VOLUME 9 NOMOR 2 DESEMBER 2022 **ISSN 2548 – 611X**

JURNAL
BIOTEKNOLOGI & BIOSAINS INDONESIA

Homepage Jurnal: http://ejurnal.bppt.go.id/index.php/JBBI

APPLICATION OF RECOMBINANT TRIACYLGLYCEROL LIPASE AND CARBOXYLESTERASE ENZYMES FROM *Bacillus velezensis* STRAIN S3 FOR POLYESTER SURFACE MODIFICATION

Aplikasi Enzim Triasilgliserol Lipase dan Karboksilesterase Rekombinan *Bacillus velezensis* Strain S3 untuk Modifikasi Permukaan Poliester

Ika Rahmatul Layly[1], Is Helianti[2]*, Rika Indri Astuti[3,4], Anja Meryandini[3,4]
[1]Microbiology Study Program, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Jl. Agatis, IPB Dramaga Campus, Bogor 16680
[2]Research Center for Applied Microbiology, OR HL, KST Soekarno, National Research and Innovation Agency. Jl. Raya Jakarta-Bogor Km. 46, Cibinong, Bogor, Jawa Barat 16911
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Jl. Agatis, IPB Dramaga Campus, Bogor 16680
[4]Center for Biotechnological Research, IPB University, Jl. Kamper, IPB Dramaga Campus, Bogor, 16680
Email: is.helianti@brin.go.id

ABSTRACT
Enzymatic polyester surface modification can be performed with lipase and esterase enzymes. In this study, the polyester fabric modification utilized triacylglycerol lipase (TGA) and carboxylesterase (CES) recombinant enzymes. The effect of these treatments was observed by determining the hydrophilicity level, dye absorption level, hydroxyl group measurement, and fiber surface morphology. The results revealed an elevated hydrophilicity level in polyester fabric, followed by dye absorption improvement and carboxyl group increase. The water absorption times required by the fabric based on the results of TGA, CES, comparative lipase, and negative control treatments were 3±0.05 seconds, 3.5±0.07 seconds, 5±0.05 seconds, and 80±11.54 minutes, respectively. Dye absorption test in polyester fabric based on these groups mentioned above were 52±0.5, 58±0.5, 178±0.5, and 2968±290 seconds. The total hydroxyl group measurement in polyester fabric was observed at 30.9±0.09, 30.5±0.05, 28.6±0.09, and 3 meq/100 g. The SEM observation showed that the enzymatic hydrolysis could alter the porous structure and surface of the fibers.

Keywords: *hydrolysis, recombinant carboxylesterase, surface modification, polyester, recombinant triacylglycerol*

ABSTRAK
Modifikasi permukaan poliester secara enzimatis dengan enzim lipase dan esterase mengubah poliester menjadi hidrofilik. Pada penelitian ini, modifikasi permukaan poliester menggunakan enzim rekombinan Triasilgliserol lipase (TGA) dan Karboksilesterase (CES). Parameter uji meliputii hidrofilisitas, penyerapan warna, pengukuran gugus hidroksil, dan morfologi permukaan serat. Hasil hidrolisis menunjukkan peningkatan hidrofilisitas, penyerapan warna dan gugus karboksil kain poliester. Waktu penyerapan air oleh kain hasil perlakuan enzim TGA, CES, lipase pembanding dan kontrol negatif berturut-turut adalah 3±0,05, 3,5±0,07, 5±0,05 detik dan 80±11,54 menit. Uji serapan warna kain poliester pada kelompok tersebut diatas berturut-turut adalah 52±0,5, 58±0,5, 178±0,5 dan 2968±290 detik. Pengukuran jumlah gugus hidroksil pada kain poliester perlakuan TGA, CES, enzim lipase pembanding dan kontrol negatif adalah 30,9±0,09, 30,5±0,05, 28,6±0,09 dan 3 meq/100 g. Hasil SEM menunjukkan adanya perubahan struktur pori dan permukaan serat.

Kata Kunci: *hidrolisis, karboksilesterase rekombinan, modifikasi permukaan, poliester, triasilgliserol rekombinan*

Received: *08 August 2021* ***Accepted:*** *22 August 2022* ***Published:*** *01 December 2022*

INTRODUCTION

Polyester is a polymer with an ester functional group as its main chain, made from ethylene-glycol and terephthalic acid polymerizing through a condensed-polymerization process (Purohit et al. 2012). Polyester has high elasticity modulus and strength, thermoplastic and wrinkle-resistant properties, resistance to weak acid and base compounds, and resistance to oxidation, alcohols, and ketones. Production of polyester in large quantities is relatively simple and cheap. These characteristics result in the use of polyesters in many textile and non-textile industries (Ibrahim and El Salam 2012).

Demand for polyester in the global market is gradually increasing. In 2019, 57.1 metric tons/year was recorded, which increased by 57.7 metric tons/year in 2020 (Fernandez 2021). Apart from the advantage of polyester characteristics, hydrophobic and low moisture content characteristics become its drawbacks, causing pilling (rolling fiber on the rotating rollers) during the spinning process and an electrostatic condition between fiber or machine that results in choking and difficulty to dye. These drawbacks can reduce the production process and the comfort properties of textile raw material. For improving the comfort level and simplifying the coloring process, a modification on the fiber surface is necessary to elevate its hydrophilicity level (Zhao et al. 2017).

