

**ISOLATION AND MOLECULAR IDENTIFICATION OF BIODETERIOGENS  
ISOLATED FROM PAINTED CLASSROOM WALL SURFACES IN UNIVERSITY OF  
PORT HARCOURT, NIGERIA**

**Abstract**

Painted wall surfaces provide breeding ground for diverse microbial flora capable of causing deterioration of the building and progressively impact on the health of inhabitant. This study was undertaken to determine the bacterial and fungal biodeteriogens of painted classroom wall surfaces in University of Port Harcourt, Nigeria. Bacterial and fungal biodeteriogens were isolated from classroom wall surfaces showing visible signs of deterioration and dampness. Internal Transcribed Spacer (ITS) region of the rDNA was used in the identification of the isolates. The bacterial deteriogens were identified as *Myroides odoratus*, *Bacillus subtilis*, *Bacillus* sp. and *Alcaligenes aquatilis*, while fungal deteriogens were identified as *Aspergillus nomius* and *Trametes polyzona*. Presence of microorganisms in classroom wall surfaces can impact on air quality of the learning environment.

**Keywords:** Biodeteriogens, ITS region of rDNA, deterioration, painted classroom

**INTRODUCTION**

Buildings are inescapably exposed to environmental factors which can cause their deterioration. Biodeterioration is any undesirable change in the intrinsic properties of materials stemming from the activities of biological agents [1,2]. Buildings are susceptible to deterioration because some of the materials used in building construction can provide nutrients for colonizing organisms [3,4].

Among biological agents microorganisms are the major culprits implicated in building deterioration. Their activities could include chemical interaction with substrate on the building surface, biofilm formation, enhancement of porosity, chelation of ions which could lead to weakening of structure and disaggregation of material [1,2]. Manifest discoloration of building surfaces appearing as greenish, reddish-brown, yellowish-brown or black patches is taken to indicate the presence of microbial [5]. Aside from loss in aesthetic value, more worrisome damage can be caused by microorganisms to materials of value which cannot easily be refurbished or replaced such as historic buildings and materials [6,7].

Previous investigations from different locations reported a number of bacterial species of the genera *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Micrococcus*, *Serratia*, *Enterobacter*, *Proteus* and *Escherichia* as common biodeteriogens of painted buildings [8,9,10]. Fungi are considered important biodeteriogens of building surfaces because of their metabolic versatility and ability to thrive in oligotrophic environment with little nutrient [11]. Among frequently isolated fungi from biodeteriorated painted buildings are members of the genera *Aspergillus*, *Fusarium*, *Alternaria*, *Trichoderma*, *Rhizopus* and *Penicillium* [8,10,12-14].

Microbial activities are potential threats to the life cycle of buildings and monuments globally. Very few studies have been conducted in Nigeria on the microbiota of painted classroom wall surfaces using molecular method to identify isolates, thus the true microbial community is indefinable. Knowing the true microbial diversity of deteriorating painted building surfaces would help towards their control. This study aimed to determine the genomic identities of bacterial and fungal isolates from deteriorating painted classroom wall surfaces in University of Port Harcourt, Nigeria.

## **MATERIALS AND METHODS**

### **Collection of Samples**

Classroom wall scraping samples were collected from visibly deteriorating surfaces in the University of Port Harcourt under aseptic conditions by scraping off superficial material to a depth of 2-5 mm. Samples were immediately moved to the laboratory for microbiological analyses.

