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Impact of physico-chemical parameters on synthesis of Polyhydroxybutyrate (PHB) produced from *Bacillus toyonensis* KUMBNGBT-62

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Abstract

The biosphere is seriously threatened by the various very hazardous chemicals employed in the manufacturing of synthetic plastic. A bio-derived and biodegradable polyester polymer, polyhydroxybutyrate (PHB) is created by a variety of species, including P3HB, P4HB, PV, PH, and PHO, as well as their copolymers. The PHB-producing bacterium was isolated from soil sample collected at garbage site in Jogfalls, Sagar Taluk, Shivamogga District, Karnataka, India. The bacteria was identified based on its physical characteristics and screened by using several staining techniques and species level identification was achieved using the 16S r-RNA gene sequencing method. A variety of circumstances were used to analyse the fabrication of PHB. Low-cost agro based products were used to produce PHB on a large scale, and UV-visible spectrophotometric analysis was used to calculate the amount of PHB contained in the removed bacterial cells. *Bacillus toyonensis* KUMBNGBT-62 was gram positive, motile and spore forming bacterium and confirmed by using 16S r-RNA sequence and deposited to GenBank, NCBI and assigned with accession No. OK136177. Additionally, nutritional broth medium, a 72-hour incubation period, 37°C temperature, pH 7.0, glucose as a carbon source, ammonium chloride as a nitrogen source and a 4:1 carbon-nitrogen ratio supports highest accumulation of PHB. At λ-max of 290 nm and liquid hydrolysate of feed stock, the highest level of PHB formation was detected. *Bacillus toyonensis* is demonstrated to maximize PHB production using low-cost residues, reducing production costs and serving as a model organism for large-scale bio-based plastics production.

Keywords: Polyhydroxybutyrate; *Bacillus toyonensis*; Agro-industrial by-products; liquid hydrolysate and UV-visible spectrophotometer

1. Introduction

The global increase in plastic manufacturing and consumption, resulting in over 400 million metric tons of plastic waste annually, poses environmental challenges and impacts living organisms. Governments are exploring alternatives like organic biopolymers and microbial derived polymers for simpler synthesis and purification processes [1, 2, 3].

Different microbial species and plants produce different amounts of chlorophyll accumulation when it comes to producing polyhydroxybutyrate (PHB). The high cost of production makes production and marketing difficult. Developing modified strains, maximizing growing conditions, streamlining fermentation procedures, and employing reasonably priced substrates are some strategies to reduce expenses [4, 5, 6].

Bacillus spp. are commonly used bacteria to produce PHB co-polymers from various renewable agro-industrial waste products, such as cotton cake, coconut cake, castor cake, feed stock, sugar cane bagasse, arecanut husk and rice bran [7].

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PHB granules can accumulate in more than 70 different bacterial genera, such as *Alcaligenes*, *Azotobacter*, *Achromobacter*, *Bacillus* and *Pseudomonas*. When the situation is favorable, soil bacteria can produce PHB. Under stressful conditions, *Bacillus* species create biopolymers such as PHB, which have potential uses in biomaterials, drug delivery, and biodegradable plastic [8, 9].

The current study focused on improving strain performance to produce PHB biopolymer by utilizing agro-industrial wastes as a more cost-effective source of raw materials. The research entails the utilization of *B. toyonensis*, a bacterium known for its ability to produce PHB. PHB biopolymer manufacturing is a viable strategy for mitigating the adverse consequences associated with synthetic polymers, which contribute to environmental contamination due to their non-biodegradable properties.

2. Material and methods

2.1. Sample collection and isolation of PHB producing bacteria

Production of PHB using bacteria was achieved by isolating it from a soil sample obtained from a garbage site in Jogfalls, Sagar Taluk, Shivamogga District, Karnataka. The sample was serially diluted up to 10^{-9} dilutions using sterile distilled water. After that, 10^{-6}, 10^{-7} and 10^{-8} dilutions were applied onto a nutrient agar medium using spread plate technique and incubated at 37 °C for 24 h. Based on their shape and color, the colonies were chosen and maintained on nutrient agar slants and stored at 4 °C in refrigerator as working stock [10].