Hydrophobic polyester surface characteristics are formed because of the ether-oxygen bond (C-O-C), while the base fiber part is formed by the ester-oxygen bond (C=O) (Samanta et al. 2014). The hydrophilicity level of the fiber surface can be increased using a conventional method with a strong base solution at high temperatures, which can alter the surface characteristics from hydrophobic to hydrophilic, causing fiber strength, resistance, depreciation, and shrinkage reductions. Waste disposal and high temperatures in the hydrolysis process impose environmental and industrial costs. A biocatalytic modification method is performed using the enzymatic hydrolysis reaction to reduce the use of chemical compounds. Hydrolase, cutinase, and oxyreductase enzymes hydrolyze the ester bonds (Kumar and Kumar 2020). Lipase (triacylglycerol-hydrolase) and esterase (carboxylester-hydrolase) are examples of hydrolase enzymes that hydrolyze ester bonds, producing the carboxyl and polar hydroxyl groups, causing a polar interaction or a hydrogen bond with water molecules and increasing the water absorption and penetration (El Ola et al. 2013). Lipase and esterase are the most applicable enzymes for polyester surface modification.

Several industrial commercial enzymes originated from plants, animals, and microbes. Many industries have used enzymes from microbes, such as bacteria and fungi, with a stable character, more variative catalytic part, high yield level, and easy production and recovery from the liquid culture. Naturally, their availability is less fulfilling in industrial applications, resulting in recombinant protein use for enzyme production (Kardas et al. 2014). The protein or recombinant enzyme product has a high productivity level, followed by the stability condition against the organic solvent, wide substrate specificity, high regio- and stereoselectivity, and appropriateness for the characteristic requirements in the industry (Pan et al. 2013).

Enzymes produced from bacteria are preferable to enzymes from fungi or molds as bacteria have a shorter generation time, a simpler nutrient requirement, and an easier screening procedure (Jaeger and Eggert 2002). Several lipase-producing bacteria are from the *Bacillus*, *Pseudomonas*, and *Burkholderia* genera (Verma et al. 2012). Because of its diversity, lipase from *Bacillus* has been widely used in the industrial biotechnology applications. *Bacillus* has diverse habitats, distributed over various landscapes from hot springs to poles. This wide habitat causes the lipase produced from *Bacillus* to have thermophilic, hyperthermophilic, psychrophilic, alkalophilic, and acidophilic characteristics that can be applied widely (Khurana et al. 2017).

One of the bacterial culture collections from the Laboratory for Development of Industrial Agro and Biomedical Technology (*Laptiab* – National Research and Innovation Agency) is recombinant *E. coli* DH5α isolate carrying the triacylglycerol lipase (TGA) and carboxylesterase (CES) genes from *Bacillus velezensis strain S3*. Both gene products are

formed as triacylglycerol lipase and carboxylesterase enzymes. These enzymes are hydrolase enzymes widely used for polyester fabric surface modification. This study applied recombinant triacylglycerol and carboxylesterase enzymes to modify the polyester fabric surface and obtain hydrophobic polyester fabric, which easily absorbs dye and water. The objective of this study was to identify whether the recombinant triacylglycerol lipase and carboxylesterase enzymes could elevate the hydrophilicity level of polyester fabric to absorb dye and water easily.

MATERIALS AND METHODS

Location and time

This study was conducted at the Laboratory for Development of Industrial Agro and Biomedical Technology (Laptiab), Puspiptek Area, Serpong, South Tangerang, Banten. This study was performed from January until May 2021.

Materials

Materials used were recombinant *E. coli* DH5α isolate carrying the Triacylglycerol lipase (TGA) and Carboxylesterase (CES) genes from *Bacillus velezensis strain S3*, Luria-Bertani media (LB), Ampicillin, Na-Phosphate buffer, β-mercaptoethanol, P-Nitrophenyl palmitate substrate, Isopropanol, Gum Arabic, Triton X-100, Tris-HCl buffer, polyester fabric, recombinant Triacylglycerol lipase enzyme (TGA), recombinant carboxylesterase enzyme (CES), non-recombinant lipase enzyme from *Bacillus velezensis strain S3*, commercial lipase enzyme (*Amano*), NaOH, distilled water, Reactive dye, HCl, and NaCl. Equipment used in this study were shaker incubator, centrifuge, sonicator, UV-Vis spectrophotometer, Thermomixer, ultrafiltration chamber, Laminar Air Flow, inoculation loop, laboratory glasswares, microtubes, micropipette, and scanning electron microscope (SEM).

Recombinant lipase enzyme production

Two colonies of *E. coli* DH5α containing recombinant TGA and CES were picked up and inoculated into the Luria-Bertani (LB) media as a starter. Each colony was inoculated into each starter of 50 mL liquid LB media with 100 µg mL^{-1} Ampicillin. Then, the samples were incubated at 37ºC with 150 rpm agitation overnight. The starter was transferred into 1000 mL of liquid LB production media with 100 µg mL^{-1} ampicillin. The production media were incubated at 37ºC with 150 rpm agitation overnight. Pellets were harvested with 8000-rpm centrifugation at 4ºC for 15 minutes. Each pellet was suspended in 0.05 M Na-phosphate buffer, pH 7, containing 1 mM β-mercaptoethanol. The suspended pellets were sonicated to break the *E. coli* cells and release the intracellular lipase enzyme. The sonication was performed for 10 minutes at 45% amplitude for 10 seconds ON and 10 seconds OFF. The sonicated product was centrifuged to obtain the supernatant material as an intracellular enzyme in Na-phosphate buffer.

Non-recombinant lipase enzyme

Using an inoculation needle, a loopful of *Bacillus velezensis strain S3* colony was inoculated into 50 mL of liquid LB media as a starter and incubated at 37ºC with 150 rpm agitation overnight. The starter was then transferred into 1000 mL of liquid LB production media and incubated at 37ºC with 150 rpm agitation. The enzyme was harvested by centrifuging the production media at 8000 rpm and 4ºC for 15 minutes. The supernatant was used for further testing, while the pellet was discarded.

Concentrated lipase enzyme production

Recombinant triacylglycerol lipase, carboxylesterase, and non-recombinant lipase enzymes from *Bacillus velezensis strain S3* were concentrated with an ulfiltration method by 350 mL *Sartorius Vivaflow 50/ 50R/200* ulfiltration chamber. The 350 mL enzyme supernatant was passed through the 10 kDa ultrafiltration membrane at a flowing rate of 10 mL^{-1} minutes. The peristaltic pump was set to regulate the flowing rate and separate the retentate (concentrated product) and permeate (withdrawn product). The lipase enzymes were concentrated 7×, whereas 350 mL supernatant could produce 50 mL permeate.

