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**RESEARCH ARTICLE**

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# Molecular-based analysis of the bacterial community structure of spoilt latex paints in Nigeria

**ABSTRACT:**

Biodeteriorated paints showing discolouration, loss of viscosity and foul odour have resulted in substantial economic losses in Nigeria. The present study focused on enumeration and identification of bacteria isolated from spoilt paints made in Nigeria using a non-selective nutrient agar (NA) and a semi-selective Pseudomonas Isolation Agar (PIA). The bacterial population density in a fresh paint sample was 3.0 x $10^3$ CFU/ml while that of five samples of spoilt paint ranged from 1.0 x $10^3$ to 1.2 x $10^6$ CFU/ml on NA. The fresh paint had no growth on PIA while spoilt paint bacterial counts ranged from 2.0 x $10^3$ to 8.0 x $10^5$ CFU/ml. The most diverse colony types from the spoilt paint samples were randomly selected for purification. Nine isolates were subjected to sequencing of a portion of the 16S rRNA gene and 14 isolates to whole-cell fatty acid methyl ester profiling using the MIDI-FAME system. The most prevalently identified were *Klebsiela* and *Providencia* species. A subset of isolates was examined by transmission electron microscopy. Other detected genera included Bacillus, Brevibacillus, Lysinibacillus, and the Nocardioforms. Our results show that spoilt latex paints made in Nigeria can harbour diverse bacterial species which can cause biodeterioration.

**KEY WORDS:**

Biodeteriorated paints, 16S rRNA, molecular methods, PCR, FAME

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## INTRODUCTION:

Architectural paints do not only protect surfaces from sunlight, moisture, abrasion, dust and damage but also add color and sheen, thereby improving their appearances. Therefore, they perform a largely decorative function. When paints are applied to various surfaces, they adhere to the surfaces as they dry, creating an aesthetically pleasing coated surface. Paints contain several types of substances such as polymer binder, primary pigment, extenders, thickeners, colorants and surfactants (Kostansek, 2007). Studies have shown that a variety of natural and synthetic pigments are used in the paint manufacturing process (Alabi and Omojola, 2013). A consequence of this formulation is the potential degradation of the physico-chemical properties of the paint and the film it forms. Exposure to extreme physical and chemical conditions such as high humidity and temperature, which are prevalent in tropical climates like Nigeria, also favor microbial degradation of paints (Lucchesi, 2003). In addition, latex paints, by virtue of their aqueous nature are highly susceptible to microbial spoilage (La Rossa *et al*., 2008).

Most microorganisms contaminating paints come from the processing water. These microorganisms produce biofilms which adhere to the inner surfaces of plumbing systems of tanks containing water for paint production and are generally very resistant to biocides (Winkowski, 1999). Previous studies have shown the occurrence of several bacterial species such as *Pseudomonas, Klebsiella, Enterobacter*, *Bacillus* and *Alcaligens* in paint biodegradation (Arquiaga *et al*., 1995; Obidi *et al*., 2010). Amongst the bacteria associated with the deterioration of packaged paints, species of *Pseudomonas, Flavobacterium, Escherichia coli* and *Bacillus* have been particularly identified in the degradation and alteration of paint characteristics (Fazenda, 1995). These microorganisms hydrolyze the paint thickeners, as well as other organic constituents, leading to loss in paint viscosity (Dey *et al*., 2004) which significantly alters the paint's adhesive properties (Bardage and

Bjurman (1998). Increased biocide levels have thus, been used to inhibit microbial spoilage of paints to prolong their shelf-life (Brady, 2000). In Nigeria, despite the fact that spoilt paints have resulted in huge economic losses, there has been very few reports assessing or evaluating microorganisms associated with spoilt paints. Therefore, considering the paucity of information on microbes associated with paint degradation and the subsequent economic losses in the Nigerian coating industry, we report in this article, the identity of bacteria isolated from spoilt Nigerian latex paints.

## MATERIAL AND METHODS:

### Sample collection and isolation procedures:

One fresh and five spoilt paints samples were obtained in sterile containers from a private paint manufacturer in Lagos, Nigeria. One gram of each sample was serially diluted (10-fold) in sterile Nanopure water and 0.1 mL aliquots of the dilutions were plated on Nutrient agar (NA) (Peptone: 5 g; yeast extract: 3 g; sodium chloride (NaCl): 3 g and agar: 2%, pH: 7.4) and Pseudomonas isolation agar (PIA) (Peptone: 20.0 g; magnesium chloride: 1.4 g; potassium sulfate: 10.0 g; irgasan: 0.025 g; agar: 13.6 g; glycerol: 20.0 ml; pH: 7.0 ± 0.2). Plates were incubated aerobically at 37ºC for 24 h prior to enumeration of colonies. A diverse range of colony morphotypes were selected for isolation, maintained in 10% (v/v) glycerol at -70ºC and freshly subcultured before each experiment.

