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Isolation of Polyhydroxyalkanoates (PHAs) Accumulating Bacteria from Waste Dumpsites

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Abstract
Polyhydroxyalkanoates (PHAs) are biodegradable and biocompatible polyesters (bioplastics) produced by some microorganisms. Their 100% biodegradability means they have nontoxic residual products and low environmental permanence and their biocompatibility means they are not harmful to living tissue and therefore can and have been utilized in a variety of medical and surgical applications. In this study standard procedures were used to isolate bacteria from soils obtained from various waste dump sites. Isolates were screened for PHA production using Sudan Black B dye. Isolates were identified at molecular level using 16SrRNA amplification, sequencing and analysis. A total of twenty-six (26) bacterial isolates were obtained. Of these twenty-six (26) isolates, nine (9) each were isolated from cassava mill waste dump site and palm oil mill waste dump site soils while abattoir waste dump site soil yielded eight (8) isolates. Thirteen (13) isolates were remarkably positive for Sudan Black B stain test for PHA accumulation. The PHA yield from the thirteen (13) selected isolates as determined by spectrophotometric analysis of the crotonic acid that the PHA was converted to, ranged from 1.95mg/l to 10.79mg/l. The isolate with the highest yield was from the abattoir dump waste site. Molecular studies of the best ten (10) PHA yielding isolates showed that their extracted genomic DNA was of high quality and high molecular weight. The 16SrRNA gene was amplified at 1500bp according to the primers used for amplification. The PHA synthase gene amplified from genomic DNA of the bacterial isolates was amplified at 239bp. Sequence analysis of the amplified 16SrRNA genes of the selected isolates revealed their identities as Gayadomonas joobiniege AAU1, Providencia vermicola AAU2, Muricauda pacifica AAU3, Proteus mirabilis AAU4, Hafnia psychrotolerans AAU5, Photorhabdus luminescens AAU6, Xanthovirga aplysinae AAU7, Methylomarinovum caldicuralii AAU8, Photobacterium aquimaris AAU9 and Proteus terrae AAU10 with corresponding GenBank assigned accession numbers of OP776063 - OP776072 respectively. This study has elucidated bacterial species that can produce PHA from waste dumpsites. This is very important for industries producing bioplastics, to greatly reduce cost of production as these new bacterial isolates can be utilized in the production of PHA, thereby reducing the high cost of production.

Key words: *Dumpsites, waste, Sudan Black B dye*

Introduction

Our world without plastics is almost unimaginable. Plastics are synthetic polymers which are widely used in our daily lives. Plastics have been an integral part of our lives (Olaniyan, 2015). Most of these materials have historically been derived from oil and face potential problems with increasing fossil fuel costs, potential scarcity and customer demands for alternatives. Conventional plastics are synthetic carbon-based polymers that are made from mostly petroleum, which is a non-renewable source. The demand of plastics is ever-growing, due to their relatively low cost, ease of manufacture and flexibility (Cruz *et al*., 2022). Their non-biodegradable nature is the major limitation associated with its usage. The production of plastics in high quantities makes their disposal a major issue to worry about among environmentalists and health department (Mitra *et al*., 2022). The extensive use of plastics is due to their versatile properties especially durability thereby causing severe problem in waste management, hence affecting the aesthetic quality of water bodies and natural areas (Ekeogu, 2024a). Plastics are man-made therefore they are not recognized by microorganisms. Hence, they take very long time to degrade that is about 450 years on average for degradation of a plastic bottle (Koushic *et al*., 2024). Moreso, disposal of these non-biodegradable (petrochemical derived) plastics poses a threat to our environment. Plastic debris also poses considerable threat by choking and starving wildlife, distributing non-native and potentially harmful organisms, absorbing toxic chemicals and degrading or broken down into micro-plastics that may subsequently be ingested. Also, due to high cost of recycling, plastics are rarely recycled leading to crammed up landfills (Ding *et al*., 2019). For eradication of these and various other problems such as carbon emission during incineration, biodegradation of plastic is a must (Ekeogu, 2024b). Hence, need for new alternative, bioplastics (Nanda *et al*., 2022).

In accordance with the literature data, more than 250 different species of bacteria possess the ability to synthesize PHAs as intracellular storage materials, when the carbon source available is in excess, which can be extracted and then formulated and processed for plastic production, (Stanley *et al*., 2018). PHAs have a great potential because of their interesting properties, high biodegradability, biocompatibility and their recyclable nature.

