Preparation and Characterization of Antimicrobial Activity Bacterial Cellulose Membrane

Wei-Hua TANG[2], Shi-Ru JIA[1], Yuan-Yuan JIA[1]

[1]Key Laboratory of Industrial Microbiology, Ministry of Education, Tianjin University of Science and Technology, Tianjin 300457, China.

[2]Department of Chemistry and Chemical Biology, Tianjin Modern Vocational Technology College, Tianjin 300350, China.

Keywords: Bacterial cellulose, ε-poly lysine, Antimicrobial activity, Characterization.

Abstract. Bacterial cellulose (BC) shows good potentiality as active packaging material. However, bacterial cellulose itself has no antimicrobial activity to prevent microbial contamination. To achieve antimicrobial activity, ε-poly lysine (ε-PL) was incorporated into BC by immersing BC in ε-PL solution. Investigations into the effects of ε-PL concentrations and contact times on incorporation of ε-PL into cellulose membranes showed that the lowest ε-PL concentration and the shortest time for production of an effective antimicrobial cellulose membrane (ε-PL/BC) were 400 mg/l and 2 hours, respectively. The antimicrobial cellulose membrane was characterized by FTIR, XRD and AFM. It was found that ε-PL is incorporated into the BC network, and the antimicrobial cellulose membrane is not a simple mixture of BC and ε-PL. In this study ε-PL/BC membranes are demonstrated to have potential applicability as antimicrobial packaging films.

Introduction

Cellulose (ß-1→4-glucan) is the main component of plant cell wall. It is also can be produced by some *Acetobacter* strains, represented by *Gluconacetobacter xylinus* (=*Acetobacter xylinum*), called bacterial cellulose (BC) [1]. Compared with plant cellulose, BC is receiving great attention and presently being widely investigated as a new type of material due to its fine fiber network, biocompatibility, high water holding capacity and high tensile strength [2].

Base on the unique absorption properties of BC, many researchers incorporated materials into bacterial cellulose films so as to enhance the performance of the bacterial cellulose. Maneerung [3] impregnated the silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. Nguyen [4] developed BC membrane containing Nisin by immersing bacterial cellulose in Nisin solution. Luo [5] prepared a novel collagen-bacterial cellulose (COL/BC) composite by adding collagen to the culture medium of *Acetobacter xylinum*.

ε-Poly-L-lysine (ε-PL) is a basic homo-polymer that consists of 20 to 30 residues of L-lysine with an ε amino group-carboxyl group linkage [6]. ε-PL shows a wide range of antimicrobial activity and is stable at high temperatures and under both acidic and alkaline conditions. As a kind of natural food preservative, ε-PL become more and more popular for its natural, innocuous and antimicrobial property [7, 8]. Bacterial cellulose is an interesting material for active packaging films. But bacterial cellulose itself has no antimicrobial activity to prevent microbial contamination. In this study, the authors aim to prepare antimicrobial BC membrane by immersing BC in ε-PL for serving as higher value products.

Materials and Methods

Bacterial Strain

Gluconacetobacter xylinus (TCCC No.12003), *Escherichia coli* and *Staphylococcus aureus* in this study was obtained from Tianjin University of Science & Technology Culture Collection Center (TCCC), China.

Preparation of BC Membranes

The medium for inoculums preparation and membrane formation used consisted of the following (v/v): carbon source 2.5 %, peptone 0.75 %, yeast extract 1 %, disodium phosphate 1 %, acetate acid 1 %, initial pH value 6.0. For seed culture, colonies of *G.xylinus* were inoculated into 100 ml of medium in a 500 ml flask shaken at 160 rpm and cultured at 30 ℃ for 24-30 hours. Fermentation culture performed in 90 mm (i.d.) petridishes containing 25 ml medium, was incubated statically at 30 ℃ for 5 days. After incubation, BC membranes were harvested and washed with water, and then boiled in 0.1 M alkali for 30 min, neutralized with 1 % acetic acid and washed with distilled water, successively. It was then dried in an oven at 80 ℃.

ε-PL Absorption

The purified cellulose membranes were soaked in 50 ml of ε-PL solution in 250 ml bottles to allow absorption of ε-PL. After soaking, the cellulose pieces were removed from the ε-PL solution and immersed briefly in sterile distilled water to remove non-absorbed ε-PL [4]. The membrane were then immersed briefly in 15% glycerol to make them flexible and dried at 80 ℃.

