Resistance of Natural Bamboo Fiber to Microorganisms, and Factors that May Affect Such Resistance

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This study investigates the relative ability of natural bamboo fiber used in textile manufacturing to resist attack by bacteria and fungi. These tendencies were determined with the dynamic test method for evaluating antibacterial activity and were compared with the bacterial and fungal resistance of other textile fibers, such as cotton, jute, flax, ramie, and regenerated bamboo fiber. The bacteria studied were *Escherichia coli* (8099) and *Staphylococcus aureus* (ATCC 6538), and the fungal species was *Candida albicans* (ATCC 10231). The relationships between the bacteriostatic ability of natural bamboo fiber and its physical state, hygroscopicity, and extractives were tested to explore the possible influencing factors. The results show that natural bamboo fiber has no natural antibacterial properties, as compared with natural cotton bacteriostatic rates against the bacteria were all zero. The physical state did not impact the natural resistance of natural bamboo fiber to the bacteria and the fungus. The resistance of the plant fiber may be related to its hygroscopicity, and some extraction methods could improve the ability of natural bamboo to resist microorganisms.

Keywords: Natural bamboo fiber; Antibacterial properties; Influencing factors

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INTRODUCTION

As an abundant resource in China and an eco-friendly and multifunctional plant, bamboo has been used in architecture, agriculture, furniture, and papermaking for thousands of years. Recently, research on the production of textile fiber from bamboo has been conducted. According to different preparation techniques, bamboo fiber for textile use has been divided into two types: natural bamboo fiber and regenerated bamboo fiber (Li 2003). The natural bamboo fiber is usually in the form of a fiber bundle that is produced by unique chemical and physical techniques (Wang *et al*. 2005; Zhang *et al*. 2007). The regenerated bamboo fiber is made from bamboo pulp in a processing method similar to that used to produce ordinary viscose fiber (Zhou and Zhong 2003).

Clothing manufactured from regenerated bamboo fiber has entered the textile market and is touted for its antimicrobial properties. However, there is no scientific evidence to support this claim (Afrin *et al*. 2009). The successful separation techniques involving natural bamboo fiber have prompted reports that tout the high resistance of natural bamboo fiber against some kinds of bacteria (Xing and Liu 2004). According to online reports from some company, bamboo has a unique anti-bacteria and bacteriostasis bio-agent named "bamboo kun" which bonds tightly with bamboo cellulose molecules during the normal process of bamboo fiber growth and this feature gets retained in bamboo fabrics too. In addition, the antibacterial agent has been identified as an anthraquinone compound with α and β phenolic hydroxyl groups (Sun 2007). However, results from other studies have indicated that natural bamboo fiber has no significant antibacterial effect (Zhou and Deng 2005), and even if it does, it is just because of certain natural micro-structures, not any antibacterial constituents (Zhu *et al*. 2008). It is also noticeable that information regarding natural bamboo and regenerated bamboo fiber products is often confusing.

The main aim of this paper was to determine whether the claims that natural bamboo fiber have inherent antibacterial properties or resistance to microbial decay as a result of some kind of a chemical or the natural micro-structures can be proved. The first objective was to investigate and compare the resistance of natural bamboo fiber, cotton, jute, flax, ramie and regenerated bamboo fiber to selected microorganisms and to determine whether the resistance shown by the natural bamboo fiber is different from that of the ordinary plant fibers or the regenerated bamboo fiber. The second objective was to research the influence of some physical or chemical factors on the antimicrobial performance of natural bamboo fiber to establish whether these factors could contribute to improve the resistance of natural bamboo fiber.

EXPERIMENTAL

Materials

The materials used in the tests are shown in Fig. 1. Natural bamboo fiber used in this study was produced from *Neosinocalamus affinis* and prepared as follows: splitting → alkali degumming → acid rinsing → water rinsing → dewatering → shaking → drying → combing. Cotton, jute, flax, ramie, and regenerated bamboo fiber were purchased from a market. Some cotton fiber was treated with the antibacterial agent SCJ-2000 (produced by Beijing Jlsun High-tech Co. Ltd.), while the remaining cotton and all other fibers were untreated.

