# *Original Research Article*

# Geneticand phenotypic characterization of Polyhydroxyalkanoate producing bacterial isolates from hypersaline water body, Atlantic Ocean, Nigeria

**Abstract**

**Aim:**To compare bacteria identification methods of phenotypic and genetic identification for marine sourced effective polyhydroxylalkanoate (PHA) production.

**Sturdy design:**Randomised design was employed for PHA producing bacterial isolates.

**Place and duration of sturdy:**Samples containing PHA-producing bacteria from the hypersaline water body of Nigerian Southern Atlantic Ocean ($6.35^{O}$ N $3.28^{O}$ E -St1; $6.35^{O}$ N $3.40^{O}$ E -St2; and $6.36^{O}$ N $3.47^{O}$ E -St3); water depths (0.07m (top), 50m (middle) and 100m (bottom) were collected and investigated between August, 2016-September, 2019.

**Methodology:** Bacterial isolates was characterized using standard microbiological biochemical tests for the phenotypes and 16S rDNA for the genotypes. Isolates were also screened for PHA potentials using specific primers.

**Results:**Genetically identified *Bacillus cereus strains LB17* and *Bacillus paramycoides SA27* were phenotypically identified as *Bacillus badius*. Similarly, *Acinetobacter calcoaceticus JL11,* five strains of *Alcaligenes faecalis*, seven strains of *Bacillus spp.*, *Enterobacter cloacae*, *Falsochrobactrumovis*, *Ochrobactrumciceri*, *Providencia stuartii including* two species of *Pseudomonas* and *Bordetella trematum*were all phenotypically identified as *Corynebacterium kutsceri*. Invariably, *Alcaligenes faecalis* (strains *PSD10* and DEP8), *Pseudomonas aeruginosa* (*H47921)* and *Vagococcusfluvialis* (*AWW1)* were identified as *Staphylococcus species*. All 34 bacterial isolates expressed *phaC* genes for PHA potential, while *Alcaligenes faecalis strains* and *Bacillus humi (NBPP9)* expressed *phaC1* for short PHA chain.

**Conclusion:** Variations were recorded in the genetical identity of phenotypes; *Corynebacterium kutsceri*, *Enterobacter amnigenus*, *Micrococcus luteus*, *Micrococcus varians*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. while, synchronization recorded in the genotypic and phenotypic identity of bacillus species. Varietal differences of PHA potential from the same species are also seen. It is therefore pertinent to rely on the genetic identification of bacteria for the effective determination of PHA-producing character as displayed in *Bacillus* spp.

**Keywords:**16S rDNA, bacteria-phenotype, hypersaline-water, Polyhydroxyalkanoates, Nigeria.

## Introduction

Polyhydroxyalkanoates (PHAs) are produced in nature through the fermentation of sugar or lipids. Living cells may store them as carbon or expend as utilized energy, they are alsobiocompatible and completely biodegradable (Albuquerque & Malafaia, 2018; Brigham*et al*., 2011; Sun, 2018). PHAs exhibitbio-plasticity, thermo-plasticity or elastomer material characters with a temperature range between 140°C to 180°C(Bugnicourt*et al.,*2014). There are over 150 monomers with the potential to combine within this family that results to materials of extremely different properties(Bugnicourt *et al*., 2014; Dhandayuthapani Iet al., 2011; Valappil *et al*., 2007).However, organisms such as bacteria are specific to the type of PHA they synthesize(Hawkins*et al.,* 2021; Jendrossek & Pfeiffer, 2014; Steinbüchel & Lütke-Eversloh, 2003).

The preference for bioplastic over synthetic plastics ishinged on the fact that the latter is non-biodegradable with disposal methods of burning which results in air pollution through the emission of toxic fumes that are inimical to human and animal health. Consequently, this action results in the disruption of the ecosystems and eventual global warming(Bagheriasl, 2013). However, biopolymers(PHA) will not contribute to environmental pollution at the end of their use when recycled due to their elastomeric property (Margesin & Schinner, 2001; Sriroth & Sangseethong, 2005; Numata*et al.,* 2009).These characteristics make them superior to synthetic plastics. Thus, the present extensiveuse of plastics today is based on their durability which is pivoted on their thermal and mechanical properties. Plastics are malleable at certain temperatures and so are rapidly replacing metals, glass and wood in various engineering applications(Nawrath*et al.,*1994). PHAs are of immense importance in the applications of medicine, engineering, agriculture, telecommunications, transportation and household utilities(Aditi *et al*., 2015; Zhang *et al*., 2015; Manikandan & Senthilkumar, 2017).

The categories of PHAs are Short Chain Length (SCL)3-5 carbons (R)-hydroxyalkanoates and characterised by their high degree of crystallization and stiff crystals thermoplastic(Koller*et al*., 2013; Wu *et al*., 2003); Medium Chain Length (MCL)are 6-14 carbon polyhydroxylalkanoateswith low degree of crystallization, hydrophobicity, low melting temperature and biodegradable(Ashby*et al.,* 2002; Solaiman*et al.,* 2006); Short Chain Length copolymers with medium chain length (SCL-MCL - Copolymers)are 4 to 14-carbon monomers, and characterized by a range of physical properties that are based on the percentage molar composition of monomers that make up the polymer. Hence copolymers having a low percentage of monomers of SCL are more elastomeric(Greene*et al.,* 2002; Gumel *et al.,* 2012; McChalicher & Srienc, 2007; Tsuge *et al*., 2005). There are other complex categories PHA that differ from the aforementioned such as low chain length-medium chain length PHAs (LCL-MCLPHA), LCL-PHAs (copolymersproduced by *Pseudomonas aeruginosa*) of,SCL-PHAs, copolymers of SCL-MCL and SCL-LCL-PHAs(Saranya*et al.,* 2017).