2.2. Screening of PHB producing isolates

The bacteria were grown on nutrient agar (Himedia laboratories-India) medium, supplemented with glucose (2 % w/v) as the exclusive carbon source. The isolates that produced PHB were confirmed by using Sudan black B (SBB) staining method.

2.3. Morphological and biochemical classification of PHB producing bacterium

Bacteria responsible for PHB production was identified based on its morphological and biochemical properties. The form, size, texture and color of the isolate were examined using the guidelines established in Bergey's manual of determinative bacteriology [13]. Under ideal conditions, the bacteria were grown on nutrient agar medium. The study concentrated on microscopic characteristics, specifically endospore and easy staining methods. Various biochemical tests were employed for bacterium identification [14].

2.4. Genomic characterization of PHB producing bacterium

For the purpose of amplifying the 16S rRNA gene by PCR, 20 µg/µL of isolated genomic DNA was utilized. This DNA was certified using gel electrophoresis using the Chromous Biotech gDNA minispin kit and CTAB methods. Universal primers, namely 27F (5′-GAG AGT TTG ATC CTG GCT CAG-3′) and 1492R (5′-AAG GAG GTG ATC CAG CCG C-3′).were used for the sequence amplification [15]. In order to examine the 16S rRNA sequence, the National Centre for Biotechnology Information (NCBI) supplied the Basic Local Alignment Search Tool (BLAST). Phylogenetic tree construction was done using maximum likelihood analysis in Bio-edit software, after pairwise alignment was carried out with ClustalW. Bootstrapping at 500 was used when analyzing the phylogenetic tree with FigTree (v1.4.4) software [16, 17].

2.5. Optimization of cultural conditions for PHB production

2.5.1. Optimization of different culture media at different incubation period for PHB production

A selection of five distinct broths, namely nutritional broth (NB), tryptone soya broth (TSB), minimal salt broth (MSB), minimal broth (MB) and luria-bertani broth (LB) were chosen to produce PHB. The isolate *B. toyonensis*, was subjected to inoculation and incubation for various durations spanning 24 to 96 h. PHB development and production analysis was conducted at 24 h intervals, with the subsequent recording of PHB yield [18].

2.5.2. Effect of temperature on PHB production

The impact of temperature on the generation of PHB was assessed by subjecting the process to various temperature conditions. The culture of *B. toyonensis* was inoculated and subjected to incubation at various temperatures, specifically 4 °C, 15 °C, 25 °C, 37 °C and 42 °C for 72 h [18].

2.5.3. Effect of pH on PHB production

The choosen isolate was cultivated in the nutritional broth supplemented with the most suitable carbon and nitrogen sources to optimize the pH conditions. These sources were selected to cover a range of pH values, specifically 3.0, 5.0, 7.0, 9.0 and 11.0. The cultivation process was carried out at the temperature that had been previously determined as optimal [19].

2.5.4. Effect of different carbon sources on PHB production

The impact of various carbon sources on the formation of PHB was assessed by cultivating the chosen isolates in 100 mL of nutrient broth medium, supplemented with different carbon sources, including glucose, fructose, sucrose, maltose and lactose, at a concentration of 2 % (w/v). The chosen isolate was introduced into a liquid medium and incubated at 37 °C for 72 h. The selection of the optimal carbon source was determined based on the yield [19].

2.5.5. Effect of different nitrogen source on PHB production

The impact of various nitrogen sources was assessed by cultivating the chosen isolate in a 100 mL nutritional broth medium that was supplemented with diverse nitrogen sources, including ammonium chloride (NH_4Cl), peptone, sodium nitrate ($NaNO_3$), urea and yeast extract at a concentration of 1 % (w/v). The bacterial culture was introduced into a liquid medium enriched with various nitrogen sources and then incubated at a temperature of 37 °C for 72 h. The selection of the optimal nitrogen source was determined based on the yield obtained from this analysis [18]

2.5.6. Effect of different carbon-to-nitrogen ratio (C:N ratio) on PHB production

The impact of the carbon-to-nitrogen (C: N) ratio on the formation of PHB was assessed by supplementing the nutritional broth with varying carbon-to-nitrogen ratios. Specifically, the ratios tested were 1:1, 2:1, 4:1, 8:1 and 16:1. These experiments were conducted under optimal pH and temperature conditions. Subsequently, the optimal carbon-nitrogen ratio was calculated based on the resulting yield [19].

