UTILIZATION OF XANTHAN GUM AND HPLC DETERMINATION OF OXALIC ACID PRODUCTION BY MICROORGANISMS ISOLATED FROM BIODETERIORATING PAINTED WALLS

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Abstract: Biodeterioration of painted walls by organisms producing various organic acids leads to undesirable loss of aesthetics. The potential of these organisms to utilize xanthan gum, a paint rheology modifier as sole carbon source for oxalic acid production was investigated. Fourteen (14) organisms isolated from grossly biodeteriorated painted walls in Lagos, Nigeria were identified to be species of the genera *Bacillus, Paenibacillus, Aspergillus* and *Penicillium* using the Sanger sequencing of the 16S rRNA gene for bacteria and the Internal Transcribed Spacer (ITS) DNA in the rRNA operon for fungi. The bacterial population ranged from 3.00 x 10^3 to 2.63 x 10^4CFU/g while the fungal population ranged from 1.00 x 10^3 to 1.63 x 10^4 SFU/g, respectively. HPLC analysis confirmed that all isolates produced oxalic acid ranging from 2.69 to 19.0447 mg/g. Descriptive statistical analysis of mean concentration of oxalic acid produced shows that *Bacillus litoralis* strain CECRI-26/07 was the highest producer on the average, while *Paenibacillus azoreducens* strain DW-TSB-4 was the lowest. Optimization studies of *B. litoralis* strain CECRI-26/07, showed 37^0C and pH 6 as optimal producing conditions. Higher level of discoloration due to oxalic acid production is expected in the tropics by virtue of the prevailing environmental condition of 37^0C which supports optimal growth of *B. litoralis* strain CECRI-26/07, a major culprit in the discoloration process.

Keywords: Paint, oxalic acid, painted wall, xanthan gum, HPLC, biodeterioration

Introduction

Oxalic acid is a strong organic acid belonging to the dicarboxylic acids family. It sometimes occurs as a free acid but more commonly as oxalates of calcium during the growth of fungal films (Son *et al*., 2000; Verma and Devi, 2015). Its biosynthesis has long been known to occur in a variety of organisms such as bacteria, fungi, plants, and animals (Magnuson and Lasure, 2004; Verma and Devi, 2015). Nevertheless, filamentous fungus, *Aspergillus niger*, remains the microorganism of choice for maximum oxalic acid production due to easy handling, ability to ferment a variety of cheap raw materials and high yields (Emeko *et al*., 2015). Interestingly, the chemical deterioration of wall paintings has been linked to production ofcertain organic acids such as oxalic acid during microbial metabolic activity (Colombini *et al*., 2002). Microbial growth on the surface of painted walls has been reported to damage and discolor the painting not only through pigmentation and enzyme production, but also by excretion of metabolic products such as organic acids (Andre *et al*., 2010; Verma *et al*., 2011; Unkovic *et al*., 2018). Heterotrophic bacteria can use organic compounds from the paint layer as growth substrates to produce acids, which results in the discoloration of the paint layer or change in its consistency (Sauer *et al*., 2008).Organic acids such as succinic acid, fumaric acid and oxaloacetate in varying amounts by different organisms (Yin *et al*., 2015) are capable of reacting with the various wall painting constituents either by solubilization of the cations or by chelation with metal ions that are present in the pigments. The carbon dioxide produced by fungi as a result of respiration, may change in an aqueous/humid environment to carbonic acid (Mookerjee *et al*., 2015), capable of dissolving the calcium carbonates of the plaster layer to form soluble calcium bicarbonates. In addition to direct attack on wall paintings, oxalic acid production favours the growth of acidophillic fungal species as secondary colonizers, thus hastening the decay process.

