Egypt. J. Rad. Sci. Applic. **Vol. 35,** No. 1-2, pp. 69-81 (2022)

Egyptian Journal of Radiation Sciences and Applications
http://ejrsa.journals.ekb.eg/

EGYPTIAN ATOMIC ENERGY AUTHORITY

Molecular, Physiological and Biochemical Identification of Polyhydroxybutyrate Producing *Streptomyces* Isolated from Egyptian Localities

Mahmoud A.A. Soliman[(1)], Doaa A. Emam[(1)], Hisham M. Swailam[(1)], Mhmoud A. Swelim[(2)], Samar A. Rezk[(2)]

[(1)]Department of Radiation Microbiology, National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority (AEA), Cairo, Egypt; [(2)]Botany and Microbiology Department, Faculty of Science, Benha University, Egypt.

THE AMOUNT of chemosynthetic plastic waste increases every year and the exact time for its degradation is unknown. Polyhydroxybutyrates (PHBs) are macromolecules synthesized by bacteria. Because of their fast degradability under natural environmental conditions, PHBs were selected as alternatives for the production of biodegradable plastics. In the present study, soil samples were collected from the rhizosphere region of seven different crops commonly grown in Egyptian soils and used to collect *Streptomyces* isolates from Benha, Al-Qalioubia Governorate. A total of 50 representative actinomycete isolates (6 from lettuce, 5 from garlic, 9 from onion, 6 from zucchini, 9 from eggplant, 8 from wheat, 7 from alfalfa rhizosphere soil) were collected and refined. Screening was performed by staining colonies of isolates with Sudan Black B. The dye Sudan Black was used as a screening tool for PHB production. *Streptomyces* isolates were identified by morphological, biochemical and molecular methods. A phylogenetic tree constructed based on the resulting 16S rRNA-encoding gene sequences was examined. A *Streptomyces* isolate turned out to be *Streptomyces incanus* BK128. PHB-purified extract of *Streptomyces incanus* BK128 was characterized using FTIR in comparison to standard PHB.

Keywords: PHB, Purified product and SEM, Rhizosphere, *Streptomyces incanus* BK128, 16s rRNA.

Introduction

Plastic materials that have been generally used in our daily lives are now causing dangerous environmental problems. Millions of tons of these non-degradable plastics accumulate in the environment per year. Petroleum-based plastics have serious ecological and social impacts because of their non-degradable nature and the leaching of carcinogenic substances when exposed to scratch or heat. Biopolymers are a type of producs that can help to overcome problems caused by petrochemical polymers. Biopolymers are generated from renewable natural sources and are often biodegradable and nontoxic (Singh & Sharma, 2016).

Natural products are compounds or substances made by living organisms that exist in nature. Natural products can also be prepared by chemical synthesis (both semisynthetic and total synthetic) and have played an important role in the development of the field of organic chemistry by providing challenging synthetic targets (Phil et al., 2018).

[#]Corresponding author e-mail: actinohallo@yahoo.com,
Received 04/11/2022; Accepted 30/01/2023
DOI: 10.21608/EJRSA.2023.167331.1140

Soil is an intensively used ecological niche. Its inhabitants produce many useful natural biologically active products. Actinobacteria are abundant producers of antibiotics and important suppliers to the pharmaceutical industry. They can produce various secondary metabolites. Actinomycetes are widely used in the pharmaceutical industry because they are ubiquitous in nature and can produce an unlimited number of secondary metabolites with different chemical structures and biological activities (Soliman et al., 2021).

Natural substances, especially in the field of organic chemistry, are often defined as primary and secondary metabolites. A more restrictive definition, limiting natural products to secondary metabolites, is commonly used in medicinal chemistry and pharmacology (Bansal et al., 2011).

Actinomycetes are the most abundant microbial source of all kinds of bioactive metabolites. Over 1,000 actinomycete secondary metabolites have been identified. The considerable interest in phytochemistry and medicinal properties has been drawn by Gouda & Das (2015).

Biological repair with microorganisms is believed to be safe and efficient. Actinomycetes, the most important group of microorganisms, are involved in the degradation and transformation of organic and metallic substrates and also have important bioremediation potential. It can break down high-dose pesticides, chemical complexes, and heavy metals. Actinomycetes, especially Streptomyces sp. Toxins can be used as carbon sources to synthesize commercially viable antibiotics, enzymes, and proteins (Kannabiran, 2017).

PHBs form a group of thermoplastic polyesters with properties similar to some common plastics used in the petrochemical industry, offering thermoplastic properties and water resistance compared to synthetic polymers. PHAs are biodegradable and are synthesized by bacterial means from renewable raw materials such as pure sugar, fatty acids, or fermentation broth from waste. A key property of PHB is crystallinity, which defines the physical and mechanical properties of the polymer. The crystalline state of a material is described as having a set of structures, so polymers with this property are said to be semi-crystalline. It is so named because its long polymer chains exhibit one crystalline phase and another amorphous phase and never fully crystallized (Wen et al., 2011; Mikkili et al., 2014).

The crystalline phase of PHB depends on the regularity of its structure, which in turn depends on the route that this was synthesized. O isotactic PHB has chiral carbon in absolute configuration R, and is obtained by means of bacterial fermentation, while the syndiotactic PHB is synthesized from monomers with setting R and S track synthetic route (Michel & Billington, 2012).

The present study aims at isolating a current strain capable of producing PHB as a natural product with commercial uses.

Materials and Methods

Samples collection

Soil samples were collected from the rhizosphere areas of seven different crops commonly grown in the Benha region of Qalioubia Governorate, Egypt and are presented in Table 1. Collected samples were air-dried at room temperature and gently ground using a pestle and mortar for further analysis. Ten grams of each soil sample was suspended in 90 ml of sterile saline, shaken vigorously, and serially diluted in sterile saline. Dilutions were made in the range of 10 -5 to 10 -7 and 1 ml of the dilutions were inoculated onto starch nitrate agar and incubated at 30°C for 7 days.

