Journal of Applied Biology & Biotechnology Vol. 11(4), pp. 178-184, Jul-Aug, 2023
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2023.11514

Utilization of agro-industrial wastes as raw materials for the biosynthesis of polyhydroxy butyrate biopolymer from *Bacillus paramycoides* strain KUMBNGBT-33

G. Nandish, S. Akarsh, D. Manjunatha, H. V. Sowmya, B. Thippeswamy*

Department of P. G. Studies and Research in Microbiology, Bioscience Complex, Jnanasahyadri, Kuvempu University, Shankaraghatta, Karnataka, India.

ARTICLE INFO

Article history:
Received on: January 06, 2023
Accepted on: March 01, 2023
Available online: June 04, 2023

Key words:
PHB biopolymer,
Sudan b black staining,
Bacillus paramycoides,
Agroindustrial substrates and
Hydrolysate

ABSTRACT

Bio plastics are natural biopolymers and synthesized by various microorganisms and these materials are non- toxic and have more advantage compared to petroleum-based conventional plastics. The present research involves the isolation, screening, characterization, optimization, and quantification of polyhydroxy butyrate (PHB) producing bacterium by using different agroindustrial waste substrate. The bacterium was isolated from dump yard of Ranganathapura, Shivamogga district, Karnataka, India. The isolated bacterium was Gram-positive, rod shaped, spore forming, and motile bacilli. The Sudan b black staining confirms the presence of PHB granules in the bacterium. Based on phenotypic and genotypic characters, the isolated bacterium was identified and confirmed as *Bacillus paramycoides* and its partial 16s rRNA gene sequence was deposited to GenBank, NCBI, and obtained Accession no. MW056185. The optimum conditions required for the maximum PHB production includes, nutrient broth medium, 72 h incubation time, 37°C temperature, pH = 7.0, glucose as carbon source, ammonium chloride as nitrogen source, and carbon-nitrogen in the ratio 8:1. The PHB producing bacterium was cultivated on different agroindustrial waste substrates among them maximum quantity of PHB production was found in hydrolysate of feed stock. The Bio-spectrophotometric analysis provides the ʎ-max of 319 nm, quantifies and confirms the production of PHB. All these findings reveals that the isolated *B. paramycoides* is an efficient PHB producer which can be exploited for the production of PHB biopolymer.

1. INTRODUCTION

The plastic components are very essential products used in daily circumstances as a basic need but they are creating many environmental complications because these plastics did not degrade in the environment [1]. Petroleum-based products are extensively used in covering materials, carry bags, clothing, water bottles, toys, domestic and industrialized goods, and constructing materials [2]. The plastics produced by utilizing non degrading components will never degrade and remains in the soil for many decades. These reprocessed plastics are more destructive to the atmosphere compared to original goods due to the usages of many non-degrading additives for the production. To overcome these ecological and scrap disposal difficult, the degradable bioplastics are developed. These bioplastics are degradable in nature and converted by many microbes into carbon dioxide and humus. Most reliable biodegradable polyesters are polyhydroxyalkanoates (PHAs), polyhydroxy butyrate (PHBs), aliphatic polyesters, and polysaccharides [1,3].

Biodegradable plastic or PHB is macromolecular compounds produced by numerous natural microorganism. Numerous different pathways for fermentative production of PHB have been used in landscape and they are suitable based on their natural habitation [4]. The biopolymer was naturally produced by many microorganisms obtained in the natural flora. These organisms are capable to produce the biodegradable polymers retort to their unfavorable growth circumstances using available carbon rich components. *Bacillus, Pseudomonas, Achromobacter, Sphingobacterium,* and *Rhizobium* produces PHB as an energy standby [5]. More than 200 different forms of bacteria have the ability to produce PHB naturally. Apart from these usual PHB producers, many genetically altered microbes are also used for large scale production of PHB. Even though various species of bacteria are documented as good PHB producers. However, still many research was undergoing to know the potent bacteria which is responsible for the production of PHB in different enhanced conditions [6]. PHB is acts as carbon storage particles produced by a number of microorganisms during the period of nutritional hassle. Many bacterial species can produce PHB including *Bacillus paramycoides*, *Bacillus subtilis*, *Bacillus thurengenesis, and B. paramycoides that was* considered to be very

**Corresponding Author:*
B. Thippeswamy,
Department of P. G. Studies and Research in Microbiology,
Bioscience Complex, Jnanasahyadri, Kuvempu University,
Shankaraghatta - 577 451, Karnataka, India.
E-mail: thippeswamyb205 @ gmail.com

© 2023 G. Nandish, *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution License -NonCommercial-ShareAlike Unported License (http://creativecommons.org/licenses/by-nc-sa/3.0/).

suitable organism to study the PHB biosynthesis [7]. PHB matrices are observed for biomolecules encapsulation, such as tumor medications and disinfectants, are used to treat the damaged tissues [8]. PHB has potential applications in various sectors due to their characteristics features of biodegradability and biocompatibility. These polymers are had wide applications in Biomedical, pharmacological, environmental, packaging, veterinary, industrial, and agricultural fields [9].