The survival andoptimal growth of halophilic bacteria in their environments necessitate the production of one or more PHAs, enzymes, metabolites, exo-polysaccharides, endo-polysaccharides, pigments and compatible solutes(Jay*et al.,* 2008; Rothschild & Mancinelli, 2001; Krulwich*et al.,* 2011; Mesbah & Wiegel, 2012) of which their products may be of high commercial value. Also, the bio-molecules produced by halophilic bacteria are stable (Oren, 2013); therefore, their metabolites exhibit significant potential in the needs or actual use by different industries such as agriculture, cosmetic, chemical, environmental, pharmaceutical, etc.Unfortunately, there have been reported cases of wrong or miss-identification of bacteria obtained from the extreme environment (Abdulaziz, 2013; Bryan-Thomas*et al.,* 2018; Sharaha *et al*., 2019), of which is noticed to be associated with their peculiar isolation requirement as well as their genetic response to their environment. In recent times man has improved in his genetic technological skills as compared with the former traditionally (phenotypic) identification methods. However, this research is charged with the characterisation of PHA-producing bacteria into their proper nomenclature by comparing the genetic relatedness and phenotypic (biochemical) techniques. Therefore, the expected results will help to determine and ease the search for environmentally-compatible alternatives to petrochemical-based plastics in extreme hypersaline environments.

## Methodology

### Sample collection

Water samples from Lagos State, Nigeria marine water bodies were collected aseptically by modified sampling methods(Bugnicourt *et al*., 2014). This was done with the aid of a water sampler, andat three different depths of the surface of the water (0-7cm), middle depth(50M) and bottom depth(100M) below the water surface. The points of the water collections wereobtained and recorded from the Geographic Positioning System (GPS) as locations of (1) latitude 6.35$^{O}$, longitude 3.28$^{O}$; (2) latitude 6.35$^{O}$, longitude 3.40$^{O}$; and (3) latitude 6.36$^{O}$, longitude 3.47$^{O}$. The aseptically collected water was transferred into a 1-litre plastic container; the temperature was taken insitubefore storage for transportation in a cooled box to the laboratory for further laboratory analysis.

### Culture and isolation of bacteria

The Plate Count Agar (PCA) medium was prepared according to the manufacturer's directions (Lab M) and poured into Petri plates, allowed to solidify whilethe sampled water was serially diluted from $10^{-1}$ to $10^{-7}$ and then cultured by spread plate method on the prepared agar plates; incubated at 35 °C within 24-48 hour.

**Enumeration and isolation of pure cultures from samples**

The standard enumeration method was employed for the determination of microbial load. Bacteria with the same cultural characteristics were isolated and cultured on PCA by considering the following parameters of each growth colony which are; margin, smooth or entire of the surface, wavy, lobate, irregular, ciliate, branching, woolly, threadlike, 'hair look-like, elevations, flat, raised, convex, drop like umbonate, in-growing into the medium.

**Phenotypic identification**

**Identification of Bacterial isolates**

The phenotypic identification was carried out according to the methods of Bargey's Manual of Determinative Bacteriology(Bergey*et al.,* 1939)which includes;

**Cultural characterization:** This was determined by a process known as plate reading by visual observation of a standard chartand observation withX10 hand lens on isolate culture plate to determine colony shape.

**Cellular characterization**:The cellular characterization was carried out by microscopy of simple Gram's stain preparations (Carter & Cole Jr, 2012)to determine the cellular structures and arrangements.

**Biochemical tests:** these were carried out by spore staining (Perez*et al.,* 2011), acid-fast staining(Koh *et al*., 2003); catalase (Reiner, 2010), oxidase, citrate (Drahota*et al.,* 2004), nitrate reduction (Bhusal & Muriana, 2021), strict anaerobic (Dione*et al.,* 2015), starch hydrolysis (Agama-Acevedo, Islas-Hernández, Pacheco-Vargas, Osorio-Díaz, & Bello-Pérez, 2012), sugarfermentation(Barrett & Gibson, 2012), Vogues Prokauer (VP) (Cappuccino & Sherman, 2005) and 6.5% sodium chloride assay (Zhang *et al*., 2021).

**Deoxyribonucleic Acid (DNA) extraction**

Quick DNA Fungi/Bacterial Mini prep kitwas used for the DNA extractionaccording to the manufacturer's instructions. The isolates were grown overnight in plate count agar (PCA) medium prior to the DNA extraction.

