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PRODUCTION OF SOME BIODEGRADABLE POLYMERS BY SOME BACTERIAL ISOLATES

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ABSTRACT

Several bacterial isolates were isolated from Dakahlia and Damitta Governorates. The isolates were purified, identified and tested for PHB production. The tested isolates were 17 isolates of *Azotobacter chroococcum*, 8 isolates of *Azospirillum lipoferum*, 22 isolates of *Alcaligenes eutrophus*, 3 isolates of *A. latus,* 3 isolates of *Bacillus subtilis*, 2 isolates of *B. cereus*, 5 isolates of *B. megaterium*, 2 isolates of *B. coagulans*, 3 isolates of *B. polymexa*, 4 isolates of *B. thuringiensis*, 2 isolates of *Pseudomonas fluorescens*, 2 isolates of P. *putida*, 2 isolates of P. *alcaligenes,* 2 isolates of *P. aeruginosa*, 4 isolates of *Rhizobium leguminosarim*, 2 isolates of *R. meliloti*, one isolate of *R. japonicum* and 12 isolates of *Streptomyces albus*. Also, the occurrence of alginate in different isolates of *Azotobacter* and *Pseudomonas* were investigated. All isolated strains proved to be PHB producers. The amount of PHB produced by *Bacillus* isolates varied from 0.02 to 0.16 g/l, while by *Azospirillum* isolates the PHB amounts ranged from 0.12 to 0.58 g/l. Using *Rhizobium* isolates, the highest amount of PHB (0.36 g/l) was produced by *R*. *leguminasarim* No 3 , while the lowest amount (0.06 g/l) was obtained by *R*. *leguminosarim* No 2. Usig two media, *Alcaligenus eutrophus* No. 20, seemed to be the most active PHB producer among all *Alcaligenes* isolates (0.52 g/l). By *Pseudomonas* isolates, *Pseudomonas fluorescens* No. 2 produced 0.30 g/L of PHB while *P. aeruginosa* No. 2 produced 0.10 g/L. Generally the isolate of *Azotobacter chroococcum* No. 16 showed the highest values. The cell dry weight, PHB concentration and the yield of PHB were 2.28 g/l, 0.78 g/l, 34.21%, respectively. And the highest amount of alginate concentration (g/l) was 0.44 by *Azotobacter chroococcum* No. 14.

Keywords : Poly-β-hydroxybutyrate, alginate and bioplastic

INTRODUCTION

The problem of environmental pollution caused by indiscriminate dumping of plastic waste has assumed global proportions. These conventional plastics that are synthetically derived from petroleum are not readily biodegradable, It is considered as environmentally harmful wastes. In the search of environmentally friendly materials to substitute for conventional plastics, different biodegradable plastics have been developed either by incorporating natural polymers into conventional plastics formulations, by chemical synthesis, or by microbial fermentations. However, physical limitations of these materials still exist (Kahar *et al.*, 2004).

Among the variety of biodegradable plastics a family of more than 40 poly-hydroxy alkanoates (PHAs) and their co-polymeric derivatives has emerged as very attractive materials due to their complete biodegradability. A number of bacteria accumulate these polymers or co-polymers as an intracellular carbon reserve when unfavorable environmental and nutritional

conditions are encountered. Poly-b-hydroxybutyrate (PHB) is a microbial polyester produced by many bacteria and stored in cells in the form of granules. It is a candidate for the synthesis of environmentally benign, biodegradable plastics. Much efforts has been spent in optimizing the poly-β-hydroxybutyrate (PHB) production using pure substrates and pure cultures. The cost of this (PHB) is still around ten times higher than that of conventional plastics (Wang and Lee, 1997).

PHA has been identified in more than 20 bacterial genera, including *Alcaligenes* (Khanna and Srivastava, 2005), *Azotobacter* (Pozo *et al*., 2002), *Bacillus* (Law *et al*., 2003), *Pseudomonas* (Sheu and Lee, 2004), *Rhizobium* (Todd *et al*., 2002) and *Streptomyces* (Verma *et al*., 2002).

