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### RESEARCH ARTICLE

#### ISOLATION, IDENTIFICATION AND SCREENING OF HALOPHILIC BACTERIA FOR POLYHYDROXYALKANOATE (PHA) IN HYPERSALINE LAGOS WATER BODY, NIGERIA.

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#### Abstract

This study aims to identify bacteria associated with hypersaline water body in Lagos State, Nigeria, screen them for their potential to produce polyhydroxyalkanoates (PHA) and determine the physicochemical properties of the water. Enumeration, identification and testing for growth at 3% w/v NaCl were carried out using standard microbiological techniques. Screening for PHA production potential of the bacteria was carried out using Sudan Black B staining techniques. Physicochemical analysis of the water (at depths of 0-0.07m, 50m and 100m) was determined using the methods of Association of Official Analytical Chemists. The highest and lowest bacterial loads of  $20.66 \pm 0.47$  Cfu/mL and  $10.00 \pm 0.82$  Cfu/mL were obtained from depth 50m respectively. Eighteen (18) bacteria namely *Micrococcus varians*, *M. luteus*, *Bacillus badius*, *B. macquariensis*, *B. megaterium*, *Corynebacterium kutscheri*, *Mycobacterium delbrueckii*, *Mycobacterium smegmatis*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Lactobacillus delbrueckii*, *Klebsiella pneumoniae* subsp. *pneumoniae*, *Neisseria veillonella*, *Enterobacter intermedius*, *Enterobacter amnigenus*, *Serratia marcescens* and *Yersinia pestis* were phenotypically identified from this study using Bergey's Manual of Systematic Bacteriology. The test for growth at 3% w/v NaCl revealed that *Bacillus badius* had the highest optical density (OD) (2.84) at 520nm, while *Serratia marcescens* had the lowest OD (2.61) at 520nm. The physicochemical parameters of the hypersaline water ranged from 0.17 to 37.4NTU, 37300 to 43500 $\mu$ S/cm and 10 to 130mg/mL for turbidity, conductivity and alkalinity respectively. The values obtained for Dissolved Oxygen, Chemical Oxygen Demand and Biochemical Oxygen Demand ranged from 4.08 to 4.42mg/L, 4.21 to 3699mg/L and 4.21 to 924.75mg/L respectively. Findings from this study revealed that 88.1% of the bacteria tested positive to PHA production and the presence of PHA producing bacteria has been established in the hypersaline water body of Lagos State, Nigeria.

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## Introduction:-

### Halophilic bacteria as an extremophile

Halophilic bacteria are extremophiles that live or survive harsh environments (Zhang *et al.*, 2015) characterized by saltiness, they either tolerate certain salt concentration range or require it for growth. High salt concentration is one of the area factors that determine or limit the biodiversity of microorganisms. High salts water bodies otherwise known as hypersaline environment (e.g. marine water bodies). Hypersalinity in water bodies are associated by low oxygen concentrations (depending on the geographical area), high or low temperatures and are most times highly alkaline (Ollivier *et al.*, 1994).

Halophiles are categorized into four, which are non-halophilic organisms, slight halophiles, moderate halophiles and extreme halophiles (Kushner, 1978). Non-halophilic is that organism that requires less than 1% (w/v) NaCl for their growth, but despite the fact that this group of microbes do not need high salt concentration for their optimal growth, they may tolerate it and so, they are considered as halotolerant microorganisms (de Lourdes *et al.*, 2013). With respect to halophilic microorganisms, slight halophiles (for example, marine bacteria) grow best in media with 1% to 6% (w/v) NaCl, whereas the optimal salt concentrations for moderate halophiles range between 7% and 15% NaCl; on the other hand, extreme halophiles show optimal growth in media containing 15% to 30% (w/v) NaCl (Madigan and Martinko, 2006; Manikandan and Senthilkumar, 2017).

The biomolecules such as polyhydroxyalkanoate (PHA) produced by halophilic microbes are stable due to their ability to live under extreme conditions (Oren, 2013; Parthasarathi *et al.*, 2013; Santini and Warren, 2015).

Polyhydroxyalkanoates (PHAs) are biopolymers and may be produced by bacteria in order to overcome environmental stresses that are characteristic of hypersalinity. The vast attention towards PHAs is mainly attributed to its properties that resemble some petrochemical plastics, as well as its biocompatibility and complete biodegradability. Microbial activity in the natural environment breaks down such bioplastics to release carbon dioxide and water or methane under aerobic and anaerobic conditions (Naik *et al.*, 2008). PHAs are biodegradable and of immense importance in the applications of medicine, engineering, agriculture, telecommunications, transportation and household utilities (Aditi *et al.*, 2015; Zhang *et al.*, 2015; Manikandan and Senthilkumar, 2017). They are inevitably important as components of production for razors, utensils, diapers, feminine hygiene products, cosmetics containers, shampoo bottles and so on. PHAs can be incorporated into packaging components such as coatings, laminations and biodegradable printing inks (Reddy *et al.*, 2003; Mojaveryazdia and Rezaniac, 2013). PHA structures include rigid thermoplastics, thermoplastic elastomers and grades useful in waxes, adhesives and binders. Properties range from elastomeric to resins as stiff as nylon 6 or polycarbonate (Hall, 1981; Board, 2004; Olabisi and Adewale, 2016). Information gathered on PHA's producing bacteria ( DasSarma *et al.*, 2010; Saranya and Shenbagarathai, 2011; Chao, *et al.*, 2013; Saharan *et al.*, 2014) revealed that little or no information has been found on the water bodies of Nigeria. It is therefore imperative to investigate the types of halophilic bacteria and their PHA producing potentials in Nigerian water bodies.