**Polyclonal Chain Raction (PCR) Analyses**

PCR technique was used to amplify the conserved region of the 16S rRNA gene using specific 16S rRNA Forward primer (5'AGAGTTTGATCCTGGCTCAG3') and 16S rRNA Reverse primer (5'ACGGCTACCTTGTTACGACTT3'), The PCR mix for this analysis comprises of 1 µL of 10X PCR buffer, 0.4µL of 50mM MgCl$_2$, 0.5µL of 2.5mM dNTPs 0.5µL each of 5mM 16s rRNA Forward and reverse primers 0.05ul of 5units/µL of Taq polymerase enzymeand 5.05µL of distilled water. The PCR mix was made up to 10µL final volume with 2ul of the DNA template.TheMJ Research (PTC-200) thermal cycler was set to 30 cycles of 94°C for 60 seconds,72 °C for 120 seconds and a final extension temperature of 72 °C for 5 minutes and 10 °C hold.

**Gel Electrophoresis**

Agarosegel electrophoresis was used to resolve the amplified fragments of the DNA using 1% agarose solution. Five microliters (5uL) of each sample were resolved at 80V for 2 hours and viewed under UV trans illumination for appropriate documentation.

**Genes encoding the synthesis of PHAs by halophilic bacteria**

The multiplex PCR technique was used to determine the PHA classes of the isolates. The three primers specific for the 3 classes (*phaC*, *phaC1* and *phaC2)* of PHAs were used (Table 1). Primer sequence for the *phaC*gene was derived from*B*. *megaterium*, and *phaC1*primer was obtained from*Pseudomonasaeruginosa*(Conte *et al*., 2006; Hoffmann & Rehm, 2004; Nayak *et al*., 2013)while, *phaC2* primer was obtained from*Pseudomonasputida(Sharma, et al., 2017)*.

Each laboratory identification number of the pure bacterial isolates was maintained and assayed for PHA-producing potential using the primers as shown in table 1.

**Multiplex PCR analysis**

MultiplexPCRanalysiswas performed by preparing PCR master mix comprising 1 µL of 10X concentration PCR buffer mix, 0.4 µL of 50 mM of MgCl$_2$, 0.5 µL of 2.5mMdNTPs, 0.5 µL of each of the forward and reverse *phaC*, *PhaC1* and *phaC2* 100 µM primers (table 1) and 2 µL of 5 unit/µL of Taq polymerase before 1.1 µL of ultra-pure water and 2 µL of template DNA added. The preparation was prepared in PCR tubes and subjected to Thermocycler Model MJ Research (PTC-200) analysis using the following PCR conditions. The PCR condition used is initial denaturation temperature of 92°C for 3 minutes, followed by 25

Table 1: Sequence of Polyhydroxyalkanoate (PHA) primers

| S/No. | Primer Name | Reaction | sequence | Base number |
|---|---|---|---|---|
| 1 | *pha*C | forward | 3'CGTGCAAGAGTGGGAAAAAT5' | 20 |

| | | | | |
|---|---|---|---|---|
| 2 | *pha*C | reverse | 5’TCGCAATATGATCACGGCTA3’ | 20 |
| 3 | *pha*C1 | forward | 3’GGAGCGTCGTAGATGAGTAACAAGAA5’ | 26 |
| 4 | *pha*C1 | reverse | 5’AGGTTGGCGCCGATGCCGTTGAA3’ | 23 |
| 5 | *pha*C2 | forward | 3’TGCTGGCCTGGCGCATTCCCAA5’ | 22 |
| 6 | *pha*C2 | reverse | 5’AAGTGGTAGTAGAGGTTGCC3’ | 20 |

cycles of 92°C for 60 seconds, 57 °C for 180 seconds and a final extension temperature of 57 °C for 5 minutes and the 10 °C hold.

**Gel electrophoresis**

Three (3) microliters of each of the amplicons obtained from the multiplex analysis were mixed with 2 µL of 1X working concentration loading dye and then loaded into the wells created by the comb on the already prepared agarose gel. In addition, 3 µL of 1kbp ladder was also loaded into the first which acts as the molecular marker. The samples were electrophoresed at 80V for 2 hours after which the gel documentation unit was employed for viewing and photography in the presence of a UV light source embedded in the unit.

**Gene Sequencing for Bacteria identification**

*Purification of PCR products for sequencing*

The amplicons for each isolate were purified using 2M Sodium Acetate wash techniques (Oberacker *et al*., 2019). Briefly, 10 µl of the PCR product was added to 1µl of 2M NaAct pH 5.2 and 20 µl absolute ethanol and incubated at 4 $^{\circ}$C for 1 hr. The solution was centrifuged at 10,000 rpm for 10 minutes and washed with 70% ethanol and air dried. Then pellet formed after drying was re-suspended in 5 µl distilled water and stored at 4$^{o}$C for sequencing.

*Preparation of sample for Gene Sequencer (ABI 3130xl machine)*

The 10ulcocktail mix is a combination of 9 µl of Hi Di Formamide with 1µl of purified sequence. The samples were loaded on the genetic analyser and the chromatogram with the sequence was released(Pascucci *et al*., 2014).

