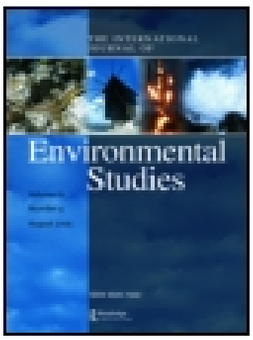

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Studying the cellulolytic activity of microorganisms isolated from stained painted walls with reference to certain factors: a cross sectional study

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ABSTRACT

Investigations of enzymatic activities amongst microbial species on stained painted walls are not common especially in the tropics. Meanwhile, an organism's enzymatic activity is a key index of its biodegradation potential. We conducted a cross-sectional study to assess the cellulolytic profiles of microorganisms isolated from stained painted buildings. A total of eight microorganisms comprising one bacterium [*Pseudomonas aeruginosa* CH01 (KY511067.1)] and seven fungi [*Meyerozyma guillermondii* MB14B1 (LT615287.1), *Meyerozyma caribbica* CBS:5674 (KY104219.1), *Aspergillus aculeatus* A1.9 (EU833205.1), *Aspergillus* sp. SL2 (KC178662.1), *Fusarium proliferatum*2705 (EU272509.1), *Cerrena* sp. N10CC2a (FJ010208.1) and *Candida tropicalis* UZ31_13 (KM361510.1)] were utilized for the study. The results showed that all isolates possessed exoglucanase and endoglucanase activities ranging from 0.174 to 1.554 IU/ml and 0.062 to 2.120 IU/ml, respectively. Analysis of the interplay of organism cellulolytic activity at various environmental conditions showed that endoglucanase activity was optimal in *Cerrena* sp., while the highest exoglucanase activity was recorded in *P. aeruginosa*.

KEYWORDS

Discoloration; carboxymethyl cellulose; painted walls; optimisation; cross-sectional analysis

Introduction

Cellulases have been classified as the second largest group of carbohydrases that have been harnessed, mainly due to their high rate of degradation, specificity as well as their potential biotechnological application in various industries [2]. Incidentally, they are also synthesised by a large diversity of microorganisms including fungi and bacteria during their growth on cellulosic materials such as paints and coatings [3]. Fungi are referred to as the most suitable cellulase producers by virtue of their ability to produce a complete cellulase system [4]. Cellulolytic fungi produce and secrete a complex of extracellular cellulases which facilitate the hydrolysis of the cellulose macromolecule into small water soluble sugars which

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can subsequently, be processed by the fungi [5]. Fungal hyphae in particular, constitute a major threat to painted walls because they penetrate deeply into painted matrix releasing extracellular enzymes that facilitate loss of aesthetics and obvious mechanical damage [6,7]. Previous studies have also reported that persistent enzymatic degradation over a long period of time contributed significantly to visible surface discolorations [8,9]. Therefore, painted films are highly susceptible to fungal colonisation and subsequent biodeterioration.

Hydroxyethyl cellulose (HEC), a non-ionic, water soluble polymer and a major thickener for both interior and exterior latex paints is widely used in paint manufacture because of its outstanding ability to thicken, suspend, bind, emulsify, form films, stabilize, disperse, retain water, and provide protective colloid action [10]. Interestingly, microbial cellulases are able to decompose both HEC as well as carboxymethyl cellulose (CMC) which is also used as a paint thickener [11]. Endoglucanases degrading HEC have been described in several fungi [12] and is a major enzyme component of *Aspergillus niger* [13,14]. *Aspergillus niger* has also been previously reported as the most important source of industrial cellulases [10].

Cellulose from paint pigments accumulating on the painted surfaces in the air, acts as a source of micro-nutrients for a variety of microbes. Cellulolytic components such as endoglucanase, exoglucanase and β-glucosidase effectively depolymerize cellulose chains in lignocellulosic paint substrates to produce smaller sugar units that consist of cellobiose and glucose [4]. Cellulose of canvas materials, glue, linseed oil, paint pigments, dust, dead or living cells accumulating on the painted surfaces all act as sources of micro-nutrients to facilitate microbial proliferation and subsequent cellulolytic activities [2]. Cellulases which have been studied in *Trichoderma*, *Aspergillus*, *Fusarium*, and *Penicillium* may be divided into 3 types: endoglucanase (endo-1, 4-β-D-glucanase, EG, EC 3.2.1.4); cellobiohydrolase or exoglucanase (exo-1, 4-β-D-glucanase, CBH, EC 3.2.1.91) and β-glucosidase (1, 4-β-D-glu-cosidase, BG, EC 3.2.1.21) [15,16]. These cellulases enhance the digestibility of cellulose and loss of viscosity in paints [17–19].

