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Biodegradation of Polylactic Acid by Proteolytic and Lipolytic Bacteria

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ABSTRACT

Biodegradable plastics opened a new way for waste management strategies since these materials are intended to degrade under various conditions. Two complementary parts were conducted to study the ability of bacteria to degrade bioplastic (polylactic acid) (PLA) under *in vitro* conditions. The first part was to screen seven bacterial strains adopted on their ability to produce proteolytic and lipolytic enzymes. As well as, use lactic acid (LA) as a sole carbon source by estimate dehydrogenase activity (DHA), optical density (OD), dry weight (DW), consumed and residual LA of the medium. The second part was incubation of those strains into media supplemented with PLA films as a sole carbon source for two months. Changes in pH, accumulated LA and weight loss were estimated periodically every 15 days during two months. The tested seven bacterial strains showed high ability to produce protease and lipase and able to use LA as a sole carbon source. Concerning PLA biodegradation, results also showed that pH was gradually decreased from the initial period to reach their maximum after 60 days of incubation. Whereas, the accumulated LA and loss of PLA films weight were increased and the highest records were observed after 60 days in medium inoculated with *Serratia marcescens* (ZH5) followed by *Pseudomonas fluorescence* (ZH6). Optical and scanning electron microscopy were conducted to observe changes in PLA films. In this respect, results showed roughening of the outer surface, formation of holes and cracks in addition changes in color.

Key words: Bioplastic, PLA, Biodegradation, bacteria, proteolytic enzymes, lipolytic enzymes

Introduction

Polylactic acid (PLA) is the first biocompatible and biodegradable polymer produced from renewable resources and is commercially available. PLA has high elasticity modulus and stiffness, thermoplastic behavior, biocompatibility and good shaping and molding capability. These properties encourage the global attention for PLA and so it has been utilized successfully in surgical-implant materials, drug delivery systems, packaging materials and agricultural applications (Chaisu *et al*., 2012).

Most of these synthetic biodegradable polymers contain hydrolyzable linkages like ester along the polymer chain. The mechanism by which PLA polymers degrade depends on the biological processes by both microbial and their enzymatic activities (Prema and Uma, 2014). The biodegradation process by bacteria, fungi, yeasts and their enzymes leading to significant changes in the chemical structure. Biodegradable plastics should break down cleanly, in a defined period, to simple molecules found in the environment such as carbon dioxide and water but it is important to note that the degradation of polymer can rarely be complete because a small portion of the polymer will be incorporated into microbial biomass, humus and other natural products (Ghosh *et al*., 2013). Two categories of enzymes, extracellular and intracellular depolymerases, are involved in biological degradation of polymers (Gu *et al*., 2000 b). During degradation, the polymers are broken down to smaller/short chains (oligomers) by exoenzymes. These are small enough to pass through the semi-permeable outer bacterial membranes and then to be utilized as carbon and energy sources (Prema and Uma, 2014). When the product evolved is primarily the monomer, the process is called depolymerization. Whereas, the process is called mineralization when the products evolved are inorganic species like carbon dioxide, methane or water (Sivalingam *et al*., 2003). Many researches demonstrated that DL-lactic acid is one of the final intermediate products of PLA degradation, the other one is glycolic acid, which is known to behave similarly from a microbial viewpoint (Torres *et al*., 1996 and Li *et al*., 2008). The enzymatic degradation of low molecular weight PLA was by using esterase-type enzymes such as lipase (Prema and Uma, 2014) and proteases (Oda *et al*., 2000). Lipases are hydrolases and exert their activity on the carboxyl ester bonds of triacylglycerols and other substrates. Their natural substrates are

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insoluble lipid compounds prone to aggregation in aqueous solution. Proteolytic enzymes (proteases) catalyze the hydrolysis of peptide bonds and the related hydrolysis of an ester bond. Therefore, the objective of the current study was to screen bacterial strains for their ability to degrade PLA under *in vitro* conditions and use it as sole carbon and energy sources.

Materials and Methods

Bacterial strains:

Bacillus sp. (ZH1), *Bacillus* sp. (ZH2), *Bacillus licheniformis (*ZH3)*, Serratia marcescens* (ZH4), *Serratia marcescens* (ZH5), *Pseudomonas fluorescence* (ZH6) and *Pseudomonas fluorescence* (ZH7), were provided from Agricultural Botany Department, Agricultural Microbiology Branch, Faculty of Agriculture, Benha University, Moshtohor, Toukh, Qalyubia, Egypt.

Polyplastic (polylactic acid):

Bi-OPL® films which produced from polylactic acid (PLA is made of degradable materials and compostable in accordance with DIN EN 13432) were obtained from Oerlemans Plastics BV manufactor, UK, www.oerlemansplastics.nl (Mostafa and Abdelrahman, 2014).