In spite of these interesting properties, industrial production of PHB is still not well established because its high production cost due to expensive carbon substrates. This has made it incapable to compete with conventional plastics in the commercial market (Matos *et al*., 2021). Therefore, the applications of cheap carbon sources have been explored. The PHB content Process economics reveal that the use of inexpensive and renewable carbon substrates viz. agro-industrial wastes, municipal waste and byproducts as PHA feedstock can contribute to as much as 40-50% reduction

in the overall production cost (Zhang *et al*., 2022). Bacterial strains, fermentation strategies and recovery processes also influence the total production cost (Sunena *et al*., 2023).

There are currently a variety of methods for detecting and identifying microorganisms that synthesize and accumulate intracellular PHAs granules. These include both phenotypic and genotypic detection methods (Wang *et al*., 2022). Sudan Black staining, Nile Blue A staining and direct staining of bacterial colonies by fluorescence (Ekeogu, 2012). Genotypic methods use various Polymerase Chain Reaction (PCR) protocols to detect and amplify the PHA synthase gene(s).

Agricultural waste is otherwise called agro-waste. It is estimated that about 998 million tonnes of agro-waste are produced yearly (Olayiwola *et al*., 2021). Human population is ever growing, needs food and energy. This creates higher and deeper pollution of the environment. Without taking preventive measures of these harmful tendencies, the planet soon would face an ecological catastrophe (Strong *et al*., 2016). Politicians and scientists from all over the world are looking for different solutions to avoid this chaos (Bibi *et al*., 2023). Considerable importance has been given to recycling of waste and residues as it offers new resources of raw materials and also leads to decrease of pollution. Raw materials make up about 50% of the production cost in which carbon sources account for 70-80% of the total cost (Anjum *et al*., 2016). Cheap and readily available feed stocks such as industrial, agricultural, municipal and food –based waste that are rich in carbon are desirable feed stocks for PHA production (Reddy *et al*., 2022) conversion of these wastes into PHA would create profit and solve waste disposal problems (Bibi *et al*., 2023).

This research is focused on isolation of bacteria from various waste dump sites and screening the bacterial isolates for PHA accumulation using Sudan Black B dye. Extract the DNA of the Sudan Black B positive bacterial isolates and amplify PHA Synthase gene (phaC) in the extracted DNA using appropriate primer sets.

MATERIALS AND METHODS

Study Area

The study area conducted is Ekpoma, Esan West Local Government Area, Edo State. Ekpoma is a town in Edo state, Nigeria. Ekpoma lies on the geographical coordinate of latitude 6^{0}08E/ 6.450^{0}N East of Edo state, Nigeria. It is home to the Ambrose Alli University.

Materials

The experiment was set up in the laboratory using a completely randomized design. All inocula were standardized, using 0.5McFarland turbidity Standard. The media used are nutrient agar, minimal salt medium (MSM) supplemented with glucose, Luria Bertani broth and Tryptone soya agar. Sudan Black B dye was used.

Sterilization of Materials

Glass wares such as Petri dishes, conical flask, beaker, test tubes were washed, and air dried, wrapped in aluminum foils and sterilized using an autoclave at 160°C for 1hr. The working surface of the equipment was disinfected by cleaning with cotton wool soaked in 70% ethanol. The wire loop was also be sterilized by heating to redness before and after each use.

Media

The media (Tryptone soya agar, nutrient agar, minimal salt medium and Luria Bertani broth) were sterilized using an autoclave at 121°C for 15min. and prepared according to the manufacturer's instruction.

Sample collection

Using a garden rake, the wastes and debris were removed and a hand trowel was used to dig the soil under the waste dump. The soil samples were taken at about 5cm depth with an auger and put in properly labeled sterile sample containers. A total of 15 samples were collected from various dump sites (abattoir, cassava mill and palm oil mill), Five (5) samples each from cassava mill, palm oil mill and abattoir site. All samples were taken to the laboratory immediately for analysis. The samples were refrigerated where delay before analysis was inevitable.

Soil samples were collected from three (3) sites.

Site A = Palm oil waste dump site soil (POWS). Five (5) soil samples randomly collected.

Site B = Abattoir waste dump site soil (AWS). Five (5) soil samples randomly collected.

Site C = Cassava mill waste dump site soil (CMWS). Five (5) soil samples randomly collected.

Soil samples collected from same site were thoroughly mixed before isolation of bacteria.