### **Isolation and Identification of Biodeteriogens**

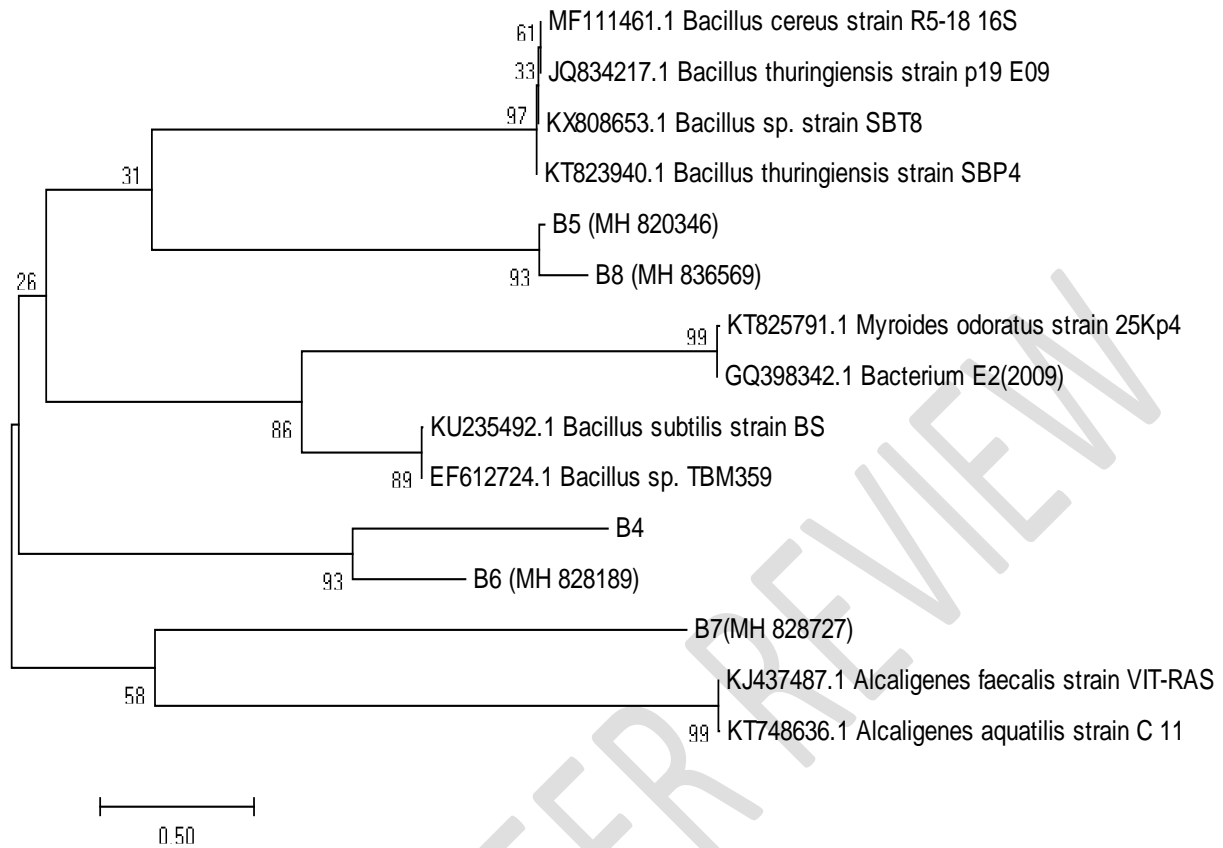
Isolation was done by plating samples on nutrient agar and potato dextrose agar for bacterial and fungal isolates respectively. Plates were incubated at 37°C for 24 and at 27<sup>0</sup>C for 48-72 hours for bacterial and fungal isolates respectively. DNA was extracted from pure culture of isolates using Zymo Quick DNA Fungal/Bacterial Kit following the protocol as describe in Ire and Eruteya [15]. The DNA was subjected to the following cocktail mix and condition for the PCR; 10× PCR buffer 1.0 µL; 25mM Mgcl<sub>2</sub> 1.0 µL; 5pMol forward primer 0.5 µL; 5pMol reverse primer 0.5 µL; DMSO 1.0 µL; 2.5Mm DNTPs 0.8 µL; Taq 5u/ul 0.1 µL; 10ng/µl DNA 2.0 µL; Water 3.1 µL. Primers used were F: ITS4 TCCTCCGCTTATTGACATGS and R: ITS5 GGAACTAAAAGTCGTAACAAGG. Amplified products were analyzed by electrophoresis in 1.5% agarose gel. Ethidium bromide was used to stain the PCR products and visualized under ultraviolet trans-illuminator. Invitrogen 1kb plus ladder was used as molecular weight marker. The 3130xl genetic analyzer from Applied Biosystems was used for gene sequencing. The phylogenetic analysis followed the method described by Ire and Eruteya [15].