Screening of examined bacterial strains:

Microbial enzymes activity:

Protease activity was estimated qualitatively on skim milk agar plates (Rupali, 2015). The plates were incubated at 30ºC±2 for 2 days. The protease producing bacteria were selected based on the zone of clearance and then subjected to quantitively assay using the modified method of Suganthi *et al*. (2013) as U/ml. One unit of protease activity was defined as the amount of enzyme required to release trichloroacetic acid-soluble casein fragments giving blue colour equivalent to 1.0µg of tyrosine per min.

Lipase activity was qualitatively detected using agar plate assay on nutrient agar medium supplemented with sterile tween 20 (1%) as lipase substrate, the formation of clear zone around the colony on the plate was considered as positive for lipolytic strains (Prasad, 2014). Then, quantitively assay of lipase was estimated as described by Lopes *et al*. (2011) as U/ml. One unit of lipase activity was defined as the amount of enzyme which liberated 1 µmol of fatty acids per minute.

Lactic acid as a sole carbon source:

The basal medium composed of (g/l): $MgSO_4.7H_2O$ (0.2); $CaCl.2H_2O$ (0.02); KH_2PO_4 (1.0); K_2HPO_4 (1.0); $(NH_4)_2SO_4$ (1.0); $FeCl_3$ (0.05) was used. Lactic acid (LA) (85% aqueous solution) was obtained from Sigma Chemical Company, Cairo, Egypt and used in this experiment. LA was added as a sole carbon source in culture medium as rate of 10 g/l before sterilization and the pH was adjusted to 7.0 ± 0.2 with 1*N* NaOH solution (Chomchoei *et al*., 2011). In addition, medium was supplemented with 0.1% Triphenyl tetrazolium chloride (TTC) and incubated with each strain of the tested bacteria. After incubation at 30ºC, the following determinations were estimated:

- Dehydrogenase activity (DHA) was estimated after 24 h of incubation as µg TPF/ml culture/24 h as described by Glathe' and Thalman (1970).
- After 7 days of incubation, bacterial growth was determined using spectrophotometer at 650 nm (OD_{650}) following centrifugation and washing of the cells. Then, the dry weight was determined as g/l (Coelho *et al*., 2011).
- The consumed and residual lactic acid (%) were measured by titration after 7 days of incubation using the following equation (ADMI, 1990).
-

$$\% \text{ LA} = \frac{ml \text{ x } N \text{ x } 90 \text{ x } 100}{V \text{ x } 1000}$$

Where, ml = ml 0.1 NaOH used, *N* = Normality of 0.1 N NaOH, *V* = ml bacterial filtrate used.

Biodegradation of PLA by the tested bacteria:

The experiment was conducted in 150 ml Erlenmeyer flasks containing 50 ml of basal medium supplemented with 1.0% (w/v) PLA (Difco) as a carbon source. Bacterial strains were cultured overnight on Tryptic Soy broth medium (TSB) (Difco) at 30°C, 180 rpm until each inoculum has a cell density of 10^6 CFU/ml (Chaisu *et al*., 2012). After that, each flask was inoculated with 5 ml of 24h bacterial inoculum then, incubated on a rotary shaker (180 rpm) at 30ºC for 60 days. The following determinations were estimated periodically every 15 days.

Determinations:

Periodically changes of pH in growth medium:

The pH of the liquid media was measured periodically every 15 days after inoculation with bacterial strains using a JSR digital pH meter.

LA (%) accumulation in growth media during incubation period:

Lactic acid accumulation was estimated periodically every 15 days in liquid medium supplemented with PLA films and inoculated with each bacterial strain using the equation as previously described (2.3.2.).

Weight loss of PLA films:

The loss of PLA weight (%) was determined using %WL equation (Chuensangjun *et al*., 2013).

$$\%\, Wl = \frac{Wo - Wt}{Wo} * 100$$

Where, *Wl* = Weight loss, *Wo* = initial weight (g), *Wt* = residual weight after incubation (g)

Microscopy of PLA films:

Optical microscopy:

At the end of experiment, PLA films were taken and examined the outer surface under image analyzer microscope (Carl Zeiss) to detect the degradation in each treatment compared with control (Phukon *et al*., 2011). This examination was carried out at Microbial Biotechnology and Fermentation Laboratory, Central Research Laboratories, Faculty of Agriculture, Benha University, Moshtohor, Toukh, Qalyubia, Egypt.