Sample preparation and analysis for bacteria

Soil samples from various dump sites were collected aseptically from different localities in Ekpoma, Edo State. For bacterial isolation, 1g of homogenized soil was dissolved in 9ml of sterile distilled water. From the solution, ten-fold serial dilutions were prepared. An aliquot (1ml) of sample dilutions from 10^{-5} and 10^{-1} were inoculated onto nutrient agar plates and incubated at 37°C for 24hr, according to the method of (Ekeogu, 2012). Various distinct colonies were individually picked and sub-cultured 3-4 times on nutrient agar plates. The bacterial colonies were streaked on nutrient agar slants, incubated at 30°C overnight and then stored at 4°C for further use.

Screening with Sudan Black B

All the bacterial isolates were qualitatively tested for PHA production following the viable colony method of screening using Sudan Black B dye (Yatim *et al*., 2017; Anjum *et al*., 2016). For rapid screening of PHA producers, tryptone soya agar (TSA) medium supplemented with 2% glucose was sterilized by autoclaving at 121°C for 15min. The medium was poured into sterile Petri dishes and allowed to solidify. The bacterial isolates were spot inoculated on the plates and the plates

were incubated at 37ºC for 24hr after which ethanolic solution of Sudan Black B (0.02%) was spread over the colonies and the plates were kept undisturbed for 30min. The excess dye was then decanted carefully drawn off using a filter paper. They were then washed with ethanol (96%) to remove excess stain from the colonies. The ethanol in the stain evaporates in about 5-10min. The dish was then flooded with Xylene until it completely decolourized. The dish is counter-stained with Safranin. After 10s, the dish was washed with running tap water and air dried, then viewed using oil immersion. Colonies unable to incorporate the Sudan Black B appeared white, while PHB producers appeared bluish black. The dark blue coloured colonies were taken as positive for PHA production. All the positive isolates were scored according to the level of dark blue colouration (i.e; +++, ++ or +) while those that showed no dark blue colour (negative) were scored as minus sign (i.e; -).

Preparation of standard curve

Standard curve of PHB was prepared using commercially purchased PHB**,** according to the manufacturer's instructions

Quantification of PHA Production By Bacteria

All the Sudan Black B positive isolates that showed deep bluish black colour (+++) and medium bluish black colour (++) were subjected to quantification of PHA production using the method, (Yatim *et al*., 2017) and (Anjum *et al*., 2016). The bacterial inocula used were of McFarland turbidity Standard. The isolates were grown in minimal salt medium (MSM) [containing (g/l) 10.0 NaCl, 0.42 $MgSO_4.7H_2O$, 0.29 KCl, 0.83 KH_2PO_4, 1.25 Na_2HPO_4, and 0.42 $NaNO_3$, supplemented with glucose 2% at 37ºC for 48hr. Ten milliliter (10ml) of the broth containing the bacterial cells was pipetted into eppendorf tube for quantification. The bacterial cells containing the polymer were pelleted at 10,000rpm for 15min using Sorvail legent Micro 17 centrifuge (Thermo scientific) and the pellet washed with acetone and ethanol to remove the unwanted materials. The pellet was resuspended in equal volume (10ml) of 14% percent Sodium hypochlorite and incubated at room temperature (30ºC) for 2hr. The whole mixture was centrifuged again at 5,000rpm for 15min and the supernatant discarded. The pellet containing PHA was washed again with acetone and ethanol. Finally, the polymer granules were dissolved in 5ml boiling chloroform and evaporated leaving only PHA, then 5ml concentrated Sulfuric acid (H_2SO_4) was added and heated at 80ºC. The addition of Sulfuric acid converts the polymer into Crotonic acid which is brownish in colour. The absorbance of the solution was measured spectrophotometrically at 235nm against a Sulfuric acid blank using UNISPEC SM7504UV (Uniscope England). By referring to the standard curve, the quantity of PHA produced was determined.

Extraction of Genomic (Chromosomal) DNA from positive bacterial isolates

The bacterial isolates were grown in Luria Bertani (LB) broth medium for 24hr at 37ºC and the genomic DNA of the bacterial isolates was extracted using "Quick-DNA Fungal/Bacterial Miniprep kit" (Zymo Research, USA) according to the manufacturer's instructions. Thereafter, the concentration of the extracted DNA was determined using NanoDrop 2000 Spectrophotometer (Thermo scientific, USA), while the quality of the DNA was determined by Agarose gel electrophoresis.

