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Screening and Optimization of Polyhydroxybutyrate Production by *Streptomyces* sp. 3MGH Isolated from the Egyptian Soil

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Abstract:

The increasing dependence on non-biodegradable plastics poses significant environmental challenges, driving the need for sustainable alternatives like polyhydroxybutyrate (PHB). This study aimed to isolate and optimize PHB production from *Streptomyces* sp. 3MGH, a bacterium isolated from Egyptian soil. Soil samples were collected from various locations, leading to the isolation of 25 *Streptomyces* species. Qualitative screening using Sudan Black B staining identified 14 PHB-producing isolates. Among these, *Streptomyces* sp. 3MGH demonstrated the highest PHB yield of 3.34 g/L. Molecular identification via 16S rRNA sequencing confirmed its close phylogenetic relationship to known *Streptomyces* strains. Optimization experiments evaluated the effects of carbon and nitrogen sources, incubation temperature, time, and shaking speed on PHB production. The results indicated that optimal conditions for PHB accumulation were achieved at an incubation temperature of 30 °C, with 150 rpm agitation, and a 7-day incubation period using fructose as carbon source and yeast extract as nitrogen source. These findings suggest that *Streptomyces* sp. 3MGH presents significant potential for industrial-scale PHB production, contributing to the development of biodegradable plastics.

Keywords: PHB, Sudan Black B, *Streptomyces*, polymer, bioplastic

1. Introduction:

The intensive use of plastics has become prevalent in different aspects of our daily life and industrial applications [1]. Currently, petroleum, a nonrenewable resource, is the primary source for plastic manufacturing [2]. The dependence on petroleum for plastic production presents challenges due to its limited availability, high costs, and environmental issues linked to non-biodegradability of the produced plastics [3]. Hence, there is a need for new alternatives to the traditional plastic and the increasing demand for biodegradable polymers has made their development a priority for industry sector [4]. Polyhydroxyalkanoates (PHAs) are among the most promising bioplastics, as they are fully degradable by different microorganisms [5].

PHAs exhibit physical properties similar to those of synthetic plastics, making them works as effective substitutes [6]. Within the PHA family, polyhydroxybutyrate (PHB) closely mimics the characteristics of conventional polymers like polyethylene and polypropylene [7]. These properties allow PHB to be processed using common manufacturing techniques such as extrusion, molding, fiber spinning, and film casting [8]. Additionally, PHB can be blended with synthetic polymers to enhance its functionality. It is well-regarded for its biocompatibility, ease of processing, and non-toxic nature, making it an ideal material for different applications [9–15]. These biodegradable polymers have found applications in tissue engineering as scaffolds for cell growth, as well as in sutures and tissue implants [16]. Additionally, PHB has been used in various roles such as barrier materials and drug delivery systems [17, 18].

PHB is produced through microbial fermentation, primarily from reducing sugars like hexoses and many microorganisms synthesize PHB as intracellular granules, which serves as energy reserves under environmental stress conditions such as nitrogen, oxygen, or phosphate limitation in presence of excess carbon [19]. Efforts to produce PHB through microbial systems including bacteria and algae have been explored [20, 21]. However, large scale production of PHB is hindered by its high production cost as raw material, particularly the carbon sources required for PHB production are expensive [22]. Alongside known PHB bacterial producers such as *Cupriavidus necator*, *Bacillus* sp., *Pseudomonas* sp., and *Escherichia coli* transformants [23–26], certain actinomycetes are capable of accumulating PHB granules. Previous research has documented PHB production and

degradation by *Streptomyces* sp. as a predominant genus within the actinomycetes [27, 28].

Microorganisms isolated from nutrient-poor environments, such as lake water and soil, tend to exhibit greater resilience compared to those from more nutrient-rich environments. *Streptomyces* species are prime examples of such microorganisms [29, 30]. As members of the actinomycetes group, *Streptomyces* produce around 10,000 metabolites and are easily recognized in culture due to their pigmentation and diverse colony morphology [31]. These species thrive in a pH range of 6.5 to 8.0 and are mostly mesophilic, growing at temperatures between 10 and 37 °C [32]. So, the aim of the current study was to isolate different *Streptomyces* species from the Egyptian soil and evaluate their ability to accumulate PHB using the staining technique, followed by identification of the most potent PHB producing isolate. Additionally, the study aimed to optimize the effective parameters for the maximum productivity of the polymer.

2. Material and methods:

2.1. *Streptomyces* isolation:

Soil samples were collected from different regions of governorates in Egypt including, El-Bajur city (30° 25' 25.13"N, 31° 2' 14.26"E), Zefta city (30° 42' 59.99" N, 31° 14' 60.00" E), Zagazig city (30° 35' 15.65" N, 31° 30' 7.20" E), and Abu Simbel city (22° 20' 12.5628" N, 31° 37' 31.9152" E) as shown on the map (Fig. 1). A 5 g sample of the collected soil was air dried, then suspended in 45 mL of saline solution (0.85% NaCl) and this suspension was serially diluted up to 10^{-5} [33, 34]. A 100 µL aliquot of the dilutions 10^{-4} and 10^{-5} was plated onto starch nitrate medium composed of (g/L): starch 10.0, K_2HPO_4 1.0, $MgSO_4·7H_2O$ 0.5, NaCl 0.5, KNO_3 2.0, $CaCO_3$ 2.0, $FeSO_4·7H_2O$ 0.01, and agar 20.0, all dissolved in 1 L of distilled water and the pH of the medium was adjusted to 7.0 [35]. The inoculated plates were incubated at 28 ± 2 °C for up to 7 days. *Streptomyces* isolates were selected based on their colony morphology, purified and maintained on starch nitrate agar slants at 4 °C for further testing [35].

