IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

PLASMID PROFILE OF BACTERIA ISOLATED FROM PAINTED CONCRETE WALLS FROM PARTS OF SOUTHERN, NIGERIA

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Abstract: Sum total of eighty samples were collected from Eleme and Port Harcourt for this study using forty (40) sterile swap sticks for each location. Two (2) different media (MacConkey Agar and Chocolate Agar) were used for the culture isolation of the microorganisms. Each swab stick was introduced on the surface of the media by streaking on the solid agar for microbial identification and biochemical analysis was done for specific identification of the microbes. Plasmid profiling of the isolated microorganisms to evaluate plasmid DNA was done by culturing in LB media with antibiotic selective pressure. This study identify four (4) microorganisms with *Escherichia coli* having the highest occurrence percentage of 46.30% from both location while 3.45% of *Serratiam arcescen*, 11.82% of *Bacillus cereus* and 38.43% of *Staphylococcus aureus* were isolated from both locations. The population of *Escherichia coli* isolated from Eleme and Port Harcourt was significantly high with percentage occurrence of 19.21% and 27.09 % respectively. This study implicated *Escherichia coli* and *Staphylococcus aureus* to be responsible for degradation of coated surface. Isolated from Eleme had plasmid of molecular weight 23.13kbp while Port Harcourt had plasmid of molecular weight 23.13kbp, and 4.361kbp showing significant change on their plasmid morphology.

Index Terms - **Bacteria, Painted walls, Plasmids, Agar, Microscopic .**

I. INTRODUCTION

Paint is a liquid of mastic composition, which on application to a surface in a thin layer converts to a solid film which is most commonly used to protect, decorate or provide texture to surfaces such as buildings (Adeleye, 1999). Paint are synthetic products of organic and inorganic essence which can be applied to various surfaces (stone walls, wood metals and among other) with a brush, roller or spray gun, and as such provides diverse ecological niches that may be subjugated by a huge selection of microorganisms. These materials are biodegradable; hence microorganisms that occur on painted surfaces use them for their growth (Gillatt, 1992). Several paints of different chemical composition have been useful for domestic and industrial purposes. The painted surfaces undergo damage due to natural weathering or growth and activity of the microorganisms (Imperi *et al*, 2007). Microorganisms contribute to mineralization of paints on the surfaces like stone buildings, causing aesthetic and structural damage (Ogbulie and Obiajulu 2004).

Various types of organisms are involved in paint deterioration and they include bacteria, fungi, algae, protozoa and cyanobacteria and the connections between these organisms can promote or reduce, generally the rate of biodegradation (Thern and Cloete, 2004). Biofilm development on coated surfaces often starts with phototropic organisms while heterotrophic organisms (most bacteria and fungi) need some organic source for their growth and thus feed by metabolites of phototropic organisms or by air-borne deposition. Interestingly, Gillatt, (1992) and Corro *et al*, (2000) reported that nutrient requirements heterotrophic microorganisms may as well be fulfilled by airborne deposition from polluted air and rain or animal remains and secretions. Thus environmental factor and climatic condition plays a vital role in deterioration and degradation of painted surfaces by microorganisms.

Generally, paints have four (4) major components which include pigment, binders, solvent and additives (Grant et al, 1993). The pigments are the substances which are responsible for the color of the paint and from natural source like clay, herbs, nuts, berries, barks, charcoal and soot. Impurities are removed in pigments by boiling before usage for paint production and also have the characteristic of reflecting and changing the colour of light due to selective absorption of colour. A substance is recognized as a pigment, because of its ability to remain stable in solid state at ambient temperatures while the binder form the building block of the paint, by holding the paints together and carries the pigment. The manufacturer's choice of binder depends on the required quantity of paints and the surface to be coated (Bailey and Sand, 1993).