Amplification of 16SrRNA Gene Sequence

The 16SrRNA gene fragment of the extracted DNA was amplified using an automated PCR thermocycler (Strong *et al*., 2016). The isolated genomic DNA was subjected to 16SrRNA gene amplification using universal bacterial primers (27F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R: 5′-GGTTACCTTGTTACGACTT-3′). These primers were synthesized at Inqaba Biotech Ltd (Pretoria, South Africa). The amplification reaction was carried out by preparing a 25µl reaction mix consisting of: 12.5 µl of 2X ready master mix (containing the dNTPs, Taq DNA polymerase, $MgCl_2$, and the reaction buffer); 2 µl of template genomic DNA; 1 µl each of forward and reverse primers (5 µm), and 8.5 µl of nuclease free water. The PCR thermal cycling programme used was as follows: initial denaturation at 95^{O}C for 5min; 30 cycles of denaturation, annealing and extension at 94^{O}C, 52^{O}C and 72^{O}C for 30s, 30s and 1min 25s respectively, followed by a final extension at 72^{O}C for 10min and kept at a hold temperature of 4^{O}C. The size of the amplicons was verified by electrophoresing the products on 2% Agarose gel stained with ethidium bromide and viewed under a UV transilluminator.

Amplification of PHA Synthase Gene (*phaC*)

The PHA synthase gene fragment of the extracted DNA was amplified using an automated PCR thermocycler following a modification of the procedures previously described by Strong *et al.*, (2016) and Ding *et al*., (2019). The *phaC* gene was amplified using the primer set *pha*C F: 5'-CCGCCSTGGATCAACAAGT–3' and *pha*C R: 5'- GTGCCGCCGAYGCAGTAGCC–3'. These primers were synthesized at Inqaba Biotech Ltd (Pretoria, South Africa). The PCR reaction was performed in 0.2 ml microcentrifuge tubes by adding 2.0 µl of template DNA, 1.0 µl of forward primer (5.0 µm), 1.0 µl of reverse primer (5.0 µm), 5.0 µl of PCR master mix (5X) and 16.0 µl of nuclease free water for a 25.0 µl reaction volume. The PCR thermal cycling programme used was as follows: initial denaturation at 95°C for 5min, 35 cycles of denaturation at 95°C for 20 sec, annealing at 61°C for 30sec, extension at 72°C for 20sec, followed by a final extension at 72°C for 10min and kept at a hold temperature of 4ºC. PCR products were observed by electrophoresis

in 2% Agarose gel stained with ethidium bromide and viewed under a UV transilluminator to determine the size of the amplicons.

Sequence Determination of PCR Amplified Products

The 16SrRNA gene sequence of the PCR amplified products was determined with a Dye Terminator Sequencing Kit and the product was analyzed with an ABI Prism DNA Sequencer (Stanley *et al.,* 2018). The sequencing service was provided by Inqaba Biotech Ltd (Pretoria, South Africa).

Isolation of bacteria

A total of twenty-six (26) bacterial isolates were obtained from the various samples/sites. Of these twenty-six (26) isolates, nine (9) each were from cassava mill waste dump site soil and palm oil mill waste dump site while abattoir waste dump site yielded eight (8) isolates. This is shown in Table 4.1.

All isolates picked were coded according to the site of soil samples from which they were isolated.

Table 1: Bacterial isolates

Serial Number	Isolate Code
1	CMWS1
2	CMWS2
3	CMWS3
4	CMWS4
5	CMWS5
6	CMWS6
7	CMWS7
8	CMWS8
9	CMWS9
10	POWS1
11	POWS2a
12	POWS2b
13	POWS3
14	POWS4
15	POWS5
16	POWS6
17	POWS7
18	POWS8
19	AWS1
20	AWS2
21	AWS3
22	AWS4
23	AWS5
24	AWS6
25	AWS7a
26	AWS7b

KEY
CMWS=Cassava mill waste soil
POWS=Palm oil waste soil
AWS=Abattoir waste soil

Rapid screening for PHA accumulation

The uptake of Sudan Black B dye by the isolates is shown in Plates 1. Thirteen (13) isolates were remarkably positive for Sudan Black B stain test for PHA accumulation, as shown in Table 2. All the positive isolates were scored according to their level of dark blue colouration (i.e; +++, ++ or +) while those that showed no dark blue colour (negative) were scored as minus sign (i.e; -).

Plate 1: Uptake of Sudan Black B by Bacterial cells

Table 2: Sudan Black B Stain Test for PHA accumulation by bacterial isolates

Serial Number	Isolate Code	Isolate score
1	CMWS1	+
2	CMWS2	+++
3	CMWS3	-
4	CMWS4	-
5	CMWS5	+++
6	CMWS6	-
7	CMWS7	+
8	CMWS8	+
9	CMWS9	-
10	POWS1	++
11	POWS2a	-
12	POWS2b	++
13	POWS3	++
14	POWS4	+

15	**POWS5**	**+++**
16	**POWS6**	**+++**
17	**POWS7**	**+**
18	**POWS8**	**++**
19	**AWS1**	**+++**
20	**AWS2**	**++**
21	**AWS3**	**++**
22	**AWS4**	**+**
23	**AWS5**	**-**
24	**AWS6**	**+++**
25	**AWS7a**	**+++**
26	**AWS7b**	**-**

KEY:

+++ = Deep dark blue colour
++ = Medium blue colour
+ = Light blue colour
- = No blue colour

Preparation of standard curve

Standard curve of PHB was prepared following the methods of Yatim *et al*., (2017) and Anjum *et al*. (2016). Commercially purchased PHB was used to prepare the standard curve.