Isolation and screening of PHB producing actinomycetes using Sudan Black dye

Serial dilutions of each soil sample were performed and the total population of actinomycete isolates was enumerated by plating appropriate dilutions onto starch nitrate agar and incubating at 30 °C for 5 days. Based on morphological differences in colony characteristics (size, shape, color and texture), a total of 50 representative actinomycete colonies were selected, purified and maintained as pure cultures for further screening. The Selected actinomycete isolates were streaked onto nitrate starch agar plates and incubated at 30°C for 24 hours. Sudan Black B was then used to screen PHB-producing isolates, plates were soaked in the dye for 30 minutes and washed with ethanol (96%) to remove the dye and excess dye. PHB-producing isolates were selected based on colony color, with black colonies assigned to his PHB-producing isolates and colorless to non-

PHB-producing isolates using the methods in Chanaporn et al. (2021) were recommended.

Cultural, physiological and biochemical properties of Actinomycete isolate

The morphological, cultural, and physiological characteristic and following the diagnostic simple working keys for the classification and identification of Normal Taxa such as Szabo key and following the description of the Streptomyces species included in the International Streptomyces Project (ISP)1,2, the methods were reported by Soliman et al. (2021).

Molecular identification and phylogenetic profile of the selected Actinomycete isolate

Gene-based 16SrRNA identification was used to describe the Actinomycete genus chosen as the PHB producer. According to Kieser et al. (2000), PCR is one of the most sensitive methods for detecting and identifying the bacterial targets because of its rapid amplification of nucleic acid targets from relatively low starting materials.

Estimation and determination of PHB production by staining

The commercial poly--hydroxybutyrate (Sigma-Aldrich) standard curve was used to calculate the quantity of PHB produced after the PHB polymer was extracted. The cell growth of each isolate containing the polymer was pelleted by centrifugation at 10,000 rpm at 4°C for ten minutes on all PHB-positive Actinomycetes isolates. To get rid of any undesirable substances, the pellet was washed with ethanol and acetone, resuspended in an equal volume of 4% sodium hypochlorite, and incubated for 30 minutes at room temperature. The mixture was centrifuged for ten minutes at 10,000 rpm for the purpose of sedimentation of the lipid granules. The cell pellet was carefully washed with ethanol and acetone after the supernatant was removed. After being previously treated with hot chloroform, the produced pellet granules were dissolved in hot chloroform and filtered through Whatman No.1 filter paper. 10 milliliters of hot concentrated H2SO4 were added to the filtrate, which transformed the polymer into crotonic acid and produced a brown solution. On a UV-VIS spectrophotometer, the absorbance was measured at 235 nm against a concentrated H_2SO_4 blank after the solution was cooled. The standard curve was used to determine the amount of PHB produced and the PHB content was defined as the ratio of PHB concentration to cell concentration given in as a percentage. The isolate with the highest PHB production was then chosen for subsequent research. PHB polymer was extracted according to detailed method of Law & Slepecky (1961), Shah et al. (2014) and Nehra et al. (2015).

Preparation of standard curve of commercial PHB

The PHB standard curve was created using pure PHB (Sigma, USA). By heating 10 ml of chloroform in a water bath at 65-70 degrees Celsius until the solution was clear, 0.01 grams of commercial PHB could be dissolved. A 1 mg/ml PHB stock solution is produced by this. To make a 100 g/ml PHB stock, 1 ml of the 1 mg/ml stock is pipetted into a new tube using 9 ml chloroform, and the tube is heated to (650 -700ºC). To obtain PHB in a variety of dilutions, the 100 g/ml stock is transferred into new tubes. The standard graph was created by plotting the various concentrations on the x-axis and the corresponding optical densities on the y-axis against a concentrated H_2SO_4 blank at 235 nm on a UV-VIS spectrophotometer. The Actinomycete isolate's PHB yield was estimated using the standard curve (Law & Slepecky, 1961).

Infrared absorption spectrum

The Streptomyces purified extract was assessed with FTIR, using potassium bromide discs technique on shimadzu IR 110 Spectroscopic, NCRRT, Cairo, Egypt.

Preparation of PHB film

PHB produced by selected Actinomycete isolate was mixed with 20 ml of chloroform under boiling conditions. The solution was poured on the pre-autoclaved petri plates and plates were incubated in a vacuum desiccator for 2-3 days for proper film making. PHB is as such brittle in nature and has a higher crystalline behavior, thus the brittleness of PHB can be reduced by blending it with other suitable polymers to increase its application spectrum. Conventional solvent cast technique was used for PHB film preparation according to the methods recommended by Sridewi et al. (2006).

Scanning electron microscope (SEM)

Scanning electron microscope exhibited the morphological shape of Actinomycete isolate and PHB film based on the methods recommended by Tawfik et al. (2015).

Results and Discussion

Bioplastic, Polyhydroxybutyrate (PHB) is well known for its environmental friendliness and complete decomposition into water and carbon dioxide by microorganisms. The main drawback of PHB commercialization is its high production cost which is 10 times higher than that of synthetic plastic. Thus, the present research work is mainly focused on the fermentative production of PHB by Actinomycetes. From the obtained results illustrated in Table 1 it was observed that out of 50 isolates, as many as 9 isolates from different rhizospheric localities (one from the rhizospheric area of lettuce; one from garlic rhizosphere; one from onion rhizosphere; two from zucchini rhizosphere; two from eggplant rhizosphere; one from wheat rhizosphere; and one from alfalfa rhizosphere soil) were found to accumulate PHB, exhibiting blue/ black color on the plates upon staining with Sudan Black. All the positive isolates were assigned code numbers based on their source of isolation. Shah (2014) reported that the staining of colonies with Sudan black B had been used for detection of PHB granules. Sudan black B staining results in dark blue/ blue black appearance of PHB granules. Bacillus subtilis G1S1 was screened for PHB production using Sudan black B staining. Another study reported by Soam et al. (2012) who found that out of 40 isolates, as many as 28 isolates were found to produce PHB, exhibiting blue black color upon staining with Sudan Black.

