

## Analysis of Microbiological Contamination in the Chosun Dynasty Textiles Exhumed from Hwasung Kupori Burial

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### Abstract

*The purpose of this research was first to analyse and compare the types of microbes inherent among the unwashed and washed Kupori textiles, and second to investigate whether there is a difference between unwashed and washed Kupori textiles on the susceptibility of contamination by microorganism when exposed to the same microbial environment. Microbial identification procedure and the Shake Flask Test for investigating the effect of exposure to microbial environment were carried out separately. The result of microbial identification procedure indicated that a variety of bacteria and fungi were inherent in both unwashed and washed textiles and that the population of contaminated microorganism became more diverse after washing. The result of Shake Flask Test indicated that given the same exposure condition, the unwashed textiles tend to be more susceptible to bacterial contamination than the washed textiles. The results of the present study supported the current conservation procedures adopted in Korean museums which include washing and humigation procedures before long-term storage or display of exhumed textiles.*

*Key words : exhumed textiles, Hwasung Kupori burial, contamination of microorganism, Shake Flask Test, textile conservation.*

### I. Introduction

Among many different relics unearthed from archaeological sites, a textile remain is one of the most fragile object which can easily lose its original appearance after it is recovered to the living environment. A sudden exposure to sunlight and handling after a prolonged burial condition, conservation treatments at the museum, a long-term display or storage, all contribute to physical and chemical degradation to different degrees on the

part of the textile. Partial extension, tearing, and (or) embrittlement may occur as well as color change such as fading into a lighter tone or a change of hue. Major factors which affect such degradation are light, humidity, heat, chemicals or activities of microorganism.<sup>1)</sup> The factors the textile remain is affected most is dependent upon the type of environment the textile had been buried, the type of conservation treatments, and the museum display or storage conditions.

Conservation treatments given to the exhumed textiles include washing, mending, resto-

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<sup>1)</sup> J. W. S. Hearle, B. Lomas, W. D. Cooke, and I. J. Duerdon, *Fibre Failure and Wear of Materials: An Atlas of Fracture, Fatigue and Durability*. (New York: John Wiley & Sons, 1989), 407-427.

ration, and disinfection, Among these, washing can be specifically classified into surface cleaning in which surface debris are eliminated with a light mechanical action, wet cleaning using water or detergent solution, and dry cleaning using solvent<sup>2)</sup>. Surface cleaning is carried out from simple brushing to vacuum cleaning. This method, when done correctly, is free of a sudden change of pH or fiber damage which are expected when detergents or chemicals are used<sup>3)</sup>. However, the exhumed textiles are in most cases severely soiled that surface cleaning alone is insufficient for restoring cleanliness. When the soils are not well-removed, additional degradation by microorganism may occur during long-term storage. However, one of the drawbacks encountered with wet cleaning the textile remains is the possible fiber damage which may result in the weakening of tensile strength and additional color loss.

Studies have shown that wet cleaning is more effective than dry cleaning, and detergent solution more effective than water alone in removing the soils in an exhumed textile<sup>4,5)</sup>. However it has also been reported that wet cleaning causes more damage to the tensile strength and color loss, and detergent solution causes more damage than water alone<sup>6)</sup>. Therefore, a conservator of a textile object must consider both problems inherent in washing and unwashing the textile and select the type of washing technique appropriate for each piece. In order for the conservator to

select an appropriate conservation treatment, diverse data such as the effect of washing or environmental causes should be offered by active research efforts. This research focused on the bacterial contamination of exhumed textiles in whether or not there is a difference in the susceptibility of bacterial contamination between washed and unwashed textiles. The textiles examined in this research were exhumed on June 9th, 1994 from a Chosun Dynasty burial located at Kupori Hwasung-kun, Kyunggi-do<sup>7)</sup>.

The Kupori textiles which date back to the late 17th century were exhumed by the cooperative work of Sukjoosun Memorial Museum and the Central Museum of Dankook University. Nearly 50 pieces of clothing were recovered from the wooden coffin which had a double casket construction with a firm lacquer coating. The clothing were preserved in a fairly good condition with little visual destruction of the form. The staffs of the museum granted the author the permission to collect washed and unwashed samples from the Kupori textiles. Prior investigation of the Kupori samples using optical microscopy and Fourier Transform Infrared Spectroscopy (FTIR) confirmed that the samples were all made of silk and the microbiological analysis of the unwashed samples indicated that the samples were heavily contaminated with microbes<sup>8)</sup>. Additional microscopic analysis on the Kupori textiles in comparison to the modern household-stored textiles suggested diff-

<sup>2</sup> Sheila Landi, *The Textile Conservator's Manual*, 2nd ed. (Oxford, Butterworth-Heinemann, 1992), 79-97.