*Nucleotide blast*

A basic local alignment search tool (BLAST) program was used to align the nucleotide sequence with other sequences available on the sequence data bank of the National Centre for Biotechnology Information (NCBI) (Fleminger & Goldacre)) web site at https://www.ncbi.nlm.nih.gov, and the corresponding bacteria identity or similarity match for each set of sequences were recorded. Similarity percentage and accession number were also documented.

## Results

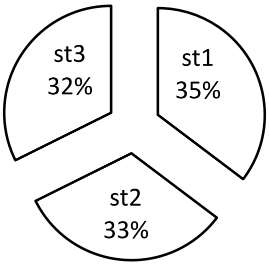

**Figure 1: Combined PHA amplified genes distribution from Bacterial obtained from Nigerian Southern Atlantic Ocean**

Keys

St1=6.35$^{O}$ N 3.28$^{O}$E; st2=6.35$^{O}$ N 3.40$^{O}$ E; and st3=6.36$^{O}$ N 3.47$^{O}$ E

The combined distribution of PHA encoding gene amplification obtained from 3 different locations of st1 (6.35°N 3.28°E), st2 (6.35$^{O}$ N 3.40$^{O}$ E) and st3 (6.36$^{O}$ N 3.47$^{O}$ E) of Nigerian Southern Atlantic Ocean is shown in Figure 1. At locations st1, st2 and st3, 35%, 33% and 32% amplified genes that encode for PHA synthesis were recorded respectively. But in figure 2, at st1, 25%, 42% and 33% amplified genes that encode for PHA synthesis were recorded at the top (0.07 m below the water surface), middle (50 m below the water surface) and bottom (100 m below the water surface) respectively. While in figure 3, at st2, 46%, 36% and 18% were recorded for top, middle and bottom respectively. Also figure 4, at st3 shows 46%, 18% and 36% distribution records at the top, middle and bottom respectively.

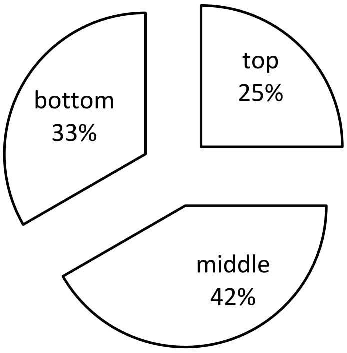

**Figure 2: PHA amplified genes distribution from Bacterial obtained at 6.35$^{O}$ N 3.28$^{O}$ E (St1) location of the Nigerian Southern Atlantic Ocean**

Keys:
top= 0.07 m below water surface; middle= 50 m below water surface; and bottom= 100 m below water surface

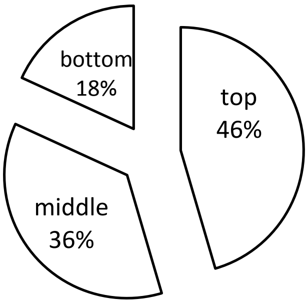

**Figure 3: PHA amplified genes distribution from Bacterial obtained at 6.35$^{O}$ N 3.40$^{O}$ E (st2) location of the Nigerian Southern Atlantic Ocean**

Keys:
top= 0.07 m below water surface; middle= 50 m below water surface; and bottom= 100 m below water surface

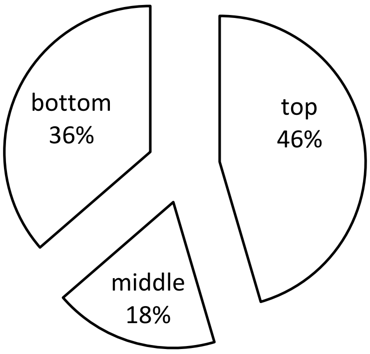

**Figure 4: PHA amplified genes distribution from Bacterial obtained at 6.36$^{O}$ N 3.47$^{O}$ E (st3) location of the Nigerian Southern Atlantic Ocean**

Keys:
top= 0.07 m below water surface; middle= 50 m below water surface; and bottom= 100 m below water surface

Table 2: Assays for Phenotypic identification of Bacterial isolates from hypersaline water body of Lagos, Nigeria.

| **Characteristics** | Isolates | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cultural** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| **Configuration** | round | round | round | round | rhizoid | wrinkle | round | round | round | round | Wrinkle | round | wrinkle | round | round | wrinkle | round | wrinkle |
| **Margin** | wavy | smooth | wavy | wavy | wooly | irregular | margin | margin | wavy | smooth | Wooly | smooth | wavy | smooth | smooth | irregular | wavy | lobate |
| **Elevation** | flat | flat | raised | flat | flat | flat | raised | raised | flat | flat | Flat | raised | raised | drop-like | flat | flat | raised | raised |
| **Morphology** | | | | | | | | | | | | | | | | | | |
| Gram's Reaction | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | =ve | +ve | +ve | +ve | -ve | -ve | -ve | +ve | +ve | +ve |
| Cellular Shape | rod | rod | rod | rod | cocci | cocci | rod | rod | rod | cocci | cocci | cocci | cocci | cocci | rod | rod | rod | rod |
| **Biochemical Tests** | | | | | | | | | | | | | | | | | | |
| Acid fast | | | | | | | +ve | -ve | | | | | | | | | -ve | |
| Catalase | +ve | +ve | +ve | -ve | +ve | +ve | +ve | -ve | +ve | +ve | +ve | +ve | +ve | +ve | -ve | +ve | +ve | +ve |
| Oxidase | +ve | -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 | +ve | +ve | +ve | | | | | | | | | | | | | | +ve | |
| Manitol fermentation | | | | -ve | -ve | | | -ve | | +ve | -ve | | | | | | | |
| Methyl red (MR) | | | +ve | | | -ve | | | +ve | | | | +ve | | +ve | | | -ve |
| Vogues prokauer (VP) | -ve | -ve | -ve | | | | | | -ve | | | | -ve | | +ve | | | +ve |
| Citrate utilization | -ve | +ve | | | | | | | | | | | | | | | | |
| Yellow pigment | | | | | +ve | | | | | | | | | | | | | |
| Novobiocine | | | | | | | | | | +ve | | -ve | | | | | | |
| Glucose fermentation | | | | | | | | | | | | | | | | | | |
| Lactose fermentation | | | | | | | | | -ve | | | | +ve | | +ve | -ve | | +ve |
| Arabinose fermentation | | | | | | | | | | | | | | | | | | |