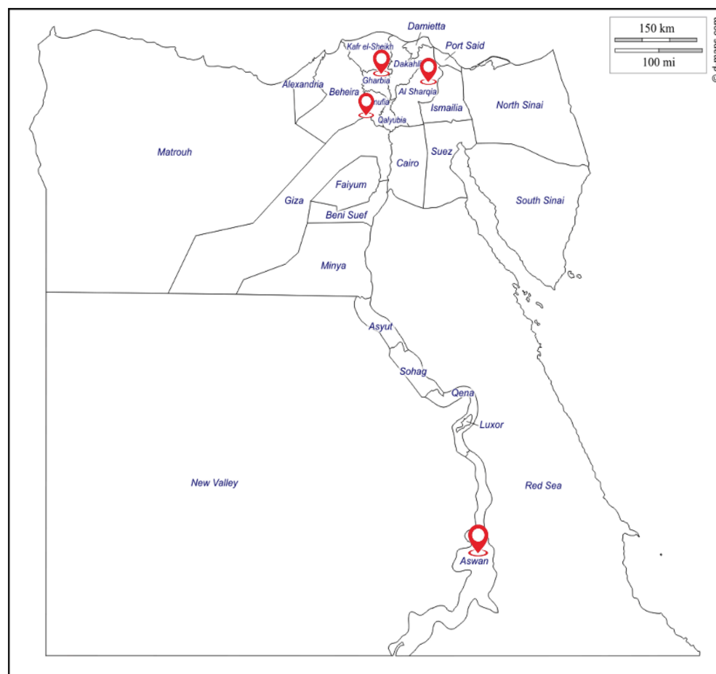

Figure 1: A map of Egypt showing the geographical locations of soil samples collection from different governorates.

2.2. Qualitative screening for PHB production by selected *Streptomyces* isolates:

The selected *Streptomyces* isolates (25 isolates) were qualitatively screened for the ability to PHB production on starch nitrate agar medium using staining method with Sudan Black B. Briefly, Sudan Black B (0.3 g) was dissolved in ethanol (70%) and the resulted solution was applied to 7-days old culture plates, which were then incubated at room temperature for 30 minutes in a static position. Following the incubation period, the excess dye was washed off using absolute ethanol (99.9%). PHB-positive isolates were identified as those with bluish-black color, whereas PHB-negative isolates showed no color change [36–39].

2.3. PHB extraction and quantitative screening in selected *Streptomyces* isolates:

2.3.1. Culture condition for PHB production:

Isolates that showed a bluish-dark color after Sudan Black B staining were selected for further assessment confirmation. Briefly, a 50 mL of starch nitrate broth medium was inoculated with a loopful of 7-days old culture and incubated in a shaker incubator with 150 rpm at 28 °C for 7-days. After incubation, cells were collected through

centrifugation at 15,000 rpm for 15 minutes at 4 ℃, then used for PHB extraction [40, 41].

2.3.2. PHB extraction:

The extraction of PHB was carried out using a modified sodium hypochlorite-chloroform technique. In brief, the harvested cells were resuspended in 10 mL of sodium hypochlorite solution (4 % w/v) and incubated at 37 ℃ for 30 minutes with continuous stirring to allow lysis od cells (22). Following the lysis step, the suspension was centrifuged at 10,000 rpm for 30 minutes to precipitate the PHB in the bottom layer. The PHB polymer was further precipitated using a mixture of acetone and ethanol (1:1) followed by dissolving in hot chloroform then, allowed to evaporate at 35 ℃ for 24 h to obtain PHB powder [37, 40]. The percentage of the produced PHB in terms of dry cell weight was calculated using the following formula [42, 42]:

$$Accumulated\ PHB\ (\%) = \frac{Dry\ weight\ of\ Extracted\ PHB\ \left(\frac{g}{L}\right)}{Dry\ cell\ weight\ \left(\frac{g}{L}\right)} X\ 100$$

2.3.3. Quantification of the extracted PHB:

To quantify the extracted PHB, 10 mL of concentrated sulfuric acid was added to the dried PHB and boiled at 100 ℃ for 10 minutes using water bath to convert PHB into crotonic acid. The release of the crotonic acid, indicated by the browning of the solution, was measured spectrophotometrically at 235 nm, using sulfuric acid as a blank [40, 43, 44]. The concentration of PHB was determined by comparing the absorbance to a standard curve (Fig. 2). Standard curve of crotonic acid was created by preparing different concentrations of crotonic acid (0.12 – 0.75 mg/mL) based on the principle that one gram of PHB is equivalent to one gram of crotonic acid [45].