Scanning electron microscopy:

The PLA films obtained after 60 days of incubation with bacterial strains were coated by gold sputter coater (SPI-Module, USA). Then, observed by scanning electron microscope (Model:JSM-5500 LV; JEOL Ltd-Japan) by using high vacuum mode as described by Phukon *et al*. (2011). This examination was carried out at the Regional Center of Mycology and Biotechnology, Cairo, Egypt to examine the degradation in PLA films under all treatments.

Results and Discussion

Screening of examined bacterial strains:

Proteolytic and lipolytic enzymes activity:

Data in Table (1) indicated that all tested bacteria except *Ps. fluorescence* (ZH7) showed qualitative protease activity on nutrient medium plates supplemented with skim milk as a substrate for protease. Whereas, all bacterial strains except *Bacillus* sp. (ZH1) showed qualitative lipase activity on nutrient medium plates supplemented with tween 20 as a substrate for lipase. Regarding the quantitative assay of two enzymes, data in Table (1) indicated that all tested bacteria could produce protease and lipase with various quantities. Protease quantity ranged from 90-240 U/ml, the highest production was by *Serratia marcescens*

(ZH5) followed by Bacillus sp. (ZH2) and Bacillus licheniformis (ZH3). Also, data showed proved that lipase quantities ranged from 9.30-13.2 U/ml, the highest and lowest quantity were observed by Pseudomonas fluorescence (ZH6) and Bacillus sp. (ZH1), respectively.

Table 1: Qualitatively and quantitatively assays for protease and lipase

Bacterial strains	Protease activity		Lipase activity	
	Qualitative	Quantitative (U/ml)	Qualitative	Quantitative (U/ml)
Bacillus sp. (ZH1)	+	100	-	9.30
Bacillus sp. (ZH2)	+	210	+	12.1
Bacillus licheniformis (ZH3)	+	200	+	11.4
Serratia marcescens (ZH4)	+	100	+	10.7
Serratia marcescens (ZH5)	+	240	+	12.8
Pseudomonas fluorescence (ZH6)	+	190	+	13.2
Pseudomonas fluorescence (ZH7)	-	90	+	11.0

(-): negative (+): positive

These results were agreed with Prema and Uma (2013) who reported that many genera of bacteria such as *Bacillus, Bordetella and Pseudomonas* able to produce protease as PLA depolymerase. Also, Oda *et al*. (2000) stated that several enzymes viz. pronase, proteinase K and bromelain can degrade PLA polymer. In addition, they examined the enzymatic degradation of PLA using commercially proteases and found that acid and neutral proteases had a little or no effect on L-PLA degrading activity but some alkaline proteases derived from *Bacillus* sp. showed ratable PLA degrading ability. In addition, Tribedi *et al*. (2012) observed the efficacy of a new strain *Pseudomonas* sp. in the biodegradation of bioplastic and found that the degradation was mediated by esterase activity. Also, Gu *et al*. (2000 b) reported that two categories of enzymes extracellular and intracellular depolymerases are actively implicated in biodegradation of polymers. Moreover, Prema and Uma (2015) stated that the enzymes such as lipases cleave the ester bond of aliphatic polyesters viz. PLA. The activity of lipase is strongly dependent on the microorganism source. The relationship between esterases and PLA degradation has been reported for PLA depolymerase from *Bacillus sp.* (Shigeno *et al*., 2003). Also, Shah *et al*. (2008) reported that the ester linkages in PLA are sensitive to both chemical hydrolysis and enzymatic chain cleavage.

Lactic acid as a sole carbon source:

Regarding to study the ability of tested bacterial strains to use lactic acid (LA) as a sole carbon source, dehydrogenase activity (DHA) and bacterial growth (OD_{650}, dry weight) were estimated as an evidence for this ability. In this respect, data presented in Table (2) indicated that all examined bacterial strains gave DHA activities after 24h. The highest DHA was recorded in culture medium of *S. marcescens* (ZH5) followed by *Ps. fluorescence* (ZH6) then *Bacillus* sp. (ZH 2). Whereas, the lowest activity was observed in culture medium of *Ps. fluorescence* (ZH7). In addition to DHA, bacterial growth as optical density (OD) and dry weight was estimated as a guide for bacterial strains ability to assimilate LA. Data in Table (2) also showed that the highest OD was recorded by *S. marcescens* (ZH5) followed by *Ps. fluorescence* (ZH6) then *B. licheniformis* (ZH3) whereas, lower activities were recorded by other examined bacterial strains. Results also indicated that the highest and the lowest dry weight were observed in *S. marcescens* (ZH5) and (ZH6), respectively. Moreover, *Ps. fluorescence* (ZH6) and *Bacillus* sp. (ZH2) gave higher records of dry weight compared to other tested bacteria.

Table 2: Dehydrogenase and bacterial growth of the tested bacteria.

