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# Isolation, Screening, and Characterization of PHA-Producing Bacteria from Dumpsite Fadama Soil

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**ABSTRACT**

The growing environmental burden caused by persistent petroleum-based plastics has intensified the search for biodegradable alternatives such as polyhydroxyalkanoates, which are microbially synthesized biopolymers with promising industrial applications. This study focused on isolating and screening efficient polyhydroxyalkanoate-producing bacteria from dumpsite fadama soil and evaluating their potential to accumulate bioplastics. Bacteria were isolated from soil samples and screened for PHA accumulation using Sudan Black B and Nile Blue staining and UV fluorescence microscopy. The competent isolates were subjected to morphological, biochemical, and molecular 16S rRNA) identification. A total of 112 isolates were recovered, of which 59 showed positive Sudan Black B staining. Subsequent Nile Blue screening identified eight potential producers, and three isolates demonstrated strong fluorescence, indicating high intracellular polymer accumulation. Molecular identification using partial 16S ribosomal RNA sequencing revealed the isolates as *Shigella flexneri SMI1, Priestia megaterium SMI2*, *and Bacillus subtilis SMI3*. The sequences were deposited in GenBank under accession numbers PX567900, PX567901, and PX567902, respectively. These species, particularly *Bacillus subtilis* and *Priestia megaterium*, exhibited strong traits associated with polyhydroxyalkanoate biosynthesis and accumulation. The findings highlight dumpsite fadama soil as a rich source of metabolically versatile microorganisms with the capacity for efficient intracellular biopolymer biosynthesis. This work provides a foundation for further optimization of culture conditions and bioprocess scale-up to support sustainable bioplastic production.



## 1. INTRODUCTION

The global proliferation of recalcitrant petroleum-derived plastics has intensified the demand for biodegradable and bio-based alternatives that can integrate into existing material streams without contributing to long-term environmental persistence. [1]. Polyhydroxyalkanoates (PHAs) are a diverse family of intracellular microbial polyesters synthesized mainly under unbalanced growth conditions, typically when cells experience macronutrient limitation in the presence of excess carbon [2], [3]. These biopolymers are structurally categorized into short-chain-length and medium-chain-length subgroups, whose monomeric variation influences crystallinity, tensile strength, and degradation kinetics [4]. Their physicochemical versatility, along with demonstrated biocompatibility, positions PHAs as leading candidates for next-generation sustainable materials, with applications spanning biomedical implants, packaging, controlled release systems, and agricultural films [2], [5].

The urgency of this shift is underscored by the scale of the underlying waste problem: petroleum-derived plastics continue to accumulate in terrestrial and aquatic environments faster than existing waste-management infrastructure can process them, a

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pressure felt acutely in rapidly urbanising regions of sub-Saharan Africa where formal recycling capacity remains limited [1]. Bio-based alternatives such as PHAs are therefore attractive not only for their end-of-life biodegradability but also because their production can, in principle, be decoupled from petrochemical feedstocks entirely, aligning with circular-economy and green-chemistry policy goals now being adopted across multiple jurisdictions [6]. Realising this potential at scale nevertheless depends on identifying robust, locally available microbial chassis capable of accumulating PHAs efficiently under low-cost cultivation conditions, which is the motivation for the bioprospecting approach adopted in this study.

Despite their promise, PHA commercialization remains constrained by high production costs, largely due to substrate expense and low carbon-to-polymer conversion yields in conventional processes [2], [6]. Consequently, recent bioprocess advances have shifted toward the valorization of lignocellulosic and agro-industrial residues, which provide complex carbohydrate streams and micronutrients capable of supporting microbial metabolism without extensive pre-treatment [7], [8]. Feedstocks such as sugarcane bagasse, wheat bran, and banana peel offer high fermentable sugar content and reduced carbon costs, making them increasingly relevant for PHA biorefineries aligned with circular bioeconomy models [7], [9].

The search for novel microbial factories capable of efficiently converting heterogeneous waste-derived substrates into PHAs has intensified [7], [9]. Fadama soils, which are periodically flooded, nutrient-rich hydromorphic systems, represent reservoirs of taxonomically diverse bacteria adapted to fluctuating carbon and moisture conditions [10], [11]. The periodic flooding and drying cycles create alternating aerobic and anaerobic conditions, coupled with pulses of organic matter decomposition. Such environmental stress favours microorganisms that can store carbon and energy as intracellular polyesters (PHAs), which serve as reserves during nutrient limitation or anoxia [12].

