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Phenotypic and Genetic Characterization of Polyhydroxyalkanoate Producing Bacterial Isolates from Hypersaline Water Body, Atlantic Ocean, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author FSO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AMO and FEN managed the analyses of the study. Author OAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The importance of polyhydroxylalkanoate (PHA) producing bacteria is hinged on the basis that PHA itself possess environmentally advantages over synthetic plastics therefore, the proper identity of these bacteria is inevitable.

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Aim: To determine the bacteria identification methods of phenotypic and genetic identification for marine sourced effective polyhydroxylalkanoate (PHA) production.
Study Design: Randomised design was employed for PHA producing bacterial isolates.
Place and Duration of Study: 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 and 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*, *Falsochrobactrum ovis*, *Ochrobactrum ciceri*, *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 *Vagococcus fluvialis* (*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: This study established variations in the genetical identity of *Corynebacterium kutsceri*, *Enterobacter amnigenus*, *Micrococcus luteus*, *Micrococcus varians*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* when compared with the phenotypic identification methods. But, there was synchronization in the genotypic and phenotypic identity of bacillus species. Records of varietal differences of PHA potential were also obtained from same species in this research. 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.

1. 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 also biocompatible and completely biodegradable [1,2 and 3]. PHAs exhibit bio-plasticity, thermo-plasticity or elastomer material characters with a temperature range between 140°C to 180°C [4]. There are over 150 monomers with the potential to combine within this family that results to materials of extremely different properties [4,5 and 6]. However, organisms such as bacteria are specific to the type of PHA they synthesize [7,8 and 9].

The preference for bioplastic over synthetic plastics is hinged 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 [10]. However, biopolymers (PHA) will not contribute to environmental pollution at the end of their use when recycled due to their elastomeric property [11,12 and 13]. These characteristics make them superior to synthetic plastics. Thus, the present extensive use 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 [14]. PHAs are of immense importance in the applications of medicine, engineering, agriculture, telecommunications, transportation and household utilities [15,16 and 17].

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 [18 and 19]; Medium Chain Length (MCL) are 6-14 carbon polyhydroxylalkanoates with low degree of crystallization, hydrophobicity, low melting temperature and biodegradable [20 and 21]; 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 [22,23,24 and 25]. There are other complex categories of PHA that differ from the aforementioned such as, 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 [26].

The survival and optimal 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 [27,28,29 and 30] of which their products may be of high commercial value. Also, the bio-molecules produced by halophilic bacteria are stable [31]; 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 [32,33 and 34], 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.

2. METHODOLOGY

2.1 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, and at three different depths of the surface of the water (0-0.07M), middle depth (50M) and bottom depth (100M) below the water surface. The points of the water collections were obtained and recorded from the Geographic Positioning System (GPS) as locations of (1) latitude 6.35^{O} N, longitude 3.28^{O} E; (2) latitude 6.35^{O} N, longitude 3.40^{O} E; and (3) latitude 6.36^{O} N, longitude 3.47^{O} E. The aseptically collected water was transferred into a 1-litre plastic container; the temperature was taken in situ before storage for transportation in a cooled box to the laboratory for further laboratory analysis.

2.2 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 while the 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.

2.2.1 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.

2.2 Phenotypic Identification

2.2.1 Identification of bacterial isolates

The phenotypic identification was carried out according to the methods of Bargey's Manual of Determinative Bacteriology [35] which includes;

Cultural characterization: This was determined by a process known as plate reading by visual observation of a standard chart and observation with X10 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 [36] to determine the cellular structures and arrangements.

Biochemical tests: These were carried out by spore staining [37], acid-fast staining [38]; catalase [39], oxidase, citrate [40], nitrate reduction [41], strict anaerobic [41], starch hydrolysis [42], sugar fermentation [43], Vogues Prokauer (VP) [44] and 6.5% sodium chloride assay [45].

2.3 Deoxyribonucleic Acid (DNA) Extraction

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

2.4 Polyclonal Chain Reaction (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 enzyme and 5.05µL of distilled water. The PCR mix was made up to 10µL final volume with 2uL of the DNA template. The MJ 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.