Quantification of PHB production

The method described by Law & Slepecky (1961) was used to determine the PHB yield of all nine isolates, and the results are shown in Table 2.

TABLE 1. Isolation of PHB producing Actinomycetes from the Rhizoshperic area of different crops

Sample no.	Type of sample	Actinomycetes isolates	No. of PHB Positive Isolates
1	Lettuce	6	1 (L_3)
2	Garlic	5	1 (G_1)
3	Onion	9	1 (O_1)
4	Zucchini	6	2 (Z_2, Z_4)
5	Egg plant	9	2 (E_5, E_7)
6	Wheat	8	1 (W_3)
7	Alfalfa	7	1 (A_7)
Total	**7**	**50**	**9**

TABLE 2. Screening for Polyhydroxybutyrate (PHB) production by Actinomycetes isolate

Isolates no.	CDW(g/l)	PHB (g/l)	PHB % yield
42	0.1967	0.068	34.5%
44	0.326	0.047	14.5%
1	0.25	0.092	36.8%
2	0.198	0.0382	19.29%
21	0.258	0.0524	20.3%
10	0.117	0.0286	24.4%
29	0.1331	0.0519	38.9%
31	0.1417	0.0336	23.7%
39	0.3262	0.071	21.7%

PHB is a biodegradable thermoplastic polyester and analogous to many conventional Petro-derived plastics currently in use. In addition, the production of PHB by microorganisms has obtained much attention in recent years due to its numerous applications in medicine, agriculture and marine fields (Deshwal & Chaubey, 2014). PHB yield of all the 9 isolates positive for PHB production were determined using the method described by Law & Slepecky (1961), the results of which are detailed in Table 2. The results exhibited that the yield of the isolates was found to vary between 14.5 g/l to 38.9 g/l, among the 9 PHB positive isolates obtained from the rhizospheric area of different crops, the highest PHB producers were observed to belong to the eggplant rhizosphere isolates (showing an average of 38.9 g/l of PHB), followed closely by the isolates belonging to the zucchini rhizosphere (with an average PHB yield of 36.8 g/l). Actinomycetes isolates from the rhizospheres of onion, garlic, and lettuce exhibited almost a similar pattern, with a few isolates showing a high PHB yield, whereas the others were of a lower yield; having an average PHB yield of 34.5, 24.4, and 23.7 respectively. The lowest PHB yield was exhibited by the isolates belonging to the wheat and alfalfa rhizosphere. Although the PHB yield was found to be different for the isolates belonging to different crop rhizospheres, several Gram-positive bacteria viz. *Clostridium, Corynebacterium, Nocardia, Bacillus, Rhodococcus, Streptomyces* and *Staphylococcus* have been reported for PHB production (Singh et al., 2009). Rajendran et al. (2013) also found that *Streptomyces* sp. is capable of producing PHB in a good amount from groundnut oil cake.

Morphological and biochemical characterization of positive isolate

Among fifty isolates from different soil samples only one most potent isolate (E7) has the ability to produce much intracellular polyhydroxybutyrate (PHB). The *Streptomyces* isolate was characterized and identified according to the methods employed by the International keys and by the description listed by the International Streptomyces project (ISP). The soil dilution plate technique was used for the isolation of Actinomycete colonies. Gram staining of the isolate revealed that the isolate was Gram positive in nature. In literature, gram positive bacteria have been reported to produce PHB (Preiss, 1989).

According to the above mentioned results of morphological, cultural, and physiological; and following the diagnostic simple working keys for the classification and identification of Normal Taxa such as Szabo key and following the description of the *Streptomyces* species included in the International *Streptomyces* Project (**ISP**)[1,2] this isolate was characterized to be likely *Streptomyces* isolate. The results described before are illustrated in Tables 3-7. The isolates exhibited straight to rectus sporophore and smooth Spore surface (Fig. 1, 2). The isolate was negative for melanin pigment production on both Peptone yeast extract iron agar and Tryptone yeast extract broth. Culture properties in our study illustrated that the type of growth observed was distinctive on all media. Moreover, the color of aerial mycelium and the type showed gray aerial mycelium was produced on all media used except on peptone- yeast extract iron agar, czapek,s agar and starch ammonium sulfate agar, which exhibits a white and powdery type of growth. While malt yeast extract agar exhibits a yellow and leathery type of growth. The color of substrate mycelium also revealed a brown color on starch nitrate agar, Glucose nitrate agar, Starch casein agar, Glucose starch peptone agar, and Nutrient agar, and showed a white color in Starch ammonium sulfate agar and Czapek's agar and exhibits a gray color in all media. The color in the medium indicated a diffusible brown pigment on Starch nitrate agar, nutrient agar and glucose starch peptone agar while no pigment could be detected in other media. The results declared that Streptomyces isolate was able to tolerate high NaCl concentrations up to 10 %. The results indicated that the starch nitrate medium was the best medium for both growth and PHB production. The main nitrogen sources in these media were sodium nitrate while, starch represents the sole carbon source. The obtained results are found to be in agreement with those of Hori et al. (2002), who found that in *Streptomyces porvutuls,* PHB content in the cell reached the maximum level after growth with sodium nitrate.

Molecular identification and phylogeny of Streptomyces incanus

Molecular studies are very essential for proper identification of the organism. Taxonomy and phylogeny studies of bacteria with the help of oligonucleotide signature sequences by far have been the most widespread housekeeping genetic marker. It occurs in almost all bacteria and often presents as a multi-gene family or operons (Mizunoa et al., 2010). The right Lane 1 shows the PCR of *Streptomyces incanus* DNA and right Lane M was for the standard ladder. The nucleotide sequence

of the DNA from the isolated actinomycete was determined. Comprising nucleotides as indicated in Table 8 when deposited in the NCBI (National center for Biotechnology Information) Gene Bank sequence databases, the actinomycete isolate found to be *Streptomyces incanus*. A phylogenetic tree constructed on the basis of the obtained 16S rRNA coding gene sequences of the isolate under study and the nearest relatives were described in Fig. 3. It was reported by Liu et al. (2014) that identification of bacterial isolates based upon 16S rRNA gene sequence analysis had widely been used.

