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Full Length Article

Poly-β-hydroxy butyrate Depolymerase from *Streptomyces lydicus* MM10, Isolated from Wastewater Sample

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Abstract

Plastics are an integral part in our daily life activities and it is difficult to conceive how we could function without them. Burning or chemical treatments of these plastic materials destroys the environment and have many dangerous effects on, water air and human health. Thus, other sources of plastic (biodegradable plastic) must be introduced. Under unfavorable growth conditions Poly ß-hydroxybutyrate (PHB) is accumulated intracellular by many bacteria and the principal enzyme for the degradation of PHB is PHB depolymerase. A number of thermophilic actinomycetes have been screened for PHB degradation on agar plats. All these isolates were recovered from soil, sand and wastewater samples on starch nitrate agar at incubation temperature of 45. Five isolates (50%) out of 10 showed degradation activities, detected by the diameter of the clear zone. The Actinomycete isolate MM10 was the most PHB degrader and it was identified using morphological, physiological and biochemical characters. According to 16SrRNA analysis, it was identified as *Streptomyces lydicus*MM10. Growth at 45°C, in minimal medium (pH7.0) containing glucose and0.3% PHB as enzyme inducer, enhanced enzyme production and maximum activity was obtained after 24 h of incubation. The PHB depolymerase was precipitated and purified using column chromatography. The optimum temperature and pH of the pure enzyme were 45°C and pH 8, respectively. However, production of bioplastics is cost effective as compared to synthetic plastics they were non-toxic and completely biodegradable by some actinomycete isolates.© 2015 Friends Science Publishers

Keywords: Depolymerase; *Streptomyces*; PHB; Degradation; Bioplastics

Introduction

Petroleum derived synthetic plastic is one of the major non-biodegradable toxic pollutants, during its production and disposal caused serious damage to the environment. Bioplastics (polyhydroxyalkanoates) are considered good substitute for plastics due to similar properties to synthetic polymers, complete biodegradability after disposal, production by several bacterial genera and complete degradation to CO_2 and water under natural environment (Anderson and Dawes, 1990; Verlinden *et al*., 2007; Aly *et al*., 2013a).

In natural environments, degradation of PHAs was by microorganisms including bacteria and fungi (Jendrossek *et al*., 1996). *Bacillus, Pseudomonas, Comamonas, Alcaligenes*, and *Streptomyces* in addition to 95 genera of fungi have excellent role in PHB degradation (Jendrossek *et al*., 1996; Tokiwa *et al*., 2009). Degradation of PHB at higher temperature is more effective in recycling biodegradable plastics, thus isolation of thermophilic PHB-degraders is essential (Tokiwa *et al*., 2009; Hsu *et al*., 2012).

There are two types of PHB depolymerases: intracellular and extracellular PHB depolymerase, which degraded native and denatured PHB granules, respectively. At first, extracellular depolymerase binds to the polymer substrate and catalyzes the hydrolytic cleavage converting the polymer to many small water soluble units of β-hydroxybutric acid, which can be transported directly through the cell wall (Gilmore *et al*., 1990). The monomers are small enough to diffuse through the cell wall where they are metabolized to water and carbon dioxide (Scott, 1999) or methane under anaerobic conditions (Luzier, 1992). Several PHB depolymerases have been isolated and purified from *Pseudomonas* (Schober *et al*., 2000), *Alcaligenes* (Bachmann and Seebach, 1999) and *Comamonas* (Kasuya *et al.,* 1994). Biochemical properties of some PHB depolymerases have been studied and further extensive studies are necessary to find out the factors affecting the enzymes production by these polyester-degrading bacteria. The aim of this study was to isolate thermotolerant PHB degrading actinomycete and to optimize the culture conditions for maximum PHB degradation.

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Materials and Methods

Sample Collection and Bacterial Isolation

Twenty different samples of soil, sand and wastewater were heat treated to isolate heat resistant microbes on starch nitrate agar samples containing antibiotic at 45°C. Soil and sand samples were collected from the different places of Jeddah. Wastewater samples were collected from Bani Malek Station, Jeddah, Saudi Arabia. Soil suspension, sand, wastewater samples were heated to 100°C for 15 min and used to isolate thermostable actinomycetes. About 0.1 mL of each sample dilution was spread on starch nitrate agar (Shirling and Gotlieb, 1966) with sterile streptomycin and amphotericin B (5 µg/mL) as described by Aly *et al*. (2011a). All plates were incubated at 45°C for 7days and the purified isolates were maintained on agar slants of the same medium at 4°C until used.

Screening for PHB Degradation

Ten thermotolerant actinomycete isolates were screened for PHB degradation using plate-clearing technique on Turbid medium containing PHB as carbon source (Augusta *et al*., 1993). Turbid medium was composted of (g/L): PHB 1, KH_2PO_4, 0.7, K_2HPO_4, 0.7, $MgSO_4$, 0.7, NH_4NO_3, 1, Agar 15, pH 7. About 0.1 mL of PHB degrading bacterium was used to inoculate the agar plate and all plates were incubated at 45°C for 7days until the presence of a clear zone which indicated PHB hydrolysis (Klich, 2002).