Because only a few of the many species of brown seaweed are suitable, as a results of abundance and location, for commercial alginate production, there is at present interest in the bacterial production of alginate-like polymers. Alginate was reported first in the opportunistic pathogen *Pseudomonas aeruginosa* and then in three nonpathogenic species of *Pseudomonas,* including *P. mendocina, P. putida,* and *P. fluorescens, Azotobacter vinelandii* appears to be highly appropriate for commercial bacterial alginate production (Bakkevig *et al*., 2005). Also, *A. chroococcum,* was used for alginate production (Pecina *et al*., 1999). In previous studies, several collection isolates of *Azotobacter* and *Pseudomonas* were screened on 3 different media.

Alginates have various industrial uses as viscosifiers, stabilizers and gel-forming, film-forming or water-binding agents. These applications range from textile printing and manufacturing of ceramics to production of welding rods and water-treatment. These properties are utilized in the food industry in products like custard creams and restructured food. The polymer is also used as a stabilizer and thickener in a variety of beverages, ice-creams, emulsions and sauces. The pharmaceutical industry uses alginates as wound dressings and dental impression materials. The polysaccharide is also used as a tablet binder or disintegrant, and by carefully choosing the optimal alginate quality one can obtain controlled release of the drug. In recent years alginates have been used for encapsulation of cells and enzymes (Bucko *et al*., 2005 and Dentini *et al*., 2007).

The aim of this work was isolation, identification and test the isolates for PHB and alginate production.

MATERIALS AND METHODS

Isolation and purification:

Different soil samples were taken from different regions in Damitta and Dakahlia Governorates, Egypt. The soil samples were collected from rhizosphere of different plants (*Zea mayz*, *Vicia faba* and *Trifolium alexandrinum*) from 0-15 cm layer. 10 gram of each sample was suspended in 90 ml of sterile distilled water and shaked vigorously, serially diluted in sterile distilled water, and the dilution from 10^{-1} to 10^{-6} were plated on specific media. Plates were incubated at 30°C for 48 h.

Azotobacter isolates were isolated and maintained using modified Ashby's medium (Abd El-Malek and Ishac, 1968) and PHB production was in (Pozo *et al*., 2002) medium which supplemented with 1% fructose as carbon source. But the alginate production was in (Clementi *et al*. 1995) medium, (Jimenez *et al*.1999) medium 2005 and (Lange *et al*., 2002) medium.

Azospirllium isolates were isolated and maintained using nitrogen deficient medium (Dobereiner, 1988) and PHB production was in (Sun *et al*., 2002) medium which supplemented with 0.5% malic acid as carbon source.

Alcaligenes isolates were isolated using mineral salt medium (Khanna and Srivastava, 2005); maintained using nutrient rich medium (Du *et al*., 2001) and PHB production was in AL1 medium (Wang and Lee, 1997) which supplemented with 3% sucrose as carbon source and (Beaulieu *et al*., 1995) medium which supplemented with 3% glucose as carbon source.

Bacillus, *Streptomyces* and *Pseudomonas* isolates were isolated and maintained using nutrient agar medium (Difco, 1977)

PHB production by *Bacillus* isolates was in (Aslim *et al*., 2002) medium which supplemented with 2% glucose as carbon source.

PHB production by *Pseudomonas* isolates was in (Qiang *et al*., 2001) medium which supplemented with 1% glucose as carbon source . But the alginate production was in (Bakkevig *et al*., 2005) medium .

Rhizobium isolates were maintained using yeast extract mannitol agar medium (Tavernier *et al*., 1997) and PHB production was in the same medium which supplemented with 0.6% fructose as carbon source .

PHB production by *Streptomyces* isolates was in yeast extract malt extract medium (YEME medium) (Verma *et al*., 2002) which supplemented with 2% glucose as carbon source.

***Rhizobium* spp:**

7 isolates of *Rhizobium leguminasarum*, *meliloti* and *japonicam* were obtained from Microbiology Department, Fac. of Agric., Mansoura Univ.

Cultivation system

The inocula were prepared in 250 ml conical flasks containing 20 ml of different media, inoculated with a loop of tested cultures and incubated in a rotary shaker at 200 rpm at 30°C for 48 h. Then the inocula were transferred into 250 ml conical flasks containing 50 ml of the production medium and incubated in a rotary shaker at 200 rpm at 30°C for 48 h. (Khanna and Srivastava, 2006).