Previous studies had focused on the metabolic and degradative mechanisms of enzymes on degraded surfaces. However, there is a paucity of information on specific analysis using cross-sectional means. The aim of this work was to investigate the activities of microbial endoglucanases and exoglucanases in discolouration using a cross-sectional analysis. The present study is the first demonstration of the application of cross-sectional analysis in validating the activities of microbial endoglucanases and exoglucanases in discoloration.

Materials and methods

Isolates

Eight cultivable and pigment producing microbes obtained by swabbing from stained painted buildings and identified with the 16S rRNA sequencing analysis in a previous study were used for this study [20]. The isolates include *Pseudomonas aeruginosa* CH01 (KY511067.1) and seven fungi [*Meyerozyma guillermondii* MB14B1 (LT615287.1), *Meyerozyma caribbica* CBS:5674 (KY104219.1), *Aspergillus aculeatus* A1.9 (EU833205.1), *Aspergillus* sp. SL2 (KC178662.1), *Fusarium proliferatum* 2705 (EU272509.1), *Cerrena* sp. N10CC2a (FJ010208.1) and *Candida tropicalis* UZ31_13 (KM361510.1)] (Data not shown).

Enzyme production

The ability of the isolated strains to produce endoglucanase and exoglucanase was evaluated. Sterilized nutrient broth medium for bacteria and potato dextrose broth medium for fungi (300 ml each in 500 ml conical flasks) were inoculated with bacterial and fungal cell suspensions adjusted to 0.5 McFarland standard corresponding to approximately 1.5×10^8 CFU/ml. Specific amount of specific substrates viz: (5 g CMC for endoglucanase activity and 5 g Whatmann filter paper No. 1 for exoglucanase activity) respectively were introduced into the flasks. The flasks were plugged with non-absorbent cotton wool and subsequently incubated aerobically for 24 h at 37 °C for bacteria, and 25 °C for 5 days for fungi in an incubator shaker at 150 rpm. Thereafter, cells were centrifuged at 15,000 rpm for 20 min. The culture supernatants were used as the crude enzyme extracts for extracellular enzymes assay [21].

Enzyme assay

Total cellulase activity of isolated cellulolytic bacterial and fungal strains was determined as described by [22,23]. For exoglucanase, an aliquot of 0.5 ml of cell-free culture supernatant from each bacterial and fungal culture was taken in a sterile test tube and 1 ml of Sodium citrate buffer (0.336 g of citric acid and 2.470 g of trisodium citrate in 100 ml of distilled water) (pH 5.8) was added. At the temperature of 50 °C, one strip of Whatmann no. 1 filter paper 1.0 × 6.0 cm (~50 mg) was introduced into each tube and incubated for 1 h. Tubes were vortexed till the filter paper settled at the bottom of the tube. After incubation, 3.0 ml of Dinitrosalicyclic acid (DNS) was added to each tube and mixed well. The endoglucanase activity was determined by the colorimetric method as described by [23]. Samples were prepared by mixing 250 µl of culture supernatant, 0.9 ml of (1% w/v) CMC solution in 50 mM sodium phosphate buffer (pH 7.0) and 250 µl of distilled water. The mixture was incubated at 40 °C in a water bath for 30 min.

Effect of various conditions on enzyme production

The effect of incubation time on individual enzyme production was determined by employing different incubation times (24, 48 and 96 h). The culture filtrates were collected at respective time interval and assayed for the enzymes activities. To determine the optimum pH for enzyme production, the pH of the medium containing 1% of the substrates was adjusted to 2, 6, and 9 with 1 N NaOH and 1 N HCl. The assay was carried out after 120 h of incubation for each isolate. The optimum temperature for individual enzyme production was studied by carrying out the assay at 15, 25 and 37 °C incubation temperature after 120 h. In order to determine the optimum substrate concentration for enzyme production, the individual media with different concentrations of substrates (0.01, 0.1 and 1%) were prepared and incubated with culture broths inocula at fixed pH and temperature, the samples were withdrawn after 120 h for each isolate. The reducing sugar concentrations for exoglucanase and endoglucanase assay were calculated according to glucose standard curve (Figure 1).