Bacterial strains	DHA as µg TPF/ml culture/24h	Bacterial growth	
		OD_{650}	Dry weight (mg/l)
Bacillus sp. (ZH1)	8.81	7.15	110.1
Bacillus sp. (ZH2)	9.97	14.8	110.6
Bacillus licheniformis (ZH3)	8.98	15.1	110.0
Serratia marcescens (ZH4)	8.58	8.82	109.6
Serratia marcescens (ZH5)	10.1	18.6	120.5
Pseudomonas fluorescence (ZH6)	9.99	15.5	110.8
Pseudomonas fluorescence (ZH7)	8.54	6.06	110.3

These results were confirmed by results of many researches who demonstrated that DL-lactic acid is one of the final intermediate products of PLA degradation, the other one is glycolic acid, which is known to

behave similarly from a microbial viewpoint (Torres *et al*., 1996 and Li *et al*., 2008). In addition, many microorganisms could use DL-LA as sole carbon and energy sources Torres *et al*. (1996). Also, Prema and Uma (2013) showed that the final biomass production was the highest in case of polylactic acid degradable microorganisms. The activities of microorganisms can be calculated by measuring the turbidity of solution (Matsuda *et al*., 2005).

In view of the ability of bacterial strains to consume LA in their culture media, results graphically illustrated by Fig (1) clearly indicated that there was variance between all examined strains in this ability. Also, results showed that *S. marcescens* (ZH5) could consume the highest amounts of LA and gave the lowest residual amounts of LA compared to all other strains and control (media without bacterial inoculum). In addition, the two strains *Ps. fluorescence* (ZH6) and (ZH7) gave approximately equal amounts of residual LA. This trend of results was true in the three strains belong to genus Bacillus. These results were in harmony with Prema and Uma (2013) who stated that final pH values were high with microorganisms able to assimilate LA type compounds to some extent. Whereas, pH values were lower in lactic acid oligomer-containing media confirming that the oligomers were transformed in LA.

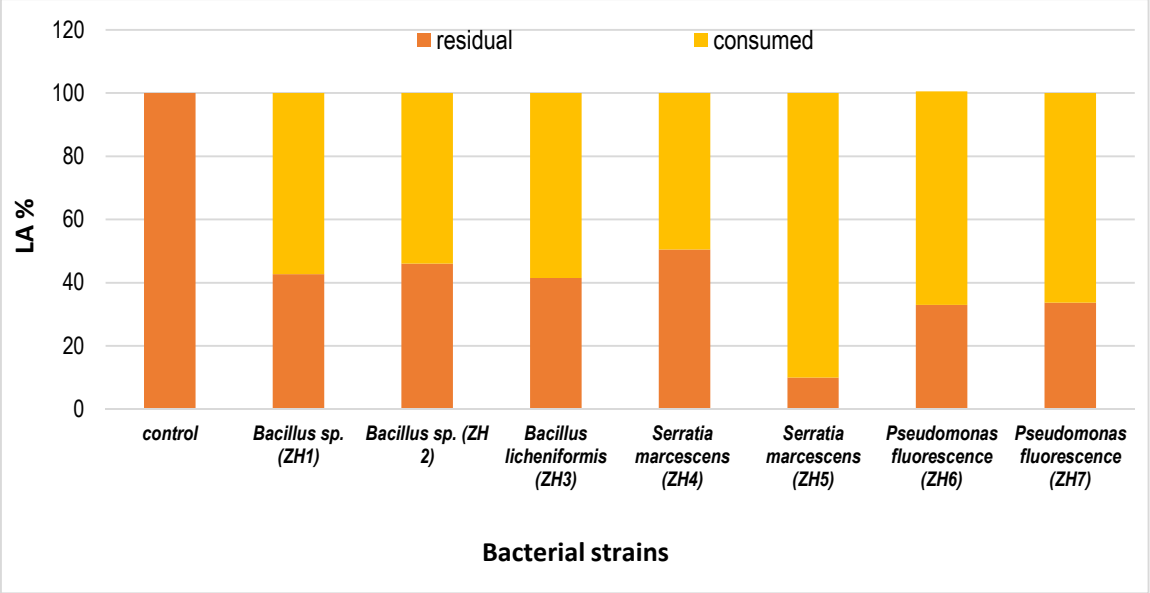


Fig. 1: The consumed and residual LA (%) by the examined bacterial strains in culture medium

Degradation of PLA films in growth medium during two months:

Periodically changes of pH

Data presented in Table (3) indicated that the pH degree by various strains were gradually decreased from the initial time to reach their maximum decrease at 60 days after inoculation. The obtained results also indicated that Bacillus sp. (ZH1) gave lower changes in pH during all determination periods, this result may be due to low protease and lipase activities compared to other bacteria (Table 1) which decrease the microbial activity in medium containing PAL. Data also indicated that pH was significantly decreased with the increasing of incubation period, moreover, the highest rate of pH decrease was recorded in medium inoculated with S. marcescens (ZH5), Ps fluorescence (ZH6) and Bacillus sp. (ZH2). This can be inferred as a result of their ability to produce protease and lipase enzymes which can degreed bioplastic (polylactic acid) and produce lactic acid.