2.5 Gel Electrophoresis

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

2.5.1 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 *Pseudomonas aeruginosa* [46,47 and 48] while, *phaC2* primer was obtained from *Pseudomonas putida* [49].

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.

2.6 Multiplex PCR Analysis

Multiplex PCR analysis was 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 2.5mM dNTPs, 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 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.

2.7 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.

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

2.8 Gene Sequencing for Bacteria Identification

2.8.1 Purification of PCR products for sequencing

The amplicons for each isolate were purified using 2M Sodium Acetate wash techniques [50]. 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 ˚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 4ºC for sequencing.

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

The 10uL cocktail 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 [51].

2.8.3 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.

3. RESULTS

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 Fig. 1. At locations st1, st2 and st3, 35%, 33% and 32% amplified genes that encode for PHA synthesis were recorded respectively. The result also shows that irrespective of the depth of the Atlantic Ocean in each of st1, st2 and st3 mentioned locations, the percentages of non PHA encoding gene bacteria accounts for 65-68% respectively. But in Fig. 2, data obtained from the different depths of st1 (6.35°N 3.28°E) location's depths of top (0.07 M below the water surface), middle (50 M below the water surface) and bottom (100 M below the water surface) are 25%, 42% and 33% respectively for amplified genes that encode for PHA synthesis. While in Fig. 3, at st2 (6.35^{O} N 3.40^{O} E), 46%, 36% and 18% were recorded for top, middle and bottom respectively. Also Fig. 4, at st3 shows 46%, 18% and 36% distribution records at the top, middle and bottom respectively.

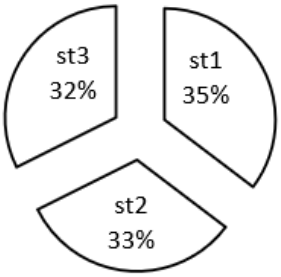

Fig. 1. Combined PHA amplified genes distribution from bacterial obtained from Nigerian Southern Atlantic Ocean

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

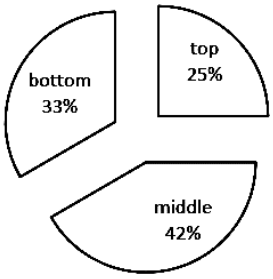

Fig. 2. PHA amplified genes distribution from Bacterial obtained at 6.35^{O} N 3.28^{O} E (St1) location of the Nigerian Southern Atlantic Ocean

Keys: Top= 0.07 M below water surface; Middle= 50 M below water surface; and bottom= 100 M below water surface

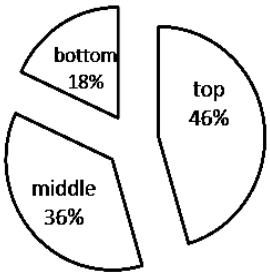

Fig. 3. PHA amplified genes distribution from bacterial obtained at 6.35^{O} N 3.40^{O} E (st2) location of the Nigerian Southern Atlantic Ocean

Keys: Top= 0.07 M below water surface; middle= 50 M below water surface; and bottom= 100 M below water surface

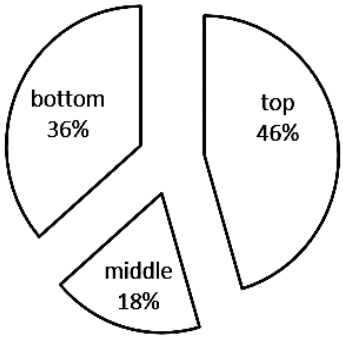

Fig. 4. PHA amplified genes distribution from bacterial obtained at 6.36^{O} N 3.47^{O} E (st3) location of the Nigerian Southern Atlantic Ocean

Keys: Top= 0.07 M below water surface; middle= 50 M below water surface; and bottom= 100 M below water surface

4. 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 [35]. However, apart from structural and the expressed biochemical secretions, the bacterium may possess or harbour other sets of genes not yet reported [50 and 51] that may be triggered for expression under a completely different environmental factor [52]. 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 [53 and 54]. In this study, the genetically (16S rDNA) identified *Bacillus cereus strains LB17* and *Bacillus paramycoides SA27 are both phenotypically identified* as *Bacillus badius*. This is in agreement with Gaballa *et al*. [55] 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 [56 and 57] borne out the expressed genes. Similarly, *Bacillus cereus strains WD-2, Bacillus thuringiensis strain B131, Lysinibacillus fusiformis* strain 28XG99 and *Ochrobactrum anthropic* strain S14D also exhibit 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 [53]. 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* (Fig. 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 [58,59 and 60]. 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 [61,62 and 63] 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 [64,65 and 66].