Table 2 (contd.): Assays for Phenotypic identification of Bacterial isolates from hypersaline water body of Lagos, Nigeria.

| | Isolates | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Characteristics Biochemical Tests (contd.)** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| Sorbitol fermentation | | | | | | | | | | | | | | | | | | -ve |
| Motility | | | | | | | | | +ve | | | | -ve | | | | | |
| Indole | | | | | | | | | -ve | | | | -ve | | | | | |
| Lysin decarboxylate | | | | | | | | | | | | | | | | | | |
| Pigmentation | | | | | | | | | +ve | | | | | | | | | |
| Urease | | | | | | | | | -ve | | | | | | | -ve | | |
| Orthinine decarboxylase | | | | | | | | | | | | | | | | -ve | | |
| Hydrogen sulphide | | | | | | | | | -ve | | | | -ve | | | | | |
| Coagulase | | | | | | | | | | +ve | -ve | -ve | | | | | | |
| **Probable bacteria** | *B. badius* | *B.megaterium* | *B.maquariensis* | *L.delbrueckii* | *M. varians* | *M. luteus* | *M. smegmatis* | *M.delbrueckii* | *S. marcescens* | *S. aureaus* | *S. epidermidis* | *S. saprophyticus* | *K. Pneumoneae subsp pneumoneae* | *N.veillonella* | *E. intermedius* | *Y. pestis* | *C. kutscheri* | *E. amnogenus* |

