

Title: Persuading of virulence factors production in *Pseudomonas aeruginosa* by artificial food coloring azo dyes

Author: Trinankur Mitra, Thulasiram Subramanian, Abinaya Kanakaraja, Allwyn Vyas Gopalakrishnan, Subash C.B. Gopinath, Pachaiappan Raman

Abstract: Azo dyes are added as coloring agents in food, beverage, cosmetics, and pharmaceutical products owing to their economic viability, aesthetic appeal, and ability to maintain color integrity. Opportunistic bacteria of Gram-negative, *Pseudomonas aeruginosa* employ N-acyl-homoserine lactone (AHL) communication to coordinate their collective behaviors, enhancing their ability to invade and subsequently establish dominance over higher host species. The regulation of pathogenic activities by quorum sensing (QS) mechanism is a crucial determinant of the virulence exhibited by *P. aeruginosa*. This study investigates the potential anti-QS effects of commercially available food coloring azo dyes: malachite green, methyl orange, tartrazine, and sunset yellow FCF against *P. aeruginosa* at determined MIC and sub-MIC concentrations. The research findings revealed that the selected azo dyes inhibited the QS mechanism of *P. aeruginosa*, diminishing its production like biofilm, EPS, pyocyanin, pyoverdine, rhamnolipid, protease, and elastase. The gene expressed for these activities is quantified by RT-qPCR, which demonstrated a substantial downregulation of QS genes, specifically the malachite green and methyl orange inhibited in the higher percentages. The impact of malachite green on the QS system gene expression suggests its potential importance as a QS inhibitor. Though adding synthetic dyes to foods and other products receives more impact, it proves to have potential anti-QS activity. Thus, this research paves the way for the effective consideration of the selected azo dyes for the anti-quorum sensing candidates against *P. aeruginosa*.

Keywords: Azo dyes, Quorum sensing (QS) inhibition, *Pseudomonas aeruginosa*, N-acyl-homoserine lactone (AHL), Anti-QS activity, Biofilm inhibition

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