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Production of Polyhydroxyalkanoates by Bacteria Isolated from the Gut of the Larvae of African Palm Weevil (*Rhynchophorus phoenicis*)

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ABSTRACT: The indispensability of plastics in everyday life has made the search for environmentally sustainable alternatives to these nonbiodegradable polymers more earnest. Polyhydroxyalkanoates (PHAs) are a class of biodegradable, biocompatible plastics comprising of polyesters of R-hydroxyalkanoic acids. They are accumulated intracellularly as polymeric granules upon cultivating several gram-positive and gram-negative bacteria in nutrient-limiting conditions. Hence, the objective of this paper is to investigate the production of polyhydroxyalkanoates by bacteria isolated from the gut of the larvae of African Palm Weevil (*Rhynchophorus phoenicis*) using agricultural-waste substrates. Two bacteria isolated from the gut of polystyrene-fed *R. phoenicis* larvae were utilized for the production of PHA using brewery waste, bean hull, cassava peel and palm pith, as substrates. The bacteria were identified as *Lysinibacillus macriodes* and *Pantoea dispersa*. Isolate *P. dispersa* accumulated PHAs ranging from 0.89- 1.44 g/L from agricultural waste and 4.24 g/L from glucose, equivalent to 30.8%-46.7% and 71.9% yields respectively. *L. macrolides*, accumulated PHAs ranging from 1.11-1.50 g/L from agricultural waste and 3.89 g/L from glucose equivalent to 40.4%-48.1% and 69.2% yields respectively. The isolates can be desirable candidates for PHAs production under optimized conditions.

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The upsurge in plastic pollution has become a global concern, despite increasing efforts to mitigate plastic waste pollution through incineration, landfilling and recycling a sizeable amount of plastic waste is still disposed of directly in the environment (Peng *et al*., 2018; Attah *et al*., 2022). Also, these conventionally accepted plastic waste management methods have notable drawbacks. Incineration of plastic waste produces secondary pollutants such as dioxins, carbon monoxide, and nitrogen oxides. Landfilled plastic wastes are broken into monomers and oligomers which leach into the soil and water bodies (Immanuel and Isaiah, 2023). Additionally, plastic waste landfilling requires large land space (Kale *et al*., 2015). Recycling plastic waste is not effective for plastic waste that has been consumed by people usually mixed with organic and inorganic waste (Ru *et al*., 2020). To shift from dependence on synthetic plastics, bioplastic materials such as polyhydroxyalkanoate (PHA) are gaining research and application traction (Castro-Aguirre *et al*., 2016). PHA is a biopolymeric material synthesized by a variety of microorganisms as a form of carbon and energy storage under conditions of stress, with carbon surplus and low phosphorus and nitrogen (Matias *et*

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al., 2009; Wu *et al*., 2009; Reis *et al*., 2011; Oliveira *et al*. (2022). During nutrient-limiting conditions such as the absence of nitrogen, acetyl-CoA instead of entering into the Krebs cycle is channeled in the direction of PHAs synthesis (Dienye *et al*., 2022). PHAs are easily degraded within days by several microorganisms in the environment (Cooper, 2013; Lambert and Wagner, 2017; Meereboer *et al*., 2020).

Researchers over many years have considered PHA to be a promising biomaterial for applications such as packaging plastics, medical materials, drug carriers, biofuel and food additives (Chen, 2009; Philip *et al*., 2007). Diverse PHA-synthesizing microorganisms have been isolated from compost, soil, water, waste and guts of insects (Haas *et al*., 2008; Castro-Aguirre *et al*., 2016; Dienye *et al*., 2022; Immanuel and Isaiah, 2023). Despite the abundance and diversity of PHA accumulating, the high cost of raw materials and product recovery have diminished the prospect for commercialization. Substantial cost can be reduced by using carbon substrate feedstock from waste streams (Koller *et al*., 2013).

Much effort has been expended in boosting PHA production with selected strains and unconventional substrates for the polymer, but the production cost remains the core impediment to the profitable production of PHA (Lee, 1996; Reddy *et al*., 2003). However, with microorganisms adapted to use less expensive substrates such as agricultural waste for accumulation of PHA, coupled with improved strategies for product recovery, the cost can be reduced.

The process of PHA production by microorganisms utilizing agricultural waste requires the conversion of the complex organic compounds present into simpler carbon sources, such as glucose or fatty acid via microbial fermentation.

