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Comparative Analysis of the Reliability of Six Bioinformatics Software for Topological Prediction of Bacteriophage Membrane Proteins

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Membrane proteins (MPs) play crucial roles in cellular functions such as energy generation, transmembrane transport, cell signaling, and bacteriophage (phage) infectivity and replication. Thus, it is important to identify MPs in phage genome annotation because deciphering the relationships between their sequences and functions could increase our knowledge of phage biology and the utility of bacteriophages in therapy, bioremediation, wastewater, and food industry biocontrol. Previously, the SEA-PHAGES program recommended using TMHMM and/or SOSUI software, but inconsistencies in results have been observed and TMHMM is now obsolete. Our aim was to investigate the reliability and efficiency of DeepTMHMM, TOPCONS, CCTOP, SOSUI, Philius, and Phobius software in detecting MPs in subcluster M1 phages. All hypothetical proteins, endolysins, and holins were analyzed for the presence of putative transmembrane domains (TMs). A comparative analysis of the number of signal peptides (SPs) and TMs detected by each software in endolysins, holins and hypothetical proteins was done. Efficiency was determined by recording the average time each software took to run. Additionally, the lengths of the analyzed amino acid sequences were recorded to determine whether there could be a manual method of saving time by ruling out the likelihood of having a membrane protein in a sequence based on sequence size. Preliminary data show that, in terms of reliability, with DeepTMHMM as the reference gold standard, CCTOP comes in ahead of the rest with (97%) of calls matching DeepTMHMM. Topcons and Philius tied at (90%), followed by Phobius (87%), and SOSUI (84%). However, SOSUI did not specifically identify SPs. Each of the software took varying lengths of time to detect the presence of transmembrane domains with SOSUI having the shortest average time of (1.06 sec), Philius (2.4 sec), Phobius (12.1 sec), CCTOP (23.1sec), Deep TMHMM (47.0 sec) and Topcons with the longest average time of (173.0 sec). Apart from holins which were larger, genes with TMs generally fell within the size range of 57-62 amino acids. All software (except Philius) detected the holin with at least one transmembrane domain, suggesting the presence of a class III holin in the analyzed subcluster M1 phages. This study describes the application of the collected data to elucidate the reliability and efficiency of the above six bioinformatics software for detecting MPs and discusses some potential applications for phage membrane proteins.