Immobilized lipase from *Lactobacillus plantarum* in meat degradation and synthesis of flavor esters

Sita Ramyasree Uppada\(^a\), Mahesh Akula\(^b\), Anupam Bhattacharya\(^b\), Jayati Ray Dutta\(^a,\ast\)

\(^a\) Department of Biological Sciences, BITS Pilani, Hyderabad Campus, Jawahar Nagar, Shameerpet Mandal, Hyderabad 500078, Telangana, India  
\(^b\) Department of Chemistry, BITS Pilani, Hyderabad Campus, Jawahar Nagar, Shameerpet Mandal, Hyderabad 500078, Telangana, India

**ABSTRACT**

Microbial lipases owing to their broad substrate specificity are widely used in various industrial applications like food processing, organic synthesis, detergent formulation and oil manufacturing. In the current study the immobilized lipase from *Lactobacillus plantarum* was found novel in degrading meat which can be applied in medical field and also in synthesizing different short chain fatty acid esters like 2,3,4-hydroxybenzyl acetates and triazole ester which makes a great impingement in natural flavor industry. The 4-hydroxybenzyl acetate obtained can also be used in cosmetics.

**Keywords:**  
*Lactobacillus plantarum*  
Lipase  
Esterification  
Flavor esters

1. Introduction

Lactic acid bacteria gained prominence in recent times due to their potential to produce probiotics. They are gram positive belonging to phylum Firmicutes [1]. They are widely used in fermentation and food industry [2]. Different industrial products like acetic acid, lactic acid, bacteriocins, enzymes, aroma compounds and ethanol that are produced by these bacteria are advantageous and generally regarded as safe [3]. The ubiquitous appearance of them in food, their potential application in production of different industrial products [4] and their contribution to healthy microflora in human and application in bio-medical treatment made them to study and exploit more. Though several lactic acid bacteria have been exploited for production of enzymes [5–7] yet a lot remains to be explored.

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are one of the largest groups of industrial enzymes which find use in diverse range of industries like detergents, pharmaceuticals, beverages, cosmetics, dairy, degreasing formulations, paper and biofuel [8]. Microbes serve as an excellent source of lipases compared to plant, animal and human because of their rapid growth, limited space for cultivation, withstanding various temperatures and easy genetic manipulation to generate high yields desirable for various applications [9].

2. Materials and methods

2.1. Microorganisms

*L. plantarum* (MTCC 4461) was used as source of lipase.

2.2. Immobilization using sodium alginate method

Immobilization of lipase was done using 3% sodium alginate suspension. The effect of different parameters like pH and temperature on enzyme activity was studied. The effect of pH on the activity of immobilized lipase was determined by incubating the enzyme in different buffers with pH 5–5.5 (acetate); pH 6–7 (phosphate) and pH 8–8.5 (Tris-HCl) at 4 °C for 24 h. Similarly the effect of temperature was determined by incubating the enzyme at different temperatures ranging from 25 to 50 °C in Tris-HCl buffer with pH 8.0 at an interval of 5 °C and the kinetic parameters V\(\text{max}\) and K\(\text{m}\) were also determined from Lineweaver-Burk plots [10].

2.3. Application of immobilized lipase in fat degradation

Generally lipases catalyze degradation of fat, oil and grease [11–13]. This property made them use in treating lipid containing environment. Hence 10 g of adipose tissue (fat) from chicken was weighed and autoclaved. 0.5 ml of enzyme was added to the tissue
and incubated at 37 °C. A control was also kept where no enzyme was added and for every 24 h, weight was taken to check the meat degradation process.

2.4. Application of lipase in ester synthesis

Short chain fatty acid esters have high importance in food industry. For this study esterification reaction was carried out in glass vials containing the solvent tetrahydrofuran (THF) varying from (500–1000 μl) in vinyl acetate (varying from 100 to 500 μl) with different substrates (ranging from 50 to 200 mg) like 2-hydroxybenzylalcohol (2-HB), 3-hydroxybenzyl alcohol (3-HB), 4-hydroxybenzylalcohol and glutotriazole (GTRI). Reaction was initiated by addition of immobilized L. plantarum lipase (ranging from 50 to 200 mg). Samples were placed at different time intervals (varying from 24 to 72 h) in an orbital shaker at different temperatures (4 °C, 37 °C, 60 °C) and rpm (100–300) along with the respective controls without immobilized lipase. Solvents were dried using standard methods and distilled before use. Visualization on TLC was achieved by use of UV light (at 254 nm). Column chromatography was performed for purification on silica gel (100–200 mesh, SRL, India) using ethyl acetate and hexane as eluent. The samples were analysed by IR (infrared spectroscopy) and NMR (Nuclear magnetic resonance spectroscopy).

