Production of bio plastic (polyhydroxyalkanoates) from cheese whey by isolated *Bacillus cereus*

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ABSTRACAT

Bioplastic products represent one of the most important eco-friendly alternatives to petrochemical plastics. Therefore, the study aims at producing polyhydroxyalkanoates (PHAs) using industrial wastes by the most potent bacterial isolate. Seventy-eight isolates were obtained from different localities in Arab Dairy Products Company (Panda, Qalyubia-Egypt). These isolates were subjected to qualitative and quantitative screening tests. Out of these isolates, bacterial isolate BZU-B2 showed the highest production of PHA. The selected strain was identified biochemically as *Bacillus cereus* by biochemical characterization. Nutritional and physiological factors that influence PHA productions were optimized. Temperature 37 ℃, whey concentration of 50%, and ammonium chloride 1.5g L were chosen as the best factors in achieving the highest production of PHA 1.63 g/L with a recovery yield of 33.42 (%, w/w) after 96h. The characteristics of extracted PHA were analyzed using FTIR-spectroscopy techniques. This study focused on improving the ability of *Bacillus cereus* to give a highly purified yield of PHA from whey (a low-cost carbon source).

Keywords: Cheese whey, Recycling, Bioplastics, PHA, *Bacillus cereus*.

INTRODUCTION

Increasing the production of the conventional plastics (Khattab *et al.*, 2021), which accumulate in the environment and are derived from non-renewable resources like fossil fuels and need hundreds of years to degrade (Abraham *et al.*, 2020), has encouraged various researchers to develop eco-friendly plastics (Ojumu, *et al.*, 2004). The importance of bioplastics is not only for their biocompatible, renewable, non-toxic, and biodegradable characteristics (Muhammadi *et al.*, 2015), but also because they can be obtained from various waste materials (*as* polysaccharides and cellulose) (Mostafa *et al.*, 2020). PHAs are a kind of bioplastics that can be obtained biologically by different strains of bacteria as intracellular particles (Koller *et al.*, 2017). Similarly, PHAs have Physico-chemical features like synthetic plastics such as flexibility and toughness (Das *et al.*, 2018).

On the other hand, PHAs have a lot of advantages over other bioplastic types and conventional plastics; PHAs are water-insoluble and can resist hydrolytic degradation. In addition, they are resistant to the UV radiation with less permeability for oxygen, and have a large area of glass transition temperatures, and melting temperatures according to the polymer molecular weight and crystallinity (Raza *et al.*, 2018). The multilateral nature of PHAs has qualified them to be prospect candidates in a broad zone of applications such as household products, packaging, and medical uses (Dietrich *et al.*, 2017). Although there are many sorts of PHAs called Poly-3-hydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV), poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), poly-3- hydroxyhexanoate poly-3-hydroxyheptanoate and 3-hydroxyoctanoate, *etc.*, PHB is the most famous kind of PHAs type (Page *et al.*, 1995). It is included in multifarious technologies such as automotive components, containers, coating materials, packaging, and disposable substances (Reddy *et al.*, 2003). Furthermore, PHB has been used in medical applications via antimicrobial materials biosynthesis by composing silver nanoparticles with PHB nanocomposites (Castro-Mayorga *et al.*, 2017).

Despite the superior characteristics and environmental merits of PHAs to petrochemical plastic, the major problem that reduces the industrial production of PHAs is the great cost (Das *et al.*, 2018),as the raw material cost is up to 40-50% of the total production cost (Schmidt *et al.*, 2016). Interestingly, exploiting waste feedstocks for carbon sources as industrial, or agricultural by-products for microbial growth may be considered a good solution for reducing the waste disposable and the PHAs production costs (Nielsen *et al.*, 2017). Among the wastes of the dairy industry, cheese whey (CW) *represents* the main waste from the cheese manufacture, considering 70–90% of the milk

transformed volume, and its application can minimize production costs by about 50% (Koller *et al.,* 2012). It has been mentioned that each year, 120 MT *per annum* of CW are obtained globally (Israni *et al.,* 2020). During the process of ultrafiltration, nearly 50% of CW is held as whey protein for animal feed supplements and human foods. Whereas whey permeates or the whey filtrate (lactose rich) is thrown as wastewater causing a toxic impact on the environment because of its high organic contents (Montiel-Jarillo *et al.,* 2017).

