Int.J.Curr.Microbiol.App.Sci (2016) *5*(1): 10-22

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 1(2016) pp. 10-22**
Journal home page: http://www.ijcmas.com

Original Research Article doi: http://dx.doi.org/10.20546/ijcmas.2016.501.002

Production of Bioplastic from some selected Bacterial strains

Jehan Mohamed El-Mohamedy Hawas[1]*, Tarek El-Said El-Banna[1], Elsayed Belal Abdelmonteleb Belal[2] and Ahmed AhmedAbd El-Aziz[3]

[1]Professor and Chairman of Pharmaceutical Microbiology, Faculty of Pharmacy, Tanta University, Egypt
[2]Professor of Agriculture Microbiology, Faculty of Agriculture, KafrEl-Sheikh University, Egypt
[3]Professor of Pharmaceutical Microbiology, Faculty of Pharmacy, Tanta University, Egypt
**Corresponding author*

Keywords

Poly-β-hydroxybutyrates (PHB),
PHV,
PHA

Article Info

Accepted:
22 December 2015
Available Online:
10 January 2016

A B S T R A C T

Polyhydroxybutyrates are biodegradable polyesters synthesized by many bacteria Biodegradable polymer plays a predominant role as a biodegradable plastic due to their hydrolysable ester bonds. In the present study fromthirty two bacterial isolates collected from clay soil rhizopher of maiz, wheat and trefoil eighteen PHB producers.PHB is highly produced from microorganisms under optimum conditions such as physical conditions (pH, temperature, incubation times),Nutritional conditions(Carbon, Nitrogen sources and C/N ratio and Biochemical conditions. Due to their biological origin it is an advantage of PHB is, they are degraded naturally and completely to carbon dioxide and water under natural environment by the enzymatic activities of microbes. The present study reports the isolation and screening of soil bacteria and subsequent PHB production under normal conditions. It was observed that clay soil was able to produce maximum yield of PHB.

Introduction

Synthetic polymers-designated as plastics-are applied in a wide range of packaging film,containers, household, agricultural, marine and medical applicationas surgical pins and sutures and bonen replacements. Example for biodegradable polymer materials are Polyhydroxyalkanoates (PHAs) (Page, 1992b; Zhang *et al*., 2003). The main member of the PHA family is Polyhydroxybutyrate (PHB). PHB is accumulated inside in numerous bacteria under nutrient-limiting conditions with excess carbon. Many references show that number of microorganisms like *Alcaligenes eutrophus, Azotobacter beijierinckia, Pseudomonas Oleovorans, Rhizobium* sp. etc., produce PHAs as reserve food material. PHB degrade naturally and completely to CO2 and H2O under natural environment by different microorganisms (Holmes, 1985; Bonartseva *et al*., 1994; Dahi *et al*., 1995; Lee, 1996; Yu *et al*., 2000; Morita *et al*.,

2001; Mahishi *et al*., 2003; Das *et al*., 2005; Philip *et al*., 2007and panigrahi *et al*.,2013).

The study focused on the producing of poly-β-hydroxybutyrate (PHB) granules by strains isolated from different soil samples. There were Screening, isolation, and optimization techniques done for the bacteria by using various techniques. The poly-β-hydroxybutyrate (PHB) granules production was tested by using various sources of C & N, C:N ratios, concentrations of C& N used, and the effect of pH, incubation times,different fermentation media and different temperature.

It was noticed that maximum density of PHB granules was recorded from the clay soil strains.

Materials and Methods

All chemicals were purchased from s d fine chemicals and were of analytical grade.

Medium: Sucrose/Yeast extracts (Bormann *et al*., 1998).

Sample Collection and Isolation of Pure Cultures

Clay soil sample was collected in clean bags. One gram of soil sample is dispensed in 10ml of sterile distilled water. This is mixed vigorously and 1ml from this is taken and added to another tube with 9ml sterile distilled water to get a dilution of 10^{-1}. This serial dilution is repeated to get dilutions of10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, $10^{-6}$and 10^{-7}.
For the isolation of organisms, 0.1ml of each dilution was plated onto a nutrient rich medium by spread plate method for the propagation of microbial growth. The plates were incubated at 30^{0}c for 48 hours. Colonies with different characteristic features were maintained as pure cultures on nutrient agar slants and stored at 4°C.

Screening of PHB Producing Bacteria

All the bacterial isolates were qualitatively tested for PHB production following the viable colony method of screening using Nile red Dye (Rhem and Valla 1997 and Spiekermann *et al*.,1999). For this screening of PHB producers, 20 µl was spread onto sterilized pre-made (sucrose/yeast extract agar media) plates to reach a final concentrations of 0.5 µg Nile red /ml medium. After inoculation, the plates were incubated overnight at 30 ºC subsequently.

The prepared clay soil samples were sub-cultured by 0.1 ml samples were spread out with a sterilized glass rod over the surface of sucrose/yeast extract agar media. The plates were incubated at 30 ºC for 48 hrs. Colonies with pinkish pigment indicated PHB production isolates were exposed to ultraviolet light (312 nm) to detect the accumulation of PHB according to The lighted plates were recorded positives, after that these isolates were picked up and purified by sub-cultured on the same media.