Xanthan gum, a high molecular weight polysaccharides employed in paint industry as a rheology modifying agent, stabilizer and emulsifier because of its compatibility with other thickeners and water-based emulsions (Silva *et al*., 2009; Vega *et al*., 2015). It prevents sagging and allows easy and even manipulation (Lachke, 2004). As a polysaccharide, it serves as an excellent substrate for various microorganisms. Decay may proceed either from the painted surface to the substrate or vice versa, depending also on the localization of the organisms. Physical decay of wall paintings is mainly due to the physical action of the growth of fungal hyphae or the fruiting bodies either on or below the surface. Microorganisms such as *Aspergillus niger*, *Ascotricha charatarum*, *Fusarium solani*, *Aspergillus* spp, *Penicillium* spp., *Cladosporium* spp., *Phoma* spp., *Stachybotrys* spp. *Ascospora* spp., *Curvularia* spp. and *Alternaria* spp. have been implicated in indoor walls of residential/ commercial constructions (Garg *et al*., 1995).

The production of organic acids by such organisms has been reported to play a significant role in biodegradation (Chunduri, 2014). The need to improve the aesthetics of painted walls cannot be over-emphasized. Therefore, it is necessary to put effective preservation strategies in place to eliminate target organisms. Unfortunately, there is dearth of information regarding the types of organic acids produced by microorganisms on painted walls. Consequently, the present study focused on the ability of organisms on deteriorated painted walls to utilize xanthan gum, a paint viscosity modifier to produce oxalic acids. The study evaluates the varying quantities of oxalic acids produced by the isolated organisms as well as the optimum production conditions.

Materials and Methods

Isolation of organisms

Samples from the discoloured painted walls were taken by a non-invasive sampling procedure (Obidi and Okekunjo, 2017) of using sterile swab sticks moistened with peptone water to swab an area of 2 cm^2 on three biodeteriorated painted walls showing visible discolorations (Fig. 1). These samples were collected from Abaranje (E1), Ijegun (E2) and Ikotun (E3) areas of Lagos state of Nigeria. The swab sticks were immediately taken to the laboratory where they were each immersed into 50 ml sterile peptone water to dislodge the

attached microbes. Aliquots of 0.1 ml were subsequently plated out in duplicates onto nutrient agar (NA) (Oxoid) and potato dextrose agar (PDA) (Oxoid) mediaprepared according to the producer's specification. The population density was determined by colony forming unit (CFU/g) considering weight of swab stick which was ~2 g. 50 mg/L of chloramphenicol and nystatin were introduced to the PDA and NA to inhibit bacterial and fungal growth respectively (Biswas *et al*., 2013). The NA plates were incubated aerobically for 24 h at 37^0C while the PDA plates were incubated for 72 h at 28^0C. The morphology of the developed isolates was determined based on the physical properties of the colonies and preliminary biochemical tests (Cheesbrough, 2003). Subsequently, isolates were subjected to confirmatory molecular identification tests using Sanger method of sequencing (Sanger, 1977).

Fig. 1: Points of isolation of samples from discolored painted walls from Abaranje (E1), Ijegun (E2) and Ikotun (E3), respectively

DNA extraction and sequencing

DNA extraction from the isolates was carried out using the Zymo Fungal/Bacteria DNA extraction kit according to manufacturer's instructions. Polymerase chain reaction (PCR)was carried out to amplify the ITS gene of the fungi isolates using the primer pair ITS-1 (5'-TCCGTAGGTGAACCTGCGG) and ITS-4 (5'-TCCTCCGCTTATTGATATGC), while it was carried out to amplify the 16SrRNA encoding gene of the bacteria using the primer pair 27F- 5′- AGAGTTTGATCCTGGCT CAG -3', and 1492R 5'-GGTTACCTTGTTACGACTT -3'. All PCR products were purified with Exo sap and sent to Epoch Life science (USA) for Sanger sequencing. The corresponding sequences were identified using the online blast search at http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Oxalic acid bioproduction in submerged fermentation

Inoculum preparation

Fungal spores of *Aspergillus* and *Penicillium* were grown on PDA for 5–7 days at 30°C. Subsequently, for inoculum preparation, the spores were aseptically harvested into a flask containing 100 ml of sterile distilled water. In the same vein, overnight bacterial isolates in NAplates were harvested into 100 ml sterile distilled water and the turbidity was adjusted to 0.5 McFarland Standard (Torey and Sasidharan, 2011). Subsequently, 5 ml of the standardized inoculum were used for the fermentation procedure.