Afterwards, the microorganisms can assimilate the converted carbon sources and convert them into PHA through a series of enzymatic reactions. The accumulated PHA can then be recovered by the sodium hypochlorite method as crystals (Hahn *et al*., 1994; Sayyed *et al*., 2009).

The gut of the African palm weevil (*Rhynchophorus phoenicis*) larvae is colonized by a diverse group of microorganisms, with fundamental genes for degradation depending on the diet of the host, as they often exhibit a symbiotic relationship with their host (Friedrich, 2013; Immanuel *et al*., 2022; Immanuel and Isaiah, 2023).

Hence, the objective of this paper is to investigate the production of polyhydroxyalkanoates by bacteria isolated from the gut of the larvae of African Palm Weevil (*R. phoenicis*).

MATERIAL AND METHODS

Isolation of Bacteria: Larvae of *Rhynchophorus phoenicis* were purchased from the Toru-Orua community, Bayelsa State, Nigeria. Larvae were fed with polystyrene foam for 21 days.

The hindgut of the insect was afterwards dissected into Ringer's solution. The gut content was homogenized and plated on a mineral salt medium (MSM) composed of; 0.2 g KH_2PO_4; 0.6 g $K_2HPO_4.3H_20$; 0.2 g $(NH_4)_2SO4$; 0.2 g $MgSO_4.7H_2O$; 15g agar, supplemented with 2.0mL per litre of trace element solution containing 0.02 $NiCl_2.6H_20$; 0.03 g $Na_2MO_4.2H_20$; 0.3 g H_3BO_3; 0.03 g $MnCl_2.4H_20$; 0.01 g $CuCl_2.2H_20$; 0.2 g $CoCl_2.6H_20$; 0.10 g $ZnSO_4.7H_20$, dissolved in 1000 mL of distilled water. Pure isolates were obtained by repeated subculturing in nutrient agar.

Characterization and Identification of Isolates: Pure cultures of the bacteria were identified based on their 16S rRNA sequences.

Preparation of Substrate: Cassava peel, palm pith, brewery waste and bean hull were subjected to two types of pre-treatments viz. direct infusion of powdered substrate in MSM and acid treatment. In the acid treatment, 20% (*w*/*v*) of the substrate was treated with 5% H_2SO_4 (solid: liquid, 1:5) for 60 min. The culture medium was autoclaved at 121°C for 30 min. Afterwards, the clarified supernatant was separated and used as a source of carbon for PHA production.

PHA Production: Inoculum used was 24 24-hour culture of bacteria isolated from the gut of the larvae. Carbon sources (powdered substrates and glucose) at a concentration of 2.0% (w/v) were supplemented individually in 50 mL of sterile MSM and inoculated with 1 mL of the inoculum (0.5 McFarland standard).

The acid hydrolysate was used at a concentration of 10 % (v/v) and was also inoculated with 1 mL volume of inoculum. The bioreactors were placed on a shaker incubator with shaking at 120 rpm for 72 hours at 30^{o}C.

Extraction of PHA: The PHA was recovered using sodium hypochlorite-chloroform extraction as per Marjadi and Dharaiya (2014).

Fourier Transform Infrared (FTIR) Spectroscopy (Agilent T) was utilised to confirm the functional

groups of the extracted PHA. Spectra were read in triplicate at 400 to 4000 wave-numbers cm^{-1}.

RESULTS AND DISCUSSION

The PHA accumulating bacteria were identified based on 16s rRNA sequences as *Lysinibacillus macriodes* and *Pantoea dispersa*. Table 2 shows dry cell weight (g/L), PHA produced (g/L) and yield of PHA (%) with *P. dispersa*. The PHAs yields by *P. dispersa* with agricultural waste as substrate ranged from 30.8%-46.7% whereas with glucose the yield was 71.9%. Table 3 shows dry cell weight (g/L), PHA produced (g/L) and yield of PHA (%) with *L. macroides*. The PHAs yields by *L. macroides* with agricultural waste as substrate ranged from 40.4%-48.1% whereas with glucose the yield was 69.2.

