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Synthesis of polylactic acid polymer by using lipase isolated from *P. aeruginosa*

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ABSTRACT

The increasing of the lipases enzymatic activity is triggered by certain lipase rearrangements structure. The present study aimed to study the ability of purified lipase in the synthesis chemical compounds application. The results showed that the purified lipase isolated from *Pseudomonas aeruginosa* had the ability to achieve the synthesis of chemical compound such as polylactic acid by using the organic compound lactic acid monomer. FTIR spectroscopy used to characterize polymer PLA. The results showed that the absorption peaks of the PLA samples spectrum showed the bands are fully consistency with the corresponding absorption bands of the standard PLA spectrum. The results of the determination the number average molecular weight (Mn) of the polymerization product PLA were showed that the maximum molecular weight of the produced PLA was (4950 Da).

Keywords: *Pseudomonas aeruginosa*, FTIR, Polylactic acid polymer.

I. INTRODUCTION

Biocatalysts is the process that used enzymes to catalyze the biochemical reactions by conversion the substrates into products this can achieved by lowering activation energy of the reaction [1]. Lipases have distinct status among biocatalysts owning to their ability to catalyze a broad variety of reactions and an important group for biotechnologically relevant enzymes [2]. Lipases have many favorable properties as biocatalysts that make them appropriate for specific applications when they are compared with chemical catalysts, because of their broad substrate specificity and high region and/or stereo selective characteristics whereas chemical processes are typically more non-specific and the production of undesired products in the waste stream can be avoided [3-6].

Furthermore, lipases can react under mild temperature a nd pH conditions, thereby increasing the energy required to direct reactions at extreme temperatures and pressure s. The stability In organic solvents and a wide variety of temperature and pH are Certain factors for bacterial lipases biotechnological abil ity are To be involved without the assistance of cofactors [7, 8]. Lipases ware utilized in industry by growing bacteria producing lipase strains i n the medium containing a suitable substrate in particula r (in the food industry), so called "in situ application" Or the use of pure enzymes that are immobilized (Fine chemicals manufacture especially), of "ex situ application" [3, 9]. Recently, many uses for petroleum - based plastic materials and synthetic polymers which are non - degradable materials serve as waste materials have resulted in a severe environment pollution which effected widely the human life on earth [10].

Enzymatic polymerization in vitro catalyzed by an isolated enzyme by non-biosynthetic pathways. This line is proven to be effective in the natural polymers synthesis and has been extended to the synthesis of biodegradable polymers [11, 12]. One of the important polymers is Polylactic acid (PLA), it is biodegradable and bio

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absorbable polymer based on renewable resources [11, 13].

Polylactic acid have more attention as the green materials owing the process to get PLA which was derived from polymerization of lactic acid which obtain from renewable resources. additionally, there were some works to synthesize PLA by the bioprocess using an enzyme based catalysts as lipases instead of using chemical processes [13-16], However, PLA is real green material entirely and non-toxic is produce from lipase-catalyst polymerization resulted in low-molecular weight [17-19]. Due to the high activity and stability of produced lipase at different ranges of pH, temperature and different organic solvents, the present study aimed to achieve the synthesis reaction of poly lactic from lactic acid monomer.

II. METHODS AND MATERIAL

Lipase used in the reaction was produced by *P. aeruginosa* isolate as in [20]. The ability of purified lipase was examined during the contribution of produced lipase in the synthesis reactions.

The polymerization reaction (synthesis reaction) was performed according to [18]. The polymerization experiments include the of use 50mL - flask containing, 1-5 m mol of Lactic acid 85%, purified lipase sample by gel filtration and lyophilized in a fixed amount of 50mg/m mol of enzyme was added. Toluene solvent, 8mL and bidistilled water, 1% (v/v) was supplemented to the mixture. The mixture was kept at the selected temperature (55- 60)℃ under magnetic stirring for 96hrs. The produced polymer was purified according to [21].

Characterization the product of the polymerization reaction:

Determination the spectroscopy of the Fourier Transform Infrared (FTIR) of the polymerization product was assaying for recording transmission spectra in the range of 4000-400 cm^{-1} depending on [17]. On the other hand, determination of the number average molecular weight (Mn) of the polymerization product was determined by end-group analysis according to settled method [10].