Experimental set – up

For each organism, 12.5 g of xanthan gum as substrate/carbon source was measured into 250 ml sterile distilled water in a 250 ml conical flask. Yeast extract, 0.4 g/L; KCl, 0.00625 g/L; $NaNO_3$, 0.375 g/L; $MgSO_4.7H_2O$, 0.00625 g/L; KH_2PO_4, 0.125 g/L were added as nutrients (Emeko *et al*., 2015). 5 ml volume of standardized inoculum was added aseptically to the flask containing the substrate and the nutrients. The pH of the suspension was adjusted and maintained at 6.0. The flasks were then incubated in a shaker incubator at 30°C for 11 days at 150 rpm. After the fermentation process, the whole set-up was subjected to HPLC analysis.

HPLC analysis of oxalic acid in culture

Extraction of oxalic acids from fermented broth

20 ml of each of the fermented broth were measured into an amber bottle. Subsequently, 20 ml of acetonitrile was added to it and both were homogenized vigorously for 30 min. After mixing, the aqueous end was run off while the organic end was collected into 25 ml standard flask and made up to the mark with acetonitrile (ASTM-D5052). Same procedure was repeated for the remaining set – ups after which they were analyzed as described by the American Standard Test Methods (ASTM-D5052).

HPLC procedures

The HPLC analysis was performed with Shimadzu (Nexera MX) HPLC equipment (Length -100, ID 4.6 mm, thickness, 7 μm Ubonda Pak C18)using Acetonitrile/water as the mobile phase. The flow rate was 0.8 ml min^{-1} (water) and 5 ml/min (Acetonitrile). Sample injection was at 5 μl, UV detection was at 254 nm (Diode Array Detector DAD) and pump pressure, 15 mpa. Standard form of analytes profile were first injected into the HPLC to generate a chromatogram with a given peak area and peak profile. This was used to create a window in the HPLC in preparation of the test sample analysis. Then, aliquot of the extracted test sample was injected into the HPLC to obtain a corresponding peak area and peak profile in a chromatogram. The peak area of the sample was compared with that of the standard relative to the concentration of the standard to obtain the concentration of the sample (ASTM-D5052).

Optimization of fermentation conditions

HPLC Chromatograms generated revealed the highest oxalic acid producing organisms to be *Bacillus litoralis* strain CECRI-26/07. It was therefore, selected for optimal studies to determine optimal oxalic acid production conditions. The fermentation was carried out with variations in temperature (25, 30 and 37^0C) and pH (2, 6 and 9) of the fermentation broth.

Results and Discussion

Discolored painted walls with arrows indicating sites of collection of the isolates are shown in Fig. 1. Molecular identification of isolates with Sanger sequencing revealed the following: *Bacillus flexus* strain KLBMP 4941, *Paracoccus carotinifaciens* strain E-396, *Bacillus litoralis* strain CECRI-26/07, *Bacillus litoralis* strain IB-B8, *Bacillus pumilus* strain CZS-20 4B, *Bacillus cereus* strain YB3, *Paenibacillus azoreducens* strain B45, *Paenibacillus chibensis* strain R6-311-1, *Paenibacillus azoreducens* strain DW-TSB-4, *Lysinibacillus fusiformis* strain HS5-MRL, *Aspergillus niger* strain SAF8-EGY, *Aspergillus welwitschiae* isolate 1051, *Aspergillus tubingensis* strain MS7 and *Penicillium chermesinum* strain PCHE-1, respectively. The bacteria isolates were identified as different species of *Bacillus* and *Paenibacillus* except the *Paracoccus carotinifaciens* strain E-396. All the fungi isolates were also identified as species of *Aspergillus* except *Penicillium chermesinum* strain PCHE-1 (Tables 1 and 2). The bacterial population density ranged from 2.30 x 10^4 to 2.63 x 10^4; 3.00 x 10^3 to 3.25 x 10^3 and 1.55 x 10^4 to 1.70 x 10^4 cfu/g while the fungal population ranged from 1.00 x 10^3 to 4.25 x 10^3, 0.83 x 10^4 to 1.63 x 10^4 and 0.25 x 10^3 to 1.25 x 10^3 cfu/g for Abaranje, Ijegun and Ikotunareas, respectively. Fig. 2 shows the mean microbial population density from the three areas.