Cell Dry Weight

After 48hrs incubation at 37^{0}C, culture medium was collected and centrifuged at 10,000 rpm for 15min. Supernatant was discarded and the cell pellet was washed twice in deionized water, recovered (for 4 min at 10000 rpm at 4^{o}C).The cell pellet was dried 24 hr at 100^{o}C then the total bacterial cell dry weight was determined as g/L: (Kuniko *et al*., 1988, Boweker *et al*., 1981, Ishizaki *et al.*, 1991 and Du *et al.*,2001).

Extraction and Quantification of PHB

All the Nile red positives isolates were

subjected to quantification of PHB production as per the method of Williamson and Wilkinson, 1958 and Arnold *et al*., 1999). The bacterial cells containing the polymer were centrifuged at 10,000 rpm for 10 min and the pellet was re suspended into alkaline sodium hypochlorite (pH 10.0-10.5 NaOCl content 5.25%-5.5%) and incubated at room temperature for 1 hr. The whole mixture was again centrifuged at10,000 rpm for 10min.and the supernatant was discarded. The cell pellet containing PHB was again washed with water, alcohol and acetone. Finally, the polymer was dried for 2 hr. at 105^{o}C and then weighed. Dry weight of extracted PHB was estimated as g/L. Residual biomass was estimated as the difference between dry cell weight and dry weight of PHB (Zakaria *et al*., 2010**)**.

Estimation of PHB from the Tested Isolates

The percentage of intracellular PHB accumulation is estimated as the percentage composition of PHB present in the dry cell weight.

PHB accumulation (%) = Dry weight of extracted PHB (g/L) × 100 / DCW (g/L)

Identification of PHB Producing Isolates

PHB producing strains were identified and characterized by morphological and biochemical characterization according to the Bergey's Manual of Systematic Bacteriology.

Morphological Characterization

Morphological features were identified by growing the cultures on nutrient agar media and gram staining was performed.

Biochemical Characterization

Different Biochemical tests were carried out includes, Catalase test, Oxidase test, Pigment production and 16S r DNA Cataloging.

Optimization of Cultural Parameters for Maximum PHB Production

Different factors affecting PHB production by the selected bacterial isolates were optimized.

Effect of Different Temperatures

The medium sucrose /yeast extract broth medium were prepared and adjust pH at 7.0. The bacterial isolate J1 were grown in conical flask (250 ml) containing 100 ml of medium were sterilized at 121^{o}C for 20 min. The cultures were incubated on a rotary shaker at 20, 30 and 40^{o}C and 150 rpm for 48 hr. and PHB was quantified.

Effect of Different pH

The bacterial isolate J1 were grown in conical flask (250 ml) containing 100 ml media and were sterilized at 121^{o}C for 20 min.. The medium sucrose /yeast extract broth medium were prepared with different pH ranging from (6, 7, 8 and 9) and the inoculated flasks were incubated at 30^{o}C at 150 rpm for 48 hr. and PHB was quantified.

Effect of Different Incubation Times

The bacterial isolate J1 was grown in conical flask (250 ml) with 100 ml sucrose /yeast extract broth medium and adjust pH at 7.0 were sterilized at 121^{o}C for 20 min The inoculated flasks were incubated at 30^{o}C at 150 rpm under different incubation times (24, 48, 72, 96 hr.).After 48 hr., PHB produced were quantified.

Effect of Different Media

The different media sucrose/yeast extract

medium M1 Bormann *et al*., (1998), M2 Burdman *et al.*, (1998), M3 (Banziger and Tobler, 2001), and synthetic medium M4 Wang *et al*., (2007) were used. The bacterial isolate J1were grown in conical flask (250 ml) containing 100 ml of each previous media and were sterilized at 121^{o}C for 20 min. The inoculated flasks were incubated at 30^{o}C at 150 rpm for 48 hr. and PHB was quantified.

Effect of Different Carbon and Nitrogen Sources

The selected bacterial isolate J1 were grown in 250 ml conical flasks containing 100 ml Sucrose yeast extract broth medium with different carbon sources like glucose, sucrose, mannitol, lactose, whey and molasses with different concentrations (1%, 2%, 3% w/v). The flasks were incubated at 30^{0}C on a rotary shaker (150 rpm) for 48 hours. After incubation, PHB produced by the isolates were quantified according to Miller (1959), Santimano *et al.* (2009), Ghate *et al.* (2011).

Effect of Different Nitrogen Sources on PHB Production

The bacterial isolate J1 were grown in 250 ml conical flasks containing 100 ml sucrose yeast extract broth medium with the best carbon source, and different 'N' sources were used like ammonium sulfate, ammonium chloride, and yeast extract, all at different concentrations (0.5, 1, 1.5 g/L). After 48 hr., PHB yields were quantified.

Effect of Different C:N Ratios on PHB Production

The bacterial isolate J1 were grown in 250 ml conical flasks containing 100 ml sucrose yeast extract broth medium with different C:N ratios i.e. 15:1, 20;1 and 25:1 using the best C and N sources and incubated on a rotary shaker (150 rpm) at 300C. After 48 hr., PHB yields were quantified according to Khanna *et al*., 2005, Belal 2013 and Panigrahi *et al*., 2013).

