National Institute of Technology Rourkela

Synopsis Seminar

Seminar Title	: Regulation of phenotypic heterogeneity and pleomorphic adaptation in bacteria through RelA-mediated stringent response during biofilm development
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Venue	: Life Science Seminar Room
Date and Time	: 21 Jul 2025 (10.00 AM)

Abstract : Bacteria predominantly inhabit spatially structured communities known as biofilms, where metabolic activities create physiologically distinct microenvironments. In natural ecosystems, bacterial pleomorphism variation in cell shape and size facilitates adaptation to fluctuating environmental conditions. This study investigates whether pleomorphic traits are also spatially patterned within biofilms, positing that the biofilm itself serves as a fundamental ecological niche driving morphological plasticity. Using *Bacillus subtilis* NCIB 3610 and *Escherichia coli* NCTC 9001 as model organisms, we observed distinct zonal differences in cell morphology across developing colony biofilms. Biofilm formation by *B. subtilis* NCIB 3610 was marked by concentric architectural zones (inner, middle, outer) distinguished by wrinkle boundaries and dynamic expansion. Between 6 and 24 h, the outer zone area significantly expanded from 6.622±0.381cm ^{2} to 38.250 ± 4.001 cm^{2} ($P < 0.005$), while inner zones remained comparatively stable. In contrast, *E. coli* NCTC 9001 formed smooth, less complex biofilms with minimal expansion. Microscopic analysis revealed pronounced spatial heterogeneity in cell size during early biofilm stages. For *B. subtilis* NCIB 3610, mean cell lengths at 18 h were highest in the outer zone (3.279µm), decreasing progressively inward (2.792µm middle, 2.296µm inner). Similar trends were noted for *E. coli* NCTC 9001 at 36 h. As biofilms matured, this size variation diminished, corresponding with reduced growth of inner regions. Upon dispersal, cells from both species reverted to elongated, planktonic forms. Geometric ratios decreased significantly during biofilm maturation, suggesting structural compaction. Gene expression analysis indicated that cytoskeletal regulators (e.g., *ftsZ*, *mreB*, *rodZ*) were downregulated in biofilm cells but restored upon dispersal. For instance, in *B. subtilis* NCIB 3610,*ftsZ* expression increased from 0.479±0.028 (biofilm) to 1.018±0.057 (planktonic). These findings highlight the reversible nature of morphological adaptations. Environmental factors, particularly high cell density, acidic pH, and oxidative stress, significantly influenced cell size. High-density conditions reduced cell length (2.59±0.77µm vs. 8.11±2.41µm in controls, $P<0.0001$), while pH mapping showed a strongly acidic biofilm core, correlating with reduced cell size and increased ROS generation. Antioxidant treatment with NAC reversed ROS-induced size reduction. Cells in the biofilm&rsquos inner regions displayed higher stress resilience, maintaining viability under 300ppm Ni^{2+} stress and showing elevated SOD and catalase activity. A key regulatory role was identified for *relA*, which encodes the (p)ppGpp synthetase central to the stringent response. Acidic conditions upregulated *relA* (2.125±0.134 at pH 5.5) and decreased membrane potential, suggesting pH-induced activation of stress responses. Supplementation with Mg^{2+}, a cofactor of RelA, stabilized biofilms, reduced dispersal gene expression, and decreased cell length. Conversely, &Delta*relA* mutants exhibited elongated, narrow cells, impaired biofilm formation, and deregulated motility, underscoring role of RelA in coordinating stress responses, membrane dynamics, and morphological regulation. This study demonstrates that bacterial pleomorphism within biofilms is a spatially and temporally dynamic process mediated by environmental gradients and stringent response signaling. RelA emerges as a critical regulator linking environmental cues to morphological and physiological adaptation, providing foundational insights for strategies targeting biofilm resilience in clinical, environmental, and industrial contexts.