PHB Degrading Bacterium in Liquid Media

The bacterial isolate with maximum PHB degradation on agar plate was cultivated in 50 mL of PHB emulsified mineral Turbid broth medium in 250 mL Erlenmeyer flasks. Each flask was inoculated with a 2 mL (4×10^6CFU/mL) of the bacterial suspension, previously grown in starch nitrate medium for 2 days at 45°C and 120 rpm. The inoculated flasks were incubated at 45°C and120 rpm for 7 days. Finally, bacterial cells were collected by centrifugation at 10,000 rpm for 15 min and the cell-free supernatant was used for PHB depolymerase assay.

Identification of PHB Degrading Actinomycete

The isolate with the highest clear zone on PHB emulsified agar plates was identified on the basis of colony morphology, microscopic examination using light and electron microscopy, sensitivity to different antibiotics and biochemical and physiological tests (Aly *et al*. 2011a, b; 2012). Whole-cell sugar composition and analysis of diaminopimelic acid isomer were as described by Hasegawa *et al*. (1983). The present phospholipids and the methyl esters of the fatty acids (Butte, 1983) were determined using by two-dimensional thin-layer chromatography (Hoischen *et al.,* 1997) and gas chromatography, respectively.

Phylogenetic Analysis of 16S rDNA Sequence

The selected isolate DNA was obtained using QIAamp DNA Mini Kit and two primers were designed based on the 16S rDNA highly conserved region for various bacteria (Weisberg *et al.*, 1991). The 16S rDNA gene was amplified by DNA thermal cycler PCR (Perkin Elmer, USA) using forward primer 5' AGTTTGATCATGGTCAG-3' and reverse primer 5' -GGTTACCTTGTTACGACT 3'.The purified PCR products (QIAquick PCR purification kit, QIAGEN) of 1360 bp was sequenced using big dye terminator cycle sequence kit, analyzed using DNA sequence ABI PRISM 310 genetic analyzer (Perkin Elmer, USA) and compared to the GeneBank database in the National Center for Biotechnology Information (NCBI) using the BLAST program.

Optimization of Conditions for PHB Depolymerase Production

Different cultures conditions for the maximum production of PHB depolymerase were optimized. All the experiments were performed in 250mL Erlenmeyer flasks using Turbid medium supplemented with 0.1% PHB as a sole carbon source. All flasks were incubated at 120 rpm for 7days. Samples were drawn aseptically after every 24 h, centrifuged at 10,000 rpm for 10 min and then supernatant was taken as a crude enzyme extract to determine the enzyme activity, protein content and relative activity. To study the effect of temperature, inoculated flasks were incubated at different temperatures ranging from 25- 55°C for 7 day.

Flasks containing PHB emulsified Turbid medium with different pH values (5-10) were prepared and inoculated with 2 mL of preculture. After incubation, the flasks were incubated at 45°C for 7 days and PHB depolymerase production was measured every 24 h as described earlier. Moreover, different carbon sources such as Glucose, Starch, Sucrose and Maltose (1% w/v) were added to PHB emulsified Turbid medium at pH 7 and inoculation was carried out using 2 mL bacterial suspension. Furthermore, to detect the effect of substrate concentration on PHB degradation, Turbid medium with different concentrations of PHB (0.1– 0.5 %) were prepared at pH 7 and inoculated with 2 mL spore suspension then incubated at 45°C for 7 days.

PHB Depolymerase Assay

After centrifugation at12,000 rpm at 4°C for 15 minutes, culture supernatant was used for measuring the PHB depolymerase activity using PHB as a substrate according to the method described by Kobayash *et al.* (1999). About 0.3% Poly (3-hydroxybutyrate) in 50mMTris-HCl buffer, pH 7.5 was sonicated for 20 min in ultrasonic water bath (35KHz285W) prior to the dilution to 0.03% in the same buffer. To 0.1mL of culture supernatant, 0.9 mL of the

substrate suspension was added and the mixture was incubated for 24 h at 30°C. The decrease in OD_{650}nm was measured using spectrophotometer, against substrate buffer blanks. One unit of the enzyme is the decrease in OD_{650}nm per 24 h (Kobayashi *et al*., 1999). Protein concentration estimation was performed for each enzyme sample using method suggested by Lowry *et al.* (1951). Color developed during the assay procedure was read at 650 nm. Finally concentration of protein in the culture supernatant was determined using standard curve of bovine serum albumin.

Enzyme Purification and Molecular Weight Determination using Gel Electrophoresis

The extracellular PHB depolymerase enzyme was obtained from the culture filtrate after 80% $(NH_4)_2SO_4$ precipitation, dialysis andcollection from affinity chromatography using concanavalin-A. The fractions with high protein content and enzyme activity were used as depolymerase enzyme (Shivakumar, 2013). Gel electrophoresis was carried out using 15% SDS polyacrylamide electrophoresis at room temperature where 20–30 µL (40 µg protein/well) from the protein standard (Merck) were applied and the slab gels were stained with Coomassie brilliant blue R-250.