Results and Discussion

Isolation of microorganisms

Microorganisms were isolated from clay soil sample was obtained by serial dilution. A total of 32 bacterial colonies were selected and the numbers were given to each colony. These colonies were streaked on enrichment and nutrient agar medium plates and preserved for further studies.

Screening of PHB Producing Bacteria

Among 32 colonies, 18 colonies showed positive pinkish colony for Nile red staining. Table (2) and Figure (1) showed the values of PHB according to dry cell weight and referred that the intensity of fluorescence according to PHB amount. The percentage of PHB from 1.50 -30.40% and the highest productivity PHB (30.40%) was obtained by bacterial isolate designated as J1.

Extraction and Quantification of PHB

The best method of PHB extraction was by sodium hypochlorite and it was selected in the further experiments. The percentage of intracellular PHB accumulation was estimated as the percentage composition of PHB present in the dry cell weight. Residual biomass was estimated as difference between the dry cell weight and dry extract of PHB.

Identification of PHB Producing Isolates

Morphological and Biochemical Characteristics

The J1 isolate was gram negative, non-spore

forming, the cell was short rods. And the biochemical studies indicated that these strains should be classified into *Pseudomonas* sp. J1as shown in table (3).

The16S r DNA Cataloging

The 16S r DNA sequence can be clearly seen that the isolated bacterial strainJ1 shown in Figures (2) and closely related to the species *Pseudomonas boreopolis*. It showed that the highest sequence similarities with 97%for *Pseudomonas boreopolis*.

Table.1

source of microorganisms	No. of collected samples	Producer PHB	Non producer PHB
Clay soil Rhizosphere of: maize wheat trefoil	 13 11 8	 9 4 5	 4 7 3
Total	32	18	14

Table.2

Code No. of isolate	DCW (g / L)	PHB (g/L)	Yield of PHB %
J1	5.46 ± 0.23	1.55±0.11	30.40%
J3	5.51±0.244	0.41±0.101488916	7.80%
J4	5.43±0.089	0.5±0.02	9.20%
J5	6±0.32	0.09±0.01	1.50%
J6	5.62 ±0.35	0.4±0.09	6.92%
J7	5.32±0.171	0.33±0.02	6.20%
J8	5.62±0.244	0.45±0.11	8.00%
J9	5.26±0.212	0.4±0.05	7.60%
J10	5.37±0.106	0.70±0.10	13.40%
J11	5.506±0.22	0.66±0.09	12.00%
J12	5.34±0.130	0.48±0.18	7.30%
J13	5.47±0.097	0.63±0.1	11.50%
J14	5.46±0.269	0.53±0.11	9.70%
J15	5.43±0.148	0.63±0.12	11.40%
J16	5.52±0.119	0.72±0.12	12.70%
J17	5.536±0.069	0.77±0.08	12.20%
J18	5.83±0.0898	0.7±0.21	12.00%
J19	5.38±0.187	0.21±0.09	3.90%

DCW: Dry cell weight PHB: polyhydroxybutyrate

Table.3 Morphological and Physiological Characteristics of the Efficient PHB Production Isolates (J1)

Test	Result
Shape of cell	Short rods
Sporulation	Non-spore former
Motility	Motile
Gram reaction	Gram-ve
Aerobic growth	Facultative
Anaerobic growth	-
Pigment production	+
Oxidase test	+

+Growth- No growth

Table.4 Effect of Different Temperature on PHB Production by *P. Boreopolis* J1

Temperatures	***P. boreopolis* J1**		
	DCW **(g/L)**	PHB **(g/L)**	**Yield of** PHB **(%)**
20 °C	2.76 ± 0.2	1.2±0.11	43%
30 °C	5±0.2	3.5±0.13	70%
40 °C	0±0	0±0	0%

DCW: Dry cell weight PHB: polyhydroxybutyrate

Table.5 Effect of Different pH on Production of PHB by *P. Boreopolis* J1

pH	***P. borepolis*J1**		
	DCW **(g/L)**	PHB **(g/L)**	**Yield** PHB**(%)**
pH6	1.9 ±0.11	0.8 ± 0.1	42%
pH7	5.4 ±0.13	3.3 ± 0.12	61%
pH8	1.3 ± 0.1	0.5 ± 0.14	38%
pH9	1± 0.2	0.3 ± 0.1	20%

DCW: Dry cell weight PHB: polyhydroxybutyrate

Table.6 Effect of Different Incubation Times on PHB Production by *P. Borepolis*J1

Incubation times	***P. boreopolis* J1**		
	DCW (g/L)	**PHB (g/L)**	**Yield PHB(%)**
24 hr	4.76±0.3	2.1±0.2	44%
48 hr	3.9±0.3	2.9±.0.16	74%
72 hr	4.766±0.198	1.9±0.2	39.50%
96 hr	3.4±0.21	1±0.2	29%

DCW: Dry cell weight PHB: polyhydroxybutyrate

Table.7 Effect of Different Fermentation Media on PHB Production by *P. boreopolis*J1

Media	*P. boreopolis* J1		
	DCW (g/I)	PHB (g/I)	Yield PHB(%)
M1	4.6±0.1	2.8±0.11	61%
M2	1.2±0.1	0.5±0.2	42%
M3	3±0.11	0.5±0.03	16%
M4	3.11±0.12	1.25±0.1	40%