## Materials and methods:-

### Collection of water samples

Marine water samples of three (3) replicates from each location of Lagos State, Nigeria Southern Atlantic Ocean water body were collected aseptically with the aid of a water sampler using the modified methods of water sampling by Bugnicourt *et al.*, (2014). The water samples were collected at three different depths (surface: 0-0.07m, middle: 50 meters and bottom: 100 meters below water surface) into a 1 litre separate sterile plastic container. The locations considered for the depths of the water collection were recorded from the Geographic Positioning System (GPS) as (1) latitude 6.35° N longitude 3.28° E (ST1); 2) latitude 6.35° N longitude 3.40° E (ST2); and 3) latitude 6.36° N longitude 3.47° E (ST3). The temperatures of the collected water samples were recorded in situ.

### Enumeration, isolation and identification of bacterial isolates

The sampled water was serially diluted from  $10^{-1}$  to  $10^{-7}$ , cultured by spread plate method on plate count agar (PCA) and incubated at 35°C for 24 to 48 hours. The colonies were counted.

Bacteria were characterized based on cultural characteristics which are; margins, elevation, growth shape. The methods of Holt (1994) were employed for the morphological characteristics of the cells and these includes gram stain reaction, cells spore, acid fast staining reaction, cell shape and cellular arrangements. The biochemical assays in order to determine the absence/presence of catalase, oxidase; citrate utilization, nitrate reduction, the probe

of the bacteria to determine if it is a strict anaerobe or not, the ability to hydrolyze starch. Also, included are assays to determine sugar fermentation ability, Vogues Prokauer (VP) and ability to grow in 6.5% sodium chloride. At the end of the assays on each of the isolates, each organism was identified with the Bergey’s Manual of Systematic Bacteriology as utilized by Tissari, *et al.* (2010).

**Determination of the effects of 3% (w/v) NaCl salt concentration on bacterial isolates**

Minimal salt medium (MSM) was prepared with the following constituents: K<sub>2</sub>HPO<sub>4</sub> (0.5g/L), KH<sub>2</sub>PO<sub>4</sub> (0.04g/L), MgSO<sub>4</sub> (0.05g/L), Ammonium dihydrogen phosphate (0.04g/L), Ferrous sulphate (0.001g/L), glucose (8% w/v) and NaCl (3% w/v). The medium was sterilized in the autoclave at 121°C for 15 minutes at 15 psi, allowed to cool in the respective distributed test tubes, after which inoculation of pure isolates at 1×10<sup>4</sup> cell per mL was carried out aseptically, then subsequently incubated at 37°C for 2 to 72 hours before absorbance was taken at 520nm with the aid of Spectrophotometer.

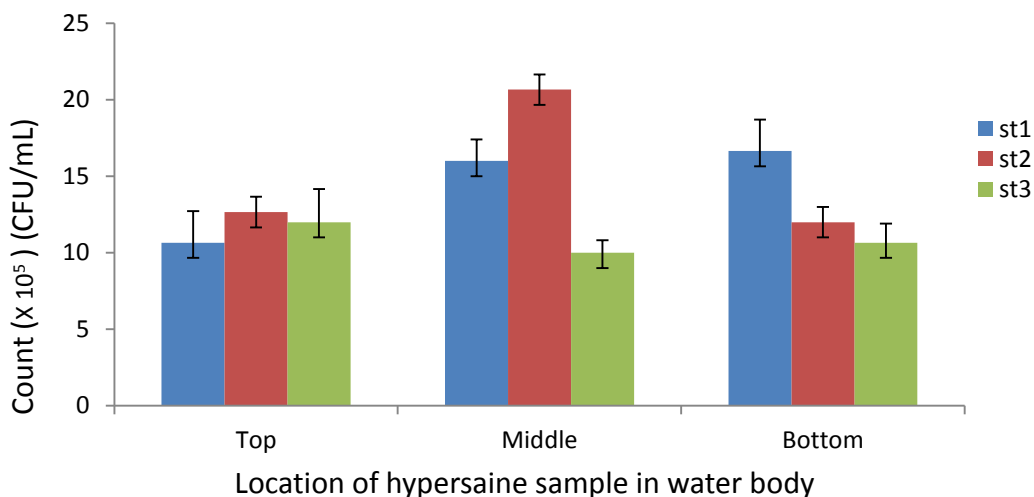
**Physicochemical analysis of water samples**

Physical, chemical and trace/heavy metal analyses were conducted on the marine water samples according to Bugnicourt, *et al.* (2014). The physical parameters temperature, pH, turbidity, conductivity and total suspended solids were studied according to Galinha, *et al.*, (2018). The chemical parameter total acidity, total alkalinity, chloride by titrimetric method, nitrate, sulphate were also determined with the aid of spectrophotometry methods (Germer *et al.*, 2014). Dissolved oxygen and chemical oxygen demand were determined by Ibtisam and Karim, (2012) methods, while biological oxygen demand were determined according to Kosseva and Web, (2013) methods.