Fig. 1. Materials used in the tests. A: Natural bamboo fiber; B: Bamboo bundle; C: Bamboo powder; D: Regenerated bamboo fiber; E: Cotton fiber; F: Jute fiber; G: Flax fiber; H: Ramie fiber

The bamboo bundle used in the study came from the first step of the above process, and bamboo powder was obtained by grinding the natural bamboo fiber into 40- to 60-mesh powder. In this study, three specimens of each type of fiber were prepared for the series of tests.

Microorganisms and Media

The test organisms used were as follows: *Escherichia coli* (*E. coli*, 8099), *Staphylococcus aureus* (*S. aureus*, ATCC 6538), and *Candida albicans* (*C. albicans*, ATCC 10231), a gram-negative bacteria, a gram-positive bacteria, and a fungal species, respectively. A nutrient broth and nutrient agar culture medium were prepared for the bacteria, and Sabouraud's agar culture medium was used for the fungi culture. A phosphate buffer solution was used for dilution (PBS, 0.03 mol/L, pH 7.2 to 7.4).

Evaluation of Fiber Hygroscopicity

First, the moisture content of the fiber samples was conditioned to 20 °C and 65% RH, referring to National Standard GB 6529-86, *Textiles Standard Atmospheres for Conditioning and Testing*. Then, the samples were dried to a constant weight at 105±2 °C, referring to National Standard GB/T 9995-1997, *Determination of moisture content and moisture regain of textile-Oven-drying method*. The moisture regain was evaluated using Eq. 1,

$$W = \frac{G - G_0}{G_0} \times 100\% \tag{1}$$

where W is the moisture regain (%), G is the wet weight of the textile fiber (g), and G_0 is the dry weight (g).

Extraction

The determination method of extractives in fibrous raw material stipulated in National Standard GB/T 2677 was used to remove the extractives inside the natural bamboo fiber. The extraction solvents used in the test were cold water (room temperature), hot water (95 to 100 °C), benzene, ethanol, a 2:1 mixture of benzene and 95% ethanol (v/v), and 1% NaOH.

Antibacterial Test

The antibacterial activity was tested with a shake flask test, referring to National Standard GB/T 20944. 3-2008 *Textiles-Evaluation for antibacterial activity-Part 3: Shake flask method*. The effect of the bamboo's physical state on the antibacterial properties of natural bamboo fiber was investigated; untreated cotton was used as the negative control sample, and the antibacterial cotton was used as the positive control sample. The microbial resistance properties were evaluated by determining the bacteriostatic rate using Eq. 2. The effect of extractives on the antibacterial and resistance properties of natural bamboo fiber was also investigated; the bacterial growth condition in the flasks containing the extracted and unextracted natural bamboo fiber was compared. The effect of the extractives was evaluated by determining the antibacterial efficiency using Eq. 3. Negative numbers in the results were represented as 0,

$$Y = \frac{W_t - Q_t}{W_t} \times 100\% \quad (2)$$

where Y is the bacteriostatic rate (%), W_t is the average CFU (colony-forming unit) per milliliter for the flask containing the negative control sample after 18 h contact, and Q_t is the average CFU per milliliter for the flask containing the test sample after 18 h contact.

$$E = (1 - \frac{D_t}{D_0}) \times 100\% \quad (3)$$

In Eq. 3, E is the antibacterial efficiency (%), D_t is the average CFU per milliliter for the flask containing the extracted natural bamboo fiber after 18 h contact, and D_0 is the average CFU per milliliter for the flask containing the untreated natural bamboo fiber after 18 h contact.

RESULTS AND DISCUSSION

Antibacterial Characterization

The results of the antibacterial tests are shown in Table 1. The untreated cotton as the negative control sample was not effective against bacteria, while the antibacterial cotton was very effective against all test bacteria, with a bacteriostatic rate of over 99% against *E. coli* and 100% against *S. aureus* and *C. albicans*, indicating the dependability of this test. The results showed that natural bamboo fiber was not effective against *E. coli*, *S. aureus*, and *C. albicans*, as the bacteriostatic rate against all of them was 0. Therefore, the claims that natural bamboo fiber have inherent antibacterial properties as a result of "bamboo kun" could not be substantiated in this investigation. By comparison, the bacteriostatic rate of ramie against *S. aureus* was over 90%, and that of regenerated bamboo fiber was 75.8%. Jute and flax had bacteriostatic rates against *C. albicans* of 48% and 8.7%, respectively.