DCW: Dry cell weight PHB:polyhydroxybutyrate

Table.8 Effect of Different Concentration of Carbon Sources on PHB Production (*P. boreopolis* J1)

Carbon sources	1 %			2 %			3%		
	DCW (g/L)	PHB (g/L)	Yield of PHB (%)	DCW (g/L)	PHB (g/L)	Yield of PHB (%)	DCW (g/L)	PHB(g/L)	Yield of PHB (%)
Sucrose	2.85±0.35	1.3±0.4	42%	3.7 ±0.3	3.1±0.3	83.7 %	2.013±0.315	0. 9±0.2	42.8 %
Glucose	3.01±0.11	1.93±0.075	64%	2.37±0.085	1.47 ± 0.095	62 %	2.666±0.110	1.543±0.09	57.8%
Mannitol	4.01±0.13	2.75±0.095	69%	3.72±0.3	3.036±0.056	81%	4.096±0.115	3.013±0.100	74%
Whey	1.91±0.18	0.93±0.075	49%	2.38±0.05	0.93±0.35	55%	2.83±0.152	1.05±0.06	57%
Lactose	2.0±0.11	0.6±0.03	30 %	2.32±0.13	0.060±0.92	40 %	1.8±0.90	0.45±0.020	25 %
Molasses	2.95±0.240	1.95±1.95	66%	3.31±0.295	2.6±0.183	78%	3.61±0.195	2.65±0.105	73%

DCW: Dry cell weight PHB: polyhydroxybutyrate

Table.9 Effect of Different Nitrogen Sources on PHB Production by *P. Boreopolis*J1

Conc. of / Nitrogen sources	0.5g/l			1.0g/l			1.5 g/l		
	DCW (g/L)	PHB (g/L)	Yield PHB%	DCW (g/L)	PHB (g/L)	Yield PHB (%)	DCW (g/L)	PHB (g/L)	Yield PHB (%)
$(NH_4)_2SO_4$	3.11±0.105	1.88±0.12	60%	3.92±0.12	2.83 ± 0.10	72%	1.88±0.15	0.92±0.1	49%
NH_4Cl	2.99±0.12	1.2±0.1	40%	3.8±0.08	1.9±0.11	50%	1.9±0.1	0.7±0.15	37%
Yeast extract	2.2±0.12	0.8±0.1	36%	2.6±0.2	1.06 ± 0.230	46%	1.5±0.35	0.3±0.028	33%

DCW: Dry cell weight PHB: polyhydroxybutyrate

Table.10 Effect of Different C:N Ratios of Medium on PHB Yields By *P. Boreoplois* (J1)

Strains	carbon source	15:1			20:1			25:1		
		DCW (g / L)	PHB (g/L)	Yield PHB (%)	DCW(g /L)	PHB (g/L)	Yield PHB (%)	DCW (g/L)	PHB (g/L)	yield PHB(%)
P. boreopolis J1	**Ammo-nium sulfate**	4.01 ±0.13	2.75 ±0.09	69%	3.72 ±0.3	3.036 ±0.056	81%	4.096 ±0.115	3.013 ±0.1001	74%

Figure.1 Correlation of PHB Producing Bacteria Isolated from Clay Soil and DCW

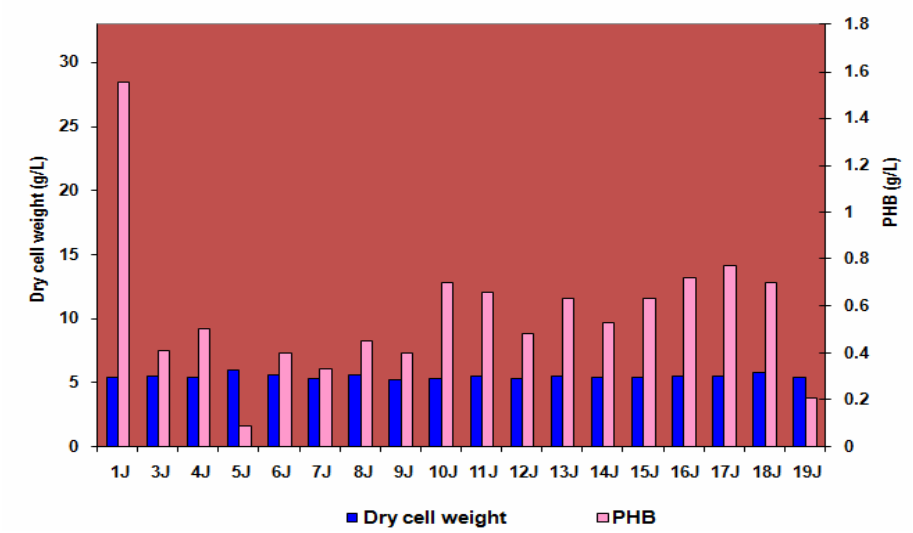


Figure.2 Dendogram illustrating the genomic relationship among fourteen isolates belonging to genus Pseudomonas revealed by UPGMA cluster analysis. The label at the internal nodes shows the distance and the bar 0.02 represents substitution

