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Production and Optimization of Green Plastics (*Polyhydroxybutyrate*) by Local Isolated Strain of *Enterobacter cloacae* DSM 30054 Using Whey Medium

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ABSTRACT

There is a growing interest in using biodegradable green plastics polyhydroxybutyrate (PHB) produced by bacteria, which is seen as the most suitable alternative to conventional synthetic plastics. Also, using cheap agro-industrial wastes have attracted research and commercial interest worldwide. The present study aimed to isolate potent PHB producing bacteria and using whey as a low cost PHB production medium. Out of 20 bacterial isolates, 10 isolates were found to be PHB positive based on Sudan Black staining method. One potent bacterial isolate with maximum PHB (870 mg/l) was selected upon screening using whey medium which identified as *Enterobacter cloacae* DSM 30054 based on 16S rRNA sequence analysis. The culture medium and growth parameters of *E. cloacae* DSM 30054 were optimized for maximum PHB production. Maximum PHB production (2186 mg/l) was achieved at 48 h when whey medium was supplemented with 3% sucrose and 1% sodium nitrate at pH 7.0, incubation temperature of 35°C, and inoculum size (2%).

Keywords: *Enterobacter cloacae*, PHB and whey

INTRODUCTION

Many environmental problems have been caused by the accumulation of the nearly 25 million tons per year of synthetic, plastics derived from petroleum substances, due to their nonbiodegradable nature (Fiechter 1990, Plastics Europe 2015, 2017). This is crucial to search for other sources of plastic that is biodegradable and friendly for environment. Bioplastic, Polyhydroxybutyrate (PHB), is well known for its environmental friendliness and complete decomposition into water and carbon dioxide by microorganisms, can be produced by some living organisms such as plants, fungi and bacteria (Mahitha and Madhuri 2016). Bioplastics can be used in many industrial, agricultural, medical and pharmaceutical applications as an alternative to synthetic plastics.

Prokaryotic microorganisms produce a group of biocompounds called the PHA includes polyhydroxybutyrate (PHB), polyhydroxyvalerate (PHV), polyhydroxyhexanoate (PHH) and polyhydroxyoctanoate (PHO). The PHB is the most common biodegradable polymer in the prokaryotes among these compounds (Verlinden *et al.*, 2007). Many bacterial strains accumulated PHB as a stored carbon and nitrogen sources and used it when exposed to starvation, such as *Ralstonia eutropha*, *Enterobacter* sp., *Pseudomonas* sp., *Azotobacter* sp., and *Bacillus* sp. Which can be isolated from different sources such as; soils contaminated with oil, plant rhizosphere and high salinity environments and known for their high efficiency in PHB production (Shodhganga 2011).

Several factors affecting the production of PHB process, such as incubator temperature, pH and speed of shaking in parallel with both the excess carbon source and the limitation of nitrogen source, were taken into consideration when selecting the best isolates for production. (Contreras *et al.*, 2013 and Saharan *et al.* 2014). The depletion of nitrogen source stops the protein synthesis, which in turn causes the inhibition of the biosynthesis of citrate and isocitrate dehydrogenase enzymes, and consequently, TCA cycle slowdown, then Acetyl-CoA turns into Polyhydroxybutyrate synthesis (Shivakumar 2012).

Approximately, 50% of the PHB production cost is the carbon source cost (Akaraonye *et al.*, 2010 and Nielsen *et al.*, 2017). Therefore, the use of wastes for production of polyhydroxybutyrate doesn't only lead to the disposal of waste which acts as an environmental problem, but also has commercial and economical values. Hence, it has become clear the importance of using a whey waste in the production of the polymer, in terms of reducing the cost of production in conjunction with solving the environmental problem of the waste (Amaro *et al.,* 2019).

Whey is the main by-product of the dairy industry. Only about 50% of 120 million tons of whey, which produced annually all over the world, are used as human food and animal feed products (Nikodinovic-Runic *et al.*, 2013). The composition of whey is mainly proteins (27–60 g/L), lactose (39–60 g/L), fats (0.99–10.58 g/L), and mineral salts (4.6–8 g/L) (Prazeres *et al.*, 2012 and Colombo *et al.*, 2016). Due to this high level of lactose sugar in whey, most of the biochemical oxygen demand is

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returned; as a result, it is necessary to use this waste to produce important biological materials (Carvalho *et al.*, 2013 and Nikodinovic-Runic *et al.*, 2013). Hence, it has become clear the importance of using whey waste in PHB production, in terms of reducing the cost of PHB production and simultaneously solving an environmental problem

Hence, the current study aims to isolate a bacterial strain from the soil that is highly efficient in PHB production, as well as to use a cheap and available whey as a medium. Also, cultural and environmental conditions such as incubation period, carbon and nitrogen sources, incubation temperature, pH, and inoculum size were optimized for maximizing the production of PHB.