Lipase enzyme activity test

The recombinant and non-recombinant lipase enzyme supernatants were tested for their activities with a spectrophotometry method using *p*-nitrophenylpalmitate as the substrate (Vidanarachchi et al. 2015). The substrate contained solution A and solution B. Solution A was generated by mixing 30 mg *p*-nitrophenylpalmitate in 10 mL isopropanol. Solution B was made by dissolving the 100 mg Gum Arabic and 400 mg Triton x-100 with 90 mL of 50 mM Tris-HCl buffer (pH 8.0). The substrate was produced by homogenously mixing solutions A and B. Subsequently, 100 µL lipase (0.1 mg mL^{-1} protein content) was mixed with 900 µL substrate. The blank was created by replacing the lipase with 50 mM Tris-HCl buffer (pH 8.0). The lipase-substrate and blank mixtures were then incubated in a thermomixer for 30 minutes at 37ºC. The absorption was measured with a UV-Vis spectrophotometer at 410 nm wavelength. An enzyme unit was presented as 1 µmol *p*-nitrophenol that was released enzymatically from the substrate each minute. The absorption measurement results were converted into the standard curve equation of *p*-nitrophenol, namely:

$$y = ax + b$$

Where *y* was the absorbance value and *x* was the activity value (µmol). The activity was converted to gain lipase activity value (unit mL^{-1}) under the following equation:

$$U = y \cdot V/t$$

Where *U* was the lipase activity (unit.mL.minutes^{-1}), *t* was reaction time (minutes), and *V* was lipase enzyme volume (mL).

Enzyme protein content measurement

The protein content was measured using the Bradford reagent 1×, in which 30 µL sample was added with 1.5 mL Bradford reagent and incubated for 30 minutes. Then, the measured absorption value was converted into the lipase activity based on the BSA standard curve:

$$y = ax + b$$

Where *y* was the absorbance value and *x* was the protein content

The specific activity was calculated by dividing the enzyme activity and the lipase-protein content.

$$Spesific\ avtivity = \frac{Lipase\ activity\ (\frac{U}{mL})}{Protein\ content\ (\frac{mg}{mL})}$$

Polyester fiber surface modification

The polyester fiber surface was modified enzymatically by following the method of El-Shemy et al. (2016). The 100% polyester fabric was cut at 2 cm × 4 cm. There were four treatment groups with four replications: group 1 (fabric soaked in recombinant TGA enzyme), group 2 (fabric soaked in recombinant CES), group 3 (positive control treatment, fabric soaked in non-recombinant lipase enzyme from *Bacillus velezensis strain S3*), and group 4 (negative control, fabric soaked in distilled water). Group 1, group 2, and group 3 were soaked with the enzymes at 40ºC, while group 4 as a negative control treatment was soaked with distilled water. All treatment groups were soaked for 30 minutes at 100 rpm agitation. The enzymes used in groups 1 – 3 had a similar activity of 1.5 U mL^{-1}. After the reaction, the polyester fabric from all treatment groups was washed using distilled water three times and air-dried at room temperature.

Polyester fabric hydrophilicity level

The hydrophilicity level of polyester fabrics was determined by the Wickability test based on El-Ola et al. (2013). The polyester fabrics from all treatment groups were soaked in distilled water at 1 cm height from below the fabric. Then, the time for distilled water to reach 3 cm height was recorded from below the fabric part (in minute unit). This test was performed four times as the recorded time average showed the hydrophilicity level.

Polyester fabric dye absorption

Dye absorption was measured by the Wettability test based on El-Shemy et al. (2016) using *Reactive Red 120* dye. The dye was prepared at 1% and dropped on the polyester fabric middle point in all treatment groups. Dye absorption time was recorded

from when the dye was dropped at the middle point until it covered all the surface parts of the fabric with a size of 2 cm × 4 cm (in the second unit). This test was performed four times as the recorded time average showed the ease of the fabric in absorbing dye.

Carboxyl contents

The carboxyl content was measured following El-Shemy et al. (2016). The carboxyl content was estimated through the total alkali contents combined in the polyester fiber. The polyester fabric samples from all treatment groups were soaked in 2% HCl for 3 hours at 100 rpm agitation, then taken away for washing using the ethanol-water mixture (60:40), and finally dried at room temperature.

The polyester fabric sample was weighed at 0.5 g, transferred into an Erlenmeyer flask, added with 50 mL of 0.1 N NaOH containing 5% NaCl, and incubated overnight at room temperature and 100 rpm agitation. The fabric sample was removed from the Erlenmeyer flask and the residual solution was titrated with 0.05 N HCl using a phenolphthalein indicator. The blank sample was a polyester fabric sample in the negative control treatment group. The carboxyl content was determined as follows:

$$Carboxyl\ content = \frac{X - Y}{W} \times 100\ meq/100g$$

Annotation:
X: Volume of HCl in the blank (mL)
Y: Volume of HCl in the sample (mL)
W: Sample weight (g)

SEM observation

To determine the morphological alteration in polyester surface, the treated polyester fabrics (incubated with recombinant lipase enzymes), positive control group (incubated with NaOH), and negative control group (incubated with distilled water) were observed under the *Thermoscientific Quanta 650* electrone microscope at 60, 100, and 500 times magnification.

