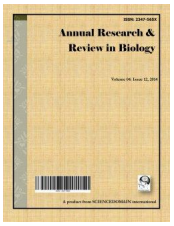

Annual Research & Review in Biology

27(5): 1-8, 2018; Article no.ARRB.42630
ISSN: 2347-565X, NLM ID: 101632869

Isolation and Characterization of Microorganisms Associated with Deteriorated Books

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Authors' contributions

This work was carried out in collaboration between all authors. Author EEE designed the study. Author IAY performed the statistical analysis. Author EEE wrote the protocol and wrote the first draft of the manuscript. Authors EEE and IAY managed the analyses of the study. Author EOA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2018/42630
Editor(s):
(1) Paola Angelini, Department of Applied Biology, University of Perugia, Perugia, Italy.
(2) Dr. George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Femi Ayoade, Redeemer's University, Ede, Nigeria.
(2) K. Immaculate Jeyasanta, Suganthi Devadason Marine Research Institute, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/25569

Original Research Article

Received 8th June 2018
Accepted 9th July 2018
Published 16th July 2018

ABSTRACT

This study was carried out to identify microorganisms associated with deterioration of books from three (3) libraries in Makurdi Benue state. Thirty-four (34) swabs collected from spoilt books from Francis Idachaba Library, College of Science and Veterinary Medicine Libraries, all of University of Agriculture, and Benue State Library Board, from different sections of the libraries were cultured using the pour plate technique. Cellulase activity of isolates was estimated by measuring clear zones of cellulose hydrolysis on Carboxymethylcellulose agar. Results showed that the average count of bacteria ranged from 4.5×10^7 Cfu/ml to 1.6×10^8 CFU /ml and the fungal count ranged from 3.8×10^7 Cfu/ml to 1.4×10^8 CFU /ml. The predominant bacteria species were *Pseudomonas sp.* 5 (23.81%) and *Bacillus sp.* 5 (23.81) and the predominant fungi species were *Chaetomium sp.* 3 (27.27%), *Aspergillus sp.* 3 (27.27%). *Providencia sp.* 1 (4.76%), *Acinetobacter sp*. 1 (4.76%) and *Klebsiella sp.* 1 (4.76%) were the least occurring bacteria while *Penicillium sp.* 1 (9.09%), *Geotrichum sp.* 1 (9.09%) and *Bipolaris sp.* 1 (9.09%) were the least occurring fungi species. *Penicillium sp*. showed the highest cellulase activity with cellulase index of 3.20 while *Aspergillus sp*. showed the least with cellulase index of 1.64. Similarly, *Bacillus sp*. had highest cellulase index of

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2.50 while *Pseudomonas sp.* had the least cellulase index of 1.20. All measurements were made at 37°C. Statistically, there was no significant difference between the activities of microorganisms to their load count ($x^2 \geq 0.05$). Findings from this study showed that Francis Idachaba Library of University of Agriculture, Makurdi had the highest bacterial load of 36.64%, and fungal load of 46.47%. Generally, the level of microbial deterioration of books was high in these libraries and authorities must take prompt steps to ensure the safety of books and book users as well as librarians.

Keywords: Isolation; deterioration; cellulase activity; cellulase; index.

1. INTRODUCTION

Books have served as a social tool for the transmission of ideas, facts, intelligence, and culture from one generation to another. “Books are the carriers of civilization”. Without books, history is silent, literature dumb, science crippled, thought and speculation at a standstill. Without books, the development of civilization would have been impossible. They are engines of change, windows of the world. “They are companions, teachers, magicians, bankers of the treasures of the mind” [1]. From them a people discover itself; its identity, history and maybe, its fate. Books are human identity. [2] agreed with this when he wrote, “the human race may have converted books or the essence of books anthropomorphically into human beings… Books are human beings”.

Since writing and civilization began, the attacks on books and libraries have been on in various ways, caused by various nefarious agents. Among the agents causing damage to books and paper materials include biological agents, natural disasters, environmental factors, chemical factors and human factors [3]. This is because books are made of paper which is an organic material; many microbes find it useful as a source of carbon under favourable conditions [4]. Identify fungi as the main cause of damage to objects of cultural heritage made or supported on paper.

Microbial damage of paper comes in form of spots, patinas and stains [5]. These are usually seen on old books and even new books that have not been well conserved. Another phenomenon discovered lately is foxing. [6] described it as the brown spots and stains seen on affected papers which may be fungal in origin or may also be caused by chemical impurities in the paper.

