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NEW BIOACTIVE COMPOSITES BASED ON BACTERIAL CELLULOSE AND NATURAL PRODUCTS

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Abstract

Bacterial cellulose (BC) has a variety of applications in biomedical fields. However, the native BC lacks certain properties, which limits its applications in various fields. The trend is nowadays towards the development of organic polymers using natural materials. In this sense, the possibility of obtaining new composite biomaterials with improved properties will prove as an interesting solution for achieving green composites BC, satisfying the need to explore minimal cost, biodegradable and renewable materials. Following these principles, this paper presents some of our studies carried out in order to obtain new eco-friendly composite biomaterials based on BC and valuable natural products such as sericin, propolis and royal jelly with appropriate biomedical applications. The main objective consisted in the biosynthesis and characterization of bacterial cellulose-based composites (BC) combined with natural products with antimicrobial properties.

Key words: *eco-friendly composites, bacterial cellulose, antimicrobial activity.*

INTRODUCTION

Bacterial cellulose (BC) has received a substantial interest owing to its unique structural features and impressive physical-mechanical properties. BC has a variety of applications in biomedical fields, including use as biomaterial for artificial skin, artificial blood vessels, vascular grafts, scaffolds for tissue engineering and wound dressing. However, the native BC lacks certain properties, which limits its applications in various fields. Therefore, synthesis of BC composites will be conducted to removing these limitations.

As like other polymers, BC has limitations which restrict the application to a certain extent. For example, BC is an excellent material for dressing as it provides a moist environment that allows rapid healing of the wound, however, it has not any antimicrobial properties to prevent infection (Maneerung et al., 2007; Maria et al., 2010; Saibuatong et al., 2010; Zhang et al., 2011).

Therefore, it cannot be used directly in treating infections but should be treated with bioactive ingredients in order to form polymer composites.

The trend is nowadays towards the development of organic polymers using natural materials. Thus, the possibility of replacing synthetic polymer composites, with ecological composite materials obtained from natural polymers, harmless environment and human health, represents an important objective for the current period. Following these principles, this paper presents some of our studies carried out in order to obtain new eco-friendly composite biomaterials based on BC and valuable natural products such as sericin, propolis and royal jelly with appropriate biomedical applications. The main objective consisted in the synthesis and characterization of bacterial cellulose - based composites (BC) combined with natural products, functionalized for biocompatibility, antimicrobial properties and healing chronic wounds.

In this sense, the possibility of obtaining new composite biomaterials with improved properties will prove as an interesting solution for achieving green composites BC, satisfying the need to explore minimal cost, biodegradable and renewable materials.

MATERIALS AND METHODS

Bacterial Cellulose composite materials

In the present study, we have explored a novel biomaterial, and we prepared different bacterial cellulose composites (BC); 1) Pure BC, 2) BC with sericin, 3) BC with propolis and 4) BC with royal jelly.

Bacterial cellulose nanocomposites were obtained by immersion of sericin, propolis, royal jelly into culture media (in situ).

Bacterial cellulose (BC) has been obtained as pellicle in our laboratory, from *Acetobacter xylinum* DSMZ-2004. The culture medium used for the fermentation of *A. xylinum* DSMZ-2004 (German Collection of Microorganisms and Cell Cultures) contained an extract obtained from inadequate quality apples, 7.5% glucose, 2% glycerol, 0.2% ammonium sulfate, 0.5% citric acid, and various amounts of sericin, propolis and royal jelly, with the pH being adjusted to 5.5 by acetic acid.

The culture media prepared in 500 mL Erlenmeyer flasks was sterilized by autoclaving at 121°C, for 15 min and then it was inoculated with 10% (v/v) *A. xylinum* DSMZ-2004 inoculum. A single *A. xylinum* colony grown on agar culture medium was transferred to a Petri dish filled with liquid glucose medium and incubated for two days to create a cell suspension. Then, the cell suspension was introduced into the sericin, propolis, royal jelly-dispersed culture medium at 30°C and incubated for 14 days. That sericin, propolis, royal jelly-incorporated BC membrane which was biosynthesized in the medium (in situ) was purified by 1N sodium hydroxide for 2 days at 30°C, in order to remove the cells included in the pellicles. The pellicles were then immersed in water solution of NaN_3 (0.02%) to reduce microbial contamination, neutralized with 1% acetic acid and washed repeatedly with distilled water until its pH was 7.0 and finally, stored at 4°C.

BC/sericin, BC/propolis, BC/royal jelly membranes were obtained with 1%, 1%, and respectivelly 3% sericin, propolis and royal jelly content.

Microbiological studies

Test organisms

The tests were carried out on three microorganisms: a Gram-negative bacterium (*Pseudomonas aeruginosa* ATCC 9027) and two Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 12228).

Inoculum preparation

The three test-strains were grown on Casein soya broth agar medium (CaSoA). Before each experiment, the strain was activated by passaging the cells on CaSoA and incubated for 18-24 hours at 30-35ºC. When the bacterial culture was optimal, with a sterile loop, it was added in sterile purified water in order to obtain a bacterial suspension with a concentration of 108-109 colony forming units (CFU) /mL.