MATERIALS AND METHODS

Substrate

Whey was obtained from the dairy factory in Faculty of Agriculture, Mansoura University. The whey was used as a low cost medium for PHB production.

Isolation and purification of bacterial strains

Soil samples were collected to isolate the PHB producing bacteria, then used to make serial decimal dilutions in sterile saline (0.85% NaCl). One ml of suitable dilutions was inoculated in nutrient agar medium enriched with 1% glucose and incubated at 30˚C for 48h. The differently featured colonies that grown on the plats were picked up and re-streaked for purification. The slants of pure bacterial isolates were maintained on nutrient agar slants and stored at 4°C for further studies.

Screening of isolates for PHB production

Screening for PHB-production using Sudan Black B dye on nutrient agar:

Sudan Black B (SBB) staining method was used to detect the potential of pure isolates for PHB production. The tested bacterial isolates were inoculated in plates containing nutrient agar medium enriched with 1% glucose. Then, solution of 0.3% SBB in ethanol 70% was spread over the plates of grown tested isolates, completely soaking them and the plates were kept at room temperature for 30-60 min. Then, the bacterial colonies were destained by washing with ethanol (97%) to get rid of the excess stain. Colonies that maintain black color of SBB after destaining represent the PHB producing strains (Murray *et al.*, 1994 and Mohamed *et al.*, 2012).

Screening for PHB-production using Sudan Black B dye and light microscope:

Microscope slides of thin smears of tested bacterial isolates were prepared, air dried, then stained with solution of 0. 3% w/v SBB in 70% ethanol for 10-15 minutes. The slides were washed with distilled water, then were stained with solution of 5% w/v safranin in distilled water for 5 minutes. The slides were examined under oil immersion microscope at 1000x magnification for PHB granules. Bacterial cells that appear black or blue violet under the microscope were considered as positive isolates for PHB production while the others were negative (Murray *et al.*, 1994, Aneja, 2001 and Legat *et al.*, 2010).

Inoculum preparation

Fifty ml of nutrient broth medium supplemented with 1% glucose were inoculated with one loop full of 24h of selected isolate. The flasks were incubated at 30 °C on the shaking incubator at 150 rpm for 24 h. One ml of inoculum (10^6 CFU/ml) was added to 50 ml of culture medium.

Culture medium

Whey solution was boiled for 5 min then filtered after cooling to remove the aggregates (Yellore and Desai, 1998), then it was used as a culture medium for production of PHB. Each Erlenmeyer flasks (250 ml) containing 50 ml of whey medium (pH 7) was inoculated with one ml of fresh inoculum and incubated at 30°C for 48h on the shaking incubator at 150 rpm. Samples to estimate the PHB production were collected at time interval.

Efficiency of PHB production by positive selected isolates on whey medium

The efficiency of PHB production by selected bacterial isolates from Sudan black B screening method, were determined by cultivating on whey medium. One ml of bacterial inoculum were added to 50 ml of whey medium (pH 7) and incubated at 30°C on the shaking incubator at 150 rpm for 48h. The potential of PHB production was determined calorimetrically according to Law and Slepecky (1961), and the bacterial isolate which showed the highest PHB producing potential was selected for further studies.

Morphological and molecular identification of PHB producing isolate

The selected PHB producing isolate was characterized by morphological characterization and molecular identification was carried out by 16S rRNA sequencing.

Optimization of culture medium conditions

Effect of incubation periods

Whey medium was inoculated with most potent PHB producing bacterial isolate and incubated at each time point (24, 48, 72, 96 and 120h) at 30°C on shaking incubator at 150 rpm. The samples were collected at an interval 24h to estimate the production of PHB.

Effect of carbon sources

Five different carbon sources which are; glucose, sucrose, starch, lactose and fructose at 1% concentration were used to supplement the whey medium. The cultures were incubated for 24 and 48 h at 30˚C on the shaking incubator at 150 rpm. The best carbon source for maximum PHB yield was selected for further studies. Whey medium without carbon source were used as control.