Analytical methods

Biomass determination

Cells from 50 ml culture broth were pelleted by centrifugation (5000×*g*, 10 min), washed twice with sterile distilled water, dried for 24 h at 100 °C and used for total cell dry weight determination (Khanna and Srivastava, 2005).

Poly-ß-hydroxybutyrate determination

For the quantitative estimation of PHB, cells of culture broth were collected by centrifugation (5000×*g*, 10 min). 10 ml of hot chloroform was added in cells at 70 °C 10 min and incubated at 30 °C for 24 h. The resulting solution was collected, allow to the chloroform to evaporate, dried at 100 °C for 24 h and cell dry weight was determined (Pozo, *et al*., 2002).

Alginate measurement:

A 10-ml sample of culture broth was centrifuged at 5000 rpm for 20 min. The supernatant was added to 30 ml propan-2-ol and the resultant precipitate was filtered through a Whatman filter-paper, dried to 70°C for 24 h until constant weight and weighted (Jimenez *et al*., 2005)

RESULTS AND DISCUSSION

The identification of isolates :

Seventeen isolates of *Azotobacter* were isolated from different soil samples. The isolates showed that the cells are oval, negative to Gram-stain, motile, capsulated, formed dark brown pigments in old cultures, catalase positive, not hydrolyzing gelatin, starch or casein, indole negative, M.R. positive, V.P. test positive, acid produced from glucose, fructose, galactose, arabinose, maltose, sucrose, xylose, mannitol, sorbitol and ribose. Acid not produced from lactose. All the isolates were identified as *Azotobacter chroococcum*.

Eight isolates of *Azospirillum* were isolated from different soil samples. The isolates showed that the cells were vibroid, 1-2 x 4-6 μm, negative to Gram stain, very active motile with spiral movement, colonies are round, white, slightly viscid , convex and translucent, catalase positive, M.R., V.P. and indole tests negative, not hydrolysing starch, gelatin and casein, acid produced from glucose, fructose, galactose, arabinose, xylose, mannitol, sorbitol and ribose and acid not produced from maltose, sucrose and lactose. The isolates were designated as *Azospirillum lipoferum.*

Twenty five isolates of *Alcaligenes* were isolated from different soil samples. The isolates showed that the cells were rods and cocobacilli, negative to Gram stain, motile, capsulated, catalase positive, oxidase positive, indole negative, M.R. negative, V.P. negative, citrate negative, not hydrolyzing gelatin, starch or casein, acid not produced from glucose, Lactose, mannitol, maltose, sucrose, xylose, anaerobic growth with nitrate positive and anaerobic growth with nitrite negative. These isolates were were involved in the species of *A. eutrophus* . The isolate Nos.11, 19 and 22 anaerobic growth with nitrate negative and anaerobic growth with nitrite positive. The three isolates were placed under the species of *A. latus* .

Several bacterial cells were isolated from from different soil samples. The cells were positive to Gram stain, endospore, motile, catalase positive, M.R. positive, hydrolyzing starch and casein. Three isolates produced acid from glucose, arabinose, xylose, manitol, not produced gas from glucose, oxidase positive, hydrolyzing gelatin, indole negative, V.P. test positive, and citrate utilization positive. These isolates were identified as *B. subtilis*. Two isolates not produced acid from glucose, arabinose, xylose, manitol, not produced gas from glucose, oxidase positive, hydrolyzing gelatin, indole positive, V.P. test positive, and citrate utilization negative. These two isolates were identified as *B. alvei*. Two isolates not produced acid from glucose, arabinose, xylose, manitol, not produced gas from glucose, oxidase positive, hydrolyzing gelatin, indole negative, V.P. test positive, and citrate utilization

