Title of thesis:

STUDIES ON CERTAIN MICROBIAL POLYMERS

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5. SUMMARY

Studies on certain microbial polymers

PHAs are an aliphatic microbial polyesters produced by a variety of bacteria as a carbon and energy-storage material. The most commonly occurring PHA is polyhydroxybutyrate (PHB), which is comprised of condensed hydroxybutyrate monomers. Alginate is a linear copolymer composed of 1-4 linked β-D-mannuronic acid and α-L-guluronic acid. Alginate is produced from two genera of bacteria *Pseudomonas* and *Azotobacter*.

The aim of this work was isolation, identification of some local bacterial isolates and testing their for PHB and alginate production. Also, factors affecting PHB production by the active microorganism and spectroscopic analysis of PHB were studied.

This study divided into three parts :

The first part : Isolation and Identification of the isolates produced biopolymers :

Azotobacter, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Streptomyces* and *Alcaligenes* were isolated on specific culture media from the rhizosphere of several plants. The isolates were purified and identified.

1- Seventeen isolates of *Azotobacter* were isolated from different soil samples and were identified as *A. chroococcum* .

2- Eight isolates of *Azospirillum* were isolated from different soil samples and were identified as *A. lipoferum*.

3- Twenty five isolates of *Alcaligenes* were isolated from different soil samples, twenty two of them were identified as *A. eutrophus* and the last three were identified as *A. latus*.

4- Twenty one isolates of *Bacillus* were isolated from different soil samples. Three of them were identified as *B. subtilis*, two isolates were identified as *B. alvei*, two isolates were identified as *B. cereus*, two isolates were identified as *B. coagulans*, five isolates were identified as *B. megaterium*, three isolates were identified as *B. polymyxa* and the last four isolates were identified as *B. thuringiensis*.

5- Eight isolates of *Pseudomonas* were isolated from different soil samples. Two of them were identified as *P. putida*, two isolates were identified as *P. fluorescence*, two isolates were identified as *P. aeruginosa* and the last two isolates were identified as *P. alcaligenes*.

6- Eleven isolates of *Streptomyces* were isolated from different soil samples and were identified as *S. albus*.

7- Four isolates of *Rhizobium leguminosarum*, two isolates of *R. meliloti* and one isolate of *R. japonicam* were obtained from Microbiology Department, Fac. of Agric., Mansoura Univ.

The second part : Efficiency of the bacterial isolates for PHB and alginate production :

For screening the most active isolates which can produce PHB and alginate, and to achieve this propose all isolates were cultivated in 250 ml Erlenmeyer flasks, each containing 50 ml of sterile different specific media and inoculated with standard inoculum.

1. PHB production :

1- Seventeen isolates of *A. chroococcum* were tested for PHB production on **Pozo *et al.* (2002)** medium. The best isolate of PHB production was *A. chroococcum* No. 16 (0.78 g/l).

2- Eight isolates of *Azospirillum lipoferum* were tested for PHB production on **Vanstockem *et al.* (1987)** medium. The best isolate of PHB production was *A. lipoferum* No. 5 (0.58 g/l).

3- Twenty two isolates of *Alcaligenes eutrophus* and three isolates of *A. latus* were tested for PHB production on two different media **(Wang and Lee (1997)** medium and **Beaulieu *et al.* (1995)** medium. The best isolate of PHB production was *A. eutrophus* No. 21 on **Wang and Lee (1997)** medium (0.52 g/l).

4- Three isolates of *Bacillus subtilis*, two isolates of *B. alvei*, two isolates of *B. cereus*, two isolates of *B. coagulans*, five of *B. megaterium*, three isolates of *B. polymyxa* and four isolates of *B. thuringiensis* were tested for PHB production on nutrient broth medium supplemented with 2 % glucose as carbon source. The best isolate for PHB production was *B. megaterium* No. 5 (0.16 g/l).

5- Two isolates of *Pseudomonas putida*, two isolates of *P. fluorescence*, two isolates of *P. aeruginosa* and two isolates of *P. alcaligenes* were tested for PHB production on **Qiang *et al.* (2001)** medium. The best isolate for PHB production was *P. fluorescence* No. 1 (0.30 g/l).