Effect of nitrogen sources

Five different nitrogen sources (ammonium sulphate, ammonium chloride, ammonium nitrate, sodium nitrate and calcium nitrate) at 1% concentration were added to whey medium supplemented with best carbon source, pH was adjusted to 7 and incubated for 24 and 48 h at 30°C. The best nitrogen source for maximum PHB yield was determine and used for further studies.

Effect of carbon source concentration

Different concentrations (0.5, 1.0, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 %) of the best carbon source were used to determine the optimum concentration for maximum PHB production. Whey medium supplemented with 1% of the best nitrogen source was used and incubated at 30 ºC for 48h.

Effect of pH

Whey medium supplemented with the best concentration of both carbon and nitrogen source, was adjusted at different pH values (5, 6, 7, 8 and 9). Cultures were incubated for 48h at 30°C on the shaking incubator at 150 rpm. The optimum pH for maximum PHB yield was determine and used for further studies.

Effect of temperatures

The effect of different temperatures (20, 25, 30, 35, 40 and 45 °C) on PHB production were studied. Whey medium supplemented with the best concentration of carbon and nitrogen source and adjusted at optimum pH was applied. The optimum temperature for maximum PHB yield was selected for further studies.

Effect of inoculum size

The effect of varying inoculum size (from 1 to 10%) on PHB production by the selected isolate was studied. Whey medium supplemented with the best concentration of both carbon and nitrogen source, and adjusted at optimum pH was used. The culture incubated at optimum temperature. Based on the maximum PHB production, the optimum inoculum size was chosen.

Quantification of PHB production

PHB production was quantified by using the method of Law and Slepecky (1961). The culture growth of the selected isolate was centrifuged at 6000 rpm at 4°C for 10 min. The pellets were then washed with equal volume of acetone and ethanol to dispose undesired components. Equal volume of 4% sodium hypochlorite was used to resuspend the pellets and kept at 50°C for 60 min. Then, to precipitate the lipid granules in the mixture, centrifugation at 6000 rpm for 10 min was carried out. The resultant pellets were washed by using equal amount of acetone and ethanol and the supernatant was disposed. The pellets were resuspended in hot chloroform and filtrated through Whatman No. 1 filter paper (treated with hot chloroform). By adding 10 ml of hot concentrated H_2SO_4 to the filtrate, PHB turned into crotonic acid, which gives a brown color. after cooling, PHB was quantified as a crotonic acid by measuring the absorbance on UV-VIS spectrophotometer at 235 nm using a concentrated H_2SO_4 as blank (Soam *et al*., 2012). Quantification of the PHB was performed by comparing to the standard curve of poly-β-hydroxybutyrate (Sigma-Aldrich).

RESULTS AND DISCUSSION

Isolation of bacterial strains

Twenty bacterial isolates were isolated from soil samples. Bacterial colonies with different features were selected and subjected to a screening of PHB production.

Screening of bacterial isolates for PHB production by Sudan Black B dye

For detecting the formation of PHB in the bacterial isolates cells on petri palates, the method of Sudan Black B staining as well as under the light microscope were performed. Ten isolates were positive, which showed the ability to form a PHB inside their cells while the others showed negative reaction. The positive bacterial isolate showed black colonies on the plates, and black granules appeared inside the cell under the microscope. Several researchers have used Sudan Black dye to detect the formation of PHB as a screening method as Ceyhan and Ozdemir (2011) who used Sudan Black dye for the detection of PHB accumulation in *Enterobacter aerogenes*. Lathwal *et al.,* (2015) who used Sudan Black dye for screening bacterial isolates for PHB production. Also, Musa *et al*., (2016) used Sudan Black method for detection granules of PHB.

Efficiency of PHB production by bacterial isolates on whey medium

The efficiency of PHB-production by the ten selected isolates from Sudan Black B staining method, were determined by using submerged fermentation on whey medium after 48 h of incubation (Fig. 1). The presented results show that, the PHB yield varied between 158 to 870 mg/l and the most potent PHB producer was isolate No. 10 (870 mg/L) after 48 h of fermentation. Isolate No..10 was selected for further studies as the superior PHB producer. Although the whey is considered a favorable growth medium for many microorganisms, a few microbes have been able to produce PHB from the whey. Obruca *et al*., (2011) found that *Bacillus megaterium* CCM 2037 produced 0.5 g/l PHB when cultured in whey medium. Also Berwig *et al*., (2016) mentioned that *Alcaligenes latus* was able to use whey lactose and produce PHA. While, Marangoni *et al*., (2002) and Pais *et al*., (2016) reported that *Cupriavidus necator* and *Haloferax mediterranei*, were able to produce PHAs when grown in hydrolyzed whey lactose.