positive. These isolates were identified as *B. cereus*. Two isolates produced acid from glucose, arabinose, xylose, not from manitol, not produced gas from glucose, oxidase negative, not hydrolyzing gelatin, indole negative, V.P. test positive, and citrate utilization positive. These isolates were identified as *B. coagulans*. Five isolates produced acid from glucose, manitol, xylose, arabinose, produced gas from glucose, oxidase positive, hydrolyzing gelatin, indole negative, V.P. test negative, and citrate utilization positive . These isolates were identified as *B. megaterium*. Three isolates produced acid from glucose, manitol, xylose, not from arabinose, not produced gas from glucose, oxidase positive, hydrolyzing gelatin, indole negative, V.P. test positive, and citrate utilization negative. These isolates were identified as *B. polymyxa*. Four isolates produced acid from glucose, not from manitol, xylose, arabinose, not produced gas from glucose, oxidase positive, hydrolyzing gelatin, indole negative, V.P. test positive, and citrate utilization positive. These isolates were identified as *B. thuringiensis*.

Eight *pseudomonas* were isolated from different soil samples. The cells were short rods, nigative to Gram stain, non endospores, motile, catalase positive, oxidase positive, not hydrolyzing starch, indole negative, V.P. negative, citrate utilization negative, acid produced from fructose and not produced from lactose and maltose. Two isolates produced acid from glucose, not produced from sucrose, xylose, manitol, not hydrolyzing gelatin and M. R. test was positive. These isolates identified as *P. putida*. Two isolates produced acid from glucose, sucrose, xylose, manitol, hydrolyzing gelatin and M. R. test was positive. These two isolates identified as *P. fluorescence*. Two isolates produced acid from glucose, manitol, not produces from sucrose, xylose, hydrolyzing gelatin and M. R. test was positive. These isolates identified as *P. aeruginosa*. Two isolates produced acid from manitol, not produces from glucose, sucrose, xylose, not hydrolyzing gelatin and M. R. test negative. These isolates identified as *P. alcaligenes* .

Eleven isolates of *Streptomyces* showed filamentous cells, positive to Gram stain, non motile, catalase positive, oxidase positive, hydrolyzing gelatin, starch and casein, indole negative, M.R. positive, V.P. negative, acid not produced from fructose or lactose, acid not produced from glucose, maltose, sucrose and xylose. The isolates identified as *Streptomyces albus*.

Efficiency of the isolated microorganisms for PHB production :

Data in Table 1 showed that all isolates of *Azotobacter* produced PHB. The cell dry weight varied from 0.54 to 2.28 g/l, the PHB concentration ranged from 0.12 to 0.78 g/l and PHB% ranged from 8.33 to 34.21%. *A. chroococcum* No. 16 showed the highest values of PHB, the cell dry weight, PHB concentration and the yield of PHB were 2.28 g/l, 0.78 g/l, 34.21%, respectively.

PHB level was higher than the level recorded by Cho *et al*., (2001) who studied the production of PHB by *Azotobacter* and found that the PHB concentration was 0.69 g /L when the medium employed for PHB production was supplemented with 20 g glucose. Kim, (2000) used an inexpensive substrate *i.e*. starch, to produce PHB in fed-batch culture of *Azotobacter* .

Fermentation was carried out in a 2.5 liters stirred tank fermentor and the mximum production was 46% PHB.

Table (1): PHB production by *Azotobacter* isolates

Isolates No.	C.D.W (g/l)	PHB (g/l)	PHB (%)
A. chroococcum No. 1	1.08	0.18	16.67
A. chroococcum No. 2	0.94	0.20	21.28
A. chroococcum No. 3	1.72	0.36	20.93
A. chroococcum No. 4	1.48	0.40	27.03
A. chroococcum No. 5	2.3	0.50	21.74
A. chroococcum No. 6	1.6	0.42	26.25
A. chroococcum No. 7	1.72	0.46	26.74
A. chroococcum No. 8	1.88	0.18	9.58
A. chroococcum No. 9	1.06	0.28	26.42
A. chroococcum No. 10	1.44	0.12	8.33
A. chroococcum No. 11	0.98	0.16	16.33
A. chroococcum No. 12	0.72	0.12	16.67
A. chroococcum No. 13	1.1	0.12	10.91
A. chroococcum No. 14	1.14	0.14	12.28
A. chroococcum No. 15	0.54	0.12	22.22
A. chroococcum No. 16	2.28	0.78	34.21
A. chroococcum No. 17	1.46	0.14	9.59