Scanning electron microscope (SEM) of morphological shape of Actinomycete isolate

The scanning electron microscope (SEM) is one of the best suited out of a variety of procedures to visualize the external appearance of *Actinomycete. Actinomycete* lives in various environments and their preparation for SEM thus takes their nature into consideration. The basic principles of isolation, fixation, dehydration, drying, mounting, and photographing have many variations. Scanning electron microscope can be used for viewing microorganisms under study; however, the concentration of cells are critical. The results from Figs. 1, 2 showed the morphological shape of *Actinomycete* isolate.

TABLE 3. Culture characteristic of the experimental isolate

Medium	Growth	Color of aerial mycelium	Color of substrate mycelium	Soluble pigment	Type of growth
Malt yeast extract agar	Moderate	Yellow	Yellow	Negative	Leathery
Glucose nitrate agar	Moderate	Gray	Brown	Negative	Powdery
Starch nitrate agar	Heavy	Gray	Brown	Brown	Powdery
Starch casein agar	Heavy	Gray	Brown	Negative	Powdery
Starch ammonium sulfate agar	Moderate	White	White	Negative	Powdery
Nutrient agar	Heavy	Gray	Brown	Brown	Powdery
Inorganic salt starch agar	Moderate	Gray	Dark gray	Negative	Leathery
Glucose starch peptone agar	Heavy	Gray	Brown	Brownish	Powdery
Czapek's agar	Moderate	White	White	Negative	Powdery
Peptone yeast extract iron agar	Heavy	White	Gray	Negative	Leathery
Glucose asparagine agar	Moderate	White	White	Negative	Powdery

TABLE 4. Physiological properties of the experimental Isolate

Physiological tests	Growth
Starch hydrolysis	Positive
Casein hydrolysis	Positive
Cellulose decomposition	Positive
Gelatin Liquefaction	Positive
Coagulation of milk	Positive
Reduction of nitrate to nitrite	Positive
Production of H_2S	Positive

TABLE 5. The Nacl tolerance for the experimental isolate

Concentration of Nacl %	Temperature	
	28°C	45°C
2.5	Heavy growth	Heavy growth
5	Heavy growth	Moderate growth
7	Moderate growth	Weak growth
10	Weak growth	Negative

TABLE 6.-Effect of temperature on growth of the experimental isolate

Temperature	Growth	Color of aerial mycelium	Color of substrate mycelium	Soluble pigment	Type of growth
25°C	Weak	White	White	Negative	Powdery
28°C	Moderate	White	White	Negative	Powdery
37°C	Moderate	White	Brownish	Negative	Leathery
45°C	Negative	Negative	Negative	Negative	Negative

TABLE 7. Utilization of different carbon sources of the experimental isolate and production of gas

Carbon source	Ferment sugar	Production of gas
Lactose	Positive	Negative
Mannose	Positive	Negative
Fructose	positive	Positive
Glucose	Positive	Negative
Galactose	Positive	Negative
Rhaminose	Negative	Negative
Mannitol	Positive	Negative

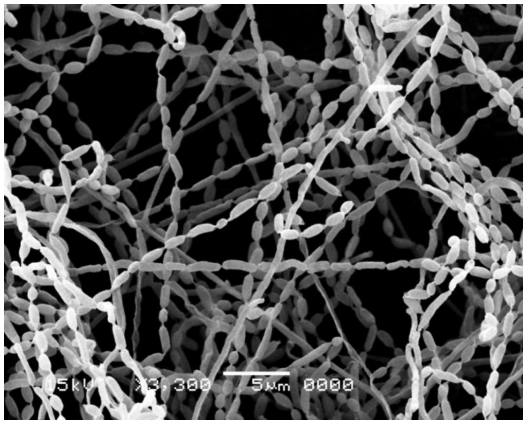

Fig. 1. SEM micrograph showing straight sporophore of *Streptomyces* isolate

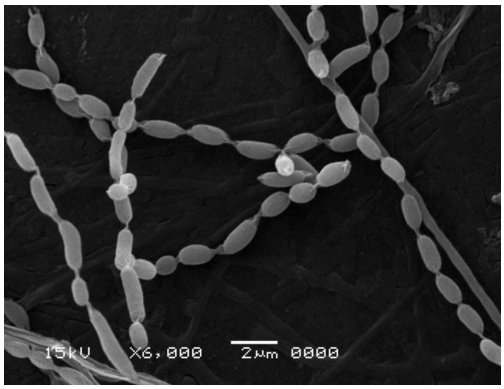

Fig. 2. SEM micrograph showing smooth spore surface of *Streptomyces* isolate

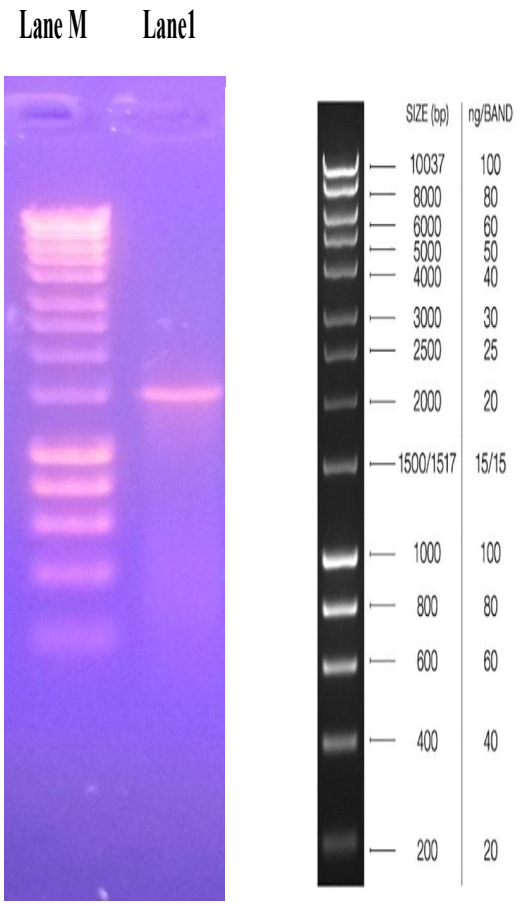

Fig. 3. Gel electrophoresis of genetic material of *Streptomyces incanus* locally isolated from eggplant Egyptian agriculture soil sample