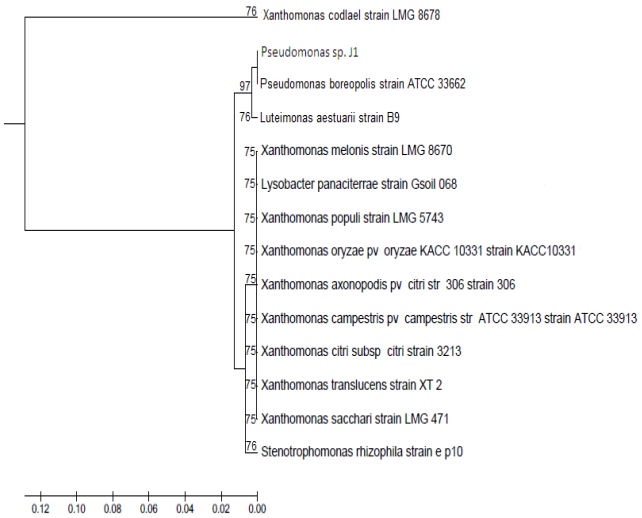


Figure.3 Effect of different temperature on PHB production by *P. borepolis*J1

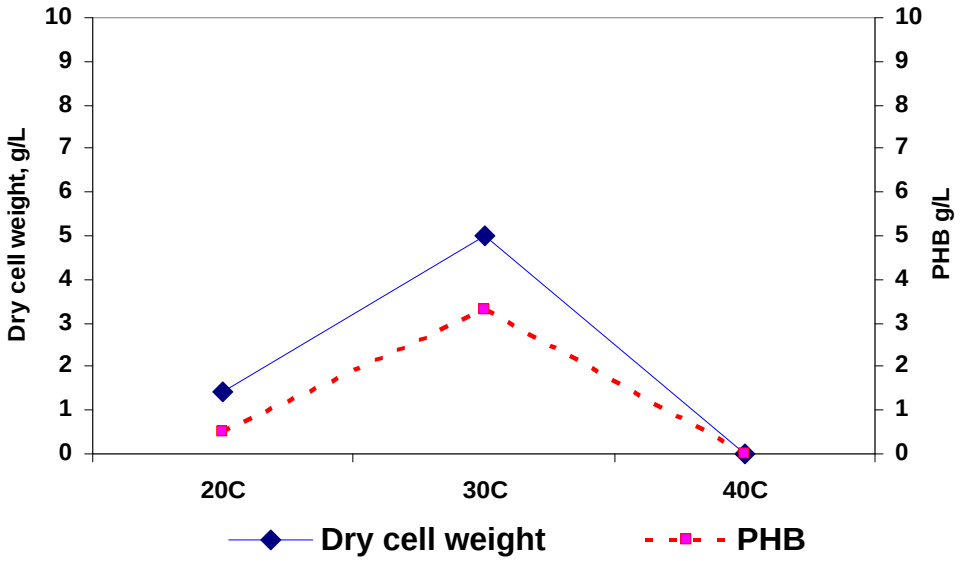


Figure.4 Effect of different pH on production of PHB by *P. boreopolis* J1

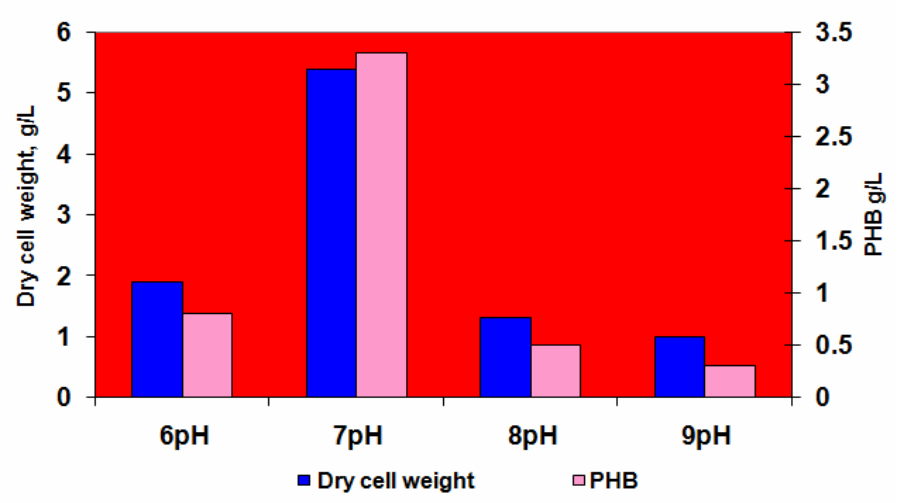


Figure.5 Effect of different Incubation times on PHB Production by *P. boreopolis* J1

