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Production of antimicrobial textiles by cotton fabric functionalization and pectinolytic enzyme immobilization

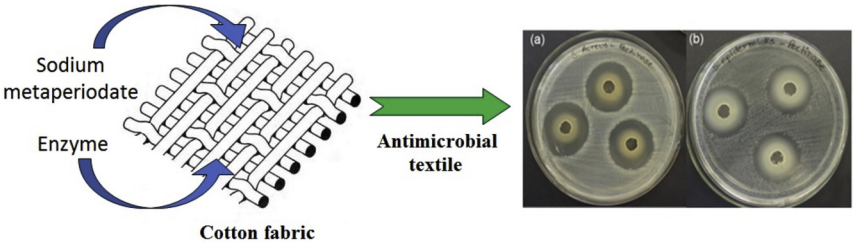
Michaela Coradi [a], Micheli Zanetti [a, b], Alexsandra Valério [a], Débora de Oliveira [a, *], Adriano da Silva [a], Selene Maria de Arruda Guelli Ulson de Souza [a], Antônio Augusto Ulson de Souza [a]

[a] Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, SC, 88040-900, Brazil
[b] Area of Exact and Environmental Sciences, Universidade Comunitária da Região de Chapecó, Chapecó, SC, 89809-000, Brazil

HIGHLIGHTS

- A novel antimicrobial textile was synthesized.
- Termamyl® 2X and Bioprep® 3000L were investigated for antimicrobial activities.
- The periodate precursors have been coated to cotton fabrics to enzyme immobilization.
- Coated fabrics showed antimicrobial properties against *S. aureus*, *S. epidermidis*, *E. coli* and *C. albicans*.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, Termamyl® 2X and Bioprep® 3000L were characterized and investigated for their antimicrobial activities by agar diffusion and Minimum Inhibitory Concentration (MIC). Bioprep® 3000L enzyme showed higher antimicrobial activity compared to the Termamyl® 2X against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Candida albicans*, and was partially active for *Pseudomonas aeruginosa*, showing a high potential of application to antimicrobial textiles production replacing the synthetic compounds. In addition, Bioprep® 3000L enzyme was immobilized by covalent bond on the chemically modified cotton fabric surface via periodate reaction (20% immobilization yield) and its antimicrobial activity was investigated.

1. Introduction

Natural fibers can be used as materials for finishing and insulation in the automotive, construction industry, and particularly suitable for sports textiles production, non-implantable medical products, hygiene and health care products, underwear, shoe linings and packaging industry [1–4]. However, natural fiber fabrics are more susceptible to microorganism attack than synthetic fiber fabrics due to the hydrophilic porous structure that retains water, oxygen and nutrients, providing an ideal medium for the microorganism proliferation [5].

Currently, many antimicrobial technologies are available in the textile industry, being used in the most different applications to prevent the growth of microorganisms. Due to the biological activity of the antimicrobial compounds, the safety evaluation of these substances is a subject of constant research. Antimicrobial compounds applied in textile materials should present low toxicity to consumers, be effective against a large spectrum of

* Corresponding author.
E-mail address: debora.oliveira@ufsc.br (D. de Oliveira).

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microorganisms and selectively eliminate unwanted microorganisms [6,7].

Many different compounds are used to confer antimicrobial functionality, from synthetic organic compounds such as triclosan, quaternary ammonium compounds, metals and metal salts such as copper, silver and zinc, titanium dioxide and synthetic dyes [7–11], to antimicrobial compounds derived from natural substances, as chitosan, essential oils and enzymes [12–17].

The constant increase of microorganisms resistance to antibiotics increased the search for the new compounds, biodegradable, non-toxic and eco-friendly antimicrobial agent due to the possible harmful effects of synthetic antimicrobial agent are being increasingly researched [18]. With the increase of microorganisms resistance to antibiotics, antimicrobial enzymes, common in nature, are under intense investigation [19]. According to Thallinger et al. [19] several enzyme-based products have already been marketed with applications in the healthcare, food and biomedical industries. Enzymes are able to directly attack microorganisms, interfere with formation or destroy biofilms, and catalyze reactions that result in the production of antimicrobial compounds investigated [12,19–21].

In some cases, antibacterial enzymes are an established technology. For example, liquid formulations of antimicrobial and anti-biofilm enzymes are often exploited for cleaning surfaces. Enzymes may be incorporated or grafted in and out of polymeric materials to prevent microbial colonization. The formulations may contain one or more enzymes, or enzymes combined with other antimicrobial or anti-biofilm agents [22]. Although widely researched in the area of food, detergents and pharmaceuticals, the use of antimicrobial enzymes in the textile field is a poorly explored topic [12,20,21]. Even though it is a promising alternative, there is still much to investigate, and there is no commercial application yet.