The problem of microbial invasion of libraries is a worldwide phenomenon, especially in libraries whose collections are paper-based. Only a few libraries hold their collections in electronic forms, the rest of the libraries, especially in developing countries like Nigeria, have paper-based collections. In such libraries, microbial deterioration is inevitable. The microorganisms causing paper deterioration have different kinds of enzymes that break down the macro components of paper. Example of cellulolytic microbes such as *Aspergillus fumigatus, Botrytis cinerea, Chaetomium globosum, Trichoderma viride, Rhizoctonia solani, Clostridium thermocellum, Ruminicoccus albus, Bacteroides succinogens, Eubacterium celulosolvens, Cellvibrio flavescens*, degrade cellulose yielding simpler products for other microorganisms to digest [7]. This breaks the bonds of paper, weakening it. In the same vein, lignin, another component of paper is degraded by fungal species such as *Polystictus versicolor, Stereum hirsitum* and *Phanerochaete chrysosporium*. Some fungal species such as *Pleurotus ostreatus, Ganoderma applanatum, Polyporus adastus, Armillaria mellea* attack lignin and cellulose simultaneously [7]. The environmental conditions in Nigeria favour microbial invasion of libraries, especially, relative humidity and temperature. Sekete [8] opined that the rate of deterioration of documents is more alarming in developing countries particularly in sub-Saharan Africa due to the interplay of factors not very prominent in developed countries.

The destruction of books by microorganisms is the major cause of paper deterioration and threat to human culture and civilization [9,10]. Microbial deterioration of books and paper materials has been a problem in libraries, archives and museums, and even homes from times immemorial. However, many people are still unaware of the damage microbes pose to books as repositories of knowledge, and our proud heritage, especially, in a developing country like Nigeria. Although similar studies have been carried out in developed countries and some parts of this country, information on

microorganism associated with book spoilage is still lacking in Benue state. This study is expository in nature as many people, particularly in third world countries like Nigeria, are grossly ignorant that microorganisms are a great threat to books. This study dwells on the destruction of books caused by microorganisms, the major cause of paper biodeterioration. The aim of this study is to determine the microbial agents associated with paper deterioration.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Francis Idachaba Library of University of Agriculture, Makurdi, Benue State Library Board, College of Science and Veterinary Medicine Libraries of University of Agriculture, all in Makurdi, in the month of October 2016.

Makurdi is the capital city of Benue State in Nigeria, a tropical country in Africa. Benue State has two seasons; the rainy and dry seasons. The rainy season is from April to October, while the dry season runs from November to March. Temperature ranges from 17.5°C to 37°C, with mean relative humidity of 78% [11]. The town lies between latitude 7°30' to 8°00' N and longitude 8°30' to 9°00' E

2.2 Sample Collection

A total of 34 samples were randomly collected from Francis Idachaba Library, College of Science and Veterinary Medicine Libraries, all of University of Agriculture, and Benue State Library Board, all in Makurdi Benue state from different sections of the libraries. The selection was based on the level of spoilage physically seen on the books. From each section, samples were collected from books with visible damage by swabbing with a sterile swab stick moistened with saline water. A book with no visible spoilage was swabbed from each section of the libraries. This served as control for comparison of microbial load. The swab sticks were well labeled and conveyed to the Biological Science laboratory of University of Agriculture Makurdi.

2.3 Preparation of Media and Samples

Three media (Carboxymethylcellulose agar, Nutrient Agar and Potato Dextrose Agar) were prepared and used for processing of the samples collected. The media were prepared based on the manufacturer's instructions. They were all sterilized by autoclaving at 121°C for 15 minutes, pressured for 15 Psi. The media were allowed to cool to a temperature of about 45°C and were poured into the Petri dishes (25 ml each). This was done close to the Bunsen burner flame to avoid contamination from the surrounding environment.

2.4 Isolation of Cellulolytic Fungi

The sample-laden swabs were placed inside 10 ml of saline water, well mixed, followed by decimal dilutions [12]. 0.1 ml from the 5^{th} dilution was inoculated into Petri-dishes, containing potato dextrose agar supplemented with chloramphenicol to inhibit bacterial growth. The plates were incubated at 27°C for 72 hours.