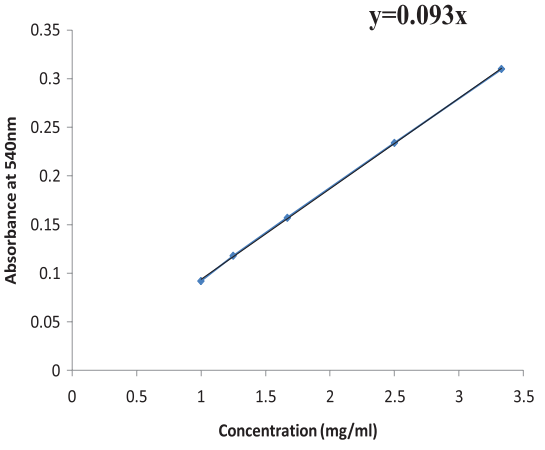

Figure 1. Glucose standard curve at 540 nm.

Results

Eight cultivable and pigmented microbes isolated from stained painted walls by swabbing and identified with the 16S rRNA sequencing analysis in a previous study were used for this study. The isolates include: *Pseudomonas aeruginosa* CH01 (KY511067.1) and seven fungi [*Meyerozymaguillermondii* MB14B1 (LT615287.1), *Meyerozymacaribbica* CBS:5674 (KY104219.1), *Aspergillus aculeatus* A1.9 (EU833205.1), *Aspergillus* sp. SL2 (KC178662.1), *Fusarium proliferatum*2705 (EU272509.1), *Cerrena* sp. N10CC2a (FJ010208.1) and *Candida tropicalis* UZ31_13 (KM361510.1)]. We found that all investigated isolates were able to produce exoglucanase and endoglucanase.

Cross-sectional effect of various conditions on enzyme production

Effect of incubation time

The results showed that the enzyme activity was optimum for exoglucanase activity in 75% of the microorganisms after 24 h (Figure 2A), while endoglucanase activity was optimum at 96 h for 87.5% of the microorganisms. Only *P. aeruginosa* showed an optimum incubation time of 24 h for both endo and exoglucanase activities. *M. caribbica*and *C. tropicalis* showed an optimum incubation time of 96 h for both endo and exoglucanase activities. Optimum incubation time for other microbes (*M. guilliermondii*, *A.aculeatus*, *F.proliferatum*, *Aspergillus* sp., *Cerrena* sp.) varied as it was 24 h for exoglucanase activity, but 96 h for endoglucanase activity.

Effect of pH

Among the various pH ranges, endo and exoglucanasess howed their optimum activities at 6 and 9 in 93.75% of the microorganisms (Figure 2B). Both *Cerenna* sp. and *P. aeruginosa* showed an optimum pH of 6, while *F. proliferatum* showed an optimum pH of 9 (for both endo and exoglucanase activities). *M. guilliermondii*, *A. aculeatus* and *Aspergillus* sp. showed an optimum pH of 6 for exoglucanase activity and an optimum pH of 9 for endoglucanase activity. On the other hand, the optimum pH for *M. caribbica* was 2 and 9

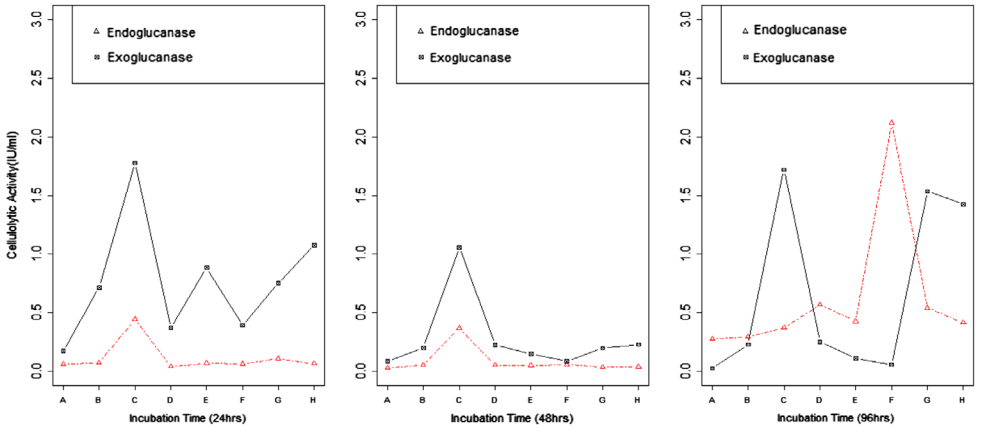

Figure 2A. Cross-sectional effect of time on cellulolytic activity.