### Fatty acid analysis:

The extraction process was carried out as described by MIDI (Newark, DE, USA). Overnight cultures of isolates were grown on Trypticase Soy Broth Agar at 28ºC. Total cellular fatty acids were extracted from cells with a 15% NaOH solution made in 1:1 methanol and water. The fatty acids were methylated in a 6 N HCl-methanol solution (3.25: 2.75) for 10 min at 80ºC. FAMEs were extracted with a 1: 1 mixture of hexane and methyl *tert*-butyl alcohol. Flame ionization detection gas chromatography was performed using the MIDI system (Newark, Del.) and in accordance with the manufacturer's recommended materials and protocols. Peaks were compared

### Preparation of Bacterial Smear for Transmission Electron Microscopy:

Three µl of overnight bacterial suspension of the test organism was dispensed onto a carbon coated grid for 1 minute, allowing contents to settle. Most of the supernatant was wicked away and 3 µl of 2% uranyl acetate was applied for 30 seconds as a negative contrast stain. The grids were wicked and allowed to dry. Images were captured using a JEOL 2100 transmission electron microscope (Japan Electron Optic Laboratories USA, Peabody, MA) with an Ultrascan 1000 digital camera and software (Gatan Inc., Warrendale, PA) (Burghardt and Droleskey, 2006).

### 16S rRNA gene sequencing:

Genomic DNA was extracted from pelleted overnight cultures using the Promega Wizard Genomic purification kit (Madison, WI, USA). The DNA concentrations were determined using a NanoDrop 2000c spectrophotometer (ThermoFisher). The genomic DNAs were used as template for PCR amplification of the 16S rRNA gene using primers 8F (5'-AGAGTTTGATCCTGGCTC-3') and 1404R (5'-GGGCGGWGTGTACAAGGC-3') (Marchesi *et al*., 1998). The PCR reaction mixture was made up of Master Mix in a total volume of 30 µl containing 100ng template DNA, 0.8 µM of each primer, 0. 2 mM of dNTP, 2.5 U Takara ExTaq DNA polymerase, and 1 X PCR buffer. Amplification was carried out in a PTC 200 Peltier Thermal Cycler using a PCR program modified from Dang and Lovell (2000) with initial denaturation at 94ºC for 5 min and subsequent 40 cycles of denaturation at 95ºC for 30 secs, annealing at 45ºC for 30 secs, and extension at 72ºC for 1 min. This was followed by final extension at 72ºC for 5 min. The resulting PCR products were analysed by agarose gel electrophoresis (1.0% wt/vol) with 103 V for 60 min (EC High Voltage Apparatus Corporation), stained with ethidium bromide and visualized under UV transillumination (Kodak digital science, Image station 440 cf). PCR products were sequenced at the ISU DNA facility. Sequences were subjected to the basic local alignment search tool (BLAST) program (http://www.ncbi.nlm.nih.gov/BLAST) and compared to GenBank to identify the closest phylogenetic match. Molecular Evolutionary Genetic Analysis Version 6 (MEGA6) was used to align sequences and to generate exploratory tree's (Tamura *et al*., 2013).

## RESULTS AND DISCUSSION:

The bacterial population density obtained on NA from the fresh paint sample was $30.0 \times 10^2$ CFU/ml while that of five spoilt samples ranged from $10.0 \times 10^2$ to $120.0 \times 10^4$ CFU/ml. On Pseudomonas Isolation medium, the population density of spoilt paint samples ranged from $20.0 \times 10^2$ to $80.0 \times 10^4$ CFU/ml. In contrast, we did not detect any culturable bacteria in the non-spoiled paint sample on PIA. In our assay the limit of detection was 100 CFU/ml. Substances such as starch, protein and various synthetic compounds that are used as binders in the paint industry serve as nutrients for growth of microbial contaminants in paints (Da Silva, 2003). In