RESULTS AND DISCUSSION

Production of recombinant enzymes

At the end of the production time for 18 hours, harvesting was conducted on the production media of recombinant *E. coli* TGA and CES to obtain the cell pellets. The recombinant TGA and CES intracellular enzymes were obtained from the breakdown of cells using ultrasonic waves. According to Rodriguez-Carmona et al. (2011), a recombinant protein or other biological products produced by *E. coli* will be secreted intracellularly in the periplasmic space or inclusion body, therefore unable to be secreted extracellularly or in the media. In the lipase production media, *Bacillus velezensis strain S3* was harvested at 18 hours to obtain the supernatant as a crude lipase enzyme. Lipase produced by *B. velezensis strain S3* was an extracellular enzyme, produced in the cytoplasm and secreted out of the cell or into the media. Lipases produced by bacteria are either extracellular, intracellular, or membrane-bond lipases. The extracellular lipase is more widely used for industrial applications because of easier downstream processing (Chandra et al. 2020).

Enzyme activity test

The recombinant intracellular and non-recombinant extracellular enzyme activities were measured using a method proposed by Vidanarachchi et al. (2015). Enzyme activity

Table 1. Lipase enzyme activity

Lipase enzyme	Act (U/ml)		Specific Act (U/mg)	
	Before being Concentrated	After being Concentrated	Before being Concentrated	After being Concentrated
Recombinant *E. coli* DH5 α TGA	0.27±0.0006	1.50±0.02	3.07±0.03	4.28±0.21
Recombinant *E. coli* DH5 α CES	0.26±0.0008	1.49±0.04	3.06±0.34	4.12±0.18
Non-recombinant *B. velezensis strain S3* lipase	0.18±0.003	1.04±0.014	2.04±0.02	3.11±0.16

was measured from the total enzyme activity, and specific enzyme activity before and after the enzyme was concentrated using an ultrafiltration membrane. The enzyme activity of recombinant TGA, CES, and non-recombinant *B. velezensis strain S3* extracellular lipase before being concentrated were 0.27, 0.26, and 0.18 U/mL, respectively, while the enzyme activities measured after being concentrated were 1.5, 1.49, and 1.04 U/mL, respectively. The enzyme activity level before or after being concentrated indicates that the recombinant enzymes have a higher activity than the non-recombinant *B. velezensis strain S3* lipase enzyme. The enzymes investigated in this study were concentrated using an ultrafiltration membrane that could concentrate the enzymes 5x more. The concentration caused elevated total and specific activities of lipase enzymes (Table 1). These results were similar to a study by Li et al. (2019), who used a 10 kDa ultrafiltration membrane to concentrate the *Aureobasidium pullulans* extracellular lipase enzyme, increasing the specific activity from 0.98 U/mg to 1.22 U/mg. This condition was because of the remaining protein concentrate after the enzyme passed through the ultrafiltration membrane, while the solvent was withdrawn with a size smaller than 10 kDa.

The specific activity was obtained by dividing the total activity and total protein content measured. The total protein contents in the recombinant TGA and CES enzymes before being concentrated were 0.063 and 0.064 mg/mL, while the total protein content in the non-recombinant *B. velezensis strain S3* extracellular lipase was 0.059 mg/mL. After being concentrated, the protein contents in three enzyme types increased to 0.486, 0.402, and 0.508 mg/mL, respectively.

The specific activity of recombinant TGA, CES, and non-recombinant lipase enzymes after being concentrated 5× seemed to increase compared to before, as proven by the increased protein content and its specific activity. The recombinant TGA, CES, and non-recombinant *B. velezensis strain S3* lipase concentration used an ultrafiltration membrane with 10 kDa pore size. This pore size could hold similar protein molecules or protein molecules with a mass of more than 10 kDa. Measurement results of the retentate showed a smaller protein content than the permeate, even the value was almost the same as the blank, which indicated the enzyme was successfully concentrated using an ultrafiltration membrane by removing the water that passed through the membrane and holding the molecules that passed through the membrane. The lipolytic lipase and carboxylesterase enzymes had 20-70 kDa size, so using a 10 kDa membrane pore could hold the enzyme protein, resulting in a concentrated enzyme. A similar condition was reported by Ariono et al. (2018), who also applied the mechanism of protein concentration or separation using an ultrafiltration membrane based on molecular size and electrostatic charge. In the separation process, the pore size and distribution in the membrane are influenced by the solution pH, salt content, and hydrodynamic system. The specific activity of recombinant TGA, CES, and non-recombinant lipase enzymes describes the enzyme purity level. The more purified enzyme protein is, the higher enzyme activity produced becomes. Edupuganti et al. (2017) purified the *Staphylococcus epidermidis MTCC 10656* extracellular lipase with gel filtration and obtained a higher enzyme activity after the purification than before the purification.

Polyester fabric surface modification

The enzymatic treatment on the polyester fabric surface modification showed different results from the negative control group. The hydrophilicity test on polyester fabrics showed the different times to absorb water. The difference was noticeable in the negative control group compared to other groups. In contrast to the enzyme treatment groups, there was no hydrolysis of ester bonds on the fabric surface in the negative control group. This condition can be shown by the time required for the polyester fabric to absorb water (Table 2).

In the negative control group, the absorption time was 80 minutes. Meanwhile, the absorption time in TGA, CES, and non-recombinant lipase enzyme treatment groups was insignificantly different at 3, 3.5, and 5 minutes, respectively. The recombinant TGA and CES enzymes

produced a better hydrophilicity level than the non-recombinant lipase enzyme because of the higher productivity and effectiveness of recombinant enzymes than non-recombinant lipase, indicated by the total and specific activity of the recombinant enzymes that was greater than that of the non-recombinant enzyme.