2.6. Production of PHB using low cost agro-industrial wastes as substrates

2.6.1. Oil waste substrates

The production of PHB was conducted using low-cost substrates such as feed stock, cotton cake, groundnut cake, coconut cake and castor cake. The basal media was prepared by suspending 150g of oil waste in 1200 mL distilled water and boiling at 100 °C for 30 minutes. The residual substances were extracted and filtered to obtain liquid hydrolysate. The hydrolysate was supplemented with glucose and ammonium chloride, heated to 70 °C, sterilized and used for PHB-producing bacteria cultivation. The selected PHB-producing bacterial isolate was inoculated into 1000mL liquid hydrolysate and incubated at 37 °C for 72 hours. The spectrophotometric measurement of PHB produced by bacteria using various oil waste liquid hydrolysate was conducted using optical density at 540 nm. [20, 21].

2.6.2. Agricultural wastes

The drying and powdering of agricultural wastes like sugarcane bagasse, rice bran and areca nut husk. The substrates were hydrolyzed using 1N sulphuric acid and heated at 60 °C for 10 minutes. The liquid hydrolysate was filtered and pH was adjusted to 7.0. The basal media were autoclaved at 121 °C for 20 minutes. A PHB-producing bacterial isolate was inoculated into the liquid hydrolysate and incubated at 37 °C for 72 hours. The amount of PHB produced by bacteria on basal media was examined spectrophotometrically by determining the optical density at 570 nm [22].

2.7. Characterization of the Polyhydroxybutyrate biopolymer

The chemical nature of the PHB biopolymer produced by *Bacillus toyonensis* was analysed and characterized by Fourier transform infrared (FT-IR) spectroscopy and Gas chromatography-mass spectrometry (GCMS) analysis

3. Results

3.1. Isolation of PHB producing microorganisms from scrap yard soil samples

The production of PHB using bacteria was achieved by isolating it from a soil sample obtained from a garbage site in Jogfalls, Sagar Taluk, Shivamogga District, Karnataka as it is considered a reliable source for isolating bacteria with a high potential for making PHB. A total of six distinct bacterial strains were obtained from a dilution of 10-7 on a nutrient agar plate ((Fig. 1)).

Figure 1 Geographical mapping of sample collection site located at Jog falls, Sagar Taluk, Shivamogga district, Karnataka

3.2. Screening of PHB producing isolates

The confirmation of the synthesis of PHB in the six isolates was predominantly conducted using SBB staining. The presence of polymer inclusion structures within the bacterial cells was noticed during the SBB staining process. All of the isolates exhibited positive results indicating the presence of PHA granules, which were seen as black inclusion bodies (Fig. 2). The screening results revealed the presence of intracellular PHB granules within the bacterial cells.

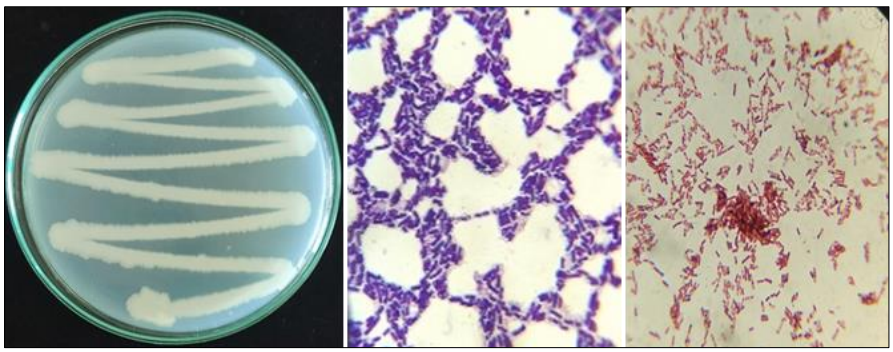

Figure 2 Isolation and screening of bacterial isolate using Gram staining and Sudan black B staining technique