Table 1: Bacterial isolates identified using Sanger sequencing method of the 16S rRNA gene

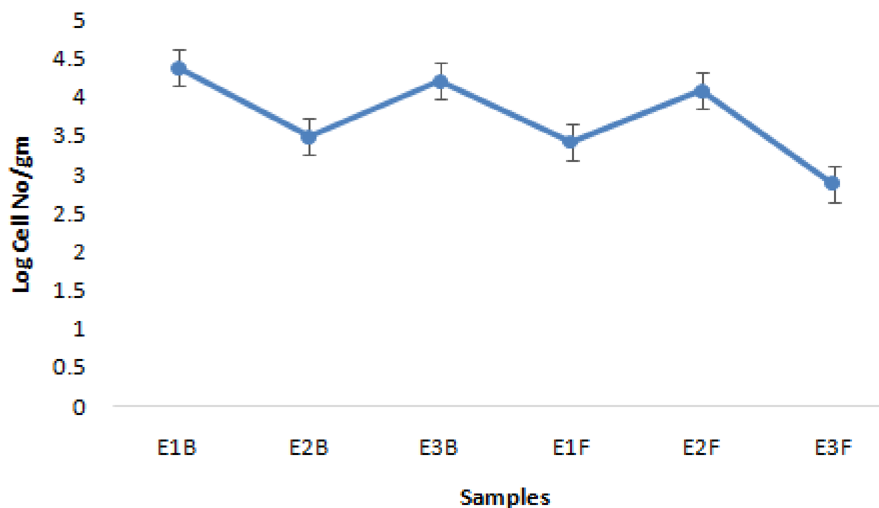
Code	Confirmed Identity	Identity (%)	Sequence ID/Accession Number
E1A1	*Bacillus flexus* strain KLBMP 4941	100	gi|1154267282|CP016790.1
E1B1	*Bacillus* sp. strain YB3	95	gi|1198370137|KX578018.1
E2A1	*Lysinibacillus* sp. strain HS5-MRL	95	gi|1048203673|KX128922.1
E2A2	*Paracoccus carotinifaciens* strain E-396	99	gi|1349733461|MG988383.1
E2A3	*Bacillus litoralis* strain CECRI-26/07	100	gi|145578089|EF535595.1
E2B1	*Bacillus pumilus* strain CZS-20 4B	81	gi|451616004|KC333913.1
E2B2	*Paenibacillus chibensis* strain R6-311-1	97	gi|381217434|JQ659795.1
E3A1	*Bacillus litoralis* strain IB-B8	99	gi|12830432|AJ309559.1
E3A2	*Paenibacillus azoreducens* strain DW-TSB-4	99	gi|401713018|JX290553.1
E3B1	*Paenibacillus azoreducens* strain B45	99	gi|939460486|LN890221.1

E1, Isolate from Abaranje, E2, Isolate from Ijegun; E3, isolate from Ikotun;A, first sample, B, duplicate sample; 1, first isolate; 2, second isolate; 3, third isolate; 4, fourth isolate; 5, fifth isolate

Table 2: Fungal isolates identified using Sanger sequencing method of the ITS and their accession number

Code	Confirmed Identity	Identity (%)	Sequence ID/Accession Number
E1B1F	*Aspergillus welwitschiae* isolate 1051	94	gi|1052541074|KT826647.1
E2A1F	*Aspergillus niger* strain SAF8-EGY	92	gi|694179300|KM222496.1
E2B1F	*Aspergillus tubingensis* strain MS7	93	gi|1279336935|MG551279.1
E3B1F	*Penicillium chermesinum* strain PCHE-1	99	gi|1127925890|KY019234.1

E1, Isolate from Abaranje, E2, Isolate from Ijegun; E3, isolate from Ikotun; A, first sample, B, duplicate sample; F, fungi; 1, first isolate; 2, second isolate



