

Discriminating multi-species populations in biofilms with peptide nucleic acid fluorescence in situ hybridization (PNA FISH).

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Abstract

Our current understanding of biofilms in the environment and in health indicates that these structures are typically composed of many different microbial species. However, the lack of reliable techniques for the discrimination of each population that composes a biofilm has meant that studies focusing on multi-species biofilms are scarce and typically generate qualitative rather than quantitative data. In here, we employ novel peptide nucleic acid fluorescence in situ hybridization (PNA FISH) methods to quantify and visualize mixed biofilm populations. As a case study, we present the characterization of *Salmonella enterica*/*Listeria monocytogenes*/*Escherichia coli* single, dual and tri-species biofilms in seven different support materials. Ex-situ, we were able to relate quantitatively the populations of ~56 mixed species biofilms up to 48h, regardless of the support material. In situ, a correct quantification remained more elusive, but a qualitative understanding of biofilm structure and composition is clearly possible for most support materials by confocal laser scanning microscopy (CLSM) at least up to 192h.

Combining the data obtained from PNA FISH with data from other established techniques such as crystal violet (to assess exopolymer production), and from calculated microbial parameters such as growth rate, we were able to develop a model for this specific tri-species biofilm. The higher growth rate and exopolysaccharide production ability of *E. coli* led this microorganism to outcompete the other two [average cell numbers (cells/cm²) for 48 hours biofilm: *E. coli* $2,1 \times 10^8$ ($\pm 2,4 \times 10^7$); *L. monocytogenes* $6,8 \times 10^7$ ($\pm 9,4 \times 10^6$); and *S. enterica* $1,4 \times 10^6$ ($\pm 4,1 \times 10^5$)]. This overgrowth was confirmed by CSLM, with two well-defined layers being easily identified: the top one only with *E. coli*, and the bottom one with mixed regions of *L. monocytogenes* and *S. enterica*.

While PNA FISH was used here to quantify and localize only bacterial populations, the flexibility of the method, as observed in clinical diagnostics, indicates that in the future it can be adapted with little effort to detect populations from any of the three Domains (Fungi, Archaea or Bacteria).