Session: Tools & modelling

Single molecule FISH – Novel tool for identifying extracellular RNA distribution in biofilms

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The phenotype of resistance and viscoelasticity is attributed to extracellular polymeric substances (EPS) such as extracellular nucleic acids, proteins, and polysaccharides. These polymers are secreted by the bacteria because of environmental stress and nutrient deprivation (Peterson et al., 2015). Once released into the biofilm matrix, these extracellular polymers for example extracellular DNA can self-assemble into networked motifs through formation of higher order structures such as G-quadruplexes, Holliday junction intermediates and Z-form DNA (Seviour et al., 2019; Buzzo et al., 2021; Devaraj et al., 2019). A recent study shows that G- quadruplex eDNA is found to be responsible for conferring viscoelasticity to *P.aeruginosa* biofilms (Seviour et al., 2019).

The idea of extracellular RNA in biofilms is emerging. Our preliminary results suggest the co-existence of extracellular RNA along with eDNA in the biofilm matrix of *P.aeruginosa.*This could potentially explain the role of certain RNA sequences extracellularly in higher order DNA structure formation.Due to exceedingly higher number of nucleotide combinations in microbial genome, it is difficult to identify which nucleotide sequences underpin DNA viscoelasticity in biofilms. To address this problem,we have employed a novel approach known as single molecule FISH (smFISH) confocal microscopy in biofilms (Tsanov et al.,2016). To our knowledge, this is the first time a FISH protocol has been used to locate extracellular RNA in biofilms which so far has only been used to detect microbial populations in a consortium. The primary probes are unlabeled and fluorescently labelled secondary oligonucleotide probes can be altered for simultaneous multi color detection of different mRNA sequences distributed in biofilms. In our study, RNA sequencing identified RNA hits that are present in the viscoelastic eDNA extract from *P.aeruginosa* biofilms. By using smRNA microscopy approach, we show that designed oligonucleotide probes specific to certain mRNA sequences colocalize along the eDNA fibers in the extracellular biofilm matrix of *P.aeruginosa* and clinical biofilm. The structural versatility of RNA might possibly explain their role in aiding viscoelasticity to eDNA. Hence understanding the nature and distribution of extracellular RNA is a major step towards explaining *P. aeruginosa* biofilm matrix assembly and the viscoelasticy of *P.aeruginosa* biofilms, that will potentially inform new biofilm control strategies.