6- Four isolates of *Rhizobium leguminosarum*, two isolates of *R. meliloti* and one isolate of *R. japonicam* were tested for PHB production on **Tavernier *et al.* (1997)** medium. The best isolate for PHB production was *R. leguminosarum* No. 3 (0.36 g/l).

7- Eleven isolates of *Streptomyces albus* were tested for PHB production on YEME medium. The best isolate of PHB production was *S. albus* No. 10 (0.12 g/l).

2. Alginate production :

The present experiments deal with evaluation the production of alginate by eight isolates of *Pseudomonas* spp. on **Bakkevig *et al.* (2005)** medium. The best isolate for alginate production was *P. aeruginosa* No. 2 (0.38 g/l), and seventeen isolates of *Azotobacter chroococcum* grown on three different media (**Clementi *et al.* (1995)** medium, **Jimenez *et al.* (2005)** medium and **Lange *et al.* (2002)** medium). The best isolate for alginate production was *A. chroococcum* No. 14 on **Clementi *et al.* (1995)** medium (0.44 g/l).

It could be observed that the best producer for PHB was *A. chroococcum* No. 16 on **Pozo *et al.* (2002)** medium, thus this isolate and this medium were employed for PHB production in the following experiments .

The third part :

1- The influence of environmental conditions on the accumulation of PHB in *Azotobacter chroococcum* :

The purpose of this part is to investigate the optimal conditions for PHB production by *A. chroococcum* No. 16 using **Pozo *et al.* (2002)** medium. The experiments were carried out in factorial arrangement.

1- The best time course was after 54 hours of fermentation and the PHB production was 0.85 g PHB /l.

2- The optimum initial pH was 7.5 and the PHB production was 1.06 g PHB /l.

3- Thirteen different carbon sources were used for PHB production. The highest amount of PHB was obtained with glucose syrup being 1.76 g/l, biomass was 3.92 g/l, consumed sugars concentration was 5.61 g/l, PHB % was 44.90 % and PHB conversion coefficient was 31.37%.

4- To investigate the effect of glucose syrup (as total sugars) concentration on PHB production, an experiment was designed using different concentrations of glucose syrup which the sugar content varied from 5 to 30 g/l. Every one was supplemented with the inorganic nutrients of the basal medium. At 12.5 g/l sugar concentration PHB reached its maximum being 1.81 g/l, cell dry weight was 4.39 g/l, consumed sugars concentration was 7.99 g/l, PHB % was 41.30 % and conversion coefficient was 22.67 %. Therefore, this concentration of total sugars (12.5 g/l) which proved to be the optimum, was used in the following experiments, (12.5 g/l from total sugar equivalent about 29.76 g/l from glucose syrup).

5- Fifteen different nitrogen sources were investigated. Because of *A. chroococcum* is a N_2 fixer, one treatment was used without nitrogen source. The highest growth and PHB production were obtained with NH_4NO_3 as a nitrogen source (6.54 g/l and 2.16 g/l, respectively) followed by $(NH)_2CO$ and $NH_4H_2PO_4$. PHB production in the case of organic nitrogen sources (peptone, yeast extract, malt extract, beef extract and CSL) was lowest than inorganic sources.

6- The effects of NH_4NO_3 on PHB production were investigated by increasing or reducing NH_4NO_3 concentrations. The highest value of PHB concentration, 2.66 g/l, was obtained with 1.25 g NH_4NO_3/l. Also at 1.25 g/l NH_4NO_3 biomass was 6.28 g/l, consumed sugars concentration was 8.27 g/l, PHB % was 42.39 % and conversion coefficient was 41.19 %.

7- The effect of K_2HPO_4 on PHB production was investigated by increasing or reducing K_2HPO_4 levels. The highest value of PHB concentration 3.04 g/l was obtained with 0.20 g/L K_2HPO_4. Also at 0.20 g/l K_2HPO_4 biomass was 8.24 g/l, consumed sugars concentration was 9.10 g/l, PHB % was 36.89 % and conversion coefficient was 33.41 %.