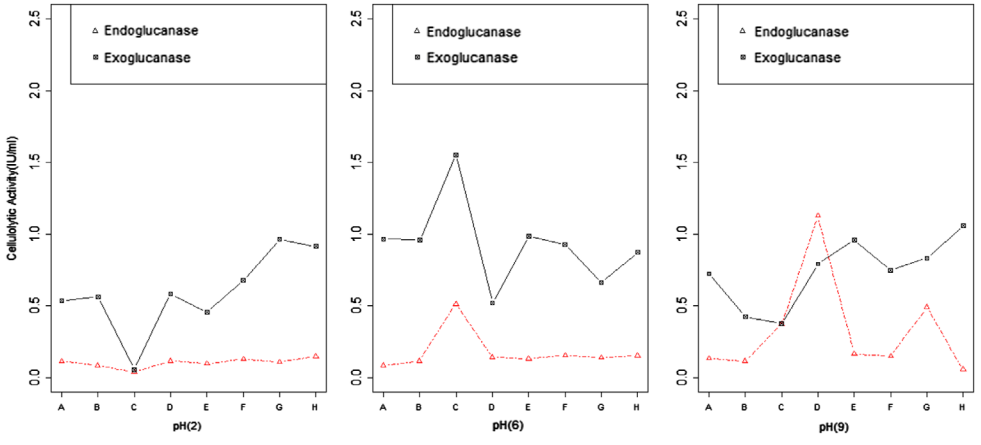

Figure 2B. Cross-sectional effect of pH on cellulolytic activity.

Table 1. Exoglucanase activity in IU/ml at optimum incubation time, pH, temperature and substrate concentration.

Microorganisms	Enzymatic activity (IU/ml)/Incubation time (h)	Enzymatic activity (IU/ml)/pH	Enzymatic activity (IU/ml)/Temperature (°C)	Enzymatic activity (IU/ml)/Substrate concentration (g)
M. guilliermondii	0.174/24	0.967/6	0.548/25	0.337/1
A. aculeatus	0.716/24	0.960/6	0.493/25	0.317/1
P. aeruginosa	1.777/24	1.554/6	3.077/25	2.265/1
F. proliferatum	0.309/24	0.793/9	0.544/25	0.319/1
Aspergillus sp	0.887/24	0.988/6	0.505/37	0.343/1
Cerrenasp	0.393/24	0.928/6	0.534/25	0.347/1
M. caribbica	1.536/96	0.964/2	0.626/37	0.322/1
C. tropicalis	1.423/96	1.059/9	0.504/25	0.536/0.1

for exo and endoglucanase, respectively, while that of *C. tropicalis* was 9 and 6 for exo and endoglucanases, respectively (Tables 1 and 2).

Table 2. Endoglucanase activity in IU/ml at optimum incubation time, pH, temperature and substrate concentration.

Microorganisms	Enzymatic activity (IU/ml) time	Enzymatic activity (IU/ml)/pH	Enzymatic activity (IU/ml)/Temperature	Enzymatic activity (IU/ml)/Substrate concentration (%)
M. guilliermondii	0.277/96	0.136/9	0.301/37	0.094/1
A. aculeatus	0.296/96	0.116/9	0.313/37	0.062/0.01
P. aeruginosa	0.443/24	0.514/6	0.507/37	0.837/0.1
F. proliferatum	0.568/96	1.129/9	0.181/37	0.082/1
Aspergillus sp	0.424/96	0.168/9	0.162/37	0.079/1
Cerrenasp	2.120/96	0.156/6	0.170/37	0.081/1
M. caribbica	0.540/96	0.493/9	0.149/37	0.087/1
C. tropicalis	0.415/96	0.153/6	0.137/37	0.086/1

