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Prokaryotic Expression and Enzymatic Properties of Lipase from *Schizochytrium* Pombe

Siqi Liu and Jun Li*

Hebei Normal University of Science & Technology, Qinhuangdao, China

ABSTRACT

Based on the codon preference of *Escherichia coli*, the gene of lipase from *Schizochytrium* pombe was optimized and the codon adaptation index was 0.8 and the gene was expressed in *Escherichia coli* as a His-tag fusion protein using pET-30a as the expression vector. The recombinant strain with OD600 about 0.6 was induced with 1 mmol/L isopropyl-β-D-thiogalactoside at 30 °C for 12 h. The purified recombinant lipase STGL3 had a molecular mass of 32 kDa, and the purified lipase had a specific enzyme activity of 2.29 μmol/(min•mg), which was 2.3 times higher than before, and a protein content of 650.00 mg/L. The recombinant STGL3 showed the highest hydrolysis activity at 40 °C and pH 7.5, respectively. The effect of different metal ion concentrations on enzyme activity varied, lipase STGL3 was stable in the presence of Ca^{2+}, while Mg^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+} and EDTA had an inhibitory effects on lipase activity. Different types of surfactants and organic solvents inhibited the enzymatic activity of recombinant lipase to different extents. The recombinant lipases showed a preference towards pNP-esters with medium and longer acyl-chains. (C12, C14 and C16) and the recombinant lipases showed relatively weak lipase activity towards the shorter carbon chains. The kinetic constants of lipase were 0.29 mmol/L for K_m, 2.28 mmol/(L•min^{-1}) for V_{max}, and 6.19 S^{-1} for K_{cat}. The present study not only achieved the expression and characterization of enzymatic properties of lipase from *Leptosphaeria japonica*, but also provided some basis for the study of enhancing the quality and quality of algal oil by means of genetic engineering, and further exploring the potential industrial applications of *Leptosphaeria japonica* lipase.

***Corresponding author**

Jun Li, Hebei Normal University of Science & Technology, Qinhuangdao, China.

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Introduction

Lipases (E. C. 3. 1. 1. 3, triacylglycerol acyl hydrolase) belong to the α/β hydrolase family and are a class of biocatalysts that can catalyze the hydrolysis or synthesis of natural oil and fat substrates at the oil-water interface. Lipases have a conserved pentapeptide sequence with an active centre - G - X - S - region and are widely used in oil and fat processing, fine chemicals, textiles, pharmaceuticals, food leather, detergents and other fields [1-3].

Schizochytrium is a marine microorganism, unicellular and spherical in shape. The oil content of Schizochytrium can reach more than 50% of the dry weight and the DHA content can reach more than 35% of the total fatty acids [4]. DHA is an omega-3 polyunsaturated fatty acid, which plays an irreplaceable role in the vision and intellectual development of infants and children [5]. Currently, microalgae oil is widely used as a source of DHA in the food industry, such as infant and maternal foods, dairy products, functional foods and other food products [6, 7].

Schizochytrium, an unicellular heterotrophic microalgae, is considered as one of the most ideal strains for the industrial production of docosahexaenoic acid (DHA) because of its intracellular accumulation of high-safety, high-purity oils and fats [5, 8]. The current researchs on DHA production in *Leptospira* was mainly focused on the screening of high-yielding strains, optimization of fermentation conditions and mutagenesis breeding, but the regulatory mechanism of intracellular lipid metabolism is not yet understood, which hinders the further development of DHA production in *Leptospira* [9-11]. constructed *Schizochytrium* sp acyltransferase (AT) and its homologs, showed the important role of the AT structural domain in the synthesis of *Schizochytrium* sp and substituted its regulation in fatty acids, with a significant decrease in total fatty acid content from 49.52% to 35.2% in AT-deficient strains. bi Z Q et al used transcriptome sequencing technology (RNA-seq) to the transcriptome analysis of four stages of *Schizochytrium* sp DHA fermentation was compared to find potential genes in *Schizochytrium* sp that may be related to lipid accumulation and lipid back-consumption in the late fermentation stage, but it was not possible to study them in depth, and the study proved that redox reaction plays an important role in lipid back-consumption in the late fermentation stage, and other genes such as signal transduction and cellular transport are related to lipid metabolism, and these Cheng found that the enhanced green fluorescent protein gene and neomycin phosphotransferase gene were not significantly different from wild type in terms of biomass and lipid content, indicating that the efficient expression of exogenous genes was achieved in *Leptospira* [12, 13].