Herein, this work's goal is to exploit *Bacillus cereus* feeds on the CW filtrate as the only carbon source to give a high PHAs yield. The production conditions of PHAs were optimized using the factor-by-factor (FBF) method. Then, the structural identification of PHA was analyzed by using Fourier transform-infrared spectroscopy (FTIR).

MATERIALS AND METHODS

Isolation sources and medium

The sources used for isolation were collected from different localities in Arab Dairy Products Co. SAE (Arab Dairy; Panda) (Qalyubia Governorate, Egypt). Enrichment medium for bacterial isolation include the following components (g/L) Na_2HPO_4, 9.0; KH_2PO_4, 1.5; $MgSO_4.7H_2O$, 0.2; NH_4Cl, 1.0; $CaCl_2.2H_2O$, 0.02; Fe(III)NH_4-Citrate, 0.0012; and 1ml of trace elements solution containing (g/L): EDTA, 50.0; $FeCl_3$, 8.3; $ZnCl_2$, 0.84; $CuCl_2.2H_2O$, 0.13; $CoCl_2.6H_2O$, 0.1; $MnCl_2.6H_2O$, 0.016; H_3BO_4, 0.1. Glucose was supplemented at 20 g/L as sole carbon source. The pH of the medium was adjusted to 7.0 using 1 N NaOH and 1 N HCl (Zohri *et al.,* 2019).

Screening tests for PHA-producers

The purified isolates were subjected to a primary screening test for the selection of the PHA-producing microorganisms. This test was constructed by viable-colony staining method using Nile Red stain as follows: 0.5 µg Nile Red /mL of solid medium was prepared, sterilized, and poured into Petri plates. The obtained isolates were streaked on agar plates and incubated at 37 ℃ for 3 days then examined under UV light. The lighted colonies were recorded as positive. In secondary screening, chosen bacterial isolates were cultivated on a medium supplemented with whey. The media were incubated for 72 hrs. at 37°C and 150 rpm. The polymers were extracted and quantified *(*Spiekermann *et al.,* 1999*).*

Identification of selected isolate

Morphological, physiological, and biochemical properties of the most potent isolate BZU-B2 were examined as described by Abdel-Rahman *et al.* **(2016)**.

Morphological studies (Williams and Davies, 1965).

The morphological characteristics of the colonies of purified bacterial isolates and their color on different agar media were illustrated.

A simple spot dye was prepared and stained according to (Khattab, 2016). While Gram stain was prepared according to Hucker's Modification (Desouky *et al.,* 2014).

Biochemical Tests.

A potassium hydroxide (KOH) test was performed by mixing two drops of a 3% solution of potassium hydroxide with a 2.0 ml loopful of fresh bacterial growth and stirring for 30 seconds. the solution becomes very viscous and mucoid with Gram-negative bacteria, while no reaction with Gram-positive bacteria occurs (Shushan *et al.,* 1981).

Catalase activity was analyzed by putting drops of 3% hydrogen peroxide into the slant of the bacterial isolates (Benson, 2001). Appearing oxygen bubbles indicate that the isolate can produce catalase enzyme and degrade Hydrogen peroxide into water and Oxygen.

According to Collins *et al.* (1995), carbohydrate fermentation was performed. Xylose, glucose, galactose, mannitol, maltose, sucrose, fructose, cellobiose, inulin, inositol, and myo-inositol were used for such purposes. The prepared medium was inoculated with the isolated organism and incubated at 37°C for 48 hrs. A positive reaction was recorded when the broth turns yellow, and gas was collected in an inverted Durham tube.

The urease production medium was prepared and dissolved by heating, pH was adjusted to 6.8 filtered, and sterilized at 115°C for 20 min. One gram of glucose and six ml of phenol red (0.2% solution) were added and 100 ml of Urea (20 % aq. soln.). The urease production medium was distributed aseptically into sterile test tubes and inoculated heavily. The tube was examined after 4 hrs. incubation and daily for 5 days, red color was indicated as positive (Desouky *et al.,* 2017).

Citrate utilization medium was prepared (Abdel-Rahman *et al.,* 2017). It contained (g/L):

NaCl, 5.0; $MgSO_4.7H_2O$, 0.2; (NH_4). HPO_4, 1.0; K_2HPO_4, 1.0. The salts were dissolved in the distilled water, Sodium citrate, 2.77 g/L; Bromothymol blue 0.08 g/L. The salt solution was added at pH 6.9, filtered through a sintered glass funnel, and sterilized at 115°C for 20 min. Then, the medium was inoculated by a single colony of the isolated microbe and examined daily for up to 7 days for growth and color change. The appearance of blue color around the streak of growth means citrate utilization, while the original appeared green color means that citrate was not utilized.

