

Microbial Physiology, a mandatory course for Biotechnology undergraduates, traditionally includes six lab practices over two months. Due to the pandemic, a revised approach using *Pseudomonas aeruginosa* PAO1 was developed to efficiently cover key concepts within a shorter timeframe. This approach uses *P. aeruginosa* PAO1, a Gram-negative opportunistic pathogen, whose virulence factors are regulated by its Quorum Sensing (QS) system, involving autoinducers like N-acyl homoserine lactones (AHLs). Three interconnected QS systems (LasI-LasR, RhlI-RhlR, and PQS) regulate gene expression through autoinduction loops. Quorum Quenching (QQ), the interference with QS systems, is demonstrated using a *aiiA* gene (encoding an AHL-degrading enzyme) expressed in *P. aeruginosa* PAO1. The reduction in AHL levels is observed using biosensor strains: *Chromobacterium subtsugae* CV026 (detects C4-C8 AHLs, producing violacein) and *P. putida* F117 (pKR-C12) (detects C10-C14 AHLs, producing green fluorescent protein). These experiments, detailed in Figure 1, integrate bacterial conjugation, bioactive molecules, signaling/regulation, and macromolecule degradation, offering a comprehensive and interconnected learning experience within the limited lab time.