Characterization of the Purified Enzymes

Effect of temperature (20–60ºC) on the purified enzymes was determined. Samples of the heated enzyme were internally withdrawn, cooled in ice bath and finally assayed immediately as described above. The pre-incubated sample at 30°C was used as a reference. Effect of different pH values on depolymerase activity was determined at pH values ranging from 3 to 9 using the standard assay conditions. The pure enzyme was suspended in different buffers with different pH values, followed by the enzyme assay. The buffers used were 0.2 M acetate buffer (pH 3–6) or 0.2 M $Na_2B_4O_7.10\ H_2O/H3BO_3$ buffer (pH 7–10). The relative activities of the crude PHB depolymerase were measured as mentioned earlier. Effect of different metal ions on the enzyme assay was studied in the presence of various metal ions at 1 mM. The relative activities were compared with the activity obtained in the metal free enzyme reaction (El-Sabbagh *et al.,* 2003).

PHB Sheet Degradation

The selected *Streptomyces* isolate was grown in liquid medium with 0.3% PHB for 24 h, and cells were collected by centrifugation at 5000 rpm for 5 min. A PHB film (wt 0.01 g) was immersed in the obtained supernatant for 7 days and compared with control (sheet in sterile medium). Moreover, similar PHB film (wt 0.01 g) on 2% sterile water agar plate was sprayed with 30 µg of the purified depolymerase enzyme, incubated at 45°C for 7 days and compared with control sheet, incubated in the same conditions without the enzyme.

Statistical Analysis

Mean of three replicates ±standard deviation were recorded and difference between mean values was determined using Student's t-test. Differences were considered significant when probability was less than 0.05.

Results

Ten actinomycetes isolates were obtained from heated samples on starch nitrate agar containing antibiotics. Out of 10 thermotolerant actinomycetes, only five isolates recorded different growth ranging from high (+++), moderate (++) or low (+) using PHB as carbon source and maximum diameter of the zones of hydrolysis was observed after 7 days (Table 1). The isolate MM10, obtained from wastewater sample from Bani Malik wastewater treatment plant, Jeddah, was the most active PHB depolymerase producing isolate (Fig. 1).

Identification of the Selected Isolate

Based on the screening data, the isolate MM10 was selected for more detail experiments. It was characterized by some morphological, physiological and biochemical properties which presented in the Table 2, 3, 4 and 5. The composition of cell wall constituents such as peptidoglycan and the presence of characteristics lipids, sugars and fatty acids were summarized in Table 6. The isolate MM10 was belonging to filamentous Gram +ve bacteria with dark gray color on starch nitrate agar. The growth was ranged from heavy, moderate to poor on different agar media (Table 2). Table 3 and 4 summarized the morphological and physiological characters of the selected isolate. Microscopic observation of the isolate MM10 showed substrate and aerial mycelia bearing a spiral chain of conidia, which had hairy surface (Fig. 2). Analyses of bacterial cell wall and whole cell hydrolysates revealed the presence of only L-isomer of diaminopimelic acid, no diagnostic sugar, phosphatidylinositol and saturated fatty acids with no mycolic acids. Comparison between the characteristics of the active actinomycete isolate MM10 above described with the former isolates indicated that it belongs to the genus *Streptomyces.* The 16S rDNA sequence was compared to the GeneBank database in the National Center for Biotechnology Information (NCBI) using the BLAST program (Fig. 3). The isolate was similar to genus *Streptomyces* and identified as *S. lydicus.*

The Best Conditions for Depolymerase Production

Depolymerase production varied with incubation temperature, incubation period, medium initial pH, PHB concentration and presence of carbon source. On Turbid medium with 0.1% PHB, the most suitable temperature for growth and depolymerase production was 45°C after 24 h of incubation (Fig. 4). The effect of initial pH of the medium on depolymerase production was shown in Fig. 5.

Table 1: Source, colony color and PHB degradation by some actinomycete isolates at 45°C

No. of bacterial isolate	Source of isolation	Colony color	Cell characters	PHB degradation at 45°C	
				Bacterial growth*	Presence of clear zone (cm) after 7 days
MM1	Compost soil	Black	Gram +ve, Filamentous	++	1.0
MM 2	Compost soil	Gray	Gram +ve, Filamentous	-	ND
MM3	Soil	White	Gram +ve, Filamentous	-	ND
MM4	Soil	Yellow	Gram +ve, Filamentous	-	ND
MM5	Soil	Gray	Gram +ve, Filamentous	-	ND
MM6	Sand	Gray	Gram +ve, Filamentous	-	ND
MM7	Wastewater	Pale gray	Gram +ve, Filamentous	++	1.1
MM8	Wastewater	White	Gram +ve, Filamentous	++	2.3
MM9	Wastewater	Pink	Gram +ve, Filamentous	++	2.4
MM10	Wastewater	Gray	Gram +ve, Filamentous	+++	4.1

*: Bacterial growth on Turbid medium with 0.1% PHB, -: No growth, +: Weak growth, ++: Moderate growth, +++: High growth, ND: No clear zone