Table 2 shows the production of PHB by *Azospirillum* isolates. Cell dry weight of the used isolates varied from 0.30 to 5.8 g/l, the PHB concentration ranged from 0.12 to 0.58 g/l and PHB% ranged from 3.33 to 53.33 %. *Azo. lipoferum* No. 5 had the highest values of PHB and the cell dry weight, PHB concentration and the yield of PHB were 1.44 g/l, 0.58 g/l, 40.28%, respectively. PHB levels were in the range obtained by Sun *et al*., (2002) who studied the production of PHB by *Azospirillum*. They found that *Azospirillum* can accumulate even more PHB up to 40% of the cell dry.

Table (2): PHB production by *Azospirillum* isolates

Isolates No.	C.D.W (g/l)	PHB (g/l)	PHB (%)
A. lipoferum No. 1	0.30	0.16	53.33
A. lipoferum No. 2	0.30	0.12	40.00
A. lipoferum No. 3	1.40	0.24	17.14
A. lipoferum No. 4	1.42	0.16	11.27
A. lipoferum No. 5	1.44	0.58	40.28
A. lipoferum No. 6	4.20	0.14	3.33
A. lipoferum No. 7	2.02	0.14	6.93
A. lipoferum No. 8	1.58	0.28	17.72

Table 3 showed that, cell dry weight of *Alcaligenes* isolates varied from 0.40 to 7.30 g/l. The PHB production ranged from 0.02 to 0.52 g/l and PHB% ranged from 0.87 to 21.76%. *A. eutrophus* No. 21 recorded the highest amount of PHB (0.52 g/l).

Several investigators studied the production of PHB from *Alcaligenes* isolates. Khanna and Srivastava (2005) found that, *R. eutropha*

exhibited a maximum biomass (3.25 g/l) with a PHB concentration of 1.4 g/l in 48 h. and Beaulieu *et al*., (1995) found that the yield of PHB was 26%, but Kim *et al*., (1995) found that, PHB concentration was 3.15 g/l.

Table (3): PHB production by *Alcaligens* isolates

Isolates No.	Medium No. 7			Medium No. 8		
	C.D.W (g/l)	PHB (g/l)	PHB (%)	C.D.W (g/l)	PHB (g/l)	PHB (%)
A. eutrophus 1	3.88	0.20	5.16	2.48	0.22	8.87
A. eutrophus 2	3.14	0.20	6.37	2.44	0.16	6.56
A. eutrophus 3	2.80	0.14	5.00	1.98	0.28	14.14
A. eutrophus 4	2.90	0.38	13.10	1.30	0.14	10.77
A. eutrophus 5	2.80	0.14	5.00	2.62	0.36	13.74
A. eutrophus 6	3.34	0.18	5.39	2.28	0.10	4.386
A. eutrophus 7	2.98	0.14	4.70	2.90	0.14	4.83
A. eutrophus 8	3.16	0.16	5.06	3.10	0.28	9.03
A. eutrophus 9	3.06	0.14	4.58	1.56	0.08	5.13
A. eutrophus 10	4.40	0.16	3.64	2.20	0.30	13.64
A. eutrophus 11	3.92	0.48	12.25	2.08	0.12	5.77
A. eutrophus 12	1.92	0.24	12.50	0.40	0.02	5.00
A. eutrophus 13	5.48	0.28	5.11	2.30	0.02	0.87
A. eutrophus 14	2.46	0.18	7.32	1.64	0.22	13.42
A. eutrophus 15	1.98	0.28	14.14	1.72	0.08	4.65
A. eutrophus 16	7.30	0.36	4.93	3.68	0.12	3.26
A. eutrophus 17	2.62	0.46	17.56	2.48	0.28	11.29
A. eutrophus 18	5.10	0.48	9.41	1.98	0.28	14.14
A. eutrophus 19	2.20	0.30	13.64	2.30	0.36	15.65
A. eutrophus 20	2.98	0.52	17.45	2.28	0.40	17.54
A. eutrophus 21	2.40	0.52	21.67	2.90	0.44	15.17
A. eutrophus 22	1.64	0.22	13.42	2.10	0.18	8.57
A. latus 1	2.06	0.10	4.85	1.90	0.16	8.42
A. latus 2	2.90	0.14	4.83	2.40	0.18	7.50
A. latus 3	1.90	0.16	8.421	2.62	0.16	6.12