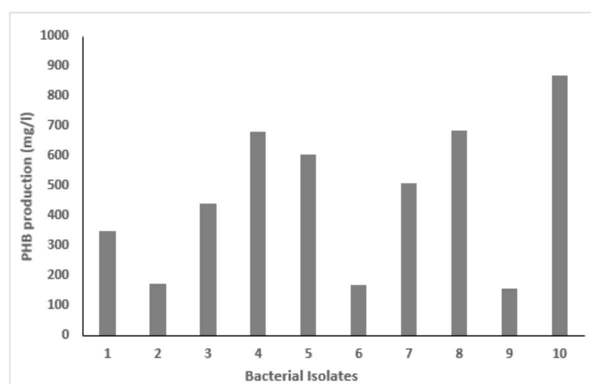

Fig. 1. PHB production (mg/l) by the bacterial isolates after 48h. incubation

Morphological and molecular identification of PHB producer bacterial isolate

The morphological characteristics of isolate No. 10 were examined, the results showed that isolate No.10 was facultative anaerobic, Gram negative, motile and rod shaped bacteria. In addition, molecular identification by 16S rRNA gene sequence analysis was performed and it was assigned as *Enterobacter cloacae* strain DSM 30054. (Fig. 2). *E. cloacae* DSM 30054 has been used for the production of PHB.