**Assay for PHA Production**

Each bacterial isolate was grown in 100 mL minimal salt medium supplemented with 8% (w/v) glucose in a 250 mL conical flask and incubated for 37 °C for 72 hours 100 rpm in an incubator. Thereafter, PHA production was investigated using Sudan Black B staining methods (Khanna and Srivastava, 2005). Data were recorded based on the positive observation of black colouration of the cells and negative observation of pink coloration of the cells. All numerical data were subjected to statistical analysis using versions of IBM SPSS 2.0 and Microsoft Excel 2010 software.

**Results:-**



**Figure 4.1:-**Bacterial load of hypersaline water body in Lagos, Nigeria Legend.

St1= latitude 6.35° N longitude 3.28° E; St2= latitude 6.35° N longitude 3.40° E; St3= latitude 6.36° N longitude 3.47° E; Top= 0.07m water surface; Middle= 50m below water surface; Bottom= 100m below water surface

Charact																			
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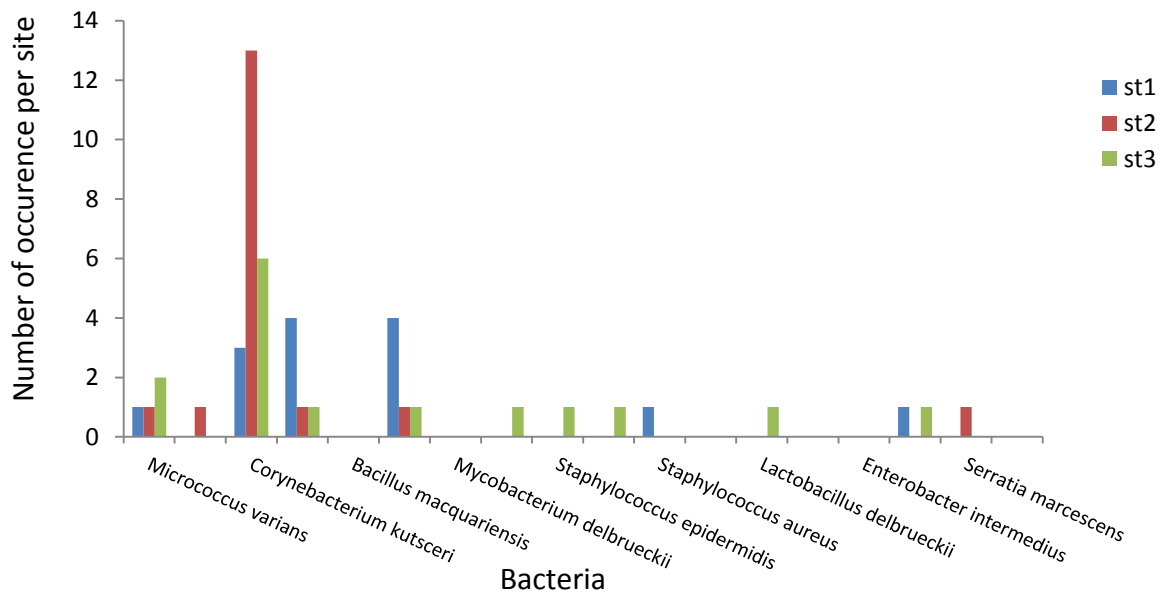
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Configuration	rou nd	rou nd	rou nd	rou nd	rhiz oid	wrin kle	rou nd	rou nd	rou nd	rou nd	wri nkle	rou nd	wri nkle	rou nd	rou nd	wrin kle	rou nd	wri nkle
Margin	wa vy	smo oth	wa vy	wa vy	wo oly	irreg ular	mar gin	mar gin	wa vy	smo oth	woo ly	smo oth	wav y	smo oth	smo oth	irreg ular	wa vy	loba te
Elevation	flat	flat	rai sed	flat	flat	flat	rais ed	rais ed	flat	flat	flat	rais ed	rais ed	dro p- like	flat	flat	Rai sed	Rais ed
Morphology																		
Gram's Reaction	+v e	+ve	+v e	+v e	+ve	+ve	+ve	+ve	+v e	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Cellular Shape	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod
Biochemical Characteristics																		
Acid fast							+ve	-ve										+ve
Catalase	+v e	+ve	+v e	+v e	+ve	+ve	+ve	-ve	+v e	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Oxidase	+v e	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Spore formation				-ve			-ve	-ve										-ve
Starch hydrolysis	+v e	+ve	+v e															+ve
Manitol fermentation				-ve	-ve			-ve		+ve	-ve							
Glucose fermentation																		
Lactose fermentation									-ve				+ve		+ve e	-ve		+ve
MR			+v e			-ve			+v e				+ve		+ve			-ve
VP	-ve	-ve	-ve						-ve				-ve		+ve			+ve
Citrate utilization	-ve	+ve																
Yellow pigment					+ve													
Novobiose										+ve		-ve						