III. RESULTS AND DISCUSSION

The synthesis reaction (polymerization reaction):

The figures (1) was shown the results of monomer lactic acid was polymerized to PLA in presence of produced purified lipase under the conditions of synthesis procedure. The results showed that the viscosity of reaction mixture was increased with the increasing of reaction time during the formation of polymer as a result of increasing the molecular weight of the polymer.

Fig 1: The synthesis reaction of PLA polymer.

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Determination of the Fourier Transform Infrared spectroscopy (FTIR) of the polymerization product:

The results in the figure (2) was showed the transmission spectra of FTIR spectroscopy of the produced polylactic acid sample, figure (3) the monomers Lactic acid (control) and figure (4) the standard PLA. All the spectra of the absorption peaks are compared, suggesting the PLA product being formed under the effect presence of lipase during the polymerization. The results of spectrum of the absorption peaks of the PLA samples showed the bands located at 2926, 2856 cm^{-1} of C-H from CH_3, 1743CM^{-1}→ C=O, C-O-C → 1220 ,1166 cm^{-1}, 3450 Cm^{-1} stretching of OH group are wholly consistent with the comparable absorption bands of the standard PLA spectrum.

The spectrum of polylactic acid polymer was shown the bands at 2926, 2856 $c\bar{m}^{1}$ from asymmetric and symmetric valance of C-H from CH_3, respectively. It's possible to observe a band shift associated to the C=O stretch in the spectrum of monomer lactic acid in 1728 $c\bar{m}^{1}$ to 1743 $c\bar{m}^{1}$ in the spectrum of polymers, these bands that show Shifts of monomers to polymers, also the results was showed a difference in the absorption peak intensity which suggests the arrangement of molecules in the polymer. Bands corresponding to the bending vibration of CH_3 (asymmetric and symmetric) found at 1462, 1529, 1546 $c\bar{m}^{1}$. C-O-C asymmetrical and symmetrical vibration found at 1220$c\bar{m}^{1}$ and 1166$c\bar{m}^{1}$. Finally the band around

3450 cm̄¹ is associated to the stretching of (OH) group and this decrease from the monomer compound to the polymer as result to polyesterification reaction was formed the ester bond that consumers the (OH) groups when they react with the acid groups.

The increasing of the lipases enzymatic activity is triggered by certain rearrangements of structural of the lipase active-site position, this characteristic feature is supported by the lipases crystal structures, with the transition state of structural analogs, and it is contributed by the residues of amino acid of the lipase itself composition. The results in the present study agree with study of [22-24] and the study of [25] whose showed that lipases are characterized by wide substrate specificity and regiospecificity, thus lipolytic enzymes could be used in hydrolysis and the synthesis in many fields of industry. The results in the present study agree with [26] and [15, 27]. The study of [17, 28], whose showed that it has particular interest especially as PLA synthesized by a green route using lipases and the polymerization of PLA using lipase as catalyst affects several reaction conditions include, kind of reaction, lipase natured, reaction time and type of solvent. Most of lipases express higher catalytic activity under such conditions than in aqueous solutions with the dissolve substrate molecules, by the so called interfacial activation phenomenon [29, 30]. When lipases act in the existence of organic solvents (although in sometimes the reaction with a small requirement of water) they have the ability to perform the reverse reactions as ester synthesis, "Esterification" or the exchange of acyl-group "Transesterification", is categorized into classes according to the chemical species which react with the ester.

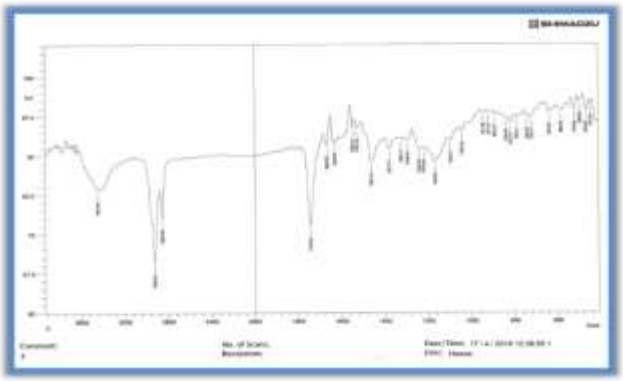

Fig 2: FTIR spectrum of PLA produced by reaction lipase - catalyzed.

