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Regular Article

An alternative anionic bio-sustainable anti-fungal agent: Investigation of its mode of action on the fungal cell membrane

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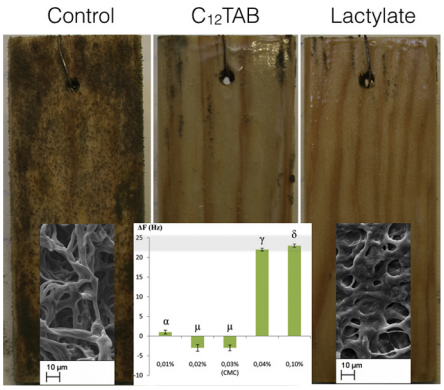
GRAPHICAL ABSTRACT



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ABSTRACT

The potential of a lactylate (the sodium caproyl lactylate or C10 lactylate), a typical food grade emulsifier, as an anionic environmental friendly anti-fungal additive was tested in growth medium and formulated in a protective coating for exterior wood. Different laboratory growth tests on the blue stain fungus *Aureobasidium pullulans* were performed and its interactions on a model fungal cell membrane were studied. Promising short term anti-fungal effects in growth tests were observed, although significant but less dramatic effects took place in coating test on wood panels. Scanning electron microscope analysis shows clear differences in the amount of fungal slime on the mycelium of *Aureobasidium pullulans* when the fungus was exposed of C10 lactylate. This could indicate an effect on the pullulan and melanin production by the fungus. Moreover, the interaction studies on model fungal cell membranes show that C10 lactylate affects the phospholipid bilayer in a similar manner to other negative charged detergents.

1. Introduction

Today there is an immediate need for a new generation of innovative and highly durable, protective coatings and preservative systems for wood among other surfaces, especially for processed materials used in construction. These protective treatments are

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needed to prevent microbial wood degradation when wood is exposed outdoors. Current wood coatings are fully or at least partially fossil based, containing toxic biocides such as 3-Iodoprop-2-yn-1-yl butylcarbamate (IPBC) that are regulated by the Biocidal Products Directive (BPD) [1–3]. The coating industry dependence on fossil resources must be eliminated and replaced by sustainable, non-fossil biosources that - at the same time - constitute environmentally friendly alternatives as described in EU White paper, European Bioeconomy 2030 [4]. Many efforts are now placed into developing a bio-sustainable strategy for non-toxic biocides that can be incorporated into 100% bio-sustainable coating systems including coatings for wood.

Lactylates are commonly used in the food and cosmetic industry as emulsifiers due to their non-toxic properties to humans [5,6]. They are organic compounds and biodegradable [7,8]. Due to the safety and versatile functionality of these lactylates, it is interesting to explore their ability as non-toxic bio-sustainable preservatives in eg. protective coatings such as wood preservative systems. Specifically, lactylates are esters of lactic acid in which the C2-hydroxy group of lactic acid is esterified to a fatty acid. Both lactic acids and fatty acids, the components of the fatty acid-based lactylates (FAL), are used as antimicrobial components. For example, lactic acid and various types of lactic acid bacteria (LAB) are used as antifungal agents [9] while lactic acid is known to inhibit *Salmonella* and *Campylobacter* contamination of broiler carcasses during industrial processing [10]. Moreover, FAL are used as antifungal agents against molds and sapstains in wood protection systems [11]. Indeed, earlier studies indicate that small chain (C2-C8) and middle chain (C9-C14) FAL have an inhibitory effect on specific fungal germination in asco- and basidiomycetes [12,13].

In this work, we perform a systematic study of the antimicrobial activity of a specific lactylate: the sodium caproyl lactylate (C10 lactylate), see Fig. 1. This lactylate is non-irritant to humans, according to Goodguide.com, and can be found in commercial cosmetic products like Dove Face Care and Life-Flo Baby Shampoo. As test organism, we selected *Aureobasidium pullulans (de Bary) Arnaud, strain P268* since this organism is widely used in European Standard tests (e.g. EN 927-6:2006) of fungal stain attacks, such as Blue Stain, on exterior wood [14]. We perform growth tests to estimate the lactylates impact on the fungal growth on agar plates and on wood panels. In order to identify a possible mechanism of action for C10 lactylate in terms of its antimicrobial activity, we investigate their effect not only on the agar plates by Scanning Electron Microscopy (SEM) but also on simple model cellular membranes for fungi by Quartz Crystal Microbalance with Dissipation measurements (QCM-D) [15,16]. Supported lipid bilayers (SLB) are commonly used as simple model systems for cell membranes for instance in relation to possible targets for anti-microbial agents [17]. Here, we used a simple lipid mixture composed of 75 mol% 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 25 mol% 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-l-serine (POPS), *see* Fig. 1. This mixture matches the fluidity and charge of the phospholipid composition in ascomycetes (*Candida albicans*) cellular membrane [18]. Finally, we compared the results for C10 lactylate to two other amphiphiles typically used in industry as emulsifiers and antimicrobial agents: Sodium dodecyl sulfate, SDS, and dodecyltrimethylammonium bromide, C_{12}TAB, see Fig. 1. Our results suggest that C10 lactylate is a promising anionic, bio-sustainable anti-fungal agent that attacks the integrity of the cellular membrane in a detergent like manner and possibly the production of pullulan and melanin.