E1, Abaranje; E2, Ijegun; E3, Ikotun B, bactrerial isolates; F, Fungal isolates

Fig. 2: Mean log cell number/g of isolates from different locations

Table 3 reveals that all the organisms possess the ability to produce varying levels of oxalic acid, at different environmental conditions. The highest oxalic acid-producing organism *Bacillus litoralis* strain CECRI-26/07 was isolated from the sample obtained from Ijegun at a temperature of 27^0C/81^0F and relative humidity of 82%, while the lowest oxalic acid-producing organism *Paenibacillus azoreducens* strain DW-TSB-4 was isolated from Ikotun at a temperature of 29^0C/82^0F and relative humidity of 82%. Fig. 3 shows the concentration of oxalic acid produced by each of the isolates. Amongst the bacteria isolates, *Bacillus litoralis* strain CECRI-26/07 from Ijegun produced the highest quantity of oxalic acid (18.8692 mg/g); followed by *Paracoccus carotinifaciens* strain E–396 (17.5287 mg/g) also from Ijegun. On the other hand, *Paenibacillus azoreducens* strain DW-TSB-4 from Ikotun had the least (2.8767 mg/g) oxalic acid production. For the fungi isolates, *Aspergillus niger* SAF8-EGY from Ijegun produced the highest quantity of oxalic acid (16.0851 mg/g) while *A. welwitschiae* 1051 from Abaranje had the least (9.0029 mg/g) oxalic acid production (Fig. 3).

Among the bacterial isolates, *B. litoralis* strain CECRI-26/07 had the highest peak area, followed by *Paracoccus carotinifaciens* strain E–396. For the fungal isolates, *Aspergillus niger* SAF8-EGY had the highest peak area indicating the highest quantity of oxalic acid (16.0851 mg/g)(data not shown). Fig. 4 shows chromatogram of *B. litoralis* strain CECRI-26/07 at optimized temperature of 37^0C and pH 6.

Table 3: Environmental conditions during isolation of organisms and the quantity of oxalic acid produced

Code	Location	Relative humidity/ dew point	Temperature ^{0}C/^{0}F	Confirmed identity	Quantity of oxalic acid (mg/g)
E1A1	Abaranje	82%/17^{0}C	27^{0}C/81^{0}F	*B. flexus* strain KLBMP 4941	3.8737
E1B1	Abaranje	82%/17^{0}C	27^{0}C/81^{0}F	*B. cereus* strain YB3	7.4627
E2A1	Ijegun	82%/17^{0}C	27^{0}C/81^{0}F	*L. fusiformis* strain HS5-MRL	10.4999
E2A2	Ijegun	82%/17^{0}C	27^{0}C/81^{0}F	*P. carotinifaciens* strain E-396	17.5287
E2A3	Ijegun	82%/17^{0}C	27^{0}C/81^{0}F	*B. litoralis* strain CECRI-26/07	18.8692
E2B1	Ijegun	82%/17^{0}C	27^{0}C/81^{0}F	*Bacillus pumilus* strain CZS-20 4B	3.4221
E2B2	Ijegun	82%/17^{0}C	27^{0}C/81^{0}F	*P. chibensis* strain R6-311-1	4.7381
E3A1	Ikotun	82%/17^{0}C	29^{0}C/82^{0}F	*B. litoralis* strain IB-B8	18.8664
E3A2	Ikotun	82%/17^{0}C	29^{0}C/82^{0}F	*P. azoreducens* strain DW-TSB-4	2.8767
E3B1	Ikotun	82%/17^{0}C	29^{0}C/82^{0}F	*P. azoreducens* strain B45	5.9008
E1B1F	Abaranje	82%/17^{0}C	27^{0}C/81^{0}F	*A. welwitschiae* isolate 1051	9.0029
E2A1F	Ijegun	82%/17^{0}C	27^{0}C/81^{0}F	*A. niger* strain SAF8-EGY	16.0851
E2B1F	Ijegun	82%/17^{0}C	27^{0}C/81^{0}F	*A. tubingensis* strain MS7	13.9587
E3B1F	Ikotun	82%/17^{0}C	29^{0}C/82^{0}F	*P. chermesinum* strain PCHE-1	11.9673