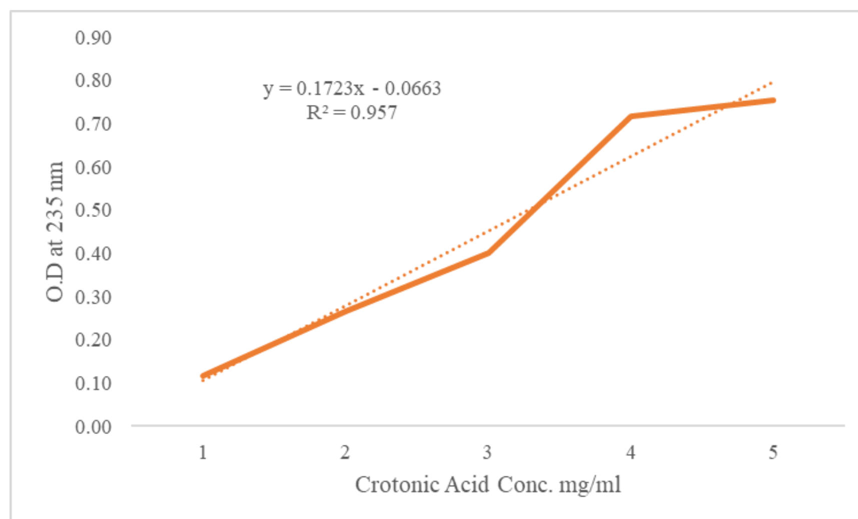

Figure 2: Standard curve of crotonic acid.

2.4. Molecular identification and characterization of the most potent *Streptomyces* isolate for PHB production:

The isolate with the highest ability for PHB production was identified through 16S rRNA gene sequencing according to previous work [46]. Briefly, genomic DNA was extracted, and 16S rRNA gene was amplified through polymerase chain reaction (PCR) using the following primers pair: F (5′ CCGAATTCGTCGACAACAGAGTTTGA TCCTGGCTCAG 3′) and R (5′ CCCGGGATCCAAGCTTAAGGAGGTGA TCCAGCC 3′) [47]. The PCR reaction mixture consisted of 25 µL of 2x my Taq PCR MasterMix, 4 µL of extracted genomic DNA, 1 µL of each primer (forward and reverse), and sterilized nuclease free water to a final volume 50 µL. The running condition for PCR was as following: Initial denaturation at 94 °C for 5 minutes, followed by 45 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and elongation at 72 °C for 60 seconds, with final elongation at 72 °C for 7 minutes and hold at 4 °C. the resulted amplified PCR product was separated on 1% agarose gel using 1X TBE buffer and 100 bp DNA ladder. Then, the correct band was selected and purified using gel purification kit according to the manufacturer's protocol and the purified product was sequenced using the same primer set. The obtained sequence was subjected to BLAST search tool in the NCBI database to identify the homologous sequences. Phylogenetic analysis was conducted using neighbor-joining method with MEGA 11 software [48], with alignment performed using

Clustal muscle algorism [49]. Sequences that showed highest the highest similarity to the 16S rRNA gene of the *Streptomyces* isolate were aligned to construct the phylogenetic tree.

2.5. Optimization of key parameters for PHB production:

During the current study, the impact of five parameters including carbon and nitrogen sources, incubation temperature, incubation time and shaking speed on PHB production was assessed and optimized to achieve the highest productivity by *Streptomyces* sp. 3MGH according to the previous studies [50–53]. Each parameter was investigated separately, while the other parameters were kept constant [35, 54]. The effect of different carbon sources such as glucose, fructose, sucrose, maltose, arabinose, and xylose was assessed [55]. Additionally, the effect of different nitrogen sources such as yeast extract, malt extract, peptone and ammonium sulphate was tested [56, 57]. The effect of incubation temperature (20, 25, 30, 35, and 40 °C) and incubation time (3, 5, 7, and 9 days) was investigated while other parameters were fixed [58, 59]. Finally, the effect of shaking speed as a controlling factor for PHB production was assessed by testing different speeds (0 (static incubation), 100, 150, 200, and 250 rpm) [59]. For all experiments, PHB was extracted, and its concentration was assessed as previously mentioned. Table 1 outlines the tased parameters with their respective values.

Table 1: Key parameters for PHP production and their ranges used for optimization

Parameter	**Value**
Strain	*Streptomyces* sp. 3MGH
C-sources	Glucose, Fructose, Sucrose, Maltose, Arabinose, Xylose
N-sources	Yeast extract, Malt extract, Peptone, Ammonium sulphate
Incubation time (days)	20, 25, 30, 35, 40
Incubation temperature (°C)	3, 5, 7, 9
Shaking speed (rpm)	0 (static), 100, 150, 200, 250

2.6. Statistical analysis:

The data were analyzed using one-way ANOVA in GraphPad Prism Version 10. Results are presented as mean of three replicates ± standard deviation.

3. Results:

3.1. Qualitative screening for PHB production by isolated *Streptomyces* species:

The *Streptomyces* isolates were initially screened using Sudan Black B staining,

employing colony staining method for rapid detection of PHB [60]. In total 25 *Streptomyces* isolates were isolated from different location in Egypt. Out of the 25 isolates tested, 14 were stained by Sudan black B, indicating accumulation within the cells as shown in figure 3, contains examples for *Streptomyces* isolates before and after Staining by Sudan black B. The appearance of different *Streptomyces* isolates across different habitats can be influenced by the dominant environmental and culture conditions [61, 62]. Soli may provide a unique source for isolation of different bacterial strain with unique characteristics as the soil is exposed to different factors that may leads to changes in ecosystem and microbial communities [43]. These results further validated the effectiveness of Sudan Black-B as a staining technique in identifying *Streptomyces* isolates capable of producing intracellular lipid biopolymers. Colonies stained dark blue with Sudan Black B were taken as positive indicators of PHB production in previous studies [43, 63]. Sudan Black-B staining, known for its high sensitivity in PHB screening, effectively revealed intracellular lipid inclusions or cytoplasmic materials that were previously undetectable [64]. Our investigations into PHB-producing bacteria highlighted that *Streptomyces* species isolated from different soil at different locations differ between each other and this may be illustrated as different soil may provide a unique environment that promotes microbial diversity, enabling certain species to accumulate PHB as a survival strategy under nutrient-limited conditions [65].