**Table 4.1:-**Phenotypic identities of bacteria isolated from hypersaline water body of Lagos, Nigeria

**Table 4.1(contd.):-**

Characters																		
Culture	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Biochemical Tests (contd.)																		
Sorbitol fermentation																		- ve
Motility									+v e				- ve					
Indole									-ve				- ve					

Lysine decarboxylase																		
Pigmentation								+ve										
Urease								-ve									-ve	
Ornithine decarboxylase																	-ve	
Hydrogen sulphide								-ve					-ve					
Coagulase									+ve	-ve	-ve							
Probable bacteria	<i>B. badius</i>	<i>B. megaterium</i>	<i>B. maquariensis</i>	<i>L. delbrueckii</i>	<i>M. varians</i>	<i>M. luteus</i>	<i>M. smegmatis</i>	<i>M. delbrueckii</i>	<i>S. marcescens</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. saprophyticus</i>	<i>K. pneumoniae</i>	<i>N. veillonella</i>	<i>E. intermedius</i>	<i>Y. pestis</i>	<i>C. kutscheri</i>	<i>E. amnigenus</i>

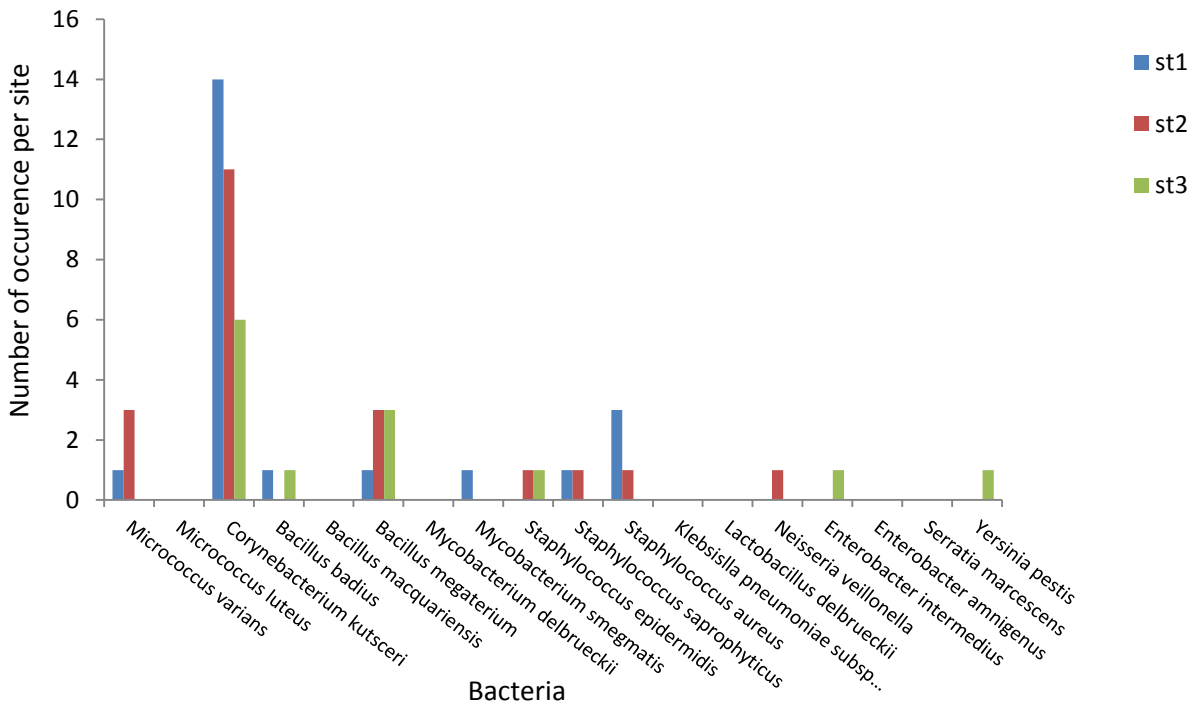


**Figure 4.2:-**Distribution of bacteria isolated from 0-0.07m (surface) of hypersaline water bodies of Lagos, Nigeria. Legend.

St1= latitude 6.35° N longitude 3.28° E; St2=latitude 6.35° N longitude 3.40° E; St3= latitude 6.36° N longitude 3.47° E

Bacterial loads from each other at the three locations were non-significantly different ( $p < 0.05$ ). *Corynebacterium kutscheri* as the most frequently occurred (13 times) bacterium in location St2 and 6 times in site 3. This was followed by *Bacillus badius* and *B. megaterium* that occurred 4 times each in St1, but in St2 and St3 *Bacillus badius* and *B. megaterium* occurred 1 time each. The occurrence of *Micrococcus varians* was twice in St3, once in St1 and St2 respectively. *Micrococcus luteus* from St2, *Mycobacterium smegmatis* from St3, *S. epidermidis* and *S. saprophyticus* also obtained from St3 including and *S. aureus* from St1 all occurred once. *Lactobacillus delbrueckii* and *Enterobacter amnigenus* including *Serratia marcescens* all obtained from St1 and St3 occurred once each.

Howbeit, *Mycobacteria delbrueckii*, *Klebsiella pneumoniae* subsp *pneumoniae*, *Neisseria veillonella* and *Yersinia pestis* were not isolated from 0-0.07 m of the three locations sampled.