The isolated fungal samples were placed in universal bottle containing sterile distilled water and shaken thoroughly. This solution was allowed to settle and the supernatant decanted off into a separate tube. This served as the stock culture. From the stock culture, a drop of the solution was applied to the surface of carboxymethylcellulose (CMC) agar plates and incubated for 72 hours. After incubation, the surface of the medium was flooded with 1% Congo red solution [13] and [14]. This reagent precipitates undegraded CMC (or other long chain polysaccharides), leaving a clear zone where CMC has been degraded.

2.5 Isolation of Cellulolytic Bacteria

About 0.1 ml were picked from the 5^{th} dilution and inoculated into Petri dishes containing nutrient agar mixed with asparagine to inhibit fungal growth [13]. The plates were incubated at 37°C for 48 hours. The bacterial isolates were suspended in distilled water to produce slight turbidity. A drop was made on plates containing CMC and incubated for 48 hours at 37°C. After incubation, the agar medium was flooded with an aqueous solution of Congo red (1 mg/ml) for 15 minutes. The Congo red solution was poured off, and plates containing CMC were further treated by flooding the agar with 1 M NaCl for 15 minutes. 1 M HCl was also flooded over the plates to enhance colour contrast and maintain the plate's longer [15]. Clear zones appeared around bacterial colonies indicating cellulose hydrolysis [14].

3. IDENTIFICATION AND CHARACTERIZATION OF ISOLATES

3.1 Identification and Characterization of Fungal Isolates

Isolated colonies were observed both macroscopically and microscopically. Macroscopic examination was based on colonial characteristics such as colony, shape, elevation and edge. For microscopic identification, scrapings from the pure cultures were eased on clean grease-free glass slides and stained with lactophenol cotton blue and examined microscopically using 40 X objectives for their characteristic features such as type of hyphae (whether septate, coenocytic or branched) and asexual reproductive structures (whether borne on sporangia, conidia, in chains or single).

3.2 Identification and Characterization of Bacterial Isolates

The bacterial isolates were differentiated first on the basis of colonial morphology (shape, colour, edge, translucency, elevation, size and surface texture), followed by microscopic biochemical examination. All the isolates were identified according to Bergey's manual of Determinative Bacteriology [16].

3.3 Statistical Analysis

Data obtained were analyzed using the chi-square (χ^2) to find out any relationship between the microbial loads (CFU/ml) of the different sections of the libraries and any relationship in microbial loads (CFU/ml) between the different libraries under study.

4. RESULTS

Table 1 compared the bacteria load from the different sections of the three libraries studied. Binding section from the different libraries all recorded the highest bacteria load with a count of 1.1×10^8 CFU/ml and 1.6×10^8 CFU /ml in BSLB and FIL – UAM respectively. College of Veterinary Medicine UAM has no binding section so had no record for that section. While bacteria count from the control sample for BSLB were too few to count (TFTC), control sample from FIL – UAM had count of 6.1×10^7 CFU /ml. Total fungal count was highest in the binding section of FIL – UAM 8.2×10^7 CFU /ml as compare to that of BSLB with a count of 4.4×10^7 CFU /ml as seen in Table 2.

5. DISCUSSION

The studies on the microbial deterioration of books reveal that, the Binding section of FIL – UAM produced the highest bacterial count, accounting for 22.04% of the total microbial load. The reason could be that most if not all of the books found in this section are there for repairs because of physical damage or microbial attack. The Science and Technology section of Benue State Library board had the least bacterial count.

It was also noted that the Science section of Francis Idachaba Library of University of Agriculture, Makurdi produced the highest fungal count, accounting for 24.73%, while the Cataloguing and Classification section of Benue State Library board had less fungal load,

Table 1. Bacteria load from all Libraries

Section	Load (CFU /ml)	Percentage (%)
Binding BSLB	1.1×10^8	15.15
Cataloguing and Classification BSLB	3.4×10^7	4.68
Science and Technology BSLB	3.1×10^7	4.27
Arts and Social Sciences BSLB	9.0×10^7	12.40
Control BSLB	TFTC	
Binding FIL – UAM	1.6×10^8	22.04
Science FIL - UAM	4.5×10^7	6.20
Agriculture FIL – UAM	TNTC	
Control FIL – UAM	6.1×10^7	8.40
College of Science UAM	1.2×10^8	16.53
College of Veterinary Medicine UAM	7.5×10^7	10.33
Control UAM	TFTC	
Total	7.26×10^8	100.00