\[ ^1H \text{NMR (300 MHz and 400 MHz) and } ^13C \text{ (75 MHz and 100 MHz) spectra were recorded in CDCl}_3 \text{ and DMSO-}d_6 \text{ solution with TMS as internal standard. IR spectra were recorded on KBr plates on Jasco FT/IR - 4200 instrument.} \]

3. Results and discussion

3.1. Application of enzyme in meat degradation

The immobilized enzyme was found to be active at pH 6.5 and at temperature 45 °C. The \( V_{\text{max}} \) and \( K_m \) values of the enzyme were 1.47 μmol/mg/min and 0.37 mM respectively [10]. The immobilized enzyme was applied in meat degradation and esterification reactions.

Degradation of fat is an important property of lipases. From the literature it is evident that the lipase produced from Lactobacillus sp. had the property to degrade meat in 72 h [14]. Similarly the proteolytic activity of lipase towards meat proteins in sausage system was studied and L. plantarum showed degradation of both sarcoplasmic and myofibrillar proteins in 96 h [15]. Lipase was also found to be a target for amelioration of oil pollution. Lee et al. found that the enzymes like lipases and proteases isolated from Bacillus sp. can solve environmental issues [16]. Modification of food and oil is an important aspect in food processing industry. Lipases alter the location of fatty acid chains in the glyceride and replace one with new ones, thus making a less desirable lipid into a higher value fat [17]. In this study, degradation property of lipase was studied and complete degradation of meat was observed at 72 h for L. plantarum as shown in Table 1 with strong smell and frothing. From the above results it is evident this enzyme can be applied in removing fats in medical field and also degrading lipid containing waste water preventing water pollution.

3.2. Application of lipase in ester synthesis

Esterification reactions catalysed by lipases present challenges, which if dealt successfully can result in number of compounds. In the present study, after optimization the short chain fatty acid esters were obtained with 900 μl of the solvent tetrahydrofuran
3.5. 4-Hydroxybenzyl acetate (4-HBA)

Yield 70%, colorless liquid, TLC R\(_f\) = 0.4 (EtOAc: n-Hexane, 5:5); \(^1\)H NMR (300 MHz, CDCl\(_3\) \(\delta\) 2.11 (CH\(_3\), s, 3H), 5.05 (CH\(_2\), s, 2H), 6.86 (m, 2H), 7.37–7.17 (m, 2H); \(^13\)C NMR (75 MHz, CDCl\(_3\) \(\delta\) 21.18, 66.53, 115.50, 127.63, 130.41, 156.12, 171.92; IR Wavenumber [cm\(^{-1}\)] 3401.82, 2924.52, 1793.47, 1706.69, 1593.88, 1503.24, 1455.03, 1377.89, 1256.4, 1093.57, 1039.44, 928.557, 822.491, 754.031, 609.396, 528.4, 434.869.

In addition to 2,3,4-hydroxyl benzyl alcohol, esterification reaction was carried out with triazole. Triazole is an interesting compound with three 2 hydroxy and one primary hydroxyl groups. But the disadvantage with this substrate is lack of solubility in solvents. Addition of excess vinyl acetate or enzyme did not show complete conversion.

3.5.1. 2-Phenyl-4-(D-arabino-4'-acetoxyl-1',2',3'-trihydroxybutyl)-2H-1,2,3-triazole (GTRIA)