Shi Y et al successfully overexpressed the structural domains of dehydratase (DH) and enoyl reductase (ER) located on the ORFC in *Schizochytrium* sp [14]. SR21 with increased PUFA and

saturated fatty acid contents, respectively. This suggests that the DH and ER domains play different roles in lipid accumulation. All of the above studies indicate that there are genes that play a key role in the metabolic process of *Schizochytrium*, and these studies play an important role in the study of the basic metabolism of *Schizochytrium*.The results showed that the fatty acid synthase (FAS) products (C12-C18) were preferentially hydrolyzed in the late stages of fermentation in flysis, thus promoting the intracellular enrichment of the polyketide synthase (PKS) products (C20-C22). The above research may involve the involvement of specific lipases within the cells of *Schizochytrium* [15]. In order to investigate the nature of the lipase gene in *Schizochytrium*, this study has selected a lipase gene by gene sequence analysis, and systematically analysed its enzymatic properties, after cloned and the heterologous expression, and gave some information for elucidating the molecular mechanism of lipid migration in *Schizochytrium* at the molecular level and further exploring the potential industrial application of *Schizochytrium* lipase.

In the present study, the *Schizochytrium* lipase gene STGL3 was optimized with reference to the codon preference of E. coli and recombinantly expressed in E. coli BL21 (DE3). The purification and enzymatic properties of *Schizochytrium* lipase STGL3 were studied providing a reference for explaining the molecular mechanism of lipid re-consumption in *Schizochytrium* during late fermentation.

Materials and Methods

Materials and Reagents

Escherichia coli (*E. coli*) BL21 (DE3) was purchased from Tiangen Biochemical Technology (Beijing) Co., Ltd; Kanamycin Sulfate (Kan), isopropyl-β-D-thiogalactopyranoside (IPTG), Plasmid Extraction Kit were purchased from Beijing Solexpro Technology Co., Ltd; Standard Molecular Mass Marker were purchased from Hebei Ripart Biotechnology Co., Ltd; p-Nitrophenol esters of different carbon chain lengths were purchased from Sigma, USA; other conventional reagents, Sinopharm; Ni-NTA agarose were purchased from Shanghai Biyuntian Biotechnology Co.

Microorganism

Schizochytrium sp. S31 was provided by the Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences. Seed medium: glucose 30 g/L, yeast powder 2 g/L, soybean peptone 2 g/L, sea crystals 15 g/L. Solid medium: glucose 30 g/L, yeast powder 2 g/L, soybean peptone 2 g/L, sea crystals 15 g/L, agar powder 15 g/L.Fermentation medium: glucose 80 g/L, yeast powder 4 g/L, soybean peptone 4 g/L, sea crystal 15 g/L, MgCl2-6 H2O 6.4 g/L, CaCl2-2 H2O 1 g/L, MgSO4-7 H2O 5 g/L, KCl 2 g/L.All of the above media were at natural pH, dispensed in 250 mL triangular flasks containing 50 mL of liquid, and autoclaved at 115 °C for 20 min before used.

Methods

Optimization and Synthesis of the STGL3 Codon of *Schizochytrium* Lipase

Using RARE CODON CALTOR (https://people.mbi. ucla.edu/sumchan/caltor.html#opennewindow) for rare codon analysis of the *Schizochytrium* lipase gene STGL3. The JAVA Condon Adaption Tool (https://http://www.jcat.de/) was used to codon optimize the STGL3 gene of *E. coli* as the expression host, avoiding the EcoRI and NotI cleavage sites in the optimised sequence. The optimised sequence was synthesized and the recombinant plasmid was constructed by Genscript (Shanghai, China).

Construction and Expression of Recombinant Bacteria

The recombinant plasmid was transformed into *E. coli* BL21 (DE3) competent cells, coated on LB plates containing 100 μg/mL of Kan, and incubated overnight at 37 °C. Three colonies were randomly selected and inoculated into 10 mL of LB liquid medium with a final mass concentration of 100 μg/mL of Kan and incubated at 37 °C for 8 h. The colonies were then transferred to 50 mL of LB liquid medium with a final mass concentration of 100 μg/mL of Kan at 1% of the inoculum. 1 mmol/L IPTG was added to the culture and the cultivation temperature was then reduced to 30 °C and kept shaking at 200 r/min for 12 h. The fermentation broth was collected by centrifugation at 4 °C.