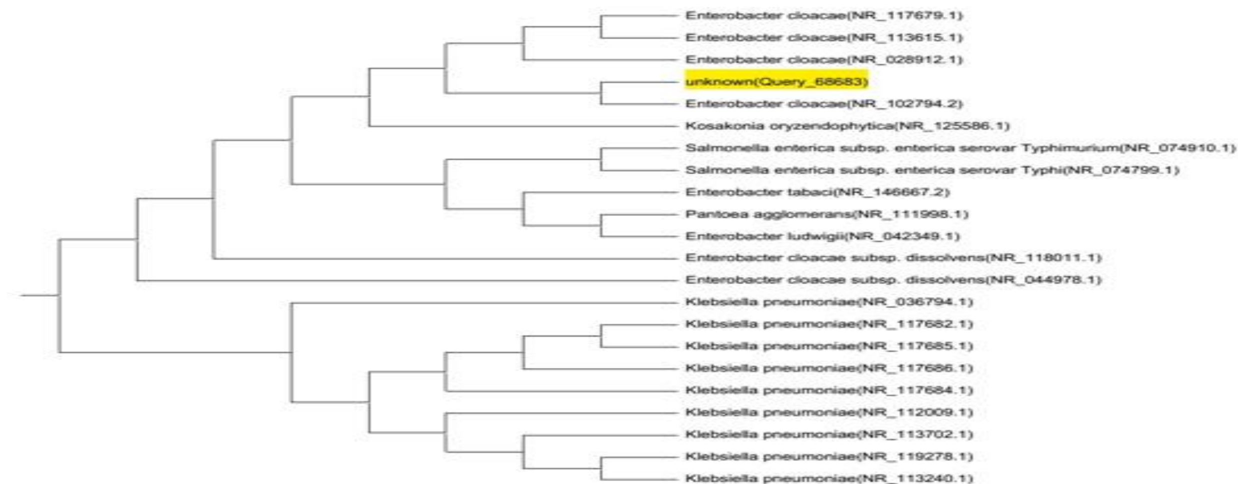

Fig .2. Phylogenetic tree of *Enterobacter cloacae* DSM 30054

Optimization of culture conditions

Effect of incubation periods

The effect of incubation periods on PHB production by *E. cloacae* DSM 30054 is shown in Fig. (3). The results show that the accumulation of PHB increased in 48 h of incubation, then PHB production was decreased. The maximum production of PHB (702 mg/l) was achieved at 48h of incubation. When more carbon sources are available, PHB are accumulated and during times of starvation are consumed to stimulate the survival of bacteria, so PHB acts as osmotic neutral reservoirs of carbon (UchiNo. *et al*., 2007 and Flora *et al*., 2010). Ceyhan and Ozdemir (2011) reported that the maximum PHB was accumulated by *Enterobacter aerogenes* in the stationary phase of growth. Hungund *et al*., (2013) showed that the synthesis of PHB was started at the log phase and continued till stationary phase of growth. A maximum of PHB production was observed at the late log phase of growth and reached maximum at 48 h of incubation of *Enterobacter cloacae* SU-1, *Burkholderia VK*-12 sp, *Bacillus subtilis* BP1 and *Bacillus subtilis* G1S (Samrot *et al.,* 2011, Kumbhakar *et al.,* 2012, Shah, 2014 and Irsath *et al*,. 2015), respectively. While Nair (2013) found 24 h was the optimum incubation period for PHB production from purified cultures of *Alcaligenes sp., Neisseria sp., Bacillus sp.* and *Pseudomonas sp*. The time of maximum PHB production varies among different bacteria and depends mostly upon cultural and environmental conditions used during fermentation and genetic potential of the microorganism (Singh *et al.,* 2013)

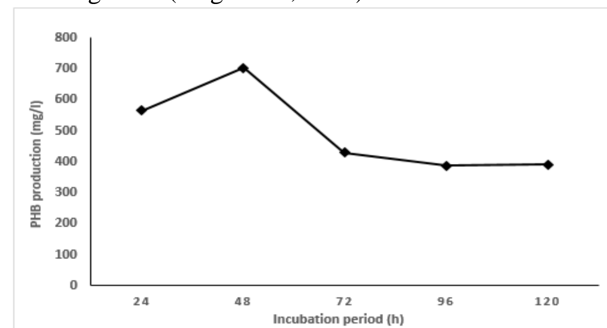

Fig. 3. Effect of incubation periods on PHB production (mg/l) by *Enterobacter cloacae* DSM 30054

Effect of carbon sources

The effect of additional different carbon sources (glucose, fructose, sucrose, lactose and starch) on PHB production by *E. cloacae* DSM 30054 is shown in Fig. (4). Data indicate that all additive carbon sources stimulated PHB production by *E. cloacae* DSM 30054. Maximum PHB yield (1127 mg/l) was obtained after 48h with sucrose addition followed by fructose, glucose, lactose and starch. Thus, disaccharide sucrose was the best carbon source for *E. cloacae* DSM 30054 that enhanced the PHB production. Also, the obtained results show that, the *E. cloacae* DSM 30054 has low capacity to convert the additional lactose into PHB (850 mg/l) comparable with the high capacity (1127 mg/l) with sucrose. On the other, at 24 h, the maximum yield of PHB was obtained with glucose (875 g/l) followed by sucrose (818 mg/l). In general, all studied carbon sources stimulated good PHB production by *E. cloacae* DSM 30054 except starch as it is a complex carbon source which is may be difficult to utilize in efficient production of PHB (Israni and Shivakumar 2015). These results are similar with those obtained by Wu et *al.,* (2001) where sucrose was found to be the best carbon source for PHB Production by *Bacillus* sp. JMa5 (25-35%, w/w). Also, Nabila and Veena (2016) reported that sucrose induced the PHB production by two halotolerant bacterial strains isolated from marine and Belal (2013) reported that, in the case of *Pseudomonas stutzeri*, sucrose was the best carbon source. On the other hand, Samrot *et al*., (2011) found that *Enterobacter cloacae* SU-1 was accumulated more PHA in the presence of lactose (87% w/w) than glucose (7.5% w/w). Also, Belal (2013) mentioned that, *Rhizobium elti* could be utilize of mannitol in efficient production of PHB.

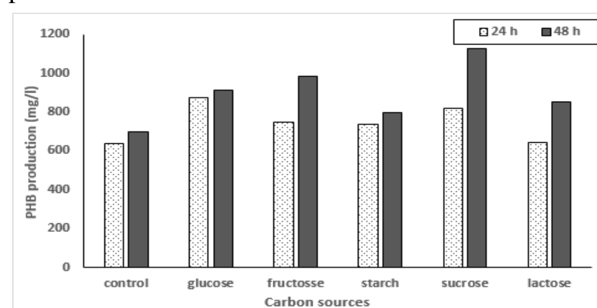

Fig. 4. Effect of carbon sources on PHB production (mg/l) by *E. cloacae* DSM 30054 after 24 and 48 h of incubation

