Molecular characterization, optimization and production of PHA by indigenous bacteria using alternative nutrient sources as substrate

ABSTRACT

Polyhydroxyalkanoates (PHA) are renewable, biodegradable biopolymer intracellularly accumulated by wide array of microorganisms as carbon reserve. This study investigates the influence of various cultural conditions on PHA production by a recently isolated local species under submerged fermentation. Six PHA producing strains were identified by 16S rDNA gene sequencing and strain *Priestiaflexa* OWO1 showed satisfactory PHA productivity. The effects of production parameters were investigated and extraction of PHA was carried out using sodium hypochlorite method and maximum amount was detected after 72h. Maximum PHA production was obtained at incubation period of 48h, pH of 7.0 and temperature of 30^{o}C. Amongst the hydrolysate of agro waste used, brewers spent grain (BSG) gave maximum yield of 3.01g/L while beans bran powder gave the highest PHA yields of 3.9 g/L amongst the organic nitrogen sources tested. Analysis of the crude PHA by Fourier Transform Infrared Spectroscopy (FT-IR) showed the presence of methyl, methylene as well as carbonyl functional groups. PHA production was higher after optimizing the production conditions as compared to basal medium therefore the utilization of these cheap renewable resources as alternative substrates for production of PHA make the process cost effective and sustainable.

Keywords: Polyhydroxyalkanoates; *Priestiaflexa;* FTIR; Submerged fermentation; hydrolysate.

INTRODUCTION

Increased demand and drive towards search for an attractive, alternative and sustainable bio-based materials to replace fossil fuel-based plastics in different industrial applications has led to the substantial interest in Polyhydroxyalkanoate (PHA) [1,2]. Polyhydroxyalkanoate (PHA) isa biocompatible and biodegradable polymer that exists in vast number of microorganisms in response to unfavorable toxic condition encountered within their environment [3,4]. PHAis stored intracellularly as granules and the size of the granules varies depending on the microorganism [5]. Like synthetic plastics, this biopolymer can be used in several industries like cosmetics, pharmaceuticals, and medical field [6].

PHA is insoluble in water and exhibit an amorphous mobile liquid state in vivo but in laboratory setting, it displays variety of mechanical and material properties, from rigid and brittle crystals to elastomers and molds [7]. PHA biosynthetic pathway is initiated by condensation of two molecules of acetyl-coenzyme-A (acetyl-CoA) by β-ketothiolaseto acetoacetyl-CoA which is then reduced to 3-hydroxybutyryl-CoA by acetoacetyl-CoA reductase and then be polymerized into PHA by PHA synthase [8]. They have proven to degrades quickly into carbon dioxide, water, methane and biomass due to the enzymatic activities of microbes [2,9].

PHA properties and qualities are dependent on monomer composition [10]. They are stratified into three classes (scl-PHA, mcl-PHA and lcl-PHA) on the basis of their monomeric unit [11]. Scl-PHA have less than 5 carbon atoms, mcl-PHA have six-fourteen carbon atoms and lcl-PHA

have more than fourteen carbon atoms but are rare and understudied [12]. Notably, its derivative PHB has been widely characterized [13].
Much of the effort these days has been made to improve the efficiency of bioprocess to allowPHA compete with synthetic plastics [14]. It is well known that 50% of total production cost is taken up by the cost of carbon source [15,16]. This has elicited a search for simple cost-effective feedstocks with biotechnological potential. This is because selection and incorporation of appropriate carbon, nitrogen and other nutrient sources play an important role in designing a cost-effective production medium [17,18]. The aim of this study is to investigate PHA production potential of some isolated bacterial strains, identify some selected possible cheap substrate and determine the effect of different cultural conditions for optimum production of PHA.

2. MATERIALS AND METHODS

2.1 Microorganism

Six PHA accumulating bacterial strain were isolated from soil, organic waste and waste water in Obio-Apor LGA, Rivers State. All strains were maintained on nutrient Agar slant at $4^{o}C$ and subculture every three weeks.

2.2 Molecular characterization and phylogenetic analysis

Extraction of bacteria genomic DNA

The six PHA bacterial accumulators coded OWO1, WW4, WW7, RD18, WW9, and WW15 were subjected to molecular identification through the extraction ofgenomic DNA using boiling methodas described by [19] with slight modification. Pure colonies of the isolated bacteria were grown on Nutrient agar media and used for DNA extraction. Five milliliters of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14000rpm for 3 min. The cells were re-suspended in 50 µLof normal saline and heated at $95^{0}C$ for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5mL micro centrifuge tube and stored at $-20^{o}C$ for other downstream reactions.

DNA quantification

Concentration and purity of Nucleic acid of the extracted DNA were quantified using Thermo-scientific Nanodrop 2000C spectrophotometer (Thermo Fisher Scientific Inc. Wilmington, Delaware, USA) which read through the DNA for 3 consecutive times and revealed their nucleic acid concentration, the maximum and minimum levels, its purity level as well as its unit.

16S rRNA Amplification

The genomic DNA of isolate was amplified using universal primers for 16S rRNA gene of bacterial. The 16SrRNA region of the rRNA gene of the isolates was amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The amplified DNA was then resolved and visualized by gel electrophoresis on a blue light transilluminator.

Sequencing and Bioinformatics analysis

The PCR products after being analyzed with 1% agarose gel electrophoresis, was sequenced by method of [20] using BigDye Terminator kit on a 3510 ABI sequencer at Inqaba Biotechnological, Pty Ltd, Pretoria South Africa. The sequences obtained were then analyzed on the Blast platform in NCBI Gene bank for similarity and the Jukes – Cantor method was used to determine the evolutionary distance of the identified species.

