPP50 and KPP95 are twelve lytic bacteriophages. They were isolated from supernatant of hospital wastewater. These phages could lyse some of ESBLs (extended-spectrum -lactamases) strains of *Klebsiella pneumoniae* (e.g. K6 or K55) and *K. pneumoniae* 10693. Under characteristic studies, they all were found to have similar basic properties such as titer (about 10^9 PFU/ml), restriction-cutting pattern of *EcoRV*, genome sizes (about 170-180 kb) and morphology of some these phages. We conclude that they are the same type of phages. KPP95 was randomized chosen for further characteristic studies and high titer of the phage (approximately 3×10^{10} PFU/ml) could be obtained. It could rapidly and violently lyse its host cell, *K. pneumoniae* 10693. Under electron microscope, it had morphology of a moderate elongated icosahedral head connected to a tail-sheathed tail and was terminated by a base plate. The genome size of KPP95 was estimated to be 170-180 kb. The DNA might have been modified on base G or C and have been glucosylated. The chemical composition of DNA of KPP95 possessed in a low G+C content situation. These properties are similar to T-type phages. Interestingly, KPP95 could also infect many ESBLs strains of *K. pneumoniae*, one cefotaxime-resisted *Klebsiella oxytoca* and one ESBLs strain of *Enterobacter agglomerans*. This suggests that it is a broad host range phage. The comparison of each KPP95 DNA fragments cloned from its genome also showed that KPP95 was much more similar to T-even or pseudo-T-even phages. SDS-polyacrylamide gel electrophoresis of the phage particles showed about 25 proteins. They included one major coat protein of 46 kDa. The N-terminal sequence of the major coat protein of KPP95 and PCR product sequence of its gene 23 were presented similarity to major coat protein (gp23) encoded by gene 23 of T-even or pseudo-T-even phages. Moreover, the alignments of gene 18, 19 and 23 of KPP95 revealed having similar modular or mosaic design with the structural genes of other T4-type phages. By the phylogenetic tree of the sequences of gene 23 of KPP95 and other 17 phages of T4-type, KPP95 should be belong to pseudo-T-even phages and much closer to phage 42.