Therefore, fadama soils—especially those impacted by organic waste (e.g., dumpsite fadama soil used in this study)—are promising habitats for isolating PHA-accumulating bacteria with enhanced metabolic plasticity. Systematic isolation and screening of microbial populations from these soils may reveal strains with improved carbon assimilation profiles, robust PHA synthase systems, and tolerance to lignocellulosic hydrolysates. Therefore, this study isolates and characterizes PHA-producing bacteria from dumpsite fadama soil in Kano, Nigeria, using a multi-stage screening approach (Sudan Black B, Nile Blue A, and fluorescence microscopy) followed by 16S rRNA-based molecular identification of native strains with high intracellular polymer accumulation, thereby contributing to the global database of microbial resources for sustainable bioplastic production.

## 2. MATERIALS AND METHODS

### *2.1 Sample Collection and Bacteria Isolation*

Soil samples were collected from three dumpsite fadama locations in Kano State, Nigeria: Bayero University Kano (BUK) New Site, BUK Old Site, and the Naibawa area. Samples were taken from a depth of 1–5 inches, passed through a 2 mm mesh sieve to remove debris, and transferred into sterile polythene bags. All samples were transported immediately to the Microbiology Laboratory of Bayero University, Kano, for processing.

For bacterial isolation, one gram (1 g) of each soil sample was suspended in 9 mL of sterile distilled water and mixed thoroughly to form the stock suspension. A ten-fold serial dilution was carried out using sterile 0.85% NaCl solution. From the $10^{-4}$ and $10^{-5}$ dilutions, 0.1 mL aliquots were aseptically spread onto Nutrient Agar plates. The plates were incubated at 35°C for 48 hours. Distinct colonies were selected based on morphological differences and purified through repeated streaking until pure isolates were obtained. The purified bacterial isolates were maintained on Nutrient Agar slants and stored at 4°C for further analyses.

### *2.2 Screening for PHA-Producing Bacteria*

*a) Sudan Stain Screening*

Bacterial isolates were screened for intracellular PHA accumulation using Sudan Black B staining. A 0.02% (w/v) alcoholic solution of Sudan Black B was poured onto bacterial colonies on Nutrient Agar plates and left for 30 minutes. The excess stain was decanted, and plates were gently washed with 100% ethanol. Colonies containing lipophilic PHA granules appeared bluish-black, whereas non-accumulators appeared white [13], [14].

*b) Nile Blue Screening*

Sudan Black B-positive isolates were further screened using Nile Blue A. The dye was added to nutrient agar at a final concentration of 0.5 mg/L before solidification. After incubation, the same plates were examined directly under UV light (365 nm). Colonies that exhibited intense orange fluorescence were recorded as PHA accumulators [15].

### *2.3 Identification of PHA-Producing Isolates*

*a) Morphological and Biochemical Identification*

Colony morphology (colour, texture, elevation, edge, pigment) was recorded. Gram staining was performed on smears using crystal violet, Lugol's iodine, 70% ethanol decolouriser, and safranin counterstain, then examined under oil immersion [16]. Biochemical tests (catalase, oxidase, citrate utilisation, indole, Voges-Proskauer, methyl red, urease, and carbohydrate fermentation with glucose, sucrose, lactose, maltose, xylose) were conducted following standard protocols [16], [17].

*b) Molecular Identification*

Genomic DNA was extracted from the bacterial isolates using the GenElute™ bacterial genomic DNA isolation kit (Sigma-Aldrich®, Shanghai, China). The purity and concentration of the extracted DNA were determined spectrophotometrically at 260 and 280 nm according to the method of Fritsch and Maniatis [18], employing a UV-1800 Shimadzu Spectrophotometer (Kyoto, Japan). DNA integrity was further assessed through electrophoresis on a 0.8% agarose gel.

PCR amplification of the 16S rRNA gene was performed using universal primers 27F and 1492R in a 20 µL reaction mixture containing 1× ReadyMix™ Taq PCR master mix (Sigma®, Bangalore, India), 10 pmol of each primer, approximately 10 ng of DNA template, and nuclease-free water. The amplification protocol consisted of an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of

denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes [19], [20]. PCR products were visualized on 1% agarose gel stained with ethidium bromide and documented using a Bio-Rad® gel imaging system (Hercules, CA, USA).