Molecular identification and phylogeny of Streptomyces isolate

The right Lane 1 shows the PCR of *Streptomyces incanus* DNA and right Lane **M** was for the standard ladder. The nucleotide sequence of the DNA from the isolated Actinomycete was determined. Comprising nucleotides as indicated in Table 8 and when deposited in the NCBI (National center for Biotechnology Information) Gene Bank sequence databases, the Actinomycete isolate was found to be *Streptomyces incanus* . A phylogenetic tree constructed on the basis of the obtained 16S rRNA coding gene sequences of the isolate under study and the nearest relatives were described in Fig. 3 and 4.

Physical characterization of purified product of PHB using FTIR

FT-IR spectrum of both purified product and standard PHB

The FT-IR analysis (Figs. 5 and 6) and Table 9 of the standard PHB revealed that the absorption band occurred at 3434 cm^{-1} representing the O-H bending. The peaks at 2969 cm^{-1} and 2927cm^{-1} show O-H stretching and the medium-CH_3 stretching groups, respectively. The medium- weak C=H bond occurred at 2358cm^{-1}. A strong C=O stretching bond occurred at 1727 cm^{-1}. A medium strong CH_2 stretching bond occurred at 1452 cm^{-1}. A medium strong C-O bond occurred at 1129 cm^{-1}. A medium strong C-H bond was found at 1377 cm^{-1}. A medium C-O-H bond occurred at 1279cm-1. A medium strong C-O was detected at 1052cm^{-1}. The results obtained from the current study declared that the polymer extracted showed the intense absorption characteristic for ester carbonyl (C=O) stretching groups and besides that, the presence of peaks due to the methyl stretching group have been observed. Furthermore, CH2 or methylene group has been observed and also CH or methane group and that were found to be congruent with that obtained by Tripathi et al. (2013) who reported that band absorption between 1720 to1740 cm-1 is a typical feature of PHB. Two strong absorption peaks of PHB extracted from *Bacillus megaterium* MTCC 8075 which were obtained at 1724.2 cm-1 and 1280.3 cm-1, matching to C=O and C-O stretching groups (Muralidharan & Radha, 2014). The region of 1675–1735 cm^{-1} was associated with the C=O stretching of the ester carbonyl bond. The FTIR spectra of PHB produced by *Bacillus circulans* MTCC 8167 showed high absorbance at 3360, 2922, 1735, and 1133 cm−1 and were supposed to be due to O-H, CH, C=O, and C-O-C, respectively (Zribi et al., 2013). It had been identified that the most apparent spectral changes during PHB crystallization are in the carbonyl band at 1740-1720 cm–1, whereas the amorphous phase presents a broad band near 1738 cm–1, while the crystalline phase band transfer to lower wave numbers near 1722 cm^{-1} (Pachekoski et al., 2013).

The commercial poly--hydroxybutyrate (Sigma-Aldrich) standard curve was used to calculate the quantity of PHB produced after the PHB polymer was extracted (Fig. 7).

Preparation of PHB film and blended films

The molecular properties of pure PHB have increasingly become an interest in forming a raw material for biodegradable plastics and serve as the most sustainable alternative to the petrochemical plastics. It can be better made with improved physical and mechanical properties by blending/copolymerization. In general, copolymerization enables the polymers to become more flexible and tougher than PHB. Additionally, they do facilitate easier degradation when discharged into the natural environment. Besides, the spectrum of possible applications also expands with the modified physical properties of the polymer blends. PHB is a semi-crystalline material with an elevated melting temperature and a high degree of crystallinity. Its mechanical properties are equivalent to those of isotactic polypropylene. Furthermore, PHB has a comparatively high glass transition temperature which is near to room temperature and thus it is rigid and fragile at ambient temperature Fig. 8.

Scanning electron microscopic observation of PHB

PHB is an ideal carbon reserve material since it exists in the cell in a high reduced state as virtually insoluble polymer exerting negligible osmotic pressure. The granules are generally spherical, sprinkled in shape and vary in size according to the organism. (Figs. 9, 10) explain that the size distribution of PHB granules ranged at different magnifications.

