DEVELOPMENT AND CHARACTERIZATION OF A SINGLE SPECIES MARINE BIOFILM EPS ASSAY

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Abstract

In natural aquatic environments, the primary mode of existence for many bacteria is in the form of surface-attached communities encased within an exopolysaccharide matrix, which are known as biofilms. Microbial biofilms provide an important survival strategy for microbes in natural aquatic systems. The formation of biofilms is of both economic and social importance as they significantly influence aquatic ecology, human health and the maintenance industrial processes. Despite this importance, there remains a lack of a direct quantitative approach to measuring biofilm production. As advances in natural sciences, as well as biofilm research, historically follow the development of new relevant techniques, this project was aimed to develop a new technique to directly quantify biofilm production. To accomplish this goal, the project explored the potential of applying the uronic acids assay, modified from Blumenkranz and Asboe (1973), to quantitatively measure biofilm production by tracking the uronic acid component of the exopolysaccharide matrix. The results demonstrated that quantitative analysis of relative abundance ratio of uronic acid content to total EPS, can be used to track biofilm EPS production in single-species biofilms. The assay was found to be simple, reproducible, and sensitive to μg levels, suggesting its potential for application as a screening technique for compounds that inhibit the production of microbial exopolysaccharide containing uronic acids. In order to investigate this potential further, the assay was first applied to biofilms produced in the presence of two universal disinfectants (e.g. sodium hypochlorite and sodium dodecyl sulfate (SDS)) known to inhibit microbial growth and biofilm formation. This data was then used to characterize the assay's performance through the statistical assessment of threshold concentrations for disinfection efficiency.

Finally, the assay was evaluated for its ability to serve as a screening tool for testing anti-biofouling activities of natural and synthetic compounds (e.g. glycosidases, halogenated furanones, and semi-crude fractions isolated from minimally fouled marine plants). Results from the EPS assay were evaluated in the context of those from conventional measures of planktonic growth and fluorescence microscopy of attached bacteria. This study suggests that the uronic acids assay can, through the use of significance thresholds, augment existing biofilm characterization methods to provide a more comprehensive description of the activity of potential antagonists on biofilm production.