2.3 Inoculum Preparation and Cultivation method

Inoculum was prepared in flasks containing nutrient broth (NB) which was inoculated with a loop full of each test isolate and incubated at 30^{o}C for 24h. For PHA production, each flask containing 50mL of sterile production medium (containing 20g/L glucose as carbon source and 0.1mLof trace element) was inoculated with known volume of inoculum (0.5 McFarland standards) and incubated at 28 ± 2^{o}C for 72h.

2.4 Selection of highest PHA producing strain by PHA and cell dry weight quantification

At the end of the fermentation period, PHA was extracted using digestion method employing sodium hypochlorite and chloroform as described by [21]. The amount of PHA produced was determined gravimetrically and the cell dry weight was measured as described by [22].

2.5 Pretreatment of organic carbon and nitrogen sources

The organic nitrogen sources beans bran (BB), fish meal (FM) and groundnut oil cake (GOC) were prepared, processed in the laboratory by drying and milled into powder and each used individually as organic nitrogen sources.

Brewer's spent grain (BSG), Roasted peanut skin (RPS) and *Chlorella vulgaris* biomass (Cv) were hydrolyzed individually with distilled water containing 1% (v/v) sulphuric acid. The mixtures were hydrolyzed at 121ºC for 30 min, cooled and filtered. The clarified supernatant was separately used as source of carbon

2.6 Selection of suitable conditions

2.6.1 Influence of different media on PHA production

Five different production media were used to maximize the production of biomass and PHA yield. Composition of media were as follows:

Medium A (g/L): $(NH_4)_2SO_4$ 0.2, KH_2PO_4 13.3, $MgSO_4$ 1.3, Citric Acid 1.7, glucose 20g/L, Trace element solution 10 mL/L, (g/L, $FeSO_47H_2O$ 10, $ZnSO_47H_2O$ 2.25, $CuSO_45H_2O$ 1, $MnSO_45H_2O$ 0.5, $CaCl_22H_2O$ 2.0, $Na_2B_4O_710H_2O$ 0.23, $(NH_4)_6Mo_7O_{24}$ 0.1, 35% HCl 10 mL) [23].

Medium B (g/L): Yeast extract 2.0, Peptone 2.0, K_2HPO_4 2.0, $(NH_4)_2SO_4$ 2.0, $MgSO_4$ 0.3, sucrose 10 [24].

Medium C (g/L) with slight modification: KH_2PO_4 1.52, Na_2HPO_4 4.0, $MgSO_47H_2O$ 0.52, $CaCl_2$ 0.02, yeast extract 0.16, urea 1.0, glucose 20, and trace element solution 0.1 mL (g/L): $ZnSO_47H_2O$ 0.13, $FeSO_47H_2O$ 0.02, $(NH_4)_6MO_7O_{24}4H_2O$ 0.06 and H_3BO_3 0.06 [25].

Medium D (g/L): NH_4Cl 0.1, NaCl 10, KH_2PO_4 1.5, Na_2HPO_4 10.2, $MgSO_4$ 0.2, $(NH_4)_2FeSO_46H_2O$ 0.01, glucose 7, trace elements solution: 10 mL of the solution (containing $ZnSO_47H_2O$ 0.1, $MnCl_24H_2O$ 0.03, H_3BO_3 0.3, $CoCl_26H_2O$ 0.2, $CuS0_4$ $5H_2O$ 0.01, $NiCl_26H_2O$ 0.01 [26].

Medium E (g/L): nutrient broth with 10% glucose [27].
The pH of all the media was adjusted to pH 7 using 0.1N HCl and 0.1N NaOH before autoclaving at 121 for 15mins. 50ml of each medium were autoclaved and inoculated with aliquot of 24 h old actively growing culture of the isolate at constant inoculum size and incubated at 30^{o}C for 72hrs. Thereafter, cells were separated by centrifugation and PHA was extracted and quantified by the method described above. Medium that produced the highest PHA yield was selected for further studies.

2.6.2 Influence of various Carbon Sources

50mL of the medium that produced highest PHA yield was employed. Different synthetic (glucose, sucrose, fructose) and organic (BSG, RPS and *Chlorella vulgaris* biomass) carbon sources at a concentration of 2.0% (w/v) were supplemented individually in the basal medium and inoculated with 1mL of the inoculum. Incubation was carried out at 30^{o}C for 72h after which the amount of PHA produced and cell dry weight was estimated as described above.

2.6.3 Influence of various Nitrogen sources

50mL of the medium that produced highest PHA yield was employed and different inorganic (urea, peptone and ammonium sulfate) and organic (bean bran, fish meal and groundnut oil cake) nitrogen sources were supplemented into the production medium individually at 1.0% (w/v). The medium was inoculated and incubated at 30^{o}C for 72h. PHA produced was extracted and amount estimated as described earlier.

2.6.4 Influence of incubation time

Varying period of incubation (24, 48, 72 and 96 h) were used to incubate the production medium to determine the influence of incubation time on PHA production and cell dry weight. Thereafter the amount of PHA and cell dry weight produced was estimated.

2.6.5 Influence of Initial pH

Varying initial pH range from 5- 8 was adjusted in the medium separately using HCl or NaOH (1M) to determine the influence of pH on production of PHA and cell dry weight. The cultivation media were incubated at 30^{o}C for 72h after which PHA yield was estimated.

2.6.6 Influence of Temperature

For studying the influence of temperature on PHA production and cell dry weight, cultures were incubated at varying temperature range of 25°C, 30°C, 35°C and 40°C. Thereafter, the amount of PHA and cell dry weight produced was estimated.

2.7 Characterization of PHA by Fourier Transform Infrared Spectroscopy (FT-IR)

The extracted PHA was mixed with potassiumbromide (KBr) powder (Spectroscopic Grade) and the infrared spectra with frequency range of 4000-600 cm^{-1} were recorded in Buck scientific M530 FT-IR spectrometer and the data collected were the average of 32 scans over the entire range.

