

# Research for the Development of New Anti-Bio Adhesive Coatings: The Strategy of Exopolysaccharides

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## Opinion

Marine biofouling is a natural process, which is initiated by the adsorption of organic and mineral molecules, followed by the adhesion of microfouling (bacteria and microalgae principally) and macrofouling (macroalgae and invertebrates). Biological fouling can bring about some unwanted and detrimental economic and environmental consequences to marine surfaces. Several ways to combat against fouling have been developed. Most of them include the use of polymeric coatings blended with metals as copper and/or toxic organic compounds. For almost 50 years, commercial antifouling (AF) paints used tributyltin (TBT), a broad-spectrum biocide formulated in copolymer paints with cuprous oxide. Unfortunately, paints are highly toxic for many aquatic organisms and severe toxic effects on marine ecosystems have been observed. In 2008, the use of TBT was banned. Today, coatings are formulated by using copper (CuSCN, Cu<sub>2</sub>O or Cu metal) and organic molecules called booster biocides. Nevertheless, these solutions are not environmentally satisfactory and the recent regulation concerning the use of biocidal agents (EU Regulation No 528/2012 known as Biocidal Product Regulation) requires a higher level of environmental protection. The current research of environmentally friendly marine antifouling coatings relate the use of biodegradable polymers to control erosion and bioactive molecules released, and natural products as bioactive ingredients [1]. Natural products from marine organisms can be used as replacements for the biocides commonly used in AF coatings [2]. Many sessile marine animals are free from biofouling and produce metabolites that demonstrate antifouling properties. AF substances have been extracted from a variety of marine organisms: micro-organisms (marine bacteria, fungi, cyanobacteria), seaweeds, aquatic plants and marine invertebrates. Different modes of actions are known inhibitor of adhesive production/release, biofilms inhibitors, quorum sensing blockers, protein expression regulators, blockers of neurotransmission or surface modifiers. However, the main difficulties are their expensive cost, their large-scale production, the maintain of their activity after formulation in paint and to retain their activity for a long time.

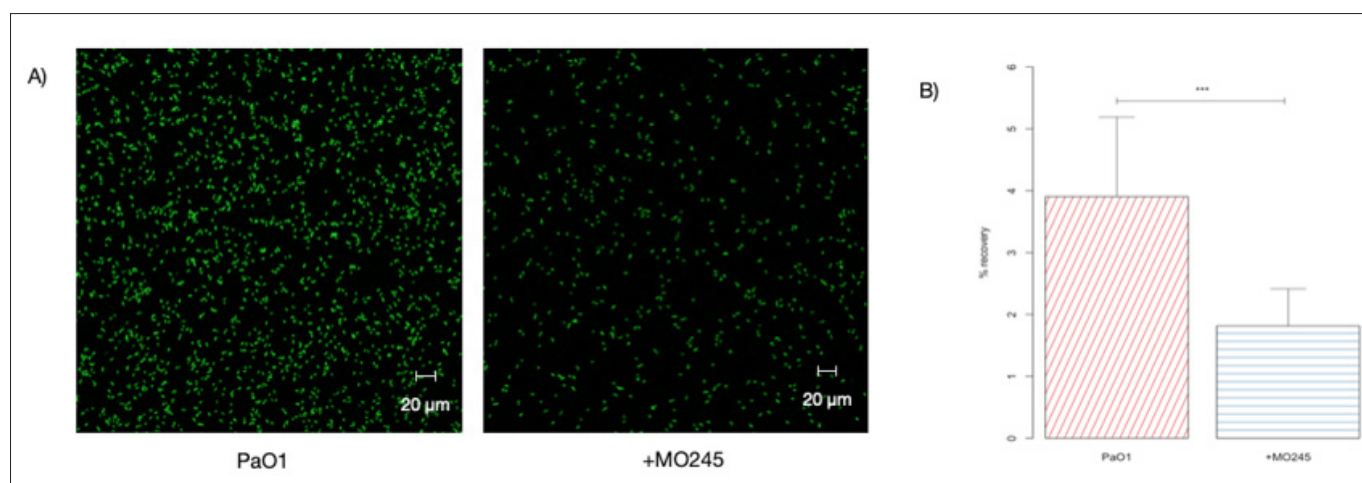
For innovative and ecological solution to marine biofouling, it is necessary to shift the research strategy to new approaches combining surfaces properties and novel surface preventing bio adhesion or promoting release [3]. Polydimethylsiloxane and fluoropolymers surfaces are an approach without biocide. The combination of low surface free energy, low roughness, and low elastic modulus together lead to low interfacial bond between organisms and the coating. Even if organisms attach on the surface, only a weak dispersive interaction will take place, resulting in the easy removal of the organisms. This concept is one of the promising antifouling alternative strategies. However, their no-toxicity remains questionable because their efficiency relies on the addition of oil. It has been reported that these “oils” are usually block copolymers that can migrate through the coatings to function as surfactants and silicone have been classified as hazardous substances [4]. Their inert oily nature and relative extreme persistence are a potential threat to aqueous environments. Moreover,