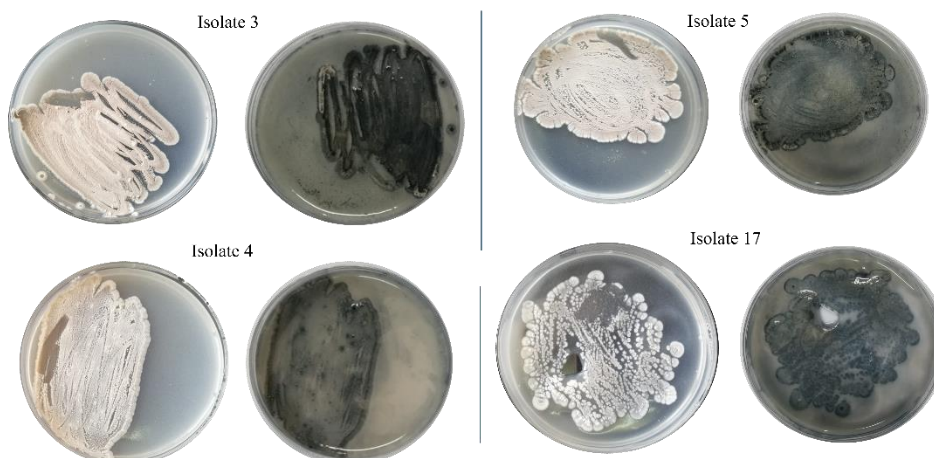

Figure 3: *Streptomyces* isolates growing on starch nitrate medium before and after staining with Sudan Black B dye.

3.2. Extraction of PHB from different *Streptomyces* isolates:

It is widely recognized that microorganisms isolated from natural environments such as soil are often limited in nutrient availability. Chandani et al. [30] reported that certain bacteria can accumulate 60-80% of their biomass as PHB under nutrient-deprived conditions. In accordance with this, the selected *Streptomyces* isolates in the current study were also able to produce satisfactory quantities of PHB using simple nutrient media containing a single carbon and nitrogen source at pH 7.0. PHB extraction using solvents such as 4% sodium hypochlorite, acetone, and ethanol proved effective in maximizing the PHB yield from cell biomass. As PHB is an intracellular product, its extraction involves several essential steps, including pretreatment to disrupt cell walls, precipitation of PHB, and purification. The first step in the process involved cell disruption using sodium hypochlorite to dissolve the cell walls and chlorophyll. Acetone and ethanol were then employed to precipitate the PHB. Finally, pure PHB was obtained through hot chloroform treatment of the precipitate. This solvent extraction method was experimentally demonstrated by Dianursanti et al. [66] and Requiso et al. [67], and has been widely used for PHB extraction. Rewate et al. [68] observed that a 5-minute treatment of cell pellets with sodium hypochlorite resulted in optimal PHB accumulation in production media. This observation was validated during the extraction process in the current study, as the PHB yield increased when the cell lysis step was extended to 5 minutes. Similarly, Aramvash et al. [69] noted that hypochlorite extraction method yields a higher quantity of PHB, likely due to its enhanced cell permeability and its ability to remove cell debris and residual lipids from the slurry. These findings confirm that sodium hypochlorite is one of the most effective agents for large scale PHB extraction from microorganism in general.

3.3. Quantitative assay of PHB production by the selected positive isolates:

The isolates that were tested positives for PHB production were further cultured in starch nitrate broth and the PHB content was quantitatively assessed following the method outlined in the materials and methods section. The amount of PHB produced by these isolates varied between 1.16 and 3.34 g/L (Table 2). The highest productivity was achieved through isolate no 3 with 3.34 g/L. Previously, Krishnan et al. [40] reported PHB production ranging from 1.79 and 4.26

g/L for *Streptomyces* sp. Also, Aly et al. [41] reported that PHB production ranged from 9% to 15.2% through using *Streptomyces* NM10. So, to compare, the current study found that the selected isolates achieved a significantly higher PHB productivity especially for isolate no. 3 that showed PHB production of 26.76% based on the dry cell weight, highlighting the potential of this *Streptomyces* isolate for industrial scale PHB production.

Table 2: Qualitative and quantitative screening of PHB by selected *Streptomyces* isolates.

Isolate no.	Qualitative screening		Quantitative screening		
	Blue-black color		Dry biomass (g/L)	PHB (g/L)	PHB % (w/w)
	Positive	Negative			
1	√		12.00 ± 0.57	2.51 ± 0.44	20.94
2		√	N/A	N/A	N/A
3	√		12.50 ± 0.42	3.34 ± 0.61	26.76
4	√		11.50 ± 0.42	2.90 ± 0.60	25.22
5	√		14.70 ± 0.71	3.17 ± 0.63	21.57
6		√	N/A	N/A	N/A
7	√		14.85 ± 0.49	3.09 ± 0.17	20.83
8	√		13.75 ± 0.64	2.22 ± 0.34	16.16
9		√	N/A	N/A	N/A
10	√		13.88 ± 0.11	1.68 ± 0.57	12.12
11	√		12.65 ± 0.35	2.40 ± 0.32	18.95
12		√	N/A	N/A	N/A
13	√		11.90 ± 0.57	1.87 ± 0.29	15.75
14		√	N/A	N/A	N/A
15	√		15.25 ± 0.92	2.51 ± 0.17	16.48
16	√		11.05 ± 0.35	1.41 ± 0.55	12.76
17	√		13.80 ± 0.57	3.27 ± 0.49	23.68
18		√	N/A	N/A	N/A
19	√		13.55 ± 0.49	1.56 ± 0.12	11.55
20		√	N/A	N/A	N/A
21		√	N/A	N/A	N/A
22		√	N/A	N/A	N/A
23	√		10.20 ± 0.57	1.16 ± 0.09	11.36
24		√	N/A	N/A	N/A
25		√	N/A	N/A	N/A

* N/A: not applicable as this isolate was negative for PHB production.