Synthetic polymers are non-degradable and cause the major environmental pollution. Due to this negative impact of synthetic polymers, researcher focused on PHB for the production of biopolymer. The biopolymers are very compatible and ecofriendly because it is easily degradable. Certain bacteria stored energy in the form of granules which is the main source of PHB. The present research encompasses the isolation, screening, characterization, optimization, and quantification of PHB producing bacterium by using different agroindustrial waste substrate. Production of PHB biopolymer from bacteria and use of biodegradable polymer products is one of the best way to counter the negative effect of synthetic polymers. *B. paramycoides* produce a good amount of PHB biopolymer and it is easily grown on agro-industrial waste substrates so, this entire work is cost-effective and creates the basement to scale up the production of PHB biopolymer.

2. MATERIALS AND METHODS

2.1. Sample Collection and Isolation of PHB Producing Bacterium

The soil sample was collected from Ranganathapura, Shivamogga district, Karnataka, INDIA in a sterile polyethylene bag and bring them into laboratory and stored at 4ºC for experimental work. The 1 g of sample was serially diluted from up to 10^{-9} dilutions and the diluted sample was inoculated on nutrient agar media using spread plate method. The plates were incubated at 37°C for 24 h. The colonies are selected based on morphological characters, the colonies are selected and maintained as pure culture on nutrient agar slants and stored at 4°C [1].

2.2. Screening of PHB Producing Bacterial Strains

The isolated bacterial colony was identified and screened by differential staining method, that is, gram staining technique and the PHB granules present in the bacterium was identified by viable colony staining technique, that is, Sudan B black staining method [10,11].

2.3. Characterization of PHB Producing Bacterium

The PHB producing bacterium was characterized based on their morphological and biochemical characters. The morphological characterization involves the cultural characters (shape, colony size, texture, and color) and microscopic characterization was done by simple and Gram's staining technique. Biochemical tests such as starch hydrolysis, gelatin hydrolysis, casein hydrolysis, citrate utilization, nitrate reduction, urease, methyl red, voges proskauer, indole, malonate utilization, H_2S production, KOH, β-galactosidase, lecithinase, lipase, catalase, oxidase, and triple iron agar tests were performed as illustrated in "Bergey's Manual of Determinative Bacteriology." The species level identification of bacterium was achieved by molecular characterization of 16S rRNA gene sequence using the primers 27F (5'-GAG AGT TTG ATC CTG GCT CAG-3') and 1541R (5'-AAG GAG GTG ATC CAG CCG C-3'). The obtained 16S rRNA gene sequence of PHB producing bacterium was compared with related sequences on NCBI, GenBank database using Basic Local Alignment Search Tool (BLAST) and the aligned sequence was submitted to GenBank, NCBI and obtained the accession no. [12].

2.4. Optimization of Parameters for PHB Production by *B. paramycoides*

2.4.1. Optimization of different media and incubation period

The 1 mL (200 CFU/mL) of PHB producing bacterial culture broth was inoculated into each 100 mL of five different broths such as nutrient broth (NA), tryptone soya broth (TSB), minimal salt broth (MSB), minimal broth (MB), and luria bertani broth (LB) tokened in 250 mL separate conical flasks and incubated at different time intervals ranging from 24 h to 96 h. After incubation at different time intervals, the culture filtrate was filtered through Whatman filter paper No. 1, bacterial biomass was washed with sterile distilled water, the purified bacterial cells were treated with hot chloroform (60°C) and transferred to separating funnel and agitated for 30 min. the separated PHB layer was collected on watch glass and dried. The yield of extracted PHB was estimated [13].

2.4.2. Influence of different temperature, pH, carbon source, nitrogen source, and carbon-nitrogen ratio (C/N ratio) on PHB production by B. paramycoides

The nutrient broth medium was prepared in five different conical flasks and 1 mL of bacterial inoculum (200 CFU/mL) was inoculated into each flask and the inoculated flasks were incubated at different temperatures, that is, 4°C, 15°C, 25°C, 37°C, and 42°C in orbital shaking incubator at 200 rpm for 72 h after incubation PHB was extracted from each flasks and yield was estimated (as described in 2.4.1). Similarly, the effect of pH (3, 5, 7, 9 and 11) on PHB production was determined with optimized temperature. The nutrient broth supplemented with different carbon source such as glucose, fructose, sucrose, maltose, and lactose at 2% concentration and different nitrogen source such as ammonium chloride, peptone, sodium nitrate, urea, and yeast extract at 1% concentration were optimized and the PHB yield was analyzed as same as mentioned above. The effect of C/N ratio on PHB production was determined by amending the nutrient broth with the top carbon and nitrogen source in the ratio of 1:1, 2:1, 4:1, 8:1, and 16:1 at optimum pH and temperature [14].