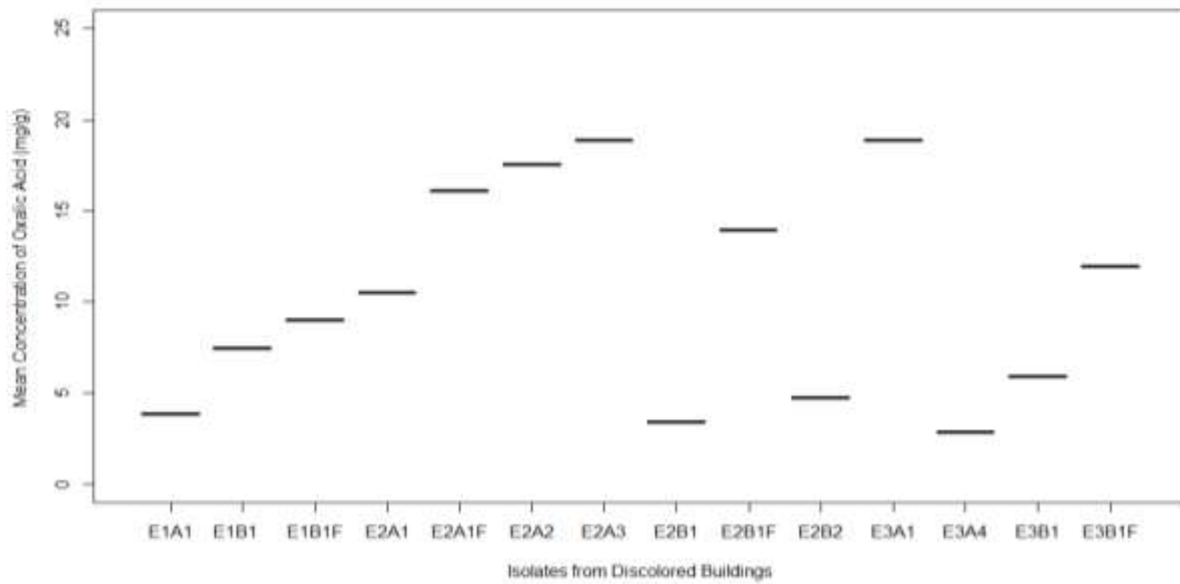
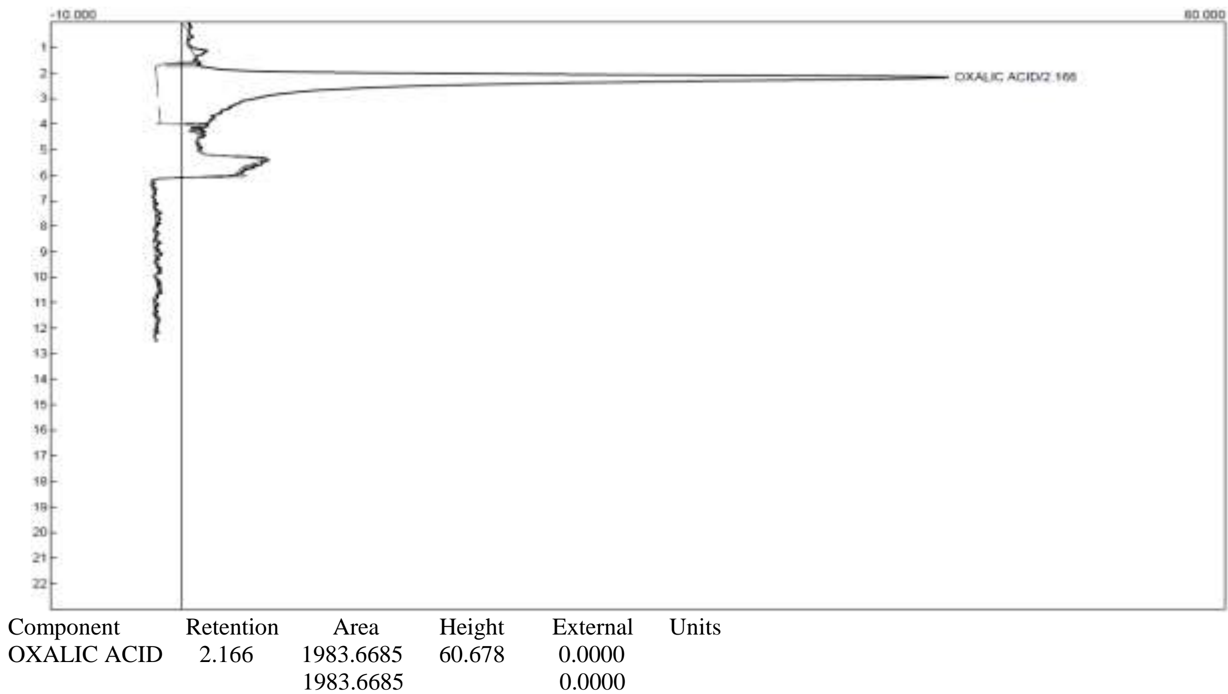