Determination of the number average molecular weight (Mn) of the polymerization product:

The experiment of measurement Molecular weight by determination the number average (Mn) of the polymerization product PLA was resulted that the maximum molecular weight of the produced PLA was (4950 Da), The present study suggested that a unique thermal treatment was employed successfully at (55-60)˚ C for 96hrs and other reaction conditions was enhanced the molecular weight increasing of PLA.

The results in the present study agree with the study of [26], who suggested that the produced PLA polymer possibly will be gotten through polymerization by using of commercial lipase Lipozyme catalyst under reaction 50˚C, has molecular weight in the range of 2600-4500Da, They reported that the rate of synthesis of PLA was depended on temperature had suggested 50℃ to be an optimal temperature for the commercial lipase was used in this work.

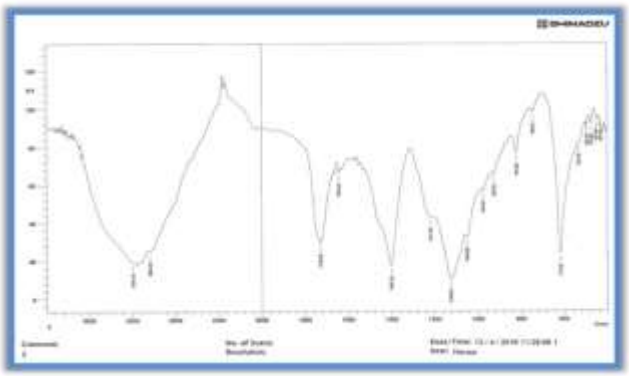

Fig 3: FTIR spectrum of Lactic acid monomer.

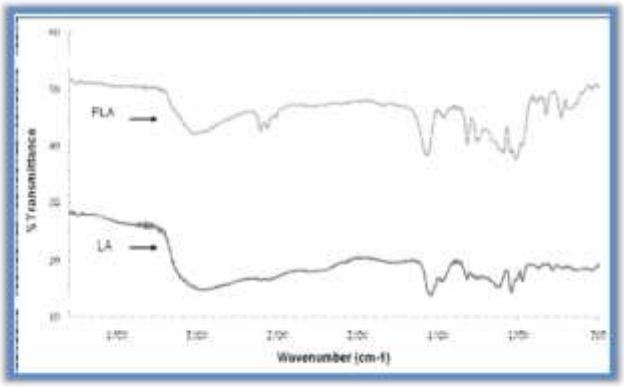

Fig 4: FTIR spectra of Standard Lactic acid monomer (LA) and Polylactic acid (PLA).

IV. CONCLUSION

The present study proved that extracted lipase from culture medium of *Pseudomonas aeruginosa* had request characteristic of significant industrial importance. The enzyme exhibited maximum stability of high temperature

range and different organic solvents, so it can be used in various chemical synthesis reaction especially the biodegradable polymer such as polylactic acid.