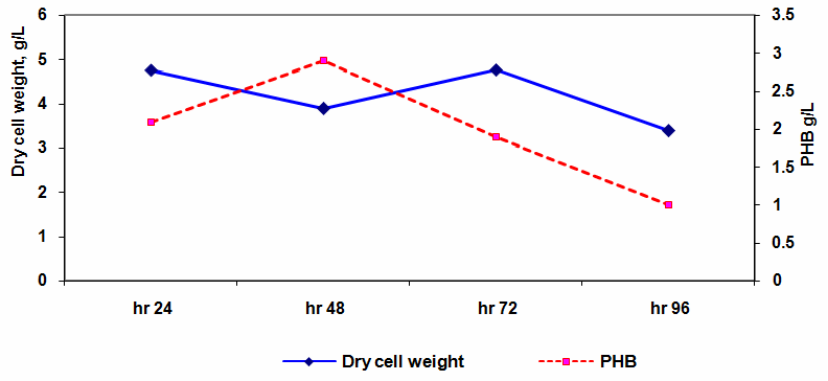


Figure.6 Effect of different Media on production of PHB by *P. boreopolis* J1

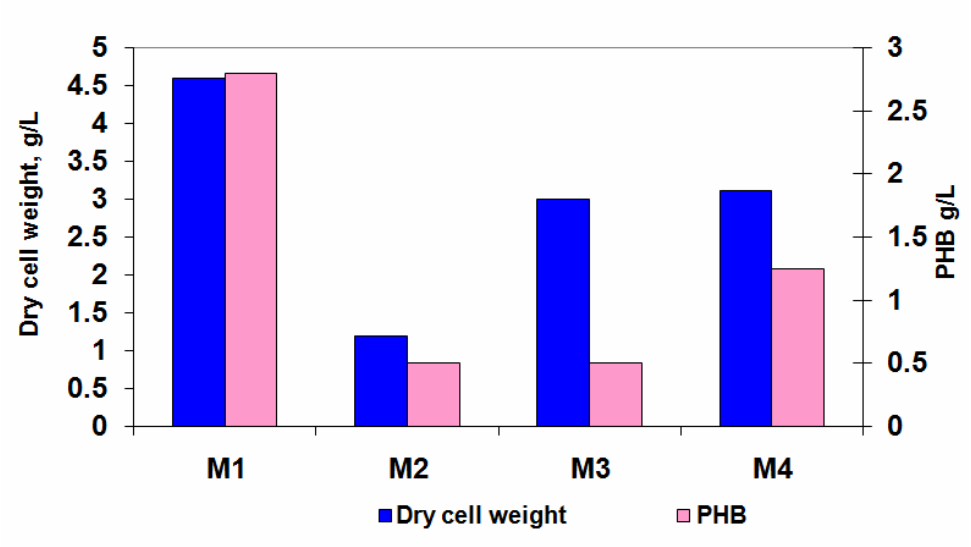


Figure.7 Effect of Different Concentration Carbon Sources on PHB Production (*P. boreopolis* J1)

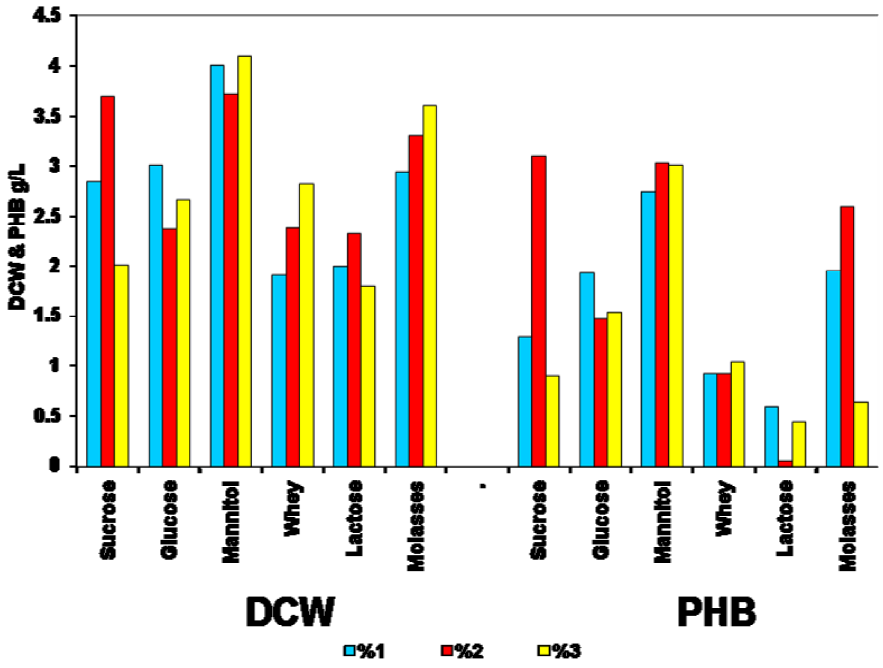
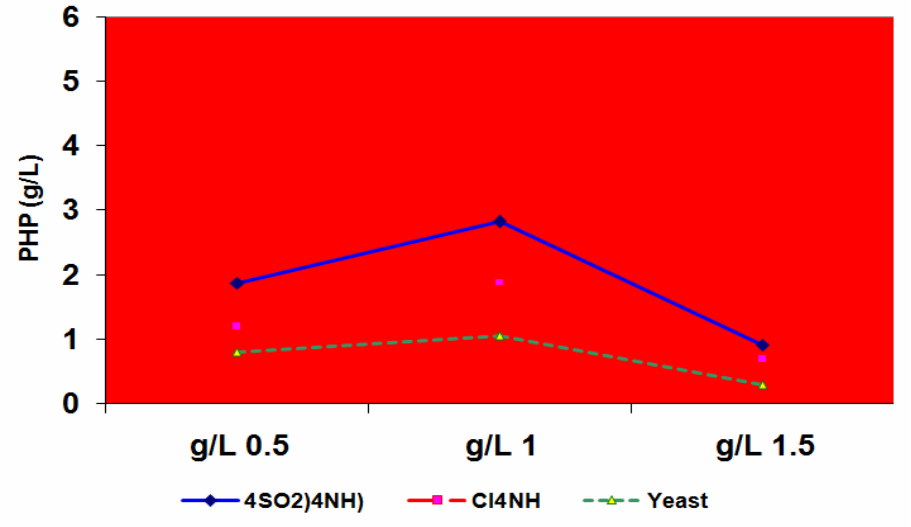