8- The effect of KH_2PO_4 concentration on PHB production was investigated by increasing or reducing KH_2PO_4 levels. The highest PHB concentration (3.12 g/l) was obtained with 0.15 g/l KH_2PO_4. Also at 0.15 g/l KH_2PO_4 biomass was 8.54 g/l, consumed sugars concentration was 10.73 g/l, PHB % was 36.53 % and conversion coefficient was 29.08 %.

9- The effect of NaCl on PHB production was investigated by increasing or reducing NaCl levels. The highest value of PHB concentration 3.56 g/l was obtained with 0.10 g/l NaCl. Also at 0.10 g NaCl/l biomass was 7.66 g/l, consumed total sugars concentration was 10.36 g/l, PHB % was 46.48 % and conversion coefficient was 34.36 %.

10- The effect of $MgSO_4.7H_2O$ on PHB production was investigated by increasing or reducing $MgSO_4.7H_2O$ levels. The highest value of PHB concentration (3.74 g/l) was obtained with 0.25 g $MgSO_4.7H_2O$/l. Also at 0.25 g $MgSO_4.7H_2O$/l biomass was 7.22 g/l, consumed sugars concentration was 10.32 g/l, PHB % was 51.80 % and conversion coefficient was 36.24 %.

11- The effect of $CaSO_4.2H_2O$ concentrations on PHB production was investigated by increasing or reducing $CaSO_4.2H_2O$ levels. The highest value of PHB concentration 3.82 g/l was obtained with 0.08 g/l $CaSO_4.2H_2O$. Also at 0.08 g/l $CaSO_4.2H_2O$ biomass was 8.00 g/l, consumed total sugars concentration was 10.12 g/l, PHB % was 47.75 % and conversion coefficient was 37.75 %.

12- The effect of $NaMoO_4.2H_2O$ PHB production was investigated by increasing or reducing $NaMoO_4.2H_2O$ levels. The highest value of PHB concentration (3.80 g/l) was obtained with 0.01 g/l $NaMoO_4.2H_2O$. Also at 0.01 g/l of $NaMoO_4.2H_2O$ biomass was 8.12 g/l, consumed sugars concentration was 11.11 g/l, PHB was 46.80 % and conversion coefficient was 34.20 %.

13- The effect of ferric citrate on PHB production was investigated by increasing or reducing ferric citrate levels. The highest value of PHB concentration 3.82 g/l was obtained with 0.02 g/l ferric citrate. Also at 0.02 g/l ferric citrate biomass was 8.14 g/l, consumed sugars concentration was 10.23 g/l, PHB % was 46.93 % and conversion coefficient was 37.34 %.

2- The spectroscopic analysis of PHB polymer :

IR spectroscopic analysis was done, the analysis of the spectrum of PHB was observed at 3400, 1639, 3018, 2978, 2842, 1216, 1044, 1164, 434 and 669-765δ, respectively, for (-OH) broad peak, (C=C) double bond, (≡C-H) acetylenic bond, ($-CH_3$) methyl group, (-S-H) thiol weak adsorption, (≡C-O-C≡) ether, (-O-C-O), (C-C=O), sulfoxide and (F-Cl) haloalkanes. The present of ether-linkage give the polymer elasticity and flexibility. The chains have the C=O and CH_3 groups in the amorphous regions is responsible for the good mechanical properties**.**

Final conclusion :

Maximum PHB obtained was 3.82 g/L. The selected strain was *A. chroococcum* No.16. The medium employed for PHB production contained glucose syrup : 29.76 g/L, ammonium nitrate : 1.25 g/L, 0.2 g K_2HPO_4; 0.15 g KH_2PO_4; 0.2 g NaCl; 0.25 g $MgSO_4.7H_2O$; 0.08 g $CaSO_4.2H_2O$; 0.01 g $NaMoO_4.2H_2O$; 0.02 g ferric citrate and pH was 7.4 at 30°C for 54 hours using shaking culture at 200 rpm. The IR analysis confirmed the polymer formation and it had a good mechanical properties.