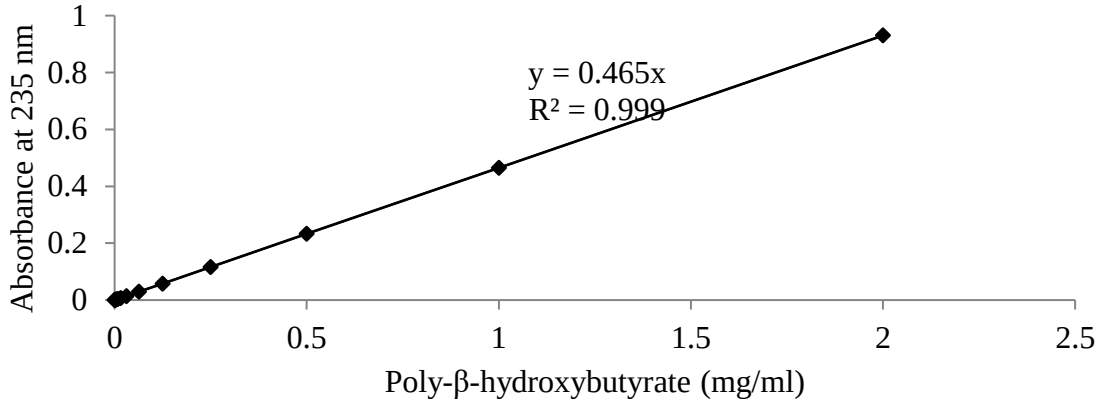


Figure 1: PHB Standard Curve

Where **X** = Concentration of Poly-β-hydroxybutyrate (mg/ml),
Y = Absorbance at 235nm, and
$\mathbf{R^2}$ = Regression coefficient.

Quantification of PHA production by bacteria

The PHA yield from the thirteen (13) selected isolates ranged from 1.95mg/l to 10.79mg/l. The isolate with the highest yield was from the abattoir dump waste site as shown in Table 3.

The best ten (10) bacterial isolates that yielded high PHA were selected for further studies

.

Table 3: Polyhydroxyalkanoate (PHA) Production by the Selected Bacterial Isolates

Serial Number	Isolate Code	PHA Yield (mg/ml)
1	CMWS2	1.95
2	CMWS5	4.17
3	POWS1	10.70
4	POWS2b	10.65
5	POWS3	8.96
6	POWS5	6.80
7	POWS6	6.46
8	POWS8	7.28
9	AWS1	6.72
10	AWS2	9.67
11	AWS3	10.79
12	AWS6	8.97
13	AWS7a	6.48

Plate 2: PHA converted to crotonic acid after addition of concentrated sulfuric acid (H_2SO

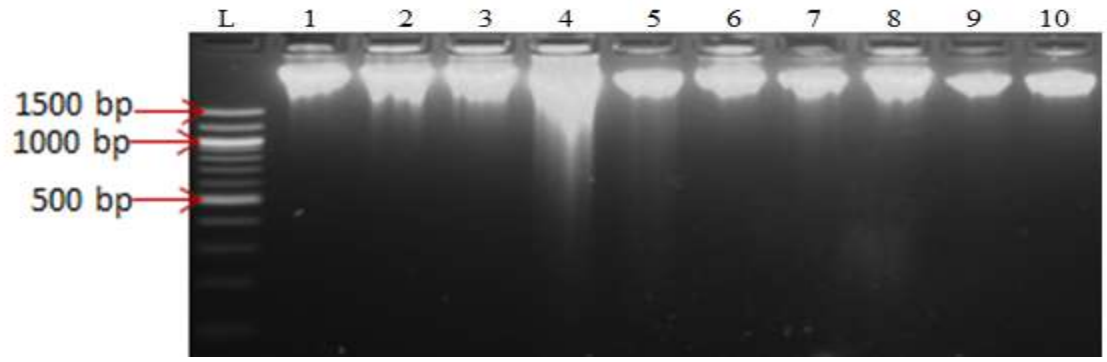
MOLECULAR CHARACTERIZATION OF THE SELECTED BACTERIAL ISOLATES

The concentration and purity of the chromosomal DNA of the best ten (10) PHA yielding bacterial isolates is shown in Table 5.

