

**Analysis of Bacterial Presence on the Surfaces of Historical Books**

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## **Abstract**

This inquiry project set out to test the diversity and presence of microorganisms, specifically bacteria, in a variety of historical books. The books were selected from the special collection of books in ACU's library and ranged from the 13th to the 20th century. Though some of these books had been carefully packaged and not available for public use, they had been handled for hundreds of years before reaching ACU. These books were published in and received from a variety of locations, allowing a diverse community of microorganisms to collect throughout their many surfaces. This project provides relevant information for the study of bacteria and their ability to remain on or within non-living objects over a wide range of years. The results demonstrate the relationship between how many bacterial cells can remain on surfaces and the time that progresses from initial colonization.

Keywords: books, colonization, diversity, microorganisms

## **Analysis of Bacterial Presence on the Surfaces of Historical Books**

Bacteria exist and survive in a wide variety of locations. Their pili and fimbriae allow them to attach to and remain on inanimate surfaces (Tuso, 2013). Though a single bacterium is only able to survive on inanimate surfaces for a few weeks to a few months, their rapid division and colonization allow them to survive for thousands of years (Kramer et al., 2006). Books are one object commonly used by most people in numerous locations, and they can serve as inanimate hosts for a wide variety of bacteria. This experiment explores the presence of bacteria and the variety of microbes found on different surfaces. It was hypothesized that bacteria with cellulolytic properties would be within these books. Because paper is abundant in starch and cellulose, this type of bacteria would be able to thrive in this environment. If this hypothesis is supported, then the bacteria swabbed should mainly be Gram-positive and rod-shaped.

### **Methods and Materials**

It was first determined that ten books would be swabbed in three different areas. Tryptic soy agar was then poured into thirty Petri dishes, and ten groups of three plates were grouped and labeled. The plates were then allowed to dry and placed in a refrigerator for 48 hours. The thirty plates and thirty swabs were then taken and used to swab the ten books. The books were carefully chosen so the selection contained books from a wide range of centuries (13th-20th centuries). Each book was then swabbed on the spine, title page, and a randomly selected page, page 27 for most. Only dry cotton swabs were used to avoid any damage to the priceless books. Each swab was used to inoculate one plate, ensuring that the entire surface of the plate was covered. The plates were then placed in a 37°C incubator for 48 hours. The plates were then taken out, and the bacterial colonies were counted and analyzed. The results were recorded and the plates that did not have any colonies were discarded. Those plates that did have colonies

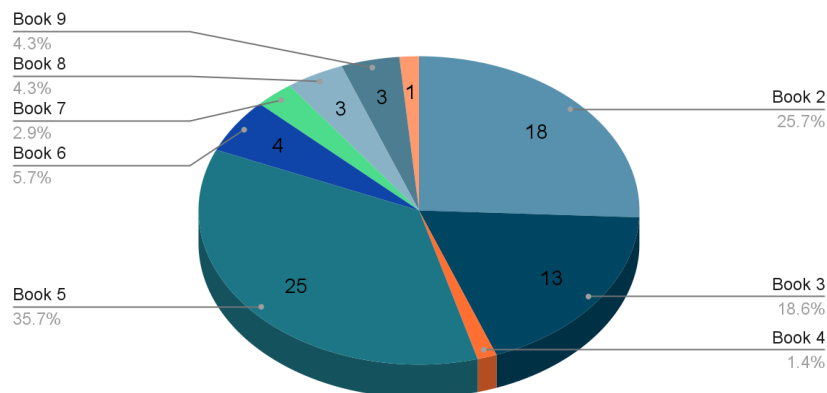
were placed in a bag and put in the refrigerator. One colony was then taken from each plate and streaked on a new agar plate for further analysis of the bacteria. The new plates were then placed in a 37°C incubator for 48 hours. After incubation, Gram stains were performed on the bacteria of each plate and the results were recorded. The data was then collected and used to draw conclusions.

## Results

By sampling ten books in three different locations, there were thirty plates of tryptic soy agar collected and analyzed with a total of seventy-one colonies grown. The majority of total colonies found were from Books 5, 2, and 3, in that order, as presented in Figure 1. The publication dates of these books are 1961, 1674, and 1580, respectively (Table 2). After counting the number of colonies produced, Gram staining procedures were completed to determine the Gram reaction of each colony. Of the original seventy-one colonies, 49 were found on the front cover or spine of the books while the title page and page 27 accounted for 28% and 20% of the total colonies, respectively (Table 1).