This may be due to their abilities to produce enzymes such as protease Prema and Uma (2013) and lipase (Liu *et al*., 2000) as PLA depolymerases. These results were also in accordance with Chomchoei *et al*. (2011) and Ghosh *et al*. (2013) who reported that microorganisms have direct mechanism for bioplastic degradation and serves as a nutritive substance for the growth of these microorganisms. The produced lactic acid from bioplastic degradation could decrease pH of medium. Similar results by Prema and Uma (2013) who stated that pH values were lower in lactic acid oligomer-containing media confirming that the oligomers were transformed in LA. Also, Prema and Uma (2015) reported that the esterase activity markedly increased from the initial period and reached to maximum at the end of incubation period. Also, he concluded that depolymerase isolated from microorganisms was an esterase and can degrade PLA under neutral pH.

Table 3: Periodically changes of pH in growth medium during two months

Bacterial strains	pH			
	15	30	45	60
	Days after incubation			
Bacillus sp. (ZH1)	6.96[a]	6.70[f]	6.57[e]	6.19[a]
Bacillus sp. (ZH 2)	6.91[b]	6.88[a]	6.63[a]	6.01[d]
Bacillus licheniformis (ZH3)	6.97[a]	6.80[c]	6.67[c]	6.03[c]
Serratia marcescens (ZH4)	6.92[b]	6.83[b]	6.61[b]	6.10[bc]
Serratia marcescens (ZH5)	6.87[cd]	6.77[d]	6.51[d]	6.00[e]
Pseudomonas fluorescence (ZH6)	6.88[c]	6.73[e]	6.58[e]	6.01[d]
Pseudomonas fluorescence (ZH7)	6.89[c]	6.77[d]	6.65[cd]	6.11[b]

Initial pH was 7.0

Periodically changes in lactic acid accumulation in the medium:

Data in Table (4) showed that all examined strains have the ability to degrade polylactic acid polymer and produce lactic acid in their culture media. These results were confirmed by results of Torres *et al*. (1996) who demonstrated that DL-lactic acid is one of the final intermediate products of PLA degradation. Also, data showed that the produced LA amounts were low after 15 days after incubation, then increased with the increasing of incubation period. This may be due to that the produced amounts of LA were enough only to the growth without any accumulation. In this respect, Shah *et al*. (2008) reported that the primary product of the biodegradation process of bioplastic was microbial biomass. Results also showed that the highest LA amounts were accumulated in medium inoculated with *S. marcescens* (ZH5) after 15 and 60 days of inoculation, this inferred their high ability to produce degradable enzymes of polylactic acid. Whereas, the lowest accumulated amounts of LA were recorded after 60 days in medium inoculated with *Ps. fluorescence* (ZH7). Additionally, higher amounts of LA were recorded in media inoculated with *Bacillus* sp. (ZH 2) and *Ps. fluorescence* (ZH6).

Table 4: Periodically changes in lactic acid accumulation in growth media supplemented with PLA during incubation period

Bacterial strains	LA (%)			
	15	30	45	60
	Days after incubation			
Bacillus sp. (ZH1)	11.3[c]	11.1[f]	18.8[e]	24.0[e]
Bacillus sp. (ZH 2)	9.00[e]	14.8[cd]	22.2[a]	28.9[b]
Bacillus licheniformis (ZH3)	11.3[c]	19.1[b]	19.9[d]	24.7[d]
Serratia marcescens (ZH4)	11.5[b]	19.9[a]	21.2[b]	24.8[d]
Serratia marcescens (ZH5)	14.3[a]	12.2[e]	20.5[c]	29.7[a]
Pseudomonas fluorescence (ZH6)	11.4[b]	15.8[c]	21.2[b]	28.3[c]
Pseudomonas fluorescence (ZH7)	11.1[d]	13.2[d]	21.8[ab]	23.7[f]

Initial LA concentration was 0.0 %

These results were in accordance with Shah *et al*. (2008) who reported that the first stage of microbial PLA degradation (15 days) is via hydrolysis to water-soluble compounds and lactic acid. Also, Li e*t al*. (2008) reported that the degradation of bioplastic would be photo, thermal or biological and the changes include formation of new functional groups. Also, microorganisms such as bacteria and fungi are involved in the degradation of both natural and synthetic plastics (Gu *et al*., 2000a). Additionally, Artham and Doble (2008) indicated that because most polymers are too large to pass through the microbial cellular membranes, these polymers could be firstly depolymerized to smaller monomers by microbial enzymes, then these monomers must be absorbed into microbial cells and biodegraded.