2.8 Data Analysis

Results obtained in this study were subjected to Analysis of variance (ANOVA) and difference between the means was examined using Duncan's multiple Range test with the help of Statistical Package for Social Sciences (version 20)

3. RESULTS

3.1 Gel electrophoresis of the 16S rRNA

The PCR products of the isolates were amplified and resolved on gel electrophoresis as depict in figure 1. DNA fragments on lanes (B1-B6) form gene band on the gel at a location corresponding to 1500 base pair (bp) against a 100 bp molecular ladder (Lane L).

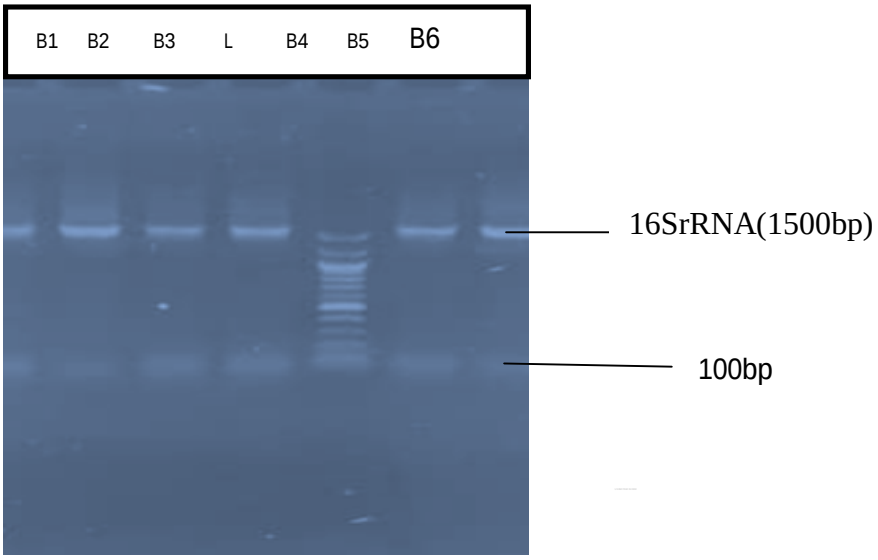

Figure 1: PCR amplification image of the 16S rRNA gene bands of the selected bacterial isolates used in the study. (Lane B1-B6 represents the 16S rRNA gene bands of the isolates; lane L represents 100bp molecular ladder).

3.2 Phylogenetic analysis

From the Phylogenetic analysis, the molecular characterization of the obtained sequences from the isolate revealed 53.4% –100% similarity and produced an exact match from the BLASTN search at the NCBI webserver revealing a close relatedness to *Priestiaflexa*, *Lysinibacilluscapsici*, *Lysinibacillusmacriodes*, *Bacillus velezensis*, *Bacillus paramycoides* and *Pseudomonasxiamenensis* respectively. Sequences of all the isolates were submitted to GenBank database (NCIB) and accession numbers were assigned as indicated in (Figure 2 and Table 1).

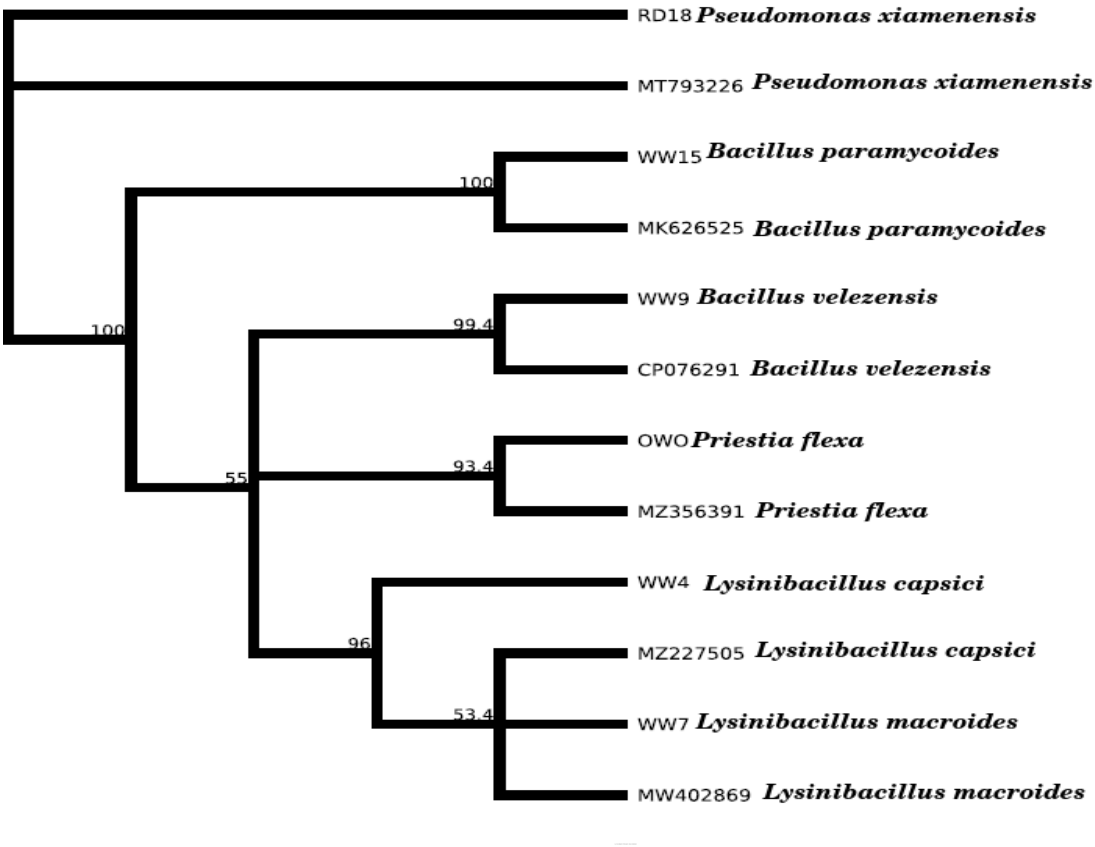

Figure 2: Phylogenetic tree showing the evolutionary distance between the bacteria strains