Purification and SDS-PAGE Analysis of Recombinant Lipase STGL3

The recombinant lipase STGL3 was purified and isolated by Ni-NTA affinity chromatography. One g of wet mass expression organism was added to 5 mL of pH 7.0 phosphate buffer (0.2 mol/L) to resuspend, which was treated with an ultrasonic cell grinder (150 W, 5 s operation, 5 s interval, 2 min treatment). The supernatant was collected by centrifugation and combined with a Ni column equilibrated in advance. 9 mL of pH 7.0 phosphate buffer (0.2 mol/L) was used to wash the heteroproteins in three portions, and the target proteins were eluted with 3 mL of equilibrated solution containing 20, 40, 80, 100, 200 and 300 mmol/L imidazole, respectively. The enzyme activity was measured, and the protein content in the supernatant was determined by the Bradford method using bovine serum protein as a standard and SDS-PAGE was performed to detect the protein purification effect [16].

Determination of Lipase Activity

Used p-nitrophenol laurate (p-NPL) as the substrate, lipase activity was determined by adding 600.0 μL of p-NPL solution in a 2.0 mL centrifuge tube, along with 25.0 μL of the enzyme solution at appropriate dilution, and 0.05 mmol/L Tris-HCl pH 8 buffer for blank control. The reaction was terminated with 500.0 μL of anhydrous ethanol, centrifuged at 12000 r/min for 5.0 min, and the absorbance was measured at 410.0 nm.

Definition

The amount of enzyme required to catalyze the hydrolysis of the substrate p-nitrophenol laurate (p-NPP) to produce 1 μmol p-nitrophenol (pNP) in 1 min is 1 enzyme activity unit (U). Where specific enzyme activity is defined as the magnitude of enzyme activity (U/mL) per unit mass of protein (mg) in U/mg.

Effects of Temperature and pH on the Lipase Activity Recombinant Lipase STGL3

The optimum activities temperature of the purified STGL3 was determined by measuring the activity using p-NPL as the substrate at 25°C, 30°C, 35°C, 40°C, 45°C, 50°C and 55°C for the purificed STGL3 using p-NPL as the substrate at pH 7.5; the optimum activities pH were determined at different pH values (4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8) at the optimum temperature.

For determining the thermal stability: the enzyme activity of STGL3 was determined after incubation at 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C at pH 7.5 for 1h and the enzyme activity was considered as 100% without the heat treatment, and then calculating of relative enzyme activity after treatment at different temperatures; For determining the pH stability, STGL3 was diluted in buffer with different pH values 4, 4.5, 5, 5.5, 6 The enzyme activity was measured after one hour of treatment at the optimum temperature at pH 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8, and the enzyme activity after one hour of treatment at the optimum pH

was considered as 100%, and the relative enzyme activity after different pH treatments was calculated.

Effect of Metal Ions and Chemical Reagents on the Lipase Activity of the Recombinant Lipase STGL3

The enzyme activity was determined by adding different 10mM metal ions, 1% sodium dodecyl sulfate (SDS) and ethylene diamine tetraacetic acid (EDTA) as the metal ion chelator to the standard reaction system. The enzyme activity was determined by adding the surface activator Tween 80 and organic reagents such as methanol, ethanol and isopropanol to give a volume fraction of 1% respectively. The enzyme activity without the addition of other chemical reagents was taken as 100%.

Effects of Different Substrate on the Lipase Activity of Recombinant Lipase STGL3

A series of p-nitrophenol fatty acid esters of different alkyl carbon chain lengths were used: p-nitrophenyl acetate (p-NPA), C2; p-nitrophenyl butyrate (p-NPB), C4; p-nitrophenyl valerate (p-NPV), C5; p-nitrophenyl caprylate (p-NPC), C8; p-nitrophenol laurate (p-NPL), C12; p-nitrophenol myristate (p-NPM), C14; p-nitrophenyl palmitate (p-NPP) C16; p-nitrophenyl stearate (p-NPS) C18 as substrates and the chain length specificity of the recombinant lipase was assayed at 35°C, in potassium phosphate buffer pH 7.5 according to standard methods. The recombinant lipase enzyme activity was measured and the relative enzyme activity was calculated and the highest enzyme activity was considered as 100% [17].