3.4. Molecular identification of the most potent *Streptomyces* isolate for PHB production:

Molecular identification of filamentous bacteria such as *Streptomyces* through amplification of the 16S rRNA gene and other genotypic approaches offers greater accuracy compared to traditional methods involving staining and biochemical tests [63, 70]. DNA extraction and PCR amplification

of the 16S rRNA gene were performed for the potent *Streptomyces* isolate and the obtained partial sequence from the amplification of the 16S rRNA gene from PHB hyper producing *Streptomyces* was compared with the existing 16S rRNA gene sequences in GenBank using the BLAST search tool. The comparison revealed a 99% similarity with *Streptomyces* sp. strains, making them the closest phylogenetic neighbors to isolate 3 (Fig. 4). The primary goal of integrating all sequences into a single tree was to explore the molecular similarities among *Streptomyces* strains that inhabit the same environment but exhibit different physiological traits. This isolate was identified as *Streptomyces* sp. 3MGH and the gene sequence for this isolate has been submitted to the GenBank and assigned the accession number PQ422119.

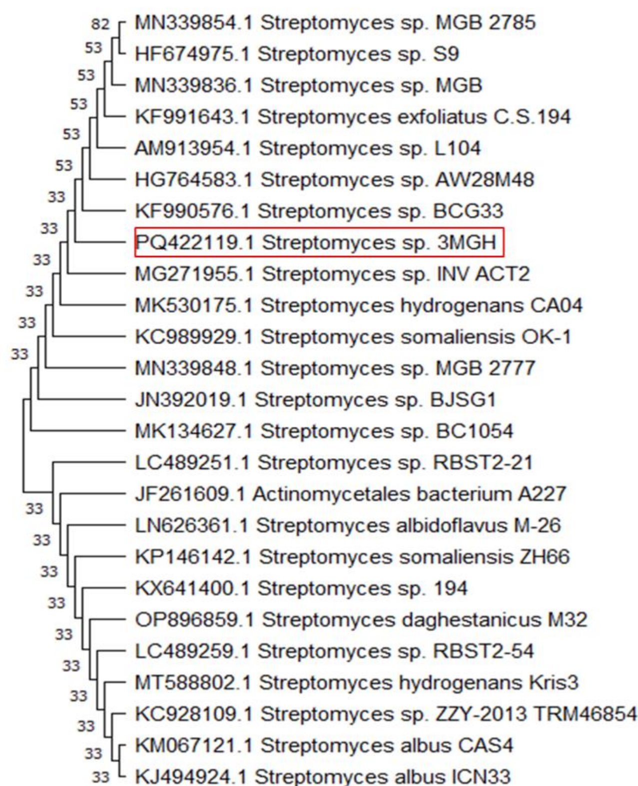

Figure 4: Phylogenetic tree based on 16S rRNA nucleotide sequences of the *Streptomyces* sp. 3MGH isolate with other sequences of published strains generated by the neighbor-joining method using MEGA 11.0 software. The numbers at the nodes indicate the levels of bootstrap support (%) based on 1000 resampled data sets.

3.5. Optimization of different factors for PHB production by *Streptomyces* sp. 3MGH:

During the current study, the growth conditions including (temperature, incubation time, and agitation speed) and nutritional requirements (various concentrations of carbon and nitrogen sources) for *Streptomyces* sp. 3MGH. The optimization was carried out using the single-factor approach, with each experiment repeated in triplicate to ensure reproducibility.

3.5.1 Effect of incubation time and temperature on PHB production:

The optimal incubation period for maximizing PHB accumulation was assessed. Experiments were conducted for 9 days under shaking conditions (150 rpm). The highest PHB yield was observed after 7 days of incubation, after which a decline in PHB production was recorded. *Streptomyces* sp. 3MGH produced a maximum of 3.23 ± 0.35 g/L of PHB from 7.40 ± 0.14 g/L of cell dry weight (CDW), equating to 43.63% PHB of CDW, after 7 days of growth (Fig. 5). This suggests that *Streptomyces* sp. 3MGH entered the stationary phase within 7 days, leading to granule formation and polymer accumulation, a finding supported by several studies [71]. In contrast, Muneer et al. optimized cultural conditions for PHB production by an indigenous isolate of *Pseudomonas sp.*, identifying 72 hours as the optimal incubation time for peak PHB accumulation [72]. Likewise, Hamdy et al. reported the same incubation time of 72 hours for *Bacillus cereus* during PHB production [73].