2.5. Growth of PHB Producing Bacteria on Agroindustrial By-products

Different agroindustrial by-products such as feed stock (FS), cotton cake (CK), groundnut cake (GC), coconut cake (CC), castor cake (CA), sugarcane bagasse (SCB), rice bran (RB), and areca nut husk (AH) were evaluated for choosing the suitable cheaper raw material for the maximum PHB production. These were converted to gelatinous form or jelly like form using gelatine and hydrolyzed as described by John *et al*. [15]. Obtained hydrolysate liquids from different substrates were filtered using muslin cloth to separate the solid residue from the liquid hydrolysate and the sugar molecule present in the hydrolysate was used as carbon source for the production of PHB by bacterium and the pH of all the liquid hydrolysate was neutralized to 7.0 using NaOH and sterilized at 121°C for 15 min. The hydrolysate was stored in refrigerator at 4°C for further analysis. The selected PHB producing bacterial culture was inoculated into liquid hydrolysate of different substrate and incubated at 37°C for 72 h. After incubation, the maximum growth of the bacteria was determined spectrophotometric ally by reading uninoculated hydrolysate liquid of different substrates served as blank. The bacterial cells were purified and used for quantitative assessment of PHB [5].

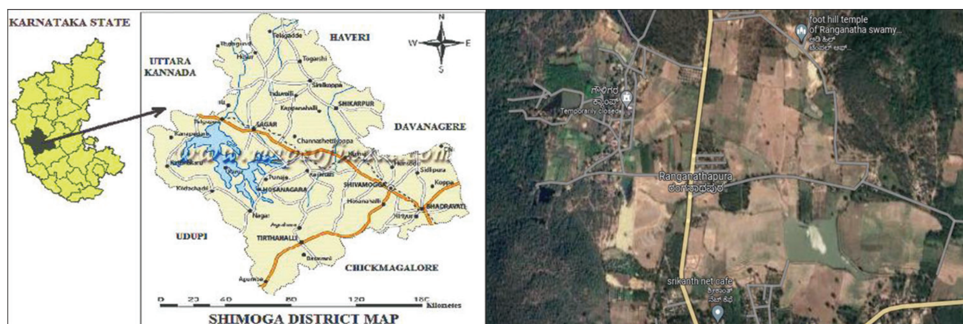

Figure 1: Sample collection site in Ranganathapura, Shivamogga district.

2.6. Quantitative Assessment of PHB

The PHB producing bacterial cells were collected by centrifugation of broth culture (liquid hydrolysate of raw material) at 11,000 rpm for 15 min and washed with acetone and ethanol. The pellet was suspended in 4% sodium hypochlorite and incubated at room temperature for 30 min. The suspension was again centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and pellet was washed with acetone and ethanol. The extracted PHB compound was dissolved in hot chloroform. 10 mL of Con. H_2SO_4 (Con. sulfuric acid) was added after the evaporation of chloroform, after addition the polymer was converted in to crotonic acid. Afterward, the compound was analyzed for the presence of PHB at 235 nm using Con. Sulfuric acid as a blank and ʎ-max was measured using Bio-spectrophotometer [12,16].

3. RESULTS AND DISCUSSION

3.1. Collection of Sample and Isolation of PHB Producing Bacteria

The dump yard comprises many dry and wet waste materials, where the microbial activity is more. It is the good source for isolation of potent PHB producing bacteria so, the soil sample was collected from dump yard site located at Ranganathapura, Shivamogga district, Karnataka, INDIA [Figure 1]. Total 65 colonies of two different bacteria were isolated from 10^{-7} dilution on nutrient agar plate [Figure 2a and b].

3.2. Screening of PHB Producing Bacterial Strains

Screening is necessary to select the potent bacteria for PHB production. Both the two isolated bacteria were Gram-positive Bacilli [Figure 3a and b]. Sudan B black staining was performed to observe the PHB granules in the bacterial cells. The observation of Sudan B black stained bacterial slides under the microscope (×100 magnification) reveals that, among the two bacteria, one contained PHB granules in their cells and another does not [Figure 4a and b]. In earlier studies of Murray *et al*., [10] and Sharma and Dhingra [17] also screened the bacterial colonies by Sudan B black staining method reveals that, the presence of black PHB granules inside the bacterial cells as similar to our study.