Table 1. Results of the Antibacterial Test

Fiber Type	Bacteriostatic Rate (%)		
	E. coli	*S. aureus*	*C. albicans*
Untreated Cotton	0	0	0
NBF	0 (-68.9)	0 (-13.2)	0 (-41.3)
Jute Fiber	0 (-15.9)	0 (-48.4)	48
Flax Fiber	0 (-45.0)	0 (-88.8)	8.7
Ramie Fiber	24.3	90.2	54
RBF	41.4	75.8	0 (-12.8)
Antibacterial Cotton	>99	100	100
Note: NBF = natural bamboo fiber, RBF = regenerated bamboo fiber (the same below)			

The microbial population density on each sample after shaking for 18 h was investigated to compare the inhibition of microbial growth of each kind of textile fiber (Fig. 2). It is clear that, compared with natural cotton, the population density of *E. coli* and *C. albicans* in natural bamboo fiber increased greatly after 18 h. This indicates that natural bamboo fiber provides a more suitable environment for the growth of the microorganisms. On the contrary, all the microorganisms' density in ramie and *E. coli* and *S. aureus* density in regenerated bamboo fiber decreased greatly. This confirms that both ramie and the regenerated bamboo fiber have an antimicrobial effect. It should be noted, however, that only a percentage of the microbes was killed and not 100%. The antimicrobial performance of regenerated bamboo fiber may mainly come from the use of a large amount of chemicals, such as sulphur, in the manufacturing process (Zhang 2008; Sun 2004), and the antibacterial performance of ramie has been attributed to pyrimidine, purine, or other antibacterial components (Shao 2000).

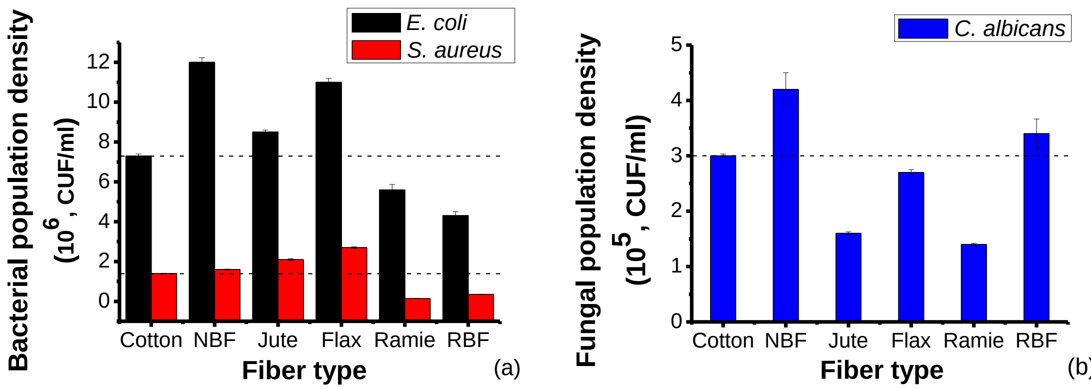

Fig. 2. Bacterial population density on each sample after shaking for 18 h

The variance analysis done on the microbial-growth counts showed that both the fiber type and the microorganisms type had a significant influence on the results (Table 2). Further statistical evaluation with multiple comparison for the factor of fibers was carried out by the Bonferroni test. The statistical result indicated that the results obtained for the natural bamboo fiber differed significantly from those obtained for other fibers except flax (Sig. =1.000) . This demonstrated, statistically, that the bacteriostatic ability of the natural bamboo fiber was similar to that of flax.

Table 2. Variance Analysis of Anti-bacterial Effect (colony count) Measured on Different Fibers on Different Microorganisms

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.35E+14 [a]	17	4.91E+13	3860.923	0.000
Intercept	5.89E+14	1	5.89E+14	46331.220	0.000
microorganisms	6.69E+14	2	3.35E+14	26302.846	0.000
fibers	8.21E+13	5	1.64E+13	1290.554	0.000
bacteria * fiber	8.37E+13	10	8.37E+12	657.722	0.000
Error	4.58E+11	36	1.27E+10		
Total	1.42E+15	54			
Corrected Total	8.35E+14	53			
a R Squared = .999 (Adjusted R Squared = .999); Confidence intervals are 95%					

Effect of Bamboo's Physical State

The bacteriostatic rates of bamboo in different physical states were all 0, as shown in Table 3, and the population density of the same bacteria with different physical states of bamboo were nearly equal (Fig. 3). It can be inferred from these data that the physical state did not have an impact on the antimicrobial activity of natural bamboo fiber.