Kinetic Parameters of Recombinant Lipase STGL3

For the determination of kinetic parameters, final concentrations of 0.25-1.5 mmol/L p-NPL were added to the enzyme activity assay system, and the kinetic parameters K_m, V_{max} and k_{cat} were calculated by fitting the meter equation curve using the kinetic module of Sigma- Plot software.

Results

Optimization of the Lipase Gene STGL3

The full length of the gene for lipase from *Schizochytrium* is 780 bp and encodes 289 amino acids. After optimizing the codon with reference to the codon preference of E. coli, all rare codons were replaced, the GC content was reduced from 57.47% to 56.55%, and the codon adaptation index was increased from 0.67 to 0.8, which is closer to the ideal value of 1.0 [18].

Inducible Expression of Recombinant Lipase STGL3

The expression strain E. coli BL21(DE3)-pET30a(+)-STGL3 was successfully constructed ; and the control was a strain containing a null plasmid, E. coli BL21(DE3)-pET30a(+). The whole bacteria, supernatant and inclusion body samples were subjected to SDS-PAGE analysis.

As shown above, the positive recombinant bacteria showed a specific band at 32 kDa compared to the original and uninduced strains. There were also no significant difference in the expression of the two proteins as indicated by the SDS-PAGE profiles. The supernatant of the induced expression organism contained the target protein, indicating that the expressed protein was present in the supernatant as a soluble protein. To further determine whether the proteins were both expressed successfully, affinity purification using Ni-NTA agarose resin was used in this paper to further verify the expression effect.

Purification and Sds-Page Analysis of Recombinant Lipase STGL3

In order to obtain higher yields and higher purity of soluble proteins, the crude enzyme solution obtained above was purified by Ni-NTA agarose resin on a Ni column, using different imidazole concentrations for elution of the target protein. The obtained eluates were used for SDS-PAGE analysis and identified by Western blot. As shown in Figure 2, the molecular mass of the recombinant enzyme was approximately 32 kDa, which was consistent with the pattern of post-expression changes of the protein, and all matched the size of the target protein bands. The specific enzyme activity of the crude and pure enzymes was found to be 2.29 μmol/(min•mg), which was 2.3 times higher than that of the crude enzyme (0.29 μmol/(min•mg)), and the protein content was 650.00 mg/L.

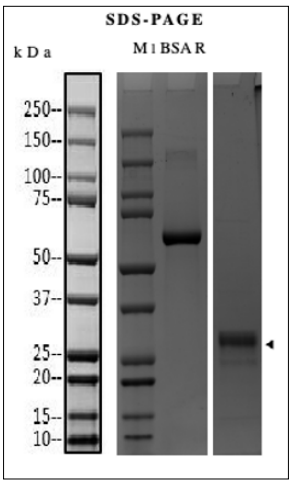

Figure 1: SDS-PAGE Analysis of STGL3 Recombinant

Lane M1: Protein marker; Lane PC1: BSA (1 μg); Lane PC2: BSA (2 μg); Lane NC: whole cells not induced; Lane 1: whole cells induced at 15°C for 16 h; Lane 2: whole cells induced at 37°C for 4 h; Lane NC1: supernatant of uninduced cell lysis; Lane 3: cell lysis supernatant induced at 15°C for 16 h; Lane 4: cell lysis supernatant induced at 37°C for 4 h; Lane NC2: cell lysis precipitate not induced; Lane 5: cell lysis precipitate induced at 15°C for 16 h; Lane 6: cell lysis precipitate induced at 37°C for 4 h

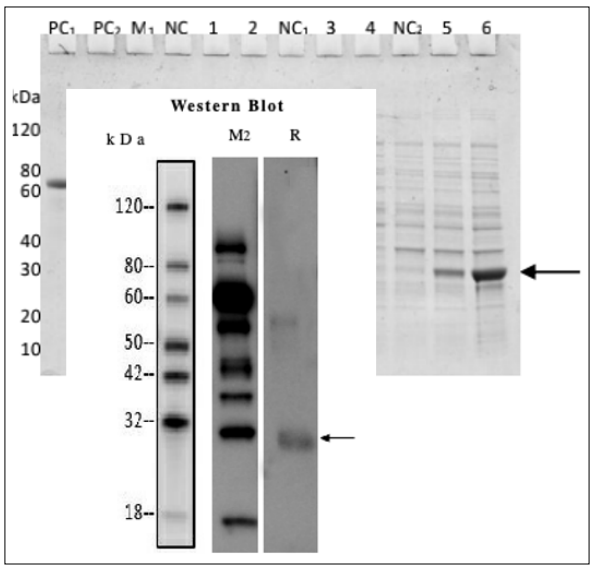

Figure 2: Purification of Inducible Expression Protein
Note: M - Protein Marker; 1 - Recombinant Lipase Purified by HistrapTM Fast Flow Ni column; 2 - crude enzyme solution filtered by semi-permeable membrane