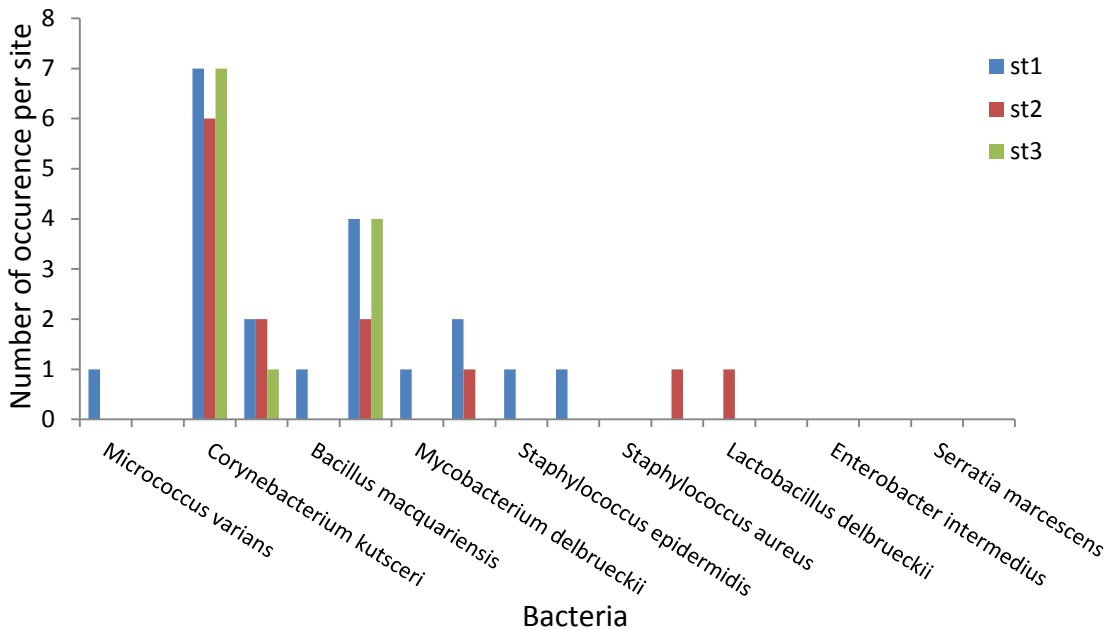
**Figure 4.2.1:-**Distribution of bacteria isolated from 50 meters below (middle) surface of hypersaline water bodies of Lagos, Nigeria.

Legend.

St1= latitude 6.35° N longitude 3.28° E; St2= latitude 6.35° N longitude 3.40° E;

St3= latitude 6.36° N longitude 3.47° E

The bacterial isolates distribution from 50 meters below surface water shows *Corynebacterium kutscheri* isolated from St1, St2 and St3's occurrences of 14, 11 and 6 times consecutively. This bacterium is followed by *B. megaterium* from St1, St2 and St3 occurring 4, 2 and 4 times consecutively as well. *B. badius* also from St1, St2 and St3 occurred in the order of 2, 2 and 1 times occurrence. *Micrococcus varians* and *S. aureus* obtained from St1 and St2 registered their occurrence with 1, 3 and 3, 1 respectively. *Mycobacterium smegmatis* isolated from St1, *S. epidermidis* from St1 and St3, including *S. saprophyticus* isolated from St1 and St2 all occurred once. However, *Micrococcus luteus*, *B. macquariensis*, *M. delbrueckii*, *Klebsiella Pneumoniae* subsp. *Pneumoniae*, *Lactobacillus delbrueckii* and *Enterobacter amnigenus*, *Neisseria veillonella* including *Serratia marcescens* were not isolated from St1, St2 and St3 50 meter depth below the sampled hypersaline surface water of Lagos, Nigeria.



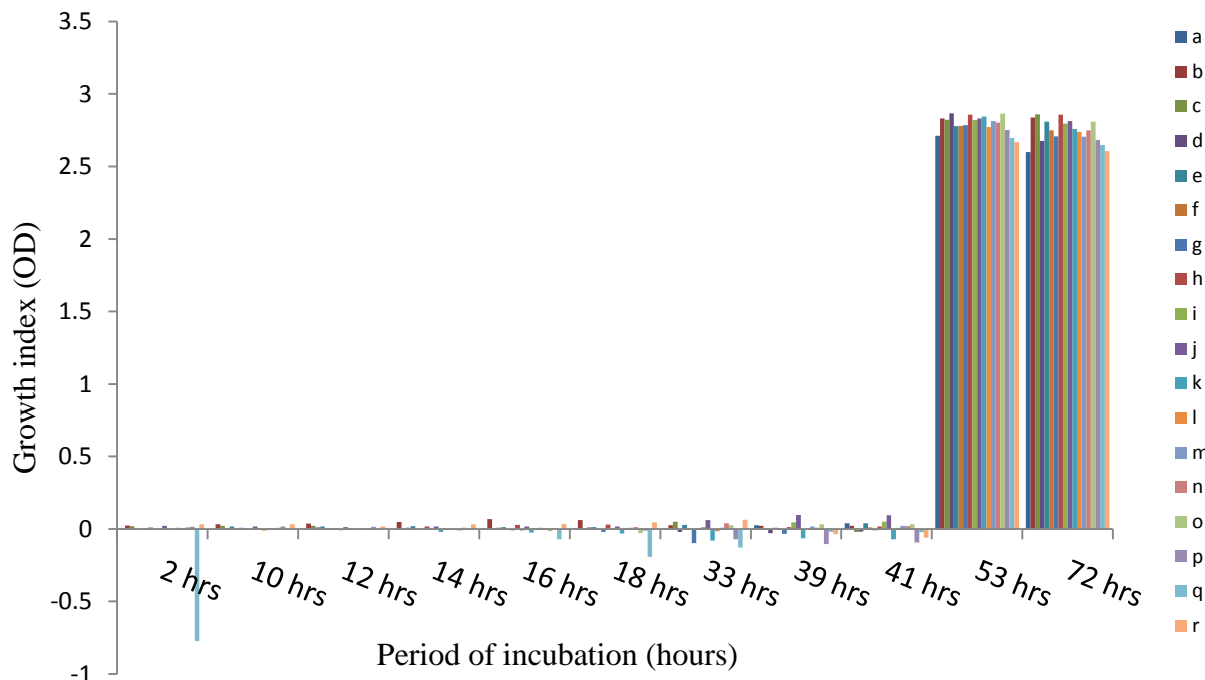
**Figure 4.2.2:-**Distribution of bacteria isolated from 100 meters below (bottom) surface of hypersaline water bodies of Lagos State, Nigeria.