3.3. Characterization of PHB Producing Isolate

3.3.1. Morphological and biochemical identification of selected isolate

The selected strain was examined using morphological and biochemical characters regarding to "Bergey's Manual of Systematic Bacteriology" [18]. The isolated bacteria were Gram-positive, spore forming, rod shaped, and motile. The colonies were circular, white colored, and 2–3 mm diameter in size and they shows a smooth and opaque texture with elevated and sticky ended. Regarding to the

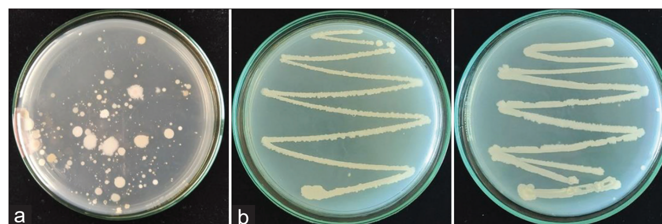

Figure 2: (a) Colonies of Bacteria on nutrient agar plate (10^{-7}), (b) pure culture of isolate 1 and isolate 2.

biochemical examinations, the bacterium showed positive results for vogues–proskauer test and triple sugar iron agar test and the bacterium has an ability to hydrolyse the starch, gelatine, casein, and utilize citrate as sole carbon source. The bacterium showed negative results for KOH, lecithinase, lipase, β-galactosidase, catalase, oxidase, urease, methyl red, indole, malonate, H_2S, and catalase [18]. The morphological and biochemical characters are summarized in Table 1. From these results, the isolated bacterium was identified as *Bacillus* sp. according to the Bergey's manual of determinative bacteriology and the obtained results are compared with the earlier findings of Hassan *et al*. [19].

3.3.2. Molecular characterization using 16s rRNA gene and analysis of phylogenetic tree

The species level identification of bacterium was achieved by molecular characterization of 16S rRNA gene sequence using the primers 27F (5'-GAG AGT TTG ATC CTG GCT CAG-3') and 1541R (5'-AAG GAG GTG ATC CAG CCG C-3'). Initial denaturation at 95°C for 2 min and Final denaturation at 95°C for 30 s and Annealing temperature was 50°C for 30 s. The PCR amplicon size was 228 base pairs and gene sequence was aligned using pair-wise alignment demonstrated maximum (100%) similarity with *B. paramycoides* isolate KUMBNGBT-33, the nucleotide sequence was deposited to GenBank NCBI and the organisms were assigned with the unique identifier, that is, MW056185 [20]. However, these outcome was compared with earlier data of Irsath *et al*. [21].

3.4. Optimization of Various Growth Conditions to Maximize the Production of PHB

3.4.1. Enhancement of different broth medium at different incubation period

The PHB producing bacterium was cultivated in different broth media by incubating at different time periods. The nutrient broth showed maximum yield, that is, 6.313 ± 0.21 g/L at 72 h time interval [Figure 5a-d]. Similar results were recorded by Sharma and Dhingra [17] and they also found that 72 h is suitable for optimum production of PHB.

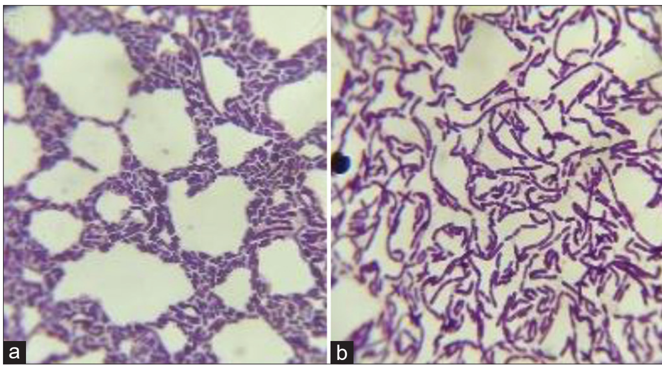

Figure 3: Gram-positive Bacilli under ×100 magnification (a) isolate 1 and (b) isolate 2.

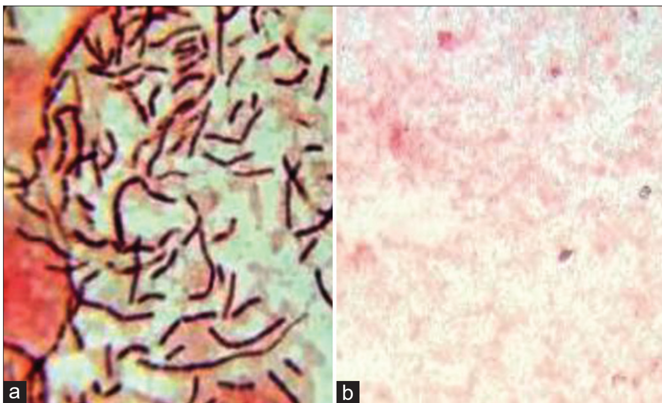

Figure 4: Sudan B black staining (a) isolate 1 (positive) and (b) isolate 2 (negative).

3.4.2. Effect of different temperature on PHB production

The maximum production of PHB was documented at 37°C after 72 h. The production of PHB decreased when the temperature exceeds above 37°C [Figure 6a]. The PHB has low polymerase activity and it affects the PHB production at high temperature [22]. The obtained result was related to earlier reports of Hamieh *et al*. [23] according to their findings optimum temperature required for the PHB production was 37°C shown by various bacteria.