Table 3. Bacteriostatic Rate with Different Physical States of Bamboo

Bamboo Shape	Bacteriostatic Rate (%)		
	E. coli	*S. aureus*	*C. albicans*
Bundle	0 (-69.0)	0 (-75.0)	0 (-45.5)
Fiber	0 (-68.9)	0 (-13.2)	0 (-41.3)
Powder	0 (-54.9)	0 (-50.0)	0 (-33.4)

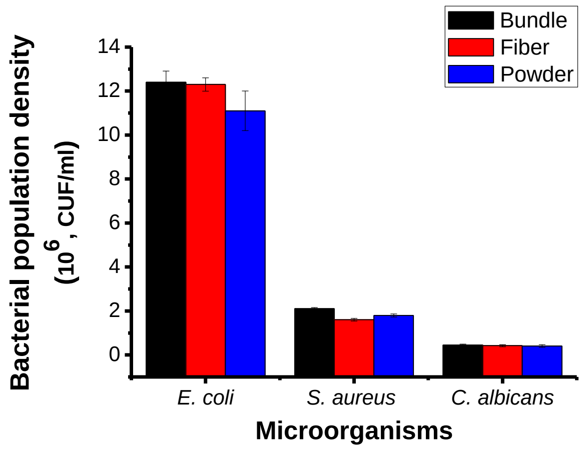

Fig. 3. Bacterial population density with different physical states of bamboo

Effect of Fiber Hygroscopicity

The moisture regains of different textile fibers are presented in Table 4, which shows that, with the exception of regenerated bamboo fiber, natural bamboo fiber exhibited the highest moisture regain amongst the plant fibers, while ramie showed the lowest moisture regain. In addition, Fig. 4 indicates the relationship between the moisture regain and bacteriostatic rate of the plant fiber. According to the figure, higher moisture regain in a fiber was correlated with a lower bacteriostatic rate against all bacteria. Some of the bacteriostatic rates had relatively good linear relationships, especially for the bacteriostatic rate against *E. coli*: its fitting correlation coefficient with moisture regain was 0.9480, with a 95% confidence level of there being a significant effect (Table 5). The moisture regain of fiber has been found to be inversely proportional to the degree of crystallization (Wang *et al*. 2009). Therefore, it could be inferred that there was a direct ratio relationship between the antibacterial property of the plant fibers and its degree of crystallization. However, the regenerated bamboo fiber did not follow this trend (there is no definite relationship between hygroscopicity and its bacteriostatic rate), which may result from its preparation process.

Table 4. Moisture Regain of Textile Fiber

Fiber	Moisture Regain (%)
NBF	9.80±0.003
Cotton Fiber	7.75±0.002
Flax Fiber	9.24±0.004
Ramie Fiber	6.81±0.001
RBF	12.09±0.010
Note: The number behind "±" means standard deviation	

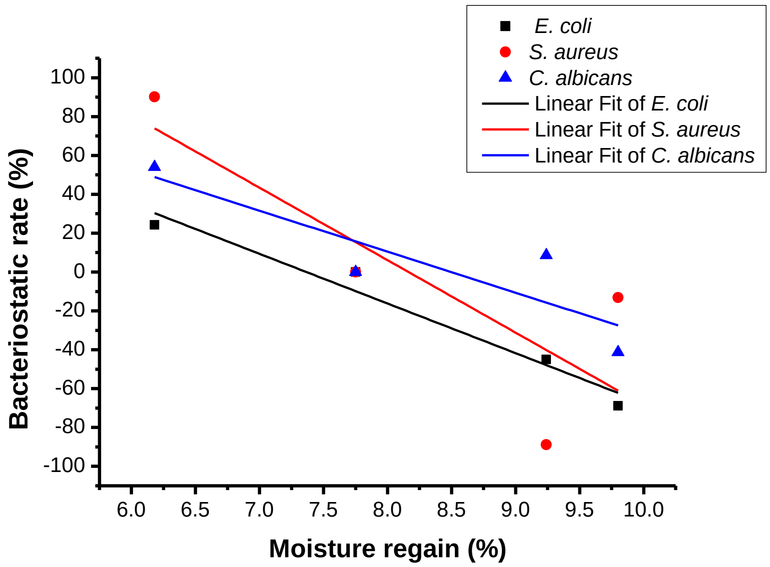

Fig. 4. The relationship between moisture regain and bacteriostatic rate

Table 5. Results of Fitting Statistics

Bacteria	R^2	F Value	Prob>F
E. coli	0.9480	55.70	0.0175
S. aureus	0.5214	4.27	0.1748
C. albicans	0.6532	6.65	0.1232

