BIOFILMS

Biofilm microanatomy

Within a biofilm, bacterial cells are thought to exist in a variety of physiological states, but an overall picture of this physiological differentiation has been difficult to obtain. Now, a report in *mBio* provides unprecedented insight into the structural and physiological complexity of *Escherichia coli* macrocolony biofilms.

The switch from a single-cell, planktonic lifestyle to a multicellular, sessile biofilm involves a number of phenotypic changes, including the production of adhesins, an extracellular polysaccharide-containing matrix and, in Gram-negative bacteria, amyloid-like curli fibres. Flagella are also required for biofilm formation, although their function within these surface-attached structures has been unclear.

On salt-free solid medium at temperatures <30 °C, *E. coli* str. K-12 substr. W3110 macrocolonies form a characteristic complex pattern of outer wrinkles and inner irregular rings. The authors found that this distinctive pattern disappeared in curli-defective mutants, suggesting that curli fibres are essential for macrocolony structure. The involvement of flagella in macrocolony structure was more nuanced, with mutant analysis indicating that flagella contribute to wrinkle development in the presence of high levels of the second messenger cyclic di-GMP. To determine the spatial distribution of curli, Serra *et al.* used fluorescence microscopy to examine cryosections of 7-day-old macrocolony biofilms grown in the presence of thioflavin S to stain the amyloid curli fibres. They found abundant expression of curli in the upper layer of the biofilm, where the fibres formed a honeycomb-like structure surrounding the cells; there was more heterogeneous expression of curli in the middle and no expression in the lower layers.

The authors went on to carry out a detailed scanning electron microscopy analysis of both 2-day-old and 7-day-old *E. coli* macrocolonies, focusing on a number of 'visual hallmarks' that are indicative of different physiological states, including cell size, cell shape, and flagella and curli expression. Broadly speaking, small, ovoid cells and curli fibres (characteristics of stationary phase) were found at the upper surface, whereas elongated dividing cells and flagella (characteristics of post-exponential growth) were found at the bottom surface. The middle region was a transition zone between the two phenotypes. Both curli fibres and flagella were shown to have core architectural functions. At the macrocolony surface, the cells are encased in a dense network of curli fibres, forming what the authors describe as "custom-moulded curli baskets". In the bottom layer of the biofilm, rotating flagella become entangled to form a dense flagellar mesh that provides support for the upper layers.

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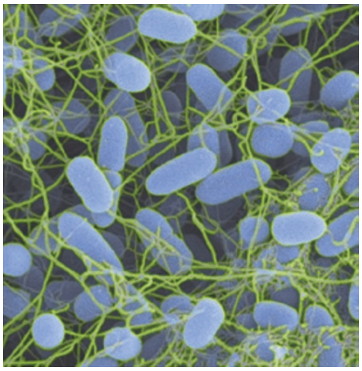

False-coloured scanning electron microscopy image of *Escherichia coli* str. K-12 substr. W3110 cells with entangled flagella, growing in the lower layer of a macrocolony biofilm close to the agar surface. Image courtesy of R. Hengge, Freie Universität Berlin, Germany.

The biofilm state is often referred to as a specialized developmental stage. This work indicates that, instead, the spatial stratification and physiological differentiation in biofilms might simply reflect a physiological response to nutrient gradients.

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