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Bioinformatics analysis of the endolysins and holin-like membrane proteins in Cluster EB phages

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Thirteen novel bacteriphages isolated from *Microbacterium foliorum* were isolated by students at La Sierra University. All thirteen phages had clear, medium size plaques indicative of lytic phages. Two phages, FinalFrontier and Slay, were chosen for further investigation. FinalFrontier and Slay have a siphoviridae morphotype based on electron microscopy analysis and both were assigned to Cluster EB after their genomes were sequenced and annotated using bioinformatic tools such as PECAAN, Phamerator, NCBI Blastp, and HHPred.

A bioinformatics approach was used to identify the proteins encoded by the genes within the lysis cassettes in the 56 phages that are part of Cluster EB, including FinalFrontier and Slay. Lysis cassettes are normally composed of one or two endolysins and one or several proteins with 1-4 transmembrane helices (TM) that might function as holins. Our analysis shows that there are 4 different endolysin phams encoded in Cluster EB and 8 different holin-like proteins. There were seven different genome organizations, each included an endolysin and either two or three holin-like proteins. Endolysin pham 73597 is present in 28 phages and it’s found in two different genome arrangements. In the first it’s followed by three holin-like membrane proteins present only in EB phages while in the second one it was followed by two holin-like proteins present only in EB phages in a third found in EB phages and in *Streptomyces* BD1-6 phages. In each case, the three holin-like proteins had four, one, and two transmembrane helices, respectively. There were three different genome organizations that included endolysin pham 4297. The endolysin, found in 17 phages, was followed by proteins with four, one, and two transmembrane helices, respectively. In the first genome arrangement, all three holin-like membrane proteins are found only in EB phages. In the second arrangement the first two proteins are exclusive to cluster EB and the protein with two transmembrane helices is not only found in Cluster EB phages but also in phages isolated from *Arthrobacter*, *Gordonia*, and *Streptomyces* species. In the third arrangement the single transmembrane protein is also found in phages from cluster EK1 and EM. Endolysin pham 62106 is present in 10 phages and followed by two holin-like membrane proteins with one, and two transmembrane helices, respectively. Finally, endolysin pham 18774 is found in only one phage, ChiliPepper, and it’s followed by proteins with four, one, and two transmembrane helices. These results show a wide organization and diversity of genes within the lysis cassette of phages and highlight that any prediction of holin or antiholin function will require future wet lab work.