## **RESULTS AND DISCUSION**

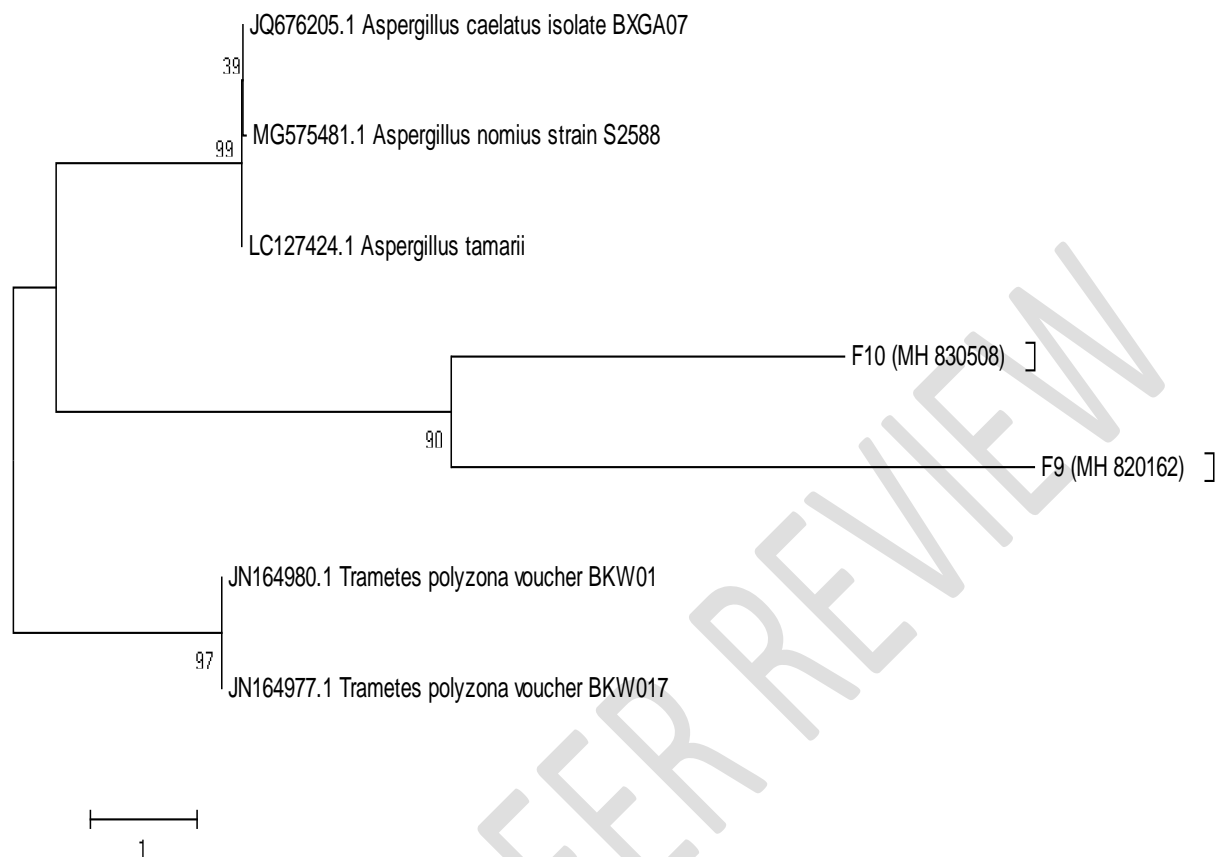
Results of partial sequences of 16S rDNA region of bacterial isolates revealed that isolates B4 is closely related to *Myroides odoratus* strain 25kp4 with percentage similarity of 99% and B6 to *Bacillus subtilis* strain BS with percentage similarity of 86%. Isolates B5 and B8 are more closely related to *Bacillus* sp. strain SBT8 with 97%. Isolate B7 is closely related to *Alcaligenes aquatilis* strain C 11 with a percentage similarity of 99%. Fungal isolates F9 and F10 were shown to be 99% closely related to *Aspergillus nomius* strain S2588 and 97% to *Trametes polyzona* respectively (Table 1). Figures 1 and 2 show phylogenic relationship of bacterial and fungal isolates obtained from deteriorating painted classroom wall surfaces respectively.