addition, latex paints, by virtue of their aqueous nature, supports diversity of microbes (Obidi *et al*., 2010). Nutrient agar, being a general-purpose medium and containing nutrients that supports the growth of a wide range of non-fastidious bacteria was probably more suitable and therefore performed better in culturing bacteria from the non-spoiled sample compared to the PIA. Visual inspection indicated there was a variety of colony morphotypes that formed on both media, suggesting that spoilt paint is comprised of diverse bacterial species. We randomly selected and struck for purity a range of colony types whose identity was determined by either sequencing the 16S rRNA gene or by whole cell fatty acid analysis using the Microbial Identification (MIDI) System. The FAME profile revealed that isolates represents at least 2 biotypes with most of the isolates belonging to the same strain with different representatives of the same biotype (Fig. 1). A total of 7 isolates were identified by sequencing 767 to 819 nucleotides of a near full-length amplicon of the 16 S rRNA gene from the 8F primer site. BlastN analysis revealed that 4 of the isolates were identified as *Provedencia sneebia* strains at 99% sequence identity while one was identified as a Provedencia species at 96 - 97% identity. Comparative analyses of DNA sequences with MEGA6 software revealed no significant variation among the sequences, suggesting that the isolates could be the same strain. The genus *Providencia,* in the family Enterobacteriaceae, is known to currently have six recognized species (Juneja and Lazzaro, 2009). Members of the genus have been repeatedly found in association with various human and non-human sources in numerous environments (Yoh *et al*., 2005; Galac and Lazzaro, 2012). One isolate was identified as *Lysinibacillus macrolides* at a 98% sequence identity and one as *Brevibacillus centrosporus* at 94% identity. An additional 14 isolates were subjected to whole-cell fatty acid analysis. Nine of the isolates were identified as members of the Enterobacteraceae, with the closest match as *Klebsiella pneumoniae*. *K. pneumoniae* is known to have wide distribution (Yong *et al*., 2009). Therefore, its occurrence in spoilt paints is not surprising. For one isolate, the closest match was Nocardia/Acinetobacteria while another isolate was Nocardia/ Rhodococcus: there was insufficient resolution to distinguish between the two genera. For two isolates the closest matches were *Bacillus sphaerecu*s and lastly for one isolate the closest match was *Brevibacillus choshinensis*. The bacteria isolated from the present study differs from those obtained by Altenburger *et al*. (1996) who observed *Pseudomonas Arthrobacter* and *Streptomyces* species although from a different paint source.

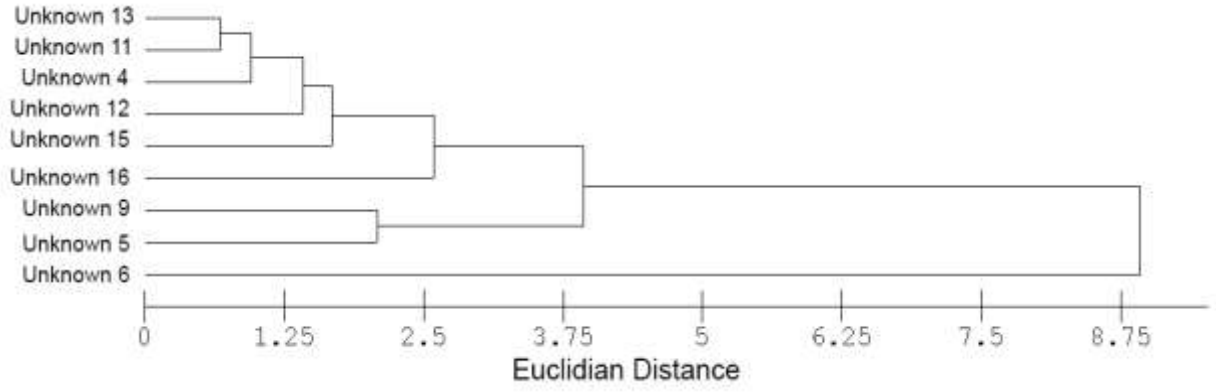
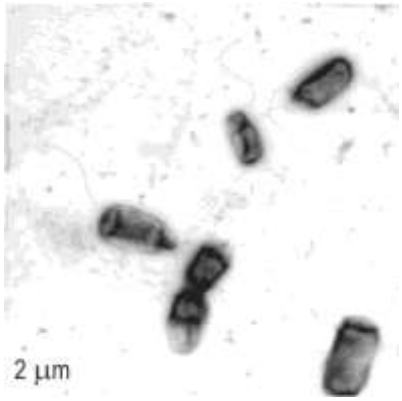



Fig. 1. FAME profiles indicating that isolates are the same strain belonging to two biotypes.