KEY: BSLB = Benue State Library Board, FIL – UAM = Francis Idachaba Library, University of Agriculture, Makurdi, UAM = University of Agriculture Makurdi, TFTC = Too Few To Count, TNTC = Too Numerous To Count

Table 2. Total fungi load from all Libraries

Section	Load (CFU /ml)	Percentage (%)
Binding BSLB	4.4×10^7	7.77
Cataloguing and Classification BSLB	3.8×10^7	6.71
Science and Technology BSLB	3.9×10^7	6.89
Arts and Social Sciences BSLB	TFTC	
Control BSLB	TFTC	
Binding FIL – UAM	8.2×10^7	14.49
Science FIL - UAM	1.4×10^8	24.73
Agriculture FIL – UAM	TNTC	
Control FIL – UAM	4.1×10^7	7.24
College of Science UAM	6.2×10^7	10.95
College of Veterinary Medicine UAM	1.2×10^8	21.20
Control UAM	TFTC	
Total	5.66×10^8	100.00

KEY: BSLB = Benue State Library Board, FIL – UAM = Francis Idachaba Library, University of Agriculture, Makurdi, UAM = University of Agriculture Makurdi, TFTC = Too Few To Count, TNTC = Too Numerous To Count

contributing only 6.71% of the total fungal load. This could be attributed to the fact that the books in Science section were mostly ancient books compared to the Cataloguing and Classification section whose collections were mostly new arrivals.

The statistical analysis carried out using Chi – Square revealed that there was no significant difference in the microbial load of the different sections of the libraries (X^2= 0.306) neither was there any in the different libraries against each other (X^2 = 0.285). Similarly, there was no significant difference in the overall microbial load observed from all the libraries (X^2= 0.261). This implies that both fungi and bacteria equally and competitively contributed to the deterioration obtained in the books sampled from these libraries.

A total of twenty – one (21) isolates of cellulolytic bacterial species were isolated in the study with *Pseudomonas spp.* and *Bacillus spp.* having highest frequency closely followed by *Proteus spp.,* Micrococcus *spp., Citrobacter spp., Providencia spp., Acinetobacter spp*., and *Klebsiella spp.* Three bacterial species could not be identified.

In a similar study in Oyo State, Nigeria, [17] obtained *Bacillus spp.* and *Pseudomonas spp.* with frequency of 43.75% and 18.75% respectively, a single *Proteus spp.* and a single *Micrococcus spp.* [18] also isolated *Bacillus spp., Streptococcus spp., Pseudomonas spp., Staphylococcus spp.,* and *Flavobacterium spp.* from deteriorated books. Similarly, [19] isolated *Bacillus sp., Staphylococcus aureus* and *Pseudomonas sp*. from deteriorated books at the University of Jos. This is also in agreement with the work of [20] who had similar isolates of *Bacillus spp., Pseudomonas* and *Proteus sp.*

A total of 11 fungi with cellulolytic activity were isolated with book deterioration in this study. *Aspergillus spp.* and *Chaetomium spp*. had highest frequency contributing 27.27% each, followed by *Fusarium spp.*18.18%. *Penicillium spp.*, *Geotrichum spp.* and *Bipolaris spp*. had a single occurrence each. Similar to the findings of [21], four fungal isolates were unidentified. *Penicillium sp*. showed the highest cellulase activity with cellulase index of 3.20, while *Aspergillus sp*. showed the least with cellulase index of 1.64. Similarly, *Bacillus sp*. had highest cellulase index of 2.50 while *Pseudomonas sp.* had the least cellulase index of 1.20. All measurements were made at 37°C.

The result of this research agrees with the research of [22] where *Penicillium sp*. had the highest cellulase activity of 0.69. [23] and [24] in separate researches agreed that *Aspergillus sp.* had the lowest cellulase activity. Likewise, [20] supports our findings, that Bacillus had the highest cellulase activity amidst *Pseudomonas sp*. and *Proteus sp*. The studies of [18] in a similar study at Olabisi Onabanjo University isolated *Aspergillus, Cladosporium, Neurospora* and *Penicillium*. In an earlier related study, [19] also isolated *Aspergillus, Chaetomium, Mucor, Trichoderma, Penicillium,* and *Rhizopus* from deteriorated books. Similarly, the findings of [25] isolated these cellulolytic organisms: *Streptomyces, Aspergillus, Penicillium, Rhizopus, Mucor, Geotrichum, Trichophyton, Chaetonium, Monilia, Nocardia, Aureosadium* and *Madurella spp*.