**Table 1:** Genomic identification of isolates collected from deteriorating classroom walls based on ITS Region of rDNA

Sample Code	Tentative Identity	Closest Relative	Accession Number	Percentage Similarity
<b>B4</b>	<i>Micrococcus</i> sp.	<i>Myroides odoratus</i>	MH820345	99
<b>B5</b>	<i>Bacillus</i> sp.	<i>Bacillus</i> sp. strain SBT8	MH820346	97
<b>B6</b>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i> strain BS	MH828189	89
<b>B7</b>	<i>Corynebacterium</i> sp.	<i>Alcaligenes aquatilis</i> strain C 11	MH828727	99
<b>B8</b>	<i>Citrobacter</i> sp.	<i>Bacillus</i> sp. strain SBT8	MH836569	97
<b>F9</b>	<i>Aspergillus nomius</i>	<i>Aspergillus nomius</i> strain S2588	MH820162	99
<b>F10</b>	<i>Penicillium</i> sp.	<i>Trametes polyzona</i>	MH830508	97



**Figure 1:** Phylogenetic relationship of bacterial isolates from deteriorating painted classroom wall surfaces



**Figure 2:** Phylogenetic relationship of fungal isolates from deteriorating painted classroom wall surfaces

Deteriorating painted building surfaces can harbour a number of bacterial species. Of the four bacterial isolates identified in this study, two belong to the genus *Bacillus*. Shinkafi and Haruna [9] identified *Bacillus alvei*, *Bacillus cereus*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus laterosporus* and *Bacillus polymyxa* among the dominant deteriogens of painted concrete buildings. Ogbulie and Obiajuru [16] listed *Bacillus* among the bacterial isolates of painted surfaces. *Bacillus* species are ubiquitous in the environment, as they are spore formers capable of withstanding adverse environmental conditions. Their presence in deteriorating painted surface could be through accumulation of dirt on wall surfaces [17]. Most *Bacillus* species are not

considered human pathogens but should however not be ignored as possible causes of infection following report of unusual bacteraemia by *Bacillus subtilis* and *Bacillus licheniformis* by Jeon *et al.* *Myroides* species are common in the environment and it was no surprise that it was isolated in this study. The little known *Alcaligenes aquatilis* has been previously isolated from marine sediment [19]. *Myroides odoratus* is an uncommon opportunistic bacterium that can cause bacteraemia and cellulitis [20]. Infections by *Myroides odoratus* are uncommon and *Alcaligenes aquatilis* is not a known pathogen.

*Aspergillus nomius* and *Trametes polyzona* were the two fungal species isolated in this study. There are several reports in literatures on the isolation *Aspergillus* sp. from painted wall surfaces [9,10,12,21]. The ability of molds to interact with materials owing to their morphology and metabolic versatility makes them very important agents of biodeterioration [6,11,22]. The colonization of buildings by fungi and subsequent deterioration is accepted to be abetted by presence of moisture [12,13]. Fungal development on painted surfaces in this study could have been aided by moisture because all sampled classroom walls were damp. Fungi are master strategies in energy utilization from different sources and in surviving in harsh nutrient poor environment [22]. They can therefore survive in building on building surfaces.