V. REFERENCES

1) Cai, Q., Hu, C., Yang, N., Wang, Q., Wang, J., Pan, H., Hu, Y., & Ruan, C. (2018). Enhanced activity and stability of industrial lipases immobilized onto spherelike bacterial cellulose. *International journal of biological macromolecules*, *109*, 1174–1181. https://doi.org/10.1016/j.ijbiomac.2017.11.100.
2) Hostacká A. (2000). Lipase activity of Acinetobacter baumannii after treatment with meropenem. *Arzneimittel-Forschung*, *50*(12), 1134–1137. https://doi.org/10.1055/s-0031-1300335.
3) Pandey, A., Benjamin, S., Soccol, C. R., Nigam, P., Krieger, N., & Soccol, V. T. (1999). The realm of microbial lipases in biotechnology. *Biotechnology and applied biochemistry*, *29*(2), 119–131.
4) Chandra, P., Enespa, Singh, R., & Arora, P. K. (2020). Microbial lipases and their industrial applications: a comprehensive review. *Microbial cell factories*, *19*(1), 169. https://doi.org/10.1186/s12934-020-01428-8.
5) Fierascu, R. C., Fierascu, I., Avramescu, S. M., & Sieniawska, E. (2019). Recovery of Natural Antioxidants from Agro-Industrial Side Streams through Advanced Extraction Techniques. *Molecules (Basel, Switzerland)*, *24*(23), 4212. https://doi.org/10.3390/molecules24234212.
6) Ramnath, L., Sithole, B., & Govinden, R. (2017). Classification of lipolytic enzymes and their biotechnological applications in the pulping industry. *Canadian journal of microbiology*, *63*(3), 179–192. https://doi.org/10.1139/cjm-2016-0447.
7) Jaeger, K. E., & Reetz, M. T. (1998). Microbial lipases form versatile tools for biotechnology. *Trends in biotechnology*, *16*(9), 396–403. https://doi.org/10.1016/s0167-7799(98)01195-0.
8) Khan, M. T., Kaushik, A. C., Rana, Q. U. A., Malik, S. I., Khan, A. S., Wei, D. Q., Sajjad, W., Ahmad, S., Ali, S., Ameenullah, & Irfan, M. (2020). Characterization and synthetic biology of lipase from Bacillus amyloliquefaciens strain. *Archives of microbiology*, *202*(6), 1497–1506. https://doi.org/10.1007/s00203-020-01869-0.
9) Manni, A., & Filali-Maltouf, A. (2022). Diversity and bioprospecting for industrial hydrolytic enzymes of microbial communities isolated from deserted areas of south-east Morocco. *AIMS microbiology*, *8*(1), 5–25. https://doi.org/10.3934/microbiol.2022002.
10) Peñas, M. I., Beloqui, A., Martínez de Ilarduya, A., Suttiruengwong, S., Hernández, R., & Müller, A. J. (2024). Enzymatic Degradation Behavior of Self-Degradable Lipase-Embedded Aliphatic and Aromatic Polyesters and Their Blends. *Biomacromolecules*, *25*(7), 4030–4045. https://doi.org/10.1021/acs.biomac.4c00161.
11) Zhou, M., Wan, G., Wang, G., Wieme, T., Edeleva, M., Cardon, L., & D'hooge, D. R. (2023). Carbon Nitride Grafting Modification of Poly(lactic acid) to Maximize UV Protection and Mechanical Properties for Packaging Applications. *ACS applied materials & interfaces*, *15*(38), 45300–45314. https://doi.org/10.1021/acsami.3c10085.
12) Singhvi, M. S., Zinjarde, S. S., & Gokhale, D. V. (2019). Polylactic acid: synthesis and biomedical applications. *Journal of applied microbiology*, *127*(6), 1612–1626. https://doi.org/10.1111/jam.14290.
13) Jeon, B. W., Lee, J., Kim, H. S., Cho, D. H., Lee, H., Chang, R., & Kim, Y. H. (2013). Lipase-catalyzed enantioselective synthesis of (R,R)-lactide from alkyl lactate to produce PDLA (poly D-lactic acid) and stereocomplex PLA (poly lactic acid). *Journal of biotechnology*, *168*(2), 201–207. https://doi.org/10.1016/j.jbiotec.2013.06.021.
14) Borkar, P. S., Bodade, R. G., Rao, S. R., & Khobragade, C. N. (2009). Purification and characterization of extracellular lipase from a new strain: Pseudomonas aeruginosa SRT 9. *Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]*, *40*(2), 358–366. https://doi.org/10.1590/S1517-838220090002000028.
15) Zhang, L., Huang, C., Xu, Y., Huang, H., Zhao, H., Wang, J., & Wang, S. (2020). Synthesis and characterization of antibacterial polylactic acid film incorporated with cinnamaldehyde inclusions for fruit packaging. *International journal of biological macromolecules*, *164*, 4547–4555. https://doi.org/10.1016/j.ijbiomac.2020.09.065.
16) Abdul Manan, F. M., Attan, N., Widodo, N., Aboul-Enein, H. Y., & Wahab, R. A. (2018). Rhizomucor miehei lipase immobilized on reinforced chitosan-chitin nanowhiskers support for synthesis of eugenyl benzoate. *Preparative biochemistry & biotechnology*, *48*(1), 92–102. https://doi.org/10.1080/10826068.2017.1405021.
17) Takahashi, Y., Okajima, S., Toshima, K., & Matsumura, S. (2004). Lipase-catalyzed transformation of poly(lactic acid) into cyclic oligomers. *Macromolecular bioscience*, *4*(3), 346–353. https://doi.org/10.1002/mabi.200300094.
18) Wang, S. L., Lin, Y. T., Liang, T. W., Chio, S. H., Ming, L. J., & Wu, P. C. (2009). Purification and characterization of extracellular lipases from Pseudomonas monteilii TKU009 by the use of soybeans as the substrate. *Journal of industrial microbiology & biotechnology*, *36*(1), 65–73. https://doi.org/10.1007/s10295-008-0473-z.
19) Singh, R. K., Zhang, Y. W., Nguyen, N. P., Jeya, M., & Lee, J. K. (2011). Covalent immobilization of β-1,4-glucosidase from Agaricus arvensis onto functionalized silicon oxide nanoparticles. *Applied microbiology and biotechnology*, *89*(2), 337–344. https://doi.org/10.1007/s00253-010-2768-z.
20) Sachan, S., Iqbal, M. S., & Singh, A. (2018). Extracellular lipase from Pseudomonas aeruginosa JCM5962(T): Isolation, identification, and characterization. *International microbiology : the official journal of the Spanish Society for Microbiology*, *21*(4), 197–205. https://doi.org/10.1007/s10123-018-0016-z.
21) Feng, Y., Ma, P., Xu, P., Wang, R., Dong, W., Chen, M., & Joziasse, C. (2018). The crystallization behavior of poly(lactic acid) with different types of nucleating agents. *International journal of biological*