Amplicons were purified (GenElute™ PCR clean-up kit) and sequenced by Eurofins Genomics India Pvt. Ltd. (Bangalore, India). Sequences were analysed with Sequencing Analysis Software v5.4 (Applied Biosystems®, USA) and BioEdit v7.2.5. BLAST searches against NCBI GenBank identified closest relatives. Representative sequences were deposited in GenBank (accession numbers **to be verified**). Phylogenetic trees were constructed using the neighbour-joining method with the Maximum Composite Likelihood model in MEGA 11.0 [21], [22].

*2.4 Production of PHB by Selected Isolates Using Mineral Salt Medium*

Polyhydroxybutyrate (PHB) production was carried out in mineral salt medium (per L): urea, yeast extract, $KH_2PO_4$, $Na_2HPO_4$, $MgSO_4 \cdot 7H_2O$, $CaCl_2$, glucose, and trace element solution ($ZnSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, $H_3BO_3$) [23]. Glucose and trace elements were filter-sterilised separately. One hundred mL of medium was inoculated with 2 mL of standardized bacterial culture ($OD_{600}$ ~0.5) and incubated at 37°C on a rotary shaker at 150 rpm for 72 hours [23].

***Biomass harvest:*** After 72 hours (not 48 h; corrected), cells were centrifuged at 10,000 × g for 20 minutes. The pellet was freeze-dried (−40°C) to obtain dry cell mass.

***PHB extraction:*** Dried biomass was mixed with equal volumes of 6% (w/v) sodium hypochlorite (NaOCl) and chloroform ($CHCl_3$), then heated at 80°C for 1 hour. After centrifugation, the lower chloroform layer was collected and filtered. PHB was precipitated by adding cold methanol (1:10 methanol: chloroform ratio, v/v). The precipitated polymer was dried at 60°C and weighed [9].. All experiments were performed in triplicate

## 3. RESULTS

*3.1 Isolation and Screening Results*

From the 112 bacterial isolates obtained from dumpsite fadama soil, 59 isolates (52.67%) showed visible intracellular granules by Sudan Black B staining. Further screening with Nile Blue A reduced the number to eight isolates (13.56% of the original; 13.56% of the Sudan Black B-positive group). Final confirmation under UV fluorescence (365 nm) identified three isolates (SMI1, SMI2, SMI3) representing 37.5% of the Nile Blue-positive group. These three isolates exhibited intense orange fluorescence, indicating high intracellular polymer content and strong metabolic capacity for PHA synthesis.

**Table 1.** Screening of PHB-Producing Bacterial Isolates from Fadama Soil

| Screening Stage | Method Used | No. of Positive Isolates (%) | Observation |
|---|---|---|---|
| **Initial Isolation** | - | 112 | Total bacterial isolates obtained |
| **Primary Screening** | Sudan Black B Staining | 59 (52.67) | Bluish-black colonies (indicating PHB granules) |
| **Secondary Screening** | Nile Blue Staining | 8 (13.56) | Positive for PHB granules |
| **Fluorescence Test** | UV Light Examination | 3 (37.50) | Colonies exhibiting fluorescence |

*Percentages for primary and secondary screening are relative to the previous stage (initial isolation count for primary; primary positive count for secondary; secondary positive count for UV test). The 37.5% represents 3 out of 8 Nile Blue-positive isolates.*

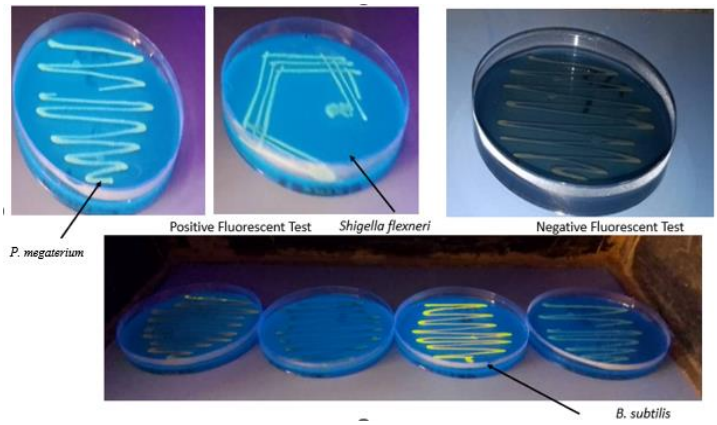


Plate 1: Qualitative detection of PHB granules in *B. megaterum*, *Shigella flexneri*, and *B. subtilis* isolates via positive fluorescent staining.