Table 2: Cultural characteristics of the actinomycete isolate MM10, grown on different agar medium at 45°C

Media used	Growth	Color of aerial mycelium	Color of substrate mycelium	Presence of soluble pigment
Starch-nitrate agar medium	Heavy	Gray	Dark gray	-
Glucose Asparagine agar medium	Moderate	Dark gray	Dark gray	-
In-organic salts-starch iron agar medium (ISP-4)	Moderate	Gray	Dark gray	-
Tyrosine agar medium (ISP-7)	Poor	Yellowish white	Pale gray	-
Yeast extract-malt extract agar medium (ISP-2)	Moderate	Pale gray	Gray	-
Oatmeal agar medium (ISP-3)	Moderate	Pale gray	Pale gray	-
Glycerol-asparagine agar medium (ISP-5)	Moderate	Black	Gray	+

+ : Soluble pigment present, - : Soluble pigment absent

Table 3: Morphological character of the selected isolate MM10

Tested character	Result
Gram stain	Gram positive
Source of isolation	wastewater
Motility of spore	Absent
Shape of spore	Cylindrical (5-7, 6-9 µm)
Spore chain	Spiral chain
Spore surface	Hairy
Number of spore/ chain	5-20
Aerial hyphae	Well developed
Substrate mycelium	Well developed
Zoospore, Sporangium, Sclerichia, Fragmented mycelium	Absent

Table 4: Physiological characteristics of the isolate MM10

Character	Reaction	Character	Reaction
Melanin pigment on Tyrosine agar	-ve	Tolerance to 10% NaCl	+
Enzyme activities:		pH range	5-10
Proteolysis	+ve	Growth temperature:	15 - 55ºC
Lecithinase	-ve	Resistance to antibiotic	
Lipolysis	+ve	Penicillin	-
Hydrolysis activities:		Cephalosporine	-
Chitin	+ve	Kanamycin	-
Gelatin	+ve	Rifampin	+
Pectin	+ve	Streptomtcin	+
H_2S Production	-ve	Resistance to 0.005 $CuSO_4$	-

-ve: Negative results, +ve: Positive results, -: Sensitive, +: Resistance

The maximum depolymerase activity was obtained when the initial pH was adjusted to 7 after 24 h. However, depolymerase production dropped significantly until pH10 where no depolymerase production was recorded. Five different PHB concentrations for depolymerase production by the selected bacterium were tested. Turbid medium with 0.3% PHB increased the enzyme production (Fig. 6). Effect of different carbon sources on depolymerase production by the selected strain of actinomyceteMM10 was illustrated in Fig.7, where glucose was the best carbon source.

Purification and Characterization of Depolymerase

Streptomyces lydicus MM10 was grown using the best conditions for depolymerase production. The cell free supernatant was precipitated with 80% ammonium sulfate,

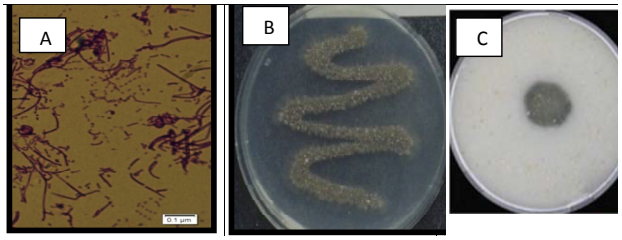

Fig. 1: The selected actinomycete isolate MM10, A: Under light microscope, B: On starch nitrate agar, C: On Turbid medium containing 0.1% PHB as carbon source after 7 days of growth at 45°C

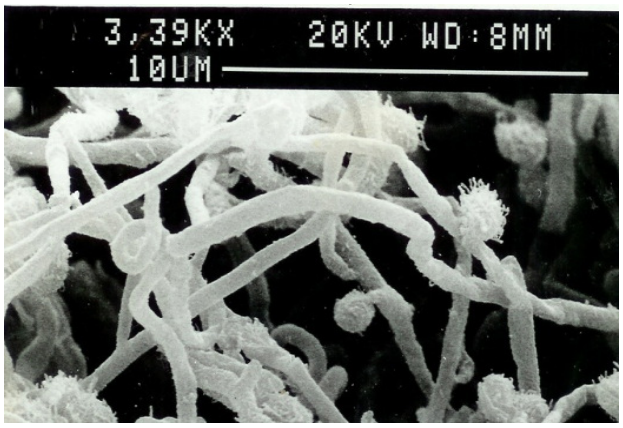

Fig. 2: The selected actinomycete isolate MM10 under scanning electron microscope