<sup>3</sup> Sheila Landi, *ibid.*, 79-80.

<sup>4</sup> C. Ahn and H. K. Cho, Analysis of nonfibrous matters in the textiles exhumed from Keumreung-Ri, Pajoo -Application of KS test method. *Journal of the Korean Society of Clothing and Textiles* 22, 6(1998): 772-780.

<sup>5</sup> S. H. Bae, A study on conservation science of exhumed textiles, Dissertation, Seoul Women's University, 1999.

<sup>6</sup> S. H. Bae, *ibid.*

<sup>7</sup> C. K. Han, Structure and artifacts of Choi Sook burial of Kupori. *Korean Costume (韓國服飾)* 14 (1996):1-25.

<sup>8</sup> C. Ahn, H. K. Cho, and J. W. Kim, Analysis of non-fibrous materials in the exhumed textiles from the Hwasung Kupori site. *Hankook Boksik (韓國服飾)* 14 (1996):34.

erences in the morphological degradation between the exhumed and the modern textiles—the degradative features of the exhumed textiles being those due to the microbial activities during long-term burial condition<sup>9</sup>. For example, the bitten hole structure and the ductile type fracture were found often among the Kupori textiles and not among the modern samples and these were highly likely evidences of microbial activity<sup>10,11</sup>. Based on the results of prior research, this research is aimed to investigate the difference in the susceptibility of bacterial contamination between washed and unwashed samples of the Kupori textiles.

Other research efforts on museum textiles and their contamination by microorganism are few in number. Min and Ahn<sup>12</sup> investigated the types and the degree of fungal contamination in the paper and textile objects and the museum display cases containing them. Among the types of fungi they identified *Cladosporium cladosporioides*, *Acremonium* sp, *penicillium*, *Aspergillus* *Trichoderma* etc. were the most dominant types<sup>13</sup>. Min<sup>14</sup> reviewed and summarized classic research results on the mechanism of chemical degradation in cellulosic fibers. Bae<sup>15</sup> reviewed previous literature on the effect of humidity and heat on the microbiological degradation of museum textiles. Kim<sup>16</sup> and Lee<sup>17</sup> explained the te-

chniques of humigation for protecting museum objects from biodegradation. Spivak and Worth<sup>18</sup> investigated the effect of pesticidal chemicals on the museum textiles themselves and found out that exposure to some of the chemicals such as  $CCl_4$ (fumigant) or dichlorvos(pest strip) affected a slight color change in natural dyed wool and also resulted in loss of breaking strength to a certain degree.

This research is part of a continuing effort on the identification and analyses of exhumed textiles represented by the Kupori textiles. Through this research the researchers hope to present an in-depth investigation of microbial contamination of the exhumed textiles by a collaborative work of professionals in two different academic fields.

## II. Research Method

The purpose of this research was twofolds. First, to analyse and compare the types of microbes inherent among the unwashed and washed Kupori textiles. And second, to investigate whether there is a difference between unwashed and washed Kupori textiles on the susceptibility of contamination by microorganism when exposed to the same microbial environment. To meet the above purposes, microbial identification procedure and the

<sup>9</sup> C. Ahn, Fiber degradation analysis of the textiles from the Hwasung Kupori Site. *Journal of Asian Regional Association for Home Economics* 4, 1(1997):10-19.

<sup>10</sup> C. Ahn, *ibid.* 16-18.

<sup>11</sup> J. W. S. Hearle, B. Lomas, W. D. Cooke, and I. J. Duerdon, *ibid.* 407-427.

<sup>12</sup> K. H. Min and H. K. Ahn, Analysis of microorganism in textile and paper heritages-Focused on the Kyungnam province. *Cultural Heritage (文化財)* 14 (1981):131-144.

<sup>13</sup> Min and Ahn, *ibid.* 141.

<sup>14</sup> Kyung H. Min, Degradation of textile artifacts by microorganism. *Conservation Science (保存科學研究)* 5 (1984):24-36.

<sup>15</sup> Sang K. Bae, A study of factors affecting fiber degradation in museum textiles. *Conservation Science (保存科學研究)* 11 (1990):3-14.

<sup>16</sup> K. S. Kim, A practical application of humigation. *Conservation Science (保存科學研究)* 5 (1984):192-205.

<sup>17</sup> H. B. Lee, Protection of heritage against biodegradation. *Conservation Science (保存科學研究)* 13 (1992):83-97.