Table 2. Assays for phenotypic identifiacation 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

Characteristics	Isolates																
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. aureus*	*S. epidermidis*	*S. saprophyticus*	*K. Pneumoneae subsp pneumoneae*	*N.veillonella*	*E. intermedius*	*Y. pestis*	*C. kutscheri*	*E. amnogenus*

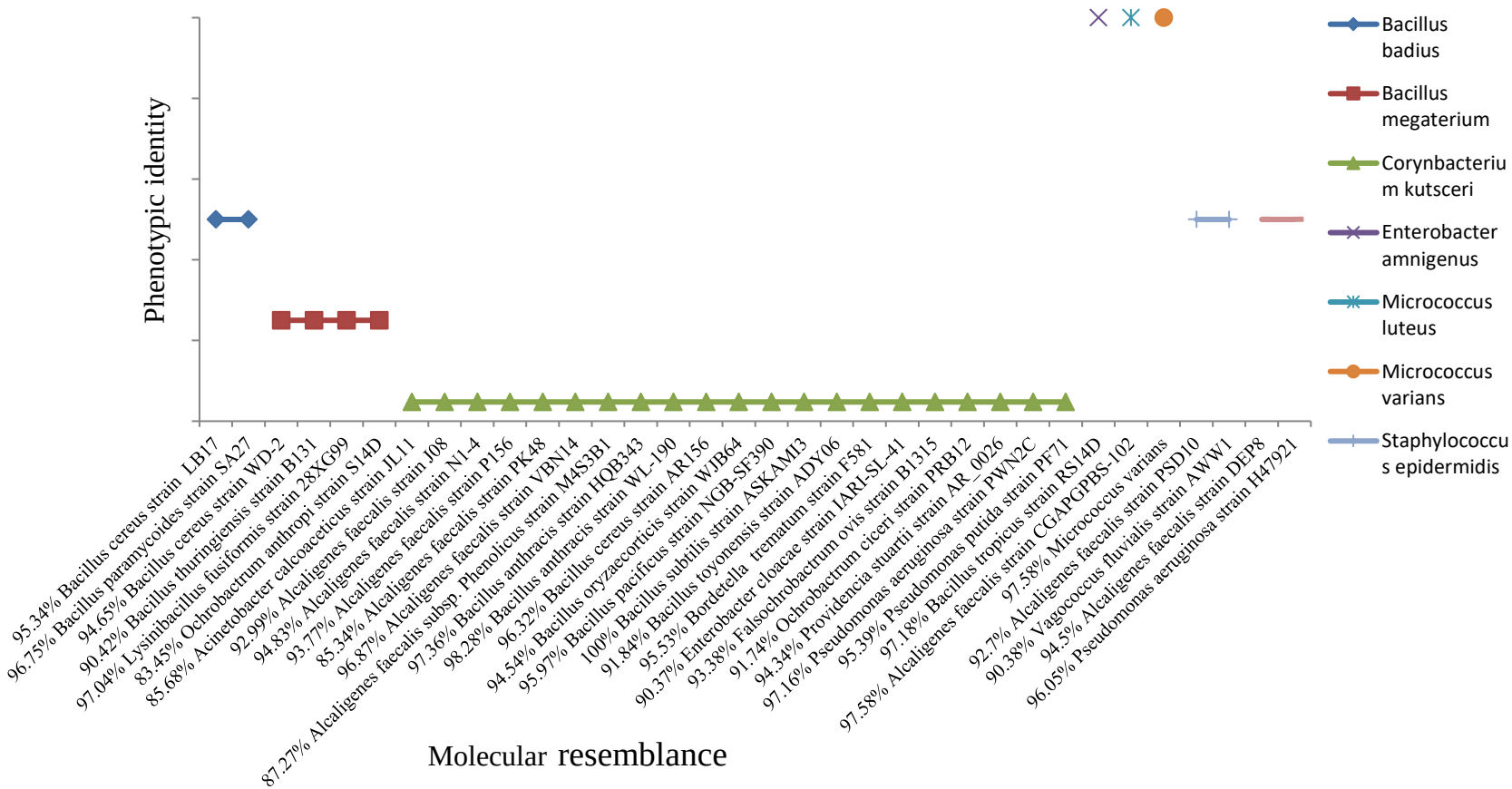

Fig. 5. Comparison of phenotypic and molecular resemblance of bacerial isolates

Table 3a. 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
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*	*Lysinibacillus fusiformis strain 28XG99*	97.04%	FJ174606.1	+ve	-ve	-ve
6	189	1151	*Bacillus megaterium*	*Ochrobactrum anthropi strain S14D*	83.45%	KT337520.1	+ve	-ve	-ve
7	187a	1063	*Corynbacterium kutsceri*	*Acinetobacter calcoaceticus 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*	*Alcaligenes faecalis strain J08*	92.99%	GQ426313.1	+ve	-ve	-ve
9	89	1132	*Corynbacterium kutsceri*	*Alcaligenes faecalis strain N1-4*	94.83%	MK972333.1	+ve	+ve	-ve
10	38	1171	*Corynbacterium kutsceri*	*Alcaligenes faecalis strain P156*	93.77%	CP021079.1	+ve	-ve	-ve
11	190	1174	*Corynbacterium kutsceri*	*Alcaligenes faecalis strain PK48*	85.34%	KU245532.1	+ve	+ve	-ve
12	130	1124	*Corynbacterium kutsceri*	*Alcaligenes faecalis strain VBN14*	96.87%	MG027661.1	+ve	-ve	-ve
13	69	1171	*Corynbacterium kutsceri*	*Alcaligenes faecalis subsp.Phenolicus strain M4S3B1*	87.27%	MH470268.1	+ve	+ve	-ve
14	80	1135	*Corynbacterium kutsceri*	*Bacillus anthracis strain HQB343*	97.36%	KT758502.1	+ve	-ve	-ve