Biochemical Characterization of Recombinant Lipase STGL3
Effect of Temperature on Lipase Activity of Recombinant Lipase STGL3

The effects of temperature on lipase activity and the thermostability of STGL3 at different temperatures were determined using p-NPL as substrate. As shown in the Figure 3, STGL3 was active in the temperature range of 25-55 □, and showed the highest activity at 40 □, and maintained above 64% activity between 35 and 45 □. As shown in Figure 3B, STGL3 still retained more than 80% of enzyme activity after 60 min of treatment at 25-40 □.

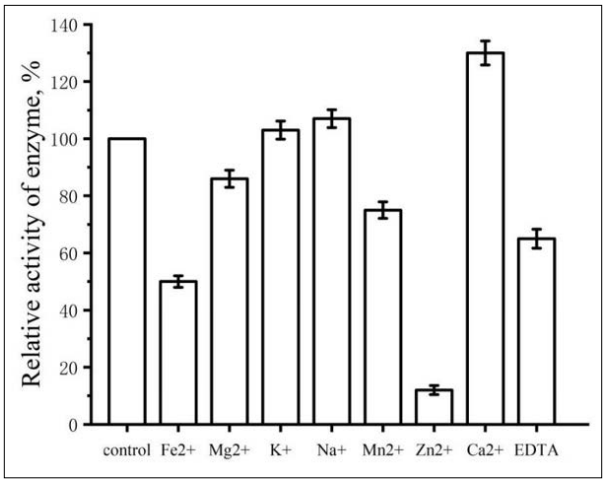

Figure 3: Optimal Temperature and Temperature Stability of Recombinant Lipase

Effect of pH on Lipase Activity of Recombinant Lipase STGL3

The effects of pH on the enzymatic activity of STGL3 were shown in Figure 4. The enzyme activity of STGL3 was measured at pH 4.0-8.0 and display the highest activity at 7.5. The residual enzyme activity of STGL3 was measured after the STGL3 kept at 40 □ for 1 h at different pH conditions, there was a loss of enzyme activity for all of the recombinant lipases.

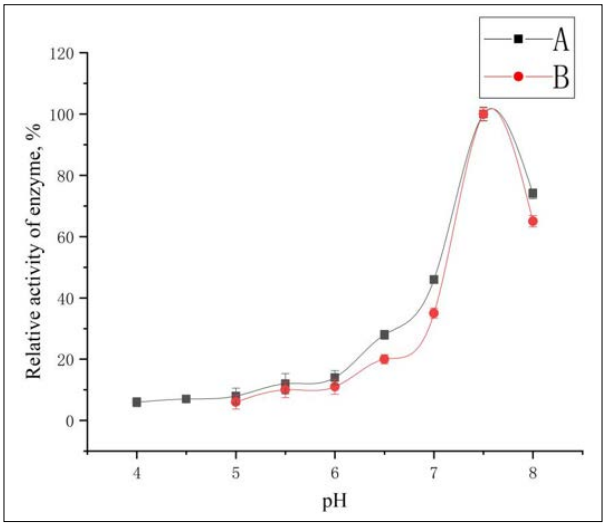

Figure 4: Optimal pH and pH Stability of Recombinant Lipase

Effect of Metal Ions and Chemical Reagents on Lipase Activity of the Recombinant Lipase STGL3

The effects of different metal ions on lipase activity are shown in Figure 5. Among these different metal ions, Ca^{2+} had a promoting effect on lipase activity, which increased lipase activity by 1.28 times; Na^{+} showed weak inhibitory effects on activity of lipase. Mg^{2+}, Mn^{2+}, Zn^{2+} and Fe^{2+} showed stronger inhibitory effects on lipase activity, with the relative enzyme activity dropping to 82%, 76%, 16% and 45%.