Cellulose derivatives have been extensively studied through theoretical and practical approaches to the immobilization enzymes due to their biocompatibility, biodegradability, chemical stability and low environment contamination risk [23]. Cellulose derivatives have hydrophilic and hydrophobic properties, non-toxic and they are chemically inert under physiological conditions, making them useful for the enzymatic activity maintenance. In addition, the hydroxyl groups on the cellulose surface are favorable for chemical reactions, thus making the cellulose materials suitable for enzymes immobilization [24].

Enzymes immobilization by covalent bonding is an efficient and stable method [25]. The enzymes support requires the specific functional groups availability. The hydroxyl groups on the cellulose surface can present poor interaction with the enzyme. Therefore, in order to obtain a strong covalent immobilization, functionalization additional steps are necessary [25–27]. Periodate oxidation is an efficient method of functionalization. It is a reaction converting 1,2-dihydroxyl (glycol) groups to a pair the aldehyde groups at the positions C2 and C3 of an anhydroglucose unit which can couple with enzyme. In addition, this method does not present significant secondary reactions and is widely used in the structural carbohydrates analysis [25,28].

Several studies have activated cellulosic textile materials for the immobilization of enzymes but none specific to pectinolytic enzymes [20,29,30] so, this study can contribute to future applications about pectinolytic enzymes immobilization in cellulose derived substrates. Girelli et al. [31] used sodium periodate as an active agent to functionalize different cellulose supports (cellulose powder, disc make up remover pads, cotton and linen tissues, cotton bud) and to immobilize lipase from *Candida rugose*. Wang et al. [32] treated cotton fabric to immobilize catalase, obtaining high enzymatic activity as a result. Nikolic et al. [33] oxidized viscose fibers for subsequent trypsin immobilization, obtaining until 83.8% of the initial enzymatic activity after 60 days of storage.

Faced in this scenario, the present work aimed the development of antimicrobial cotton textiles through the superficial modification and pectinolytic antimicrobial enzyme immobilization, providing subsidies for the development of technology for the antimicrobial textiles production, as a natural and biodegradable alternative.

2. Materials and methods

2.1. Materials

Two commercial enzymes Termamyl® 2X (α-amylase, EC 3.2.1.1, specific activity 67.68 U mg^{-1}) and Bioprep® 3000L (alkaline pectinase, EC 4.2.2.2, specific activity 390.55 U mg^{-1}) was kindly donated by Novozymes (Novozymes Latin America Ltda., Araucaria, BR). Antimicrobial activity tests were performed with strains of Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 18112); strains of Gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853); and yeast strain of *Candida albicans* (ATCC 24433).

For cotton fabric functionalization and enzyme immobilization, the following materials were used: sodium metaperiodate (Neon), pentahydrate copper (II) sulfate (Vetec), potassium sodium tartrate (Labsynth), sodium hydroxide (Vetec), 3,5-dinitrosalicylic acid (DNS) (Sigma-Aldrich), citrus pectin (Vetec).

For the antimicrobial activity, the following materials were used: Plate Count Agar (PCA) (Merck), Sabouraud agar chloramphenicol medium (Kasvi), Brain Heart Infusion (BHI) broth (Kasvi), Sabouraud broth (Kasvi), Triphenyl Tetrazolium Chloride (TTC) (Sigma-Aldrich).

2.2. Antimicrobial activity

Bacterial suspensions were adjusted to a concentration of 10^8 CFU mL^{-1} using the 0.5 McFarland scale, and diluted in sterile saline (0.85%) to the concentration of 10^4 CFU mL^{-1}. They were then sown on the surface of PCA agar. In each plate, three equidistant holes were made, with a diameter of approximately 6 mm, and the pure Bioprep® 3000L and Termamyl® 2X enzyme preparations were deposited. It was incubated at 36 °C for 24 h and after this time, the inhibition halo of microbial growth formed was measured, using a millimeter ruler. The procedure for yeast *C. albicans* was similar to the described for bacteria, with yeast (10^6 CFU mL^{-1}) seeded in Sabouraud agar chloramphenicol medium.

2.3. Determination of minimum inhibitory concentration

The determination of minimum inhibitory concentration (MIC) was performed by follow methodology describe in CSLI [34], with modifications. The microdilution test was performed on 96 microwell plates containing 100 μL of BHI broth for bacterial assays and 100 μL of Sabouraud broth for yeast assays. The assays were performed in triplicate, 200 μL of the Bioprep® 3000L solution or Termamyl® 2X was added to the wells corresponding to line A. After samples homogenization, aliquots of 100 μL were transferred successively to a new microwell, thus obtaining solutions with different concentrations, as presented in Table 1. For each concentration, 5 μL of each inoculum of bacteria or yeast investigated at the concentration of 10^4 CFU mL^{-1} and 10^6 CFU mL^{-1} were added using the 0.5 McFarland scale, respectively. The positive control was done with 100 μL of BHI and 5 μL of microorganism, in order to certify if the used broth allowed the growth of the microorganisms tested. The blank corresponds to 100 μL culture medium to ascertain the sterility.