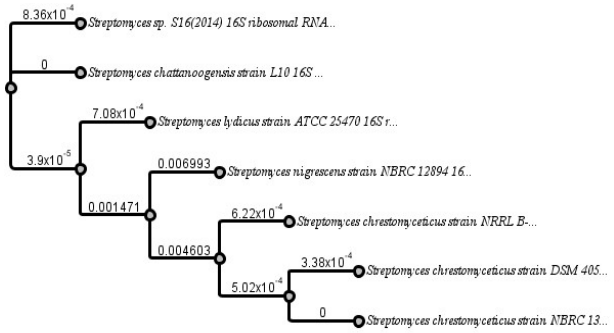

Fig. 3: Phylogenetic tree based on 16S rDNA sequence comparisons of *Streptomyces lydicus* MM10, using neighbor joining tree method, maximum sequence difference =0.002

purified and the active fractions with maximum enzyme activity were collected, lyophilized and used for enzyme characterization and molecular weight determination. The molecular weight was determined to be 65 KDa using gel electrophoresis (Fig. 8) and exhibited optimum activities at 45°C and pH 8.0 (Fig. 9, 10). Depolymerase activity was significantly affected by most of the tested metal ions at 1 mM (Table 7).

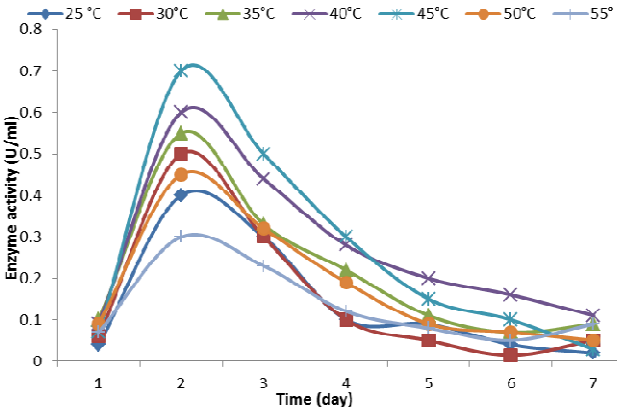

Fig. 4: Effect of different temperature on PHB degradation by the selected strain of actinomycete MM10

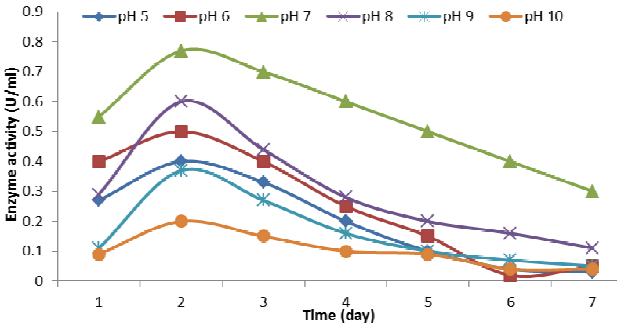

Fig. 5: Effect of different initial pH value on PHB degradation by the selected strain of actinomycete MM10

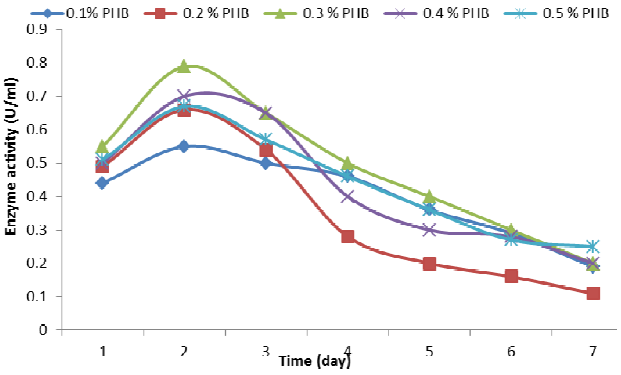

Fig. 6: Effect of different concentrations of PHB on polymerase production by the selected actinomycete isolate MM10

PHB Film Degradation in Liquid and on Solid Medium

PHB films immersed in filter sterile supernatant of the selected *Streptomyces* at 45°C was degraded and decreased in weight until disappearance. No degradation was observed for control PHB film incubated in sterile broth medium under the same previous conditions (Fig. 11). Moreover, PHB film that incubated with 30 µg of the pure enzyme at

Table 5: Utilization of different carbon and nitrogen sources using the selected isolate MM10

Utilization of different carbon source	Result	Utilization of different nitrogen source	Result
Positive control (glucose)	++	Na NO_3	++
Negative control	-	NH_4NO_3	++
D-mannitol	++	KNO_3	++
Glycerol	++	NH_4OH	++
Raffinose	-	NH_4Cl	++
D- galactose	-	$NaNO_2$	-
Sucrose	++	Phenyl alanine	++
Fractose	++	Valine	++
D-xylose	++	Peptone	++

-: No utilization. ++ Utilization

Table 6: Sugars, amino acids, phospholipids, and fatty acids of the cell wall or cell hydrolysate of the selected isolate MM10

Type of the reaction	Results	Type of the reaction	Results
Cell hydrolysate sugar		Phospholipids	
-Glucose	+	-Phosphatidylethanolamine	+
		-phosphatidylinositol mannoside	-
Cell wall amino acids		Saturated fatty acids	Branched and unbranched
-Diaminopimelic acid	+ (L-Form)	(Gas Chromotodraphy)	acids
- Alanine	+		

45°C decreased in weight with time until complete disappearance and no degradation was observed for control film, incubated in the same conditions without the purified depolymerase enzyme (Fig.12).