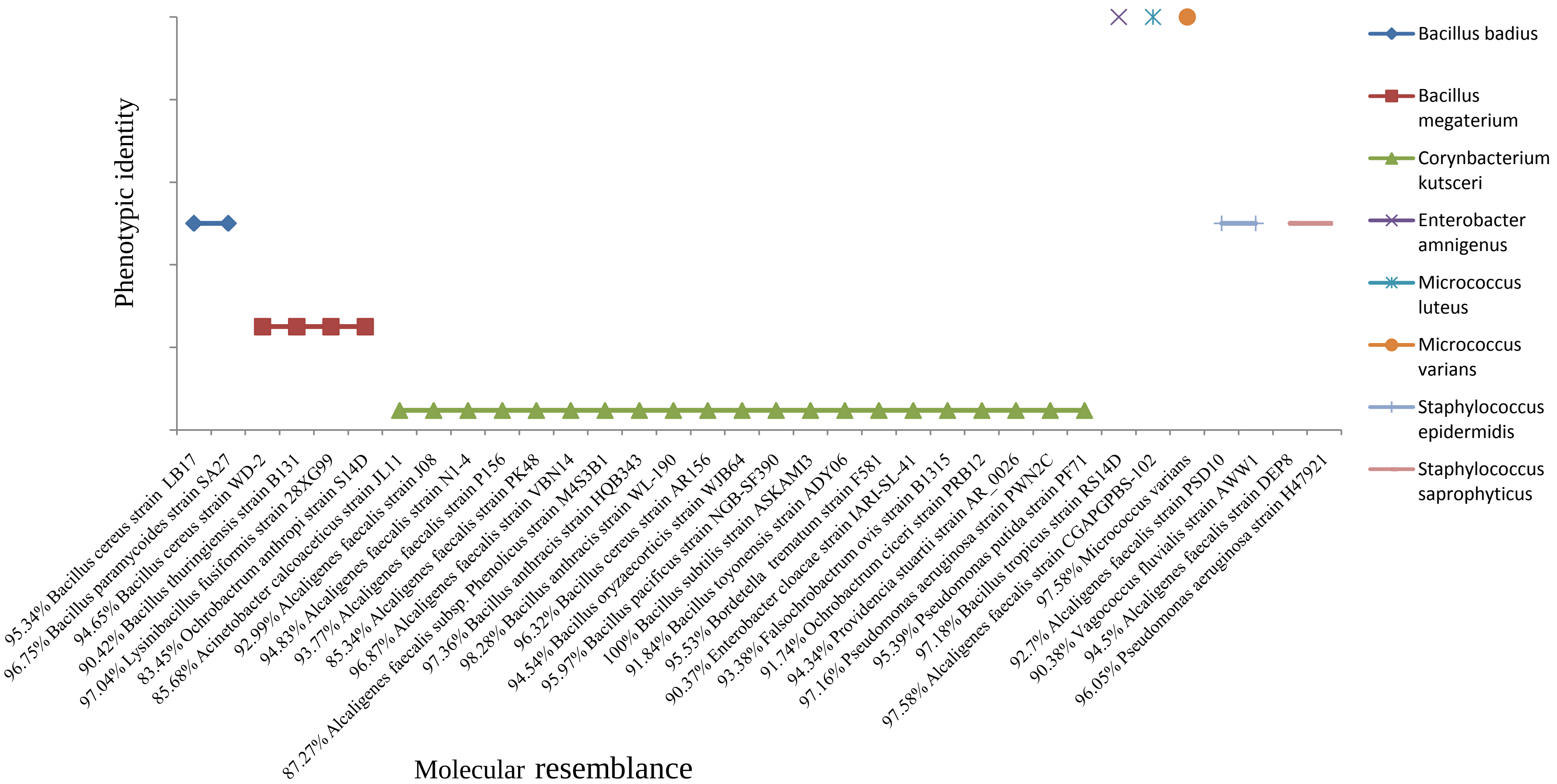

Figure 5: Comparison of Phenotypic and molecular resemblance of Bacerial isolates

**Table 3a:PHA gene potentials in bacteria obtained from Molecular and Microbiological identityassays inNigeria hypersaline water.**

| S/N | Lab No. | No. of Nucleotides | Phenotypic resemblance identity | Molecular identity resemblance | Level of Molecular resemblance | Accession No. resemblance | *phaC*status | *phaC1* status | *phaC2* status |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 102 | 1129 | *Bacillus badius* | *Bacillus cereus strain LB17* | 95.34% | MN087786.1 | +ve | -ve | -ve |
| 2 | 81 | 1107 | *Bacillus badius* | *Bacillus paramycoides strain SA27* | 96.75% | MN467588.1 | +ve | -ve | -ve |
| 3 | 163 | 1083 | *Bacillus megaterium* | *Bacillus cereus strain WD-2* | 94.65% | KJ526821.1 | +ve | -ve | -ve |
| 4 | 93 | 1076 | *Bacillus megaterium* | *Bacillus thuringiensis strain B131* | 90.42% | KX023374.1 | +ve | -ve | -ve |
| 5 | 166 | 1075 | *Bacillus megaterium* | *Lysinibacillusfusiformis strain 28XG99* | 97.04% | FJ174606.1 | +ve | -ve | -ve |
| 6 | 189 | 1151 | *Bacillus megaterium* | *Ochrobactrumanthropi strain S14D* | 83.45% | KT337520.1 | +ve | -ve | -ve |
| 7 | 187a | 1063 | *Corynbacterium kutsceri* | *Acinetobactercalcoaceticus strain JL11* | 85.68% | EU418714.1 | +ve | -ve | -ve |

**Table 3b:PHA gene potentials in bacteria obtained from Molecular and Microbiological identity assays in Nigeria hypersaline water.**

| S/N | Lab No. | No. of Nucleotides | Phenotypic resemblance identity | Molecular identity resemblance | Level of Molecular resemblance | Accession No. resemblance | *phaC*status | *phaC1* status | *phaC2* status |
|---|---|---|---|---|---|---|---|---|---|
| 8 | 187b | 1202 | *Corynbacterium kutsceri* | *Alcaligenesfaecalis strain J08* | 92.99% | GQ426313.1 | +ve | -ve | -ve |
| 9 | 89 | 1132 | *Corynbacterium kutsceri* | *Alcaligenesfaecalis strain N1-4* | 94.83% | MK972333.1 | +ve | +ve | -ve |
| 10 | 38 | 1171 | *Corynbacterium kutsceri* | *Alcaligenesfaecalis strain P156* | 93.77% | CP021079.1 | +ve | -ve | -ve |
| 11 | 190 | 1174 | *Corynbacterium kutsceri* | *Alcaligenesfaecalis strain PK48* | 85.34% | KU245532.1 | +ve | +ve | -ve |
| 12 | 130 | 1124 | *Corynbacterium kutsceri* | *Alcaligenesfaecalis strain VBN14* | 96.87% | MG027661.1 | +ve | -ve | -ve |
| 13 | 69 | 1171 | *Corynbacterium kutsceri* | *Alcaligenesfaecalis subsp. Phenolicus strain M4S3B1* | 87.27% | MH470268.1 | +ve | +ve | -ve |
| 14 | 80 | 1135 | *Corynbacterium kutsceri* | *Bacillusanthracisstrain HQB343* | 97.36% | KT758502.1 | +ve | -ve | -ve |
| 15 | 57 | 1156 | *Corynbacterium kutsceri* | *Bacillusanthracisstrain WL-190* | 98.28% | KJ210666.1 | +ve | -ve | -ve |
| 16 | 115 | 1125 | *Corynbacterium kutsceri* | *Bacillus cereus strain AR156* | 96.32% | CP015589.1 | +ve | -ve | -ve |
| 17 | 84 | 1086 | *Corynbacterium kutsceri* | *Bacillusoryzaecorticis strain WJB64* | 94.54% | KU877643.1 | +ve | -ve | -ve |
| 18 | 37 | 1145 | *Corynbacterium kutsceri* | *Bacilluspacificus strain NGB-SF390* | 95.97% | MK318260.1 | +ve | -ve | -ve |

