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Editorial

Composition and Function of Individual Microbial Biofilm Matrix Components: A Key Element to Its Control

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Abstract

Studies on the molecular characterization of extracellular polymeric substances (EPS) in biofilms are still few in the literature. Understand the role of individual EPS on biofilm's function and properties is of a great importance not only to better understand the structure and activity of microbial biofilms but also to better control such biological communities (either as contaminants or as productive structures). Thus, the main challenge on the field of biofilms passes through establishing correlations between specific EPS and environmental and operating conditions, as well as between specific EPS and the structural and functional properties and resistance to physicochemical treatments. However, advances on the determination of composition, functions and properties of individual EPS matrix are only possible after develop suitable fine analytical procedures for selective and sequential extraction, separation, purification and quantification of the molecular species within the various families of EPS, viz. polysaccharides, proteins, lipids and nucleic acids. This communication underlines the importance to focus the research of the field of microbial biofilms in the direction to find out the role of individual extracellular polymeric substances (EPS) on biofilm's function and properties.

Keywords: *Microbial biofilms; Extraction, separation and quantification; Extracellular polymeric substances (EPS); Exopolysaccharides; Extracellular nucleic acids; Proteins; Lipids; Development of analytical methods*

Microbial biofilms (or simply biofilms) are structured communities of sessile microorganisms attached to a surface and encapsulated within extracellular polymeric substances (EPS) [1-5]. The presence of microbial biofilms may be desired, for instance in wastewater treatment systems and in the production of microbial biomass and metabolites [6]. However, they are often unwanted, due to negative impact in industrial plants and biomedical settings. Microbial biofilms are associated with additional problems in domestic and industrial cleaning and disinfection procedures. At this regards, it represents a major concern in the effectiveness of sanitation in food industry – hence in combating and preventing cross-contaminations and food spoilage. Moreover, they can cause energy losses and blockages in membrane systems and heat exchangers – a phenomenon known as biofouling. Accordingly, microbial biofilms and biofouling can lead to substantial increase of the operating costs in industrial facilities as diverse as membrane systems, power and desalination plants, ship hulls and equipments for drinking water treatment. Furthermore, microbial biofilms are known for the deleterious effects in hospital settings and medical devices (e.g. catheters, contact lens and implants). In fact, estimates indicate that they are responsible for more than 60-70% of all microbial infections [2,4,7-14].

In biofilms, the extracellular material matrix is composed by different biopolymers - known as EPS - mostly produced by the existing microorganisms and which are responsible for biofilm adhesion and cohesion. Analyses of EPS present several difficulties and the composition varies greatly with factors such as microorganisms, hydrodynamics experienced, nutrient availability and composition, temperature, etc. Additionally, the role and properties of individual components from the extracellular matrix is still poorly understood

[1,3-5,15-17]. EPS (also known as matrix) are mainly polysaccharides, proteins, nucleic acids, peptidoglycan and lipids, and possess several functions in biofilms. EPS are usually classified into two fractions according to the water solubility: water-soluble polymers (many polysaccharides, proteins and nucleic acids) and water-insoluble polymers (cellulose, chitin and glycolipids such as rhamnolipids). They provide mechanical stability and mediate adhesion and cohesion phenomena. Matrix components are responsible for biofilm's structural integrity – allowing cell-cell interaction, the formation of structures to access to nutrients and inhibition of antibiotics. The extracellular matrix acts as an external digestive system – due to the retention of extracellular enzymes - and recycling centre by keeping lysed components available. EPS serve as nutrients, sorption of organic and inorganic compounds, exchange of genetic material and export of cell components, electron acceptor or donor, and creates a protection against several factors, viz.: desiccation, oxidizing or charged biocides, some antibiotics and metallic cations, UV radiation, protozoan grazers and host immune defences. Amongst exopolysaccharides - the major fraction of EPS matrix – isolated from biofilm are the homopolysaccharides glucans, fructans and levans, curdlan or dextran and cellulose, and the heteroplolysaccharides alginate, emulsan, gellan or xanthan, Pel (pellicle), Psl (polysaccharide synthesis locus). In addition to the enzymes, biofilm matrix accounts for several structural proteins, e.g. CdrA, TasA, Bap and proteinaceous appendages (pili, fimbriae and flagella) [1,3-5,15-17].

Selective extraction, fractionation and isolation of individual EPS components present several difficulties and deeper knowledge is required. As a result, role and properties of individual components from matrix on the biofilms as a whole is still poorly understood. Nevertheless, and as already stressed, to understand the role of EPS in biofilm architecture and properties, it is fundamental to recognize the composition, functions and properties of individual EPS matrix [1,3-5,15-17]. Isolation of individual EPS components requires multi-methods for extraction and separation, and has usually to be adapted according to the type of biofilm. Method protocols for extraction of EPS (usually by multi-method protocols) may involve physical (e.g. filtration, heating, blending, sonication, dialysis, lyophilization), chemical (e.g. buffers, tween, EDTA, formaldehyde, NaOH, cation exchanger resins) and enzymatic procedures (e.g. amylases, nucleases, proteases). Furthermore, fractionation, separation and quantification methodologies found in the literature encompasses a broad spectrum of techniques, e.g. ATR-FTIR spectroscopy, HPSEC, HPAEC-PAD, HPLC-SM, GC-MS, LC-MS, NMR [1,3,16,18-38]. The chemical, physical and enzymatic analytical methods used for extraction and separation of EPS have a major influence for characterisation of biofilms, thus selection of effective methods for extracting, separation, purification and quantification of extracellular polymeric substances must play a major concern on the studies of the role, composition and function of individual microbial biofilm matrix components.

To address the challenges in controlling the formation and development of microbial biofilms as well as in the resistance of the biofilms to physicochemical treatments, find out correlations between such effects and extracellular polymeric substances is required. The characterization and function of EPS on biofilm matrix has drawn an increasing attention but a deeper knowledge is essential. It is likely that the critical bottlenecks found in control of microbial biofilm growth and withstand to physicochemical treatments may effectively be overcome through the correlation with specific EPS - selectively extracted and further quantified with appropriated fine and effective analytical methods. Furthermore, it is expected that these efforts are of first importance to allow the advance of cutting-edge technologies for biofilm control, based on novel, efficient, sustainable and cost effective processes.

Microbial biofilms hold a major economic and environmental importance because of its strong impact in the industry and hospital settings. A better knowledge and control of the biofilm growth may lead to the employment of cheaper, more efficient and environmental friendly practices for its removal in the industrial processing units and medical devices. In addition, it may contribute to the reduction of associated nosocomial infections – thus playing important economical and social roles. Besides, a better characterization of microbial biofilms may contribute to the required knowledge towards the economical feasibility of its employment in wastewater treatments and/or microbial biomass production and generation of high-added by-products for several applications.

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