<sup>18</sup> S. M. Spivak and J. Worth, Assessing the effects of pesticidal chemicals on historic textiles. Preservation of paper and textiles of historic and artistic value II, *Advances in chemistry series* 193, (American Chemical Society, 1981). 333-343.

Shake Flask Test for investigating the effect of exposure to microbial environment were carried out separately.

### 1. Samples

The exhumed clothing were brought to the Sukjoosun Memorial Museum immediately after the excavation. After they were aired, the clothing were kept in hardboard boxes (covered slightly with paper) for several weeks and then washed. Except for a few pieces which were dry cleaned by a professional dry cleaner, all the clothing were wet-cleaned (without detergent) by museum personnel using running tap water, gentle hand motion<sup>19</sup>. The clothing from which the samples were taken were all wet-cleaned.

Samples from unwashed textiles were collected during the summer of 1995 before washing was done. Samples from washed tex-

tiles were collected after they were wet-cleaned. Swatches less than 3cm×6cm in size were collected either by cutting out small pieces from hidden areas of the clothes (i.e. hemline) or by collecting scattered pieces from the storage box. In some of the samples taken from unwashed clothing, dark particles of soil were attached showing a clear visual contamination. The weave and yarn characteristics of the unwashed and washed Kupori textiles are specified in <Table 1><sup>20</sup>.

Identification of microbes were conducted on 4 samples of the unwashed textiles and 3 samples of the washed samples. Shake flask test was conducted on 3 unwashed samples and 3 washed samples. Except for sample 014 (washed) all the samples were cut from shroud clothing. For unwashed samples 006 and 010, microbial identification test was conducted only since the samples were too small

<Table 1> Characteristics of the Kupori Samples

Sample No.	Weave	Density (1cm <sup>2</sup> )	Yarn twist		Yarn thickness		Test	
			A <sup>1</sup>	B	A	B	ID	Shake
006	plain weave	26 × 40	low	low	medium	medium	o	
010	plain weave	38 × 28	low	low	medium	medium	o	
Unwashed 013	plain weave	- <sup>2</sup>	low	low	medium	medium		o
023a	plain weave	11 × 20	low	low	thick	medium	o	o
023b	plain weave	13 × 15	low	low	thick	thick	o	o
014	jacquard weave	26 × 24	low	low	medium	medium	o	o
Washed 015	jacquard weave	25 × 19	low	low	medium	thin	o	o
017	jacquard weave	27 × 29	low	low	medium	thin	o	o

<sup>1</sup> Since some of the Kupori textiles lacked the information on the warp and filling direction, the terms were not used for samples description.

<sup>2</sup> Fabric density was unmeasurable since the sample was in hardened into small bundle.

<sup>19</sup> S. S. Park. A study on the clothing exhumed from Susung Choi burial. *The Excavation Report of Susung Choi burial at Kupori*, Hwasung-gun, Kyunggi-do. The Central Museum of Dankook University, 1995.

<sup>20</sup> Due to a small sample size and also due to irregular widths in several of the samples, density in this study was counted within 1 cm<sup>2</sup> area, rather than within 1 inch×1 inch or 5 cm×5 cm area. High, medium, and low values of yarn twist in Table 1 were based on the relative ranking of the amount of twist among the samples rather than on the actual numbers of twist. The true yarn twist cannot be counted in archaeological textiles because of severe fragility of the samples upon handling and also because of the need to minimize the destruction of textiles.

to carry out the shake flask test. However, the test result obtained from sample 013 should be comparable to the result which might have been obtained from samples 006 or 010 since the three textiles were all part of the shroud clothing having the same history of burial condition.

### III. Methods

In this research, two different experiments were carried out. First, the identification procedure for the microbes in the samples, and second, the Shake Flask Test for investigating the effect of exposure to microbial environment between unwashed and washed samples.

#### 1. Identification of Microorganism

Contamination of Kupori textiles by microorganisms was analyzed by isolating bacteria and fungi from unwashed and washed samples. A small piece of each sample (1cm × 1cm) was placed on an agar plate using a sterile forceps and incubated at 25 or 37°C until colonies or growth appeared on the plates. Each pure colony was transferred to a fresh agar plate and subjected to various morphological and physiological tests for identification. The tests included Gram's staining, motility, spore formation, oxygen requirement, catalase, oxidase, nitrate reduction, MR-VP, fermentation of sugars, hydrolysis of organic compounds etc. All of the tests and identification of the isolates were carried out according to Bergey's manual<sup>21)</sup>.