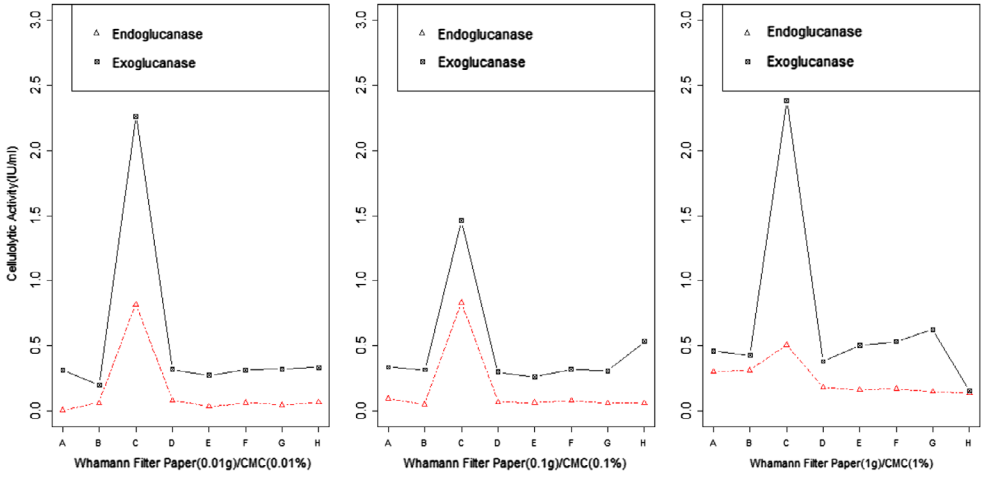

Figure 2C. Cross-sectional effect of substrate concentration on cellulolytic activity.

Effect of substrate concentration

Enzyme activity tends to increase as the substrate concentration increases. For exoglucanase, 12.5% of the microorganisms showed optimum activity at 0.1%, and 87.5% at 1% substrate concentration. For endoglucanase, 12.5% showed optimum activity at 0.01%, 12.5% at 0.1% and 75% at 1% (Figure 2C). *M. guilliermondii*, *F. proliferatum*, *Aspergillus* sp., *Cerrena* sp. and *M. caribbica* needed an optimum substrate concentration of 1% for both exo and endoglucanase activities. Although both *A. aculeatus* and *P. aeruginosa* required an optimum substrate concentration of 1% for exoglucanase activity, they differed in their optimum substrate concentration for endoglucanase activity. While the latter had an optimum concentration of 0.1%, the former had an optimum concentration of 0.01%. Regarding *C. tropicalis*, it required an optimum concentration of 0.1% for exoglucanase activity and 1% for endoglucanase activity.

Effect of temperature

Temperature played an important role in enzyme activity. All the microbes showed optimum endo and exoglucanase activities at temperature range of 25 and 37 °C (Figure 2D). While *M. caribbica* and *Aspergillus* sp. showed an optimum temperature of 37 °C for both exo

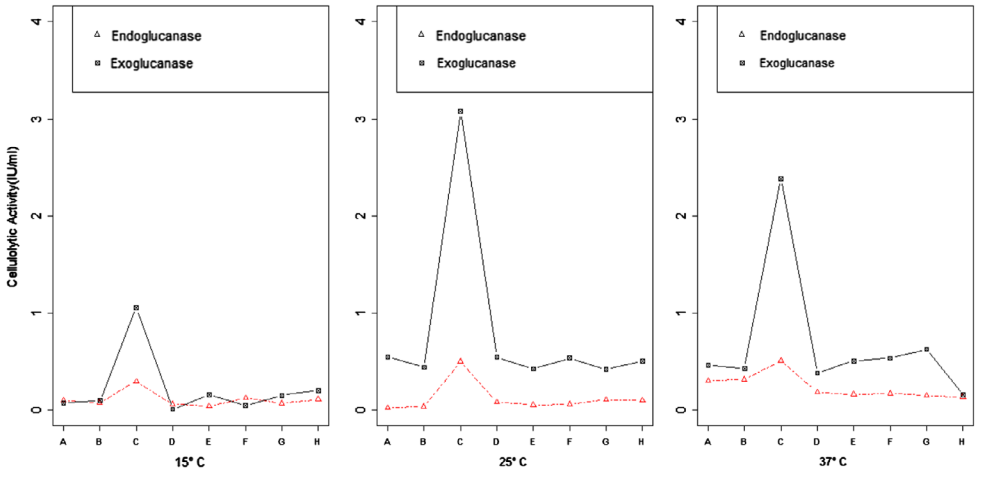

Figure 2D. Cross-sectional effect of temperature on cellulolytic activity.
Notes: A, *Meyerozyma guillermondii;* B, *Aspergillus aculeatus;* C, *Pseudomonas aeruginosa;* D, *Fusarium proliferatum*; E, *Aspergillus* sp.*;* F, *Cerrena* sp.; G,*Meyerozyma caribbica*; H, *Candida tropicalis.*

and endoglucanase activity, *M. guilliermondii*, *A. aculeatus*, *P. aeruginosa*, *F. proliferatum*, *Cerrena* sp. and *C. tropicalis* showed an optimum temperature of 25 ˚C for exoglucanase activity and 37 ˚C for endoglucanase activity.