Table 1: Accession number and reference matches

Isolate code	Similarity	Reference match	Accession number
PHA1	99.32	*Lysinibacillus macriodes*	OQ652017
PHA2	95.07	*Pantoea dispersa*	OQ652023

Table 2: Comparison of PHAs yields by *P. dispersa*

Carbon substrates	Dry cell weight (g/L)	PHA produced (g/L)	Yield of PHA (%)
Glucose	5.90	4.24	71.9
Palm pith	3.18	1.44	45.3
Palm pith (Acid hydrolysis)	3.23	1.51	46.7
Cassava peel	2.89	0.89	30.8
Cassava peel (Acid hydrolysis)	3.06	1.22	39.9
Brewery waste	3.16	1.28	40.5
Brewery waste (Acid hydrolysis)	3.20	1.34	41.9
Beans hull	2.95	1.21	41.0
Beans hull (Acid hydrolysis)	3.11	1.38	44.4

Table 3: Comparison of PHAs yields by *L. macroides*

Carbon substrates	Dry cell weight (g/L)	PHA produced (g/L)	Yield of PHA (%)
Glucose	5.62	3.89	69.2
Palm pith	3.09	1.26	40.8
Palm pith (Acid hydrolysis)	3.10	1.49	48.1
Cassava peel	3.01	1.11	36.8
Cassava peel (Acid hydrolysis)	3.12	1.26	40.4
Brewery waste	2.98	1.38	46.3
Brewery waste (Acid hydrolysis)	3.12	1.35	43.3
Beans hull	3.12	1.26	40.4
Beans hull (Acid hydrolysis)	3.17	1.50	47.3

Fourier Transform Infra-Red (FTIR) Spectroscopy Analysis of PHA: FTIR spectroscopy gave further insights into the chemical structure of the PHA granules with the presence of the C=O group and also the monomeric units. In this study, the functional groups of the polymer PHA are seen at 3365.8 m; 2922.2 cm^{-1}; 2851.4 cm^{-1}; 1640 cm^{-1}; 1449.9 cm^{-1};1375.4 cm^{-1}; 1028.7 cm^{-1} and 905.7 cm^{-1} corresponding to ester and thio esters and as well as other monomeric units.

The potential of bacteria isolated from the gut of polystyrene-fed *Rhynchophorus phoenicis* (African palm weevil) to produce PHA was explored in this study. Two bacteria were isolated from the gut of the larvae, which were found to be closely related to *Pantoea dispersa* and *Lysinibacillus mycroides.* The potential ingestion of polystyrene and likely biodegradation by *R. phoenicis* larvae was reported in the study by Immanuel and Isiah (2023). The results pointed out the obvious ability of *R. phoenicis* larvae and their gut flora to biodegrade polystyrene. In the present study, polystyrene-fed larvae, experienced a community shift favouring a selection of two bacteria adapted to nutrient-deficient conditions in the gut of the insect in the absence of its traditional diet (palm pith) the mortality rate of all the various feeding groups was closely related. Thus, it seems plausible that the larvae could use Polystyrene as their energy source.

The study reveals the effects of different carbon sources on PHA yield, as the isolate *P. dispersa* accumulated PHAs ranging from 0.89- 1.44 g/L from

agricultural waste and 4.24 g/L from glucose, equivalent to 30.8%-46.7% and 71.9% yield respectively. *L. macrolides*, accumulated PHAs ranging from 1.11-1.50 g/L from agricultural waste and 3.89 g/L from glucose equivalent to 40.4%-48.1% and 69.2% yield respectively. Dienye *et al*. (2022) isolated six PHA-producing bacteria from soil and reported yields in the range of 0.9-1.1 g/L by the isolates with organic waste (beans bran, brewer's spent grain, *Chlorella vulgaris biomass*, fish meal, peanut skin and groundnut oil) as substrates. The use of agricultural waste as substrates for PHA has been suggested as a veritable means to reduce the current cost of commercial production of PHA mainly because they are inexpensive (Getachew and Woldesenbet 2017; Stavroula *et al*., 2020). Results of this study indicat*e that the* bacterial isolates have the potential to synthesize PHAs using less expensive carbon sources such as agricultural waste with reasonable yields.