3.3. Production and extraction of PHB

An initial experiment was done to test the productivity of the six isolated strains to produce an efficient amount of PHB. The production of polymer by using six strains was achieved on NB supplemented with (2 % w/v) glucose as a carbon source and (0.2 % w/v) (NH4)2SO4) as a nitrogen source. After incubation at 37 °C for three days, cells were harvested and dry weights were obtained. Isolate PHB 5 showed the highest PHB production with 5.83±0.005 g/L CDW (Concentration of Dry Weight), which is significantly ($P < 0.05$) higher than the other isolates. On the contrary, strains PHB 1, PHB 2, PHB 3, PHB 4 and PHB 6 showed 1.13±0.005 g/L, 2.33±0.005 g/L, 2.86±0.011 g/L, 3.31±0.005 g/L, 3.37±0.005 g/L CDW respectively that reflects the wide variation in the polymer productivity among the studied isolates (Fig. 3).

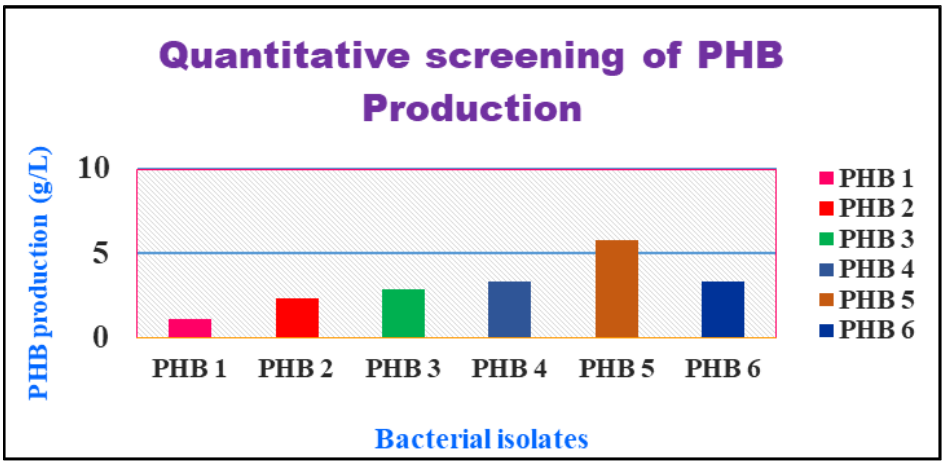

Figure 3 Quantitative screening PHB production by *Bacillus toyonensis* KUMBNGBT-62 using solvent extraction method

3.4. Morphological and biochemical characterization of PHB producing bacterium

The morphological characteristics of PHB-producing bacterium are summarized in Table 1. According to morphological characters, the isolate is gram-positive, spore-forming and motile. According to biochemical analysis, the isolate is positive for Vogues–Proskauer, KOH, lecithinase, lipase, β-galactosidase, oxidase and triple sugar iron agar test. The bacterium can hydrolyze the starch, gelatine, casein, also reduce the nitrate and utilize citrate as sole carbon source. On the divergent, it damaged urease, methyl red, iodole, malonate, H_2S and catalase tests (Table 2). From these results, the isolated strain was identified as Bacillus using Bergey's manual of determinative bacteriology [13, 23, 24].

Table 1 Morphological and microscopic characteristics of Bacillus cereus KUMBNGBT-34

Colony Morphology	**Results**
Shape	Circular
Size	5-10nm
Texture	Spongy
Color	White
Microscopic Characters	
Cell Shape	Rods
Cell size	1×3-4 µm
Motility	+
Spore formation	+

Table 2 Biochemical Characterization of Bacillus cereus KUMBNGBT-34

Biochemical tests	Results
Starch hydrolysis	+
Gelatine hydrolysis	+
Casein Hydrolysis	+
Citrate Utilization	+
Nitrate reduction	+
Urease	-
Methyl red	-
Vogues–Proskauer	+
Indole production	-
Malonate utilization	-
H_2S production	-
KOH	+
Lecithinase	+
Lipase	+
β-Galactosidase	+
Catalase	-
Oxidase	+
Triple sugar iron	+

3.5. Molecular characterization of PHB producing bacterium

The 16S rRNA gene was detected using Sanger's dideoxy nucleotide sequencing technique. The Blastn analysis yielded a significant multiple sequence alignment, with the selected strain having a length of 680 base pairs. The acquired gene sequence underwent pair-wise alignment, revealing a complete match of 100 % identity with *Bacillus toyonensis* strain KUMBNGBT-62 and deposited to the NCBI GenBank and issued the accession number OK136177. Phylogenetic tree was constructed using maximum likelihood analysis using reference sequences retrieved from NCBI and FigTree (v1.4.4) software was used to analyse the Phylogenetic tree (Fig. 4).