*3.2 Identification of PHB-Producing Bacteria*

*3.2.1 Biochemical and Morphological Identification*

Table 2 presents the biochemical characteristics. Isolates SMI2 and SMI3 were Gram-positive rods and catalase-positive. SMI2 was oxidase-negative but positive for starch hydrolysis, citrate utilisation, urease, and methyl red (MR). SMI3 was oxidase-positive and also positive for starch hydrolysis, citrate utilisation, urease, and MR. Isolate SMI1 was Gram-negative and exhibited a different profile (Table 2).

**Table 2.** Biochemical and Morphological Characteristics of the Isolates

| Biochemical Test | Isolates | | |
|---|---|---|---|
| | **NS1** | **NB1** | **NB2** |
| Cellular Morphology | **Rod** | **Rod** | **Rod** |
| Gram-reaction | - | + | + |
| Methyl Red | + | + | + |
| Catalase | + | + | + |
| Oxidase | - | - | + |
| Voges-Proskauer | - | - | - |
| Indole Production | - | - | - |
| Citrate Utilization | - | + | + |
| Starch Utilization | - | + | + |
| Urea Utilization | + | + | + |
| Organism | *Shigella* sp | *Priestia megatarium* | *Bacillus sp* |

Key: +: Positive
- : Negative

*3.2.2 Molecular Identification*

The molecular identification of the isolates was performed using partial 16S rRNA sequencing, yielding approximately 1500 base pairs per isolate. BLAST analysis against the NCBI GenBank database revealed high sequence similarity to known bacterial species. Isolate NS1 showed 98.4 percent identity with *Shigella flexneri* strain MMS08, isolate NB1 showed 99.47 percent similarity to *Priestia megaterium* strain BMJBN02 (Fig. 3), and isolate NB2 exhibited 98.2 percent identity with *Bacillus subtilis* strain AaR113. Phylogenetic analysis using the Neighbor-Joining method further supported these affiliations, with each isolate clustering firmly within its respective clade and receiving strong bootstrap support values above 95 percent (Fig. 2-4). The nucleotide sequences obtained from the three isolates were subsequently submitted to the GenBank repository to ensure public accessibility and future comparative analyses. These sequences are available under the accession numbers PX567900, PX567901, and PX567902 respectively