Table 5: Concentration and purity of chromosomal DNA determined using Nanodrop-spectrophotometer

Isolate Code	DNA conc.(ng/µl)	A_{260}/A_{280}(purity)
POWS1	**77.53**	**1.88**
POWS2b	**124.87**	**1.91**
POWS3	**107.25**	**1.97**
POWS5	**96.03**	**1.90**
POWS8	**142.28**	**1.92**
AWS1	**68.92**	**1.74**
AWS2	**128.95**	**1.91**
AWS3	**97.38**	**1.90**
AWS6	**128.29**	**1.96**
AWS7a	**107.17**	**1.95**

Plate 3: Agarose gel electrophoresis of genomic DNA of the bacterial isolates



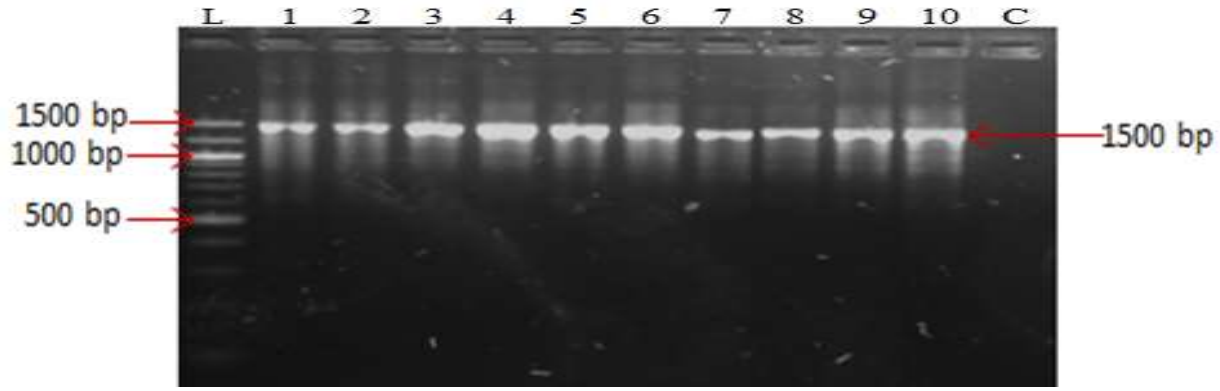
Genomic DNA was of high quality and high molecular weight as shown in Plate 3.

Key:

L: Ladder,
1: AWS1,
2: AWS2,
3: AWS3,
4: AWS6,
5: AWS7a,

6: POWS1,
7: POWS2b,
8: POWS3,
9: POWS5,
10: POWS8

Plate 4: 16SrRNA genes amplified from genomic DNA of the bacterial isolates



The 16SrRNA gene was amplified at the expected base pairs site (1500bp) according to the primers used for amplification, as seen in Plate 4.

Key:
L: ladder,
1: AWS1,
2: AWS2,
3: AWS3,
4: AWS6,
5: AWS7a,
6: POWS1,
7: POWS2b,
8: POWS3,
9: POWS5,
10: POWS8,
C: control.

Plate 5: PHA synthase genes amplified from genomic DNA of the bacterial isolates

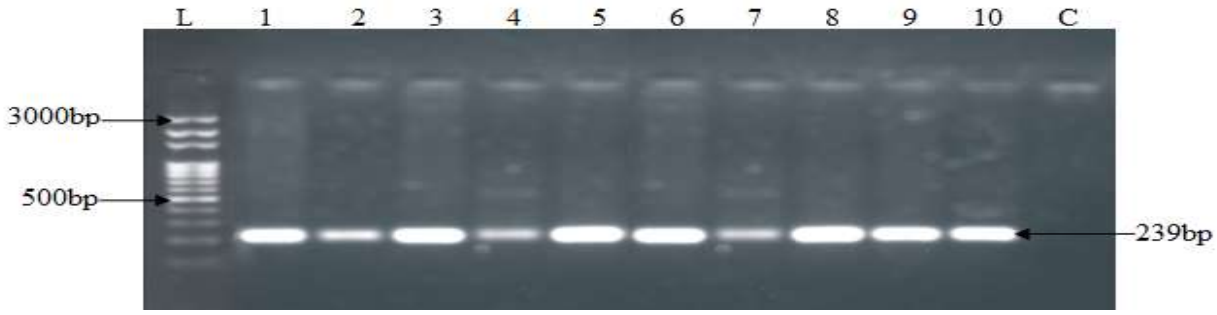


Plate 5 shows the PHA synthase gene amplified from genomic DNA of the bacterial isolates at 239 base pairs site.