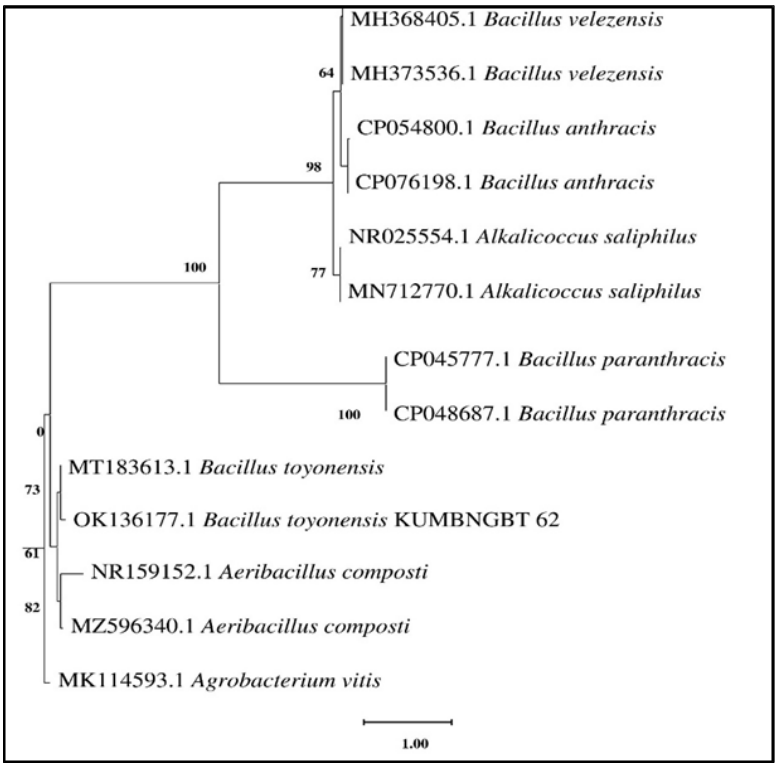

Figure 4 Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing relationships between *Bacillus toyonensis* KUMBNGBT-62 and its closely related type strains with *Agrobacterium vitis* as an out group. Numbers at the nodes indicate levels of bootstrap support based on an analysis of 500 resampled datasets; only values above 50% are given. The scale bar indicates 1.00 substitutions per nucleotide position

3.6. Optimization of cultural conditions for PHB production

3.6.1. Optimization of different culture media at different incubation period for PHB production

The *B. toyonensis* strain was cultivated using five distinct broths to assess PHB production. The NB exhibited the highest yield of 6.403±0.09 g/L, in comparison to the other broths tested, namely MSB (3.183±0.07 g/L), MB (3.566±0.09 g/L), LB (4.276±0.11 g/L) and TSB (5.136±0.11 g/L) (Fig. 3a). The appropriate incubation duration was examined by subjecting the strain to various time intervals (24, 48, 72 and 96 h) and the resulting production of PHB was documented and presented in Figure 3b [18]. The research determined that the most favorable duration for the generation of PHB by *B. toyonensis* was 72 h, resulting in a yield of 7.833±0.05 g/L. This yield was compared to various time intervals, namely 24 h (5.966±0.11 g/L), 48 h (6.833±0.05 g/L) and 96 h (6.266±0.11 g/L).

3.6.2. Effect of temperature on PHB production

Figure 3c illustrates the variation in PHB production by *B. toyonensis* across different temperatures. The bacterium *B. toyonensis* exhibited the highest production of PHB at a temperature of 37 °C, with a value of 6.333±0.05 g/L. In comparison, the production levels at other temperatures were as follows: 4 °C-0±0 g/L, 15 °C-0±0 g/L, 25 °C-4.666±0.1 g/L and 42 °C-3.466±0.11 g/L.