Enzymatic polyesters modification using lipase or cutinase has altered the polyester characteristics from hydrophobics to hydrophilics through the ester bond hydrolysis. The ester bond hydrolysis produces polar carboxyl and hydroxyl groups, causing a polar interaction or hydrogen bond with water molecules and increasing the water absorption and penetration (Kumar and Kumar 2020). The effect of enzymatic polyester fabric surface modification on the hydrophilicity level was determined by the absorption of reactive red dye. The faster the dye spreads evenly on the fabric, the higher the hydrophilicity level becomes. Reactive dyes were used to investigate dye absorption as reactive dyes are anionic, stable, and permanent in fabrics and are also often used to dye fabrics from various natural and synthetic fibers. During the dyeing process, the reactive groups of the dye form covalent bonds with the fabric fibers (Al Mamun et al. 2017). The dye absorption results showed that the dye

Table 2. Hydrophilicity level of polyester fabrics in various soaking treatments

Polyester fabric treatment groups	Time (minutes)
Negative control (distilled water)	80±11.54
Non-recombinant *B. velezensis strain S3* lipase enzyme	5±0.5
Recombinant *E. coli* DH5 α TGA enzyme	3±0.05
Recombinant *E. coli* DH5 α CES enzyme	3.5±0.07

Table 3. Dye absorption level in polyester fabrics

Sample	Time (seconds)
Negative control (distilled water)	2968±290
Non-recombinant *B. velezensis strain S3* lipase enzyme	178±4
Recombinant *E. coli* DH5 α TGA enzyme	52±0.5
Recombinant *E. coli* DH5 α CES enzyme	58±0.5

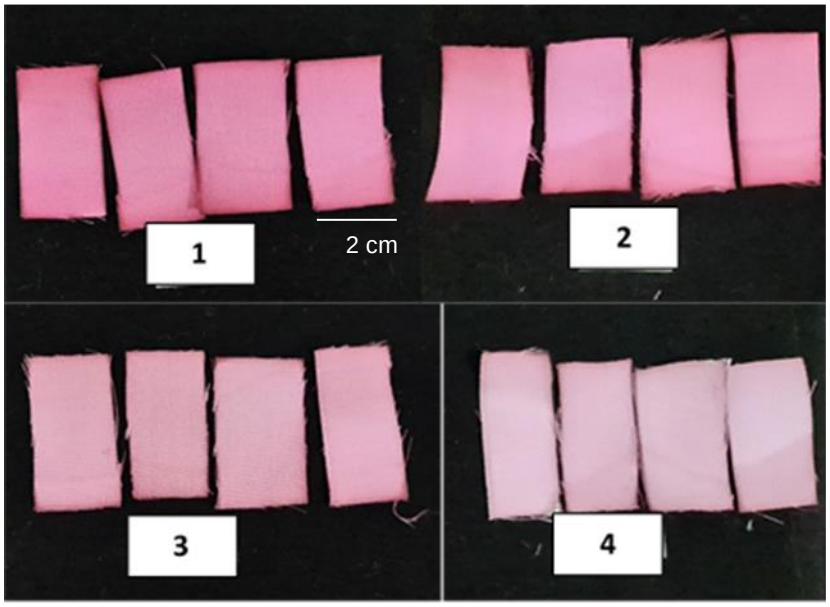

Figure 1. Reactive red dye absorption in polyester fabrics after enzymatic treatments. Note: 1 = recombinant TGA, 2 = recombinant CES, 3 = non-recombinant *B. velezensis strain S3* lipase, 4 = Negative control (distilled water)

absorption lasted for 49 minutes in the negative control group. Meanwhile, dye absorption time was faster in the enzyme treatment group (Table 3).

The recombinant TGA and CES enzyme treatment groups produced a better dye absorption in polyester fabrics than the non-recombinant lipase enzyme and negative control groups did. These results indicated that the enzymatic treatment of polyester fabric causes a hydrolysis process. Surface modification of polyester fabric hydrolyzes the ester bonds into polar carboxyl and hydroxyl groups. Anions from carboxyl groups and aliphatic hydroxyl groups become the targets of the reactive dye, forming covalent bonds between reactive dyes and fabric fibers (Sauperl et al. 2013). The polyester fabric with the highest red color intensity level was found in the recombinant TGA and CES enzyme treatment groups, followed by polyester fabrics in the non-recombinant lipase enzyme treatment group and the negative control group with the lowest red color intensity level (Figure 1). These results indicate that the enzymatic surface modification can alter the nature of the polyester surface fiber to be hydrophilic, thus easily absorbing water and dyes. The specificity and effectiveness of the surface modification were demonstrated by the recombinant CES and TGA recombinant enzyme treatment groups. The dyeing process in polyester fabrics generally involves three main processes, namely (1) dye migration from the solvent to the fabric fiber surface (adsorption), (2) dye diffusion into the fabric fiber (absorption), and (3) dye fixation by forming bonds or filling the fabric fiber pores (Shahidi et al. 2013).

The surface modification of polyester fabric fibers through the hydrolysis process of ester bonds produces carboxyl and hydroxyl groups. The more carboxyl and hydroxyl groups are formed, the higher the enzyme's hydrolysis level. As shown in Table 4, the carboxyl group content of polyester fabric in the negative control treatment group was 3 meq/100 g, which means that no hydrolysis occurred. In the polyester fabric samples treated with recombinant enzymes TGA, CES, and non-recombinant lipase, the carboxyl groups were 30.9, 30.5, and 28.6 meq/100 g, respectively. These results were similar to the results of the study conducted by El-Shemy et al. (2016), which stated that surface modification of polyester fabrics using lipase type-II and lipolase 100L-Ex obtained a carboxyl content of 28.1 and 27.5 meq/100 g, while the untreated group had the carboxyl content at 16.6 meq/100. Kawai et al. (2017) stated that the higher carboxyl group content could elevate the hydrolysis rate, so the hydrophilicity and dye absorption could improve. These results were observed in the fabrics treated with recombinant TGA and CES enzymes, which gained higher carboxyl content than fabrics treated with non-recombinant lipase enzymes. These results correspond to El-Shemy et al. (2016), who used light reflection techniques to determine dye penetration value on polyester fabrics. The 100L-EX lipolase and type-II lipase treatment groups showed higher dye penetration than the negative control group at 3.4, 3.3 and 0.3, respectively. The obtained results indicate that the enzymatic surface modification of polyester fabric can increase the fabric hydrophilicity level, resulting in easier water and dye absorption.