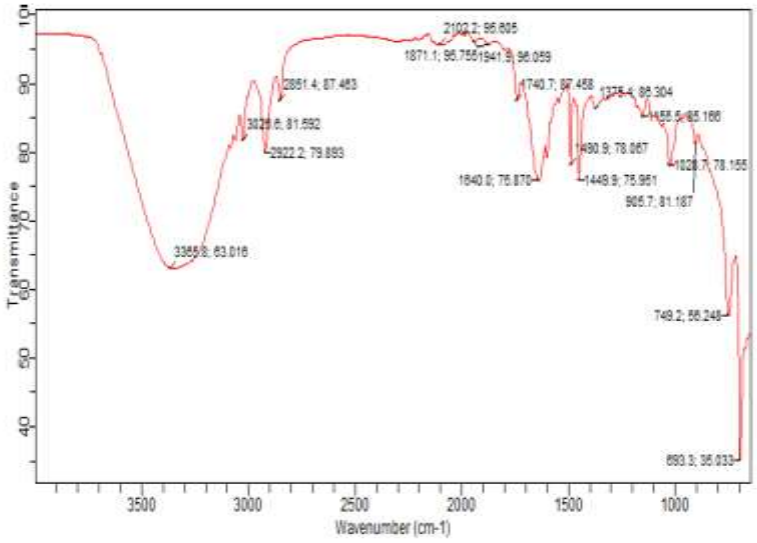

Fig 1: FTIR spectra of extracted PHA

In the present study, more PHA was produced with glucose substrate compared to the agricultural waste. Glucose is readily metabolized by microorganisms tolerant to them and would thus reasonably yield more PHA during fermentation. Getachew and Woldesenbet (2016) reported more PHAs under glucose surplus and low phosphorus and nitrogen. The limitation of nitrogen source in the present study relative to surplus carbon, favoured PHAs accumulation. This is in congruence with the report by Dienye *et al*. (2022) that nitrogen limitation plays an important role during PHAs synthesis. FTIR spectroscopy gave further insights into the chemical structure of the PHA granules with the presence of the C=O group and also the monomeric units. In this study, the functional groups of the polymer PHA are seen at 2983 m; 2922.9 cm^{-1}; 2851 cm^{-1}; 1640 cm^{-1}; 1491 cm^{-1};1302 cm^{-1}; 1028.7 cm^{-1} and 905.7 cm^{-1} corresponding to ester and thio esters and as well as other monomeric units. Similar peaks were reported by Dienye *et al*. (2022) as characteristics of PHA peaks. PHA consisting of 2-methyl-3-hydroxybutyric acid or hydroxypivalic acid and containing one or two methyl groups at the α-carbon atoms, may be considered polyhydroxybutyrate (PHB) (Shamala *et al*., 2003; Philip *et al*., 2007). Biopolymeric PHAs have been accounted for to have incredible potential as an option for oil-derived plastics. The use of less expensive carbon sources, viable maturation strategies, and altered downstream cycles can considerably reduce the production expense of PHAs. Additionally, concerning environmental issues, the serious issue of plastic waste removal all around the world may likewise be minimalized by blending or substituting their use of synthetic polymer with PHAs.

Conclusion: Pantoea dispersa and *Lysinibacillus macroides* can accumulate large amounts of PHAs in their cells using agricultural waste as their carbon source. Thus, they hold promise for the cost-effective production of PHAs under optimized conditions for production and product recovery.

REFERENCE

Attah, S; Stanley, HO; Sikoki, F.D; Immanuel, OM. (2023). Assessment of Microplastic Pollution in Selected Water Bodies in Rivers State, Nigeria. *Arch. Curr. Res. Inter*. 23(7):45-52.

Castro-Aguirre, E; Iñiguez-Franco, F; Samsudin, H; Fang, X; Auras, R. (2016). Poly(lactic acid)-Mass production, processing, industrial Applications, and end of life. *Adv. Drug Deliv. Rev.* 107: 333–366.

Chen, GQ. (2009). A microbial polyhydroxyalkanoates (PHA) based bio-and Materials industry. *Chem. Soc. Rev*. 38: 2434–2446.

Colthup, NB; Daly, LH; Wiberley, SE. (1964). Introduction to infrared and Raman spectroscopy. New York, NY: Academic Press.

Cooper, T.A. (2013). Developments in Bioplastic Materials for Packaging Food, Beverages and Other Fast-Moving Consumer Goods; *Wood head Publising Limited:* Sawston, UK, pp. 108–152.

Dienye, BN; Agwa, OK; Abu, GO. (2022). Molecular characterization, optimization and production of PHA by indigenous bacteria using alternative nutrient sources as substrate. *Microbiol. Res. J. Inter.* 32(11-12): 12-26

Friedrich, MJ. (2013). Genomes of Microbes Inhabiting the Body Offer Clues to Human Health and Disease. *JAMA*. 309 (14). 1447–1449. Doi: 10.1001/Jama.2013.2824.

Getachew, A; Woldesenbet, F. (2016). Production of biodegradable plastic by polyhydroxybutyrate (PHB) accumulating bacteria using low cost agricultural waste material. *BMC Res Notes*. 9: 1-9. https://doi.org/10.1186/s13104-016-2321-y.

