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A first report on the identification of a novel archaea, *Methanospirillum lacunae* from spoilt paints in Lagos, Nigeria using a metagenomic approach

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ABSTRACT

Paint deterioration is a serious esthetic and economic concern attributed to microbial activity.Identifying these microbes is crucial to mitigating their deleterious effects. This paper assessed the bacterial community and population of fresh and spoilt water-based paints from a reputable paint company in Lagos, Nigeria using culture-dependent and metagenomic approaches. Gas Chromatography Mass Spectrophotometry (GCMS) was used to elucidate components of the samples. The bacterial population ranged from 1.4×10^6 to 4.4×10^8 and from 2.3×10^6 to 6.8×10^8 CFU/ml in fresh and spoilt samples respectively. Culture-based technique revealed *Bacillus* spp. as the dominant bacteria in fresh paint along with *Paenibacillus macerans, Corynebacterium striatum, Enterobacter cloacae* and *Clostridium sphenoides* while *Pseudomonas* spp. were predominant in spoilt paints together with *Arthrobacter globiformis, Azotobacter chroccoccum, Burkholderia mallei, Alcaligenes faecalis, Enterobacter amnigenus* and *Bacillus megaterium*. Microbiome sequencing of extracted DNA from spoilt paint revealed a rich bacterial diversity and a surprising archaea population of *Methanospirillum lacunae* belonging to phylum Euryarchaeota. GC-MS analysis revealed esters as dominant compounds in fresh paints while acids and alcohols suggest that degradation of the components predominate in the spoilt paints. Metagenomic approach thus revealed for the first time, the co-existence of *M.lacunae*,an archaea with other bacteria in paints.

Introduction

Paints are liquefiable mastic materials that are applied on surfaces to function as strong colored layers. They are essentially used as protection against surface deterioration while also serving a cosmetic purpose. Their characteristics rely largely on their constituents, i.e., the film-forming substances used [1]. Paints encompass various kinds of components, such as polymer binder, main pigment, thickeners, dyes, extenders and surfactants [2]. Some of these components are made up of organic and inorganic compounds that can be utilized by microorganisms to increase in biomass and number [3]. This

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makes paint susceptible to deterioration by microbes as it is relatively difficult to keep paints free of exposure to organisms and their spores [2]. Water-based paints are reported to be highly prone to biodeterioration because of their aqueous nature and undergo microbial attack during the paint production phases and during storage. The water creates a conducive environment for the growth of these organisms [4]. Bacteria, fungi and algae are the main deteriogens which can cause undesirable effects such as gassing, mal-odor, loss of viscosity and discolouration of in-can paint [5]. These biodeteriogens gain access to in-can paints through water used during production, raw materials as well as plumbing lines (tanks and tubes) and other equipment vessels [6].Other factors which may impact the deterioration of paints and paint products include: the anaerobic setting in the paint can, the organic nature of the paint components, the microbial quality of the packaging materials and the level of hygiene of the processing units of the manufacturing plant. This can lead to consequences of microbial deterioration, such as foul odor, viscosity loss, discoloration and visible surface growth, with serious economic implications for the paint industry [7].Furthermore, tropical environments like Nigeria with elevated humidity and temperature may also contribute to spoilage of in-can paint [8]. Distinct bacterial species linked to paint degradation include:*Pseudomonas, Micrococcus, Sphingomonas, Thiobacillus, Mycobacterium, Clostridium, Alcaligenes, Bacillus, Flavobacterium, Arthrobacter, Gallionella, Staphylococcus, Gracilibacillus, Salibacillus Virgibacillus*, and *Shewanella,* etc. [9,10]. A wide range of anaerobic bacteria including *Bacteroides, Clostridium, Desulphovibrio* and *Bifidobacterium* have also been isolated in paints [11] where they oxidize organic matter using electron acceptors (an oxidation–reduction type of reaction) that leaves large acidic residue in the paint. These isolates were mostly identified using culture-based method which historically has been reported to only reveal less than1% of the total microbial community present in a sample [12]. However, culture-based methods can be combined with molecular-based methods to allow detailed characterization of organisms that can be isolated [13]. The limitation presented by this approach has been massively improved on with the advent of culture-independent method which can fully elucidate the diversity (number of species within microbial community), evenness (number of cells within species), total number and distribution of species in the natural environment [14]. This can be achieved by the amplification, cloning and sequencing of the 16S rRNA gene. The 16S rRNA gene is considered to be a standard marker that has long been utilized for rapid identification of microbial phylogeny including all phyla of bacterial communities. The length of 16S rRNA is about 1500bp with highly conserved and hypervariable region that permits for finer distinction between bacteria and they are approximately ubiquitous in all bacterial members [15]. Several articles have indicated the use of culture dependent method in investigating microbial deterioration of paints and paint products [7,10]. Only few studies have used culture independent method to assess the microbial community in painted surfaces [16–18]. Furthermore, there is paucity of information about the use of metagenomics in profiling the bacteria community of spoilt in-can paint and this study, therefore, reports such for the first time in Nigeria to the best of our knowledge. Thus, this study combines the use of culture-based method with metagenomic**s** in analyzing the microbial community of fresh and spoilt paint as well as the use of gas-chromatography mass-spectrometry to identify the different constituent of the paint samples.