3.4.3. Effect of pH on PHB production

Maintaining optimal pH is very difficult because the minor variation in the pH will alter the chemical reactions of the organisms and it also influences the change in pH required for the PHB production by *B. paramycoides*. Here, Figure 6b shows that the pH = 7 is suitable for the maximum production of PHB, that is, pH = 7:6.633 ± 0.05 g/L compared to other pH ranges. (pH = 3:0.833 ± 0.05 g/L; pH = 5:4.033 ± 0.05 g/L, pH = 9:5.366 ± 0.05 g/L, and pH = 11:3.133 ± 0.05 g/L). These outcomes were correlated with earlier findings of Grothe *et al*. [24]. They reported that pH range of 6.0–7.5 is necessary for microbial growth.

3.4.4. Effect of different carbon source on PHB production

Carbon is an essential component utilized by bacteria for their growth. Excess carbon stored in the form of granules so the carbon is the sole source for the production of PHB by the bacteria. In Figure 6c, the organism utilizes maximum amount of glucose for the production of PHB compared to other carbon sources. The bacterium showed the maximum production of PHB when amended with glucose as a carbon source, that is, 10.333 ± 0.05 g/L. The obtained result was correlated with the previous reports of Borah *et al*., [25]. According to their report glucose and fructose were found to be most suitable for growth and PHB accumulation.

Table 1: Morphological and biochemical characters of *Bacillus paramycoides* KUMBNGBT-33.

Phenotypic characters	Results
Colony morphology	
Shape	Circular
Size	2–3 nm
Texture	Sticky
Color	White
Microscopic characters	
Cell shape	Rods
Cell size	1.8–2.2 μm × 0.8–1.2 μm
Motility	Motile
Spore formation	+ve
Biochemical tests	
Citrate utilization	+ve
Nitrate reduction	+ve
Urease	-ve
Methyl red	-ve
Vogues–Proskauer	+ve
Indole production	-ve
Malonate utilization	-ve
H2S production	-ve
KOH	+ve
Lecithinase	+ve
Lipase	+ve
β-galactosidase	+ve
Catalase	-ve
Oxidase	+ve
Triple sugar iron	+ve
Hydrolysis	
Starch	+ve
Gelatine	+ve
Casein	+ve

3.4.5. Effect of different nitrogen source on PHB production

For selection of suitable nitrogen sources for PHB production by *B. paramycoides*, several different nitrogen sources were studied. In this study, ammonium chloride showed maximum PHB production followed by sodium nitrate, urea, and peptone [Figure 6d]. Yeast extract showed less growth and PHB accumulation. In the previous studies, Borah *et al*., [25] reported that *Bacillus mycoides* showed maximum PHB production when supplemented with beef extract which was added to a medium containing sucrose. Therefore, the different microorganisms utilize different nitrogen source for efficient growth and PHB production.

3.4.6. Effect of different carbon-nitrogen ratio

The maximum PHB production was found at 8:1. The low or higher carbon to nitrogen ratio will affect the growth of the PHB was shown

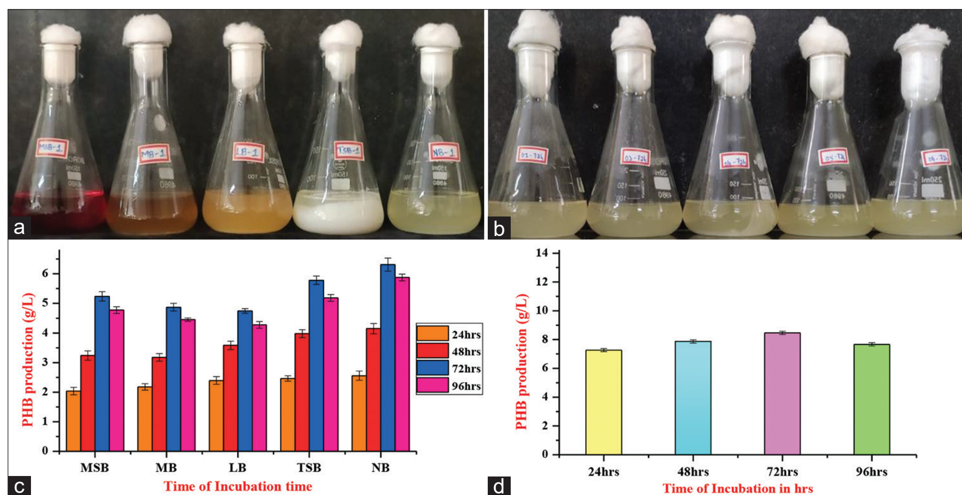

Figure 5: Optimization of isolated bacterium by different broth medium at different incubation time. In Figure 5 (a and b), nutrient broth showed maximum growth compared to other broth and in Figure 5 (c and d) 72 h indicates maximum production of polyhydroxy butyrate.