TABLE 8. *Streptomyces incanus sp*. 16S ribosomal RNA gene, partial sequence

Description	Max score	Total score	Query cover	E value	Ident.	Accession
Streptomyces incanus strain Bk 128 16s ribosomal RNA gene partial sequence	483	483	100%	3e-137	85%	NR 108498.1
Streptomyces griseorubiginosus strain NBRC13047 16s ribosomal RNA gene partial sequence	483	483	100%	3e-137	85%	NR 112350.1
Streptomyces coacervatus strain IFM11055 16s ribosomal RNA gene partial sequence	483	483	100%	3e-137	85%	NR 112916.1
Streptomyces phaeopurpureus strain NRRL B 2260 16s ribosomal RNA gene partial sequence	483	483	100%	3e-137	85%	NR 034505.1
Streptomyces phaeopurpureus strain NBRC 3930 16s ribosomal RNA gene partial sequence	483	483	100%	3e-137	85%	NR 112505.1
Streptomyces phaeopurpureus strain NBRC 12899 16s ribosomal RNA gene partial sequence	483	483	100%	3e-137	85%	NR 112327.1
Streptomyces griseorubiginosus strain LMG19941 16s ribosomal RNA gene partial sequence	483	483	100%	3e-137	85%	NR 042298.1
Streptomyces griseorubiginosus strain ISP 5469 16s ribosomal RNA gene partial sequence	483	483	100%	3e-137	85%	NR 114835.1
Streptomyces camponoticapatis strain 2H TWYE 14 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 152020.1
Streptomyces seymenliensis strain B 1041 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 134823.1
Streptomyces kebangsaanensis strain SUK 12 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 109009.1
Streptomyces herbaeceus strain BK 119 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 108496.1
Streptomyces flavidovirens strain NBRC13039 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 108009.1
Streptomyces flavidovirens strain NRRL B 16367 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 044146.1
Streptomyces termitum strain NRRL B 3804 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 115959.1
Streptomyces niveus strain NRRL 2466 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 115784.1
Streptomyces flavovanablis strain NBRC 100764 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 112589.1
Streptomyces albosporeus subsp strain NBRC 5387 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 125445.1
Streptomyces bikiniensis strain NBRC 14593 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 112436.1
Streptomyces clavuligenus strain NBRC 13307 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 112374.1
Streptomyces violaceorectus strain NBRC 13102 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 041114.1
Streptomyces longisporus strain NBRC 12885 16s ribosomal RNA gene partial sequence	477	477	100%	1e-135	84%	NR 112319.1

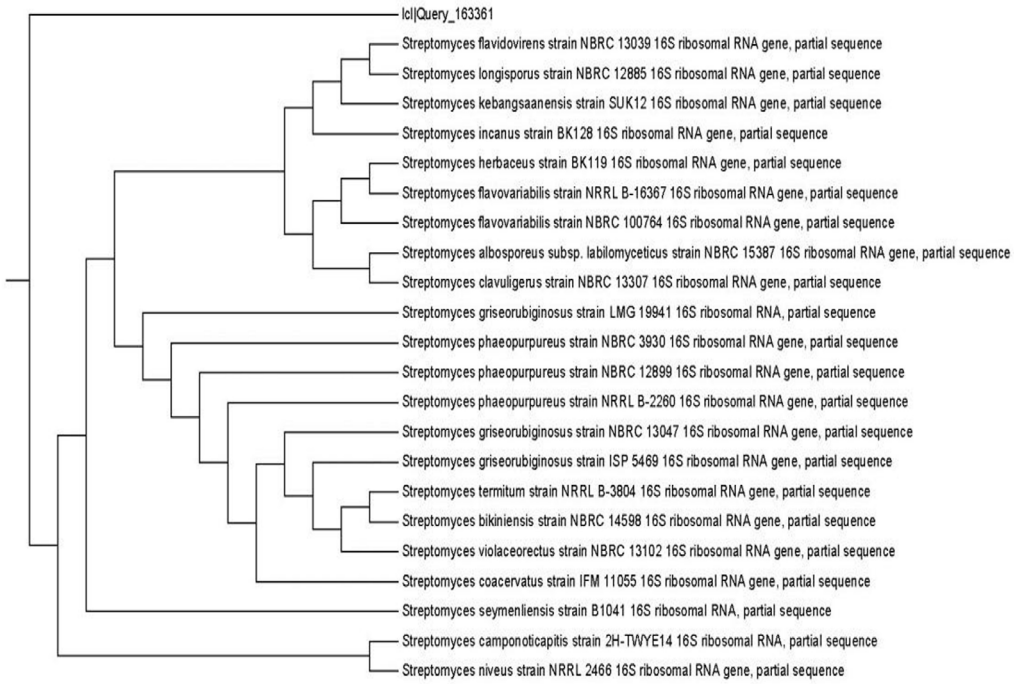

Fig. 4. Phylogenetic tree analysis of PHB producing Actinomycete (*Streptomyces incanus* strain Bk 128) constructed through neighbor joining method with boot strap values as percentage at the nodes