Starch agar medium contains 1% soluble starch, and nutrient agar medium pH was adjusted at (7–7.2), then sterilized at 121°C and 1.5 atm for 15 minutes. After cooling at 45°C, starch agar medium was distributed into sterile Petri dishes with equal amounts under aseptic conditions. Each plate was inoculated in the center with a bacterial isolate on the surface of the plate and incubated at 50°C for 48hrs . After incubation, starch agar amylase activity was visualized by flooding the starch agar plates with Logule's iodine solution prepared according to Barrow and Feltham (1993). The appearance of a clear zone around bacterial growth was investigated and taken as criteria for determining the amylolytic activity.

Gelatin agar medium was prepared for gelatinase production that was performed by Ammar *et al.* (1991). The medium contained (g/L): Gelatin, 4.0; in nutrient agar medium. The gelatin was soaked in water and when thoroughly softened, the melted nutrient agar medium was added, mixed, and sterilized at 115°C for 10 minutes. Inoculate plates of gelatin agar and incubate for 3 days. Flood the surface with 5–10 ml acid mercuric chloride solution; clear zones indicate gelatin hydrolysis. Acid mercuric chloride solution contained: $HgCl_2$ 12 g; Conc. HCl 16 ml; distilled water 80 ml. After that, the acid is added and shaked well until the solution is completed (Cowan and steel, 1993).

For cellulose production, nutrient agar medium was prepared and 1% of carboxymethylcellulose was added. Cellulose agar medium was sterilized and poured into sterile Petri plates, then inoculated with the isolate, incubate, and check (Khattab, 2016).

Pectinase enzyme production (Ammar et al., 1994), pectinolytic agar plates were inoculated singly spot-wise with bacterial isolates under study. Hydrolysis was detected after 2–4 days by flooding plates with lugol's iodine solution. The appearance of clear zones around the bacterial growth compared with the brown color in the background of the assay plate indicates pectinase production.

Optimization of PHAs Production.

Using FBF Method.

FBF strategy was adopted to identify the significant conditions required for PHAs production from *Bacillus cereus* which feed on Cheese whey. The effectiveness of *Bacillus cereus* to produce PHAs was tested using the same sugar concentration (50 g/L) of pure lactose and Cheese whey as a sole carbon source. Establishing the optimized conditions was accomplished through a series of experiments targeting the maximum PHAs concentration by *Bacillus cereus* from cheese whey.

Analytical Methods.

Cell growth was monitored by measuring the optical density (OD) at 600 nm (M-ETKAL-721 spectrophotometer). The culture medium was centrifuged at 10,000 rpm and 4 ◦C for 5 mins. The cell pellet was washed with distilled water, harvested by centrifugation, and dried at 105 ◦C overnight till a constant weight was noticed. Cell mass concentration was determined by the standard calibration curve between OD-600 and dry cell weight (Rodriguez-Contreras *et al.*, 2015).

Extraction of PHAs from the bacterial cells was initiated by centrifuging the cells at 10,000 rpm for 10 min. The pellet was treated with sodium hypochlorite solution (4% w/v), then incubated at 37 ◦C for 1 h, followed by a centrifugation step at 5000 rpm for 15 min. Finally, the pellet was rinsed with distilled water and then acetone (Montiel-Jarillo *et al.*, 2017). To assay the PHAs, the extracted pellet was transferred to a clean test tube and dissolved by chloroform C/MS 1989-2014, Agilent Technologies, Inc). The method of separation was done following a previous study (Pantazaki *et al.*, 2009; and Li *et al.*, 2012). The peaks and their MS spectra were identified using the "NIST" standard library (Suhazsini *et al.*, 2020).

Film Casting

The preparation of PHAs film was done by following the procedure of (Savenkova *et al.*, 2000). In brief, about 80 - 100 mL solution of the extracted PHAs (2.0% in chloroform) was poured onto glass plates (100 × 15 mm). Then, the solution was left on a flat and leveled surface to dry at room temperature for a minimum of 4 h and without air disturbance to

get a uniform thickness film. Finally, the film was peeled out of the plate after the complete dryness for further analysis (Savenkova *et al.*, 2000).

