

## **Amphiphilic Compounds as Potential Non-Toxic Antifouling Agents: A Study of Biofilm Formation Assessed by Micro-titer Assays with Marine Bacteria and Eco-toxicological Effect on Marine Algae**

**Authors :** D. Malouch, M. Berchel, C. Dreanno, S. Stachowski-Haberkorn, P-A. Jaffres

**Abstract :** Biofilm is a predominant lifestyle chosen by bacteria. Whether it is developed on an immersed surface or a mobile biofilm known as flocs, the bacteria within this form of life show properties different from its planktonic ones. Within the biofilm, the self-formed matrix of Extracellular Polymeric Substances (EPS) offers hydration, resources capture, enhanced resistance to antimicrobial agents, and allows cell-communication. Biofouling is a complex natural phenomenon that involves biological, physical and chemical properties related to the environment, the submerged surface and the living organisms involved. Bio-colonization of artificial structures can cause various economic and environmental impacts. The increase in costs associated with the over-consumption of fuel from biocolonized vessels has been widely studied. Measurement drifts from submerged sensors, as well as obstructions in heat exchangers, and deterioration of offshore structures are major difficulties that industries are dealing with. Therefore, surfaces that inhibit biocolonization are required in different areas (water treatment, marine paints, etc.) and many efforts have been devoted to produce efficient and eco-compatible antifouling agents. The different steps of surface fouling are widely described in literature. Studying the biofilm and its stages provides a better understanding of how to elaborate more efficient antifouling strategies. Several approaches are currently applied, such as the use of biocide anti-fouling paint<sup>6</sup> (mainly with copper derivatives) and super-hydrophobic coatings. While these two processes are proving to be the most effective, they are not entirely satisfactory, especially in a context of a changing legislation. Nowadays, the challenge is to prevent biofouling with non-biocide compounds, offering a cost effective solution, but with no toxic effects on marine organisms. Since the micro-fouling phase plays an important role in the regulation of the following steps of biofilm formation<sup>7</sup>, it is desired to reduce or delate biofouling of a given surface by inhibiting the micro fouling at its early stages. In our recent works, we reported that some amphiphilic compounds exhibited bacteriostatic or bactericidal properties at a concentration that did not affect eukaryotic cells. These remarkable properties invited us to assess this type of bio-inspired phospholipids<sup>9</sup> to prevent the colonization of surfaces by marine bacteria. Of note, other studies reported that amphiphilic compounds interacted with bacteria leading to a reduction of their development. An amphiphilic compound is a molecule consisting of a hydrophobic domain and a polar head (ionic or non-ionic). These compounds appear to have interesting antifouling properties: some ionic compounds have shown antimicrobial activity, and zwitterions can reduce nonspecific adsorption of proteins. Herein, we investigate the potential of amphiphilic compounds as inhibitors of bacterial growth and marine biofilm formation. The aim of this study is to compare the efficacy of four synthetic phospholipids that features a cationic charge (BSV36, KLN47) or a zwitterionic polar-head group (SL386, MB2871) to prevent microfouling with marine bacteria. We also study the toxicity of these compounds in order to identify the most promising compound that must feature high anti-adhesive properties and a low cytotoxicity on two links representative of coastal marine food webs: phytoplankton and oyster larvae.

**Keywords :** amphiphilic phospholipids, bacterial biofilm, marine microfouling, non-toxic antifouling

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