Some natural binders like chalk, lime (Lime has antibacterial qualities and thus is used for interior and exterior surfaces of buildings), casein (non- fat milk curds), animal or vegetable glues and oil have been used industrially for production of paints

(Warschied and Braams, 2000 and Allsopp and Gaylarde, 2004). Oil-based paints have varying combinations of solvents with aliphatic and aromatic compounds like alcohols, esters, petroleum distillates, resins etc (Gonzalez and Saiz-Jimenez, 2004). Additives are low level ingredient that provides precise paint properties such as mildew resistance, deforming, and good flow and leveling. Besides these components, paint can have a wide variety of miscellaneous additives usually added in very small amounts. Others are thickeners, coalescent solvents and biocides to fight bacteria and other microorganisms. All these components of paint both organic and inorganic contain important molecules which can be utilized by microorganisms for their growth and existence; which is the absolute reason behind their growth on painted surfaces (Gaylarde and Morton, 2002, Gary, Dhawan and Bhatuagar, 1991). This study is aimed at isolating and identifying microorganism responsible for coated surface degradation.

Materials and Methods

Sample collection

The present report describes the investigations on samples taken from partially green or black pigment found on the painted walls of Eleme and Port Harcourt Local Government Area with coordinate of 4.7994° N, 7.1198° E and 4.8156° N, 7.0498° E respectively, all in River State. The samples were collected between July and September 2018, which are the prime and peak of raining season in these areas. Eighty (80) samples were collected from the painted walls using forty (40) sterile swap sticks for each location. The painted buildings showed cumulative decay as black biofilms, some with cracks and discoloration spread in most painted building. The collected samples were transport to the laboratory in ice chest container for examination and analysis.

Figure 1. Geographic location of samples collection

Figure 1. Geographic location of samples collection

Media preparation

Two (2) different media (MacConkey Agar and Chocolate Agar) were used for the culture isolation of the microorganisms. The media was prepared using manufacturer's description. The powder form of the medium is rehydrated and stirs. The agar medium is boiled to dissolve the agar powder. The dissolved agar powder is distribute into tubes and are autoclave to sterilize the media tubes. The media tube was left to stand and then pour into sterile petri dishes.

Inoculation of the samples

Each swab stick was introduced on the surface of the media by streaking on the solid agar. Each medium were streaked accordingly starting with Nutrient Agar to MacConkey Agar and Chocolate Agar and then each inoculated plate was incubated at 37^{o}c for 24hrs.

Plasmid DNA Extraction

Microorganisms with plasmid cultured in LB media with antibiotic selective pressure, overnight on a shaker at 37°C. 1.5 ml of bacterial culture was pellet in a microfuge tube by centrifuging for 2 minutes at 10,000 rpm. The supernatant was decanted and 200 µl of the resuspension buffer was added. 250 µl of the lysis buffer was added and the tube was invert10 times to mix thoroughly. The solution becomes clear and viscous. 350 µl of the neutralization buffer was add and the tube was invert 10 times or until a precipitate forms. The precipitate is a mixture of protein and chromosomal DNA. The tubes were centrifuged for 10 minutes at 10,000 rpm. The supernatant was transferred to a microfuge tube and 0.7 isopropanol was added and was incubated at -20°C for 15 minutes. The solution was transfer to a spin column and was centrifuged for 1 minute at 7,000 rpm. The flow through

was discard. 400 µl of the wash buffer was added and was centrifuged for 1 minute at 7,000 rpm. The flow through was discard and this step was Repeat. Centrifugation was done for an additional 2 minutes at 10,000rpm to remove residual wash buffer. The column was transferred to a clean microfuge tube and 50 µl of elution buffer was added and was centrifuged for 1 minute at 10,000 rpm.

RESULTS

The percentage of *Escherichia coli* and *Staphylococcus aureus* isolated from various deteriorating coated walls in Eleme and Port Harcourt are significantly high as presented in Table 1.1 and Figure 2. This study revealed high presence of *E. coli* (19.21%) and *S. aureus* (16.75%) in Eleme while in Port Harcourt 27.09% *E. coli* and 21.68% *S. aureus* were isolated. *S. arcescen and B. cereus* were significantly low at both locations. The strength of bio-acid, quality and source of the raw materials used for paint production and the production plant could contribute to the variations of the isolated microorganism.

Table 1.1 Microorganisms Isolated From Decaying Coated Walls

Locations	*Escherichia coli*	*Serratiam arcescen*	*Bacillus cereus*	*Staphylococcus aureus*
Eleme	39(19.21%)	4(1.97%)	13(6.40%)	34 (16.75%)
Port harcourt	55(27.09%)	3(1.48%)	11(5.42%)	44 (21.68%)

The prevalence of *E coli* and *S. aureus* among the isolated microorganism as presented in Figure 2 show their significant role in deterioration of coated walls. *E coli* and *S. aureus* accounts for 35.96% of walls in Eleme while 48.77% in Port Harcourt.