Periodically loss of the PLA weight:

Data presented in Table (5) showed that the weight of PLA films was changed through the incubation period. The weight loss of PLA was periodically increased from the initial time to reach maximum at the end of incubation. This trend of results was observed in all examined strains. The mass loss of tested bioplastic films was widely applied especially in the defined synthetic medium (Witt *et al*., 2001). Data also revealed that no significant differences in loss of PLA weight were observed between *S. marcescens* (ZH5) and *Ps. fluorescence* (ZH6) after 15 days of incubation. Also, data showed that the highest loss of PLA films

in culture medium was recorded by *Ps. fluorescence* (ZH6) after 30 and 45 days after incubation. Whereas, the highest loss of PLA films after 60 days on incubation was observed by *S. marcescens* (ZH5) followed by *Ps. fluorescence* (ZH6). On contrast, at the end of incubation period (60 days) lowest loss of PLA films was observed by *Bacillus* sp. (ZH1). The degradation changes in the PLA film were determined by measuring the dry weight (Prema and Uma, 2013). Also, he reported that many genera of bacteria such as *Bacillus sp.* and *Pseudomonas sp.* have been involved in degradation of PLA. Also, Gu *et al*. (2000 b) reported that the microbial exoenzymes break down the polymers yielding monomers, that are smaller enough to inter through the bacterial membranes and then utilized as carbon and energy sources. In addition, Prema and Uma (2015) stated that the biological processes by both microbial and enzymatic activities are currently considered to be the most sustainable recycling methods for PLA degradation. Many researches proved that there were large number of microorganisms (especially bacteria and fungi) have the capacity to degrade these biopolymers by their exoenzymes in faster way (Shah *et al*., 2008 and Ghosh *et al*., 2013). Also, Li *et al*. (2008) reported that about 80% of PLA was degraded within 8 days according to the weight loss of the film.

Table 5: Periodically loss of the PLA weight during two months

Bacterial strains	Loss of PLA weight (%)			
	15	30	45	60
	Days after incubation			
Bacillus sp. (ZH1)	21.0^{cd}	39.4^{cd}	46.0^{e}	50.6^{e}
Bacillus sp. (ZH 2)	20.1^{d}	36.0^{d}	56.4^{b}	64.1^{b}
Bacillus licheniformis (ZH3)	22.2^{c}	31.5^{e}	48.8^{d}	51.3^{d}
Serratia marcescens (ZH4)	25.8^{b}	40.0^{c}	50.8^{c}	52.3^{c}
Serratia marcescens (ZH5)	30.0^{a}	44.2^{b}	55.0^{bc}	66.7^{a}
Pseudomonas fluorescence (ZH6)	30.2^{a}	49.8^{a}	61.2^{a}	64.0^{b}
Pseudomonas fluorescence (ZH7)	24.0^{bc}	29.9^{f}	46.1^{e}	52.1^{c}

