Expression and the Functional Study of Fusion Proteins -Amylase and Hemolysin- as an Application in Biofilm Polysaccharide Degradation

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Biofilm is an aggregate of consortium bacteria that adhere to each other on a surface. It is usually protected by the exopolysaccharide layer. Various invasive medical procedures, such as catheterization, endotracheal tube installation, and contact lens utilization, are vulnerable to biofilm infection. The National Institute of Health (NIH) estimates 65% of all microbial infections are caused by biofilm. Periplasmic -amylase (MalS) is an enzyme that hydrolyzes -1, 4- glicosidic bond in glycogen, starch, and others related polysaccharides in periplasmic space. Another protein called hemolysin- (HlyA) is a secretion signal protein on C terminal of particular peptide in gram negative bacteria. We proposed a novel recombinant plasmid expressing -amylase and hemolysin- fusion in pSB1C3 which is cloned into E.coli to enable -amylase excretion to extracellular for degrading biofilm polysaccharides content, as in starch agar. Microtiter assay was performed to analyze the reduction percentage of biofilm by adding recombinant E.coli into media. This system is more effective in degrading biofilm from gram positive bacteria i.e.: Bacillus substilis (30.21%) and Staphylococcus aureus (24.20%), and less effective degrading biofilm of gram negative i.e.: Vibrio cholera (5.30%), Pseudomonas aeruginosa (8.50%), Klebsiella pneumonia (6.75%) and E. coli (-0.6%). Gram positive bacteria have a thick layer of peptidoglycan, causing the enzyme to work more effectively in degrading polysaccharides.