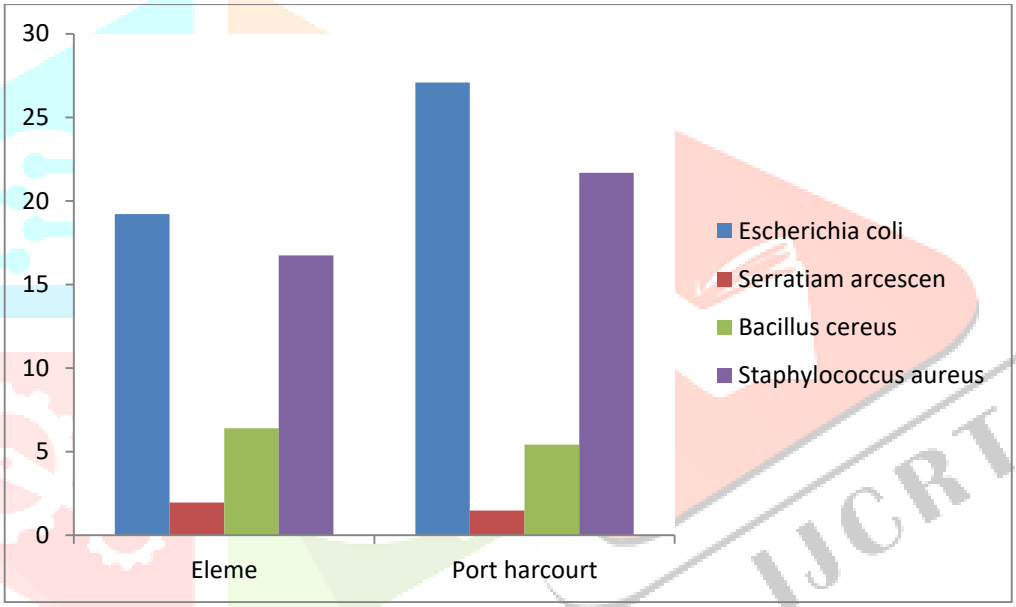

Figure 1: *E coli* and *S. aureus* account for deterioration of coated walls

Biochemical analysis of the isolated microorganism revealed that catalase, and coagulase was present in *Escherichia coli*, *Serratiam arcescens*, *Staphylococcus* and *Bacillus cereus* while oxidase and sugar fermen were absent in all the isolated microorganism revealing the specificity of the isolated bacteria responsible for deterioration of coated walls as presented in Table 1.2.

Table.1.2. summary of Biochemical Analysis

	Escherichia coli	*Serratiam arcescens*	*Staphylococcus*	*Bacillus cereus*
Gram staining	+	_	_	_
Motility	+	+	_	+
Oxidase	_	_	_	_
Catalase	+	+	+	+
Indolase	+	_	_	_
Citrate	_	+	_	_
Methyl red	+	_	+	+
Sugar fermen	_	_	_	_
Mannitol	+	_	+	+

Lactose	+	_	+	+
Nitrate reduction	_	+	+	+
Glucose	_	+	+	+
Coagulase	+	+	+	+

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Plasmid profiling of the isolated microorganism was evaluated to determine plasmid kbp of the various microorganism as shown in Figure 2 and 3. Isolated from Eleme had plasmid of molecular weight 23.13kbp while Port Harcourt had plasmid of molecular weight 23.13kbp, and 4.361kbp.