Legend.

St1= latitude 6.35° N longitude 3.28° E; St2= latitude 6.35° N longitude 3.40° E;

St3= latitude 6.36° N longitude 3.47° E

The distribution of bacteria isolated from 100 meters below hypersaline water surface of Lagos, Nigeria water bodies indicates the predominant occurrence of *Corynebacterium kutscheri* from St1, St2 and St3 with 7, 6 and 7 times consecutively, this is followed by *B. megaterium* from the same locations with the occurrence of 4, 2 and 4 times. *B. badius* from St1, St2 and St3 all occurred in the order of 2, 2 and 1 times respectively. Also, *Micrococcus varians*, *B. macquariensis*, *Mycobacteria delbrueckii*, *S. epidermidis* and *S. saprophyticus* that are all obtained from ST1 occurred once each. *Klebsiella pneumoniae* subsp. *Pneumoniae* and *Lactobacillus delbrueckii* bacteria that are also obtained from St2 occurred once each. However, *M. luteus*, *Staph. aureus*, *Neisseria veillonella*, *E. intermedius* and *E. amnigenus*, *Serratia marcescens* and *Yersinia pestis* were not isolated from St1, St2 and St3 at the depths of 100 meter below the hypersaline water body of Lagos, Nigeria samples.



**Figure 4.2.3:**-The effects of 3% salt concentration on the growth of bacteria isolated from Lagos, Nigeria hypersaline water body.

**Legend:**

a=*Yersinia pestis*; b=*Bacillus badius*; c=*Corynebacterium kutscheri*; d=*Neisseria veillonella*; e=*Bacillus megaterium*; f=*Enterobacter intermedius*; g=*Klebsiella pneumonia subsp pneumoniae*; h=*Enterobacter amnigenus*; i=*Staphylococcus epidermidis*; j=*Micrococcus luteus*; k=*Staphylococcus aureus*; l=*Micrococcus varians*; m=*Mycobacterium smegmatidis*; n=*Staphylococcus saprophyticus*; o=*Mycobacterium delbrueckii*; p=*Bacillus megaterium*; q=*Lactobacillus delbrueckii*; r=*Serratia marcescens*;

Figure shows the effect of 3% (w/v) salt on bacteria isolated from Lagos, Nigerian hypersaline water body. There was no significant ( $p < 0.05$ ) increase in the isolated bacteria growth before 41 hours of incubation. However, *Lactobacillus delbrueckii* among the other bacteria showed a notable decline in growth (OD - 0.77) after 2 hours of incubation. The highest growth (OD of 2.87) was recorded for *Neisseria veillonella* at 53 hours of incubation but, declined at 72 hours to OD 2.68. The graph generally showed that the highest growth for many of the bacterial was obtained at 53 hours of incubation before.

**Table 4.4:**-Producing polyhydroxylalkanoate ability of bacteria isolated from the hypersaline water body in Lagos State, Nigeria

	Number of bacteria	Percentage number of bacterial
Total bacterial isolates obtained	190	100%
Positive PHA potential bacterial isolates	119	66.7%
Negative PHA potential bacterial isolates	71	37.4%

Table 4.4 shows the positive PHA and negative PHA producing potentials of bacteria isolated from hypersaline water bodies of Lagos, Nigeria water body. A total of 190 bacterial were isolated from this research and 119 (66.7%) tested positive for PHA producing potential with the induction of glucose at 2% in minimal salt medium.