Yield 16.66%, White solid, TLC R\(_f\) = 0.3 (EtOAc); \(^1\)H NMR (600 MHz, DMSO-\(d_6\) \(\delta\) 2.03 (s, 3H), 3.56–3.53 (m, 1H), 3.85–3.81 (m, 1H), 7.97 (s, 1H), 5.37 (d, \(J = 7.0\) Hz, 1H), 4.85 (d, \(J = 7.9\) Hz, 1H), 7.40 (t, \(J = 7.4\) Hz, 1H), 7.57–7.55 (m, 2H), 7.99 (d, \(J = 7.7\) Hz, 2H), 4.27 (dd, \(J = 11.3, 2.5\) Hz, 1H), 4.00 (dd, \(J = 11.3, 6.5\) Hz, 1H), 5.11 (dd, \(J = 7.1, 2.0\) Hz, 1H), 5.09 (d, \(J = 6.2\) Hz, 1H); \(^13\)C NMR (151 MHz, DMSO-\(d_6\) \(\delta\) 21.33, 65.65, 66.97, 68.50, 74.30, 118.56, 127.80, 130.14, 135.80, 139.78, 153.48, 171.03; IR Wavenumber [cm\(^{-1}\)] 3392.17, 2955.38, 2916.81, 2848.35, 1734.66, 1734.66, 1519.63, 1472.38, 1462.74, 1365.33, 1231.33, 1024.98, 728.961, 719.318, 644.20.406.

4. Conclusion

The present study is focused on the catalytic property or esterification efficacy of the immobilized lipase from \(L.\) \(p\)lantarum\ in synthesizing different industrial products. The main objective of the study is to explore the efficacy of bacterial lipases derived from \(Lactobacillus\) class of probiotics, which are healthy microflora of human mucosal surfaces, towards meat degradation and ester synthesis. Due to their avirulent nature, they do not cause any secondary pollution and health hazard. \(Lactobacillus\) \(p\)lantarum has ability to adapt to different environments and substrates and it is highly versatile lactic acid bacterial strain [18]. Lipase produced from such versatile strain can be useful in bio-medical field and pollution control. The esterification reactions performed with the immobilized lipase from \(L.\) \(p\)lantarum was novel in synthesizing different short chain fatty acid esters which find use as flavoring agents in food industry. The 4-hydroxybenzyl acetate obtained as an esterification product may also be used in cosmetics as anti-tanning agent [19]. This signifies that the non-pathogenic class of lipases can be engineered towards efficient ester synthesis and meat degradation.

Acknowledgement

We acknowledge BITS-Pilani Hyderabad Campus for providing all the facilities for the present work.

Conflict of interest

The authors declare that there is no conflict of interest.

References

[1] Yusuf MA. IOSR J Pharm 2013;3:44–50.
[2] Rattanachaikunsopon P, Phumkhachorn P. Ann Biol Res 2010;4:218–28.
[3] Yang E, Fan L, Jiang Y, Doucette C, Fillmore S. AMB Express 2012;2:48.
[4] Kannmhi M, Viji N. Int J Pharm Sci Rev Res 2014;29:183–6.
[5] Patel A, Shah N, Prajapati JB. Croat J Food Sci Technol 2013;5:85–91.
[6] Zareian M, Ebrahimpour A, Abu Bakar F, Mohamed AKS, Forghani B, Ab-Kadir MSB, et al. Int J Mol Sci 2012;13:5482–97.
[7] Gurung N, Ray S, Bose S, Rai V. Biomed Res Int 2013;2013:6.
[8] Ray A. Asian J Pharm Technol 2012;2:33–7.
[9] Anbu P, Subash C, Gopinath B, Cihan AC, Chaulagain BP. Biomed Res Int 2013;2013:2.
[10] Ramyasree S, Dutta JR. Int J Chem Technol Res 2015;8:680–5.
[11] Rocha D, Gomes BM, Gomes SD, Dilcemara LS, Zenatti C. Eng Agríc 2013;33:332–40.
[12] Subash C, Gopinath B, Anbu P, LakshmiPriya T, Hilda A. Biomed Res Int 2013;2013:10.
[13] Odeyemi AT, Aderiye BI, Bamidele OS. J Microbiol Res 2013;3:43–52.
[14] Padmapriya B, Rajeswari T, Noushida E, Sethupalan DG, Venil CK. World Appl Sci J 2011;12:1798–802.
[15] Fadda S, Oliver C, Vignolo G. J Food Sci 2002;67:1179–83.
[16] Lee LP, Karbul HM, Citartan M, Gopinath SCB, LakshmiPriya T, Tang TH. Biomed Res Int 2015;2015:1–5.
[17] Clausen K. Eur J Lipid Sci Technol 2001;103:333–40.
[18] Siezen RJ, Wilson C. Microbiol Biotechnol 2010;3:1–9.
[19] Michael A, Ash I. Hand book of preservatives. New York: Synapse Info Resources; 2004.