Discussion

Different actinobacteria were obtained from heated samples on agar medium containing antibiotics. Heating of samples or addition of antibiotics to growth medium inhibit growth of unwanted microbes and allow actinobacteria to dominate (Aly and El-Sabagh, 2004, Velho-Pereira and Kamat, 2011). Similarly, initial screening was performed on modified glycerol arginine agar to isolate common actinomycetes and on modified medium to isolate rare actinomycetes (Ghorbani-Nasrabadi *et al*., 2012).

Polyhydroxyalkanoic acids or bioplastic, among the different types of biodegrable plastics have been extensively studied, because of their similarity to conventional plastics, complete biodegradability and current market domination (Verlinden *et al*., 2007). The biodegradation of plastics proceeds actively under different conditions according to microbe properties and each microbe has its own optimal growth conditions (Lucas *et al.,* 2008). All actinomycete isolates were screened for depolymerase production on solid Turbid medium containing 0.1% PHB as inducer. As it is well known, depolymerase enzyme was inducible, and presence of PHB as inducer in the medium is necessary for enzyme formation (Lodhi *et al*., 2011). Induction and expression are subjected to a complex regulation and depolymerase enzyme is not required for balanced bacterial growth and may be synthesized in response to energy or nutrient limitation (Lodhi *et al*., 2011). In this work, 50% of the screened actinobacteria were depolymerase enzyme producing and this activity was detected as clear zones accompanying the growth in solid agar medium. Similarly, 31(48%) bacterial strains out of 67 showed PHB degradation, detected as a clear zones on assay medium (Lee *et al*., 2005).

Table 7: Effect of different metal ions and EDTA on depolymerase activity

Metal	Relative activity (%)
Control	100
$CaCl_2$	100
$MgCl_2$	100
$MnCl_2$	100
$ZnSO_4$	100
$CuSO_4$	30*
$FeSO_4$	90*
EDTA	50*

*: significant difference at $p< 0.05$

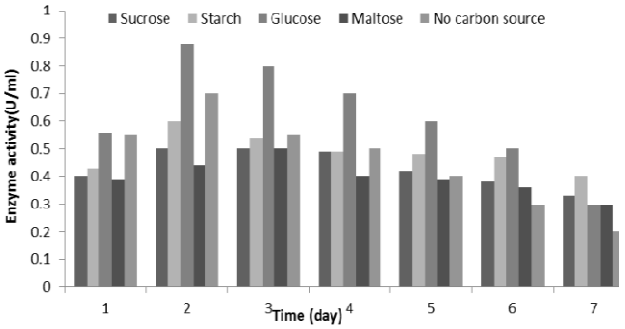

Fig. 7: Effect of different carbon sources on polymerase production by the selected strain of actinomycete MM10

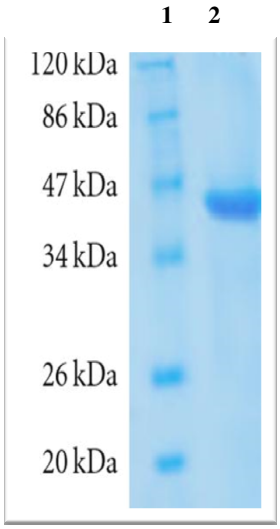

Fig. 8: SDS-PAGE profile of purified depolymerase, Lane 1: Standard protein marker, lane 2: Purified depolymerase

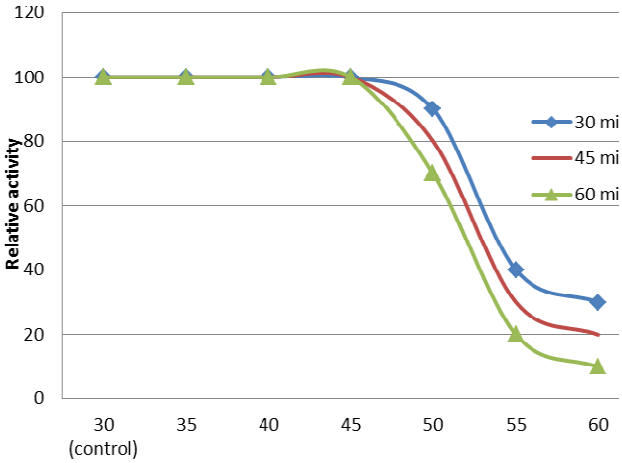

Fig. 9: Effect of different temperatures on relative depolymerase activity after 30, 45 and 60 min

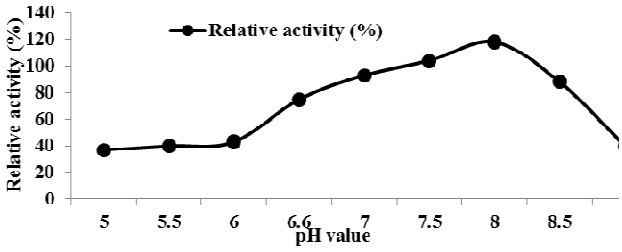

Fig. 10: Effect of different pH values on relative depolymerase activity

Depolymerase enzyme detection was obtained using plate-clearing technique and/or measuring the enzyme activity in liquid medium. Plate-clearing technique was a very simple method to detect polymer degradations in agar