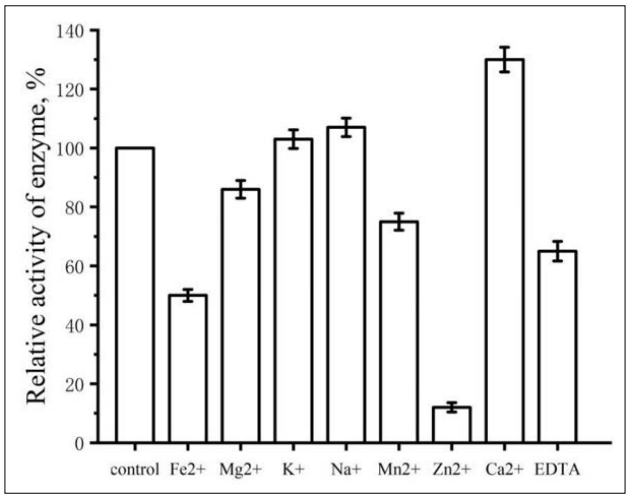

Figure 5: Influence of metal ions on recombinant lipase

EDTA is a good chelating agent, which can form chelates with metal ions. The enzyme maintained about 63% relative activity of control when treated with EDTA. Detergents can change the proportion of lipid in the interface of oil and water, affecting the access of substrate into the active site of enzyme. The activity of STGL3 was determined in the presence of 1% concentrations of several detergents, respectively. The relative enzyme activity of the control group without surfactant was taken as 100% and converted to the relative enzyme activity of each other group. The effect of surfactants on lipase was shown in Figure 6. The effect of non-ionic surfactants Tween 20, Tween 80 and Triton X-100 had an inhibitory effect on enzyme activity. Complete inhibition of STGL3 by sodium dodecyl sulfate (SDS) was observed.

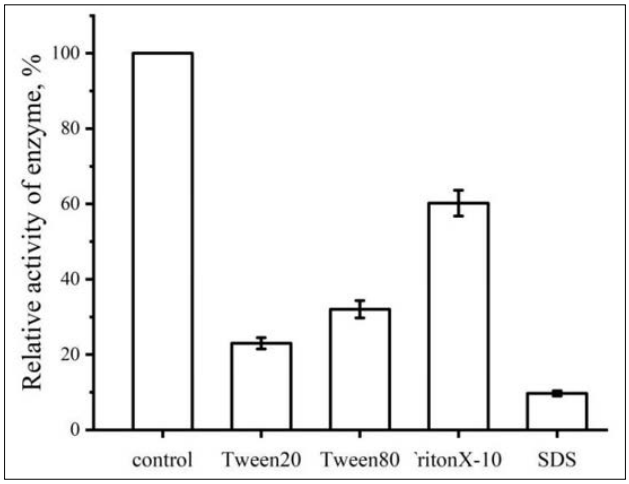

Figure 6: Effect of surfactant on recombinant lipase

The effect of organic solvents on lipase activity was shown in Figure 7, Methanol, ethanol, acetone, isopropanol and butanol decreased the enzyme activity; The STGL3 retained about 85% relative activity in the presence of 1% concentration of methanol, ethanol, butanol and isopropanol; benzene and toluene impaired the enzyme activity of recombinant lipase more. Acetone was a stronger inhibitor of lipase activity, with a 38% loss of enzyme activity after 1 hour of treatment with 1% concentration.

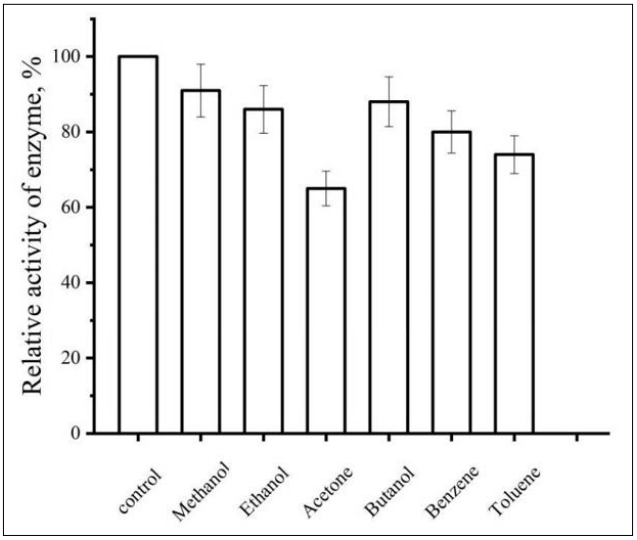

Figure 7: Effect of organic solvents on recombinant lipase

Substrate Specificity of Lipase STGL3

As shown in From Figure 8, the recombinant lipase displayed a preference toward both medium and long carbon chain (C12, C14 and C16) fatty acids, STGL3 showed the highest activity toward p-nitrophenol laurate (p-NPL) with a maximum enzyme activity of 5.40 U/mg and lower lipase activity towards C2, C4 and C8.