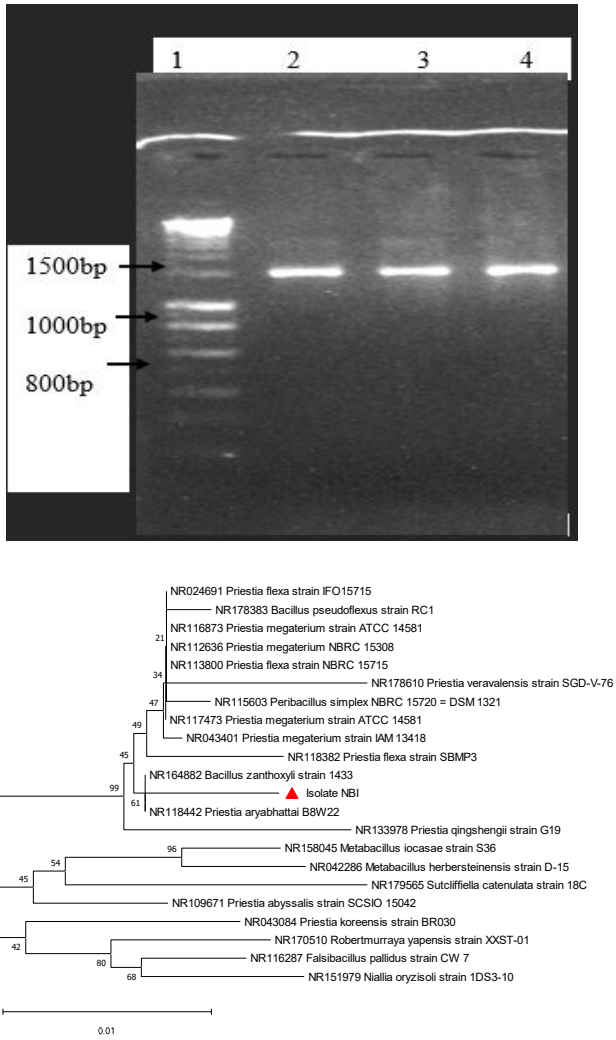


**Fig. 1.** Phylogram (neighbour-joining method) indicating the genetic relationship between Isolate NB1 and 1referenced related microorganisms based on the 16S rRNA gene sequence analysis. Accession numbers are accompanied by the specie name of their 16S rRNA sequences.

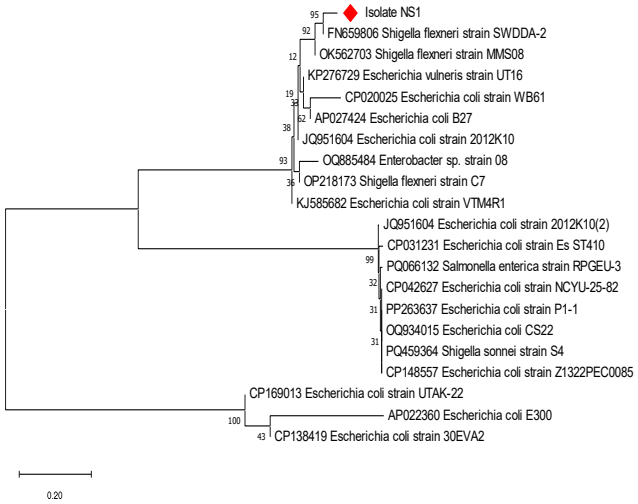


**Fig. 2.** Phylogram (neighbour-joining method) indicating the genetic relationship between Isolate NS1 and referenced related microorganisms based on the 16S rRNA gene sequence analysis. Accession numbers are accompanied by the specie name of their 16S rRNA sequences.

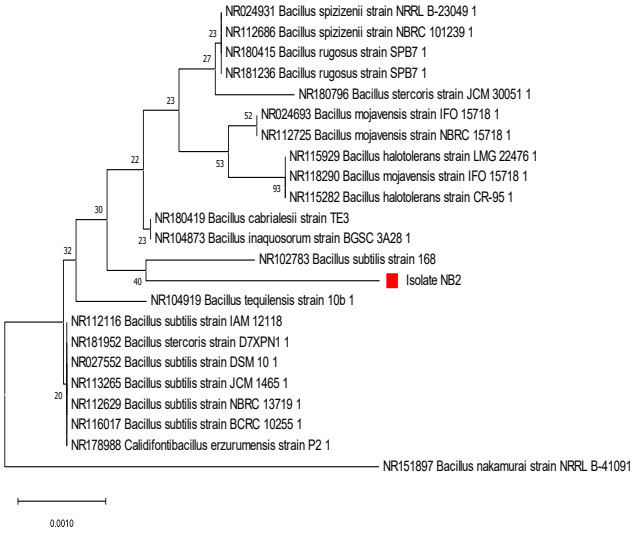


**Fig. 3.** Phylogram (neighbour-joining method) indicating the genetic relationship between Isolate NB2 and referenced related microorganisms based on the 16S rRNA gene sequence analysis. Accession numbers are accompanied by the specie name of their 16S rRNA sequences.