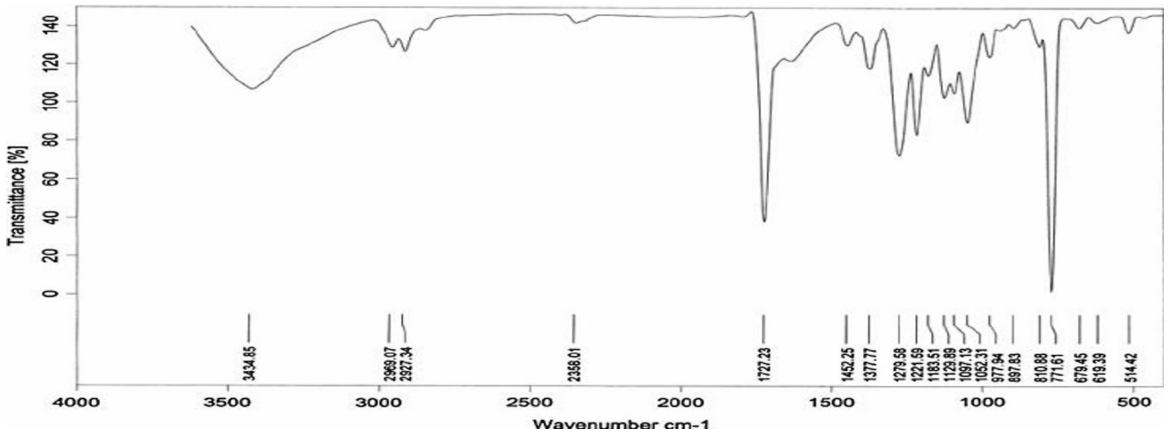

Fig. 5. FTIR peaks of the standard PHB

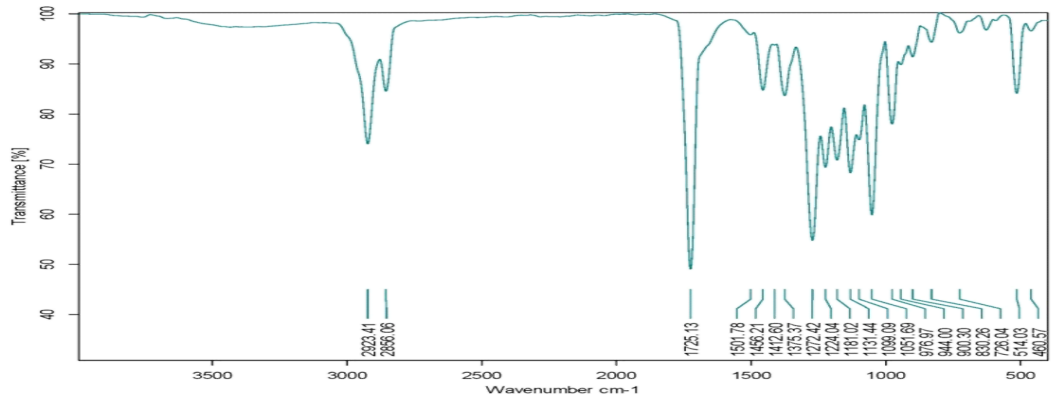

Fig. 6. Infra-Red spectrum of PHB purified product

TABLE 9. Groups absorbing in IR region for PHB purified product

Range (cm^{-1})	Assignment
2923.41	CH_3
2856.06	C-H aliphatic
1725.13	C=O
1456.21	CH_2
1412.60	O=H
1375.37	C-H
1272.42	C-O-H
1181.02	C-O
1131.44	C-O-C

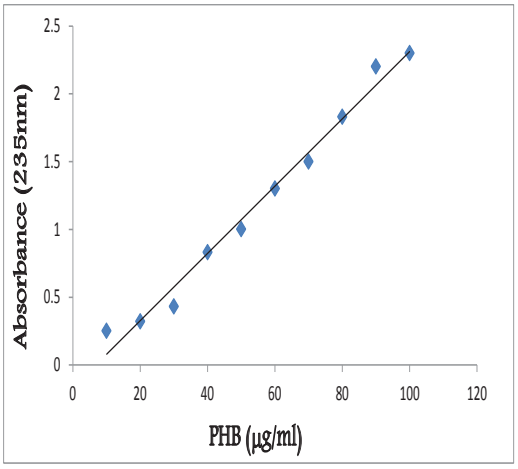

Fig. 7. Standard curve of commercial polyhydroxybutyrate

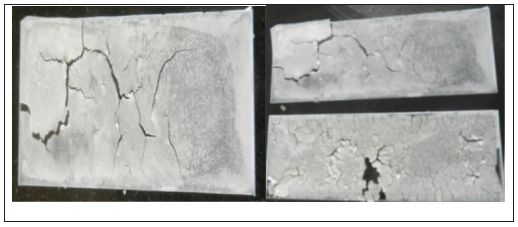

Fig. 8. The brittle PHB films produced by *Streptomyces incanus* BK 128

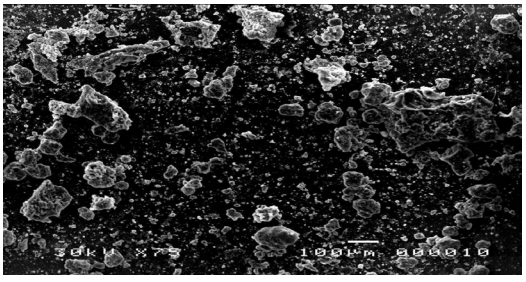

Fig. 9. Scanning electron micrograph of PHB granules obtained from *Streptomyces incanus* BK128

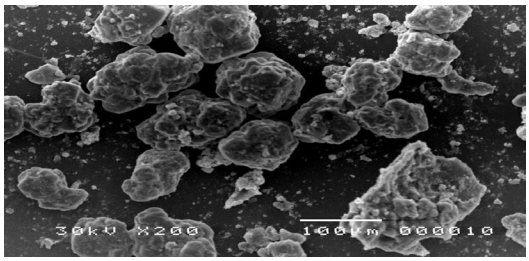

Fig. 10. Scanning electron micrograph of PHB granules obtained from *Streptomyces incanus* BK128

Conclusion

The objectives of the present study are to isolate effective local bacterial strains for PHB production and use a cheap simple medium. Depending on the results of the present study, *Streptomyces incanus* BK128was selected as an effective PHB producer.

References

Bansal, S.S., Goel, M., Aqil, F., Vadhanam, M.V., Gupta, R.C. (2011) Drug delivery systems for controlled release of therapeutics. *Cancer Prevention Research,* **4**, 1158–1171.

Chanaporn, T., Antika, B., Waraporn, A., Akihiko, K., Takamitsu, A., Kumar, S., Pilanee, V. (2021) Enhanced polyhydroxybutyrate (PHB) production by newly isolated rare actinomycetes Rhodococcus sp. strain BSRT1-1 using response surface methodology. *Scientific Reports*, **11**, Article number: 1896.

Deshwal, V.K., Chaubey, A. (2014) Isolation and characterization of *Rhizobium leguminosarum* from root nodule of *Pisum sativum* L. *Journal of Academia and Industrial Research,* **2**, 464.

Gouda, S., Das, G. (2015) Mangroves: A rich source of natural bioactive compounds. In: "*Recent Advances in Natural Products*"; Sujogya Kumar Panda, ed.; Stadium Press LLC, U.S.A., pp. 1-12.

Hori, K., Kaneko, M., Tanji, Y., Xing, X.H., Unno, H. (2002) Construction of self-disruptive *Streptomyces porvutuls,* in response to substrate exhaustion for polyhydroxybutyrate production. *Applied Microbiology and Biotechnology,* **59**, 211-216.

IAEA (1973) The effect of ionizing radiation on

bacteria. In: Manual of radiation sterilization of medical and biological materials technical reports. Series No. 149.