Effect of nitrogen sources

Five different nitrogen sources (ammonium sulphate, ammonium chloride, ammonium nitrate, sodium nitrate and calcium nitrate) at concentration of 1% were added to the whey medium along with the best carbon source (sucrose, 1%). Figure (5) shows that nitrogen sources containing ammonium ion (ammonium sulphate, ammonium chloride and ammonium nitrate) stimulated PHB production, but their effects were less compared to nitrogen sources containing nitrate ion (sodium nitrate and calcium nitrate). Also, among five tested nitrogen sources, sodium nitrate gave the maximum PHB production by *E. cloacae* DSM 30054 (1470 mg/L), followed by calcium nitrate (1335 mg/L), so sodium nitrate was chosen as the best nitrogen source for production of PHB. These results are in agreement with those of Musa *et al.,* (2016) who found that inorganic nitrogen KNO_3 was the best in the case of using *Bacillus subtilis* and *Escherichia coli* for PHB production. In addition, sufficient PHB production by *Pseudomonas stutzeri* E114 and *Rhizobium elti* E1 was observed with ammonium nitrate (Belal, 2013). Also, the obtained results are supported by the results of Beaulieu *et al*., (1995) and Elsayed *et al*., (2013) who reported that the presence of inorganic nitrogen salts is necessary to maximize the accumulation of PHB as a result to stimulate the growth. On the other hand, in contrast to these results, Shah (2014) reported that the complex nitrogen source, yeast extract increased the yield of PHB by *Bacillus megaterium.*

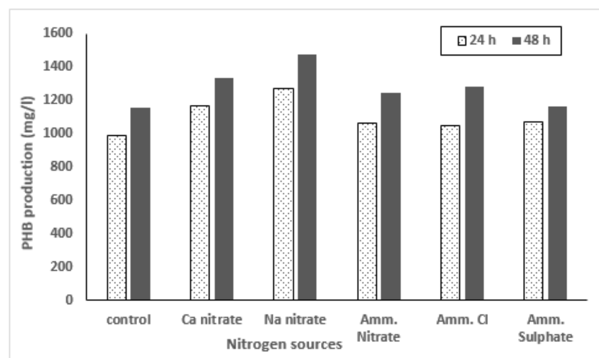

Fig. 5. Effect of nitrogen sources on PHB production (mg/l) by *E. cloacae* DSM 30054 after 24 and 48 h of incubation

Effect of concentrations of sucrose

The effect of different concentrations (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5%) of the best carbon source (sucrose) on PHB production by *E. cloacae* DSM 30054 was studied in whey medium supplemented with 1% of sodium nitrate (as the best nitrogen source) (Fig. 6). The results show that, by increasing the concentrations of sucrose up to 3% the PHB production increased. The maximum PHB (1890 mg/l) was recorded at after 48 h of cultivation in the whey medium supplemented with 3% sucrose by *E. cloacae* DSM 30054. However, higher levels of sucrose (>3%) reduced the yield of PHB. This reduction might be attributed to substrate inhibition at higher concentrations, decay in PHB biosynthesis enzyme system, depletion of nutrients along with PHB consumption by the cells (Flora *et al*., 2010). Similar observations were reported in previous studies (Belal, 2013 and Panigrahi and Badveli, 2013). Jehan *et al*., (2016) observed that sucrose at concentration 2% was maximized the yield of PHB by *Pseudomonas boreopolis.*

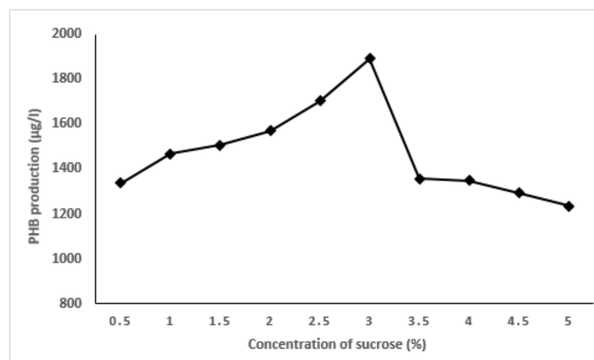

Fig. 6. Effect of sucrose concentrations (%) on PHB production (mg/l) by *E. cloacae* DSM 30054 after 48 h of incubation

