**Uncovering the Role of an Enzymatically Active Flagellin in Surface Motility and Biofilm Regulation**

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The bacterial flagellum, traditionally recognized for its role in bacterial swimming, has recently unveiled a novel enzymatic function through flagellinolysin, a metallopeptidase-containing flagellin. This enzymatic flagellin, identified in 74 bacterial species, has sparked interest in the flagellum’s biological roles beyond swimming motility. This exploratory study aims to investigate the biological significance of flagellinolysin, particularly its involvement in surface motility, adhesion and biofilm development, using Pseudoalteromonas tunicata as a model organism. Simultaneously, this study examines a putative flagellinolysin substrate within P. tunicata’s proteome, a protein deemed “VCBS,” which contains calcium-dependent adhesive domains. To explore differences in biological function, knockout and catalytic site point mutants of flagellinolysin, and knockout mutants of VCBS were constructed. These mutants, along with the wildtype strain, were cultivated on agar plates to assess differences in surface motility and grown in micro-centrifuge tubes to quantify biofilm development via a colorimetric assay. Findings from the surface motility and biofilm assays suggest antagonistic activities between VCBS and flagellinolysin in which the former promotes surface adhesion while the latter promotes surface motility and regulates biofilm-related adhesion. These results contribute new insights to the collective understanding of the role of flagella in surface motility and biofilm regulation.