Characterization of PHAs

Physicochemical techniques such as FTIR. were used for the structural identification and characterization of the extracted PHAs biopolymer.

Fourier transform-infrared spectroscopy (FTIR)

The main functional groups of pure polymeric film were investigated using ATR-technique (Mohapatra *et al.*, 2013) (Brucker Vertex 80 V spectrophotometer, Germany). The spectrum was acquired between 400 and 4000 cm–1 with a 4 cm-1 resolution.

RESULTS AND DISCUSSION

Isolation and screening of PHA-producers

Several samples of liquids and solids were collected from different places of the Arab Dairy Products Company – (Arab Dairy – Panda). These sources were used for getting the PHA-producing isolates using fermentation media containing lactose as described previously in materials and methods. Seventy-eight bacterial isolates were selected and purified. These isolates were preliminarily screened for PHA production via fluorescence using Nile red staining assay technique. Two isolates showed bright fluorescence on the plates upon exposure to UV light at 312 nm. These results ensured that these isolates have the ability to produce PHA, therefore, the two isolates were selected for further experiments. The chosen isolates were cultivated in mineral salt media provided with lactose and incubated for 72 hrs at 37 ◦ C. Cell growth was examined, then the cells were collected to extract the PHA. After that, the yield of PHA was assayed and compared. The isolate Bzu-B2 showed the highest production of PHA 0.880 g/L compared to isolate Dzu-IN1 which showed low production of PHA 0.280 g/L. Therefore, the Bzu-B2 strain was considered the best isolate and used for the identification and enhancement of the production of PHA.

Identification of Isolate BZU-B2

The isolate was obtained from dairy production Co. wastes. To identify the isolate BZU-B2, morphology (Gram's reaction, shape) and biochemical characteristics were studied. It was performed as shown in Table (2). The strain BZU-B2 is Gram-positive with a rod shape, and catalase-positive, which means the classification in the Bacillaceae family. The isolate can grow in high salt media [up to 10 % sodium chloride]. The isolate also showed the capability of gelatin, cellulose, and starch hydrolysis. Nevertheless, it could not use urea. This isolate could degrade different sugars including glucose, mannitol, fructose, lactose, and sucrose but not able to degrade xylose, galactose, maltose, inositol, inulin, myo-inositol, or cellobiose. Therefore, the isolate was suggested to be *Bacillus cereus* according to Bergey's Manual 1974.

Optimization of PHAs production

The best production of PHAs got by *Bacillus cereus* was obtained after a numbers of experiments, which measure the impact of the carbon concentration, ammonium chloride concentration, and temperature on PHA production. Furthermore, residual sugar, PHAs content, dry cell weight (DCW), and PHA productivity were analyzed.

Impact of whey concentration

Various concentrations of Cheese whey (30, 40, 50, 60, 70, 80, and 100%) were utilized as the only carbon source in the fermentation medium. The results indicated that the best range of PHAs was obtained at a concentration of 50% over other concentrations, Table (3). Moreover, the highest production of DCW was 3.73 g/L and maximum productivity 0.011 g/L/h obtained respectively, as Cheese whey concentration raised from 30 % to 50 %. On the other hand, the maximum recovery yield of PHA was shown at a Cheese whey concentration of 100%. Therefore, a concentration of 50% of sweet whey represented the best concentration for achieving the high efficiency of PHA fermentation. Whereas at higher concentrations of Cheese whey (>50%), a deficiency in the cell biomass was noted which might be because of the osmotic pressure (Obruca *et al.*, 2011). These data agree with the results mentioned by (Obruca *et al.*, 2011) and (Khattab *et al.* 2021), in which 50% CW concentration was perfect for the best PHA production from *Bacillus sp.* and that could show the high PHA productivity in the current work.

Effect of Incubation Temperature.

The influence of different incubation temperatures on PHA and DCW production was investigated. As noticed in table (4), a positive correlation was observed among

incubation temperatures studied in the range (of 30- 45℃). As incubation temperature raised up to 37 ℃, DCW increased and achieved maximum production of 5.30 g/L at 96 h. Also, maximum production of PHA 1.40 g/L, maximum recovery yield of PHA 26.49 % (w/w), and maximum value of Y (p/s) 0.064 g/g was observed at 37 ℃ at 96 h. below or above 37 ℃ decreasing in PHA accumulation and recovery yield of PHA were observed. As a result, 37 ℃ was represented the best incubation temperature for achieving high efficiency of PHA fermentation. At a higher temperature, the decrease in PHA yield was showed. It could be because the enzyme degradation which is responsible for the PHA synthesis. Grazia *et al.* (2017) observed strong PHA accumulation with raising the incubation temperature gradually from 15ºC to 30ºC. In addition, he also studied that the temperature alteration reduced the mass transfer efficiency and the level of dissolved oxygen which ultimately lower the PHA synthesis (Cho *et al.*, 2015). *Bacillus thuringiensis* SBC4 used corn cob as a carbon source to produce the maximum PHA content of 21.05%, at 37ºC, and after an incubation time of 48 hours (Odeniyi & Adeola, 2017).