3.6.3. Effect of pH on PHB production

The pH value that yielded the highest generation of PHB was determined to be pH 7, resulting in a concentration of 7.033±0.05 g/L (Fig. 3d). The yields observed at pH 5 and pH 9 were found to be moderate in comparison to the yield at pH 7, precisely weighing 3.233±0.05 g/L and 5.933±0.05 g/L, respectively. The pH levels of 3 and 11 exhibited reduced productivity, yielding 0.333±0.05 g/L and 2.433±0.05 g/L respectively. Based on the findings presented, it can be concluded that the pH levels, characterized by their acidic and essential nature, are not conducive to synthesizing PHB [25].

3.6.4. Effect of different carbon source on PHB production

The impact of various carbon sources, namely glucose, fructose, sucrose, maltose and lactose, on the yield of PHB was examined (Fig. 4a). The study evaluated various carbon sources for their impact on PHB production by *B. toyonensis*. The results indicated that media supplemented with glucose exhibited the highest PHB production (13.133±0.05 g/L) compared to other carbon sources, including fructose (10.833±0.05 g/L), sucrose (9.433±0.05 g/L), maltose (12.333±0.05 g/L) and lactose (8.233±0.05 g/L) [26]. Due to its molecular structure as a simple sugar, glucose is readily utilized and promotes the formation of PHB [27].

3.6.5. Effect of different nitrogen source on PHB production

Figure 4b illustrates the impact of various nitrogen sources, namely yeast extract, peptone, urea, sodium nitrate and ammonium chloride, on PHB synthesis. Ammonium chloride was identified as the most effective nitrogen source for producing PHB by *B. toyonensis*, yielding 9.033±0.05 g/L. In comparison, other nitrogen sources such as yeast extract (5.633±0.05 g/L), peptone (6.166±0.11 g/L), urea (6.933±0.05 g/L) and sodium nitrate (7.833±0.05 g/L) exhibited lower yields [28].

3.6.6. Effect of different carbon-to-nitrogen ratio (C:N ratio) on PHB production

The bacterium exhibited the highest PHB production at a carbon-to-nitrogen ratio of 4:1, with a measured value of 7.433±0.05 g/L. This value surpassed the PHB production observed at all other tested carbon-to-nitrogen ratios, including 01:01 (4.033±0.05 g/L), 02:01 (4.533±0.05 g/L), 08:01 (6.333±0.05 g/L) and 16:01 (5.533±0.05 g/L) (Fig. 4c).

3.7. Production of PHB using low cost agro-industrial wastes as substrates

3.7.1. Oil waste substrates

Microorganisms produce PHB using low-cost oil waste as a substrate, effectively using these materials while providing vital nutrients. The oil waste substrates chosen for analysis were examined using a bio-spectrophotometer set at a wavelength of 540 nm. Among five oil wastes, feedstock exhibited the highest level of PHB production, with a mean concentration of 1.436±0.005 g/L. Cotton cake (1.173±0.005 g/L), ground nut cake (1.306±0.01 g/L) and coconut cake (0.723±0.005 g/L) exhibited a moderate level of PHB build up in comparison to the feedstock. The synthesis of PHB from castor cake (0.096±0.01 g/L) exhibited a comparatively low yield compared to other oil wastes [21].

3.7.2. Agricultural wastes

The utilization of agro-industrial wastes as primary resources for the synthesis of PHB has the potential to effectively mitigate both the financial burden associated with production and the environmental impact stemming from pollution. *B. toyonensis* utilizes various carbon sources to facilitate the accumulation of PHB. The highest PHB production was identified in rice bran, with a concentration of 0.663±0.005 g/L. Similar level of PHB was also found in sugarcane bagasse, with a concentration of 0.493±0.005 g/L. The yield of areca nut husk (0.226 ±0.01 g/L) was comparatively lower when compared to other agricultural wastes [22].

3.8. Mass production, purification and quantification of PHB

The PHB producing bacteria was mass cultivated in oil waste substrate producing maximum yield of PHB i.e., feedstock. The obtained cells were quantified and the obtained bacterial cells showed λmax at 0.075 nm and it confirms the presence of PHB. The obtained chloform containing cells were characterized.