It is expected that any environment harbouring large number of molds could pose health risk to humans through possible inhalation of their spores. The two fungal isolates in this study *Aspergillus nomius* and *Trametes polyzona* are spore formers. Fungal spores in indoor air can trigger allergies, such as allergic rhinitis, pharyngitis and asthma [1,23]. Inhaling fungal spores can cause fungal lung infection such as aspergillosis, histoplasmosis and blastomycosis [24]. *Aspergillus* species are ubiquitous opportunistic fungi that cause pulmonary infections in immunocompromised patients. Fungi that were previously thought to be of uncertain

pathogenicity are emerging as causes of infections in immunosuppressed host [25]. For example, *T. polyzona* which is prevalent in tropical areas was recently reported to cause pulmonary infections in critically ill patients [26]. *A. nomius* has been reported as the etiological agent of breakthrough pneumonia [27].

It is common to see painted buildings showing signs of biodeterioration in form of discoloration because the painted surfaces support diverse microbial biota. Protective biocides are often added to paints to mitigate microbial proliferation. Harsh environmental conditions and prolong use may make the paint susceptible to microbial deterioration nonetheless. The indoor air quality of a classroom with deteriorating painted wall can be impacted by microorganisms inducing the deterioration. It is important that environmental protection be given to buildings where applicable and advisable to regularly repaint classroom walls as part of routine maintenance practice, to ensure a safe learning environment.

## **Conclusion**

Four bacterial and two fungal deteriogens were isolated from visibly deteriorating painted classroom wall surfaces. The biodeteriogens were identified as *Myroides odoratus*, *Bacillus subtilis*, *Bacillus* sp., *Alcaligenes aquatilis* *Aspergillus nomius* and *Trametes polyzona*. These microorganisms and their spores are capable of impacting on the classroom indoor air quality with consequential effect on the health of students and teachers.

## **REFERENCES**

1. Allsopp, D., K.J. Seal and C. Gaylarde, 2004 Introduction to biodeterioration. 2nd ed. Cambridge University Press. New York, USA.



2. Cutler, N. and H. Viles, 2010. Eukaryotic Microorganisms and Stone Biodeterioration. *Geomicrobiology Journal*, 27: 630-647.
3. Gillatt, A.C., 1992. Bacterial and fungal spoilage of waterborne formulations. *Additives*, 10: 387-393.
4. Singh, A., 2004. Biodegradation of building material. *Biodeterioration of stone surfaces*. St. Clair LL and Seaward MRD Ed., Kluwer Academic Publisher.
5. Gaylarde, C.C. and P.M. Gaylarde, 2002. Biodeterioration of historic buildings in Latin America. 9DBMC-2002; Paper 171: 1-9.
6. Milica, V.L. and B. Jelena, 2009. Role of Fungi in Biodeterioration Process of Stone in Historic Buildings. *Proc. Nat. Sci, Matica Srpska Novi Sad.*, 116: 245-251.
7. Mohammadi, P. and N. Maghbol-Balasin, 2014. Isolation and molecular identification of deteriorating fungi from Cyrus the Great tomb stones. *Iranian Journal of Microbiology*, 6(5): 361-370.
8. Ugbogu, O.C., I. Awache, D.I., Agwaranze, A.C. Ogodo, A. Ubandoma and M.N. Yakubu, 2017. Microbial Deterioration of Painted Wall Surfaces in Wukari, Taraba State, Nigeria. *American Journal of Microbiology and Biotechnology*. 4(4): 31-34.
9. Shinkafi, S.A. and I. Haruna, 2013. Microorganisms associated with deteriorated desurface painted concrete buildings within Sokoto, Nigeria. *International Journal of Current Microbiology and Applied Science*, 2(10): 314-324.
10. Obidi, O. and F. Okekunjo, 2017. Bacterial and fungal biodeterioration of discolored building paints in Lagos, Nigeria. *World Journal of Microbiology and Biotechnology*, 33:196. <https://doi.org/10.1007/s11274-017-2362-y>.