Figure.8 Effect of Different Nitrogen Sources on PHB Production (*P. boreopolis*J1)



Optimization of Cultural Parameters for Maximum PHB production

Effect of Different Temperatures on Production of PHB by *P. Boreopolis* J1

Data presented in Table (4) and Figure (3) showed that, the maximum PHB production occurred at 30^{o}C after 48 hr. of incubation at a pH 7by *P. boreopolis* J1.

Effect of Different pH on Production of PHB by *P. Boreopolis* J1

Data presented in Table (5) and Figure(4) showed that, out of different pHs of media tested, pH 7.0 was found to be optimum for maximum PHB production by *P.Boreopolis.*

Effect of Different Incubation Time

As shown in Table (6) and Figure (5), the highly production of PHB at incubation time 48 hr. for *P.Boreopolis* J1 isolate.

Effect of Different Media

The results presented in Table (7) and Figure (6) showed that the Sucrose yeast extract medium gave high yield of PHB by *P. boreopolis*J1.

Effect of different Carbon sources on PHB production by P. boreopolis *J1*

Data presented in Table (8) and Figure(7) illustrate the effect of different concentrations from carbon sources on PHB yield. The highest yield of PHB among the tested carbon sources was observed with Sucrose2% by *p. boreopolis*J1.

Effect of Different Nitrogen Sources on PHB Production

The result presented in Table (9) and figure (8) showed that the highest yield of PHB was recorded with 1.0 g/L ammonium sulfate by *P. boreopolis* J1.

Effect of Different C:N Ratio on PHB Production by *P. Boreopolis*J_1

The data are presented in Table (10) showed that, the highest yield of PHB by *P. boreopolis* J1at C:N ratio was 20:1.

In conclusion, the main aim of this present study was to isolate the PHB producing bacteria from Clay soil sample. Now Days researchers are focusing on biopolymer producing microorganisms for developing

biodegradable plastics. The medium used for the PHB isolates was simple medium and less cost effective and the PHB yield from these isolates was high compared with the earlier reports. Among 18 isolates the strain J1 is showing more production of biopolymer. The PHB produced from this strain will further be characterized by analytical techniques like Infra Red spectra and Gas Chromatography analysis.

References

Arnold, L.; Demain, J. and Davis, E. (1999): Polyhydroxyalkanoates. Manual of Microbiology and Biotechnology. Washington, Am Soc. Microbiol., 2: 616-627.

Banziger, S. and Tobler, N. (2001): The formation of reserve polymers in *Bacillus megaterium*. Microb. Ecology Course, 1-4.

Belal, E. B.(2013b):Production of Poly-β-Hydroxybutyric Acid (PHB) by *Rhizobium elti* and *Pseudomonas stutzeri*. Curr. Res. J. Biol. Sci., 5(6): 273 - 284.

Bergey's Manual of Systematic Bacteriology (2005), 2nd ed. Vol. II the Proteo bacteria., Springer Verlage.

Bonartseva, G.A.; Myshkina, V.L. and Zagreba, E.D. (1994): Poly-hydroxybutyrate content in cells of various *Rhizobium* species during growth with different carbon and nitrogen sources. Microbiol., 63(1): 45-48.

Bormann, E.J.; LeiBner, M.and Beer, B.(1998): Growth associated production of poly (hydroxybutyric acid) by *zotobacterb-eijerinckii* from organic nitrogen substrates. Appl. Microbiol. Biotechnol.,49: 84- 88.

Bowker, R.R. (1981): Manual of Methods for General Bacteriology. American Society for Microbiology. Washington, D.C

Burdman,S.; Jurkevitch, F.; Schwartshurd, B.; Hampal, M. and Okon, Y. (1998): Aggregation in *Azospirillum brasilense* : effects of chemical and physical factors and involvement of extracellular components. Microbial., 144:1989-1999.

Dahi, M.K.;D. Schmiedel and W. Hillen, (1995): Glucose and glucose -6-phosophate interaction with xylose repressor proteins from *Bacillus* spp. May contribute to the regulation of xylose utilization. J. Bacteriol., 177: 5467-5472.

Das, Q.; Chowdhury, J.U. and Anwar, M.N. (2005): Isolation, purification and characterization of biodegradable polymer producing bacteria *Pseudomonas pseudomallei*. Int. J. Agri. Biol., 7(1): 114-117.

Du, G.; Chen, J.; Yu, J. and Lun, S. (2001): Continuous production of poly-3-hydroxybutyrate by *Ralstonia eutropha* in a two stage culture system. J. Biotech., 88: 59-65.

