A Study of Cas9 Protein of Mollicutes Bacteria on the Example of M.gallisepticum

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In this work, I studied cas9 protein, one of the proteins of the CRISPR-Cas bacterial defense system. It was chosen because a genome editing system based on it is currently under development. To obtain the most effective tool, we need to understand which types of cas9 protein are found in bacteria. So the smallest bacteria called Mollicutes were selected for this study. Their systems are as elementary as possible. Moreover, the CRISPR-Cas system is particularly spread among Mollicutes, which makes them an interesting object to work with. First, I reviewed the existing literature regarding cas9 protein. Then, to analyze the structure and function of cas9 in M.gallisepticum, I found two template proteins in the SWISS-Model program and modeled four conformations of protein based on them. I built visualizations of the structures in the ChimeraX program and then compared them with their template proteins. I tracked the dynamics of cas9 domains of M. gallisepticum, calculated conservatism for individual protein domains, and drew the domain structure. The results regarding the dynamics and conservatism of the domains largely correlate with the existing research on bacteria in total. However, I found that C-terminal domains were absent in Mollicutes cas9 proteins, despite the fact that they are found in most bacteria. The function of these domains is PAM binding. PAM is a short sequence present in viruses and absent in bacteria; its binding is necessary for bacteria to defend itself. The absence of this structure simplifies the work of the entire protein. Based on these results, I suggest that using M. gallisepticum cas9 for genome editing may be more convenient than the currently used constructions based on other proteins, but this requires testing in the laboratory.