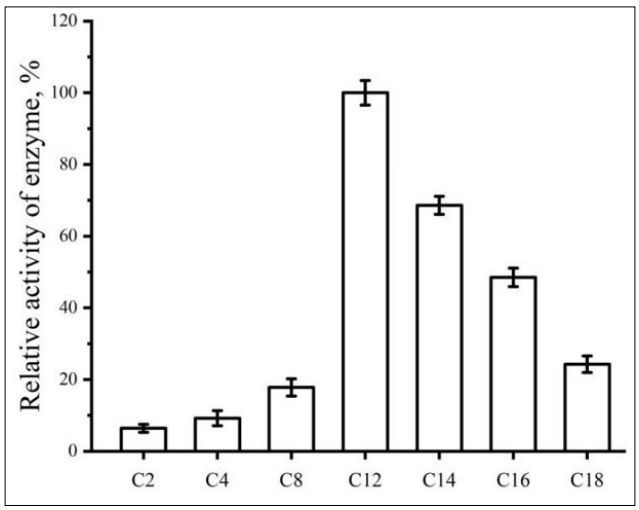

Figure 8: Substrate specificity of recombinant lipase

Kinetic Study of Lipase

The enzyme activity was determined by adding p-NPL at final concentrations of 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 mmol/L to the enzyme activity assay system under optimal conditions, and the kinetic parameters were calculated using the kinetic module of Sigma-Plot software to fit the meter equation curves. The K_m of STGL3 was 0.29 mmol /L, V_{max} was 2.28 mmol /(L•min^{-1}), and K_{cat} was 6.19 S^{-1}.

Discussion

In this study, the codon-optimized gene sequence was synthesized and cloned into, the vector pET-30a(+) was subjected to double digestion reaction using in the location between NdeI and HindIIIrestriction enzyme sites, the target gene fragment obtained after purification was inserted and transferred into *E. coli* TOP10 competent cells, and the expression vector was successfully constructed using sequencing primers for validation. The obtained positive plasmid was transformed into the expression host E.coli BL21 (DE3) and positive recombinants were screened on resistance plates for expression. Under suitable induction conditions, the positive recombinants were ultrasonically fragmented with the empty strain and the supernatant was collected for SDS-PAGE analysis. After affinity purification of the supernatant by Ni-NTA agarose resin, a theoretically consistent protein with a band molecular mass about 32 kDa was obtained.

The optimum reaction temperature for the recombinant lipase STGL3 was 40 °C, and the enzyme activity was remained above 64% between 35 and 40 °C. Above 40 °C, the enzyme activity decreased sharply. For example, the optimum temperature for lipase from *Pseudomonas* strain kb700a The result is consist with others was 35 °C, and the enzyme activity decreased sharply when the temperature was higher than 35 °C; Maharana et al isolated the cold-tolerant red yeast *Rhodotorula* sp. Y-23 lipase from an East Antarctic lake sediment sample at an optimum reaction temperature of 35°C [19, 20]. The recombinant lipase STGL3 has a higher reaction temperature, but is slightly less adaptable to temperature and less stable than lipases of microbial origin. The optimum pH of recombinant lipase STGL3 was 7.5 and it was stable at pH range 4.0 to 8.0. The activity of the enzyme was lost after 1 h of treatment. The *Pseudomonas* sp. purified lipase is active over a wide pH range (5.0-9.0) [21].

The pH optimum of *Pseudomonas fluorescens* lipase obtained by Saadatullah M I et al was 7 [22]. The pH optimum of fungal lipases such as moulds and yeasts is mostly weakly acidic to neutral, but their pH stability ranges widely. Satti S M et al found that a lipase from Bacillus licheniformis NCU CS-5 was stable between pH 6.0 and 9.0. Zhao J et al found that the lipase isolated from SJXYZ strain was able to maintain viability in the pH range of 5.0-9.0. Gao K et al found that *Streptomyces violaceus* OUC-lipase showed optimal activity at pH 9.0 [23-25].