Table 1. Isolates Accession number and reference match based on 16sRNA sequencing

Isolate code	Gene similarity (%)	Reference Match	Accession number
OWO1	93.4	*Priestiaflexa*	OKO47710
RD18	100	*Pseudomonas xiamenensis*	OKO47711
WW4	96	*Lysinibacilluscapsica*	OKO90911
WW7	53.4	*Lysinibacillusmacriodes*	OKO90912
WW9	99.4	*Bacillus velezensis*	OKO90913
WW15	100	*Bacillus paramycoides*	OKO90914

3.3 Selection of highest PHA producing strain

The six strains of bacteria were further assessed for PHA production capacity in Mineral salt medium using glucose as carbon source. Maximum PHA production of 1.1g/L was shown by

strain OWO1 with a percentage PHA yield of 31.4% cell dry weight after 72h of incubation. The amount of PHA production by other strains range from 0.9 to 1.0 g/L with a percentage PHA yield between 25.7 to 31.9 % cell dry weight as revealed in table 2.

Table 2: Quantities of PHA and cell dry weight produced

Code number of isolates	CDW(g/L)	PHA(g/L)	Yield of PHA (%)
OWO1	3.5	1.1	31.4
WW4	3.25	1.0	30.8
WW7	2.85	0.9	31.9
RD18	3.0	0.9	30.0
WW9	3.5	0.9	25.7
WW15	3.5	0.9	25.7

3.4 Optimization of cultural parameters for PHA production

3.4.1 Influence of different media on PHA production

PHA was assayed after 72h of incubation at 30^{o}C under various production media (medium A-E). Result revealed that the bacteria strain had the ability to grow and produce PHA in all the production medium with different composition. Among the five media used, maximum PHA yield (1.1 g/L), cell dry weight (5.05g/L) and PHA accumulation (35.7%) was obtained with medium C. The least PHA yield (0.21g/L) along with cell dry weight (5.0g/L) and PHA accumulation (4.1%) was obtained when medium B was used as production medium as shown in Figure 3.

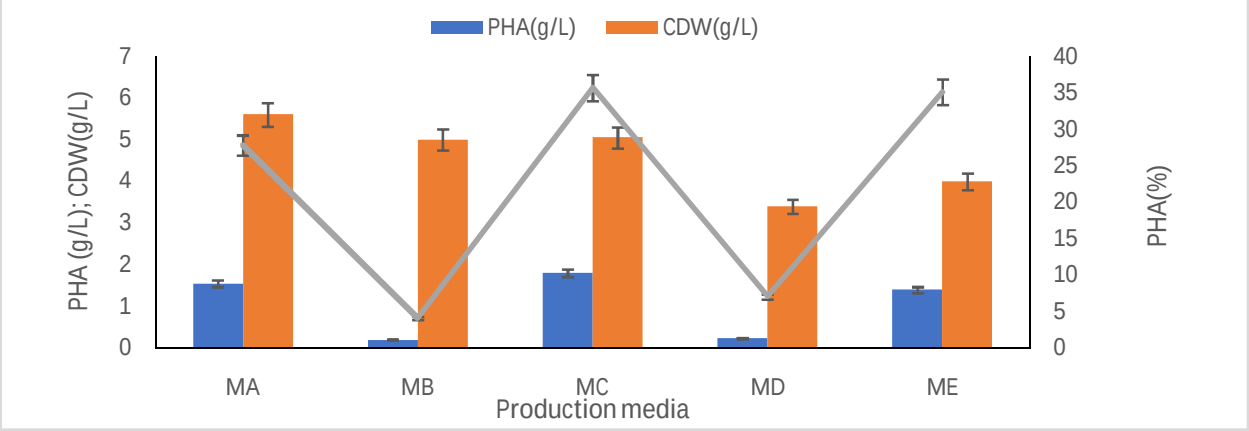

Figure 3.Influence of production media on PHA production

3.4.2 Influence of synthetic and organic carbon sources on PHA production

The effect of different synthetic and organic carbon sources was tested on PHA production and cell dry weight after 72h of incubation period at 30^{o}C. Among the tested carbon sources, maximum amount of PHA was observed in BSG hydrolysate containing medium at 3.0 g/L yield with optimum cell dry weight of 4.95g/L followed by glucose culture medium with PHA yield of 2.46 g/L, bacterial biomass of 4.85g/L and PHA accumulation of 50.7%. Roasted peanut skin gave a yield of 2.2g/L PHA and cell dry weight of 4.9g/L. The minimum amount of PHA production was observed in fructose medium with PHA yield of 1.25 g/Lwith lowest biomass of 3.01g/L as shown in Figure 4.