11. Gadd, G.M., 2004. Mycotransformation of organic and inorganic substrates. *Mycologist*, 18: 60-70.
12. Mamta, C. and S. Padma, 2012. Building deteriorating fungi as biocontaminant. *Asian Journal. Exp. Biological. Science*, 3(1): 209–213.
13. Bashir, U. and R. Hafeez, 2016. Deterioration of painted wall surface by fungal saprobes: isolation and identification. *Pakistan Journal Phytopathology*, 28 (1): 09-13.
14. Dupont, J., C. Jacquet, B. Denetière and S. Lacoste, 2007. Invasion of the French Paleolithic painted cave of Lascaux by members of the *Fusarium solani* species complex. *Mycologia*, 99:526-533.
15. Ire, F.S., and O. C. Eruteya, 2017. Antimicrobial susceptibility of spoilage bacteria of Atama (*Heinsia critana*) soup. *International Journal of Current Microbiology Applied science*, 6(4):2664-2672.
16. Ogbulie, J.N. and I.O.C. Obiajuru, 2004. Microbial deterioration of surface paint coatings. *Global Journal of Pure and Applied Sciences*, 10(4): 485-490.
17. Anele , B. C., P. O. Okerentugba, H. O. Stanley and C. J. Ugboma, 2019. Biodeterioration of classroom wall surfaces in the University of Port Harcourt, Nigeria. *Journal of Advances in Microbiology*. 15(1): 1-8.
18. Duran, R.E., B. Barra-Sanhueza, F. Salva-Serra, V. Mendrz, D. Jaen-Luchoro et al., 2019. Complete genome sequence of the marine hydrocarbon degrader *Alcaligenes aquatilis* QD168, isolated from crude oil-polluted sediment of Quintero Bay, Central Chile. *Microbiology Resource Announcements*. 8(4).

19. Jeon, Y.L., J.J. Yang, M.J. Kim, G. Lim, S.Y. Cho et al., 2012. Combined *Bacillus licheniformis* and *Bacillus subtilis* infection in a patient with oesophageal perforation. *Journal of Medical Microbiology*, 61: 1766-1769.
20. Beharrysingh, R., 2017. *Myroides* bacteremia: a case report and concise review. *IDCases*, 8: 34-36.
21. Elumalai, P., E.K. Elumalai and E. David, 2014. Fungi associated with deteriorations of painted wall surfaces: isolation and identification. *European Journal of Academic Essays*, 1(3): 48-50.
22. Gutarowska, B., 2014. Moulds in biodeterioration of technical materials. *Folia Biologica et Oecologica*, 10: 27–39.
23. Zukiewicz-Sobczac, W.A., 2013. The role of fungi in allergic diseases. *Postepy Dermatol Alergol.*, 30(1): 42-45
24. Smith, J.A. and C.A. Kauffman, 2012. Pulmonary fungal infections. *Respirology*, 17(6): 913-26.
25. Hsu, L.Y., E.S. Ng and L.P. Koh, 2010. Common and emerging fungal pulmonary infections. *Infect Dis Clin North Am.*, 24(3):557-77.
26. Gauthier, A., J. Jaubert, N. Traversier, J. Lemant, L. Balu, et al., 2017. *Trametes polyzona*, an emerging filamentous basidiomycetes in Reunion Island. *Mycoses*, Wiley, 60(6), pp. 412-415.
27. Caira, M., B. Posteraro, M. Sanguinetti, E. de Carolis, G. Leone and L. Pagano, 2012. First case of breakthrough pneumonia due to *Aspergillus nomius* in a patient with acute myeloid leukemia, *Medical Mycology*, 50(7):746-750.