#### 2. Shake Flask Test

Shake Flask Test was originally developed by the Dow Corning Company for measuring

the antibacterial function of antibacterial finished textiles. The test has been modified and adopted by the Japan S.E.K standard(學會法). Different test centers<sup>22)</sup> of Korea are utilizing the S.E.K method with the change of shaking period from original 1 hour to 12 hours in order to increase the sample-bacteria contact. In this research the Shake Flask Test was used following the S.E.K standard and the incorporation of 12 hours shaking period.

From each of the unwashed and washed samples a specimen weighting 0.75g was cut out. Then each sample was cut again into small pieces of less than 1cm × 1cm in size. Preserved *Staphylococcus aureus* was transplanted to brain heart infusion agar and cultured at 35–37°C for 24 hours and then again in nutrient broth for 6 hours by shaking culture. The shaken culture was adjusted to 52% T at 475nm using the UV/VIS Spectrophotometer. It was then diluted 1,000 times to make the inoculum. Prepared textile samples were inserted into 200ml erlenmeyer flasks filled with 70ml phosphate buffer solution, sterilized by an autoclave at 105°C (0.25lgf/cm<sup>2</sup>) for 10 minutes. The phosphate buffer solution was prepared as such. 34g of potassium monophosphate was dissolved in 500ml purified water, and 4% sodium hydroxide solution was added. Then, purified water was added to the above solution to obtain a 1,000ml of the stock solution (pH 7.2).

5ml inoculum was added to each of the sample flask and a blank flask. Blank flask was prepared in order to measure the number of living bacterial ("0" time) colonies before shaking action<sup>23)</sup>. Cotton fabric for the colorfastness of dye (KS K standard) was used for the textile specimen for blank flask. From the

<sup>21</sup> John G. Holt, Noel R. Krieg, Perter H. A. Sneath, James T. Staley, and Stanley T. Williams. *Bergey's manual of determinative bacteriology*, 9th ed., (Baltimore: Williams & Wilkins, 1994), 559-570.

<sup>22</sup> Korea Apparel Testing Research Institute and FITI Testing and Research Institute

<sup>23</sup> Japan S.E.K. (學會法). *Manual for Antimicrobial test method of antibacterial treatment for textile in S.E. K*, 1996.

blank flask 1ml of the solution was taken and a ten time dilution series were prepared. These diluted series were mixed with T.G.E. agar in petri dish and incubated at 35~37°C until the end of the experiment. These were labelled as [A].

Sample flasks and the blank flask were treated simultaneously with a Wrist Action Shaker at 25±5°C, 320 r.p.m. for 12 hours. The shaken solutions were then diluted 10 times successively with the phosphate buffer test solution to obtain a ten-time dilution series. 1ml of each diluted solution was mixed with T.G.E. agar in petri dish and incubated at 35~37°C for 24 hours. These were labelled as [B]. Using the cultured plates [A] and [B], the number of grown colonies was counted. The reduction rate of test bacteria was calculated as follows:

$$\text{Reduction rate} = \frac{A}{A-B} \times 100(\%)$$

Where A : average number of living bacterial colonies before shaking

B : average number of living bacterial colonies after shaking

#### IV. Results and Discussion

The followings are the separate results of identification procedure and the Shake Flask Test conducted on the unwashed and washed samples of the Kupori textiles.

##### 1. Identification of Microorganism

Forty-two bacterial colonies and a fungus were isolated from unwashed or washed samples, 24 from unwashed and 19 from washed (Table 2). Among them, the fungus isolated from one of the washed samples was a member of *Penicillium*. The bacterial colonies isolated from unwashed samples were identified as 12 species belonging to 3 genus

<Table 2> Microorganisms isolated and identified in this study

From unwashed samples	From washed samples
<i>B. circulans</i> (2)*	<i>B. brevis</i> (3)
<i>B. alcalophilus</i> (1)	<i>B. firmus</i> (1)
<i>B. licheniformis</i> (6)	<i>B. sphaericus</i> (1)
<i>B. subtilis</i> (4)	<i>B.adius</i> (1)
<i>B. polymyxa</i> (2)	<i>B. coagulans</i> (1)
<i>B. marinus</i> (1)	<i>S. saprophyticus</i> (1)
<i>B. macerans</i> (1)	<i>K. zopfii</i> (1)
<i>B. fastidiosus</i> (1)	<i>B. pumilis</i> (5)
<i>L. grayi</i> (1)	<i>B. lentis</i> (2)
<i>B. pumilis</i> (3)	<i>Caryophanon</i> (1)
<i>B. lentis</i> (1)	<i>Penicillium</i> (2)
<i>Caryophanon</i> (1)	
3 Genus 12 species	5 Genus 11 species