Materials and methods

Cultivation and enumeration of microorganisms

Two water-based in-can paint (fresh and spoilt) samples from a reputable paint industry in Lagos, Nigeria were analyzed. One mL of each sample was serially diluted (10-fold) in sterile distilled water and 0.1 mL aliquots of the dilutions 10^{-3}, 10^{-4} and 10^{-6} were plated out in duplicates on Nutrient agar (NA) (Peptone: 5 g; yeast extract: 3 g; sodium chloride (NaCl): 3 g and agar: 2%, pH: 7.4) and Centrimide agar (Centrimide: 0.3 g/l; Gelatin peptone: 20 g/l; Magnesium chloride: 1.4 g/l; Potassium sulfate: 10 g/l;Agar:15 g and pH: 7.0 $\pm$ 0.2). Plates were incubated aerobically at 37 °C for 24 h prior to enumeration of colonies. Bacterial count was carried out for both fresh and spoilt paint samples after incubation. A diverse range of colony morphotypes were selected for purification, maintained in agar slant and freshly sub-cultured before each experiment. Conventional biochemical tests which include catalase, indole, oxidase, Methyl red, Voges-Proskauer and sugar fermentation were carried out for isolates from fresh and spoilt samples as described by Cheesbrough, 2004 [19].

MiSeq high-throughput sequencing

Genomic DNA (gDNA) was extracted from fresh and spoilt paints using ZYMO RESEARCH BACTERIAL DNA MINIPREPTM KIT (Zymo Research Corp USA). For each sample, the integrity of the DNA was determined by electrophoresis on 1.0% agarose gels, and the concentration and purity of the DNA were measured spectrophotometrically with a NanoDrop2000 (Thermo Scientific Inc., USA). DNA samples were then PCR amplified using universal primer pairs (341F and 785R - targeting V3 and V4 of the 16S rRNA gene) [20]. Resulting amplicons were gel purified, end repaired and illumina specific adapter sequence were ligated to each amplicon. Following quantification, the DNAsamples were individually indexed, and another purification step was performed. Amplicons were then sequenced on illumina'sMiSeq platform, using a MiSeq v3 (600 cycle) kit. 50Mb of data (2 $\times$ 300 bp long paired-end reads) were produced for each sample. Reads were processed through usearch (https://drive5.com/usearch) and taxonomic information was determined based on the Ribosomal Database Project's (http://rdp.cme.msu.edu/index.jsp) 16 s database v16. Reads were clustered into OTUs using USEARCH version 7.1. Operational taxonomic units (OTUs) with a 97% similarity level were determined and α-diversity index including Berger_parker, Chao, Shannon diversity, Simpson diversity indices and Coverage analysis were used with MOTHUR (1.30.1).

Gas chromatography-mass spectrometry of spoilt and fresh paint

One mL of each paint sample was obtained and added to 5 mL of Methanol (HPLC grade) in a test tube. Liquid-to-liquid extraction was performed by vortexing for 20 min and the vortexed sample was centrifuged in a 2 ml micro centrifuge tube at 13,000 rpm for 10 min. A clear supernatant was obtained and 1 mL aliquot was then pipetted to be used for GCMS analysis. The samples were analyzed using a GC–MS-QP-2010 SE (Shimadzu, Japan) with a column Rtx–5MS type at a column oven temperature of 60 °C, injection temperature of 250°C, an ion source temperature of 230°Cwith a solvent cut time of 4.50 min and threshold of 2200, a flow rate of 1 mL/min helium carrier gas and an injection volume of 1 µl in a split mode. Flow control mode was in Linear velocity of 46.3 cm/sec with pressure of 144.5 kPa, a total flow of 38.7 mL/min, column flow of 3.22 mL/min, purge flow of 3.0 mL/min and split ratio of 10:1. The start and end time for the MS was set at 5.50 min and 22.50 min respectively with event time of 0.50 s , scan speed 1428 and threshold of 2200 [21].

Results

Total heterotrophic count of samples on nutrient and centrimide agar

The spoilt paint recorded a higher bacterial count on Nutrient agar with values ranging from 2.3×10^6 to 6.8×10^8 CFU/mL while it ranged from 6.4×10^5 to 5.3×10^6 CFU/mL on Centrimide agar. The fresh paint bacterial heterotrophic count on Nutrient agar ranged from 1.4×10^6 CFU/mL to 4.4×10^8CFU/mL however, no growth was observed on Centrimide agar.

Biochemical characterization of isolates from fresh and spoilt paint

The biochemical characterization revealed that the fresh paint was dominated by various species of *Bacillus* which accounted for 50% of the total isolates identified. Other isolates were identified as *Corynebacterium striatum, Enterobacter cloacae, Clostridium sphenoides, Paenibacillus maceran and Staphylococcus simulans* (Table 1). Forty percent of the isolates identified from spoilt paint were *Pseudomonas* species (Table 2). However, organisms like *Bacillus megaterium, Alcaligenes faecalis, Arthrobacter globiformis, Azotobacter chroccoccum, Burkholderia mallei* and *Enterobacter amnigenus* were also identified.

Bacterial diversity in spoilt paint

Illumina MiSeq data revealed the bacteria diversity in the spoilt paint sample. A read count of 8911 was obtained from the sample. The bacterial Operational Taxonomic Unit (OTUs) can be assigned into 25 phyla, 29 classes, 41 orders, 92 families, 211 genera and about 360 species [Supplementary material (Figures SM1-SM3)]. Proteobacteria was the most dominant phylum with an average relative abundance of 37.27%, closely followed by Bacteriodetes with 29.87%, Actinobacteria 29.42%, Planctomycetes 1.87% and Firmicutes with about 139 OTU. The most prevalent class was Actinobacteria (29.64%). Larger percentage of the species are relatively unknown (42.30%) and unclassified. *Dysgonomonas oryzarvi, Sphingobacterium hotanense, Comamonas terrigena, Ottowia beijingensis, Ochrobactrum oryzae, Phenylobacterium lituiforme, Brevundimonas naejangsanensis, Dysgonomonas mossii, Singulisphaera acidiphilia and Diaphorobacter nitroreducens* accounts for about 50% of the identified species. Commonly isolated species using culture based method such as *Pseudomonas* sp. accounts for just 3.41% of the total reads.