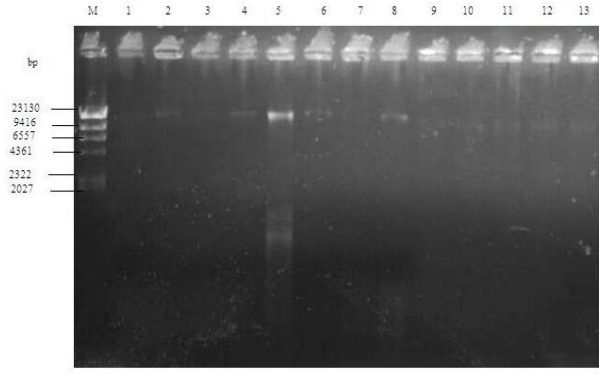

Figure 2: Plasmid size of isolates from Port Harcourt

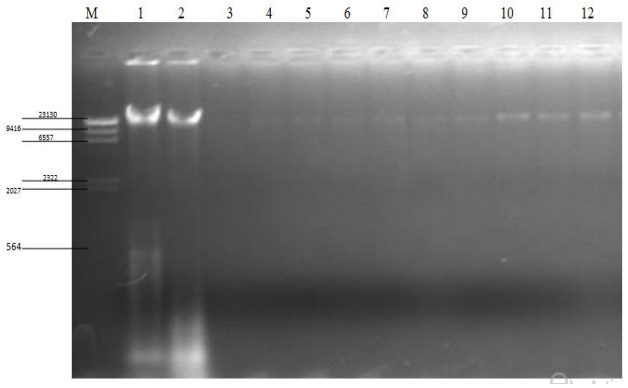

Figure 3: Plasmid sizes of isolates from Eleme

DISCUSSION

Sum total of eighty (80) samples collected from Eleme and Port Harcourt in this study identified four (4) microorganisms which include *Escherichia coli, Serratiam arcescen, Staphylococcus aureus and Bacillus cereus* and *Escherichia coli* had the highest occurrence percentage of 46.30% while 3.45% of *Serratiam arcescen*, 11.82% of *Bacillus cereus* and 38.43% of *Staphylococcus aureus* were also isolated from both locations. Microorganism isolated from decaying coated surface of the two study areas revealed that *Escherichia coli, Staphylococcus aureus and Bacillus cereus* played active roles in the deterioration of coated surface utilizing the biofilm as substrate for growth. Thern and Cloete (2004),reported that Various types of microorganisms including bacteria, fungi, algae, protozoa and cyanobacteria are involved in paint deterioration and that the connections between these organisms can promote or reduce, generally the rate of biodegradation (Thern and Cloete, 2004). The biochemical analysis performed for the isolated microorganisms revealed the presence of coagulase while sugar fermen was absent for the four microorganisms suggesting that the presence of the microorganisms especially *Escherichia coli* and *Bacillus cereus* plays a vital role in the degradation of the coated surfaces. This supports the report of Gillatt (1992), that Paint as a synthetic product, produced from organic and inorganic essence (which include animal and plant glues, sugars, proteins, polysaccharides, oil waxes etc) applied to various surfaces (stone walls, wood metals and among other) with a brush, roller or spray gun provides diverse ecological niches that may be subjugated by a huge selection of microorganisms and because these materials are biodegradable; hence microorganisms that occur on painted surfaces use them for their growth. The population of microorganisms (*Escherichia coli*) isolated from Eleme and Port Harcourt was significantly high with percentage occurrence of 19.21% and 27.09 % respectively. The high presence of *Escherichia coli, Bacillus cereus* and *Staphylococcus aureus* on the decaying walls of Eleme and Port Harcourt could be attributed to the environmental condition such as humid nature of the environment. This also support the report of Grant *et al* (1993) and O'Neil, (1986) that environmental factors and climatic conditions plays unique role in deterioration and degradation of coated surfaces. Plasmid profiling of the isolated microorganisms revealed differences in plasmid size suggesting differences due to adaptability, extreme environmental conditions, effects of some chemicals such as biocides and rate deterioration processes.

CONCLUSION

In conclusion, microorganisms consisting of bacteria are mostly responsible for the deterioration of painted walls revealing its potential in bioremediation for oil spillage in oil exploring communities like River State if properly utilized. It is recommended that microbial colonization should be taken into consideration when planning paint production and other structures even when composing building materials. Also keeping hygienically clean environment should be a crucial component of any residential area. The bacterial species were identified on the basis of colony, morphology, cultural characteristics, and pigmentation. This study identified E. *coli and S. aureus* as most prevalent microorganisms in deterioration of painted walls in the study areas, revealing the dangers it poses to human health. Thus, this calls for attention of relevant stakeholders and house owners on the importance of yearly repainting of the painted surfaces.

ACKNOWLEDGEMENT

All thanks to Almighty God for His guidance in course of this work. I also wish to acknowledge Professor Oguzie Emeka Emmanuel for his immerse contribution and encouragement and Mr. Ezeanowai Chikezie Franklin for his relentless effort through the success of this work.

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