Kannabiran, K. (2017) Actinobacteria are better bio remediating agents for removal of toxic heavy metals: an overview. *International Journal of Environmental Technology and Management,* **20**,(3/4), 129–138.

Kieser, T., Bibb, M.J., Buttner, M.J., Chater, K.F., Hopwood, D.A. (2000) "*Practical Streptomyces Genetics*" (Vol. 291, p. 397). Norwich: John Innes Foundation.

Kim, J.I., Cox, M.M. (2002**)** The RecA proteins of Deinococcus radiodurans and Escherichia coli promote DNA strand exchange via inverse pathways. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 7917-7921.

Law, J.H., Slepecky, R.A. (1961) Assay of poly-β-hydroxybutyric acid. *Journal of Bacteriology*, **82**, 33- 36.

Liu, Y., Huang, S., Zhang, Y., Xu, F. (2014) Isolation and characterization of a thermophilic *Bacillus shackletonii* K5 from a bio trickling filter for the production of polyhydroxybutyrate. *Journal of Environmental Sciences,* **26**, 1453–1462.

Michel, A.T., Billington, S.L. (2012) Characterization of poly-hydroxybutyrate films and hemp fiber reinforced composites exposed to accelerated weathering. *Polymer Degradation and Stability,* **97**(6), 870–878.

Mikkili, I., Karlapudi, A.P., Venkateswarulu, T.C., Babu, J.D., Nath, S.B., Kodali, V.P. (2014) Isolation, screening and extraction of polyhydroxybutyrate (PHB) producing bacteria from sewage sample. *International Journal of PharmTech Research,* **6**, 850-857.

Mizunoa, K., Ohtaa, A., Hyakutakeb, M., Ichinomiyab, Y., Tsugeb, T. (2010) Isolation of polyhydroxyalkanoate producing bacteria from a polluted soil and characterization of the isolated strain Bacillus cereus YB4. *Polymer Degradation and Stability*, **95**, 1335–1339.

Muralidharan, R., Radha, K.V. (2014) Biological production and characterization of polyhydroxybutyrate (PHB) by *Bacillus megaterium* MTCC 8075. *Journal of Chemical, Biological and Physical Sciences*, **4**, 1271-1279.

Nehra, K., Dahiya, N., Lathwal, P., Singh, M. (2015) Influence of nutritional and environmental parameters on PHB production by bacteria isolated from contaminated soils. *International Journal of Pharma and Bio Sciences*, **6**(1B), 676–691.

Pachekoski, W.M., Dalmolin, Carla, José, A.M.A. (2013) The influence of the industrial processing on the degradation of poly(hidroxybutyrate) – PHB. *Materials Research,* **16**(2) • Apr

Phil, S. (2018) Natural Product Total Synthesis. *Journal of the American Chemical Society*, **4**, 4751-4755.

Preiss, J. (1989) Chemistry and metabolism of intracellular reserves. In: "*Bacteria in Nature: Structure, Physiology, and Genetic Adaptability*" vol. 3. Poindexter J.S. and Lead better E.R. (Eds.). Plenum Press, New York and London. pp. 189-258.

Rajendran, R., Mekala, M., Suganya, K. (2013) PHB production by *Streptomyces* species using the cheap substrate groundnut oil cake. *International Journal of Pharmacy and Biological Sciences,* **4**, 1006-1015.

Shah, K.R. (2014) Optimization and production of polyhydroxybutyrate (PHB) by Bacillus subtilis G1S1from soil. *International Journal of Current Microbiology and Applied Sciences*, **3**, 377-387.

Singh, M., Patel, S.K.S., Kalia, V.C. (2009) Bacillus subtilis as potential producer for polyhydroxyalkanoate. *Microbial Cell Factories*, **8**, 38-48.

Singh, P., Sharma, V. (2016) Integrated plastic waste management: Environmental and improved health approaches. *Procedia Environmental Sciences,* **35**, 692–700.

Soliman, M.A., Hamza, A.F., Zahran, Nagwan F., Bassioni, Ghada (2021**)** Microbiological study and insecticidal potential of purified extract from**.** Streptomyces sp. on the larvae of *Galleria mellonella*. *Journal of Plant Diseases and Protection.* DOI: 10.1007/s41348-021-00508-0.

Sridewi, N., Bhubalan, K., Sudesh, K. (2006)

Degradation of commercially important polyhydroxyalkanoates in tropical mangrove ecosystem. *Polymer Degradation and Stability,* **91**(12), 2931-2940.

Tawfik, Z.S., El Shikh Hussein, H., Haroun, B.M., Yassin, M.M., El Sonbty, Sawsan M., Zaki, A.G., Mahmoud, A.M. (2015) Biological and histological studies of purified product from *Streptomyces janthinus* M7 metabolite. *Journal of Science and Technology,* **3**, 14 .

Trakunjae, C., Boondaeng, A., Apiwatanapiwat, W., Kosugi, A., Arai, T., Sudesh, K., Vaithanomsat, P. (2021) Enhanced polyhydroxybutyrate (PHB) production by newly isolated rare actinomycetes Rhodococcus sp. strain BSRT1-1 using response surface methodology. *Scientific Reports*, **11**, Article number: 1896.

Tripathi, A.D., Srivastava, S.K., Singh, R.P. (2013) Novel approach for optimization of fermentative condition for polyhydroxybutyrate (PHB) production by Bacillus megaterium using Taguchi (DOE) methodology. *African Journal of. Biotechnology,* **110**, 7219-7224

Weng, Y.X., Wang, X.L., Wang, Y.Z. (2011) Biodegradation behavior of PHAs with different chemical structures under controlled composting conditions. *Polymer Testing*, **30**(4), 372–380.

Zribi, M.E., Trabelsi, I., Elleuch, L., Chouayekh, H., Salah, R.B. (2013) Purification and characterization of two polyhydroxy-alkanoates from Bacillus circulans MTCC 8167. *International Journal of Biological Macromolecules*, **61**, 82-88.