*3.3 Preliminary PHB Production in Mineral Salt Media*

Fig. 5 presents preliminary PHB production using the 3 candidate isolates in MSM liquid media. Among the three isolates, *Bacillus subtilis* yielded the highest PHA concentration at 145 mg/L, followed by *Priestia megaterium* with 133 mg/L, while *Shigella spp*. produced 112 mg/L. The observed yield pattern is *B. subtilis* > *P. megaterium* > *Shigella spp.*

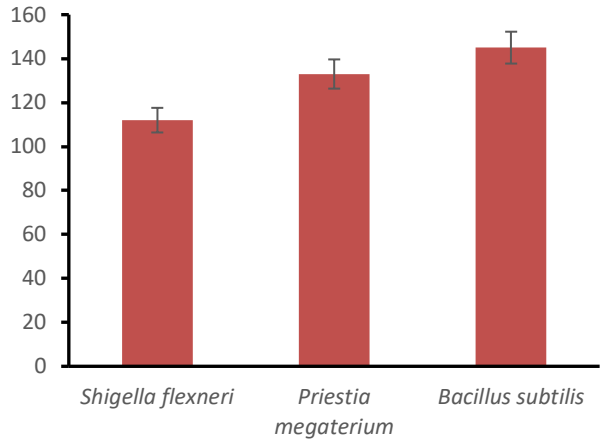


**Fig.** 4. Quantitative assessment of PHB concentration (mg/L) and biomass (g/L) for isolates SMI1 (Shigella flexneri), SMI2 (*Priestia* megaterium), and SMI3 (Bacillus subtilis). Bars represent mean ± SD (n=3).

## 4. DISCUSSION

In this study, many of the isolates obtained from the dumpsite fadama soil were initially found to be capable of accumulating PHB, with 59 out of 112 testing positive using Sudan Black B staining. However, further screening using Nile Blue A and fluorescence microscopy reduced this number to only three strong accumulators (SMI1, SMI2, and SMI3). This progressive reduction highlights the importance of using more specific screening techniques, as Sudan Black B can stain other intracellular lipids in addition to PHAs, whereas Nile Blue A provides greater specificity for polyhydroxyalkanoates. This agrees with previous reports that natural environments rich in organic matter often harbour PHB-producing microorganisms [24], [25]. However, the observed variation in PHB accumulation among isolates suggests significant metabolic diversity. Similarly, Bhuwal et al. [26] reported that although many isolates may test positive with Sudan Black B, only a small proportion are true high-level PHA producers, highlighting the need for confirmatory screening methods. The variation observed among the isolates in this study further suggests underlying metabolic diversity in PHA accumulation capacity.

The occurrence of PHB-producing bacteria in dumpsite fadama soil can be explained by the fluctuating environmental conditions, including periodic flooding, high organic load, and nutrient variability, which favour microorganisms capable of storing carbon as intracellular polymers. In addition, the dumpsite environment likely introduces increased microbial competition and substrate availability, further enriching for PHA-accumulating phenotypes [27].

Biochemical characterisation showed that SMI3 (*Bacillus subtilis*) and SMI2 (*Priestia megaterium*) were Gram-positive and exhibited positive reactions for catalase, starch hydrolysis, citrate utilisation, urease, and methyl red tests. These characteristics are consistent with the known metabolic versatility of these organisms and support their established roles in biopolymer production [9], [27] [28]. In contrast, SMI1 (*Shigella flexneri*) was Gram-negative and showed a different biochemical profile, including negative reactions for citrate utilisation and starch hydrolysis. Despite this, the isolate demonstrated the ability to accumulate PHA, suggesting the possibility of a less characterised or alternative biosynthetic pathway [29], [30].

Molecular identification confirmed that the three most efficient PHB producers were Shigella flexneri (98.4% similarity), Priestia megaterium (99.47% similarity), and Bacillus subtilis (98.2% similarity). The identification of *Bacillus subtilis* and *Priestia megaterium* aligns with previous reports that Gram-positive bacteria, particularly *Bacillus spp.*, are among the most efficient PHB producers due to their robust metabolic networks [7], [9], [31]. According to [32], bacteria of the Bacillus genus produce class IV PHB synthase, which plays a key role in PHB biosynthesis, further validating the identification of *Bacillus subtilis* as a promising candidate. *Priestia megaterium*, which was formerly classified within Bacillus, shares many of these metabolic traits and has also been reported to synthesize intracellular polyhydroxybutyrate (PHB), a common type of PHB [2], [13].