Impact of ammonium chloride concentration

The restricted concentration of nitrogen represents one of the main agents that play a critical role in PHAs synthesis and cell biomass. The impact of different nitrogen concentrations (0.5, 1.0,1.5, 2.0, 2.5, and 3.0 g/L of ammonium chloride) on the PHAs fermentation and cell biomass of *Bacillus cereus* in MSM medium with Cheese whey as the only carbon source at 37 ℃ were investigated. Data represented in Table (5) showed that there is a comparable DCW obtained in the range (4.16 – 4.88) g/L among different concentrations of nitrogen sources tested. On the other hand, the highest production of PHA 1.63 g/L, recovery yield of 33.42 g/L, and productivity of 0.017 g/L/h were obtained with 1.5 g/L of ammonium chloride after 96 hr. While above or below this point, decreases in PHA production were observed. Accordingly, 1.5 g/L was considered the optimal concentration for PHA production. Different researchers reported that ammonium chloride is the preferable source of nitrogen in PHA production (Aramvash *et al.*, 2015; Abdel-Rahman *et al.*, 2017; and El-Metwally *et al.*, 2020). Briefly, 1.0 g/L ammonium chloride causes the maximum PHA production (0.95 g/L) and PHA content (20.96%, w/w). In contrast, providing 0.25 g/L ammonium chloride led to the lowest PHA concentration (0.32 g/L) with a PHA content of 7.5%. The prior study showed that 1.5 g/L of ammonium sulfate was the best concentration (Desouky *et al.*, 2017).

Characterization techniques

Fourier transforms infrared spectroscopy (FTIR)

The spectrum of FTIR for the PHA film produced using whey by *Bacillus cereus* detected the properties of absorption bands for the PHAs, Fig. 6. The spectrum observed a very little absorption band of about 3438 cm^{-1} of the expansion hydroxyl group (–OH) (Suhazsini *et al.*, 2020). Also, the several peaks of maxima 2985, 2937, and 2882 cm^{-1} appeared with the expansion (C–H) groups. (Dhangdhariya *et al.*, 2015; and Das *et al.*, 2018).

The noticeable robust absorption band at 1727 cm^{-1} related to the carbonyl (C=O) ester group. Whereas the sharp intense bands at 1035 and 1275 cm^{-1} were associated with the expansion (O–C–O) groups of the aliphatic and the ester groups, respectively. As well as the bands at 1380 and 1452 cm^{-1} were associated with the bending (C–H) group of the alkane (CH_3) and the methylene (CH_2) groups, respectively. In addition, the range (899 - 519 cm^{-1}) in the spectrum pointed to the carbon fingerprinting zone in PHB polymer (Suhazsini *et al.*, 2020).

CONCLUSION

In the current study, the production and optimization of polyhydroxyalkanoates using Cheese whey as a cheap substrate were achieved by an Egyptian isolate *Bacillus cereus*. Whey concentration at 50 %; ammonium chloride, 1.5 g/L, and 37 ℃ incubation temperature were the optimal production conditions for PHA. In these conditions, the maximum production of PHA was 1.63 g/L at a recovery yield of 33.42 (%, w/w), which means that the production of PHA increased double fold. The chemical nature of the obtained PHA (PHB type) was investigated using FTIR. The coherence of the results ensured the possibility of using *Bacillus cereus* for PHB production from cheese whey in a pure structure and high production.

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Table 1. Quantitative screening test by two bacterial isolates for PHA production using whey.

No.	sources of isolates	Isolates code	PHA(g/L) ± SD
1	Factory floor in Panda company	Bzu-B2	0.880±0.005
2	A place for washing tools in Panda company	Dzu-IN1	0.280±0.009

Table 2: A summary of the morphological, physiological, and biochemical characteristics of the most potent bacterial isolate BZU-B2.