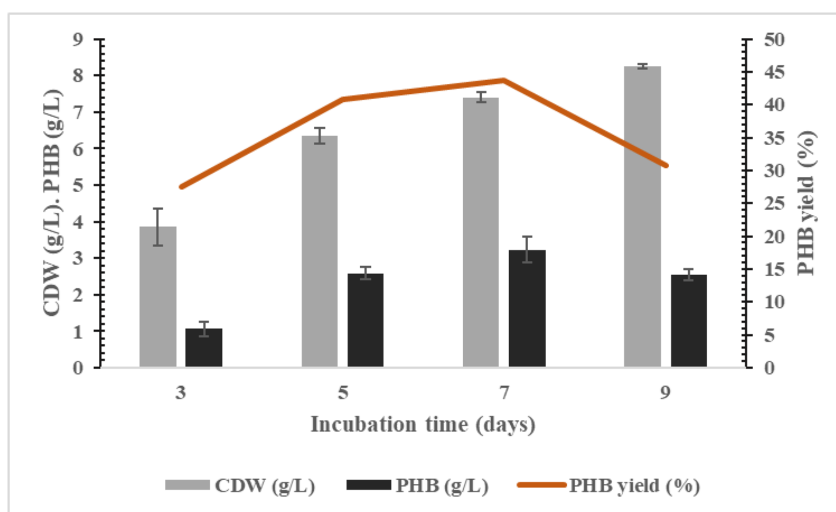

Figure 5: Effect of different incubation time on the growth and PHB production by *Streptomyces* sp. 3MGH.

Temperature is a critical factor for microbial growth and metabolic activities [74]. In this study, the highest PHB yield was achieved at 30°C, with *Streptomyces* sp. 3MGH producing 4.20 ± 0.49 g/L of polymer, representing 43.48% PHB of CDW at this temperature (Fig. 6). A reduction in PHB production was observed at temperatures above or below 35°C. This may be due to enhanced enzyme activity at mesophilic temperatures, while extreme temperatures can denature enzymes and impair their function [75]. Moayedi found 30°C to be the optimal temperature for maximum PHB production by different *Streptomyces* strains [76] . Additionally, Sasidharan et al. optimized cultural conditions for PHB production using the marine bacterium *Vibrio azureus* and identified 35°C as the optimal temperature for PHB accumulation [77]. In contrast, *Cupriavidus taiwanensis* produced PHA most efficiently at 30°C [78]. This result aligns with previous studies indicating that similar strains favor a temperature range up to 30°C, which supports metabolic activity and PHB accumulation. However, beyond this point, there is a rapid decline, likely due to reduced activity of the polymerase enzyme.

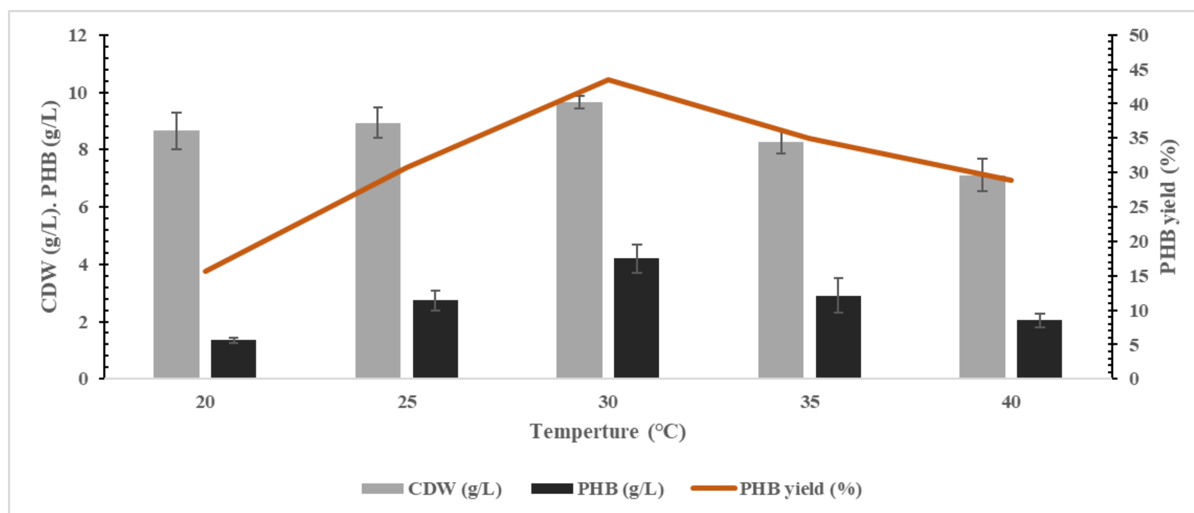

Figure 6: Effect of different temperature on the growth and PHB production by *Streptomyces* sp. 3MGH.

3.5.2. Effect of agitation on PHB production

The influence of different agitation speeds on PHB production was investigated. Optimal agitation is crucial, as it ensures proper mixing and minimizes shear stress during fermentation [79]. Excessive agitation can damage the inoculum. In this study, an agitation speed of 150 rpm was found to be optimal for *Streptomyces* sp. 3MGH, resulting in a PHB yield of

5.53 ± 0.49 g/L and a cell dry weight (CDW) of 9.65 ± 0.21 g/L, corresponding to a 49.95% PHB yield (Fig. 7). This result is away from the findings in previous studies. For instance, Biradar et al. reported significant PHB production at 200 rpm [80].