Table 4 shows that all isolates of *Bacillus* produced PHB. Cell dry weight varied from 0.22 to 2.92 g/l. The PHB concentration ranged from 0.02 to 0.16 g/l and PHB% ranged from 1.53 to 21.43 %. *B. megaterium* No. 5 showed the highest values of PHB and cell dry weight, PHB concentration and yield of PHB were 1.42 g/l to 0.16 g/l, 11.27%, respectively. Aslim *et al*., (2002) reported that PHB was produced by *B. subtilis*, *B. megaterium*, *B. firmus*, *B. sphaericus*, *B. theringiensis* and *B. pumilus*. The highest value of PHB in *B. megaterium* Y6 was 0.27 g/l and the cell dry weight was 1.04 g/l in *B. subtilis* K1, and the lowest value of PHB was 0.04 g/l in *B. theringiensis* D1 and the cell dry weight was 1.04 g/l in *B. firmus* G4.

Table 5 shows that cell dry weight of *Pseudomonas* isolates varied from 0.32 to 2.92 g/l, the PHB concentration ranged from 0.10 to 0.30 g/l and PHB% ranged from 4.00 to 43.75%. *P. fluoresens* No.1 recorded the highest values of PHB (0.30 g/l), while the cell dry weight was 1.68 g/l, and the yield of PHB was 17.86 %.

Table (4): PHB production by *Bacillus* isolates

Isolates No.	C.D.W (g/l)	PHB (g/l)	PHB (%)
B. subtilis 1	0.46	0.08	17.39
B. subtilis 2	0.98	0.02	2.04
B. subtilis 3	0.82	0.02	2.44
B. alvei 1	1.34	0.04	2.99
B. alvei 2	1.16	0.02	1.72
B. cereus 1	0.56	0.04	7.14
B. cereus 2	2.62	0.04	1.53
B. coagulans 1	1.02	0.04	3.92
B. coagulans 2	0.68	0.06	8.82
B. megaterium 1	2.92	0.12	4.11
B. megaterium 2	0.22	0.04	18.18
B. megaterium 3	0.48	0.02	4.17
B. megaterium 4	1.10	0.10	9.10
B. megaterium 5	1.42	0.16	11.27
B. polymyxa 1	0.64	0.08	12.50
B. polymyxa 2	1.14	0.04	3.51
B. polymyxa 3	0.28	0.06	21.43
B. thuringiensis 1	0.58	0.06	10.35
B. thuringiensis 2	2.56	0.06	2.34
B. thuringiensis 3	0.64	0.04	6.25
B. thuringiensis 4	0.40	0.02	5.00

Table (5): PHB production by *Pseudomonas* isolates

Isolates No.	C.D.W (g/l)	PHB (g/l)	PHB (%)
P. putida 1	1.02	0.10	9.80
P. putida 2	2.92	0.12	4.11
P. fluorescence 1	1.68	0.30	17.86
P. fluorescence 2	0.84	0.18	21.43
P. aeruginosa 1	2.46	0.14	5.69
P. aeruginosa 2	2.50	0.10	4.00
P. alcaligenes 1	0.92	0.18	19.57
*P. alcaligenes*2	0.32	0.14	43.75

Table 6 shows that cell dry weight of *Rhizobium* isolates, cell dry weight varied from 0.38 to 1.22 g/l, the PHB concentration ranged from 0.06 to 0.36 g/l and PHB% ranged from 7.38 to 29.51%. The highest values of cell dry weight, PHB concentration and the yield of PHB were obtained by *R. leguminosarim* No. 3. Similar results were obtained by Tavernier *et al*., (1997) and Encarnacion *et al*., (2002).

Table 7 shows that cell dry weights of *Streptomyces* isolates were varied from 0.90 to 3.04 g/l, the PHB concentration ranged from 0.02 to 0.12 g/l and PHB% ranged from 1.03 to 6.68%. *Streptomyces albus* No. 10 seemed to be the highest producer of PHB. Verma *et al*., (2002) studied the production of PHB by 12 different strains of *Streptomyces* and found that all the tested isolates produced PHB and wide variation in the PHB content and the time required for maximum production observed.

