Characterization of multispecies biofilms by peptide nucleic acid fluorescence in situ hybridization (PNA-FISH)

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Number: 89

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 quantification, visualization and discrimination of each population has meant that studies assessing multi-species interactions between sessile microorganisms are scarce and low-throughput.
Employing novel peptide nucleic acid fluorescence *in situ* hybridization (PNA-FISH) methods, we present here a characterization of *Salmonella enterica*/*Listeria monocytogenes*/*Escherichia coli* single, dual and tri-species biofilms in seven 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. Regarding biological behavior, composition of mixed-culture biofilm seems to be the final result of competition between microorganisms, both for available nutrients and for free surface to colonize. It is also suggested that the ability to form biofilm is mostly a species-dependent phenomenon rather than surface-dependent, as six of the materials maintained both the species profile and had similar total cell numbers. The exception was copper, that inhibited the biofilm formation for the species tested.

Our findings concluded that, using a single method, such as PNA-FISH, to confidently discriminate multispecies early-stage biofilms, researchers can infer about spatial organization, intra- or inter-specie interaction and also assess viable but not cultivable states.