Antimicrobial assay

The tests were performed in sterile Petri dishes, each of them containing 15-20 ml of culture medium previously inoculated with 104-105 CFU/ml. On each dish, 3 or 4 samples (cut with a pair of sterile scissors) of approximately 20-25 mm diameter were placed on the solidified surface of the medium. The Petri dishes were incubated 18-24 hours at 30-35°C. After the incubation period, the growth inhibition zones were measured and the microbial growth was assayed in the contact zone between the sample (BC composite) and the agar media.

The microbial growth was assayed in the following manner:

- no growth - „none”;
- some colonies, but less that the control sample - „weak”;
- same as the control sample - „important”.

RESULTS AND DISCUSSIONS

The analysis of the experimental results presented in the Table 1 shows the antibacterial effect of our BC- samples on all those three strains tested, the lowest effect being of the Sample 4 (over the two strains of *Staphylococcus*) because these samples are missing the inhibition zone, the antibacterial effect being limited to the contact area between the material and the environment culture. However, we can see a certain antibacterial effect of all the samples over *Pseudomonas aeruginosa*, especially of samples BC-1, 3 and 4, with the inhibition zone measuring between 4,0- 4,5 mm (Figures 1-4).

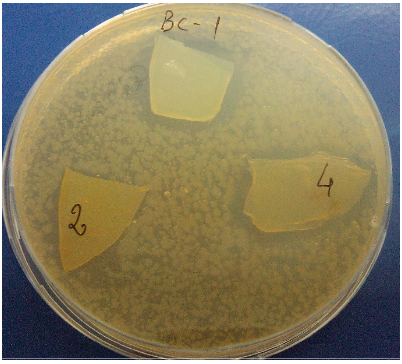

Figure 1. Antimicrobial activity of BC films

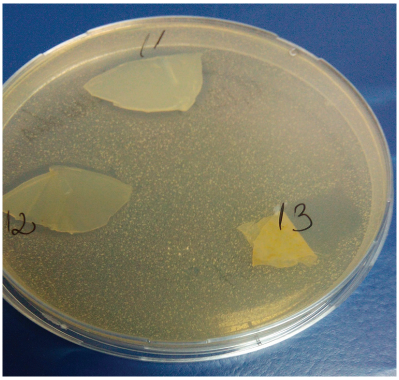

Figure 3. Antimicrobial activity of composite films BC-propolis 1%

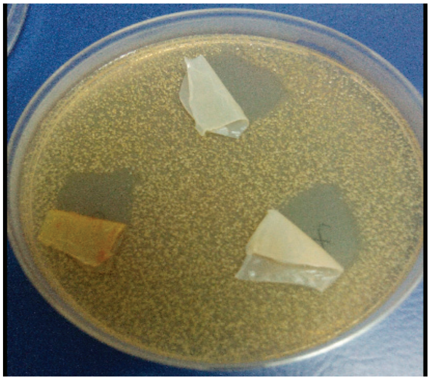

Figure 2. Antimicrobial activity of composite films BC-sericin 1%

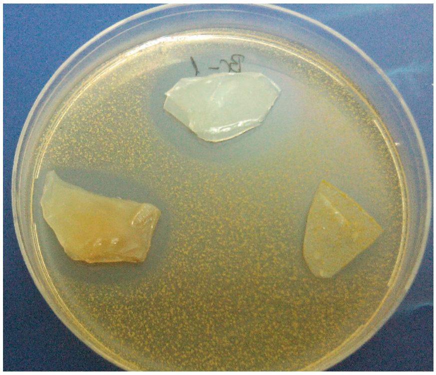

Figure 4. Antimicrobial activity of composite films BC-royal jelly 3%

Table 1. The antimicrobial activity of bacterial cellulose composites on *S. epidermidis, S. aureus* and *P. aeruginosa*

Sample	Test - Organism Bacterial strain	Inhibition Zone (mm)	Breeding microbial (contact area)
1) Pure BC BC-1	*S. epidermidis* ATCC 1228	4	None
	S. aureus ATCC 6538	6	None
	P. aeruginosa ATCC 9027	4	None
2) BC with sericin 1%	*S. epidermidis* ATCC 1228	2.5	None
	S. aureus ATCC 6538	2	None
	P. aeruginosa ATCC 9027	3	None
3) BC with propolis 1%	*S. epidermidis* ATCC 1228	3	None
	S. aureus ATCC 6538	4.5	None
	P. aeruginosa ATCC 9027	4.5	None
4) BC with royal jelly 3%	*S. epidermidis* ATCC 1228	0	Weak
	S. aureus ATCC 6538	0	None
	P. aeruginosa ATCC 9027	4	None

CONCLUSIONS

Three types of eco-friendly BC composite materials were presented in this study. The matrix of composites is the Bacterial Cellulose (BC), while the reinforcements of BC composites are represented by sericin (BC/sericin), propolis (BC/propolis) and royal jelly (BC/royal jelly).

All composites were obtained by static culture method, using valuable ingredients added directly into the biocellulose culture medium (in situ modification).

All of these three presented BC composite materials had an obviously antibacterial effect on all three strains tested.

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