Haas, R; Jin, B; Zepf, FT. (2008). Production of poly-3-hydroxybutyrate from waste potato starch. *Biosci. Biotechnol. Biochem.* 72(1): 253-256.

Hahn, SK; Chang, YK; Kim, BS; Chang, HN. (1994). Optimization of microbial poly (3-hydroxybutyrate) recovery using dispersions of sodium hypochlorite solution and chloroform. *Biotechnol Bioeng,* 44:256–261. Doi: 10.1002/bit.260440215.

Immanuel, OM; Omoko TL; Egumandi, EU. (2022). Preliminary screening of cellulase producing bacteria from the gut of African palm weevil larvae. *African Scientist*. 23(2):72-77.

Immanuel, OM, Isaiah, I.A. (2023). Polystyrene biodegradation by bacteria isolated from the gut of *Rhynchophorus Phoenicis* larvae. *Nig. J. Biotech.* 40(22) 99-106. DOI: https://dx.doi.org/10.4314/njb.v40i2.11

Kale, SK; Deshmukh, AG; Dudhare, MS; Patil, VB. (2015). Microbial Degradation of Plastic: a Review. *J. Biochem. Technol.* 6(2): 952-61.

Lambert, S; Wagner, M. (2017). Environmental performance of bio-based and biodegradable plastics: The road ahead. *Chem. Soc. Rev*. 46: 6855–6871.

Marjadi, D; Dharaiya, N. (2014). Recovery and characterization of poly(3-hydroxybutyric acid) synthesized in *Staphylococcus epidermidis*. *Afric J Environ Sci Technol*. 8: 319-329.

Matias, F; Bonatto, D; Padilla, G; Rodrigues, MF; Henriques, JA. (2009) Polyhydroxyalkanoates production by actinobacteria Isolated from soil. *Can. J. Microbiol.* 55: 790–800

Meereboer, KW; Misra, M; Mohanty, (2020). A.K. Review of recent advances in the biodegradability of polyhydroxyalkanoate (PHA) Bioplastics and their composites. *Green Chem*. 22: 5519–5558.

Oliveira, J; Belchior, A; da Silva, V. D; Rotter, A; Petrovski, Z; Almeida, PL; Lourenço, ND; Gaudêncio, SP. (2022). Marine Environmental plastic pollution: Mitigation by microorganism degradation and recycling valorization. *Front. Mar. Sci*. 7: 567126.

Peng, R. T; Xia, M. L; Ru, J. K; Huo, Y. X; Yang, Y. (2018). Microbial degradation of polyurethane plastics. *Chin. J. Biotechnol.* 34: 1398–1409.

Philip, S; Keshavarz, T; and Roy, I. (2007) Polyhydroxyalkanoates: Biodegradable polymers with a range of applications. *J. Chem. Technol. Biotechnol*. 82: 233–247.

Reis, M; Albuquerque, M; Villano, M; Majone, M. (2011). *Mixed Culture Processes for Polyhydroxyalkanoate Production from Agro-Industrial surplus/waste as feedstocks*, 2nd ed; Elsevier B.V.Amsterdam, The Netherlands.

Ru, J; Huo, Y; Yang, Y. (2020). Microbial Degradation and Valorization of Plastic Wastes. *Front. Microbiol.* 11:442. doi: 10.3389/fmicb.2020.00442

Sayyed, R. Z; Gangurde, N. S; and Chincholkar, SB. (2009). Hypochlorite digestion method for efficient

recovery of PHB from *Alcaligenes faecalis*. *Indian J Microbiol,* 49, 230–232. Doi: 10.1007/s12088-009-0036-7.

Shamala, TR; Chandrashekar, A; Vijayendra, SVN, Kshama, L. (2003). Identification of polyhydroxyalkanoates (PHA) producing *Bacillus spp*. using the polymerase chain reaction (PCR) *J Appl. Microbiol*. 94:369-374.

Stavroula, K; Simos, M; Joanne, HK. (2020). Polyhydroxyalkanoates (PHAs) from household food waste: research over the last decade. *Intl L Biotechnol Bioeng*. 6:26-36.

Wu, Q; Wang, Y; Chen, GQ. (2009). Medical application of microbial bio-polyesters polyhydroxyalkanoates. *Artif Cells Blood Substit Immobil Biotechnol*. 37:1-12.