The detection of PHA accumulation in *Shigella flexneri* is noteworthy, as this organism is primarily known as an enteric pathogen associated with bacillary dysentery. Although a few studies have reported PHA accumulation in *Shigella* under stress conditions [29], the genetic basis of this ability remains unclear. This study did not investigate the genetic mechanisms involved, so the suggestion that PHA biosynthesis genes may have been acquired through horizontal gene transfer remains speculative. Further studies involving whole-genome sequencing and comparative genomic analysis would be necessary to clarify this. Given its pathogenic nature, the use of *S. flexneri* in industrial PHA production is not advisable without extensive safety considerations, including attenuation and strict containment. This finding is mainly of scientific interest and highlights the metabolic adaptability of this organism rather than its suitability for industrial application.

The preliminary PHA yields obtained under non-optimised conditions were relatively low, with *B. subtilis* (SMI3) producing 145 mg/L, *P. megaterium* (SMI2) 133 mg/L, and *S. flexneri* (SMI1) 112 mg/L. Although these values are below industrial production levels, they are comparable to results reported in initial screening studies using wild-type isolates grown in mineral salt media. PHA yield could be improved by optimising the carbon-to-nitrogen ratio, adopting fed-batch fermentation systems, and applying statistical tools such as response surface methodology. In addition, the use of low-cost agro-industrial substrates and metabolic engineering approaches may further enhance production efficiency [8] [5].

One limitation of this study is the lack of further purification and characterization of the extracted PHA. The sodium hypochlorite–chloroform extraction method used may co-extract other cellular components such as lipids and proteins. As a result, the reported yields should therefore be regarded as crude polymer estimates. Future work should include detailed

polymer characterization using techniques such as FTIR, NMR, or GC–MS to confirm composition, purity, and material properties.

Beyond providing crude yield estimates, the findings reported here reinforce the broader case for treating waste-impacted fadama soils as an underexploited bioprospecting resource within a circular bioeconomy framework. Because these isolates were recovered from soil already burdened by anthropogenic organic waste, their native metabolism is plausibly pre-adapted to the heterogeneous, nutrient-fluctuating substrates typical of agro-industrial residues such as sugarcane bagasse and wheat bran [7], [9]. This pre-adaptation is commercially relevant, since substrate cost remains the single largest barrier to competitive PHA production, and strains capable of efficient conversion of low-cost lignocellulosic streams are more readily integrated into biorefinery pipelines than laboratory-adapted type strains [2], [8]. Consequently, the isolates characterised in this study, particularly Bacillus subtilis and Priestia megaterium, warrant evaluation on locally available agro-industrial feedstocks rather than glucose-based mineral salt medium alone, as this would provide a more realistic assessment of their industrial potential.

The comparative yield ranking observed among the three isolates (B. subtilis > P. megaterium > Shigella spp.) is consistent with the wider literature positioning Bacillus-related genera as among the most metabolically robust PHA accumulators recovered from environmental samples [7], [9], [31]. Nevertheless, the absolute yields obtained here remain modest relative to optimised bioreactor systems, underscoring that these results should be read as a baseline screening outcome rather than a final assessment of production capacity. Strain improvement strategies such as mutagenesis, adaptive laboratory evolution, and co-culture with complementary hydrolytic organisms could further enhance carbon flux toward polymer accumulation [12], [27]. Taken together, these findings support continued screening of dumpsite-impacted fadama soils across other locations in northern Nigeria, both to expand the pool of candidate PHA producers and to strengthen the region's contribution to the global inventory of microbial resources for sustainable bioplastic production.

From an applied standpoint, the pathogenicity of Shigella flexneri does not preclude its usefulness as a discovery tool. Characterising the PHA synthase machinery of an atypical accumulator can reveal alternative genetic architectures that may later be transferred into non-pathogenic chassis strains such as Bacillus subtilis or well-characterised Escherichia coli derivatives [29], [30]. Framed this way, SMI1 is best regarded as a source of novel biosynthetic information rather than as a candidate production organism, consistent with the safety caveats already raised in this study. Overall, the isolation, screening, and preliminary production data reported here position dumpsite fadama soil as a tractable, low-cost sampling environment for future PHA bioprospecting efforts across the wider West African sub-region.

An additional, often overlooked, dimension of this work is the dual character of dumpsite fadama soil as both a bioprospecting reservoir and a site of public-health relevance. The same nutrient-rich, periodically flooded conditions that favour PHA-accumulating organisms such as Bacillus subtilis and Priestia megaterium also support enteric organisms of clinical concern, as reflected in the recovery of Shigella flexneri alongside the two Gram-positive producers [10], [29]. Environmental surveillance programmes targeting dumpsite fadama soils could therefore serve a dual purpose, simultaneously supporting bioplastic feedstock discovery and providing early warning of enteric pathogen carriage in peri-urban waste-impacted land, an angle not typically considered in conventional PHA bioprospecting studies.