Antimicrobial Activity Assay

The antimicrobial activity of BC membrane after ε-PL absorption was determined by a modification of assay of Li [9]. Briefly, *Escherichia coli* or *Staphylococcus aureus* was grown in a nutrient broth (peptone 10 g, beef extract 3 g, NaCl 3 g in distilled water 1000 ml, pH 7.2-7.4) at 37 ℃ for 24 hours. Then they obtained fresh culture was ready for antimicrobial test. 0.2 ml of the fresh culture was inoculated into broth medium (9.8 ml) containing cellulose membrane (reference) or ε-PL/BC membrane (0.03±0.005 g) at 37 ℃ for 24 hours. The numbers of *Escherichia coli* and *Staphylococcus aureus* were determined by spread plating on Broth agar. Colonies were counted and the number of bacteria was expressed as $CFU.ml^{-1}$.

Morphological and Structural Characterizations

Fourier-transform infrared spectroscopy (FTIR) characterization was conducted by VECTOR 22 Fourier-transform infrared spectrometer. FTIR spectra were recorded in a spectral range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}. X-ray diffraction (XRD) analyses of samples were performed on an X-ray diffractometer (Rigaku D/max 2500). The BC membrane was directly observed by the AFM (JSPM-5200, Japan) at room temperature.

Results and Discussion

Preparation of the Antimicrobial BC membrane

Antimicrobial activities of the ε-PL/BC membranes prepared in this study against *Staphylococcus aureus* and *Escherichia coli* on broth plates can be seen in Fig. 1.

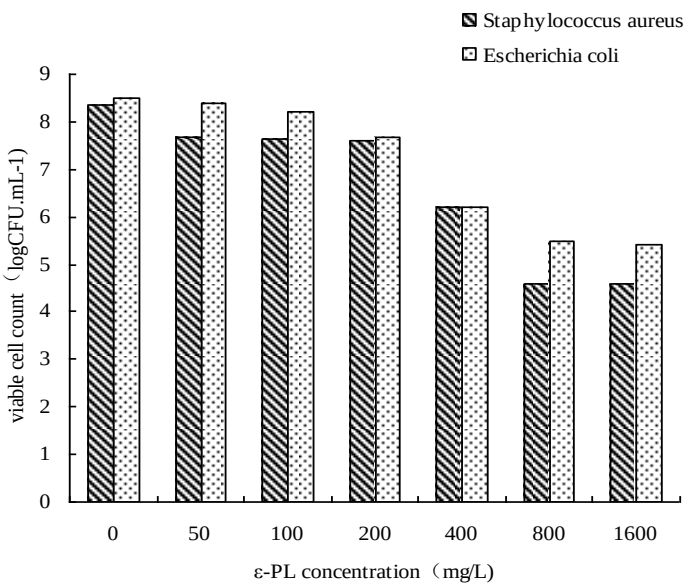

Figure 1. Antimicrobial activities of BC membranes immersed in seven ε-PL concentrations for 12 hours against *Staphylococcus aureus* and *Escherichia coli*

The viable cell count (VCC) of initial culture medium inoculums 2% of *Staphylococcus aureus* and *Escherichia coli* was ~6.39 and ~6.15 LogCFU.ml^{-1}, respectively; After 24 hours growing, the VCC was increased to ~8.34 and ~8.50 LogCFU.ml^{-1}, respectively. This shows that BC has no antimicrobial activity, see Fig. 2(a). By contrast, cellulose membrane immersing in ε-PL solution showed antimicrobial activity. When the ε-PL solution was 400 mg/l, the VCC was approximately the same with initial culture medium, this indicated that the BC membrane exhibited significant antimicrobial activity. Furthermore, with an increase in concentration of ε-PL, the LogCFU.ml^{-1} values decreased accordingly. Therefore, the lowest ε-PL concentration is 400 mg/l.

In order to investigate the effect of contact time with ε-PL solutions on antimicrobial activities of BC membranes, a solution of 400 mg/l was used with cellulose for different periods of time.

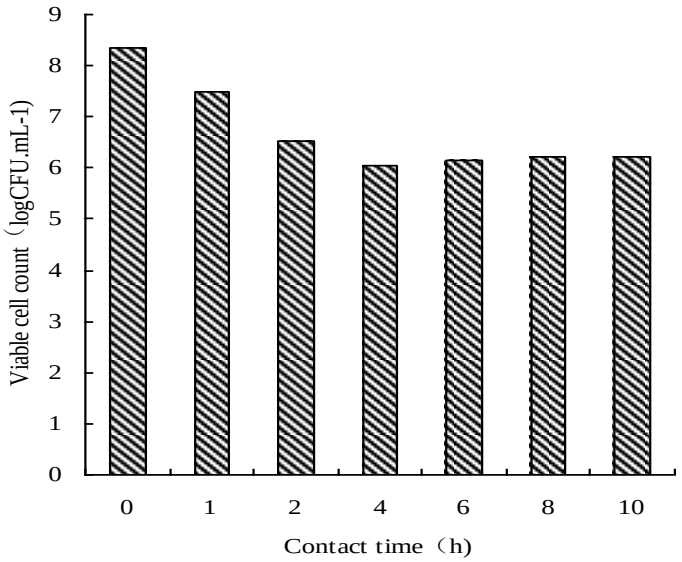

Figure 2. Antimicrobial activities of BC membranes immersed in 400 mg/l ε-PL for different periods of time against *Staphylococcu aureus* on broth medium