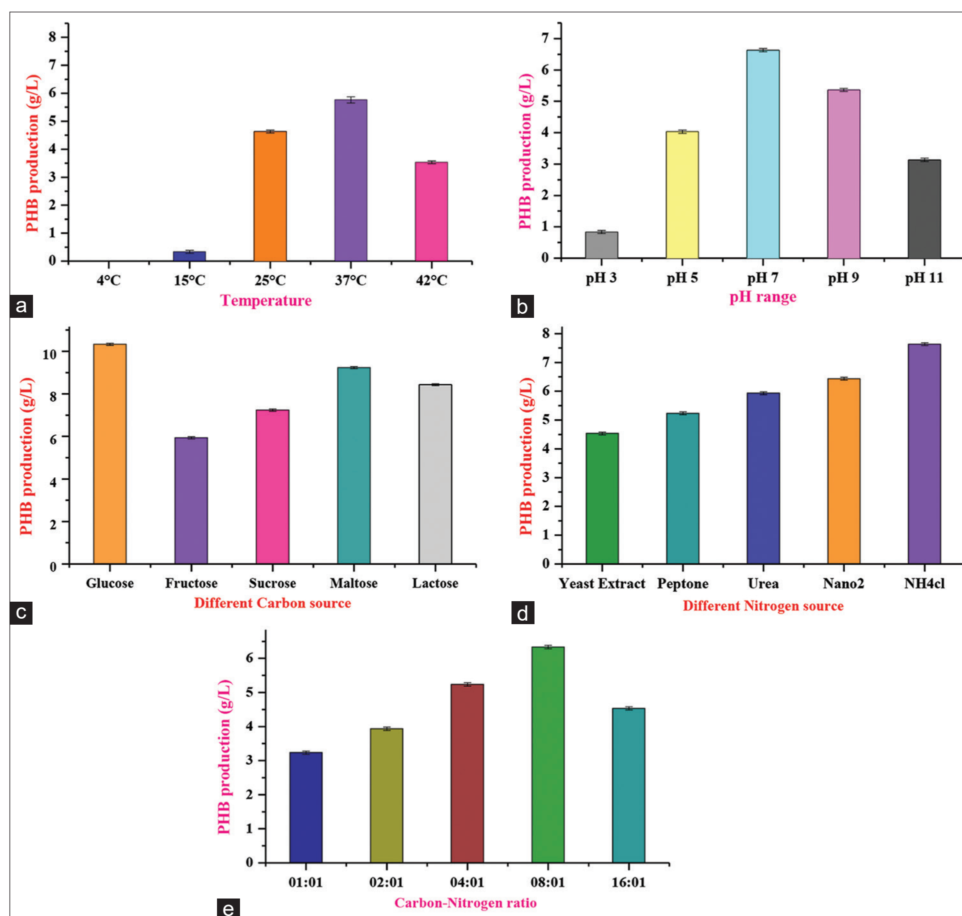

Figure 6: Production of maximum polyhydroxy butyrate. (a) effect of different temperature, (b) effect of pH, (c) effect of different carbon source, (d) effect of different nitrogen source, and (**e**) effect of carbon-nitrogen ratio (C/N ratio).

in Figure 6e. The C: N ratio increase up to 16:1 and afterward decrease in the growth was observed. Due to several enzymes, the degradable activities of the organisms decrease when the carbon to nitrogen ratio concentration increases or decreases due to their reliable nature. Similar findings were previously reported by Monika Sharma and Dhingra [17].

3.5. Production of PHB using Agro-industrial By-products

Due to their high production cost, the commercialization of bio plastic is restricted. The production cost was reduced by low cost agroindustrial wastes which acts as a sole carbon source for the production of PHB. In several reports, the different bacteria strains can utilize various carbon source was studied. According to Taidi *et al*. [26], the bacterium

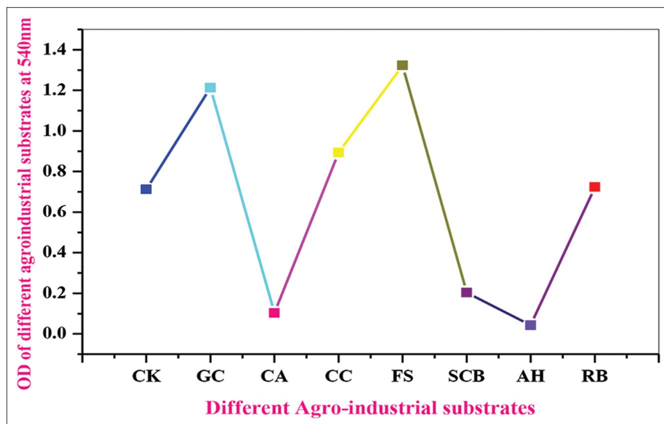

Figure 7: Growth of *Bacillus paramycoides* on hydrolysate of different agro-industrial substrate.