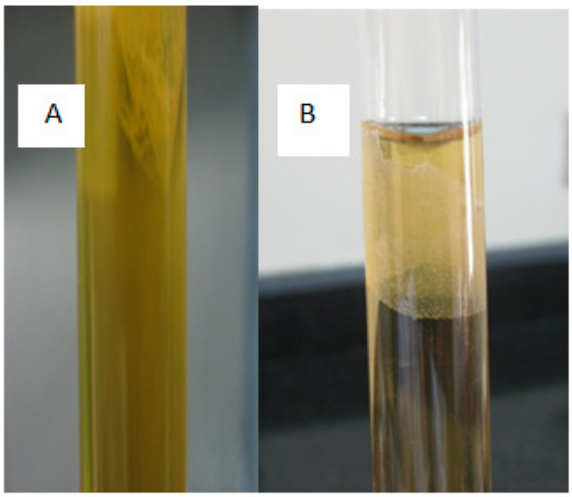

Fig. 11:PHB film immersed in filter sterile supernatant of the selected *Streptomyces* isolate after 10 days at 45°C (A), Control PHB film incubated in sterile broth medium under the same conditions (B)

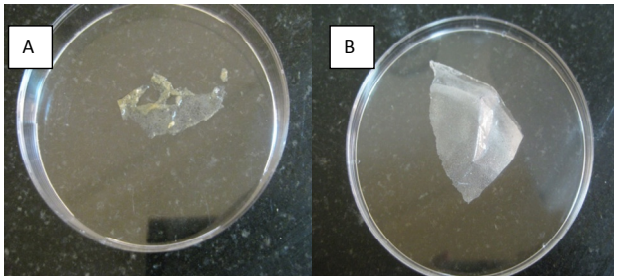

Fig. 12: PHB film on water agar sprayed with 30 µg of the pure enzyme after 7 days of incubation at 45°C (A) and control film incubated in the same conditions without enzyme (B)

medium and used to detect the PHB degradation by *Aspergillus fumigates* as a clear halo zone around the fungal colonies (Lodhi *et al*., 2011).This method is usually applied to screen microorganisms that can degrade a certain polymer in solid medium (Augusta *et al*., 1993; Nishida and Tokiwa, 1993; Abou-Zeid *et al.,* 2001). For bacteria, growth on agar medium is usually easier than in broth medium (Choi *et al.,* 2001), thus, no further assessment was conducted for isolates that could not grow or showed weakly activity on solid agar medium containing PHB as carbon source. The weak or lack of activity in some actinomycete isolates may be due to a loss of trait in the isolate or the used culture conditions was not effective in inducing the depolymerase enzyme. It is well known that depolymerase enzymes obtained by fungi are extracellular, whereas the enzymes from bacteria are either extracellular or intracellular. The extracellular depolymerase production of the isolate MM10 was selected for more detail studies.

The isolate MM10 was identified as *S. lydicus* using morphology, spore surface and physiological character (Shirling and Gottlieb, 1966; Aly *et al*., 2013b). Analysis of the cell wall indicated a wall of chemotype IV and the whole-cell sugar pattern was type A. The phospholipid pattern was type PII, and the fatty acids were pattern c. The isolate MM10 belongs to the genus *Streptomyces* (Pridham and Tresner, 1974; Williams *et al*., 1989) and was closely related to *S. lydicus.* Using16SrDNA, it was closely resample to *S. lydicus* by 93% and was identified as *S. lydicus* MM10.

Genus *Streptomyces* is one of the most famous actinomycete genera with beneficial roles inenzymes and antibiotics production (Ellaiah *et al*., 2004; Aly *et al.*, 2011b). The knowledge on the participation and role of actinomycetes in hydrolysis of PHB are extremely limited and need to be elucidated in further studies. This study is important in isolation of *S. lydicus* MM10, which produce excellent depolymerase enzyme in the culture supernatant after 2 days of growth on PHB. Although, this enzyme have a wide distribution in fungi and in some algal genera, bacterial depolymerase have considerable potential in commercial applications due to substrate specificity, resistance to proteolysis and catalytic efficiency (Shah *et al*., 2008). Extracellular PHB depolymerase has been isolated from different bacteria as *Alcaligenes faecalis*, *Rhodospirillum rubrum*, *B. megaterium, A. beijerinckii* and *Pseudomonas lemoignei*, purified and characterized (Mergaert *et al*., 1996; Panagiotidou *et al*., 2014).