Discussion

The detection of pigmented bacteria and fungi as inhabitants of stained and deteriorated painted walls, highlights the necessity to clarify their role in the biodeterioration process. Indeed, the production of the cellulolytic enzymes detected in this study could be a reason for the heavy discoloration observed on the stained painted surfaces from which the organisms were isolated. This is because microorganisms are known to synthesise both constitutive and inducible enzymes with potential ability to degrade different types of molecules. A previous study had shown the presence of a diverse group of microorganisms in liquid paints and also on painted walls [24,25]. This indicated that a major contribution to the survival of these organisms on such walls and cans is the nutritional nature of paint components which are degradable by microbial enzymes besides favorable environmental conditions especially in the tropics. In this study, we found that all the tested microorganisms which were from stained painted walls produced exoglucanase and endoglucanase optimally at 25–37 ˚C. This is expected firstly because of the prevailing climatic conditions in the tropics [26] and secondly because of cellulolytic activities of the existing microbes. [27] reported the natural occurrence of microbial cellulases in the aerobic and anaerobic environments where cellulolytic substrates are found. The study showed that various cellulolytic organisms that occupy such environments have developed necessary strategies to degrade cellulose, thereby secreting copious amounts of free cellulases which act synergistically to degrade paints and painted films. In a study by [28], cellulase activity was reported to be optimum at 35–45 ˚C in an assay carried out on *Bacillus circulans*. This suggests and corroborates cellulase contribution to the heavy discoloration at such prevailing temperature (25–30 ˚C)

at the sites of isolation [25]. The fact that cellulases are produced optimally at such temperature range shows that cellulase activity would decrease as the temperature increases above 45 °C. In this study, exoglucanase activity increased as temperature increased from 15 to 37°Cin *Cerrena* sp. and *M. caribbica* as also corroborated by [28]. An optimum temperature range of 25–37 °C was observed in our study for *Pseudomonas*. However, [29] reported an optimum temperature of 35 °C for *Pseudomonas*. *Cerrena* sp. had the highest endoglucanase activity at 37 °C and this explained their predominance on the surfaces. [20] also detected them at 0.4×10^3 CFU/g as against other microbes with population density ranging from 0.02 to 0.2×10^2 CFU/g. This shows that the prevailing temperature conditions in such tropical environments will favour their growth. [28] and [30] reported the optimum cellulase production for *Pseudomonas* and *Aspergillus* at pH 5 and 6, respectively. Contrary to this, [31] reported that the optimum pH for cellulase production by an *A. niger* strain was 4.5 and 7.5. Our study however, showed an optimum pH of 6 for *Pseudomonas* and a range of 6 to 9 for fungi. This variation could be a result of cellulase sensitivity to pH as cultivation pH greatly affects the microorganisms' morphology [7].

From the optimisation studies, we foresee a continual staining of important painted buildings in the tropics as the culprit microorganisms utilized minimal substrate concentrations (0.01–1%) for their exoglucanase and endoglucanase activities. In our study, the optimum substrate concentration for cellulase production was 1% for *Aspergillus* sp., the mostly encountered fungus on stained walls. This is particularly important since paints contain several organic and inorganic compounds which are utilizable as substrates [24,32] by this organism. Earlier researchers have shown that microorganisms on painted surfaces utilize nutrients, substrates and carbon sources which contribute to their metabolic activities resulting in gross staining on painted walls [33,34]. These organisms have been reported to digest various substrates which are constituents of the painted surface to support their growth, proliferation and enzyme production [9] despite the seeming lack of available nutrients on a dry painted wall. This colonisation based on substrate utilization, will eventually result in the production of irreversible staining of violet, red, yellow, orange, black and brown colorations [35].

Conclusion

This study has shown that microorganisms from stained painted walls produce exoglucanase and endoglucanase that play definite roles in degradation of paint layers and its subsequent discoloration. Temperature, pH, substrate concentration and incubation time all contribute at different levels to exoglucanase and endoglucanase activities on painted walls especially in the tropics. From the over-all result, it was observed that amongst the eight microbial strains, the endoglucanase activity was higher in *Cerrena* sp., followed by *F. proliferatum, M. carribica* and *P. aeruginosa* at varying environmental conditions. Similarly, the highest exoglucanase activity was recorded in each of *P. aeruginosa, M. caribbica* and *C. tropicalis*.

Disclosure statement

No potential conflict of interest was reported by the authors.

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