Diversity indices of paint sample

Alpha diversity are commonly used to charactize species diversity in a community. They account for both abundance and evenness of the species present in a sample. The obtainable valid read for bacteria and archaea were 8911 and 4 respectively while the OTU was 6427 [Supplementary material (Table SM1)]. Several diversity indices including berger_parker, chao1, dominance, Simpsons and Shannon indices were computed. Chao1, Shannon and Simpson illustrate the richness of bacteria in the sample. Simpson index is the probability that two randomly selected reads will belong to the same OTU. A value close to 1 indicates that a single large OTU dominates the sample, small values indicate that the reads are distributed over many OTUs. The dominance is the probability that two randomly selected reads will belong to different OTUs and it is calculated as 1 – simpson. The berger_parker index reflects the frequency of the most abundant OTU. A value close to 1 indicates that a single large OTU dominates the sample, small values indicate that the reads are distributed over many OTUs. Shannon index reflects the number of species and the effect of evenness [Supplementary material (Table SM1)].

Gas chromatography-mass spectrophotometry of fresh and spoilt paint

The gas chromatography mass spectrum (GC–MS) was done for both the fresh and spoilt paint samples to determine the chemical composition of both samples. Fig. 1 shows the chromatogram of the fresh paint sample. Four (4) compounds were identified from the fresh paint sample which include: 3–hydroxy-2, 4, 4-trimethylpentyl-2-methylpropanoate and 1-Hydroxy-(2,4,4-trimethylpentan-3-yl) 2-methylpropanoate which accounts for 41.87% and 56.18% of the fresh paint composition respectively (Fig. 2). 3–hydroxy-2, 4, 4-trimethylpentyl-2-methylpropanoate are plasticizers added to a synthetic

Table 1
Biochemical identification of isolated bacteria from fresh paint samples.

Isolate code	Gram reaction	Cellular morphology	Catalase test	Oxidase test	Indole test	Motility test	MR	VP	Citrate utilization	Urease activity	Casein hydrolysis	Gelatin hydrolysis	Starch hydrolysis	NO_3 reduction	MacConkey	Coagulase test	Spore test	Glucose	Sucrose	Lactose	Raffinose	Arabinose	Xylose	Galactose	Fructose	Trehalose	Maltose	Mannitol	Sorbitol	Probable identity
KAFII	+	Rods	−	+	−	−	+	−	−	−	+	−	+	+	−	−	−	+	−	−	−	−	−	−	+	−	+	−	−	*Corynebacterium striatum*
KAFI	−	Rods	+	−	−	+	−	+	+	+	−	−	+	+	+	−	−	+	+	+	+	+	−	−	+	+	+	+	+	*Enterobacter cloacae*
KAF^{2}9	+	Rods	+	−	−	+	−	+	+	−	+	+	+	+	−	−	+	+	+	−	−	+	−	−	+	−	+	+	−	*Bacillus licheniformis*
KAFVII	+	Rods	+	+	−	+	−	+	+	−	+	+	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	−	*Bacillus coagulans*
KAFV	+	Rods	+	+	−	+	−	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	*Bacillus polymyxa*
KAFVIII	+	Rods	+	+	−	+	−	−	−	−	+	+	−	+	+	−	+	+	+	+	+	−	−	−	−	+	−	+	−	*Bacillus laterosporus*
KAF9	+	Rods	−	−	+	+	+	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	*Clostridium sphenoides*
KAF7	+	Rods	+	+	−	+	+	−	−	−	−	+	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	+	−	*Bacillus brevis*
KAF5	+	Rods	+	+	−	+	−	−	+	−	−	+	+	+	+	−	+	+	+	+	+	+	+	−	−	+	−	+	−	*Paenibacillusmacerans*
KAF11	+	Cocci	+	−	−	−	−	+	−	+	−	−	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	+	−	*Staphylococcus simulans*

Table 2
Biochemical identification of Isolated bacteria from spoilt paint.

Isolate code	Gram reaction	Cellular morphology	Catalase test	Oxidase test	Indole test	Motility test	MR	VP	Citrate utilization	Urease activity	Casein hydrolysis	Gelatin hydrolysis	Starch hydrolysis	NO_3 reduction	MacConkey	Coagulase test	Spore test	Glucose	Sucrose	Lactose	Raffinose	Arabinose	Xylose	Galactose	Fructose	Trehalose	Maltose	Mannitol	Sorbitol	Probable identity
KAS6	–	Rods	+	–	–	+	–	–	+	–	–	–	–	+	+	–	–	+	–	–	–	–	–	–	+	+	–	+	–	*Arthrobacter globiformis*
KAS8	+	Rods	+	+	–	+	–	–	–	–	–	–	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	+	–	*Azotobacter chroccoccum*
$G^{-2}C$	–	Rods	+	–	–	–	–	–	+	+	–	–	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	+	–	*Burkholderia mallei*
$G^{-3}C$	–	Rods	+	+	–	+	–	–	+	–	–	–	–	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	*Alcaligenes faecalis*
KASII	-	Rods	+	–	–	+	–	+	+	–	–	–	–	+	+	–	–	+	–	–	–	–	–	–	–	+	+	+	+	*Enterobacter amnigenus*
KAGC	–	Rods	+	+	–	+	–	–	+	–	–	+	–	+	+	–	–	+	+	+	–	–	–	–	+	–	–	–	+	*Pseudomonas mendocina*
$C^{-2}C$	–	Rods	+	+	–	+	+	+	+	–	–	–	–	+	+	–	–	+	+	+	+	+	–	+	–	+	+	+	–	*Pseudomonas putida*
KAC2	–	Rods	+	+	–	+	–	–	+	–	–	+	–	+	+	–	–	+	+	+	–	+	+	–	–	–	+	+	–	*Pseudomonas aeruginosa*
KAC3	–	Rods	+	+	–	+	–	–	–	–	–	–	–	+	+	–	–	+	–	–	+	–	+	–	+	–	–	–	–	*Pseudomonas caryophylli*
KAS2	+	Rods	+	+	–	+	–	–	–	+	+	+	+	–	+	–	+	+	+	–	–	+	–	–	–	–	–	+	–	*Bacillus megatarium*

MR- Methyl Red, VP- Voges-Proskauer.