Table 3.

Sample/Criteria	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Gram	+ Cocci	- Cocci	- Rod	- Cocci	- Rod	- Rod	- Cocci	+ Rod	+ Rod	- Rod	- Rod	- Rod	+ Cocci	- Rod	+ Rod	- Rod	+ Rod	- Rod	+ Rod	- Rod	+ Rod	+ Rod	- Rod	+ Rod	- Rod
RESULTS OF BIOCHEMICAL TESTS																									
TSI																									
Slant	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Yellow	Yellow	Red	Red	Red	Red	Red	Red	Yellow	Red	Yellow	Red	Red	Red
Butt	Black	Black	Yellow	Black	Yellow	Black	Yellow	Black	Black	Black	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Gas	-	+	+	-	-	-	+	+	+	-	-	+	+	+	+	+	+	-	+	-	+	+	-	+	+
H_2S	+	+	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-
Coagulase	-	-	+	+	-	+	-	+	+	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+
Oxidase	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease	+	+	+	+	+	+	+	-	-	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+
Probable organism	*Micrococcus spp.*	*Micrococcus spp.*	*Acinetobacter spp.*	*Unknown*	*Providencia spp.*	*Proteus spp.*	*Pseudomonas spp.*	*Pseudomonas spp.*	*Unknown*	*Proteus spp.*	*Proteus spp.*	*Klebsiella spp.*	*Micrococcus spp.*	*Citrobacter spp.*	*Bacillus spp.*	*Citrobacter spp.*	*Unknown*	*Pseudomonas spp.*	*Paenibacillus spp.*	*Proteus spp.*	*Bacillus spp.*	*Bacillus spp.*	*Pseudomonas spp.*	*Bacillus spp.*	*Pseudomonas spp.*

Das and Ahmad [26] also found *Chaetomium, Aspergillus* and *Penicillium* to be predominant in his study which was carried out in India. Studies from Iran, [27] who also isolated highest frequencies of *Aspergillus, Penicillium* and *Mucor* from Astan Quds Museum Library corroborates the results of this research.

Table 4. Bacterial isolates with cellulolytic activity

Bacterial isolate	Number	Frequency of occurrence (%)
Pseudomonas spp.	5	23.81
Bacillus spp.	5	23.81
Proteus spp.	4	19.05
Citrobacter spp.	2	9.52
Micrococcus spp.	2	9.52
Providencia spp.	1	4.76
Acinetobacter spp.	1	4.76
Klebsiella spp.	1	4.76

Table 5. Cellulolytic fungal isolates

Fungal isolate	Number	Frequency of occurrence (%)
Chaetomium spp.	3	27.27
Aspergillus spp.	3	27.27
Fusarium spp.	2	18.18
Geotrichum spp.	1	9.09
Penicillium spp.	1	9.09
Bipolaris spp.	1	9.09

Most of the isolates are of great significance to man, not only because they damage books, but also because they pose serious health concerns to man. *Aspergillus spp*. produces aflatoxins, derivatives of difuranocoumarin [28] capable of causing death, cancer and immune suppression among other slow pathological conditions. Similarly, Ochratoxin A, a nephrotoxic, immunesuppressive and carcinogenic compound produced by species of *Aspergillus* and *Penicillium*. *Fusarium spp.* produces trichothecenes and zearalenone capable of depressed immune responses, nausea and vomiting [29]. The spores of *Clostridium, Bacillus* and fungi are capable of initiating asthma, inhalation fever, [30] and allergic alveolitis [31]. Species of *Klebsiella*, *Providencia*, *Pseudomonas*, *Proteus* and *Citrobacter* all cause different diseases varying from respiratory tract infections to urinary tract infections.

6. CONCLUSION

In conclusion, Pseudomonas *spp. Bacillus spp. Proteus spp.,* Micrococcus *spp., Citrobacter spp., Providencia spp., Acinetobacter spp*., and *Klebsiella spp* in addition to some fungi species were found associated with deteriorated books, and as such preventive measures such as proper aeration of the libraries, allowance of some lux of sunlight and the maintenance of a relative humidity will help a great deal.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25569