compared with other coatings, they are more vulnerable to damage. Biomimetic antifouling coatings are microstructure surfaces [5]. Their mechanisms of action are to destroy the physical attachment of organisms. Their surfaces are similar to the microstructure contained in the biological epidermis (skin of surface of sharks, shells...). The current methods of conceiving these microstructure surfaces include photolithography, laser etching, micro-contact printing, three-dimensional printing, picosecond laser texturing [6]. However, the construction and the repair of such surfaces is complicated, and their efficiency is limited in real marine environment due to the large scale of topography to be achieved (from a few  $\mu\text{m}$  to several hundred  $\mu\text{m}$ ).

For all these reasons, novel and environmentally friendly antifouling coatings are needed and the use of polysaccharides could be the key to an original strategy. Polysaccharides are widely distributed in the nature. They have a number of characteristics such as biocompatibility and biodegradability and may be commercially available. Among these polymers, bacterial extracellular polysaccharides (EPS) display antibiofilm properties with interesting modes of action. Their antibiofilm activity is due to mechanisms other than bacteriostatic or bactericidal activity [7,8]. Most of them act as surfactant molecules. Bacterial EPS modify the physical properties of abiotic as well as cell surfaces. In addition to inhibiting intercellular adhesion, bacterial EPS have been shown to inhibit binding of bacterial cells to various biotic surfaces. They may act as an interspecies cell-to-cell signal that downregulates biofilm formation. Moreover, several bacterial EPS might inhibit and/or destabilize the biofilm. Hence, surface treatment by bacterial EPS and bacteria-repellant coatings are promising approaches [9].

Among the several genus of bacteria that produce EPS, *Vibrio* is one of the more widespread [10]. A bacterium from *Vibrio* genus was isolated from bacterial Mats in the lagoon of Moorea Island (French Polynesia) and it is referred to as strain MO245. During stationary phase of growth, the isolated MO245 strain produces an exopolysaccharide (EPS) characterized by equal amounts of glucuronic acid and hexosamines (galactosamine and glucosamine in the same proportion) with a molecular weight ( $M_w$ ) of 513kDa average. It is a weak polyelectrolyte with similar conformational and physico-chemical properties than hyaluronic acid, a known antibiofilm polysaccharide [11].

The bioactivity of EPS from MO245 have been shown against a pathogenic bacteria *Pseudomonas aeruginosa* PAO1. Until 500 $\mu\text{g}/\text{mL}$ , no bacteriostatic or bactericide activities have been observed: the polymer is not toxic against *Pseudomonas aeruginosa*. However, the impact of EPS from MO245 (125 $\mu\text{g}/\text{mL}$ ) on adhesion of *Pseudomonas aeruginosa* PaO1 on glass surface at ambient temperature clearly shows an inhibition of adhesion. Confocal laser scanning microscopy observations show a significant decrease ( $p < 0,001$ ) of cells adhesion in the presence of EPS compared to the control without EPS (Figure 1): the percentage of adhered cells on the glass surface decrease from (3,90%) to (1,81%) without and in the presence of EPS respectively [12]. The potential mechanism of EPS from MO245 concerns an anti-adhesive effect rather than a biocidal effect. Hence, the conception of biobased coatings based on EPS could be an interesting approach in the development of new strategies fighting antibiofilm and antifouling. Indeed, the antifouling efficiency of bacterial EPSs have already been highlighted in natural environment as seawater [13].



**Figure 1:** Impact of EPS from MO245 ((125 $\mu\text{g}/\text{mL}$ ) on adhesion of *Pseudomonas aeruginosa* PaO1 on glass surface at room temperature (Protocol from [12]).

A) Top view of *Pseudomonas aeruginosa* PaO1gfp adhesion observed by CLSM (lexcitation: 488nm, lemission: 500-584nm).

B) Recovery percentage of *Pseudomonas aeruginosa* PaO1 adhesion analysis by Image J. \*\*\*p.value<0,001 (Man Whitney test).

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