**Table 3c:PHA gene potentials in bacteria obtained from Molecular and Microbiological identity assays in Nigeria hypersaline water.**

| S/N | Lab No. | No. of Nucleotides | Phenotypic resemblance identity | Molecular identity resemblance | Level of Molecular resemblance | Accession No. resemblance | *phaC*status | *phaC1* status | *phaC2* status |
|---|---|---|---|---|---|---|---|---|---|
| 19 | 137 | 1120 | *Corynbacterium kutsceri* | *Bacillussubtilis strain ASKAMI3* | 100.00% | FJ430566.1 | +ve | -ve | -ve |
| 20 | 129 | 1168 | *Corynbacterium kutsceri* | *Bacillustoyonensis strain ADY06* | 91.84% | MH084795.1 | +ve | -ve | -ve |
| 21 | 40 | 1081 | *Corynbacterium kutsceri* | *Bordetellatrematum strain F581* | 95.53% | CP016340.1 | +ve | -ve | -ve |
| 22 | 33 | 1175 | *Corynbacterium kutsceri* | *Enterobacter cloacae strain IARI-SL-41* | 90.37% | JX645222.1 | +ve | -ve | -ve |
| 23 | 158 | 1134 | *Corynbacterium kutsceri* | *Falsochrobactrumovis strain B1315* | 93.38% | NR_135736.1 | +ve | -ve | -ve |
| 24 | 68 | 1158 | *Corynbacterium kutsceri* | *Ochrobactrumciceri strain PRB12* | 91.74% | MH685438.1 | +ve | -ve | -ve |
| 25 | 31 | 1150 | *Corynbacterium kutsceri* | *Providencia stuartii strain AR_0026* | 94.34% | CP026704.1 | +ve | -ve | -ve |
| 26 | 168 | 1163 | *Corynbacterium kutsceri* | *Pseudomonas aeruginosa strain PWN2C* | 97.16% | MK026852.1 | +ve | -ve | -ve |
| 27 | 45 | 1132 | *Corynbacterium kutsceri* | *Pseudomonas putida strain PF71* | 95.39% | MF838694.1 | +ve | -ve | -ve |
| 28 | 94 | 1135 | *Enterobacter amnigenus* | *Bacillustropicus strain RS14D* | 97.18% | MK359039.1 | +ve | -ve | -ve |

**Table 3d:PHA gene potentials in bacteria obtained from Molecular and Microbiological identities assays in Nigeria hypersaline water.**

| S/N | Lab No. | No. of Nucleotides | Phenotypic resemblance identity | Molecular identity resemblance | Level of Molecular resemblance | Accession No. resemblance | *phaC*status | *phaC1* status | *phaC2* status |
|---|---|---|---|---|---|---|---|---|---|
| 29 | 96 | 1153 | *Micrococcus luteus* | *Alcaligenesfaecalis strain CGAPGPBS-102* | 97.58% | KY495219.1 | +ve | -ve | -ve |
| 30 | 117 | 1117 | *Micrococcus varians* | *Bacillushumi strain NBPP9* | 88.4% | EM_PRO:FJ973539 | +ve | +ve | -ve |
| 31 | 73 | 1147 | *Staphylococcus epidermidis* | *Alcaligenesfaecalis strain PSD10* | 92.70% | KP835577.1 | +ve | -ve | -ve |
| 32 | 105 | 1111 | *Staphylococcus epidermidis* | *Vagococcusfluvialis strain AWW1* | 90.38% | MH760800.1 | +ve | -ve | -ve |
| 33 | 2 | 1137 | *Staphylococcus saprophyticus* | *Alcaligenesfaecalis strain DEP8* | 94.50% | KX118704.1 | +ve | -ve | -ve |
| 34 | 145 | 1132 | *Staphylococcus saprophyticus* | *Pseudomonas aeruginosa strain H47921* | 96.05% | CP008861.1 | +ve | -ve | -ve |