Table (6): PHB production by *Rhizobium* isolates

Isolates No.	C.D.W (g/l)	PHB (g/l)	PHB (%)
R. leguminasarum 1	1.38	0.16	11.59
R. leguminasarum 2	0.66	0.06	9.09
R. leguminasarim 3	1.22	0.36	29.51
R. leguminasarum 4	1.18	0.10	8.48
R. meliloti 1	0.40	0.08	20.00
R. meliloti 2	2.44	0.18	7.38
R. japonicam	0.38	0.06	15.79

Table (7): PHB production by *Streptomyces* isolates

Isolates No.	Cell dry weight (g/l)	PHB (g/l)	PHB (%)
S. albus 1	2.12	0.04	1.89
S. albus 2	1.00	0.04	4.00
S. albus 3	3.04	0.08	2.63
S. albus 4	1.44	0.04	2.78
S. albus 5	0.90	0.04	4.44
S. albus 6	1.94	0.02	1.03
S. albus 7	3.04	0.10	3.29
S. albus 8	2.54	0.08	3.15
S. albus 9	1.84	0.06	3.26
S. albus 10	1.72	0.12	6.98
S. albus 11	1.46	0.04	2.74

Alginate production :

Table 8 shows that when *Pseudomonas* isolates were used for alginate production, cell dry weight varied from 1.36 to 2.54 g/l, the alginate concentration ranged from 0.12 to 0.38 g/l and alginate % ranged from 6.67 to 14.96 %. *P. aeruginosa* No. 2 showed the highest values of alginate (0.38 g/l). Martins *et al*., (1990) studied the production of alginate by *Pseudomonas* isolates, and Bakkevig *et al*., (2005) observed that *P. fluorescence* produced alginate after 2 days and alginate yield was 0.45 g/l.

Table (8): Alginate production from *Pseudomonas* spp.

Isolates	Cell dry weight (g/L)	Alginate (g/L)	Alginate (%)
P. putida 1	1.68	0.18	10.71
P. putida 2	2.30	0.24	10.44
P. fluorescence 1	1.98	0.22	11.11
P. fluorescence 2	1.36	0.12	8.82
P. aeruginosa 1	1.44	0.12	8.33
P. aeruginosa 2	2.54	0.38	14.96
P. alcaligenes 1	2.08	0.18	8.65
*P. alcaligenes*2	1.80	0.12	6.67

Table 9 shows that cell dry weight of *Azotobacter* isolates were varied from 0.66 to 4.00 g/l, the alginate concentration ranged from 0.02 to 0.44 g/l and alginate% ranged from 1.03 to 20.9%. *A. chroococcum* No. 14 showed the highest values of alginate (0.44 g/l) and the cell dry weight was 4.0 g/l. Castaneda *et al.,* (2000) reported that, alginate was produced by *Azotobater* isolates. Clementi *et al*., (1995) observed that, pH, phosphate and C/N ration affected on the aginate production ant it was 0.63 g/l.