3.8.1. FT-IR Analysis

The FT-IR spectra of *B. toyonensis* was recorded using KBr pellets in the region 4000-400 cm-1 on a FTIR- BRUKER IR spectrometer. In the IR spectra C=O functionality present in the region of 1658-1656 cm-1. The strong C-H absorption bands could be seen in the range between 2926-2925 cm-1 and a CH3 absorption band present in the region at 2854 cm-1 respectively. In the range of 1153–1152 cm-1, the C–O stretching functional group was confirmed (Fig. 5a).

3.8.2. Gas chromatography-mass spectrometry (GCMS) analysis

The PHB sample was subjected to methanolysis and the methylesters displayed fragmentation patterns in GCMS, allowing to identify the derivatives of PHB produced by *B. toyonensis*. The outcomes were compared to those of standard results in the PubChem database and sigma reference. The chromatogram of the biopolymer extract revealed five major peaks with retention times of 7.085 min, 8.509 min, 21.577 min, 27.316 min and 29.960 irrespectively. They are

Dimethyl Sulfoxide, Methane, tris (methylthio)-, 4-Bromo-3-chloroaniline, 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl) and 2H-1,3,4-Benzotriazepine-2-thione, 5-benzyl-1. Table 3 shows the principal peaks and supports the presence of polyhydroxy butyric acid (PHB) in *B. toyonensis* extract. The x-axis in Fig. 5b represents retention time (min) and the y-axis represents signal intensity.

Table 3 Mass spectrum analysis of *Bacillus toyonensis* KUMBNGBT-62

Peak	R Time	I. Time	F. Time	Area	Area%	Name
1	7.085	7.033	7.192	969407	74.64	Dimethyl Sulfoxide
2	8.509	8.442	8.733	172286	13.26	Methane, tris(methylthio)-
10	21.577	21.300	21.600	17676	1.36	4-Bromo-3-chloroaniline
14	27.316	27.267	27.417	14943	1.15	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)
16	29.960	29.883	30.058	24001	1.85	2H-1,3,4-Benzotriazepine-2-thione, 5-benzyl-1

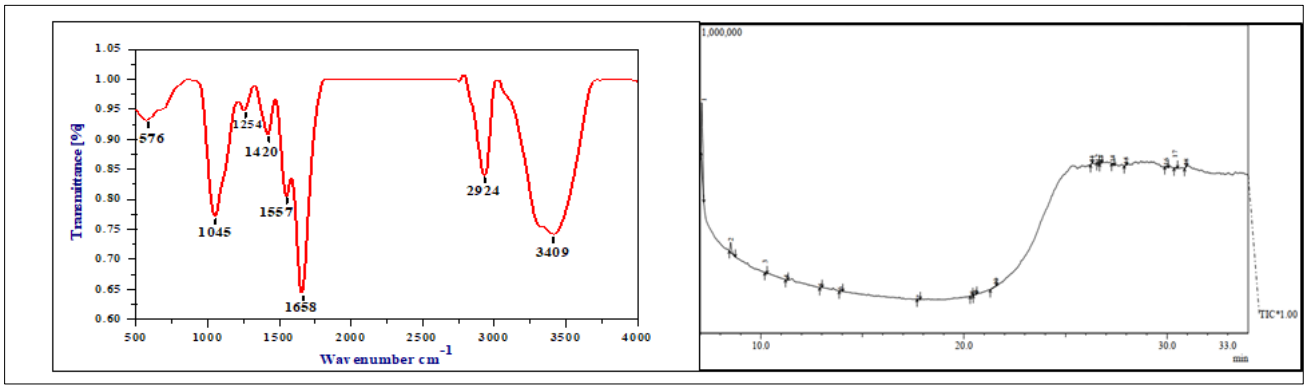

Figure 5 a. Characterization of extracted PHB using FT-IR spectrum; b. Mass spectrum of the compound extracted using *Bacillus toyonensis* KUMBNGBT-62

4. Discussion

The bacterium responsible for PHB production was found in a soil sample, typically belonging to the genus *Bacillus*, which has been identified as an efficient PHB producer.

The isolated bacterium was screened by using Sudan black B and Gram staining to identify the most potent PHB-producing bacteria. As PHB levels increased, so did the fluorescence intensity. Six isolates had negative results, while only one sample showed positive results. The bacteria were then screened again using solvent extraction after initial screening.