Subset of isolates were also examined by transmission electron microscopy. The TEM examination of the strains revealed that the Gram-negative rod-shaped *Providencia sneebia* is flagellated, rod shaped and ranging from 1.80 - 2.0 µm in length and 0.50 to 0.66 µm in diameter (Fig. 2). The Gram-negative *Providencia sp.* as observed by TEM, is rod shaped with a length of 2.0 - 2.5 µm and diameter of 0.6 - 0.8 µm (Fig. 3). Lastly, the *Bacillus sphaericus* isolate is also rod shaped but slightly smaller with a length of 1.0 - 2.0 µm and a diameter of 0.6 to 1.0 µm (Fig. 4).



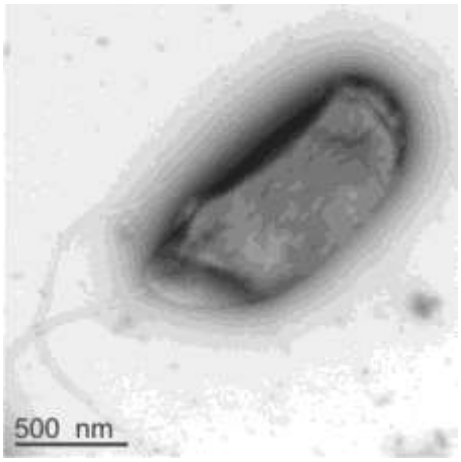



Fig. 2. TEM image of a *Providencia sneebia* isolate at various magnifications.

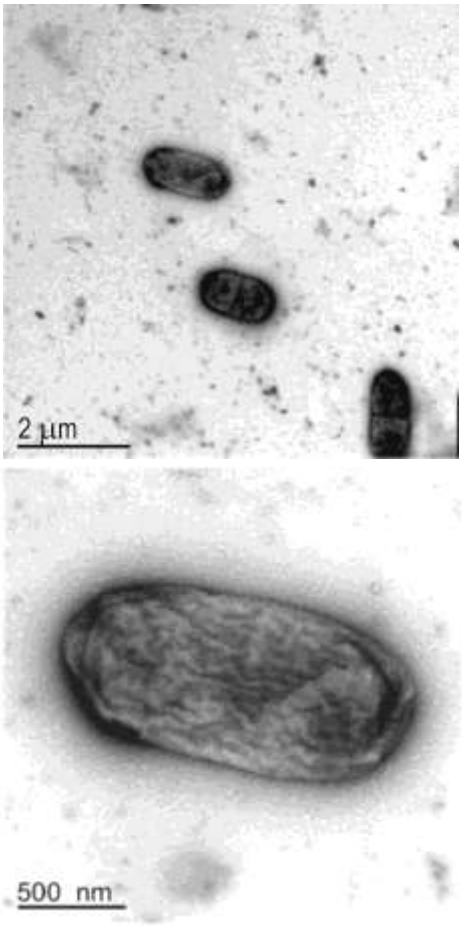



Fig. 3. TEM images of the Providencia species isolate at various magnifications.

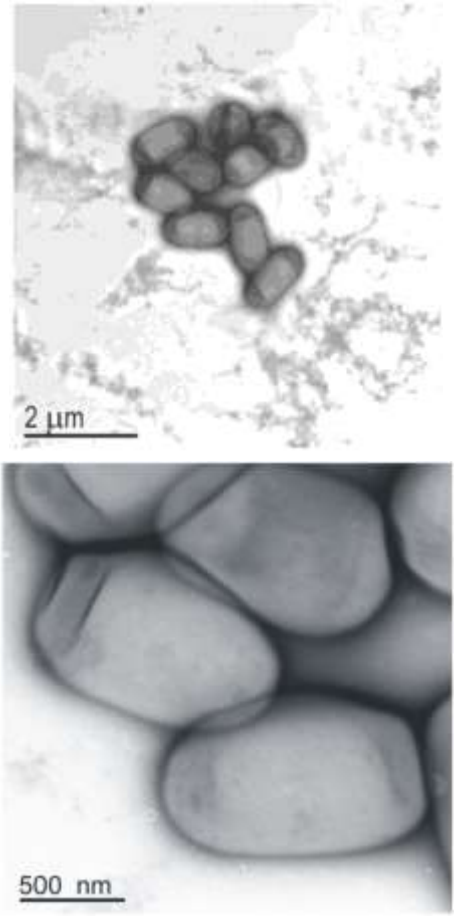



Fig. 4. TEM images of a *Bacillus sphaericus* isolate at two different magnifications.

## CONCLUSION:

The present study describes the isolation and identification of bacteria isolated from biodeteriorated latex paints produced in Nigeria. Diverse bacterial species were isolated from spoilt paints, including primarily Gram-negative as well as Gram-positive species. Knowledge of their identity will facilitate elucidating measures to prevent their introduction in the paint manufacturing process.

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