The results showed that the cellulose membrane exposed to ε-PL for less than 2 hours had no significant antimicrobial activity. While a contact time of more than 2 hours resulted in no significant change in LogCFU.ml^{-1} values, see Fig. 2. It was therefore apparent that 2 hours was the shortest period of immersing needed for preparation of antimicrobial BC membrane.

Characterization of the Antimicrobial BC membrane

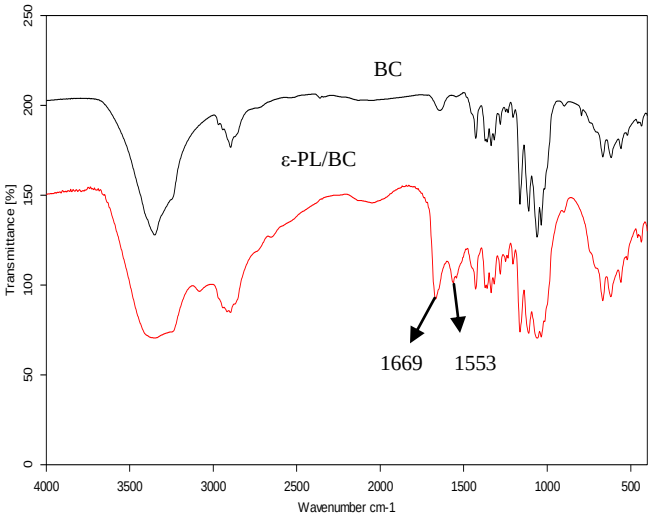

Figure 3. FTIR spectra of BC and ε-PL/BC

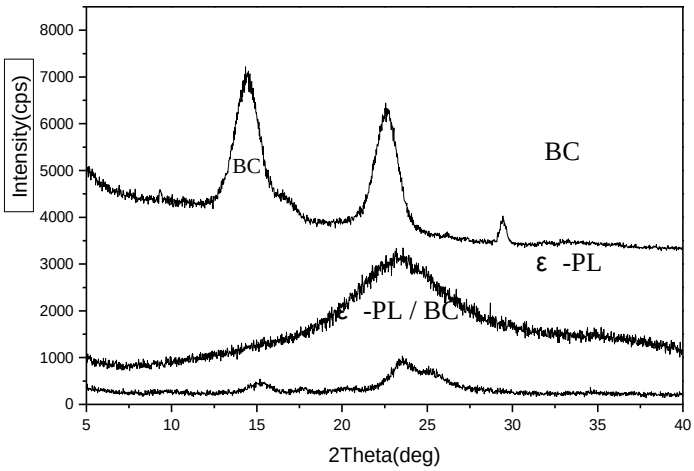

Figure 4. XRD patterns of BC, ε-PL and ε-PL /BC

FTIR spectra obtained from BC and ε-PL/BC are shown in Fig. 3. The spectrum of BC showed the absorption band assigned to the hydroxyl group and hydrogen bond at 3200–3500 cm^{-1} [10]. Usually, ε-PL displays bands at 1680-1640 cm^{-1} and 1580-1520 cm^{-1} [11]. Spectrum of ε-PL/BC in 1669 and 1553 cm^{-1} has two strong absorption peaks. This might confirm the incorporation of ε-PL into the cellulose network.

XRD patterns of various samples obtained in this study are shown in Fig. 4. The upper curve is a typical XRD pattern of BC. The three characteristic peaks locate at 14.460, 16.520 and 22.680 can be assigned to the BC planes of (1−1 0), (1 1 0), and (0 2 0), respectively. This is an agreement with the result of Keshk [12]. The broad diffraction peaks observed for BC is due to the fact that BC is not a completely crystalline material [5]. The middle curve only shows an extensive broadening peak in

the 2θ range of 20-27.5°, which is a XRD pattern of ε-PL. Note that the XRD pattern (the bottom curve) of ε-PL/BC is not a simple mixture of BC and-PL. ε-PL is incorporated into the network of BC.