**Physicochemical analysis of physical parameters of hypersaline water body sampled from Lagos, Nigeria.**

**Table 4.5:-**Physical characteristics of hypersaline water body in Lagos, Nigeria sampled sites for PHA bacterial isolates

		Location								
		0-0.07 m water surface			50 m below water surface			100 m below water surface		
<b>Parameters</b>		St1	St2	St3	St1	St2	St3	St1	St2	St3
Physical	<b>Temperature (°C)</b>	26.79±0.05	27.71±0.07	27.56±0.03	28.04±0.04	23.4±0.05	26.55±0.04	27.54±0.04	27.04±0.11	27.29±0.08
	<b>pH</b>	7.88 ± 0.02	7.96 ± 0.05	7.89±0.22	7.78±0.55	7.90±0.22	7.91±0.078	7.91±0.08	7.70±0.067	7.78±0.14
	<b>Turbidity (NTU)</b>	0.255±0.04	0.166±0.07	0.303±0.03	37.4±0.04	17.9±0.05	0.863±0.04	36.4±0.04	8.92±0.11	0.933±0.08
	<b>Conductivity (µS/cm)</b>	39000±74.56	38900±231.92	37700±380.83	41600±498.67	38700±941.55	37300±462.97	43100±731.20	43500±814.84	42000±246.07
	<b>Total suspended solids (mg/L)</b>	2±1.24	1±0.81	0±0		48±22.76	21±9.93	8±2.62467	37±2.16	9±2.44
	<b>Total dissolved solids (mg/L)</b>	21250±9229.16	21390±504.22	20730±196.90	22880±816.02	21200±889.30	20500±530.07	23705±225.03	23900±94.07	23100±2056.42

Legend.

St1= latitude 6.35° N longitude 3.28° E; St2= latitude 6.35° N longitude 3.40° E;

St3= latitude 6.36° N longitude 3.47° E

**Physicochemical analysis of chemical parameters of hypersaline water sampled from Lagos, Nigeria.**

**Table 4.5.1:-**Chemical characteristics of hypersaline water body in Lagos, Nigeria sampled sites for PHA bacterial isolates

		Location								
		0-0.07 m water surface			50 m below water surface			100 m below water surface		
<b>Parameters</b>		St1	St2	St3	St1	St2	St3	St1	St2	St3
Chemical	<b>Total acidity (mg/L)</b>	40±8.16	90±21.20	130±14.14	50±8.16	10±0.00	80±16.33	40±0.0	60±16.33	70±8.16
	<b>Chloride (mg/L)</b>	15240±214.17	15670±96.26	16760±134.91	17190±392.26	17900±81.65	15240±176.64	17720±216.49	17930±72.57	14880±200.5
	<b>Nitrate (mg/L)</b>	20.4±0.62	21.5±0.85	16.5±0.14	7.7±0.216	7.6±0.37	5.2±0.35	9.4±0.637	14±0.16	3.7±0.24
	<b>Phosphate (mg/L)</b>	0.97±0.02	0.86±0.05	0.24±0.03	0.96±0.008	0.74±0.03	0.3±0.014	0.44±0.037	0.93±0.35	0.18±0.02
	<b>Sulphate (mg/L)</b>	2750±66.83	2700±8.165	2770±21.60	2790±37.417	3380±42.426	2990±86.02	2950±64.807	3620±21.60	3220±35.59
	<b>Dissolved Oxygen (mg/L)</b>	4.21±0.31	4.42±0.05	4.09±0.05	4.15±0.155	4.08±0.043	4.26±0.042	4.38±0.107	4.27±0.02	4.24±0.04
	<b>Chemical Oxygen Demand (mg/L)</b>	4.21±8.28	1986±4.96	2174±17.56	2239±28.856	2203±70.8	2737±51.65	1984±47.633	2096±26.73	3699±173.73

Biological Oxygen Demand (mg/L)	4.21±15.77	496.5±19.85	543±16.51	559.75±8.127	350.75±5.199	684.25±5.961	496±4.967	524±12.32	924.75±18.93
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Legend.

St1= latitude 6.35° N longitude 3.28° E; St2= latitude 6.35° N longitude 3.40° E; St3= latitude 6.36° N longitude 3.47°E.

Table 4.5 shows the physical characteristics of the hypersaline water body of Lagos, Nigeria, where pH ranged between (7.70±0.067 and 7.96±0.05) for st2 at 100 m below water surface and st2 at 0-7 cm water surface; Turbidity between (0.166±0.07 and 37.4±0.04) NTU (Nephelometric unit) for 0-7 cm water surface in St2 and 50 m below water surface at St1 respectively; conductivity between (37300±462 and 97 43100±731.20) µS/cm for 100 m below water surface and 50 m below water surface in St1 and St3 respectively; and TDS between (20500±530 and 0723900±94.07) mg/L for 100 m below water surface and 50 m below water surface in St1 and St3 respectively.

Table 4.5.1 shows the chemical characteristics of the hypersaline water body of Lagos, Nigeria where highest and lowest parameters of total acidity (mg/L) of 130±14.14 and 10±0.00 records for 0-7 cm water surface at st3 and 50 m below water surface at St2 respectively. Other chemical parameters includes chloride (mg/L) 17930±72.57 and 14880±200.5 for 100 m below water surface at st2 and 100 m below water surface a st3 respectively; nitrate (mg/L) 21.5±0.85 and 3.7±0.24 records for 0-7 cm water surface and 100 m below water surface in St2 and St3 respectively; phosphate (mg/L) 0.97±0.02 and 0.24±0.03 obtained from 0-7 cm water surface in St1 and St3 respectively; sulphate (mg/L) 3620±21.60 and 2700±8.165 records for 0-7 cm water surface and 100 m below water surface in St2 and St3 respectively; dissolved oxygen (mg/L) 4.42±0.05 and 4.08±0.043 obtained from 0-7 cm water surface and 50 m below water surface in St2; COD (mg/L) 3699±173.73 and 4.21±8.28 records of 0-7 cm water surface and 100 m below water surface in St1 and St3 respectively; and BOD (mg/L) 924.75±18.93 and 4.21±15.77 obtained from 0-7 cm water surface and 100 m below water surface in St1 and St3 respectively.