Polyester is a polymer with an ester functional group as the main chain, made from the polymerization of ethylene glycol with terephthalic acid through a condensation polymerization process (Purohit et al. 2012). The surface modification of polyester fabric was performed to eliminate the hydrophobic nature of polyester fabric formed by ether-oxygen (C-O-C) and ester-oxygen (C=O) bonds (Samanta 2014). Kantouch et al. (2005) performed surface modifications using lipase type-II and lipolase 100L-Ex enzymes to remove the hydrophobic characteristics and increase the hydrophilicity and dye absorption level on wool fabrics. Kardas et al. (2014) used commercial lipase enzymes, such as amano lipase-A and lipozyme, for surface modification of gloss polyester and obtained an alteration from hydrophobic to hydrophilic characteristics, characterized by the capability of the polyester to easily absorb dye and water.

Polyester fabrics after the modification

The increased hydrophilicity level of polyester fabrics after the enzymatic surface modification treatment was related to the alteration of fabric fiber surface morphology (Figure 2). In Figure 2, the fabric fibers modified using the recombinant TGA, recombinant CES, and non-recombinant lipase enzymes had irregular fiber strands, in contrast to the fabric fibers in the negative control group. In Figure 3, the surface morphology of the polyester fiber is more visible. In Figure 3.1 and 3.2, it can be seen that the fabric fiber produced from the hydrolysis of recombinant TGA and CES enzymes have many cracks, holes, and cavities. Likewise, the fibers produced from non-recombinant lipase enzyme hydrolysis have cracks, holes, and cavities, but not as much as those in the recombinant enzyme treatment groups. In the negative control treatment group, the fabric fiber morphology was uniform, smoother, and without holes and cracks.

The enzymatic and non-enzymatic surface modifications cause morphological alterations in polyester fabric fibers. The enzymatic treatment with recombinant TGA and CES enzymes resulted in more significant morphological alterations on the polyester fabric surface than the non-recombinant lipase enzyme. The observation results of fabric fiber morphology, hydrophilicity, dye absorption, and carboxyl

Table 4. The carboxyl content of polyester fabrics after the treatment

Sample	Carboxyl content (meq/100 g)
Negative control (distilled water)	3±0.3
Non-recombinant *B. velezensis strain S3* lipase enzyme	28.6±0.09
Recombinant *E. coli* DH5 α TGA enzyme	30.9±0.09
Recombinant *E. coli* DH5 α CES enzyme	30.5±0.05

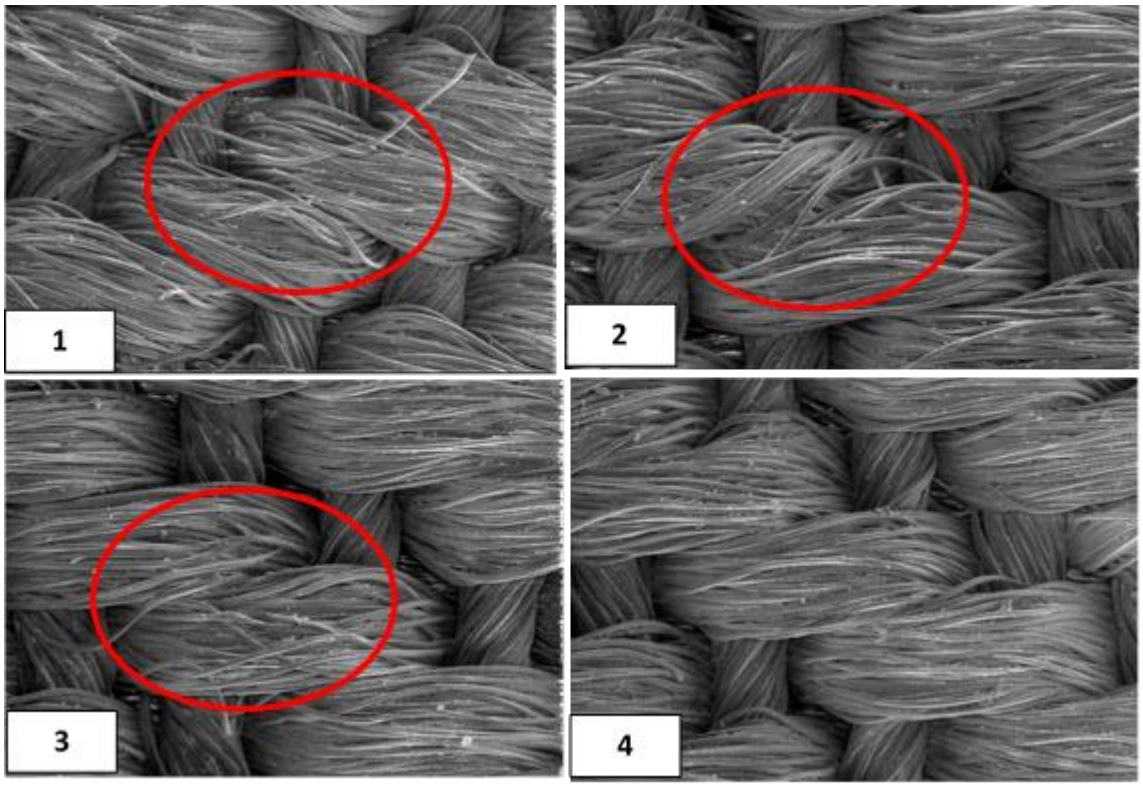

Figure 2. The fiber structure of polyester fabrics in the post-treatment period under the electron microscope at 100x magnification. Note: 1 = recombinant TGA, 2 = recombinant CES, 3 = non-recombinant *B. velezensis strain S3* lipase, 4 = Negative control (distilled water), Red circle = Irregular fiber strands in the treatment groups

content indicate that the recombinant TGA and CES enzyme applications can produce a hydrophobic polyester fabric that is suitable for clothing material.