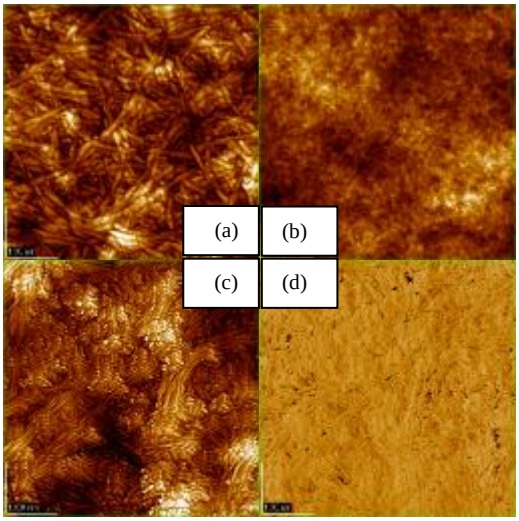

Figure 5. AFM images of BC, ε-PL and ε-PL/BC. (a) height image of BC (b) height image of ε-PL (c) height image of ε-PL/BC (d) phase image of ε-PL/BC

BC membrane is composed of highly entangled nano-fibrils with dimension of approximately 60~100nm, which is shown in Fig. 5(a). The height image of ε-PL is shown in Fig. 5(b).ε-PL uniformly distributed and embedded into the network of BC, as seen in Fig. 5(c) and Fig. 5(d). So they verified the conclusions from FTIR and XRD.

Conclusion

Antimicrobial BC membrane (ε-PL/BC membrane) has been obtained by immersing bacterial cellulose in ε-PL solution higher than 400 mg/l and least contact time 2 hours. The incorporation of ε-PL into the BC network is verified by FTIR, XRD and AFM, and the ε-PL/BC membrane is not a simple mixture of BC and ε-PL. As the ε-PL /BC membrane have dual characteristics of BC and ε-PL, it would be used as antimicrobial packaging for higher value products.

Acknowledgments

This work was funded by the National Basic Research Program of China (973. Program) under No. 2007CB714305.

Literature References

[1] Toda K, Asakura T, Fukaya M, Entani E, Kawamura Y. Cellulose Production by Acetic Acid-Resistant Acetobacter xylinum. J Fermen Bioeng, 1997, 84(3):228-231

[2] L.Hong, Y.L.Wang, S.R.Jia, Y.Huang, C.GAO, Y .Z. Wan. Hydroxyapatite/bacterial cellulose composites synthesized via a biomimetic route. Mater Lett, 2006, 60:1710-1713

[3] Maneerung T,Tokura S, Rujiravanit R.Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. Carbohyd Polym, 2008, 72(1):43-51

[4] Nguyen V.T, Gidley M.J, Dykes G.A. Potential of a nisin-containing bacterial cellulose film to inbibit Listeria monocytogenes on processed meats. Food Microbiol, 2008, 25:471-478

[5] H.L. Luo, G.G. Xiong, Y. Huang, F. He, Y.L Wang, Y.Z Wan. Preparation and characterization of a novel COL/BC composite for potential tissue engineering scaffolds. Mater.Chem.Phys, 2008, 110(2-3):193-196

[6] Shima S, Sakai H. Poly-L-lysine produced by Streptomyces. Part III. Chemical studies [J]. Agric Biol Chem, 1981, 45:2503- 2508

[7] Yoshida, T., Nagasawa T.ε-Poly-L-lysine: microbial production, biodegradation and application potential. Appl.Microbiol. Biotechnol. 2003, 62:21-26

[8] Hiraki J, Ichikawa T, Ninomiya S , Seki H, Uohama K, Seki H, Kimura S, Yanagimoto Y, J.Barnett Jr. Use of ADME studies to confirm the safety of ε-polylysine as a preservative in food. Regul.Toxicol. Pharmacol, 2003, 37:328-340

[9] Zhi Li, Xu Pin Zhuang, Xiao Fei Liu, Yun Lin Guan, Kang De Yao. Study on antibacterial o-carboxymethylated chitosan/ cellulose blend film from LiCl, N-dimethylacetamide solution. Polymer, 2002, 43: 1541-1547

[10] Choi Y-J, Ahn Y, Kang M-S, Jun H-K, Kim I S,Moon S-H. Preparation and characterization of acrylic acid-treated bacterial cellulose cation-exchange membrane. J. Chem. Technol. Biotechnol. 2004, 79(1): 79-84

[11] Shima S, Sakai H. Poly-L-lysine Produced by Streptomyces.Part II.Taxonomy and Fermentation Studies. Agric Biol Chem[J]. 1981, 45(II).2497-2502

[12] Keshk S, Sameshima K. Influence of lignosulfonate on crystal structure and productivity of bacterial cellulose in a static culture. Enzyme Micro Technol, 2006, 40: 4-8.