Temperature is one of the most critical parameters to be controlled in any bioprocess. A number of mesophilic microbes have been found to be responsible for degrading PHB in soil and aquatic environments (Mergaert *et al*., 1994; Kim *et al*., 2000) and many thermotolerant strains are capable of degrading PHB at high temperatures ≥ 40°C from soil and compost (Mergaert *et al*., 1994; Kim *et al*., 2000). Therefore, the current study is performed for the isolation of PHB degrading bacteria at high temperature for production of thermostable depolymerase enzyme. Maximum depolymerase production by *S. lydicus* MM10 was at 45°C. Similarly, *Bacillus* strain TT96 (Tansengco and Tokiwa, 1998) and *Streptomyces* strain MG (Tokiwa and Calabia, 2004) were capable of degradation at higher temperatures. *A. fumigates* was able to degrade PHB better at 45°C after 24 h of incubation in liquid medium and little information on microbial degradation of PHB at high temperatures was available (Lodhi *et al*., 2011). There was a gradual decrease in production of enzyme after 24 h, where degradation in liquid media needless time. Similarly, Papaneophytou *et al.,* 2009) reported maximum enzyme production by *Thermus thermophilus* HB8 after 24 h of incubation. The gradual decrease in the production of enzyme after 24 h was as a result of utilization of substrate and other nutrients (Shivam *et al*., 2009). On contrast, *A. fumigates* M2A needed 150 h of incubation in liquid medium to degrade PHB by extracellular PHB depolymerase (Scherer *et al*., 1999).Moreover, maximum enzyme production by *S. lydicus* MM10 was using Turbid medium at pH7. Increasing pH more than 7 affected the charges on the amino acids within the enzyme active site. Similarly, maximum production of PHB depolymerase by *A, fumigates* was observed at pH 7 after 24 h of incubation in liquid medium (Lodhi *et al*., 2011). The maximum activity of extracellular PHB depolymerase produced by *Bacillus megaterium* N-18-25-9, was observed at pH 9.0 at 65ºC (Takaku *et al*., 2006) and at pH 7.5–8.0 when sewage sludge was used as inoculum (Briese *et al*., 1994). In the present study, the maximum production of PHB depolymerase was found at 0.3% of substrate concentration as indicated by the maximum enzyme activity, while it decreased with further increase in polymer concentration which might be due to substrate level inhibition (Manna and Paul, 2000). In case of *Arthrobacter* sp. Strain W6, the optimal concentration of PHB was 0.1% (Asano and Watanabi, 2001). Production of extracellular PHB depolymerase by *T. thermophilus* HB8 was reported after 24 h of incubation using 0.1% PHB (Papaneophytou *et al.*, 2009). In the current study, presence of glucose in the growth medium along with PHB enhanced activity of PHB depolymerases. On contrast, lactose enhanced PHB degradation by *A, fumigates* (Lodhi *et al*., 2011). According to Manna and Paul (2000), degradation of PHB by bacterial strains was affected significantly when the PHB containing medium was supplemented with easily consumable carbon sources. Glucose, fructose and arabinose supplementation lowered the extent of degradation (Manna and Paul, 2000). PHB depolymerase expression is repressed in the presence of a soluble carbon source that permits high growth rates. However, after exhaustion of the nutrients, the synthesis of PHB depolymerase is derepressed (Jendrossek and Handrick, 2002).

Using the best conditions for depolymerase production, the enzyme was collected and purified using concanavalin-A which is a lectin, binds specifically to various glycolipids. The MW was approximately 45 KDa, with maximum activity at 45°C and pH8. The activity was not significantly enhanced by most of the tested metal ions. The purified PHB depolymerase from *Streptomyces* 77T-4 cells has a molecular mass of approximately 40 kDa, which is in the size range of all bacterial and fungal PHB depolymerases (Calabia and Tokiwa, 2006). Generally, temperature and pH affect enzyme activity and thermal stability. Optimum temperature and pH for enzyme activity depend on strain type. The first PHB depolymerase from a gram-positive were from *S. ascomycinicus* and the enzyme was found to be a monomer with a molecular mass of 48.4 kDa, with the highest activity at 45°C and pH 6 and the activity was increased in the presence of divalent cations (García-Hidalgo *et al*., 2013). The thermophilic *Streptomyces* sp. MG has depolymerase enzyme with 41–43 kDa and the optimum pH and temperature were 8.5 and 60°C, respectively. Most PHA depolymerases were 40–50

kDa; with no affinity to anion exchanger materials and the optimum pH was 7.5–9.8 (Shah *et al.,* 2007). Inhibitors are indicative of the various functional groups present in the active site of an enzyme. Effect of inhibitors on the enzyme activity was investigated in order to identify the active sites in the PHB depolymerase activity.

PHB film was degraded in *Streptomyces* supernatant due to the presence of depolymerase enzyme while on solid medium degradation was observed using the purified depolymerase enzyme obtained from *Streptomyces*. Similar results were obtained by García-Hidalgo *et al*. (2013). Further researches are required for exploiting the interesting characters of depolymerase enzymes and their roles in PHB degradation.

Conclusion

Bioplastics are eco-friendly products, can be used instead of plastic from petrol that are toxic and pollute the environment. Bioplastics are usually produced from bacteria and are 100% biodegradable by soil and water microbes especially actinomycetes. Depolymerase enzyme is inducible bacterial enzyme, used for Bioplastics degradation. It was isolated, purified and characterized from *S. lydicus* MM10.

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