Key:
L: ladder,
1: AWS1,
2: AWS2,
3: AWS3,
4: AWS6,
5: AWS7a,
6: POWS1,
7: POWS2b,
8: POWS3,
9: POWS5,
10: POWS8,
C: control.

Table 6: Molecular identification of bacterial Isolates based on 16SrRNA gene sequencing data

Isolate Code	Identified Bacteria	% Similarity	Assigned GenBank Accession Number
AWS1	***Gayadomonas joobiniege* strain AAU1**	**99.11%**	**OP776063**
AWS2	***Providencia vermicola* strain AAU2**	**99.32%**	**OP776064**
AWS3	***Muricauda pacifica* strain AAU3**	**98.88%**	**OP776065**
AWS6	***Proteus mirabilis* strain AAU4**	**98.81%**	**OP776066**

AWS7a	***Hafnia psychrotolerans* strain AAU5**	**99.43%**	**OP776067**
POWS1	***Photorhabdus luminescens* strain AAU6**	**98.95%**	**OP776068**
POWS2b	***Xanthovirga aplysinae* strain AAU7**	**99.33%**	**OP776069**
POWS3	***Methylomarinovum caldicuralii* strain AAU8**	**100%**	**OP776070**
POWS5	***Photobacterium aquimaris* strain AAU9**	**99.43%**	**OP776071**
POWS8	***Proteus terrae* strain AAU10**	**99.71%**	**OP776072**

The identity of the bacterial isolates was revealed based on 16SrRNA gene sequencing data with their corresponding GenBank assigned accession numbers as shown in Table 6.

Discussion and Conclusion

Bacterial polyhydroxyalkanoates (PHAs) are generally biodegradable, thermoprocessable, biocompatible and piezoelectricable, making them highly attractive as biomaterials. Despite these advantages, the widespread applications of PHAs is limited due to high cost of production, which basically resulted from the cost of the carbon substrate or source used for PHA production as it accounted for about 50% of the production costs. A total of twenty-six (26) bacterial isolates were obtained from the various samples/sites. Of these twenty-six (26) isolates, nine (9) each were from cassava mill waste dump site soil and palm oil mill waste dump site while abattoir waste dump site yielded eight (8) isolates. When screened with Sudan Black B dye thirteen (13) isolates were remarkably positive for Sudan Black B stain test for PHA accumulation, showing that bacteria isolated from waste dumpsites can accumulate PHA when grown in excess carbon. Thus previous reports by (Matos *et al.,* 2021) and (Nanda *et al.,* 2022) also confirmed the Sudanophilic nature of polyhydroxyalkanoates granules. Standard curve of PHB was prepared following the methods of Yatim *et al*., (2017) and Anjum *et al*., (2016) with commercially purchased PHB. The conversion of PHA to Crotonic acid by adding sulphuric acid is necessary before quantification of PHA yield using spectrophotometer. The PHA yield from the thirteen (13) selected isolates ranged from 1.95mg/l to 10.79mg/l. The isolate with the highest yield was from the abattoir dump waste site. The concentration and purity of the chromosomal DNA of the best ten (10) PHA yielding bacterial isolates shows that the extracted genomic DNA was of high quality and high molecular weight. The PHA synthase gene was amplified from genomic DNA of the bacterial isolates at 239 base pairs site. The 16SrRNA gene amplified products was sequenced. The final consensus sequences developed were subjected to BLAST analysis for comparison with the existing sequences in the NCBI database for identification. These sequences after identification were submitted to GenBank database at NCBI for assigning of accession numbers as shown in Table 6.

REFERENCES

Anjum, A., Zuber, M., Zia, K.M., Norren, A., and Tabasum, S. (2016). Microbial production of polyhydroxyalkanoates(PHAs) and its copolymers: a review of recent advancements. *Int. J. Biol. Macromol*., **89:** 161-174.

Bibi, N. J., Gopalakrishnan, K., Pratima, J., Seeram, R. and Shashi, K. B. (2023). Current advances and emerging trends in sustainabIe Polyhydroxyalkanoate modification from organic waste streams for material applications. *Int. J. Biol. Macromol.,* **70:** 247-253.

Cruz, R.A.P., Oehmen, A., and Reis, M.A.M. (2022). The impact of biomass withdrawal strategy on the biomass selection and Polyhydroxyalkanoates (PHA) accumulation of mixed microbial cultures. *New Biotechnol.,* **66 :** 8-15.