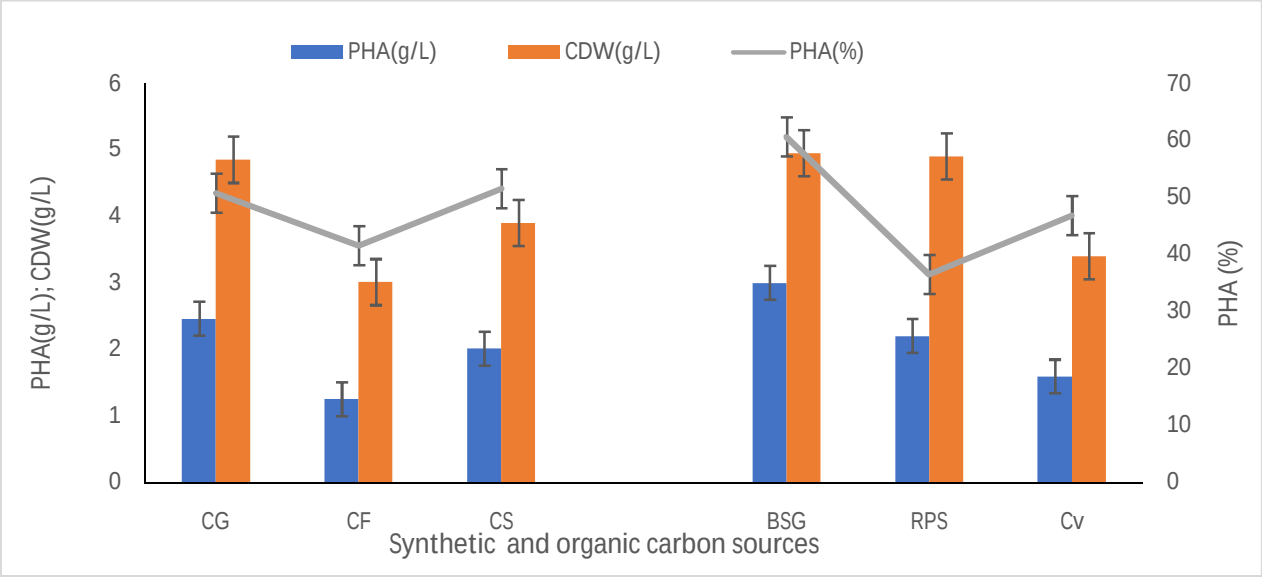

Figure 4. Influence of synthetic and organic carbon sources on PHA production

3.4.3 Influence of Synthetic and organic nitrogen sources on PHA production

All the nitrogen sources investigated in this study supported biomass and PHA production as revealed in Figure 5. Amongst the three synthetic nitrogen sources, 1.0g/L peptone containing medium produced maximum PHA yield of 1.38 g/L and optimum cell dry weight of 2.25g/L after 72 h of cultivation. Under the same condition, minimum cell dry weight of the strain (1.65g/L) was noted in urea containing medium. The synthetic nitrogen source of the medium composition was replaced by three different organic nitrogen powder individually (BB, GC and FM). Bean bran powder was however found to support the highest production of PHA 3.9 g/L at optimum cell dry weight of 6.2g/L followed by groundnut cake while the lowest production was observed in fish meal containing medium as nitrogen source.

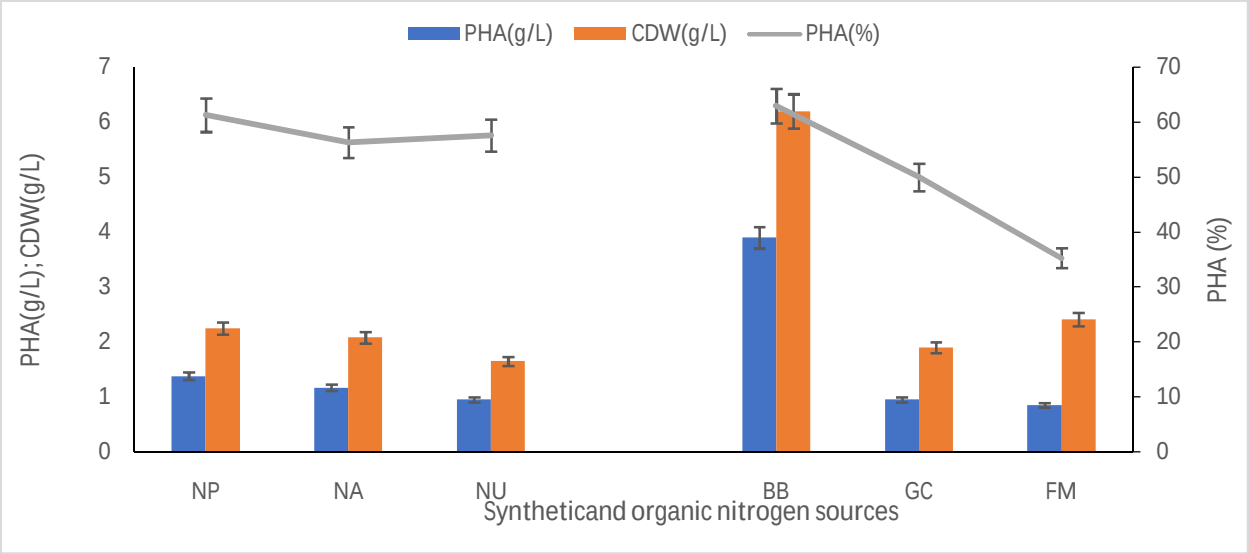

Figure 5. Influence of synthetic and organic nitrogen sources on PHA production

3.4.4 Influence of initial pH on PHA production

Initial pH of the fermentation medium was maintained in the range of 5.0-8.0. Among the various initial pH investigated maximum yield of 1.65 g/L PHA corresponding to a PHA accumulation of 33.6% was observed at initial pH 7 and highest biomass yield of 3.9g/L at initial pH 6. The least amount yield of 0.9 g/L PHA with least cell biomass of 2.7g/L was observed from lowest initial pH value 5 as shown in Figure 6.