Table (9): Alginate production from *Azotobacter chroococcum* on different media

Isolates No.	Medium No. 1			Medium No. 2			Medium No. 3		
	C.D.W (g/l)	Alginate (g/l)	Alginate (%)	C.D.W (g/l)	Alginate (g/l)	Alginate (%)	C.D.W (g/l)	Alginate (g/l)	Alginate (%)
A.chroococcum No. 1	0.66	0.04	6.06	0.70	0.02	2.86	0.74	0.14	18.92
A.chroococcum No. 2	1.28	0.24	18.75	1.34	0.14	10.45	1.34	0.28	20.90
A.chroococcum No. 3	1.66	0.30	18.07	2.16	0.34	15.74	1.16	0.14	12.07
A.chroococcum No. 4	1.98	0.16	8.08	1.76	0.16	9.09	1.76	0.14	7.96
A.chroococcum No. 5	2.46	0.38	15.45	2.80	0.34	12.14	2.36	0.30	12.71
A.chroococcum No. 6	1.48	0.10	6.76	1.16	0.14	12.07	1.94	0.02	1.03
A.chroococcum No. 7	1.96	0.14	7.14	1.34	0.14	10.45	1.16	0.16	13.79
A.chroococcum No. 8	2.44	0.40	16.39	2.46	0.32	13.01	2.38	0.28	11.77
A.chroococcum No. 9	1.60	0.16	10.00	1.32	0.14	10.61	1.52	0.14	9.21
A.chroococcum No. 10	1.84	0.12	6.52	1.18	0.12	10.17	1.16	0.18	15.52
A.chroococcum No. 11	1.74	0.10	5.75	1.28	0.16	12.50	1.92	0.16	8.33
A.chroococcum No. 12	1.58	0.14	8.86	1.34	0.14	10.45	1.14	0.18	15.79
A.chroococcum No. 13	1.64	0.14	8.54	1.92	0.18	9.38	1.78	0.10	5.62
A. hroococcum No. 14	4.00	0.44	11.00	2.74	0.32	11.68	2.16	0.34	15.74
A.chroococcum No. 15	1.64	0.14	8.54	1.46	0.18	12.33	1.90	0.14	7.37
A.chroococcum No. 16	1.80	0.14	7.78	1.00	0.14	14.00	2.16	0.22	10.19
A.chroococcum No. 17	1.00	0.14	14.00	1.76	0.12	6.82	0.90	0.02	2.22

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إنتاج بعض البوليمرات القابلة للتحلل بواسطة بعض العزلات البكتيرية

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تم عزل العديد من البكتيريا المنتجة للبوليمر الحيوى البولى بيتا هيدروكسى بيوتيرات واللألجينات وتم تنقية هذه السلالات وتعريفها واختبار قدرتها على الإنتاج. وتم عزل 17 عزلة من *Azotobacter chroococcum*، 8 عزلات من *Azospirillum lipoferum* ، 22 عزلة من *Alcaligenes eutrophus* ، 3 عزلات *A. latus* ، 3 عزلات من *Bacillus subtilis*، وعزلتين من *B. cereus* ، 5 عزلات من *B. megaterium* ، وعزلتين من *B. coagulans*، 3 عزلات من *B. polymexa* ، 4 عزلات من *B. thuringiensis* ، وعزلتين من *P. fluoresens*، وعزلتين من *P. putida* ، وعزلتين من *P. alcaligenes* ، وعزلتين من *P. aeruginosa*، 4 عزلات من *Rhizobium leguminasarim* ، وعزلتين من *R. meliloti* عزلة واحدة من *R. japonicum* و12 عزلة من *Streptomyces albus*. كما تم دراسة مدى وجود مادة الألجينات فى عزلات مختلفة من *Azotobacter* و *Pseudomonas*. وكانت كل العزلات لها القدرة على إنتاج الـPHB. وكانت كمية الـPHB المنتجة بواسطة عزلات *Bacillus* تتراح بين 0.02 – 0.16 جرام/لتر ، بينما عزلات *Azospirillum* كانت تتراوح بين 0.12 – 0.58 جرام/لتر. وتم الحصول على أعلى النتائج من *R. leguminasarim* No 3 بينما أقلها كان من *R. leguminasarim* No 2. وعن طريق استخدام بيئتين لإنتاج الـPHB بواسطة *Alcaligenus* تم الحصول على اعلى النتائج من *Alcaligenus eutrophus* No. 20 (0.52 جرام/لتر) . وفى حالة عزلات *Pseudomonas* أعطت العزلة *Pseudomonas fluorescence* No. 2 أعلى النتائج (0.3 جرام/لتر) بينما *P. aeruginosa* No. 2 كانت 0.1 جرام/لتر. وأظهرت العزلة رقم 16 من جنس *Azotobacter* أعلى النتائج حيث كان وزن الخلايا الجافة ، تركيز مادة PHB والمحصول النهائى لمادة PHB % هو 2.28 جرام / لتر ، 0.78 جرام / لتر و34.21 % على الترتيب. بينما أعلى تركيز من الألجينات تم الحصول عليها كان 0.44 جرام/لتر من العزلة رقم 14 لجنس *Azotobacter*.