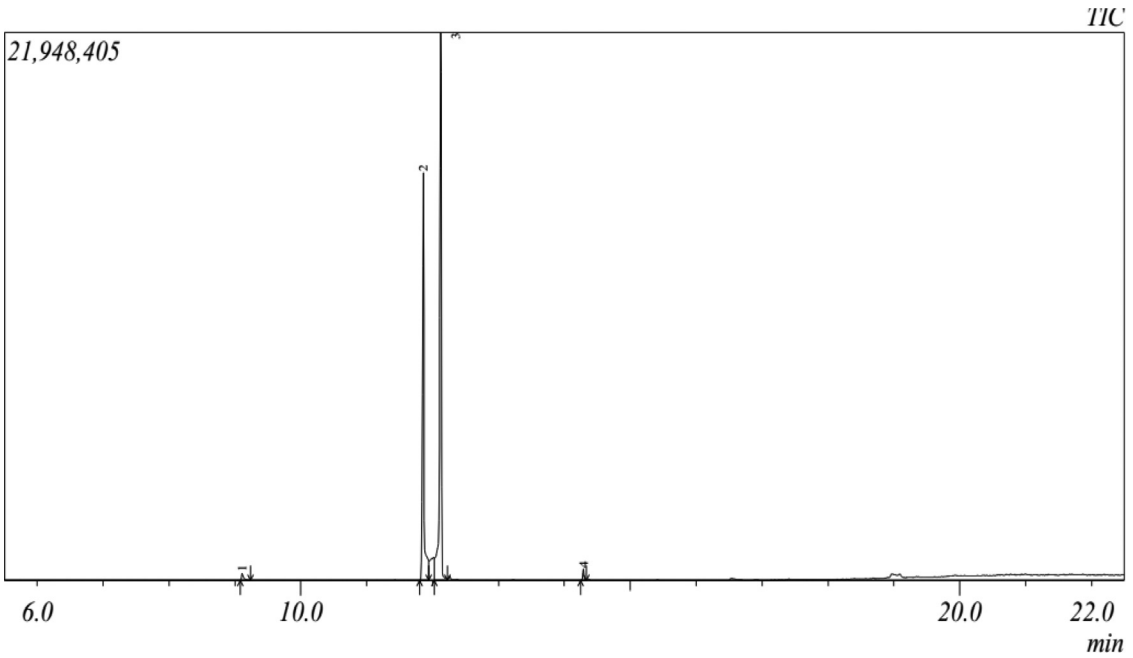

Fig. 1. Gas chromatogram of freshpaint sample.

resin to produce or promote plasticity and flexibility and to reduce brittleness. The other compounds are 2,2,4-trimethyl-1, 3-Pentanediol and Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)−2-methyl-1,3-propanediyl ester. Fig. 3 shows the chromatogram of the spoilt sample. In contrast to the fresh samples, twenty four (24) compounds were identified in the spoilt paint which include: 2-{2-[4-(1,1,3,3-T*etramethylbutyl*)*phenoxy*]ethoxy}ethanol and 10-hydroxycamptothecin responsible for 12.55% and 21.33% of the spoilt paint samples (Fig. 4).

Discussion

The bacterial population density obtained on nutrient agar from the fresh paint sample was much lower than that of spoilt samples.This is however, much lower than the figures posited by Oyeleke et al. (2005) and Obidi et al. (2017) [23] who nevertheless reported higher microbial load in spoilt in-can paint than fresh paint. However, there was no growth detected on Centrimide agar for fresh paint samples while the mean bacterial count for spoilt paint was up to 10^6 CFU/mL. The lack of growth on Centrimide agar for fresh paint which is selective for *Pseudomonas* spp is consistent with the findings of Obidi et al. (2017) [23]. Generally, contaminated raw materials used in the production of paints, lack of sterile processing water and unhygienic manufacturing processes contribute to rapid deterioration of paints [7]. However, the low population of microorganisms in the fresh samples can be linked to the effectiveness of incorporated biocides, which are still very active at the fresh state. The wearing out of the biocidal effect over time will lead to higher bacterial population levels which was observed in spoilt paints**.** Increased number of bacteria in the spoilt paint can be attributed to their utilization of the paint components (organic materials) such as cellulose used as thickener; glues, emulsifier and binders used as substrates for growth thereby leading to paint deterioration [24].

Biochemical identification of isolates from fresh paint reveals the dominance of *Bacillus* spp which accounts for about 50% of the isolates identified. *B. licheniformis, B.coagulans, B.polymyxa, B.laterosporus* and *B. brevis* were all identified. Their presence in fresh in-can paint is suggestive of soil contamination. Previous studies have implicated the presence of *Bacillus* in paints and paints product [7, 9, 11,25]. Their survival in fresh in-can paint can be linked to their ability to form spores and their versatile metabolic capabilities. The effects of *Bacillus* spp. that can grow in paint irrespective of the biocides added depends on many factors of which storage is of prime importance. They can oxidize organic matter present in fresh in-can paint through oxidation–reduction types of reaction that leaves large acidic fragments in the paint. Orehek et al. (2013)[26] studied the biodeterioration of carboxymethyl cellulose (used as thickener and moisture retention agent) by *B. subtilis* subsp. *subtilis* NCIB 3610. *B. subtilis* subsp. *subtilis* NCIB 3610 was shown to synthesize cellulases that effectively break down chemical bonds in carboxymethyl cellulose; however, it was unable to re-utilize the end products of hydrolysis [25]. *Paenibacillus macerans,* a *Bacillus*-related genera was also identified in the fresh in-can paint. This diazotrophic bacterium was reported by Gorbushina et al. (2004) [9] as part of the biodeteriogens found on the mural painting environments in Germany. Its appearance in fresh paint can be linked to soil contamination as these spore formers are abundant in soil and