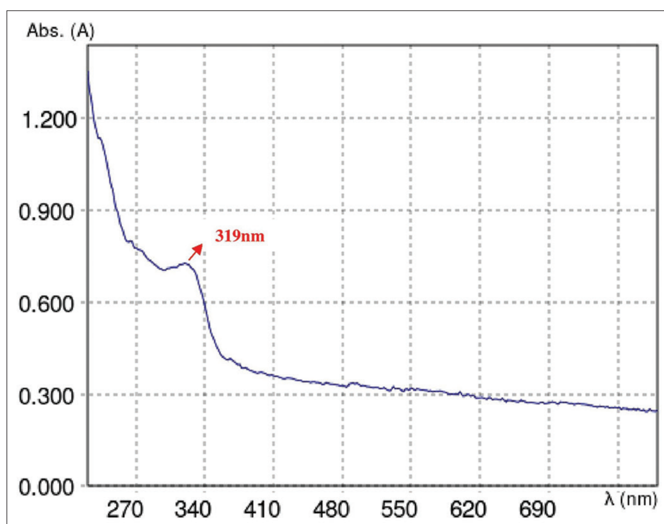

Figure 8: Quantitative assessment of isolated polyhydroxy butyrate using bio-spectrophotometer.

Ralstonia Eutropha utilizes glycerol as a carbon source [27]. Different low-cost substrates such as feeds, CK, GC, CC, CA, SCB, RB, and AH were replacing the artificial substrates used for growth of PHB producing bacteria. The low-cost substrate, that is, FS shows maximum yield for growth of PHB produced by *B. Paramycoides* compared to CK, GC, CC, CA, RB, sugar cane bagasse, and arecanut husk shown in Figure 7. These findings were previously reported by Van-Thuoc *et al*. [27]. According to their studies, wheat bran showed maximum yield for PHB production.

3.6. Quantitative Assessment of PHB

In quantification method, the breakdown of PHB polymer into its monomers because of its acidic nature present in the compound. These converted monomers into compound were measured using crotonic acid and con. H_2SO_4 was used as a standard. The ʎ-max of the PHB producing strain was 319nm measured at 235nm using Bio-spectrophotometer was shown in Figure 8. The maximum amount of PHB present in the sample was directly associated with amount of UV radiation absorbed by crotonic acid. These findings were compared with earlier results of Reshma *et al*. [12].

4. CONCLUSION

A PHB-producing bacterium *B. paramycoides* KUMBNGBT-33 was isolated from scrap yard soil sample collected from Ranganathapura, Shivamogga district and screened for PHB production using differential and viable staining techniques. In screening method, the isolated bacteria were positive for both grams staining and Sudan B black staining techniques. New bacterial isolate was screened, identified and characterized by morphological, biochemical, and molecular characters. From the morphology and biochemical analysis, the isolated organisms belong to the genus *Bacillus* spp. and confirmed by molecular characterization and identified as *B. paramycoides*. The growth conditions were optimized using various parameters such as media, incubation time, temperature, pH, different carbon, and nitrogen sources and different carbon to nitrogen ratio. The stain utilizes different optimization conditions for growth and production utilizing various carbon and nitrogen sources. Different agricultural by-products were used to maximize the production of PHB using glucose molecules. Production of PHB was quantified using spectrophotometric studies. Agricultural wastes used for cultivating the PHB can be commercialized and targeted by global market and used as a future bioplastic.

5. AUTHORS' CONTRIBUTIONS

NG: Conceptualization, data collection, investigation, writing-original draft, and editing. AS: Data rectification, investigation, review, and editing. MD: Data editing and phylogenetic analysis. S-HV: Data correction, review and editing. BT: Design and correction, supervision, review, and editing.

6. FUNDING

For the conduct of this research and experimental charges, the amount was funded by Backward Welfare Department (BCWD), Govt. of Karnataka.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

10. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

11. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional privileges in the published official affiliation.

REFERENCES

1. Mikkili I, Karlapudi AP, Venkateswarulu TC, Babu DJ, Nath SB, Kodali VP. Isolation, screening and extraction of polyhydroxybutyrate

(PHB) producing bacteria from sewage sample. Int J Pharm Tech Res 2014;6:850-7.

