

# Identifying the Role of Alternative Ribosomes in Inducing Biofilm Formation

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Bacterial biofilms are complex and structured surface-attached bacterial communities held by a matrix of extracellular polymeric substances. Biofilms pose a significant threat to healthcare as bacteria in biofilm state are 1000x more resistant than planktonic bacteria as antibiotics cannot penetrate the biofilm, accounting for over 80% of infection related deaths due to antibiotic resistance. Alternative ribosomal proteins (AltRPs) synthesize alternative ribosomes that are suggested to translate unknown sets of proteins at differential efficiency when compared to their canonical counterparts. Two strains of *Bacillus subtilis* were used in this study: Wild Type (WT) and Deletion Mutant ( $\Delta$ ). The strains were studied with the condition of zinc (Zn) availability as AltRPs are known to be expressed in low zinc conditions, which was ensured by the supplement of the zinc chelator, TPEN. WT + Zn, WT + TPEN,  $\Delta$  + Zn, and  $\Delta$  + TPEN conditions were tested. Crystal violet assays, plate culturing, and a statistical proteomic analysis were conducted to identify the role of alternative ribosomes in biofilm formation. Through the crystal violet assay, WT + Zn had the greatest biofilm formation at 1.03 AU (arbitrary units) with the least for  $\Delta$  + TPEN at 0.41 AU, but similar to WT + TPEN at 0.44 AU. It was determined by observing the Luria Broth (LB) and Low Zinc Minimal Media (LZMM) agar plate cultures that there is an active response to zinc starvation with demonstrated decreased motility, which correlates to *Bacillus subtilis*' transition to biofilm state. Moreover, this study suggests that alternative ribosomes may translate stress-response genes and genes relating to biofilm structure, but there is a need for more research in regards to the relationship between AltRPs and biofilms.