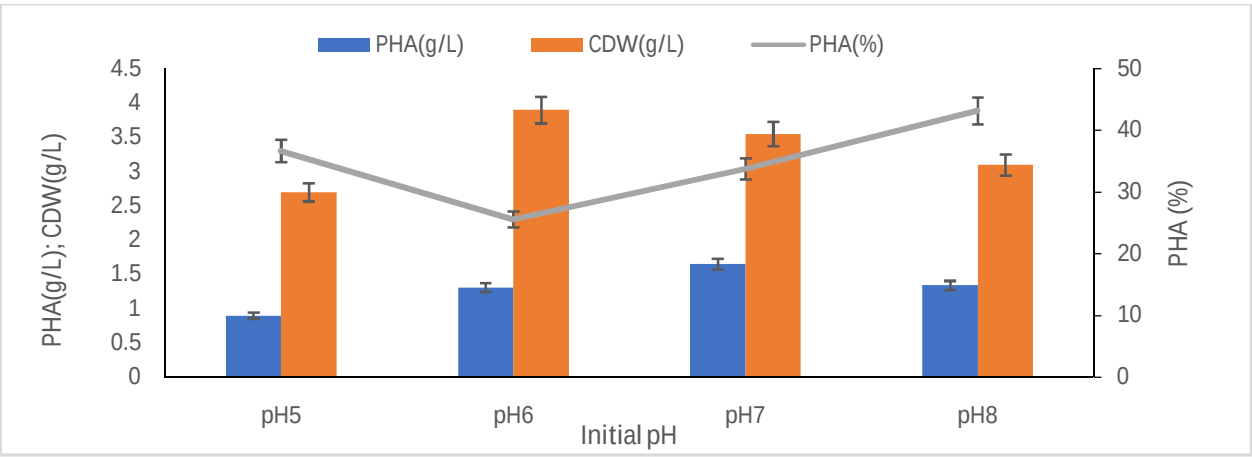

Figure 6. Influence of pH on PHA production

3.4.5 Influence of incubation time on PHA production

The influence of incubation time of the fermentation medium was considered between 24h to 96h and resultreveals that maximum PHA yield of 2.25g/L was observed at 48h with corresponding cell dry weight of 3.84g/L and PHA accumulation of 58.6%. Minimum amount of PHA production was recorded at (1.1g/LPHA yield; 1.65g/L CDW) as shown in Figure 7.

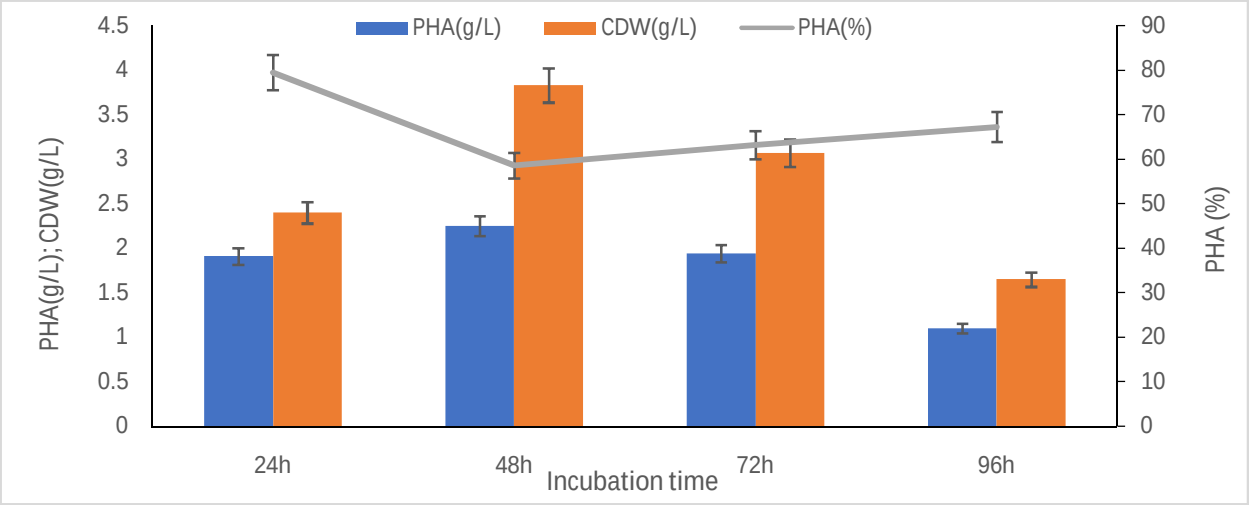

Figure 7: Influence of incubation time on PHA production

3.4.6 Influence of temperature on PHA production

Among the different temperature examined, optimum PHA yield of 2.78g/L and cell dry weight of 4.35g/L was observed at 30 ^{o}C followed by 35^{o}C were biomass yield of 3.79g/L was obtained. The lowest PHA yield of 1.1g/L was obtained at 40^{o}C as shown in figure 8