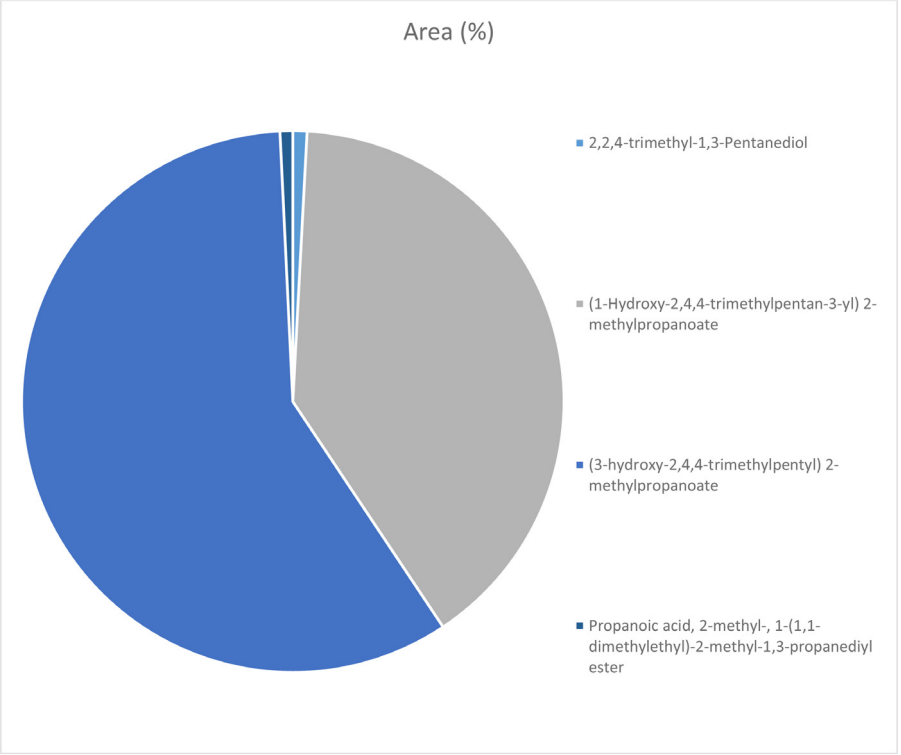

Fig. 2. Concentration of chemical component of fresh paints.

they possess a wide range of metabolic capabilities [26]. *Staphylococcus simulans* was also found in the fresh paint which is quite different from the *S. aureus* reported by previous investigators [22, 27, 28].

Heterotrophic bacteria such as *Enterobacter cloacae* and *Corynebacterium striatum* were isolated from the fresh paint sample. Obidi et al. (2010) [29] reported the occurrence of *Enterobacter*spp in-can paint deterioration but several researches have only indicated the presence of *Corynebacterium* in deteriorated painted surfaces. Anele et al. (2019) [3] reported the isolation of *Corynebacterium* spp. (10.9%) from biodeteriorated painted classroom wall surfaces. *Clostridium sphenoides*, an obligate anaerobe was also isolated. This is in agreement with findings by Tothill and Seal (1993) [30] who implicated the presence of *Clostridium sphenoides* along with *Actinomyces israelii, Bacteroides clostridiformis, B. fragilis, B. hypermegas, B. ovatus, Bifidobacterium adloscentis, Clostridium butyricum, C. subterminale, Desulfovibrio desulfuricans, Lactobacillus fermentum, Peptococcus saccharolyticus* and *Streptococcus intermedius* in the biodeterioration of waterborne paint cellulose thickeners. Its survival in in-can paint can be attributed to the anaerobic environment of the can. *Clostridium* sp. are known to produce cellulase that can degrade the thickener used in paint production and break them down into cellobiose. Anaerobic microorganisms in the finished paint function by oxidizing organic matter using electron acceptors other than oxygen for growth.The degradation activities of anaerobic organisms are the result of a hydrolytic or simultaneous oxidation–reduction-type of reaction. Biochemical identification also revealed that 40% of the isolates obtained from the spoilt paint belongs to the genus *Pseudomonas*. Previous studies by Obidi et al. (2010) [29] has shown the occurrence of several bacterial species such as *Pseudomonas, Klebsiella, Enterobacter, Bacillus* and *Alcaligenes* in paint biodegradation. Species of *Pseudomonas* and *Bacillus* have been particularly identified in the degradation and alteration of paint characteristics [6]. Similarly, Odokuma et al. (2013)[4] also reported that *Bacillus* sp. and *Pseudomonas* sp. exhibited higher predominance on painted surfaces, in-can paint and paint waste that may be attributed to different survival strategies evolved by these microorganisms. The fact that *Pseudomonas* sp.only occur in spoilt in-can paint is in tandem with earlier publications by Dey et al. (2004) and Obidi et al. (2010) [31, 29]. The persistence of *P. aeruginosa* in spoilt paint is also suggestive of its ability to resist different biocides and high level of heavy metals that accumulate in spoilt paint. Obidi et al. (2010) [29] in similar studies investigated the biodegradative potential of *Pseudomonas aeruginosa* on water-based paints and linked their biodegradative ability and antimicrobial resistance to the presence of plasmid which range in size from 0.030 to 0.112 kgbases (kb). They reported 8% decrease in the biodegradative potential of the cured strain when compared with the wild strain which exhibited 25% biodegradative potential. Also, the pH of most paints which is in range of 8–9.5 favours their growth. *P. mendocina* has been identified to completely mineralize pentachlorophenol (biocide) used in paints [32]. *Arthrobacter globiformis* was also identified in the spoilt paint. This is in agreement with Altenburgera et al. (1996) [33] who reported *Arthrobacter, Streptomyces* and *Pseudomonas* as major biodeteriogens in medieval wall paintings. *Alcaligenes faecalis* identified in spoilt paint has also