Test	Result	Test	Result
Gram stain	+	Sugar fermentations:	
Shape	Rod shape	Glucose	+
KOH	-	Xylose	-
Catalase	+	Galactose	-
Citrate utilization	+	Maltose	-
Urea hydrolysis	-	Fructose	+
Cellulose hydrolysis	+	Sucrose	+
Gelatin hydrolysis	+	lactose	+
Starch hydrolysis	+	Inositol	-
Growth at different NaCl conc.:		Myo-inositol	-
2%	+	Cellobiose	-
5%	+	Inulin	-
10%	±		

Table 3: Effect of Cheese whey concentrations on PHAs production from *Bacillus cereus* after 96 h at 37 ◦C, 150 rpm.

Different conc. of whey %	Max. DCW (g/L) ± SD	Residual sugars (g/L) ± SD	$Y_{(x/s)}$[a] (g/g)	PHA (g/L) ± SD	Recovery yield % (*w*/*w*)	$Y_{(p/s)}$[b] (g/g)	$P_{(g/L/h)}$[c]
30	3.25± 0.20(96)	2.091 ±0.72	0.251	0.61 ±0.13	14.33	0.047	0.006
40	3.47± 0.24(96)	2.710 ±020	0.201	0.90 ±0.01	15.44	0.052	0.009
50	3.73± 0.32(96)	3.829 ±0.36	0.200	1.09 ±0.01	13.20	0.051	0.011
60	3.60± 0.27(96)	5.332 ±0.10	0.166	0.80 ±0.02	14.90	0.032	0.008
70	3.62± 0.33(96)	7.747 ±0.10	0.151	0.73 ±0.00	15.98	0.027	0.008
80	3.43± 0.40(96)	7.217 ±0.26	0.105	0.70 ±0.00	15.45	0.021	0.007
100	2.96± 0.16(96)	13.285 ±0.26	0.081	0.65 ±0.00	21.71	0.018	0.007

[a] Yield of biomass-based on substrate consumed; [b] Yield of PHA based on substrate consumed; [c] Productive.

Table 4: Effect of different incubation temperatures on PHA production from sugar-cane whey by *Bacillus cereus*.

Temperature (°C)	Max. DCW (g/L ± SD at indicated time	Residual sugar(g/L) ± SD	Consumed sugar (g/L)	$Y_{(x/s)}$[a] (g/g)	PHA (g/L) ± SD	Recovery yield % (*w/w*)	$Y_{(p/s)}$[b] (g/g)	$P_{(g/L/h)}$[c]
30	5.06± 0.08(96)	4.124 ±0.67	20.88	0.243	0.98 ±0.008	19.37	0.047	0.010
35	5.23± 0.10(96)	2.539 ±0.03	22.46	0.233	1.00 ±0.005	19.16	0.045	0.010
37	5.30± 0.19(96)	3.249 ±0.06	21.75	0.243	1.40 ±0.015	26.49	0.064	0.015
40	5.17± 0.33(96)	3.853 ±0.04	21.15	0.245	1.10 ±0.006	21.25	0.052	0.011
45	4.59± 0.27(96)	8.466 ±0.08±	16.53	0.277	1.03 ±0.006	22.46	0.062	0.011

[a] Yield of biomass-based on substrate consumed; [b] Yield of PHA based on substrate consumed; [c] Productivity of PHA

Table 5: Effect of addition of different concentrations of ammonium chloride on PHA production.

Ammonium chloride conc. (g/L)	Max. DCW (g/L) ± SD	Residual sugar (g/L) ± SD	*Y(x/s)a* (g/g)	PHA (g/L) ± SD	Recovery yield % (w/w)	*Y (p/s)b* (g/g)	*P(g/L/h)c*
0.5	4.16 ±0.33	7.099 ±6.47	0.232	1.27 ±0.08	30.54	0.071	0.013
1	4.77 ±0.28	7.777 ±3.25	0.277	1.33 ±0.08	27.80	0.077	0.014
1.5	4.88 ±0.03	9.518 ±8.81	0.315	1.63 ±0.24	33.42	0.105	0.017
2	4.86 ±0.21	7.464 ±5.15	0.277	1.44 ±0.06	29.69	0.082	0.015
2.5	4.84 ±0.04	8.083 ±3.53	0.286	1.25 ±0.28	25.81	0.074	0.013
3	4.67 ±0.09	6.324 ±2.19	0.250	1.13 ±0.28	24.29	0.061	0.012