Moving from the shake-flask mineral salt medium trials reported here toward pilot-scale validation will require attention to process parameters beyond strain selection alone. Aeration and dissolved-oxygen control, feeding strategy during the polymer-accumulation phase, and downstream recovery efficiency all materially affect volumetric productivity in bioreactor-scale PHA fermentations [8], [27]. Given that Bacillus subtilis (SMI3) produced the highest preliminary yield among the isolates tested, it represents the most immediate candidate for scale-up trials, while Priestia megaterium (SMI2) and Shigella flexneri (SMI1) remain valuable for comparative and mechanistic follow-up work respectively [9].

The three genera recovered in this study, Bacillus, Priestia, and Shigella, also illustrate the taxonomic breadth that can be captured through a straightforward Sudan Black B–Nile Blue A–UV fluorescence screening cascade, without recourse to more expensive flow-cytometric or transcriptomic pre-selection methods. This is a practically important observation for laboratories in resource-limited settings, where access to advanced screening infrastructure is often the binding constraint on bioprospecting throughput rather than the availability of suitable environmental samples [13], [14]. The relatively low cost and reagent burden of the cascade used here suggest it could be adopted more widely across similar dumpsite and fadama environments in the region as a first-pass filter, reserving molecular confirmation for the smaller, pre-screened subset of isolates that pass fluorescence-based selection.

Finally, the phylogenetic placements obtained for SMI1, SMI2, and SMI3, each supported by bootstrap values exceeding 95 percent, provide a reasonably high-confidence taxonomic anchor for any follow-up genomic or fermentation work built on these strains. Depositing the corresponding 16S rRNA sequences in GenBank under accession numbers PX567900 to PX567902 also allows other groups working on fadama or comparably disturbed soils elsewhere in Nigeria and the wider Sahel to cross-reference newly isolated strains against these reference sequences, supporting cumulative, comparable bioprospecting efforts rather than isolated, non-reproducible screening exercises.

Methodologically, the stepwise narrowing from 112 initial isolates to three confirmed high-level accumulators also illustrates why single-assay screening is insufficient for bioprospecting studies of this kind. Sudan Black B is a useful, low-cost primary filter, but its sensitivity to other intracellular lipid inclusions means it will always overestimate true PHA-accumulator counts; pairing it with Nile Blue A fluorescence and, ultimately, molecular confirmation is what allowed this study to arrive at a small, high-confidence isolate set suitable for downstream production trials. Future bioprospecting efforts drawing on this workflow could shorten the pipeline by moving directly to Nile Blue A or flow-cytometry-based fluorescence

screening for larger sample sets, reserving Sudan Black B for rapid field-level triage.

Finally, the strong bootstrap support recovered for all three isolates in the neighbour-joining phylogenies adds confidence to the species-level assignments reported here and provides a reliable basis for future comparative genomic work, including whole-genome sequencing aimed at resolving the PHA synthase gene content of SMI1 in particular. Depositing the corresponding 16S rRNA sequences in GenBank ensures that these strains, and the taxonomic assignments built upon them, remain available for verification and reuse by other groups working on PHA bioprospecting from underexplored soil environments.

## 5. Conclusion

This study demonstrates that dumpsite fadama soil serves as a fertile reservoir for polyhydroxybutyrate (PHB) producing bacteria, yielding potent isolates such as Bacillus subtilis and Priestia megaterium. The unexpected detection of PHB accumulation in Shigella flexneri highlights significant metabolic plasticity within this genus; however, the absence of direct evidence for horizontal gene transfer necessitates future genomic exploration to resolve the pathway's evolutionary origin. Given its pathogenic nature, the industrial utilisation of S. flexneri is not recommended without appropriate containment or attenuation. Future optimisation should also address carbon-to-nitrogen ratios and polymer purity confirmation. Ultimately, the transition to optimised fed-batch systems and the valorisation of agro-industrial residues as carbon sources will be critical in translating these wild-type performances into sustainable, large-scale bioplastic production.

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