The selected bacterium was identified through morphological and biochemical tests. The 16s rRNA sequencing method confirmed the species level identification, confirming the bacterium's identification as *B. toyonensis*, based on various parameters and biochemical tests.

The isolate's maximum PHB production was achieved at 72 hours when cultured on nutrient agar media supplemented with carbon, indicating that PHB production is linked to growth conditions and a strain's growth, possibly due to lack of necessary nutrients. This finding aligns with Monika Sharma et al.'s [19] earlier study, which found 72 hours as the optimal incubation period for *Pseudochrobactrum asscharolyticum*.

The optimal temperature for PHB growth and accumulation is 37 °C, with PHB production increasing until 37 °C and decreasing as temperature increases. Previous studies have found 33 °C to be the optimum temperature for PHB production. Temperature variance affects PHB content, as extreme temperatures slow down metabolic activity, ultimately reducing PHB production ability [19].

The optimal pH range for PHB production by the isolate is 7, as the acidic and basic nature of the pH is not suitable for PHB production, as per the findings of Elsayed et al. [26], who found a pH of 7-7.5 as optimal.

The study found that glucose, a glucose-supplemented medium, yielded the highest PHB production after 72 hours of incubation. This finding is consistent with previous research by Khanna et al [27], who found that *Ralstonia eutropha* produced maximum biomass supplemented with fructose. Ammonium chloride was found to be the best nitrogen source for *B. toyonensis*. In Borah et al [28] study, the *Bacillus mycoides* can produce maximum PHB when supplemented with beef extract. The optimal carbon and nitrogen ratio for maximum PHB accumulation is 4:1, which is slightly related to Monika Sharma et al.'s [19] 16:1 C: N ratio, which found the 16:1 ratio optimal for PHB production.

In present study, maximum production of PHB was observed in feed stock, followed by cotton cake, ground nut cake, and coconut cake. Castor cake showed low PHB production compared to all oil waste substrates. Rice bran had the highest PHB production, while sugarcane bagasse had moderate production and arecanut husk had low PHB production. In the earlier study of Salgaonkar et al [22], they found that Hgm. *borinquense* strain E3 had the highest PHA accumulation and specific productivity (qp) of 3.0 and 2.7 (mg/g/h) using NaCl synthetic medium supplemented with 25% and 50% SCB hydrolysate, respectively.

FT-IR and GC-MS analyses of PHB biopolymer revealed strong C-H absorption bands and CH3 absorption bands. The extract showed five major peaks with retention times of 7.085 min, 8.509 min, 21.577 min, 27.316 min, and 29.960 min. These results were slightly related to the previous studies of Mandragutti et al. [30], according to their observation, The FTIR analysis of *B. paraconglomeratum* extracts revealed the presence of aliphatic C-H, =C-O, =CH, and =C-H bonds. The PHB sample underwent methanolysis, revealing fragmentation patterns in GCMS. The biopolymer extract showed four major peaks, supporting the presence of PHB with retention times of 21.140 min, 22.635 min, 30.096 min, and 35.441 min, supporting the presence of polyhydroxybutyric acid (PHB) in *B. paraconglomeratum* extracts.

5. Conclusion

Synthetic polymers possess inherent characteristics that render them environmentally unfriendly and potentially harmful. In order to mitigate the adverse effects of synthetic polymers, the utilization of biopolymers is the sole viable solution due to its biocompatibility and eco-friendly in nature. PHB, a biopolymer with higher degradation resistance, can be utilized by bacteria to accumulate surplus energy through PHB granules, enabling the production of biopolymers. The bacterium *Bacillus toyonensis* KUMBNGBT-62, when grown on inexpensive agro-industrial residue, feedstock had a high yield and demonstrated the production of PHB. Based on the findings, it has been determined that the bacterium *B. toyonensis*, when isolated, exhibits potential as a valuable source of high-quality PHB. This discovery suggests that in the future, it may be possible to enhance the production of PHB biopolymer on a larger scale by utilizing cost-effective raw materials.

Compliance with ethical standards

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Author contributions

NG: Conceptualization, data collection, investigation, methodology, validation, writing-original draft and review & editing. BT: Data rectification, investigation, methodology, review and editing, design and correction, supervision, review & editing.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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