15	57	1156	*Corynbacterium kutsceri*	*Bacillus anthracis strain 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*	*Bacillus oryzae corticis strain WJB64*	94.54%	KU877643.1	+ve	-ve	-ve
18	37	1145	*Corynbacterium kutsceri*	*Bacillus pacificus 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*	*Bacillus subtilis strain ASKAMI3*	100.00%	FJ430566.1	+ve	-ve	-ve
20	129	1168	*Corynbacterium kutsceri*	*Bacillus toyonensis strain ADY06*	91.84%	MH084795.1	+ve	-ve	-ve
21	40	1081	*Corynbacterium kutsceri*	*Bordetella trematum 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*	*Falsochrobactru movis strain B1315*	93.38%	NR_135736.1	+ve	-ve	-ve
24	68	1158	*Corynbacterium kutsceri*	*Ochrobactrum ciceri 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*	*Bacillus tropicus 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*	*Alcaligenes faecalis strain CGAPGPBS-102*	97.58%	KY495219.1	+ve	-ve	-ve
30	117	1117	*Micrococcus varians*	*Bacillus humi strain NBPP9*	88.4%	EM_PRO:FJ973539	+ve	+ve	-ve
31	73	1147	*Staphylococcus epidermidis*	*Alcaligenes faecalis strain PSD10*	92.70%	KP835577.1	+ve	-ve	-ve
32	105	1111	*Staphylococcus epidermidis*	*Vagococcus fluvialis strain AWW1*	90.38%	MH760800.1	+ve	-ve	-ve
33	2	1137	*Staphylococcus saprophyticus*	*Alcaligenes faecalis 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

The *Alcaligenes faecalis strains* (N1-4, PK 48, M4S3B1) in Table 3b and *Bacillus humi* strain *NBPP9* (Table 3d) 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* [67 and 68]. 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.

5. 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 results obtained 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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