Fig. 3: Mean concentration of oxalic acid produced by isolates from discolored buildings



Component	Retention	Area	Height	External	Units
OXALIC ACID	2.166	1983.6685	60.678	0.0000	
		1983.6685		0.0000	

Fig. 4: Chromatogram of *Bacillus litoralis* strain CECRI-26/07 AT optimum temperature of 37^{0}C and pH of 6

Discoloration is a common feature on painted walls (Cappitelli *et al*., 2007), albeit, less studied in relation to the culprit organism's ability to produce certain organic acids such as oxalic acid. In the present study, the ability of the organisms isolated from discolored painted walls to produce oxalic acid using xanthan gum as a sole source of carbon was investigated. The species isolated which included *Bacillus flexus* strain KLBMP 4941, *Paracoccus carotinifaciens* strain E-396, *Bacillus litoralis* strain CECRI-26/07, *Bacillus litoralis* strain IB-B8, *Bacillus pumilus* strain CZS-20 4B, *Bacillus* sp. strain YB3, *Paenibacillus azoreducens* strain B45, *Paenibacillus chibensis* strain R6-311-1, *Paenibacillus azoreducens* strain DW-TSB-4, *Lysinibacillus* sp. strain HS5-MRL, *Aspergillus niger* strain SAF8-EGY, *Aspergillus welwitschiae* isolate 1051, *Aspergillus tubingensis* strain MS7 and *Penicillium chermesinum* strain PCHE-1 have been previously reported to be associated with the deterioration of painted walls (Uzma and Rahila, 2016; Ugbogu *et al*., 2017). Discoloration of concrete or paint on walls or nearby surfaces is usually an indication of presences of mould colonies. Of all the biological factors, fungi have been reported to be responsible for 80% of total building materials degradation (Verma and Devi, 2015). It is noteworthy that microorganisms need various environmental conditions for their growth and subsequent oxalic acid production. Oxalic acid formation in a shaking flask experiment depends on the pH value, temperature, relative humidity and incubation time. The high microbial population density of 0.25 x 10^3 to 2.63 x 10^4 CFU/g obtained in the shake flask experiment in this study could be attributed to the high relative humidity (82%) anddew point of 17^0C (Table 3). This establishes a link between high microbial population having ability to produce oxalic acids and biodegrading the walls. Dannemiller *et al*. (2017) reported that under sustained, elevated building moisture conditions, bacterial and fungal growth occurs. In addition, the study revealed that more than 50% of airborne microbes will result from fungal growth at equilibrium relative humidity of 85% and above. Therefore, the higher the percentage of relative humidity, the more humid (moist) the air, and the more, conducive it is for microbial growth. On the other hand, a lower percentage of relative humidity results in drier air. Shinkafi and Haruna (2013) reported that moisture content of the habitat is the most imperative factor for the growth of microorganisms. Generally, the microbial development on the superficial surface of a painted area suggests absorbed moisture within the walls and sufficient organic materials to support growth. Consequently, microbial growth occurs, which becomes hazardous to humans especially when spores are inhaled. The Optimal conditions obtained in the present study for the highest (18.8692 mg/g) production of oxalic acid by *Bacillus litoralis* strain CECRI-26/07 were 37^0C and pH 6. Similarly, Bolhmann *et al*. (1998) reported the highest oxalic acid concentration reached in a shaking flask experiment to be at pH 6. The high populations of bacteria ranging from 3.00 x 10^3 to 2.63 x 10^4 CFU/g and fungi from 1.00 x 10^3 to 1.63 x 10^4 CFU/g (Fig. 2) observed in the samples show that they contribute to the ongoing deterioration and discoloration of the painted walls. Although, some of the organisms isolated did not show visible pigmentation on agar plate, results of oxalic acid production (Fig. 3) revealed that they all produce different levels of oxalic acids which contribute to subsequent discoloration and degradation (Unkovic *et al*., 2018) on substrates such as painted walls. Furthermore, the present study showed that the population density does not affect the quantity of oxalic acid produced as the highest oxalic acid producing organism *Bacillus litoralis* strain CECRI-26/07 had a population density of 3.00 x 10^3 CFU/g, which is not one of the highest values (Table 3, Fig 2). Garg *et al*. (1995) reported that microbial occurrence on painted walls is as a result of the various organic and inorganic elements present in paint which provides different nutrients that may be utilized by quite a number of microbial species. The HPLC analysis carried out in this study confirm the ability of the isolates to produce oxalic acid using xanthan gum as substrate and carbon source. The study showed that *Bacillus litoralis* strain CECRI-26/07 had the highest peak area showing the highest quantity of oxalic acid (18.8692 mg/g). For the fungi isolates, *Aspergillus niger* SAF8-EGY produced the highest quantity of oxalic acid (16.0851 mg/g) (Table 3). However, the *Penicillium species* (*P. chermesinum* strain PCHE-1) produced a lower quantity of oxalic acid (11.9673mg/g) (Table 3). Similar result was obtained by Li *et al*., (2016) when *P. oxalicum* and *A. niger* were assessed for production of oxalic acid. The total concentrations of organic acids secreted by *P. oxalicum* and *A. niger* were 4000 mg/L and 10,000 mg/L, respectively. This study establishes a link between oxalic acid production by microorganisms isolated from painted wall and degradation of the wall. Similar studies reported the microbial discoloration and mechanical degradation of painted surface occurs when the microbes leach metals from the mineral pigments of the paints. The organisms produce oxalic acids which react with the metals causing the pigments to precipitate. This precipitation occurs before discoloration, fading and eventual deterioration (Milanesi *et al.*, 2006). This was corroborated by Irazola *et al*. (2012) who also observed decaying products such as oxalates on damaged and detached paintings.