The results our study was coincide with El-Ola et al. (2013). They modified the surface of polyester fabrics using the *Bacillus* lipase enzyme and obtained fabric fibers with rough texture, cavities, holes, and pores after observing them under an electron microscope. At the same time, untreated treatment group had smooth texture fiber without any cracks. Kumar and Kumar (2020) modified the surface of polyester fabrics using the commercial lipase enzyme, namely lipolase 100L-EX. Under the electron microscope observation, the untreated enzyme treatment group had a uniform, flat, and smooth fiber surface, while the lipase enzyme treatment group had a fiber surface with rough texture, cavities, pores, and holes. Similarly, Lee and Song (2013) modified the surface of polyester fabrics using the commercial lipase enzyme called Novozyme to produce hydrophilic polyester fabrics that could easily absorb dye and water, and Kawai et al. (2017) used recombinant cutinase enzyme to modify the polyester fabrics surface and reported that the morphology of the fabric fibers was different between the negative control group and the cutinase enzyme treatment group after the electron microscope observations.

CONCLUSION

Enzymatic polyester fabric surface modification using the recombinant TGA and CES has increased the hydrophilicity level. The increased hydrophilicity level was marked by the easier absorption of water and dye, where water absorption lasted for 3 and 3.5 minutes and dye absorption lasted for 52 and 58 seconds. The recombinant TGA and CES enzyme treatments obtained carboxyl contents of 30.9 and 30.5 in the fabrics. The morphological observation in polyester fibers showed the existence of altered morphology based on the conditions of the fiber strand that had many cavities, cracks, and holes.

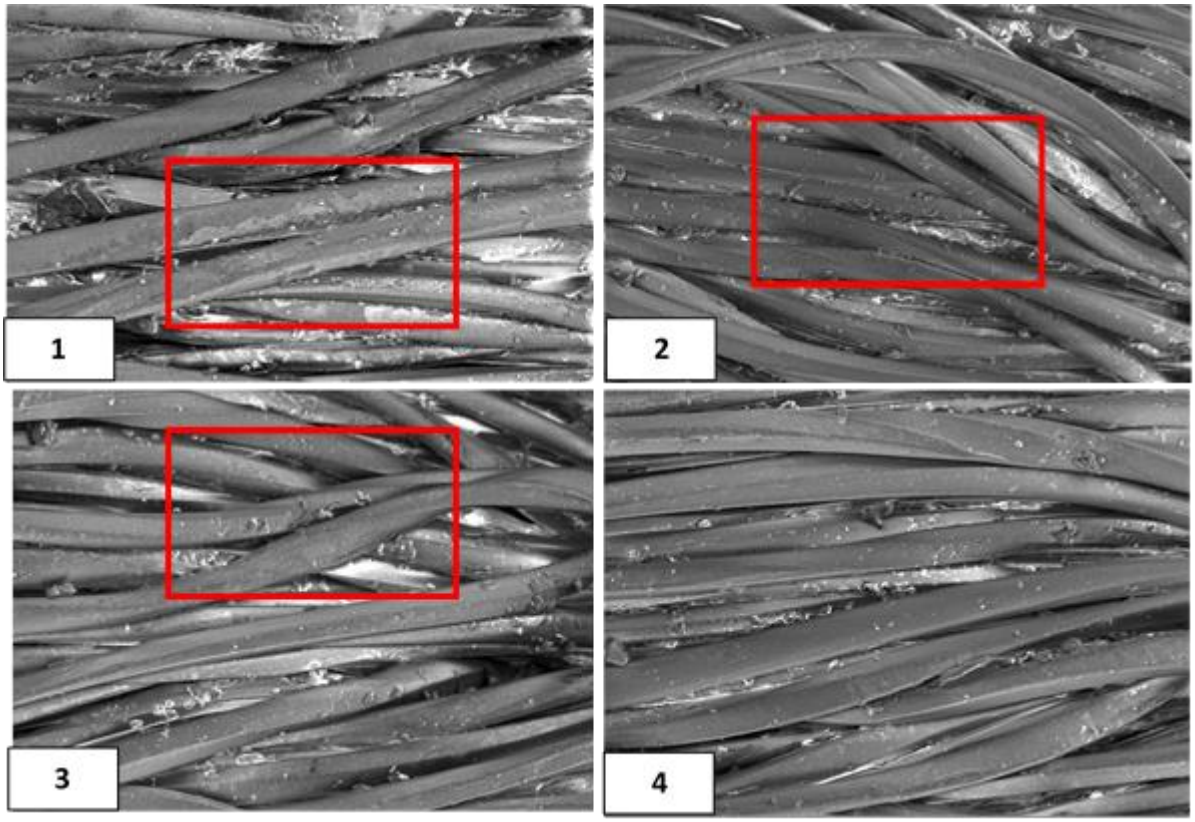

Figure 3. The fiber structure of polyester fabrics in the post-treatment period under the electron microscope at 500x magnification. Note: 1 = recombinant TGA, 2 = recombinant CES, 3 = non-recombinant *B. velezensis strain S3* lipase, 4 = Negative control (distilled water), Red rectangle: Fiber surface with cracks, pores, cavities, and fractures in the treatment groups

ACKNOWLEDGMENTS

We thank the BPPT for funding this project through the DIPA BPPT program 2018/2019, Indonesian Ministry of Research, Technology, and Higher Education for Human Resource Development in Science and Technology. We would also like to express our gratitude to the Laboratory for the Development of Industrial Agro and Biomedical Technology (*Laptiab*), which provided the facilities required for performing the experiment in the Laboratory.