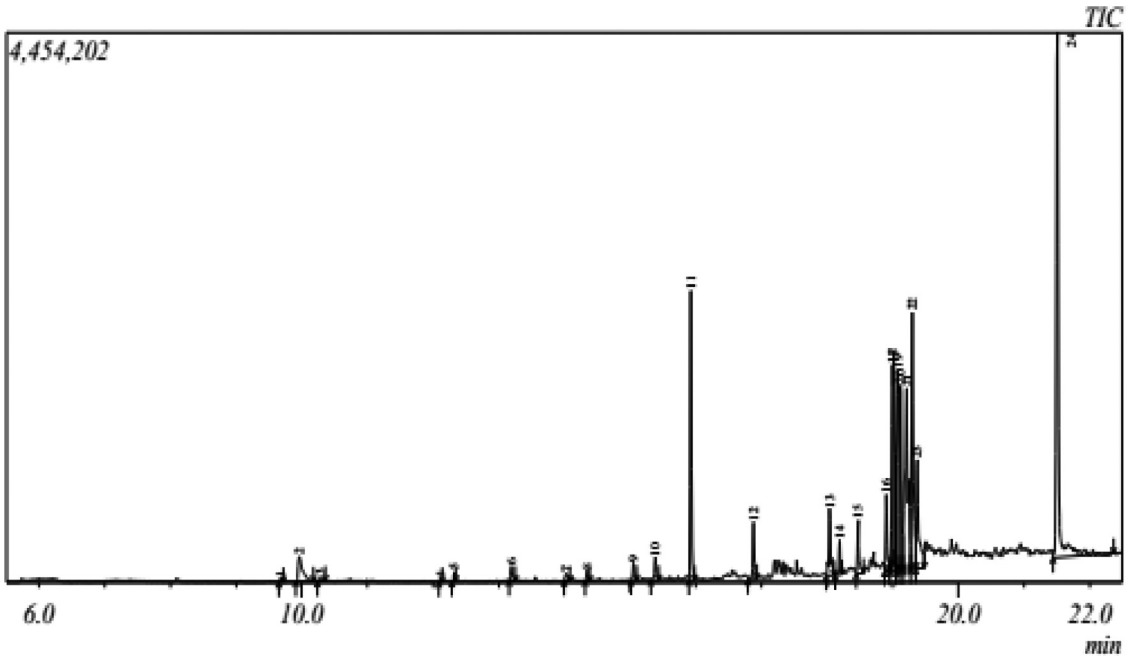

Fig. 3. Gas chromatogram (GC) of spoilt sample.

been reported in previous studies by Santos et al. (2009) and Ravikumar et al. (2012) [34, 10]. Similar study by Etim and Antai (2014) [35] reported that *Alcaligenes* population in spoilt paint scrap was just next to*Pseudomonas* and *Bacillus.* The presence of these organisms in paint is indicative of soil contamination since they are prevalent in soil. *Bacillus megaterium, Enterobacter amnigenus, Burkholderia mallei* and *Azotobacter chroccocum* were also identified in spoilt in-can paint. *Burkholderia* sp. have been reported to have biodegradative potentials [36] but their occurrence in spoilt in-can paint has been rarely reported.

Traditional methods for microbial identification rely on the recognition of differences in morphology, growth, enzymatic activity, and metabolism to define genera and species which are limited in their application. However, full and partial 16S rRNA gene sequencing methods have emerged as useful tools for identifying phenotypically aberrant microorganisms [37]. The extracted DNA purity and concentration was ascertained to be 5.2 ng/ul and 1.60 which signals effective removal of protein fraction. However, no DNA was recovered from fresh paint. This could be attributed to the fact that the biocide in a fresh paint is still very active which will greatly reduce the population of microorganism to a level that is too minute to be picked by the extraction kit. Nevertheless, growth of isolates from fresh paint on Nutrient agar recorded in this study may be due to the fact that the media composition was fortified with the adequate growth factors which encouraged the microbes to thrive. The analysis revealed a valid read of 8911 for bacteria and 4 reads for archaea. Illumina MiSeq data revealed the bacteria diversity can be assigned into 25 phyla, 29 classes, 41 orders, 92 families, 211 genera and about 360 species. In similar study by Duan et al. (2017) [38], bacterial OTUs reported were assigned into 19 phyla, 202 families and 435 genera. Identification of archaea in paint is unexpected and novel. Pinar et al. (2001) [39] identified archaeal communities belonging to well-characterized halophilic and alkaliphilic archaea in two deteriorated ancient wall paintings by combination of Denaturing Gradient Gel Electrophoresis (DGGE) of PCR-amplified DNA and construction of clone libraries. This lend credence to the fact that archaea can be found in non-extreme environment and terrestrial ecosystem. Archaea identified in this study belongs to the novel phylum Diapherotrites and phylum Euryarchaeota. *Methanospirillum lacunae,* a methane-producing archaea, identified in the spoilt in-can paint has been known to be responsible for the final step of the anaerobic degradation of organic substances [40]. This archaeon has not been identified in paints prior to this study. Phylum Proteobacteria dominates the microbial communities and accounts for about 37.27% of the bacterial community present in the spoilt paint which is in contrast with report of Duan et al. (2007) [38] where Actinobacteria was the most abundant division which account for about 53.53% of the total read. Phylum Bacteroidetes, Actinobacteria, Proteobacteria, Planctomycetia, Firmicutes, Acidobacteria and Gemmatimonadetes accounts for 29.87%, 29.64%, 1.87%, 1.56%, 0.18% and 0.07% of the bacteria OTUs present in the spoilt paint respectively. This is in contrast with similar study by Ma et al.(2015) [41] on deteriorated wall painting, where Firmicutes and Proteobacteria collectively accounted for 83.8% of all samples and the remaining seven bacterial phyla only accounted for 16.2%, with 6.0% Actinobacteria, 4.9% Acidobacteria, 2.9% Cyanobacteria, 1.7% Bacteroidetes, 0.3% Gemmatimonadetes, 0.3% Planctomycetes, and 0.2% Chloroflexi. Genera obtained from this analysis are dominated by *Streptomyces (*32.95%), *Dysgonomonas*(17.71%), *Sphingobacterium (*14.35%) and *Comamonas* (5.77%). This is in contrast with findings of Gurtner et al. (2000) [42] who used DGGE and 16S rRNA sequence to identify *Arthrobacter, Actino-*