\* numbers in parentheses represent frequency of isolation.

including *Bacillus*, *Listeria*, and *Caryophanon*. *Bacillus licheniformis*, and *B. subtilis* were detected most frequently from the unwashed samples. Those isolated from washed samples were identified as 11 species belonging to 5 genus including *Bacillus*, *Staphylococcus*, *Kurtzia*, *Caryophanon*, and *Penicillium*. One of the isolates remained unidentified due to limited information. *Bacillus pumilis* and *Bacillus brevis* were the most abundant bacteria among them. Even though different kinds of bacteria were isolated from each sample in general, three bacterial species, *Bacillus pumilis*, *Bacillus lentis*, and *Caryophanon* were isolated from both unwashed and washed samples.

The result suggested that washing did little or no effect on reducing the diversity of microorganism in the Kupori textile. The results also indicated that the number of viable microorganisms in the Kupori textiles did not decrease significantly, but the population of contaminated microorganisms became more diverse after washing. *Bacillus* spp. which

were isolated most frequently in this study are soil bacteria with the capability of surviving severe and/or limiting conditions by forming endospores. Therefore, it is possible that the contamination of the unwashed Kupori textiles by these bacteria have occurred during the time of their burial. However, since these bacterial types are those easily found floating in air, the possibility of their transmittance to the Kupori textiles via air cannot be overruled. *Staphylococcus* and *Penicillium* can withstand very dry conditions as in the air. The above result also suggests that washing treatment of the exhumed textiles is not enough to keep it free of microbial contamination.

## 2. Shake Flask Test

The result of Shake Flask Test is presented in Table 3. The average number of living bacterial colonies before wrist action shaking was about the same in both unwashed and washed samples except for sample 014 of washed textile. Higher degree of bacterial colonies was found in samples 013 and 023b of the unwashed textiles and sample 014 of the washed textile. Overall, smaller numbers of bacterial colonies were found in the samples of washed textiles than the samples of unwashed textiles. Such results suggest that given the same condition of bacterial exposure, unwashed textiles are more apt to be contaminated by microorganism than the washed, soil-removed textiles. The exception of sample 014 of the washed textile may be due to an incomplete removal of soils through washing in that particular textile. In order to examine fully of such incidence, further investigation on the comparable soil amounts in number of washed Kupori textiles needs to be carried out. As an additional explanation for <Table 3>, the reduction rates of the living bacterial colonies in are all of negative numbers. Such result is due to the fact that the Shake Flask Test is originally designed to calculate the antibacterial function in a textile treated with anti-

<Table 3> Number of bacterial colonies after Shake Flask Test

Sample No.	Before Shaking	After Shaking	A - B / A ×100(%)	
Unwashed	013	30	397	-12.2
	023a	30	297	- 8.9
	023b	30	392	-12.1
Washed	014	107	392	-9.5
	015	30	40	-0.3
	017	30	52	-0.7

bacterial finish. In a modern antibacterial finished textile, the reduction rate should show a positive number, a higher number indicating a higher degree of antibacterial function and thus the successful effect of antibacterial finish. However, the samples in this study were not antibacterial finished modern textiles nor the textiles with inherent antibacterial function. Thus the negative number of reduction rate in Kupori textiles suggests that an exhumed textile would have a higher potential for bacterial contamination when exposed to microorganism than an unsoiled modern textile represented by the KS K test fabric.

## V. Conclusion

This research focused on the bacterial contamination of the textiles exhumed from Kupori Hwasung-gun, Kyunggi-do in whether or not there is a difference in the susceptibility of bacterial contamination between unwashed and washed textiles. The identification of microorganism indicated that a variety of bacteria and fungi were inherent in both unwashed and washed textiles. It was apparent that washing did little or no effect on reducing the diversity of microorganism in the Kupori textile. Also, the number of viable microorganism did not decrease significantly, but the population of contaminated microorganism became more diverse after washing. The Shake Flask Test for measuring the sus-

ceptibility of bacterial contamination in both unwashed and washed textiles indicated that given the same exposure condition, the unwashed textiles tend to be more susceptible to bacterial contamination than the washed textiles. Such result supports the current conservational procedures taken in Korean museums for exhumed textiles. Washing seems to be a must procedure in order to lessen or eliminate the susceptibility of microbiological contamination during storage or display. The current application of humigation treatment before setting the textile relic in long-term storage seems to be part of an ideal conservation procedure. However, further studies needs to be conducted on the effect of humigation chemicals and pesticidal chemicals as well on the possible color change or fiber degradation in long-term stored or displayed textile relics.

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