## Discussion

The vast attention towards polyhydroxyalkanoates (PHAs) is mainly attributed to their properties that resemble some petrochemical plastics. Several categories of PHA including low chain length-medium chain length PHAs (LCL-MCLPHA), LCL-PHAs (copolymers produced by *Pseudomonas aeruginosa*) of, SCL-PHAs, copolymers of SCL-MCL and SCL-LCL-PHAs have been reported (Saranya *et al.,* 2017). However, apart from structural and the expressed biochemical secretions, the bacterium may possess or harbour other sets of genes not not yet reported(Kim *et al*., 2010; Cabili *et al*., 2011) that maybe triggered for expression under a completely different environmental factor (Zhu*et al., 2*014). The phenotypic otherwise known as a traditional (cultural and biochemical) method of identification as one of the two methods (phenotypic and 16S rDNA probe) employed for each of the sample isolates in this research agrees with the earlier research findings which stated that morphological characteristics may be controlled by the expression of a set of similar genes situated at a particular locus in a sequence when triggered by specific environmental condition (Albert & Kruglyak, 2015; Govindaraj *et al.,* 2015). In this study, the genetically (16S rDNA) identified *Bacillus cereus strains LB17* and *Bacillus paramycoides SA27 are both phenotypically identified* as *Bacillus badius* in this research. This is in agreement with Gaballa *et al*. (2021)reports. However, the report indicates that bacteria which belong to a group may be difficult to differentiate based on the similarities of their physiology and some vital biochemical reactions (Catoira*et al.,* 2019; Mclntyre*et al*., 2008) borne out the expressed genes. Similarly, *Bacillus cereus strains WD-2, Bacillus thuringiensis strain B131, Lysinibacillus fusiformis* strain 28XG99 and *Ochrobactrum anthropic* strain S14D alsoexhibit the phenotypic identity of *Bacillus megaterium*. However, this may also be associated with the fact that genes harboured and or acquired are expressed phenotypically while the genetic probes reveal the constitution of the bacterium identity (Albert & Kruglyak, 2015). Invariably, some individual isolates phenotypically identified as *Corynebacterium kutsceri are* genetically assayed as *Acinetobacter calcoaceticus JL11,* five strains of *Alcaligenes faecalis (*J08, N1-4, P 156, PK48 and VBN14) seven strains of *Bacillus spp. (HQB343, WL-190, AR156, WJB64, NGB-SF390, ASKAMI3 and ADY06),Enterobacter cloacae*, *Falsochrobactrum ovis*, *Ochrobactrum ciceri*, *Providencia stuartii including* two species of *Pseudomonas (P. aeruginosa* strain *PWN2C and P. putida* strain *PF71)*and *Bordetella trematum* (figure 5). Likewise, *Bacillus tropicus* strain *RS14D is phenotypically identified as Enterobacter amnigenus.*While*Alcaligenes faecalis* strains *PSD10* and *Vagococcus fluvialis* strain *AWW1* were identified as *Staphylococcus epidermidis*. *Alcaligenes feacalis* strain *DEP8* and *Pseudomonas aeruginosa* strain *H47921*are also identified as *Staphylococcus saprophyticus in this research*.

This study shows that the *phaC* a gene for PHA detection potential was expressed in all 34 bacterial isolates (Table 2). However, it is worth noting that the manipulation of biological PHA synthesis can be carried out under the choice of PHA synthase that is, the enzyme responsible for the incorporation of (D)-3-hydroxyacyl-CoA substrate into PHA ( Nomura & Taguchi, 2007; Zhuang, Wang, Liang, & Qi, 2014; Morya *et al*., 2021). Therefore, the expression of *phaC* genes in the bacteria isolates does not give us an idea of the possible type of PHA due to these research isolates when optimum conditions are attained. Furthermore, it is also important to know that organisms that carry *phaC* gene synthase have the potential to synthesize homopolymer and copolymer and this is determined by the type of carbon source utilized (Chen, 2010; Możejko-Ciesielska & Kiewisz, 2016; Możejko-Ciesielska*et al.,* 2019) hence, the expression of this gene is only an indication of the potential of an organism to produce PHA and not to predict the type of PHA it might produce (Zinn *et al.,* 2001; Koller & Rodríguez-Contreras, 2015;Koller *et al.,* 2017)

The *Alcaligenesfaecalis strains* (N1-4, PK 48, M4S3B1) in Table 2b and *Bacillus humi* strain *NBPP9* (Table 2d) in this study expressed *phaC1*which is noted for short PHA chain potential. *phaC1* and some other related PHA synthase genes are harboured in *phaC*(Tan *et al*., 2020; Lim *et al*., 2021). Hence, the expression of this gene in any organism indicates the potential type of PHA it might synthesize. However, *phaC2* gene was not expressed on the bacterial isolates which indicate that none of the bacterial isolates can produce medium chain length PHA.

## Conclusion

The results from this study revealed that there are specie variations in the phenotypically identified isolates of *Corynebacterium kutsceri*, *Enterobacter amnigenus*, *Micrococcus luteus*, *Micrococcus varians*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* to that of their genetic identity. However; species is only distinctly synchronized in bacillus genotypic and phenotypic identity and not in the variety, while the varietal differences of the type of PHA produced from the same species are recorded. Observations from this study indicate that, of all the 16S rDNA BLAST resultsobtained at NCBI website, only one (1) nucleotide sequence (*Bacillus substilis* strain ASKMI3) matched at 100%; the remaining sequences resemblance are between 74.18 and 98.28% from the thirty-four samples. It is therefore pertinent to rely on the genetic identification of bacteria for the effective determination of PHA-producing character as displayed in *Bacillus* spp. Also, the non-synchronization of genetic identities noticed in the phenotypic method employed for *Corynebacterium kutscheri*, *Acinetobacter calcoaceticus, Alcaligenes faecalis, Bacillus spp, Enterobacter cloacae*, *Falsochrobactrum ovis*, *Ochrobactrum ciceri*, *Providencia stuartii, Pseudomonas spp.* calls for further study that will be tailored to the order of standardization and validity of the identities of the aforementioned bacteria whenever a phenotypic method is to be used.

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