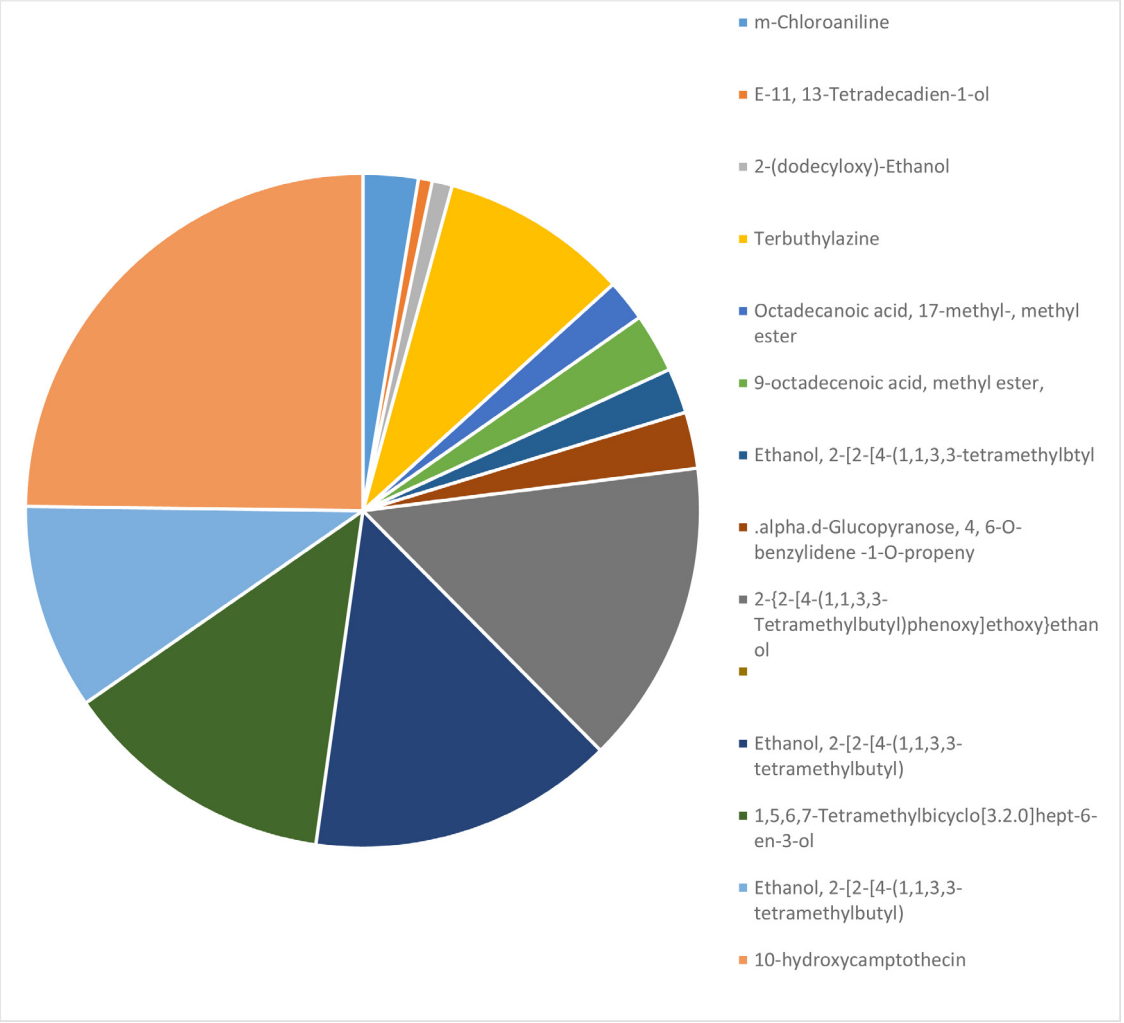

Fig. 4. Concentration of the chemical component of spoilt paint.

