Silica-nanoprobes for the study of bacterial biofilm
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The study is a collaboration at Chemistry-Microbiology interface in order to develop innovative tools for biofilms’ exploration. A complete inhibition of the biofilm formation is currently not possible. So, it’s essential to control their development. New tools for studies \textit{in situ} without biofilm destruction are required. Studies of W.J. Drury \textsuperscript{[1]} \textit{et al.} and D.de Beer \textsuperscript{[2]} \textit{et al.}, had shown that luminescent latex-particles (µm) were able to circulate inside the biofilm through channels.

By analogy to latex-particles, we use luminescent silica-nanoparticles having different surface properties as a new tool of exploration.

These characteristics making them multifunctional nanoparticles and permitting to up-date favourable particle–biofilm interactions for exploration.

We'll present the synthesis of luminescent silica-nanoparticles ranging from 20 to 200 nm. The influence of their physico-chemical properties (size, hydrophobicity and ionic charge) on their interaction with \textit{Pseudomonas aeruginosa} biofilm is in progress, the first results will be presented.

**Evaluation of different extraction methods for the capsular EPS fraction of Desulfovibrio vulgaris**

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**Abstract:** In recent years extracellular polymeric substances (EPS) attracted the attention of scientists and industry because of their possible biotechnological potential. Industry is constantly looking for EPS with novel functional properties to satisfy the need for modern technology. A particular objective of this study was to evaluate three different methods for the extraction of capsular EPS from \textit{Desulfovibrio vulgaris} in order to establish a standard extraction method. Until now almost nothing is known about the capsular EPS of \textit{D. vulgaris}. Therefore the cell pellets were treated with one chemical (EDTA) and two physical methods (Dowex 50 x 8 and Crown Ether cation exchange resins).

Results show that the amount and composition of capsular EPS was dependent upon the extraction method. Depending on the method 2% up to 20% (w/w) of total-weight of the extracts were identified. The analysis revealed that capsular EPS of \textit{D. vulgaris} are mainly composed of carbohydrates and proteins. Additionally, the EDTA- and Dowex- extract contained high amounts of iron ions. The Crown ether method was proven to be superior for extracting lipids.

A frequently recommended method for determination of DNA in EPS is the Burton method (1956). In this study the measurement was performed with Quant-iT PicoGreen dsDNA reagent. This method was proven to be functional for DNA quantification analyses and had several advantages like easy performance and time saving. Furthermore, the results indicate that the EPS content of the capsular fraction was affected, if the culture medium was either centrifuged or filtered.

**Characterization of multispecies biofilms by 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 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 \textit{in situ} hybridization (PNA-FISH) methods, we present here a characterization of \textit{Salmonella enterica}/\textit{Listeria monocytogenes}/\textit{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. \textit{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.