### Discussion:-

There is no significant difference ( $p < 0.05$ ) in the data of bacterial distribution obtained in shown in figure 1 of this research, and this agrees with the findings that 100 m depth is not enough for the spatial difference when considering other factors such as dissolved oxygen, photic effect, period of sample and frequent waves experienced by the water body (Breitburg, et al., 2018; Grasshoff et al., 2009; Tissot and Welte, 2013). Figure 4.2, 4.2.1 and 4.2.3 reveals that *Corynebacterium kutscheri* is widely distributed organism found across all sites and depths sampled in this research. It also shows that there is no significant difference ( $p < 0.05$ ) in the occurrence of this same bacterial isolates between 0-0.07m and 50 m below surface of the water but reveals tremendous difference between the aforementioned depths with that of 100 m below water surface ( $p < 0.05$ ). This may be as a result of the lower dissolved oxygen recorded at 100 m below water surface compared to the values that of 0-0.07 m and 50 m below surface water which has no significant values ( $p < 0.05$ ). Thus dissolved oxygen may be said to affect the growth of *Corynebacterium kutscheri*. This aforementioned result is in agreement with the characteristics of *Corynebacterium sp.* as salt tolerant like Micrococcus, Bacillus species as well as Staphylococcus species (Barksdale et al., 1981; Denis et al., 2001; Anihouvi et al., 2007).

Figure 4.3 shows the initiation of exponential period at 53 hours from the effects of 3% salt on the growth of bacteria isolates from Lagos, Nigeria hypersaline water body. Hence, apart from the adjustment of bacterial isolates to temperature, osmotic pressure, atmospheric pressure, pH, and moisture availability among other environmental factors (Kaye and Baross, 2004), the bacterial isolates displayed the characteristic of accumulating enough salt concentration (known as lag period of which may also be term period of growth adjustment) in order to initiate the proliferation (cellular multiplication), and this may be because their genetic makeup may require sodium chloride or salt for growth (Elabed et al., 2019). Figure 4.3 also displayed the 'salt shock' experienced of *Lactobacillus delbrueckii* where the initial inoculum size was seen to be significantly decreased ( $p \geq 0.05$ ) before 53 hour after culture when it fully recovered. Thus, the acclimatizing period of over 52 hours of accumulation may be required for 3 % of salts (NaCl) concentration for *Lactobacillus delbrueckii* for the initiation of proliferation as observed in this research. Figure 4.3 clearly shows that *Lactobacillus delbrueckii* experienced 'salt shock' at 2 hours of incubation before progressive recovery at 41 hours after incubation.

All eighteen (18) bacterial isolated conventionally are identified as *Bacillus* species 4 (22.22%) species, *Corynebacterium* specie, 1 (0.06%), *Enterobacter* 2 (0.11%), *Klebsiella* 1 (0.06%), and *Micrococcus* 2 (0.11%) species. *Mycobacterium* also recorded 2 (0.06%) species, *Neisseria veillonella* 1 (0.06%), *Staphylococcus* species 3 (0.17%) and *Yersinia* 1 (0.06%). Hence from these bacterial isolates, *Bacillus* species have been predominantly known to produce PHA under different environmental conditions and carbon source (Halami, 2009). Also, *Enterobacter* species do accumulates PHA production (Ceyhan and Guven, 2011) even when isolated from other source but may be dependent upon the conditions as well as genetic inclination for PHA production. *Corynebacterium* species is another PHA producer when circumstance triggers the production. These bacteria are members of the taxonomically related genera *Rhodococcus*, *Nocardia* and *Corynebacterium*, and it has been discovered that 3HV and 3HB monomers present in PHA is dependent on the carbon source and Laos noted that 3HV is generally the major 3-hydroxyacid produced (Anderson *et al*, 1990).

### Conclusion:-

The data available from this research hereby suggests the presence of *Corynebacterium kutscheri* proliferation at all the three depths and sites researched in this work, it also pointed that their population is reduced as the available concentration of dissolved oxygen decreases. *C. kutscheri* is a PHA producer among others viz: *Micrococcus varians*, *M. luteus*, *Bacillus badius*, *B. macquariensis*, *B. megaterium*, *Mycobacterium delbrueckii*, *Mycobacterium smegmatis*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Lactobacillus delbrueckii*, *Klebsiella pneumonia* subsp. *pneumoniae*, *Neisseria veillonella*, *Enterobacter amnigenus* and *Serratia marcescens*. However, only 2 bacterial isolates (*Enterobacter intermedius* and *Yersinia pestis*) from the sampled site did not test positive for PHA when induced with glucose.

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