Modified Techniques to Detect Antibacterial Activity of Bamboo and Natural Bamboo Fiber

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Textiles that exhibit antibacterial properties are of great importance for health, sanitation, and consumer demands. Fibers that have antibacterial properties have been the target of developmental research, especially if it is a characteristic property of a natural fiber (Budama et al. 2013; Boothroyd and McCarthy 2011; Rocky and Thompson 2018; Comlekcioglu et al. 2017; Gokarneshan, Nagarajan, and Viswanath 2017; Simoncic and Tomsic 2010; Tanaka et al. 2011). Bamboo, which can be a source of fiber, has innate antibacterial properties (Rocky and Thompson 2019; Singh et al. 2010; Tanaka et al. 2011; Keski-Saari et al. 2008). However, production of bamboo fibers with natural antibacterial property has not been achieved at an industrial level. This leaves the market open to bamboo viscose which has at times been claimed to be antibacterial. Standard test methods established by ISO, ASTM and AATCC to assess antibacterial activity on textiles are not able to test specimens in various forms, such as fibers, powders, or yarns. The current methods rely on a zone of inhibition, which is less precise as it is hard to measure irregular inhibition zones, and cell recovery assessment after cell suspension with the specimen. These methods are also typically qualitative or only partially quantitative. Existing test methods may also lead to misunderstanding among manufacturers, retailers and consumers because of inconsistent grading and labelling of products (Boothroyd and McCarthy 2011; Williams et al. 2006). This study focuses on modifying suitable antibacterial test methods to provide more quantitative results. The modified methods were applied to specimens from four bamboo species, extracted natural bamboo fibers (NBFs) from the four species, commercial bamboo viscose, and three other conventional fibers.

Methodology

Four bamboo species: Bissetii, Giant Gray, Moso and Red Margin were collected from Lewis Bamboo, Inc., AL, USA. Reagents and auxiliaries were purchased from Sigma-Aldrich, and Consos, Inc. USA. The gram-positive bacterium *Staphylococcus aureus* (ATCC No. 6538), and the gram-negative bacterium *Klebsiella pneumoniae* (ATCC No. 4352) were used for the study. Comparison samples of bamboo viscose (12), rayon (1) and cotton (3) were collected from different retailers. Extraction of bamboo fibers was as follows: splitting → rasping/scraping → pressing-crimping/crushing/pounding → soaking → delignification → modification. Processing

chemicals were NaOH (2-20 g/L), $NaHCO_3$ (6-10 g/L), H_2O_2 (4-6 L/mL, 30% solution), $Na_5P_3O_{10}$ (5-20 g/L), Na_2SO_3 (8-15 g/L), Na_2SiO_3 (10-20 mL/L), scouring agent (3-4 mL/L), bleaching agent (3-4 mL/L), and penetrating agent (4-5 mL/L) were applied at different stages with varied combination at 90 °C for periods (1-8 hours). The fibers were neutralized to account for any residual chemicals.

Antibacterial activity was tested with a modification of optical density (OD) by spectrophotometric, and viable plate counting (VPC) techniques. For OD, specimens were inoculated with overnight grown bacteria in tryptic soy broth (TSB) media followed by 24 hours incubation at 37 °C. For VPC, overnight grown culture was subjected to serial dilution, inoculation of 0.1 g samples in 10 mL culture, incubation for 1-6 h and 24 h, plating 0.1 mL on Tryptic Soy Agar (TSA) plates, followed by 20-24 h and 60-72 h incubation and counting.

Results

The OD technique was unable to provide precise distinction between the specimens and the control. VPC was able to a detect clear distinction in antibacterial activity of different samples quantitatively. *K. pneumoniae* was countable after 20-24 h incubation on TSA plates but *S. aureus* took longer (60-72 h) for colonies to grow into clearly countable sizes. Before plating on TSA plates, the incubation of inoculated culture for 1-6 h was found to be effective. Longer incubation of the culture killed almost all bacteria. After multiple repetitions, this study has shown that samples in multiple forms, such as crushed raw bamboo powder, fabrics, or NBF cut into pieces are acceptable to be used with the modified VPC technique. It was observed that 11 out of 12 commercial bamboo viscoses did not exhibit inhibition. Similar results were observed for cotton and regular rayon. While bamboo specimens showed some inhibition, it was higher for NBFs. Using the modified VPC technique it was observed that most of the bamboo and NBF specimens effectively presented a range of bacterial reduction of 8-95% against *K. pneumoniae* and 3-50% against *S. aureus* (Figure 1). Thus, our modified VPC technique would be able to detect quantitative results in antibacterial assessments for a variety of materials.

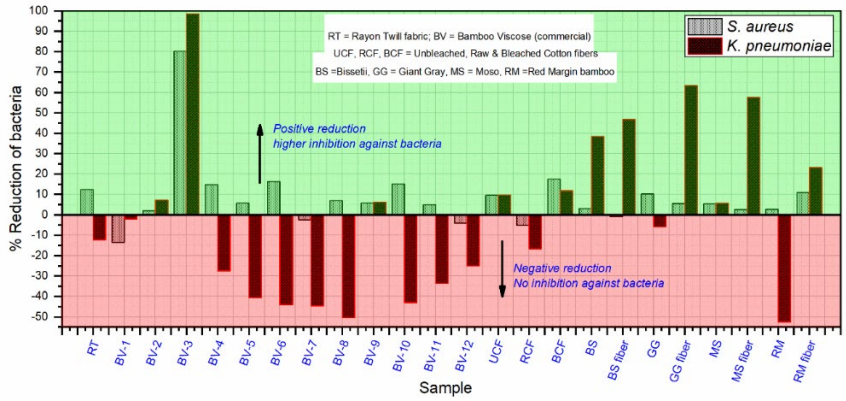

Figure 1. Antibacterial activity of bamboo, NBF, bamboo viscose and other fibers.

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