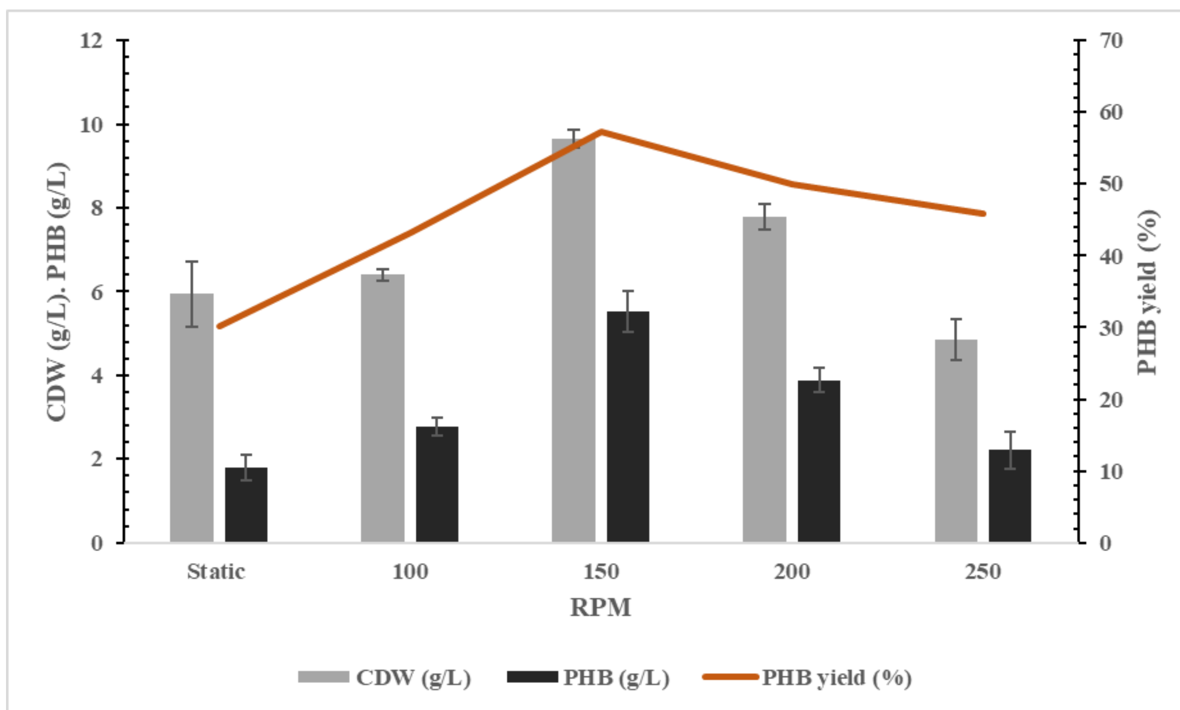

Figure 7: Effect of different agitation speed (RPM) on the growth and PHB production by *Streptomyces* sp. 3MGH.

3.5.4. Effect of different carbon sources on PHB production

The choice of carbon source plays a pivotal role in microbial growth, particularly for PHB production [81]. Supplementation with readily available sugars was introduced to enhance polymer yield. To identify the optimal carbon source and concentration for maximum PHB production, several commercial carbohydrates—including glucose, lactose, sucrose, fructose, maltose, mannose, starch, galactose, glycerol, xylose, and arabinose—were added to the medium. Among these, fructose emerged as the most effective carbon supplement. *Streptomyces* sp. 3MGH produced the highest PHB yield (5.40 ± 0.30 g/L) when supplemented with fructose, resulting in PHB accumulation of 39.45% of cell dry weight (CDW) (Fig. 8). This result suggests that fructose is the most favorable carbon source for the growth and PHB production of the *Streptomyces* sp. 3MGH. As a hexose sugar, fructose can be efficiently metabolized by certain bacteria, resulting in increased biomass and enhanced PHB accumulation.

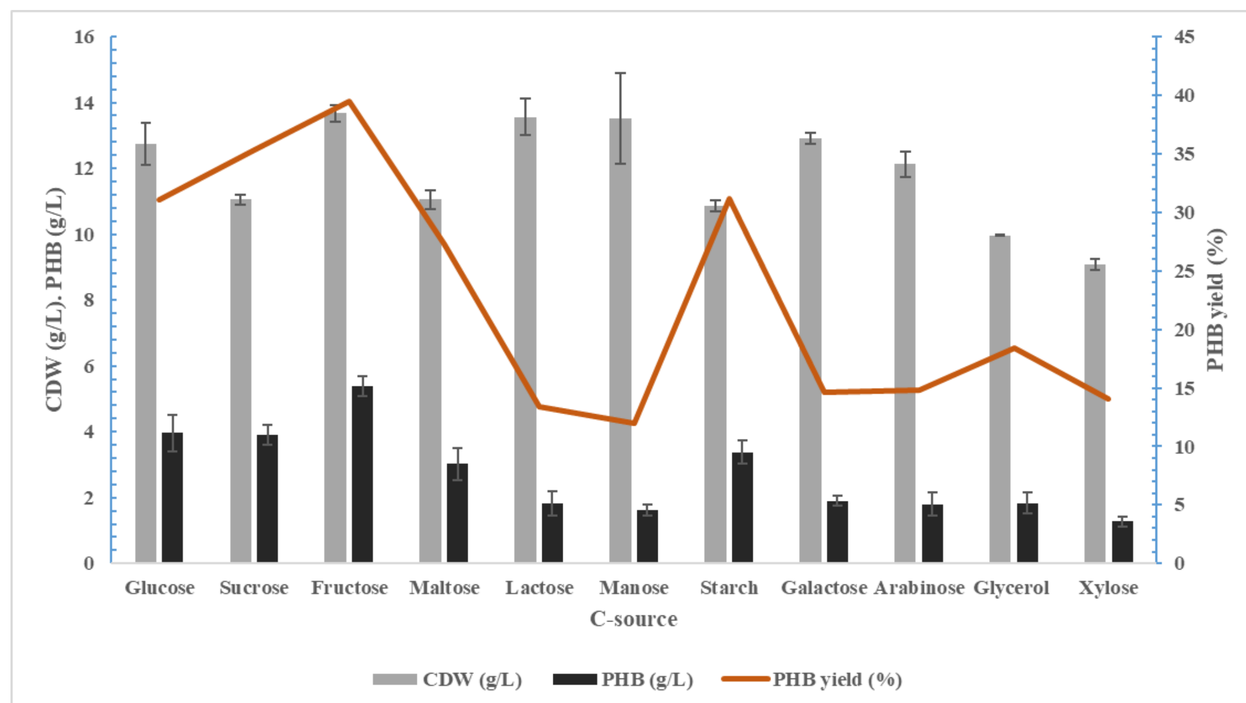

Figure 8: Effect of different carbon sources on the growth and PHB production by *Streptomyces* sp. 3MGH.