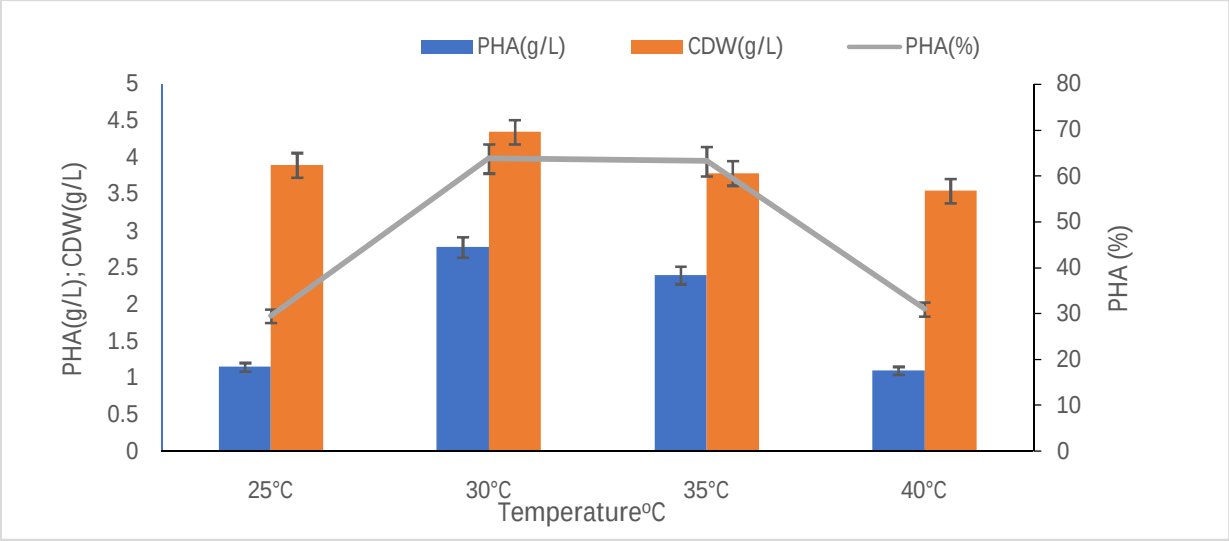

Figure 8.Influence of temperature on PHA production

3.5 FTIR

PHA extracted from Strain OWO1 was subjected to FTIR analysis for characterization. The FTIR spectra of the extracted polymer are shown in Figure 9. The band at 3320.4 and 3102.9 cm^{-1}indicates the presence of -OH groups. The absorption peak around 2900 and1256.32 cm^{-1} suggests the asymmetric methyl CH_3 groups while the peak at 2758.9 cm^{-1} was due to symmetrical methylene group. Stretching absorption band at 1720.2cm^{-1} indicates the C=O

stretching in ester group of lipids which assures the PHA nature of the extract. Absorption bands at 1256.3cm^{-1} and 1099.5 cm^{-1} corresponds to C-O-C stretching vibration while bands at 828.2cm^{-1}and 733.1cm^{-1} represent C-O and C-C stretching vibration. Therefore, the precipitated material is established as PHA by the peaks.

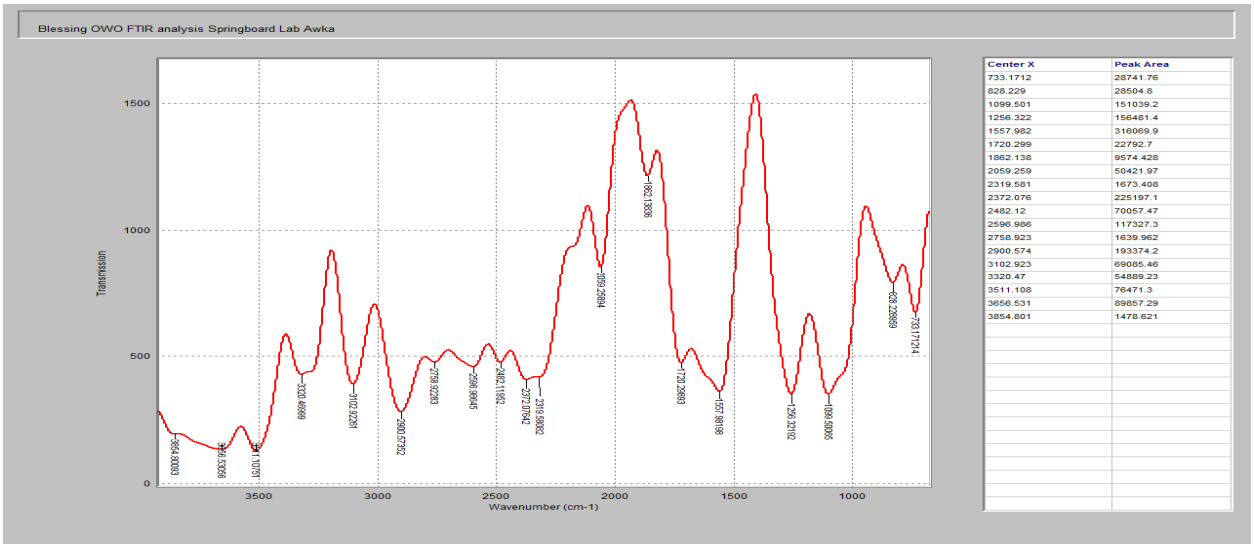

Figure 9. Fourier transform infrared spectroscopy(FTIR) of extracted polymer from *PriestiaFlexa* OWO1

4. DISCUSSION

PHA offers similar properties with conventional plastics, making it a good substitute in place of current synthetic plastics. Nevertheless, its versality and application is hampered by its high cost of production [28]. However, searching for bacteria strains with the ability of producing PHA and optimization is required to cut down the cost of production and help in its wide utilization for different applications. In the present study, six PHA accumulating strains were characterized and identified by molecular approach through their 16S rRNA gene. The phylogenetic analysis to understand the evolutionary relation of the bacteria strains to other species using Jukes-Cantor method were in agreement with the phylogenetic placement of the isolates 16S rRNA within the *Priestia*, *Lysinibacillus*, *Bacillus,* and *Pseudomonas* genera.

In a similar work, the same molecular technique has been employed to facilitate reliable identification of PHA accumulating microorganism isolated from soil and waste water. The bacteria genera (*Bacillus*sp,*Lysinibacillus*sp) found are in line with previous report by [21, 29, 30] who isolated similar strains to be PHA accumulators in different environment. Result of the study conducted by [31] reported *Lysinibacillus*sp from river effluent having the ability of synthesising PHA.

