

Analysis of Novel Multi-Enzyme Formulation's Synergistic Implications for the Enhanced Interventional Disruption of *E. coli* Biofilms

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Biofilms are intricate and stratified bacterial communities anchored on various surfaces, such as medical devices, surgical equipment, teeth plaque, etc. Contemporary treatment methods - antibiotics and sterilization - are ineffective against the rigid matrix structure consisting of various exopolymers. Thus, the microorganisms thrive and contribute to nosocomial infections, SSIs, and loss of life. This research focuses on utilizing enzymes to hydrolyze the exopolymers and disrupt the biofilm structure. This project is a continuation of a previous project validating the potential of enzymes to degrade biofilm. Now, a novel multi-enzyme formulation containing proteinase K and DNase I aimed at adhesion proteins and eDNA, respectively, is applied to explore increased potency than individual applications. A 24-well microtiter plate biofilm assay was performed and repeated three times with the enzymes applied individually and synergistically. The plate was split into six columns: blank, negative control, proteinase K, DNase I, Synergy 1, and Synergy 2 with twice the concentrations. Each assay was performed over three days with *E. coli* inoculation, enzyme application, and crystal violet staining. The CV-stained plates were read under OD540-590 for biofilm measure. The results were analyzed through the percent difference from the negative control, and the synergistic combinations showed increased disruption of the biofilms than the individual applications ($p < 0.05$). This proved that multi-enzyme formulations procure more significant disruption of biofilms, implicating their use to develop practical applications to eradicate biofilms. A one-way ANOVA and post-hoc Tukey HSD were the statistical tests utilized to affirm the differences and significance of the results.