3.5.5. Effect of different nitrogen sources on PHB production:

Various organic and inorganic nitrogen sources were tested as supplements to evaluate their effect on PHB production by *Streptomyces* sp. 3MGH under shaking conditions. According to previous studies, bacterial PHB production is often influenced by the complexity and limitation of the nitrogen source used [57]. The highest PHB yield (4.14 ± 0.52 g/L) was obtained when the medium was supplemented with yeast extract as the nitrogen source (Fig. 9). This resulted in a PHB content of 38.40% of cell dry weight (CDW), the highest among all nitrogen sources tested. Bora et al. [82]reported the highest PHB production of 1.38 g/L using *Bacillus megaterium* with glucose as the carbon source. Similarly, Mohandas et al. [83] observed maximum PHB production of 2.3 g/L from *Vibrio harveyi* MCCB 284 when glycerol was used as the carbon source. Gouda et al. [84] also reported significant PHB production using *Bacillus megaterium*, achieving maximum yield with 2% molasses as the carbon source in the fermentation medium.

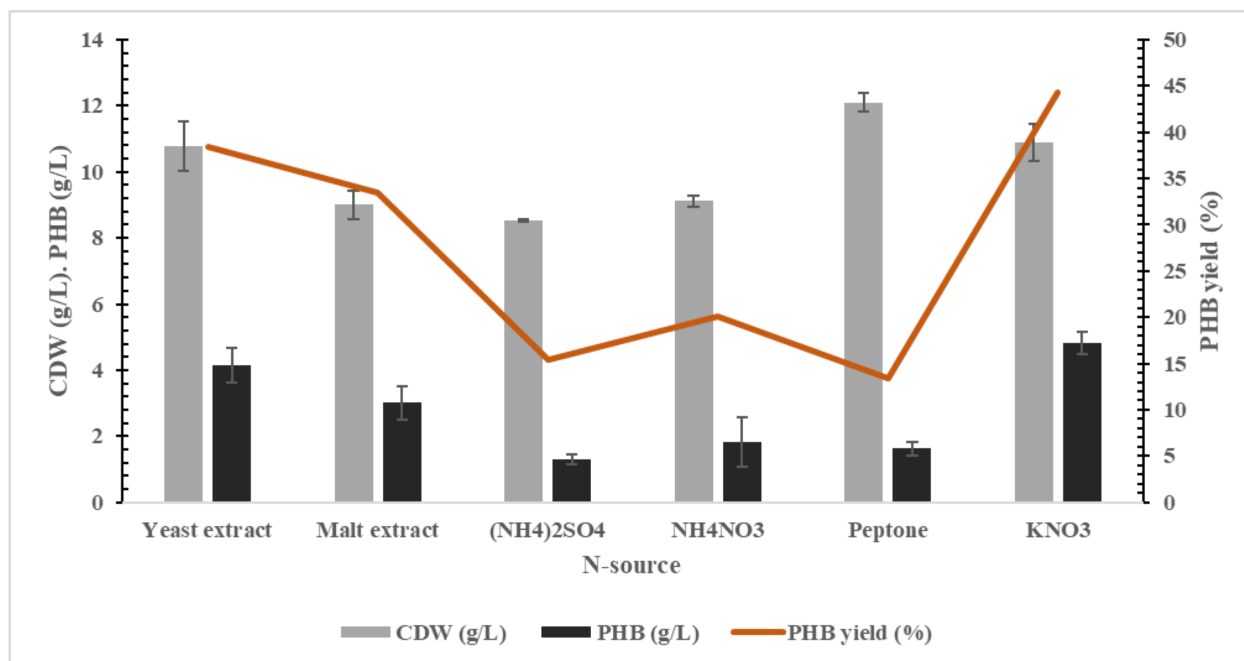

Figure 9: Effect of different nitrogen sources on the growth and PHB production by *Streptomyces* sp. 3MGH.

4. Conclusion:

In conclusion, the current research successfully isolated and characterized *Streptomyces* sp. 3MGH as a promising candidate for PHB production from Egyptian soil. The study demonstrated that environmental factors such as temperature, agitation, and nutrient availability significantly influence PHB synthesis. The optimal production conditions identified—30 °C, 150 rpm agitation, and a 7-day incubation period—resulted in a considerable PHB yield, highlighting the strain's industrial potential. As the demand for sustainable materials continues to rise, the utilization of microbial systems for biopolymer production, particularly from resilient actinomycetes like *Streptomyces*, can play a crucial role in addressing plastic pollution and promoting eco-friendly alternatives. Further research should focus on scaling up production and exploring the practical applications of PHB in various industries.

Author Contributions:

All the authors contributed to the study conception and design, material preparation, data collection and analysis. All the authors read and approved the final manuscript.

Conflict of interest:

The authors have no conflict of interest to declare.

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