**Figure 1**

*Microbial Colonies found on Historical Books*



*Note.* Each piece of the pie chart represents the total number of colonies found in all three locations swabbed in each book. Book 10 is represented by the light orange color with 1 colony documented.

Additionally, 69% of the colonies were gram-positive, and separately, 69% of the colonies had rod morphology. Looking at columns two through four, five of the thirteen colonies were gram-positive and displayed rod morphology.

**Table 1**

*Microbial Colony Quantity, Gram Reaction, and Morphology on Various Locations of Historical Books*

Book	Numbers of Colonies Present, Gram Reaction, and Morphology			Totals of Gram Reaction Results		Colony Morphology Totals	
	Front Cover/Spine	Title Page	Page 27	Gram-positive	Gram-negative	Rods	Cocci
Book 1	0	0	0	-	-	-	-
Book 2	0	18 (+) rods	0	1	-	1	-
Book 3	12 (-) rods	1 (-) rods	0	-	2	2	-
Book 4	1 (-) rods	0	0	-	1	1	-
Book 5	25 (+) rods	0	0	1	-	1	-
Book 6	3 (+) rods	1 (both) rods	0	2	-	2	-
Book 7	1 (+) rods	0	1 (+) cocci	2	-	1	1
Book 8	3 (+) cocci	0	0	1	-	-	1
Book 9	3 (-) both	0	0	-	1	1	-
Book 10	1 (+) cocci	0	1 (+) cocci	2	-	-	2
Totals (%)	49 (69%)	20 (28%)	2 (3%)	9 (69%)	4 (31%)	9 (69%)	4 (31%)

**Table 2***Titles and Publication Dates of each Book Sampled*

Book 1	<i>A Collection of Hymns For the Use of Christians</i>	1804
Book 2	<i>An Exposition on the Third, Fourth, and Fifth Chapters of the Epistle of Paul the Apostle to the Hebrews.</i>	1674
Book 3	<i>A Commentary of Dr. Martin Luther upon the Epistle of S. Paul to the Galatians</i>	1580
Book 4	<i>The Sacred Writings of the Apostles and Evangelists of Jesus Christ, Commonly Styled the New Testament</i>	1826
Book 5	<i>Biblia Sacra: Gutenberg</i>	1961
Book 6	<i>Abilene Christian College Bible Lectures</i>	1919
Book 7	<i>The Prickly Pear Volume I</i>	1916
Book 8	<i>The New Testament Imprinted at London by Christopher Barker</i>	1589
Book 9	<i>The Bible that is The Holy Scriptures contained in the Old and New Testaments Imprinted at London by Christopher Barker</i>	1599
Book 10	<i>Ethiopian Bible of the Ge'ez Coptic Church</i>	12-13th century

**Discussion**

Based on these results, our hypothesis was supported. Most of the bacteria that were present on the surface of the book and within the book were gram-positive and rod-shaped. Though it is not certain whether or not these bacteria are cellulolytic, they most likely are due to the abundance of starch and cellulose as the common food source. It is also likely that the bacteria are aerobes or facultative anaerobes due to the presence of oxygen. Obligate anaerobes would be unable to perform metabolism because oxygen is constantly present in abundance.

Based on this information, the most common bacteria found in these books are probably in the genera *Pseudomonas*, *Actinomyces*, *Bacillus*, or *Cellulomonas* (Cho et al., 2012).

The results also indicate that there is no correlation between the age of the book and the abundance of bacterial colonies. The books that contained the most bacteria were from three different centuries, demonstrating that any age of the book is equally likely to have the most bacteria present. In terms of bacterial location on the books, the front cover and spine were the most abundant in bacteria. This makes logical sense due to the books most commonly being handled at their covers and spines.

Some limitations need to be taken into account when drawing conclusions in this study. Because gloves were not worn when handling the books, it may be likely that some of the bacteria grown came from those who handled it rather than previously present bacteria. Also, most of the books were placed in protective cases and stowed carefully, inhibiting the collection of more bacteria over the years. There may have also been mistakes made during the process of Gram staining, leading to inaccurate results and conclusions about the type of bacteria found on and within the books.

Potential future research could include the identification of bacterial species found within or on the historical books through biochemical testing and isolation of colonies. Moreover, extensive DNA sequencing would be required to identify each bacterial species collected. With this proven bacterial presence, we initially thought books could be labeled as fomites harboring pathogenic bacterium species. Upon further research, however, it became evident that paper typically does not serve as a fomite in pathogen transmission (Tuson & Weibel, 2013).

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