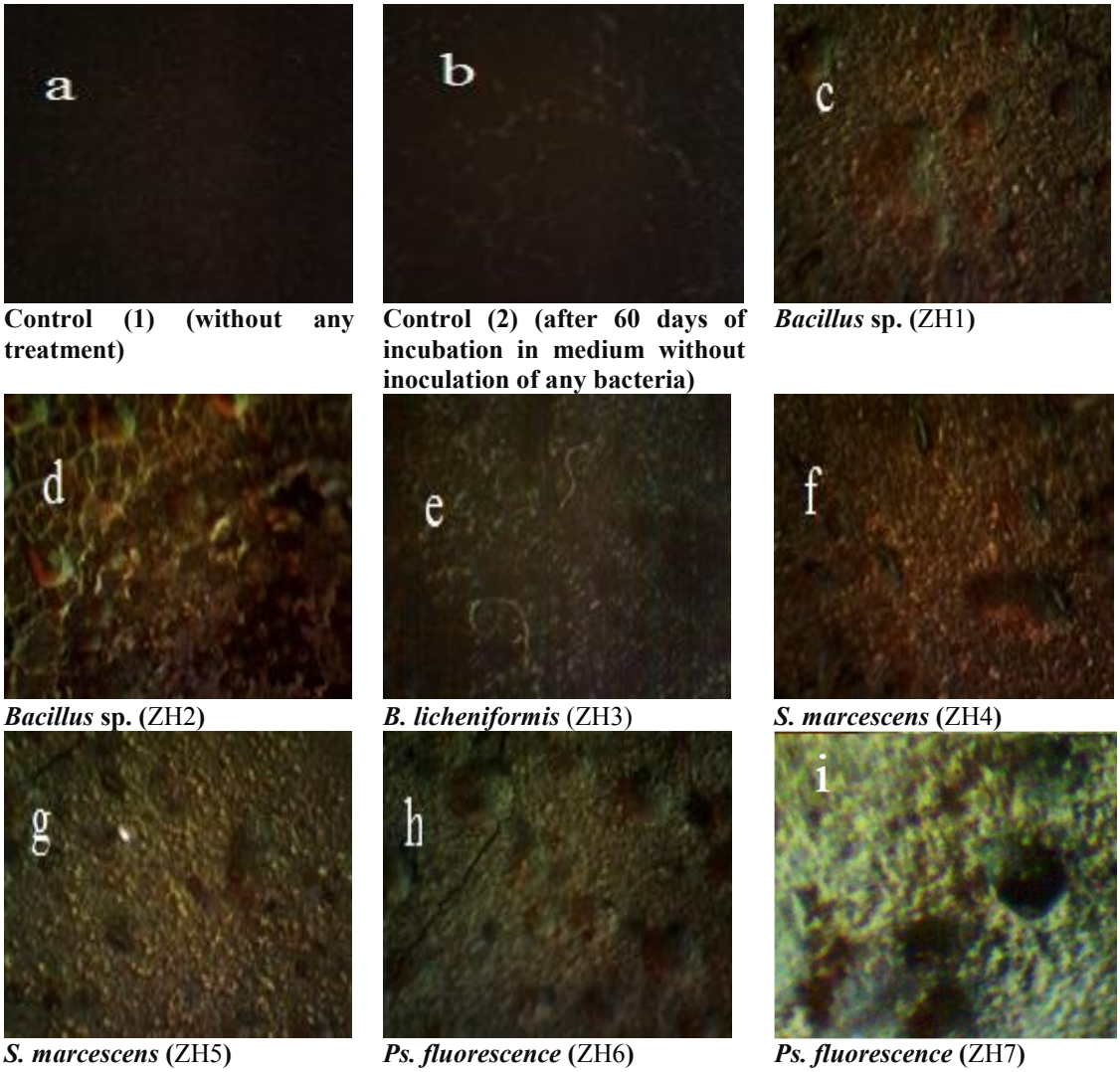
Microscopy:

Optical microscopy:

Photos (1 a – i) showed the changes in bioplastics films under optical microscope, it was clearly that all tested bacteria caused changes in the external surface compared to control. These changes included roughening of the surface, formation of holes or cracks and changes in color. These changes might prove the presence of a biodegradation by microorganisms (metabolism) and can be used as a first indication of the microbial attack (Shah *et al*., 2008). Also, he proved that PLA films become thinner because of the surface corrosion process by the biodegradation process, while the inner side of the films wasn't affected. Changes in PLA properties might be due to metabolism of the polymer material (Kikkawa *et al*., 2002).

Scanning electron microscope (SEM):

SEM was used to observe changes in PLA film after incubation with the tested bacteria for 60 days. The PLA films were recovered from the culture media to estimate the nature of the microbial growth and the surface damage done to PLA films. The film surface was smooth in the two control treatments without any treatment (Photo 1a) or after 60 days of incubation in medium without inoculation of any bacteria (Photo 1b). Whereas, the PLA film, incubated with all tested bacteria showed large number of pit holes and cracks all over the surface of the films and most of the film surface was attacked by bacterial cells (Photos 2 c-i). Also, the scanning electron micrographs of the incubated film showed evidences of degradation with surface roughening, grooves, cavities and disintegration. The microbial degradation rate of bioplastic films dependent on the degradation ability of the microorganisms colonizing the surface of incubated films (Sang *et al*., 2000).



Control (1) (without any treatment)

Control (2) (after 60 days of incubation in medium without inoculation of any bacteria)