REFERENCES

Al Mamun MH, Mostofa A, Hossain MA, Khan M, Zakaria M, Yeasmin MS (2017) Effect of reactive groups of reactive dyes on dyeing of modal fabrics. Int J Text Sci 6:158-164. doi: 10.5923/j.textile.20170606.04

Ariono D, Aryanti PTP, Wardani AK, Wenten IG (2018) Analysis of protein separation mechanism in charged ultrafiltration membrane. J Eng Technol Sci 50:202-223. doi: 10.5614/j.eng.technol.sci.2018.50.2.4

Chandra P, Enespa, Singh R, Arora PK (2020) Microbial lipases and their industrial application: A comprehensive review. Microb Cell Fact 19:169. doi: 10.1186/s12934-020-01428-8

Edupuganti S, Parcha L, Mangamoori LN (2017) Purification and characterization of extracellular lipase from *Staphylococcus epidermidis* (MTCC 10656). J Appl Pharm Sci 7:57-63. doi: 10.7324/JAPS.2017.70108

El-Ola SMA, Moharam ME, El-Bendary MA (2013) Optimum conditions for surface modification of PET by lipase enzymes produced by Egyptian bacilli in comparison with standard one. Indian J Fibre Text Res 38:165-172. Corpus ID: 100087339

El-Shemy NS, El-Hawary NS, El-Sayed H (2016) Basic and reactive-dyeable polyester fabrics using lipase enzymes. J Chem Eng Process Technol 7:271. doi: 10.4172/2157-7048.1000271

Fernandes L (2021) Polyester fiber production globally 1975-2020. https://www.statista.com/statistics/912301/polyester-fiber-production-worldwide/. Accessed 9 Augustus 2022

Ibrahim DF, El-Salam SHA (2012) Enzymatic treatment of polyester fabrics digitally printed. J Text Sci Eng 2:3. doi: 10.4172/2165-8064.1000113

Jaeger KE, Eggert T (2002) Lipases for biotechnology. Curr Opin Biotechnol 13:390- 397. doi: 10.1016/s0167-7799(98)01195-0

Kantouch A, Raslan WM, El-Sayed H (2005) Effect of lipase pretreatment on the dyeability of wool fabric. J Nat Fibers 2: 35-48. doi: 10.1300/J395v02n02_03

Kardas I, Lipp-Symonowicz B, Sztajnowski S, Wojciechowska D (2014) The influence of pet fibres surface enzymatic modification on the selected properties 14:179-186. doi: 10.2478/aut-2014-0020

Kawai F, Kawase T, Shiono T, Urakawa H, Sukigara S, Tu C, Yamamoto M (2017) Enzymatic hydrophilization of polyester fabrics using a recombinant cutinase cut 190 and their surface characterization. J Fiber Sci Technol 73:8-18. doi 10.2115/fiberst.2017-0002

Khurana J, Maan P, Cameotra SS, Kaur J (2017) Studies on recombinant lipase production by *E. coli*: Effect of media and bacterial expression system optimization. Int J Mol Biol 2:17-23. doi: 10.15406/ijmboa.2017.02.00008

Kumar JA, Kumar MS (2020) A study on improving dyeability of polyester fabric using lipase enzyme. Autex Res J 20:1-7. doi: 10.2478/aut-2019-0030

Lee SH, Song WS (2013) Effect of treatments with two lipolytic enzymes on cotton/polyester blend fabrics. J Korean Soc Cloth Text 37:1107-1116. doi: 10.5850/JKSCT.2013.37.8.1107

Li Y, Liu T, Zhao M, Zhang H, Feng F (2019) Screening, purification, and characterization of an extracellular lipase from *Aureobasidium pullulans* isolated from stuffed buns steamers. J Zhejiang Univ Sci B 20:332-342. doi: 10.1631/jzus.B1800213

Pan H, Chen Y, Yu P (2013) Advanced strategies for improving the production of industrial enzymes in heterologous host systems. Enz Eng 2:2. doi:10.4172/2329-6674.1000114

Purohit J, Chawada G, Choubisa B, Patel M, Dholakiya B (2012) Polyester Polyol derived from waste poly (Ethylene Terephthalate) for coating application on mild steel. Chem Sci J 76:1-7. doi:10.4172/2150-3494.1000053

Rodriguez-Carmona E, Villaverde A, Garcia-Fruitos E (2011) How to break recombinant bacteria: Does it matter? Bioeng Bugs 2:222-225. doi: 10.4161/bbug.2.4.15778

Samanta KK, Basak S, Chattopadhyay SK (2014) Environment-friendly textile processing using plasma and UV treatment. Textile Science and Clothing Technololgy. Springer Science Business Media. Singapore. doi: 10.1007/978-981-287-065-0_6

Sauperl O, Kostic M, Milanovic J, Zemlijc LF (2015) Chemical binding of chitosan and chitosan nanoparticles onto oxidized cellulose. J Eng Fibers Fabr 10:70-77. doi: 10.1177/155892501500100002 08

Shahidi S, Wiener J, Ghoranneviss M (2013) Surface modification methods for improving the dyeability of textile fabrics. Eco-Friendly Textile Dyeing and Finishing. Intech, New York, Chapter 2 pp 33-52. doi: 10.5772/53911

Verma N, Thakur S, Bhatt AK (2012) Microbial lipases: Industrial application and properties (A review). Int Res J Biol Sci 1:88-92. doi: 10.1186/s12934-020-01428-8

Vidanarachchi JK, Li S, Lundh AS, Johansson M (2015) Lipolytic activity on milk fat by *Staphylococcus aureus* and *Streptococcus agalactiae* strains commonly isolated in Swedish dairy herds. J Dairy Sci 98:8560–8564. doi:10.3168/jds.2015-9559

Zhao Z, Zhou J, Fan T, Li L, Liu Z, Liu Y, Lu M (2017) An effective surface modification of polyester fabrics for improving the interfacial deposition of polypyrrole layer. Preprints 2017, 2017050008. doi: 10.20944/preprints201705.0008.v1