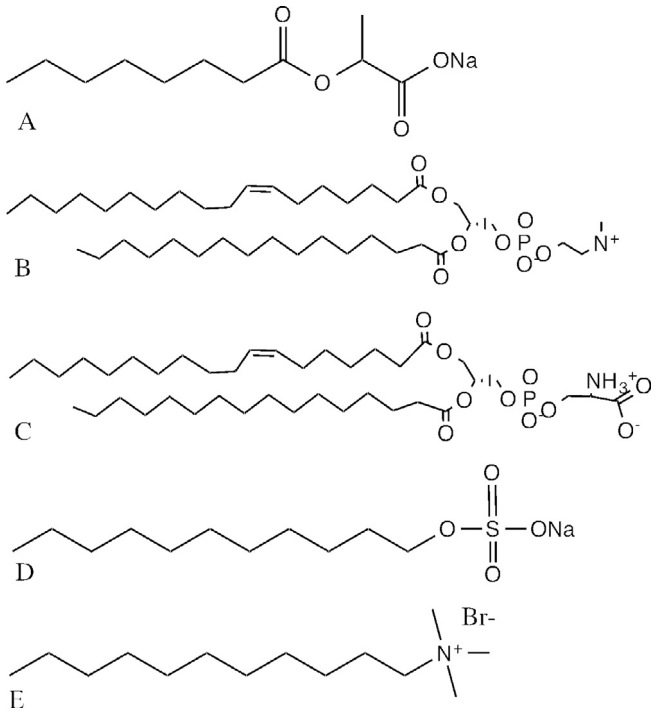

Fig. 1. Chemical structures of sodium caproyl lactylate (A), phospholipids POPC (B) and POPS (C), sodium dodecyl sulfate (D), and dodecyltrimethylammonium bromide (E).

2. Methods

2.1. Materials

Pure powder (>99%) of POPC and POPS was purchased from Avanti Polar Lipids Inc and used without further purification. SDS, $MgCl_2$, C_{12}TAB (99% Purity), potato dextrose agar/broth (PDA/PDB, respectively) were purchased from Sigma and used without further purification. The C10 lactylate was kindly provided by Palsgaard A/S, which is based on an ester between lactic acid and sodium lactate and capric acid. MilliQ purified water (MilliQ) was used in all experiments. Strains of *Aureobasidium pullulans (De Bary) Arnaud, P268* (IMI 269216) were provided by the Danish Technological Institute. Strains of *Aspergillus versicolor* (IMI 45554), *Penicillium purpurogenum* (IMI 178519) were standard strains in the laboratory.

2.2. Supported lipid bilayer (SLB)

SLB were formed by vesicle fusion, a simple method in which small unilamellar vesicles (SUV) are introduced onto an ultra-clean surface [19–21] such as the QCM-D silica sensor (Q-sense). Upon contact with the silica surface, the vesicles fuse and break forming the SLB. First, the SUV suspension was prepared following standard procedures: Briefly, POPC and POPS were dissolved to a concentration of 20 mg/mL in chloroform. Lipid films were then formed by mixing the lipids to the desired molar ratio, and by subsequent drying under N_2 flow and then under vacuum for 30 min. The films were then stored at −18 °C until use. Prior to use, the films were suspended in 2 mL MilliQ to a final concentration of 0.25 mg/mL. The suspension was then bath sonicated for 60 min at room temperature, followed by 60 s tip sonication (50% duty cycle 3 s on/off). Prior to injection into the QCM-D flow cells, 2 mL of 4 mM $MgCl_2$ were then added to the lipid suspension.