Optimization study done to determine the optimum condition for oxalic acid production by *B. litoralis* strain CECRI-26/07 revealed that the highest quantity was produced at temperature of 37^0C. This is understandable because 37^0C is the standard temperature for optimum mesophilic bacteria growth (Yoon and Oh, 2005). According to Yoon and Oh, (2005), *B. litoralis* has been found to grow optimally at 37^0C. Further analysis on optimization revealed that an almost neutral pH of 6.0 favours the growth of *B. litoralis* strain CECRI-26/07 than an alkaline or acidic pH. This is in line with the work of Wang *et al*., (2016)who reported the growth and optimum production of alginate lyase by a strain of *B. litoralis*. It can be inferred from our result that *B. litoralis* strain CECRI-26/07, *B. litoralis* strain IB-B8, *A. niger* SAF8-EGY and *P. chermesinum* strain PCHE-1 are oxalic acid producers and probably contributed to discoloration on the painted walls.

Conclusion

Although all tested organisms produce oxalic acid, *B. litoralis* strain CECRI-26/07, *B. litoralis* strain IB-B8, *P. carotinifaciens* strain E–396, *A. niger* SAF8-EGY and *P. chermesinum* strain PCHE-1 produced higher quantity of oxalic acid than the other tested organisms. Descriptive statistical analysis of mean concentration of oxalic acid produced by isolates from discolored buildings revealed that *B. litoralis* strain CECRI-26/07 was the highest oxalic acid producer on the average, while *Paenibacillus azoreducens* strain DW-TSB-4 was the least. The oxalic acid production by microorganisms that colonizes painted walls contributes to their deterioration and discolouration. The study further revealed that oxalic acid can be optimally produced by *B. litoralis* strain CECRI-26/07 at temperature of 37^0C and pH of 6.0 which is prevalent in the tropics suggesting higher levels of discoloration in the tropics.

Conflict of Interest

Authors declare that there is no conflict of interest reported in this work.

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