[a] Yield of biomass-based on substrate consumed; [b] Yield of PHA based on substrate consumed; [c] Productivity of PHA

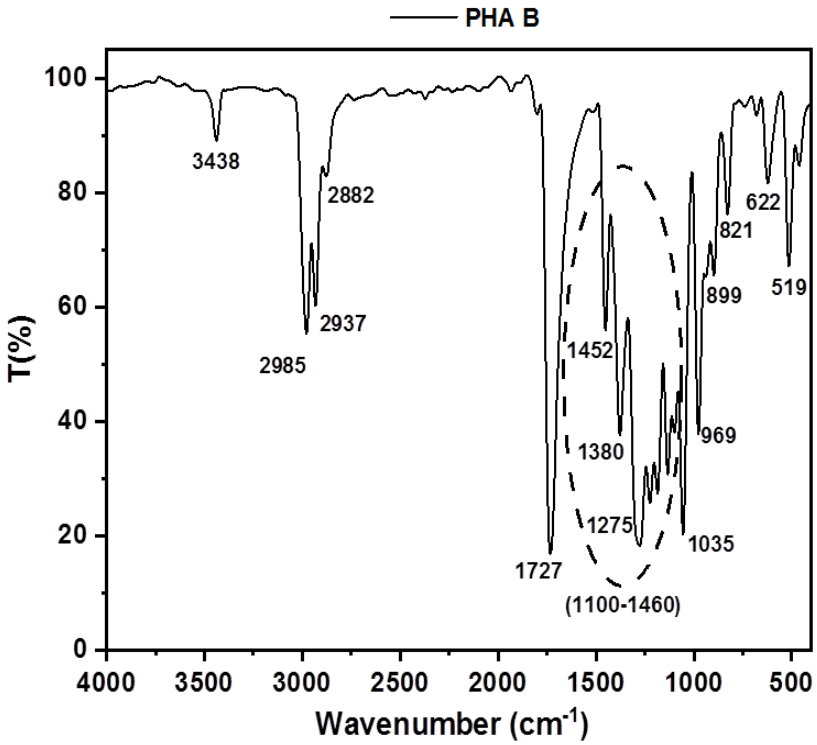

Figure 1: FTIR for cast film produced from Cheese whey by *Bacillus cereus*.

إنتاج البلاستيك الحيوي (البولي هيدروكسي الكالونات) من مصل اللبن بواسطة العزلة *Bacillus cereus*

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الملخص العربي

تمثل منتجات البلاستيك الحيوي أحد أهم البدائل الصديقة للبيئة للبلاستيك البتروكيماوي. لذلك هدفت دراستنا إلى إنتاج (Polyhydroxyalkanoates ; PHAs)، وهو لدن حراري قابل للتحلل الحيوي، يصنع من النفايات الصناعية بواسطة بعض العزلات البكتيرية. تم الحصول على ٦٧ عزلة من مواقع مختلفة في الشركة العربية لمنتجات الألبان (اراب ديري ، باندا) (محافظة القليوبية ، مصر). تم إخضاع هذه العزلات لاختبارات الفرز الكمي والنوعي. من بين هذه العزلات، أظهرت العزلة البكتيرية BZU-B2 أعلى إنتاج لـ PHA. تم التعرف على هذه السلالة على أنها *Bacillus cereus* BZU-B2 عن طريق التوصيف الكيميائي الحيوي. تم تحسين العوامل الفسيولوجية والتغذوية التي تؤثر على إنتاج PHA في عمليات التخمير على دفعات. تم اختيار درجة الحرارة ٣٧ درجة مئوية وتركيز مصل اللبن ٥٠٪ وكلوريد الأمونيوم ١,٥ جم لتر كأفضل العوامل لتحقيق أعلى إنتاج من PHA 1.63 جم / لتر مع عائد استرداد ٣٣,٤٢ (٪ وزن / وزن) بعد ٩٦ ساعة. تم تحليل خصائص البلاستيك الحيوي المستخرج باستخدام تقنيات التحليل الطيفي FTIR. ركزت هذه الدراسة على تحسين قدرة *Bacillus cereus* على إنتاج عائد جيد من PHB عالي النقاء من مصل اللبن (كمصدر كربوني منخفض التكلفة).

الكلمات الاسترشادية : شرش الجبن، البلاستيك الحيوي،الباسيلس سيريس، إعادة التدوير .