macromolecules, *106*, 955–962. https://doi.org/10.1016/j.ijbiomac.2017.08.095.

22) Lo, M., Taylor, C., Wang, L., Nowack, L., Wang, T. W., & Thompson, J. (2004). Characterization of an ultraviolet B-induced lipase in Arabidopsis. *Plant physiology*, *135*(2), 947–958. https://doi.org/10.1104/pp.103.036376.
23) Akoh, C. C., Chang, S. W., Lee, G. C., & Shaw, J. F. (2007). Enzymatic approach to biodiesel production. *Journal of agricultural and food chemistry*, *55*(22), 8995–9005. https://doi.org/10.1021/jf071724y.
24) Straathof A. J. (2003). Enzymatic catalysis via liquid-liquid interfaces. *Biotechnology and bioengineering*, *83*(4), 371–375. https://doi.org/10.1002/bit.10688.
25) Masi, C., Tebiso, A., & Selva Kumar, K. V. (2023). Isolation and characterization of potential multiple extracellular enzyme-producing bacteria from waste dumping area in Addis Ababa. *Heliyon*, *9*(2), e12645. https://doi.org/10.1016/j.heliyon.2022.e12645.
26) DelRe, C., Jiang, Y., Kang, P., Kwon, J., Hall, A., Jayapurna, I., Ruan, Z., Ma, L., Zolkin, K., Li, T., Scown, C. D., Ritchie, R. O., Russell, T. P., & Xu, T. (2021). Near-complete depolymerization of polyesters with nano-dispersed enzymes. *Nature*, *592*(7855), 558–563. https://doi.org/10.1038/s41586-021-03408-3.
27) Lan, D., Wang, Q., Xu, J., Zhou, P., Yang, B., & Wang, Y. (2015). Residue Asn277 affects the stability and substrate specificity of the SMG1 lipase from Malassezia globosa. *International journal of molecular sciences*, *16*(4), 7273–7288. https://doi.org/10.3390/ijms16047273.
28) Rodney, R. L., Allinson, B. T., Beckman, E. J., & Russell, A. J. (1999). Enzyme-catalyzed polycondensation reactions for the synthesis of aromatic polycarbonates and polyesters. *Biotechnology and bioengineering*, *65*(4), 485–489.
29) Adlercreutz P. (2013). Immobilisation and application of lipases in organic media. *Chemical Society reviews*, *42*(15), 6406–6436. https://doi.org/10.1039/c3cs35446f.
30) Khan, F. I., Lan, D., Durrani, R., Huan, W., Zhao, Z., & Wang, Y. (2017). The Lid Domain in Lipases: Structural and Functional Determinant of Enzymatic Properties. *Frontiers in bioengineering and biotechnology*, *5*, 16. https://doi.org/10.3389/fbioe.2017.00016.