Many reports have addressed the production capacities of isolates in liquid medium as the benchmark for selection of most potent producer of PHA from group of selected bacterial isolates. Out of the six bacterial strain, *Priestiaflexa* OWO1 was outstanding and was selected as

the potent producer of PHA with efficient yield in the submerged fermentation process. Lately, the genus *Priestiaflexus* was proposed as a novel genus of *Bacillus* related species by [32]. PHA production by gram-positive bacteria such as *Bacillus*sp has been reported to have several advantages over others due to absence of lipopolysaccharides layer, high protein secretion and capacity to utilize cheap raw materials [33]. Amidst 50 bacteria strains examined quantitatively by [34],*Bacillus flexus* Azu-A2 gave maximum PHA yield of 0.880 g/l and was chosen as most efficient while work of [35] obtained a yield of 0.186 g/l by a promising producer of PHA identified as *Bacillus flexus*isolated from rhizopheric soil sample.

Production of PHA was studied initially in media reported in literatures and out of the various media used, medium C suited best as it stimulated and gave maximum yield of PHA. Glucose supplemented synthetic medium was described as the most suitable medium for higher productivity of PHA [36].

To achieve maximum PHA production diverse cultural conditions of the incubation medium of the bacterium was optimized. Carbon substrate have been reported to affect PHA production medium as it is required for growth and metabolite production by microorganisms [37]. All the carbon sources investigated in this study supported PHA production at varying degree. Based on the effectiveness of the carbon sources, Brewer's spent grain hydrolysate supplementation in medium as carbon source showed good impact when compared to others screened. Similar results have also been reported in earlier literatures [38,39]. High yield observed with utilization of brewer's spent grain hydrolysate could be a function of its various composition and pretreatment method used. Several other authors [40, 41].have reported PHA synthesis using cost effective substrates produced by *Bacillus* species. Different agro industrial effluents e.g. sugar cane molasse, dairy and paper mill effluent where screened out for PHA production by *Bacillus substilis* RS1 which showed 4.5 g/L yield of PHA with the use of treated sugarcane molasse as carbon sources [42].Studies conducted by [43] investigates PHA production in a cost -effective manner with the use of molasse and other carbon source that produced PHA yield of 0.1-8.9±0.3g/L.

PHA production has been previously shown to be highly influenced by nitrogen sources concentration in the medium [44]. The most common nitrogen sources currently used for PHA production by isolates includes urea, ammonium sulphate, peptone, yeast extract. On screening synthetic and organic nitrogen sources, results were promising using bean bran powder at concentration of 1.0g/L. which led to the accumulation of remarkable amount of PHA in the medium. This may be due to the presence of some essential nutrients and co factors which could have played a vital role in accumulation of PHA. However, during growth in medium containing synthetic nitrogen sources, biomass production as well as PHA accumulation was remarkably reduced. The difference in PHA yield by the various nitrogen sources evaluated was statistically significant.

Similarly, [34] found that using corn steep liquor as source of nitrogen among others increased both growth and PHA production by *Bacillus Flexus* ME-77. Jangra andNehra(2017) also informed that *Bacillus* and *Pseudomonas* species showed different preference on mustard cake and other nitrogen substrate utilized for PHA production [33].To the best of our knowledge, for economic PHA production, few studies were conducted by using different organic nitrogen sources such as mustard cake, corn steep liquor but to mention a few, but no study at present shows the utilization of bean bran powder as nitrogen substrate for low cost PHA production.

Regarding the effect of pH on PHA production by *Priestiaflexa*OWO1, results obtained were in agreement with the findings of [40,45, 46] who reported similar trend with different isolate where they observed that these isolates could produce PHA at pH 7. Contrary, [36] expressed a different trend for PHA production by *Bacillus* strains with maximum PHA yield achieved at pH 8. The decline in PHA output at low and high pH above optimum was due to degradative enzymes playing an important role in breaking down the polymer, allowing PHB to be consume at a rate nearly equivalent to its production rate [47].

Time of incubation also had effect on PHA production. The optimum time for PHA production by bacteria strain was found to increase from 24h and peaked at 48h, after which there was a steady decline in PHA yield, cell dry weight and accumulation. The decline in PHA production might be due to bacteria using PHA as a carbon source resulting in depletion of available nutrient in the culture medium as reported by other studies [40] Similar results were supported by [48, 49] on *Bacillus* sp.

At temperature growth condition study, any increase or decrease in the culture temperature from optimum temperature (30^oC) caused a decrease in PHA production studyand cell dry weight. This is in agreement with work reported by other workers [50]. Likewise, conducted for PHA production by [51] reported maximum P HA production by *Bacilllus*at 30^oC than at 35^oC. The lowest (15^oC) and highest (50^oC) tested temperature results in decreased activity of enzymes which affected the metabolic activity of the microorganism resulting into reduced growth and PHA production [52].

The chemical structure of PHA produced by *Priestiaflexa* strain OWO1 was revealed by analysing the crude extract using Fourier Transform Infrared spectroscopy. The dried PHA isolated appeared as off-white powder and the FTIR analysis showed characteristics bands for the group CH_2, CH_3, C=O, -CH and OH. A similar pattern of FTIR was reported for the presence of PHA by [53]. The observed pattern of IR spectrum was very similar to spectrum obtained by [5] who reported the appearance of an absorption band of carbonyl functional group which was confirmed from the intense peak observed at $1720cm^{-1}$ with FTIR analysis of PHA produced by *Bacillus thuringenesis*. The analyzed results also correlate with the documentation of [52] confirming that the extracted compound was PHA.

5. CONCLUSION

The present work demonstrated the optimization of media composition along with environmental parameters to scale up the production of PHA by locally isolated *Priestiaflexa* strain OWO1 at laboratory scale. The fermentative study shows that PHA yield at pH of 7.0, temperature at 30^oC and incubation period at 48h were established as the optimal environmental condition which could be useful for large scale production. High amount of PHA was achieved through the use of brewer's spent grain hydrolysate and bean bran powder as organic carbon and nitrogen sources. These readily available renewable resources can be converted into value added products. Further studies would be carried out in elucidating the structure of the PHA produced.

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