Ghate, B.; Paandit, P.; Kulkarni, C.; Mungi, D.D. and T.S. Patel, (2011): PHB Production using novel agro-industrial sources from different *Bacillus* species. Internat. J. Pharma Bio Sci., 2(3): 242 - 249.

Holmes, P.A. (1985): Applications of PHBs: A microbially produced biodegradable thermoplastic. Phys. Technol., 16: 32-36.

Ishizaki, A. and Tanaka, K. (1991): Production of poly-ß -hydroxybutytic acid from carbon dioxide by *Alcaligenes eutrophus* ATCC17697. J. Ferm. and Bioeg.,71(4): 254-257.

Khanna, S. and Srivastava, A.K. (2005b): Statistical media optimization studies for growth and PHB production by *Ralstonia eutropha*. Process Biochem., 40: 2173-2182.

Kuniko, M.; Nakamura, Y.and Doi, Y. (1988): New bacterial coployestras produced in *Alcaligenes eutrophus* from organic acids. Polymer Commun., 29: 174-176.

Lee, S.Y. (1996): Bacterial polyhydroxyalkanoates. Biotechnol. Bioeng., 49: 1-14.

Lee, S.Y. (1996): Plastic bacteria progress and prospects for polyhydroxyalkanoate production in bacteria. Trends. Biotechnol., 14: 431-438.

Mahishi, L.H.; Tripathi, G.and SRawal,.K. (2003): Poly (3-hydroxybutyrate) (PHB) synthesis by recombinant Escherichia coli harboring *Streptomyces aureofaciens* Poly-3-hydroxybutyrate biosynthesis genes: Effect of various carbon and nitrogen sources. Microbiol. Res., 158:19-27.

Miller, G.L. (1959): Use of Dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31: 426-428.

Morita, M.; Tanji, Y.; Mizoguchi, K.; Soejima, A.; Orito, Y.and Unno, H. (2001): Antibacterial activity of *Bacillus amyloliquefaciens* phage endolysin without holing conjugation.J. Biosci. Bioeng., 91: 469-473.

Page, W.J. (1992b): Suitability of commercial beet molasses fractions as substrates for polyhydroxyalkanoate production by *Azotobacter vinelandii* UWD. Biotechnol. Lett., 14: 385-390.

Panigrahi, S and Badveli, U.(2013):Screening, Isolation and Quantification of PHB-Producing Soil Bacteria. Int. J. of Eng. Sci. Invention.,(2) 9:01-06.

Philip, S.; T. Keshavarz and Roy, I. (2007): Polyhroxyalkanoates: biodegradable polymers with a range of application. J. Chem. Technol. Biotechnol., 82: 233-247.

Rehm, B.H. and Valla, S. (1997): Bacterial alginates: Biosynthesis and applications. Appl. Microbiol. Biotechnol., 48: 281-288.

Santimano, M.C.; Nimali, N.; Prabhu, and S. Garg, (2009): PHA production using a low cost agro industrial waste by *Bacillus* sp. Strain COL1/A6. Res. J. Microbiol., 4(3): 89-96.

Spiekerman, P.; Rehm, B.H.; Kalscheuer, R.; Baumeister D.and Stenbuchel, A**.** (1999): A sensitive, viable-colony staining method using Nile red for direct screening of bacteria that accumulate polyhydroxylakanoic acids and other lipid storage compounds. Arch . Microbiol., 171: 73-80 .

Wang, Y. J.; Hua, F. L.; Tsang, Y. F.; Chan, S.Y.; Sin, S. N.; Chua, H. M; Yu, P. H. F. and Ren, N. Q. (2007): Synthesis of PHAs from waste water under various C: N ratios. Bioresource Technol., 98:1690-1693.

Williamson, D.H. and Wilkinson, J.F. (1958): The isolation and estimation of the poly-β hydroxybutyrate inclusions of *Bacillus* species. J. Gen. Microbiol., 19: 198-209.

Yu, H.; Yin, J.; Li, H.; Yang, S. and Shen, Z. (2000): Construction and selection of the novel recombinant *Escherichia coli*. Eur. J. Biosci. Bioeng., 89: 307-311.

Zakaria, M. R.; Ariffin, H.; Johar, N. A. M.; Aziz, S. A.; Nishida, H.; Shirai, Y.and Hassan M. A.(2010): Biosynthesis and characterization of poly (3-hydroxybutyrate-co-3-hydroxybutyrate) copolymer from wild type *Comamonas* sp. EB172. Polym. Degrad. Stab., 95: 1382-1386.

Zhang, S.; Kolvek, S.; Lenz, R.W. and Goodwin, S.(2003): Mechanism of the polymerization reaction initiated and catalyzed by the polyhydroxybutyrate synthase of *Ralsotonia eutropha*. Biomacromol., 4: 504-509.

How to cite this article:

Jehan Mohamed El-Mohamedy Hawas, Tarek El-Said El-Banna, Elsayed Belal Abdelmonteleb Belal and Ahmed AhmedAbd El-Aziz. 2016. Production of Bioplastic from some selected Bacterial strains. *Int.J.Curr.Microbiol.App.Sci.* 5(1): 10-22.
doi: http://dx.doi.org/10.20546/ijcmas.2016.501.002