Ding, C., Wang, X., and Li, M. (2019). Evaluation of six white-rot fungal pretreatments on corn stover for the production of cellulolytic and ligninolytic enzymes, reducing sugars, and ethanol. *Appl. Microbiol. Biotechnol.,* **6:** 1-12.

Ekeogu, M.N. (2012). Prevalence and incidence of diarrhea caused by protozoa among children of school age in Ekpoma, Edo state, Nigeria. *International Journal of Scientific Innovations and Sustainability Development,* **2(2):** 93-97.

Ekeogu, M. N. (2024). Production of biosurfactants by fungi isolated from palm oil mill effluent contaminated soil and molecular characterization of the associated organisms. *Irish Journal of Environment and Earth Sciences,* **8(5):** 1-13.

Ekeogu, M. N. (2024). Microbial Deterioration of painted wallsurfaces in the University of Delta campuses, Agbor, Delta state, Nigeria. *Advance Journal of Agriculture and Ecology,* **9(9):** 36-44.

Koushic, M.U., Lorenzo, N., Antonio, G. and Alessandro, S. (2024). Polyhydroxyalkanoates, A prospective solution in the textile industry- A review. *Polymer Degradation and Stability* **8:** 210-219.

Matos, M., Cruz, P.A.R., Cardoso, P., SiLva, F., Freites, E.B.,and Calvalho, G.(2021). Combined strategies to boost PHA production from fruit waste in a three-stage pilot plant. *ACS. Sustain. Chem. Eng.*, **9:** 8270-8279.

Mitra, R., Xu, T., Chen, G.Q., Xiang, H., and Han, J. (2022). An updated overview on the regulatory circuits of polyhydroxyalkanoates (PHA) synthesis. *Microb. Biotech.* **15:** 1446-1470.

Nanda, S., Patra, B. R., Patel, R., Bakos, J. and Dalai, A.K. (2022). Innovations in applications and prospects of bioplastics and biopolymers, A review. *Environ. chem. lett.*, **20:** 379-395. *doi. 10.1007/s10311-021-01334-4.*

Olaniyan, A. B. (2015). Maize: Panacea for hunger in Nigeria. *Afr. J. pl. sci.*, **9(3):**155-174.

Olayiwola, S.A., Mohamad, A., Ishak, K.A., Yusuf, H., and Subramanium, R. (2021). Innovative application of biopolymer composite as proton exchange membrane in microbial fuel cell utilizing real wastewater for electricity generation. *J. Clean. Prod.*, **2:** 7-12.

Reddy, V. U., Ramanaiah, S.V., Reddy, M. V., and Chang, Y.C. (2022). Review of

the development of bacterial medium-chain-length polyhydroxyalkanoate (PHA). *Bioengineering*, **9:** 22-29.

Stanley, A., Kumar, H.P., Mutturi, S., and Vijayendra, S.N. (2018). Fed- batch strategies for production of PHA using a native isolate of *Halomonasvenusta* KT832796 strain. *Appl. Biochem. Biotechnol.*, **184 (3):** 935-952.

Strong, P., Laycock, B., Mahamud, S., Jensen, P., Lant, P., Tyson, G., and Pratt, S. (2016). The opportunity for high performance biomaterials from methane. *Microorganisms* **4:**1-24.

Sunena, D., Manju, B., Ruma, R., and Minakshi, P. (2023). Polyhydroxybutyrate in nanoparticulate form improves physical and biological performance of scaffolds. *International of Bio. Macrom.* **23:** 68-75.

Wang, K., Hobby, A. M., Chen, Y., Chio, A., Jenkins, B.M., and Zhang, R. (2022). Techo-economic analysis on an industrial-scale production system of Polyhydroxylalkanoates (PHA) from cheese by-products by halophiles. *Processes*, **10:** 20-29**.**

Yatim, A.F.M., Syafiq, I.M., Huong, K.H., Amirul, A.A., Effendy, A.W.M., and Bhubalan, K. (2017). Bioconversion of novel and renewable agro-industry by-products into a biodegradable poly(3-hudroxybutyrate) by marine *Bacillus megaterium* UMTKB-1 strain. *Biotechnol.*, **98(2):** 141-151.

Zhang, X., Liu, X.Y., Yang, H., Chen, J.N., Lin, Y., Han, and S.Y. (2022). A Polyhydroxyalkanoates (PHA)-based carrier platform of bioactive substances for therapeutic applications. *Front. Bioeng. Biotechnol.*, **9:**1-16. Doi. 10.3389/fbioe.2021.798724.