Effects of Extractives

It can be seen from Table 6 that extraction was effective in improving the antibacterial performances of natural bamboo fiber against *E. coli* and *S. aureus*, except when using benzene as the extraction solvent. Extraction using hot water was the most effective method in improving the antibacterial properties against *E. coli*, with an antibacterial efficiency of nearly 70%; using 1% NaOH was the best method against *S. aureus*, with an antibacterial efficiency of over 67%. However, removing extractives from natural bamboo fiber had no effect against the fungus *C. albicans*.

Table 6. Antibacterial Efficiency of Different Extraction Methods

Extraction Solvent	Antimicrobial Efficiency （%）		
	E. coli	*S. aureus*	*C. albicans*
Cold water	64.35	10.91	0
Hot water	69.57	30.91	0
Ethanol	18.26	7.88	0
Benzene	0	0	0
Benzene/ethanol	4.35	36.36	0
1% NaOH	58.26	67.88	0

Cellulose, hemicellulose, and lignin are the major components of the natural bamboo fiber, and they account for more than 90% of the dry weight of the fiber. The contents of other chemical components are little, such as protein, fat, pectin, tannin, pigment, *etc*. Only a very minor amount of these components can be extracted by the extraction solvent. The components of cold-water soluble extract include monosaccharide, oligosaccharide, inorganic salt, water-soluble pigment, a small amount of tannin and amino acid; in addition to these, hot-water soluble extract may include some kinds of polysaccharides such as amylum and pectin; organic solvent extract usually includes a small amount of fat, wax, resins, essential oil, sterols, tannin, pigment, and fatty acids; 1% NaOH extract may include tannin, pigment, alkaloid, soluble mineral component, some kinds of carbohydrate, amylum, pectin, protein, amino acid, a part of hemicellulose and lignin, and a little of essential oil (Higuchi 1987; Li 1990; Suzuki and Itoh 2001; Jiang *et al*. 2006).

The influence of extraction on the antibacterial properties of the natural bamboo fiber may be attributed to a few reasons. First, the removal of some natural bamboo fiber extractives, such as carbohydrates and inorganic salts (which are microbial growth nutrients) (Higuchi 1987), could help slow bacterial growth and improve the fiber's microbial resistance. Secondly, the extractives may contain some antibacterial or antifungal components, and the reduction of the extractives may decrease the microbial resistance of the natural bamboo fiber at the same time. Finally, changes in chemical composition changed the pH value of the fiber, and this change could affect bacterial growth (Sun *et al.* 2006). Therefore, it can be inferred that the combination of these three aspects led to the influence of extraction on the microbial resistance and antimicrobial properties of the natural bamboo fiber.

CONCLUSIONS

1. Compared with natural cotton fibers, natural bamboo fiber has no natural antibacterial ability, which is similar to what had been found for flax fiber. Ramie fiber, by contrast, exhibits some inhibitory action against all three test bacteria, especially against *Staphylococcus aureus*, with the bacteriostatic rate as high as 90.2%. In addition, regenerated bamboo fiber has an inhibitory effect on bacterial growth but has no effect on fungi.
2. The fact that the bacterial growth on bamboo in different physical states was nearly equal indicates that the physical state do not have an impact on the microbial resistance of natural bamboo fiber.
3. The linear relationship between the moisture regain and the bacteriostatic rate suggests that the microbial resistance of the plant fiber may be related to its hygroscopicity.
4. Some extraction methods could improve the performance of natural bamboo fiber against bacteria.

ACKNOWLEDGMENTS

The authors are grateful for the support of the National Scientific and Technical Supporting 12th Five-year Plan Project No. 2012BAD23B01 and 11th Five-year Plan Project No. 2006BAD19B07.

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Article submitted: June 13, 2013; Peer review completed: August 5, 2013; Revised version received: October 25, 2013; Accepted: October 26, 2013; Published: October 31, 2013.