bispora, Amycolata, Asiosporangium, Frankia, Geodermatophilus, Nocardioides, Promicromonospora, Pseudonocardia, Rubrobacter, Streptomonospora, Saccharopolyspora, Sphaerobacter, Aquaspirillum, Chromohalobacter, Deleya, Erythrobacter, Halomonas, Porphyrobacter, Pseudomonas, Rhizobium, Salmonella and *Thermocrismum* on two different biodeteriorated wall paintings. Genus *Pseudomonas* accounts for only 3.9% of the read count which betrays what was obtained using the culture-based method in this study and in previous reports that used culture-based method [33, 1]. Large number of the species (33.25%) are unknown which may be due to limited availability of known reference genomes and insufficient sequencing coverage [43]. *Dysgonomonas oryzarvi* accounts for 13.95% of the read count. This is in sharp contrast to the result obtained using culture-based method where *Pseudomonas* spp dominates. *D. oryzarvi* has not been implicated in deterioration of paints prior to this study. It is a Gram negative, facultative anaerobic, non-motile short coccoid to short rod-shaped bacterium which was first isolated from microbial fuel cell where they degrade organic matter to generate electricity [44]. *D. oryzarvi* presence in paint can be linked to its degradative activity. Duan et al. (2016) [38] reported the biodegradative activities of *Dysgonomonas* sp. on kraft lignin with optimum conditions of pH 6.8 and temperature of 33 °C. Kita et al. (2015) [45] also detailed the ability of *D. alginatilytica* to break down alginate which is used as thickener during paint production in form of sodium alginate. *Sphingobacterium hotanense* accounts for 15.76% of the read count in this study. Its presence in spoilt paint is actually surprising because it's a non-spore former and strict aerobe with likely source being soil. The most dominant Proteobacteria in this study was *Comamonas terrigena* with 5.48%. *Comanonas terrigena* has been reported to degrade phenol [43] and dialkyl sulfosuccinates (DASS) which is used as emulsifiers in paint production [46]. Only 5 reads were reported for *Alcaligenes faecalis* and *Pseudomonas aeruginosa* which highlights the inability of culture-based method to elucidate the microbial composition of a spoilt paint. This study shows that the degradation of the paint is caused by a large consortium of microorganisms which act synergistically to degrade basic component of the paint.

Alpha diversity is the main species diversity in habitats at local scale. These diversity indices are commonly used to characterize species diversity in a community and either accounts for abundance, evenness or richness of the species present within sample obtained from a local scale [47]. This study showed 6427 Operational Taxonomic Units (OTUs) for the spoilt paint sample, which refers to clusters of (uncultivated or unknown) organisms, grouped by DNA sequence similarity of a specific taxonomic marker gene. Accurate assessment of species richness is useful for the effective analysis of biological communities [48] and the OTUs richness, which is the number of OTUs with at least one read for this sample is 52. Chao1 is a nonparametric method for estimating the number of species in a community [49] and is based on the concept that rare species infer the most information about the number of missing species. The Chao1 index for this sample is the same as the OTUs richness. The Simpson and Shannon indices provide more inference about the community composition than simple species richness or evenness. The Berger-parker measures the frequency of the most abundant OTUs and a value close to 1 indicates that a single large OTU dominates the sample, small values indicate that the reads are distributed over many OTUs [50]. The Berger-parker index reads 0.35 which indicates the reads are well saturated over many OTUs.

The Gas-chromatography Mass-spectrophotometry (GC–MS) revealed four (4) components for the fresh paint. The peaks revealed (1-Hydroxy-2, 4, 4-trimethylpentan-3-yl) 2-methylpropanoate) and (3–hydroxy-2,4,4-trimethylpentyl) 2-methylpropanoate) as the dominant compounds with relatively high peaks. These are alkyd resins which are mostly used as binders, to enhance paint performance by making them resistant to mud-cracking, washability and scrub resistance. 2, 2, 4-trimethyl-1,3-pentanediol also revealed by the GC–MS as component of fresh paint are polyester resins and plasticizers which confers special effect on the paint. A key additive in most water-based (latex) paints as reported by Corsi and Lin (2009) [51] is 2,2,4-trimethyl-1,3-pentanediol monoisobutyrate (TMPD-MIB). TMPD-MIB is added as a coalescing aid that helps to soften polymeric binder particles, a property that facilitates complete fusion when paint dries. It also enhances scrub resistance, color development, and packaging stability [51]. The presence of these compounds in the fresh paint shows the good quality of the paint compared to the spoilt paint. The Twenty-four(24) compounds revealed by the GC–MS for spoilt paint is suggestive of the degradation of paints component into different intermediates leading to separation of the paint into phases, malodour and discolouration. The peak heights of 10-hydroxycamptothecin and Terbuthylazine are the highest with 20.52% and 11.31%. Terbuthylazine, a microbicide presence in the spoilt paint showed that it was not degraded by the organism and indicated resistance to its antimicrobial activities. The high abundance of ethanol and related compounds in the spoilt paint indicates the activity of microorganism in deteriorating the fresh paints components thereby liberating alcohol as end products of metabolism. The presence of these compounds was reported by previous workers [51,52]. Carbamic acid presence in spoilt in-can paint shows that the compounds that are used as biocides for the control of growth and proliferation of microorganisms are still relatively present albeit in small quantity. They observed diminution in ester compounds of the spoilt paint as compared to the fresh is due to the attack of microorganisms that led to change in polymeric structure of esters by rupturing the ester linkages. This result is consistent with the findings of Ishfaq et al. (2015) [52] who used Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) studies to analyze paint degradation by fungi and bacteria. They reported loss in intensity of the bands for ester linkages indicated degradation of the paints through the breaking of the ester group and a loss in intensity of bands at a wavelength of 3286.87 cm^{-1} (corresponding alcoholic peak) due to breakage of alcoholic linkages. Datasets are as described by Obidi et al.,(2020) [53].

Conclusion

The study reveals a more detailed account of microbial diversity and interactions with metagenomic approach compared to the conventional isolation process. This is particularly important because the study reports for the first time the use of metagenomics in Nigeria for studying the paints' microbial composition. The GC–MS analysis complements the obtained results by providing vital information on the degradative abilities of the observed organisms.

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Data availability

The raw data required to reproduce these findings are available to download from [https://data.mendeley.com/datasets]. The processed data required to reproduce these findings are available to download from [https://data.mendeley.com/datasets].

Declaration of Competing Interest

The authors declare that there is no conflict of interest

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