Effect of pH

Different pH values (5.0, 6.0, 7.0, 8.0 and 9.0) were used to evaluate its effect on PHB production on whey medium supplemented with best carbon and nitrogen (3% sucrose and 0.1% sodium nitrate) sources. Data represent in Fig. (7) show that the maximum PHB production (1935 mg/l) was achieved at pH 7.0 by *E. cloacae* DSM 30054. Also, data show the ability of *E. cloacae* DSM 30054 to grow and accumulate PHB at a wide range of pH from pH 5 to pH 9. Low PHB production (759 mg/l) was obtained at pH 5, that is demonstrated the negative impact of the acidic medium on PHB production. While there was a slight effect for basic media (pH 9) on PHB production (1735 mg/l). Thus, pH 7.0 was optimum for PHB production. It is due to most favorable pH for bacterial growth is neutral pH (7.0), and hence contributes to higher PHB production (Shaaban *et al.*, 2012). These results are in accordance with the results obtained by Samrot *et al*., (2011) who reported that *E. cloacae* SU-1 produced maximum concentration of PHA (94% w/w) at pH 7.5. Sindhu *et al*., (2011) who found that, the maximum PHB production by *Bacillus sphaericus* NII 0838 was achieved with pH 7.0 PHB. Also, according to Kumbhakar *et al*., (2012) who reported that the maximal PHB production by *Burkholderia* isolate VK-12 was achieved at pH 7.0 and Jehan *et al*., (2016) reported that, pH 7.0 was the optimum to maximize PHB production by *Pseudomonas boreopolis.*

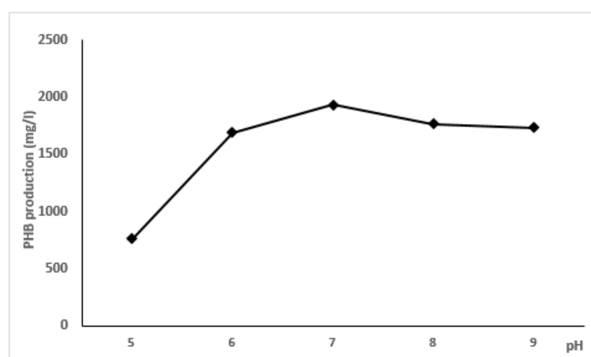

Fig. 7. Effect of pH on PHB production (mg/l) by *E. cloacae* DSM 30054 after 48 h of incubation

Effect of incubation temperatures

The effect of different incubation temperatures on PHB production by *E. cloacae* DSM 30054 is illustrated in Fig. (8). Data show that the maximum PHB production (2108 mg/l) was achieved at 35°C. Thus the 35°C was selected to be the optimum temperature. Data also show that, at 30 and 40 °C the production of PHB was slightly less than at 35°C. However, the PHB production below 30°C and over 40°C was sharply decreased. These results are in agreement with those of Grothe *et al*., (1999) who found that 33°C was the optimum incubation temperature for PHB synthesis. Also, 35°C for *E. coli and Bacillus subtilis* was reported by Gomaa, (2014).

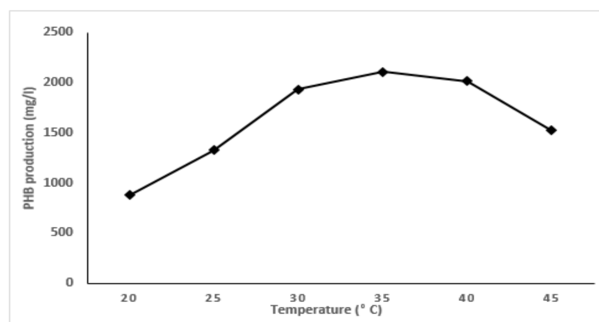

Fig. 8. Effect of incubation temperatures (°C) on PHB production (mg/l) by *E. cloacae* DSM 30054 after 48h of incubation

Effect of inoculum size

The optimization of inoculum size to maximize the production of PHB was examined. The obtained results show that the PHB production increased by increasing the inoculum size up to 2% (v/v) reached to 2186 mg/l (Fig. 9). Therefore, inoculum size 2% (v/v) was the best for maximum production of PHB by *E. cloacae* DSM 30054. While, with higher inoculum size beyond 2% (v/v) decline in PHB production by *E. cloacae* DSM 30054 was noted. Also, sharp decrease in PHB production was observed at inoculum size (0.5%). Therefore, higher or lower inoculum size of *E. cloacae* DSM 30054 resulted in reduction of PHB production, it may be due to insufficient number of microbial cells in the culture with low inoculum size while high inoculum size above 2% could cause inadequate mixing, poor aeration and fast lack of nutrients of the media (Abusham *et al*., 2009 and Prasanna *et al*., 2011). Maximum PHB production by *Bacillus sphaericus* NCIM 5149 was obtained at inoculum size 2% (Ramadas *et al.,* 2010) while *Bacillus subtilis* NG220 produced maximum PHB yield at 1% v/v inoculum (Singh *et al*., 2013).