Mg^{2+}, Mn^{2+}, Zn^{2+} and Fe^{2+} showed an inhibitory effect on lipase activity, with Zn^{2+} showing the greatest inhibitory effect. Studies showed that heavy metal ions may react with amino acid residues on the recombinant lipase and change the conformation of the lipase, resulting in a loss of enzymatic activity [26]. The promotive effect of Ca^{2+} on lipase activity has been more frequently reported in the literature [27-29]. It can be concluded that the enzymatic activity of the recombinant lipase STGL3 is not dependent on Ca^{2+}, but plays a stabilizing role in the STGL3 protein structure.

Surfactants can increase the contact surface at the oil-water interface and thus have a beneficial effect on lipase catalytic efficiency [30-32]. The specific targeting of different types of lipases would also have different effects. The non-ionic surfactants Tween 20, Tween 80 and Triton X-100 showed some inhibition of enzyme activity, which is similar in nature to that reported for the lipases produced by Pseudomonas strains and Pseudomonas cepacia strains [33, 34]. The addition of SDS (sodium dodecyl sulfate) at 1% by volume inhibited the enzyme activity stronger, possibly resulting in a change in the configuration of the enzyme's active centre.

The great stability of lipases in organic solvents is the basis for their use in biocatalytic synthesis. The mechanism of the effect of organic solvents on lipases, however, is complex and has not yet been well explained. The activity of lipases in organic solvents is not only influenced by the organic solvent, but also by the source of the lipase, the method of extraction and isolation [35]. Acetone was a strong inhibitor of recombinant lipase, with a 38% loss of enzyme activity after 1 h of treatment with 1% acetone, which was

related to the polarity of acetone. Because lipase as a whole is a typical structure of a hydrophilic protein, with a hydrophobic core and a hydrophilic surface, acetone is more polar and it can crowd out the essential water layer on the enzyme surface, changing the conformation of the catalytic centre and thus inactivating the enzyme [36].

Through substrate specificity analysis, the recombinant lipase showed higher activity towards C12, C14 and C16, which is consistent with the pattern of glyceride hydrolysis in the late fermentation stage of *Schizochytrium*, and it is hypothesized that the lipase gene STGL3 may be involved in glyceride hydrolysis in the late fermentation stage, and the preferential medium to long carbon chain fatty acids are produced in *Schizochytrium* under the action of hydrolase.

K_m indicates the affinity between the enzyme and the substrate, and the larger the K_m value, the weaker the affinity. The K_m values of STGL3 were lower than those reported in the literature for S. *mucilaginosa* (0.39 mmol•L^{-1}) [37]. The k_{cat}/K_m is considered to be the most comprehensive measure of the catalytic capacity of the enzyme, with higher catalytic efficiency for larger values. kcat/K_m for this enzyme was 21.34 s^{-1} •L•$mmol^{-1}$, which was greater than all other substrates and also higher than the recombinant lipase of *Sphaerococcus* thermophilus reported by Noro [38]. Lipase was higher. This kinetic study confirms that STGL3 has not only a wide range of substrates, but also a high catalytic efficiency.

Conclusions

A novel l lipase from marine *Schizochytrium* pombe has been characterized in this work. The recombinant lipase STGL3 protein molecular mass was about 32 kDa, and the purified lipase had a specific enzyme activity of 2.29 μmol/(min•mg), which was 2.3 times higher than before, and a protein content of 650.00 mg/L. The optimum reaction temperature and pH for STGL3 were 40 °C and 7.5. Na^+, K^+, Li^+, Mg^{2+}, Mn^{2+} and Ca^{2+} all significantly promoted to lipase activity of STGL3, whereas Ag^+, Cu^{2+} and Fe^{3+} significantly inhibited the enzyme. The recombinant lipases showed a preference toward pNP-esters with medium and longer acyl-chains.(C12, C14 and C16). , while the lipase showed relatively weak lipase activity against short carbon chains. The kinetic constants of lipase were 0.29 mmol/L for K_m, 2.28 mmol/(L•min^{-1}) for Vmax, and 6.19 S^{-1} for Kcat. These results suggests that the recombinant lipases play an important role in the hydrolysis of long-chain fatty acids in triglycerides in *Leptospira* cells, and provided information a reference for explaining the molecular mechanism of lipid re-consumption in *Leptospira* during late fermentation.

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