2. Lee KM, Gilmore FD. Formulation and process modelling of biopolymer (Polyhydroxyalkanoates: PHAs) production from industrial wastes by a novel crossed experimental design. Process Biochem 2005;40:229-46.
3. Steinbuchel A. Polyhydroxy alkanoic acids. In: Byrom D. Biomaterials. Basingstoke: Macmillan publishers Ltd.; 1991.
4. Naheed N, Jamil N. Optimization of biodegradable plastic production on sugar cane molasses in *Enterobacter* sp. SEL2. Braz J Microbiol 2014;45:417-26.
5. Ramadas NV, Singh SK, Soccol CR, Pandey A. Polyhydroxybutyrate production using agro-industrial residue as substrate by *Bacillus sphaericus* NCIM 5149. Braz Arch Biol Technol 2009;52:17-23.
6. Madison LL, Huisman GW. Metabolic engineering of poly(3-hydroxyalkanoates): From DNA to plastic. Microbiol Mol Biol Rev 1999;63:21-53.
7. Reinecke F, Steinbuchel A. *Ralstonia eutropha* strain H16 as a model organism for PHA metabolism and for biotechnological production of technically interesting polymers. J Mol Microbiol Biotechnol 2009;16:91-108.
8. Chee JW, Amirul AA, Muhammad TS, Majid MI, Mansor SM. The influence of copolymer ratio and drug loading level on the biocompatibility of P(3HB-co-4HB) synthesized by *Cupriavidus* sp. (USMAA2-4). Biochem Eng J 2008;38:314-8.
9. Dos Santos AJ, Valentina LV, Schulz AA, Duart MA. From obtaining to degradation of PHB: Material properties. Ingeniería Y Ciencia 2017;13:269-8.
10. Murray RG, Doetsch RN, Rainbow CF. Determinative and cytological light microscopy. In: Methods for General and Molecular Bacteriology. Washington, DC: American Society for Microbiol; 1994. p. 21-41.
11. Mekala M, Rajendran R, Suganya K. Comparative studies on cell growth and production of PHB (poly hydroxyl butyrate). Int J Environ Sci 2013;3:2261-76.
12. Reshma P, Sonal S, Vatsala S. Screening and isolation of polyhydroxy butyrate producers. Int J Res Appl Sci Eng Technol 2017;5:1259-64.
13. Lathwal P, Nehra K, Singh M, Jamdagni P, Rana JS. Optimization of culture parameters for maximum polyhydroxybutyrate production by selected bacterial strains isolated from rhizospheric soils. Pol J Microbiol 2015;64:227-39.
14. Chandani N, Mazumder PB, Bhattacharjee A. Biosynthesis of biodegradable polymer by a potent soil bacterium from a stress-prone environment. J Appl Biol Biotechnol 2018;6:54-60.
15. John RP, Nampoothiri KM, Pandey A. Solid-state fermentation for L-lactic acid production from agro wastes using *Lactobacillus delbrueckii*. Proc Biochem 2006;41:759-63.
16. Paul S, Sasikumar CS, Balakumuran MD. Optimization, purification and characterization of polyhydroxybutyrate (PHB) produced by *Bacillus* cereus isolated from sewage. Int J Chem Tech Res 2007;10:884-904.
17. Sharma M, Dhingra HK. Isolation and culture conditions optimization for PHB production by *Pseudochrobactrum asaccharolyticum*. Int J Sci Res 2013;4:1895-901.
18. Krieg NR, Holt, JG. Bergey's Manual of Systematic Bacteriology. 1st ed., Vol. 1. Williams and Wilkins: Baltimore; 1984.
19. Hassan MA, Haroun BM, Amara AA, Serour EA. Production and characterization of keratinolytic protease from new wool-degrading *Bacillus* species isolated from Egyptian ecosystem. Biomed Res Int J 2013;2023:175012.
20. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4:406-25.
21. Irsath H, Santhosh VH, Hemalatha V, Vikramathithan M, Dhanasekar R, Dhandapani R. Production and optimization of polyhydroxybutyrate using *Bacillus subtilis* BP1 isolated from sewage sample. Int J Pure Appl Biosci 2015;3:158-66.
22. Singh G, Kumari A, Mittal A, Yadav A, Aggarwal NK. Poly β-hydroxybutyrate production by *Bacillus subtilis* NG220 using sugar industry waste water. Biomed Res Int 2013;2013:952641.
23. Hamieh A, Olama Z, Holail H. Microbial production of polyhydroxybutyrate, a biodegradable plastic using agro-industrial waste products. Glob Adv Res J Microbiol 2013;2:54-64.
24. Grothe E, Moo-Young M, Chisti Y. Fermentation optimization for the production of poly-(β-hydroxybutyric acid) microbial thermoplastic. Enzym Microbial Technol 1999;25:132-41.
25. Borah B, Thakur PS, Nigam JN. The influence of nutritional and environmental conditions on the accumulation of poly-beta hydroxybutyrate in *Bacillus mycoides* RLJ B-017. J Appl Microbiol 2002;92:776-83.
26. Taidi B, Anderson AJ, Dawes EA, Byrome D. Effect of carbon source and concentration on the molecular mass of poly (3-hydroxybutyrate) produced by *Methylobacterium extorquens* and *Alcaligenes* eutrophus. Appl Microbiol Biotechnol 1994;40:786-90.
27. Van-Thuoc D, Quillaguaman J, Mamo G, Mattiasson B. Utilization of agricultural residues for poly (3-hydroxybutyrate) production by *Halomonas boliviensis* LC1. J Appl Microbiol 2007;104:420-8.

How to cite this article:
Nandish G, Akarsh S, Manjunatha D, Sowmya HV, Thippeswamy B. Utilization of agro-industrial wastes as raw materials for the biosynthesis of polyhydroxy butyrate biopolymer from *Bacillus paramycoides* strain KUMBNGBT-33. J App Biol Biotech. 2023;11(4):178-184.
DOI: 10.7324/JABB.2023.11514