***Bacillus* sp. (**ZH1**)**

***Bacillus* sp. (**ZH2**)**

B. licheniformis (ZH3)

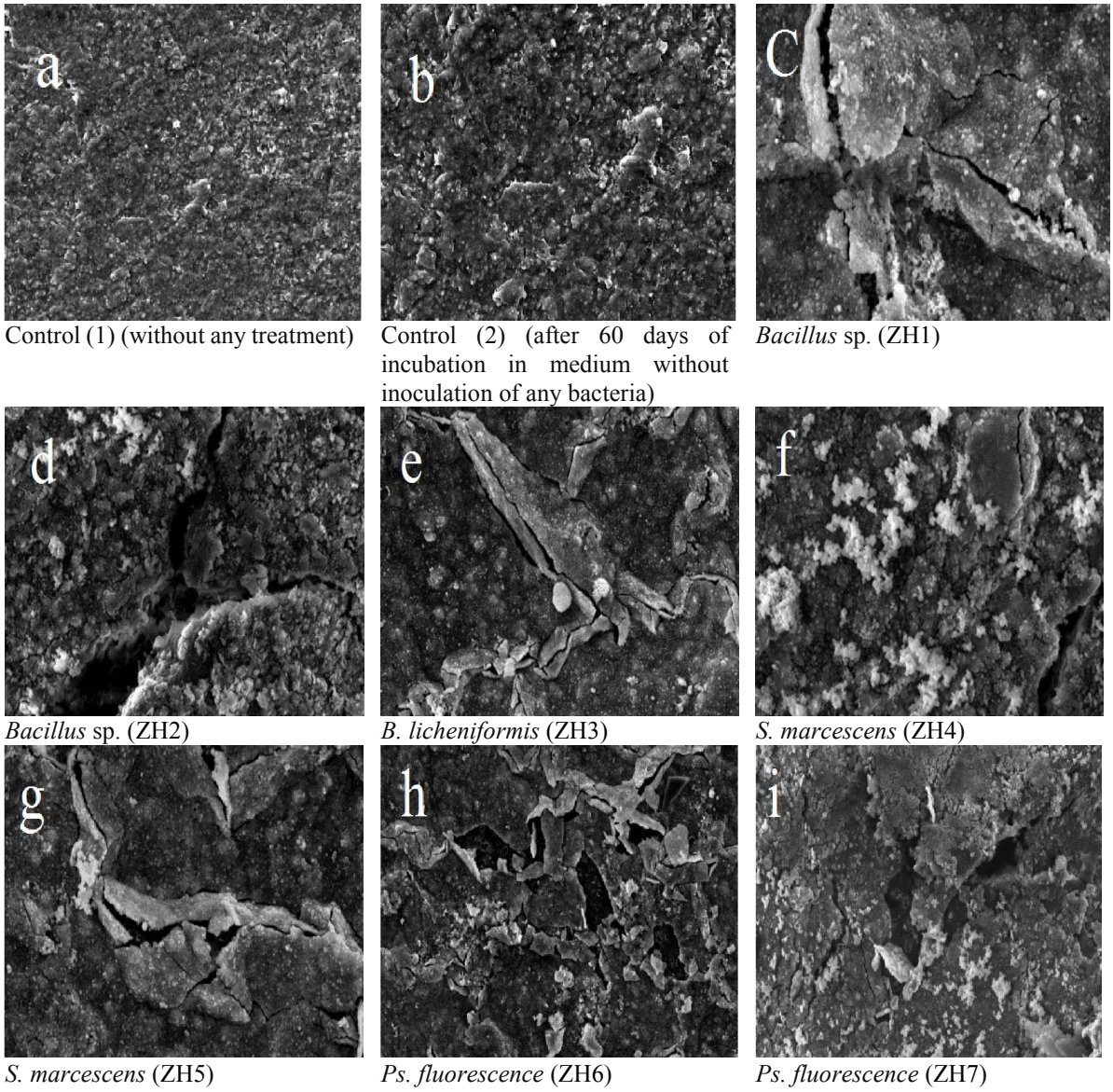
***S. marcescens* (**ZH4**)**

***S. marcescens* (**ZH5**)**

***Ps. fluorescence* (**ZH6**)**

***Ps. fluorescence* (**ZH7**)**

Photo 1: Changes observation in PLA films by optical microscopy under different treatments



Control (1) (without any treatment) | Control (2) (after 60 days of incubation in medium without inoculation of any bacteria) | *Bacillus* sp. (ZH1)

Bacillus sp. (ZH2) | *B. licheniformis* (ZH3) | *S. marcescens* (ZH4)

S. marcescens (ZH5) | *Ps. fluorescence* (ZH6) | *Ps. fluorescence* (ZH7)

Photo 2: Scanning electron micrograph (20 μm 2.000 X) of PLA film surface of control (a and b) and PLA film degraded by tested bacteria (c-i) after 60days of incubation.

Conclusion

From the current study, it can be concluded that seven tested bacteria were able to use bioplastic (polylactic acid) films as a sole carbon source in culture media during two months. PLA were significantly degraded by bacteria with protease and lipase as PLA depolymerases. The intermediate product of PLA degradation was lactic acid. Bacterial degradation of PLA films cause pit holes, cracks roughening, grooves, cavities and disintegration all over the surface of the films.

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