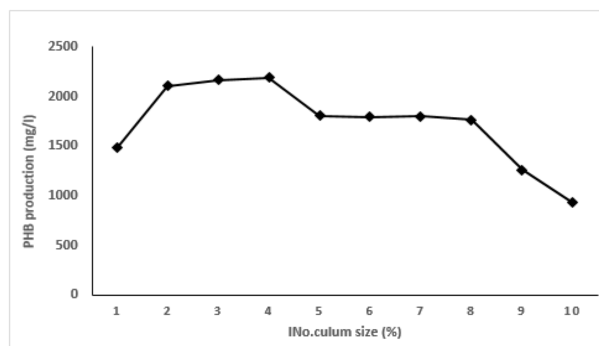

Fig. 9. Effect of inoculum size (%) on PHB production (mg/l) by *E. cloacae* DSM 30054 after 48 h of incubation

CONCLUSION

The objectives of the present study were to isolate effective local bacterial strains for PHB production and use whey as a cheap simple medium. Depending on the results of the present study, *Enterobacter cloacae* DSM 30054 was selected as an effective PHB producer. For maximizing PHB production, submerged fermentation process was carried out for 48 h on whey medium supplemented with 3% sucrose as a carbon source, 1% sodium nitrate as a nitrogen source, pH was adjusted at 7 and shaking incubation at 35°C.

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إنتاج البلاستيك الأخضر (البولى بيتا هيدروكسى بيوترات) بواسطة السلالة البكتيرية المعزولة محلياً *Enterobacter cloacae* DSM 30054 باستخدام بيئة الشرش

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تعد حماية البيئة والحد من التلوث من المهام التي تجذب الكثير من الباحثين، ولقد لاقى إنتاج البلاستيك الأخضر (الحيوي) وما يُعرف علمياً بمركب البولي هيدروكسي بيوترات اهتماماً كبيراً في الآونة الأخيرة كبديل للبلاستيك الصناعي الغير قابل للتحلل والذي يسبب مشكلة بيئية كبيرة. وقد هدفت الدراسة إلى عزل بكتيريا محلية ذات كفاءة عالية في إنتاج البلاستيك الحيوي، كما هدفت إلى استخدام الشرش كمخلف رخيص ومتوفر كبيئة للإنتاج. وقد وجد من بين 20 عزلة بكتيريه تم عزلها من عينات التربة، أن عشرة عزلات منها موجبة لصبغة أسود السودان كدليل على تخليق هذه العزلات للبولي هيدروكسي بيوترات. ثم اختبرت كفاءة هذه العزلات الموجبة في انتاج البولي هيدروكسي بيوترات على بيئة الشرش، وتم تقدير الإنتاج باستخدام الاسبكتروفوتوميتر عند طول موجي 235 نانومتر. وقد أظهرت النتائج أن العزلة رقم 10 أعطت كفاءة أعلى في الإنتاج بلغت 870 ملجم/لتر بعد 48 ساعة من التحضين.عُرّفت هذه العزلة باستخدام تحليل التتابع الجيني 16SrRNA على أنها *Enterobacter cloacae* DSM 30054. ولتحقيق أقصى إنتاج من البولي هيدروكسي بيوتيرات باستخدام هذه السلالة تم دراسة الظروف الغذائية والبيئية المثلى للإنتاج وجاءت النتائج كالتالي: تم الحصول على أقصى إنتاج من البولي هيدروكسي بيوترات (2186 ملجم / لتر) عند 48 ساعة، وذلك بعد تدعيم بيئة الشرش بسكر السكروز (3 ٪) و نترات الصوديوم (0.1 ٪) ، عند درجة الحموضة 7.0 ، والتحضين على درجة حرارة 35° م ، وباستخدام حجم من اللقاح (2 ٪).