International Journal of General Practice Nursing, 2025, Volume 3, No Special
https://ojs.bbwpublisher.com/index.php/IJGPN
ISSN Online: 2981-9423
ISSN Print: 2981-9415

Screening of Lipase-Producing Bacterial Strains and Analysis of Enzyme Production Characteristics

Chenguang Zhu[1,2]*, Yongrui Cai[2], Zheqing Zhu[3], Sinv Zheng[2]

[1]College of Health Management, Shanghai Jian Qiao University, Shanghai 201306, China
[2]School of Life Sciences, Shanghai University, Shanghai 200444, China
[3]Junior High School Department, The Affiliated School of Shanghai University, Shanghai 200444, China

**Corresponding author: Chenguang Zhu, zhuchenguang@gench.edu.cn*

Abstract: Lipase, as an important biological catalyst, hydrolyzes triglycerides into fatty acids, diglycerides, monoglycerides, and glycerol, and is widely used in food, leather, pharmaceutical, and daily chemical industries, particularly in the food sector. Lipase is abundantly present in microorganisms and animals, with lipase-producing microorganisms being suitable for large-scale industrial production due to their rapid reproduction, short cycles, and diverse species. However, since there are currently few strains capable of producing high lipase activity, the increasing demand for industrial applications has made the search for more novel lipase-producing strains increasingly crucial. In this study, from 15 bacterial strains initially isolated from soil in the laboratory, one lipase-producing strain, zjy1-5, was identified through preliminary screening on glycerol-containing plates. Through 16S rDNA sequence analysis and Gram staining microscopic observation, the strain was identified as *Rhodococcus rhodochrous*, a Gram-positive bacterium. Subsequently, the fermentation conditions for lipase production were optimized, revealing that glucose was the optimal carbon source and 37°C the optimal culture temperature for lipase production. This research provides an experimental basis for further development and utilization of bacterial lipase.

Keywords: Lipase; Bacteria; Glyceride; 16S rDNA; *Rhodococcus*

Online publication: December 31, 2025

1. Introduction

Lipase, also known as triacylglycerol hydrolase, is widely present in plants, animals, and microorganisms. It can catalyze the hydrolysis of natural substrate oils at the oil-water interface, as well as various chemical reactions such as ester exchange, ester synthesis, alcoholysis, transesterification, acid hydrolysis, and ammonolysis. It is one of the important enzymes in industrial enzyme preparations and is widely used in industries such as oil processing, food, medicine, and daily chemical products [1,2]. Microbial lipases are widely present in bacteria, yeast, and mold, with characteristics such as diverse species, short cycles, fast reproduction, susceptibility to genetic variation, wide

temperature and pH range of action, and good substrate specificity [3,4]. They are suitable for industrial production and can produce high-purity products. They have significant value in both basic theoretical research and practical applications. Microbial lipases have become the main source of lipase for industrial production [5–7].

Lipases derived from microorganisms are generally secreted extracellular enzymes, and the main fermentation microorganisms include *Aspergillus niger*, *Candida albicans*, etc. [8,9]. A small number of bacteria are used as commercial sources, such as colorless bacteria, alkali-producing bacteria, tuberous bacteria, spore-forming bacteria, and *Pseudomonas* [10–16]. Compared with fungi, the lipase activity of bacteria is relatively low, and currently, no bacterial species with lipase activity comparable to fungi, such as *Aspergillus* and yeast, have been identified [17–20]. Due to the existence of a large number of bacterial species attached to special habitats in nature, there is still a lot of exploration space to continue searching for efficient lipase-producing bacterial strains.

Our preliminary work isolated 15 strains of bacteria from various environments such as soil, water, and trees. In this study, we plan to screen lipase-producing bacteria from these bacteria using the flat plate glycerol ester hydrolysis circle method, identify and analyze the strains, and optimize the fermentation conditions related to enzyme production characteristics. This will provide effective strains and reference experimental data for the industrial development and utilization of lipases in the future.

2. Materials and methods

2.1. Preliminary screening of lipase-producing bacteria using an enzyme-producing culture medium

Use a solid agar plate to screen the culture medium, sterilize the prepared 300 mL of culture medium, and place it on a clean bench for operation. The formula of culture medium was described in the reference [21], which includes 0.3% (w:v) of yeast extract, 0.5% (w:v) of trypton, 1% (w:v) of glucose, 1% (v:v) of triglyceride butyrate emulsion, 1.5% (w:v) of agar powder. Take clean culture dishes, mark the date and bacterial number, pour 15–20 mL of culture medium into each dish, and let it solidify. Then draw lines and culture separately to complete the inoculation operation. Seal the tablet with sealing film and place it upside down in a 37°C constant temperature incubator for cultivation. After 48 hours of cultivation, remove and confirm the growth of the strains in each culture dish, and observe whether there are obvious hydrolysis transparent circles around the colonies. Record the growth of bacteria and the presence and size of hydrolysis rings in each culture dish, select strains with transparent hydrolysis rings, and store them in glycerol tubes. Subsequent experiments will be based on this strain to conduct a preliminary analysis of its enzyme production characteristics.

2.2. The influence of different carbon sources on the enzyme production of bacterial strains

Different carbon sources, such as glucose, sucrose, maltose, starch, and corn flour, were used as fermentation media to test the effect of different carbon sources on the enzyme activity of the experimental strain. The formula of fermentation medium, which was described in the reference [22], includes 1.5 g of trypton, 0.1 g of MgSO4·7H2O, 0.1 g of K2HPO4, 1 g of carbon source, 100 mL. Take 5 mL of each prepared liquid culture medium with different carbon sources and transfer them to sterilized and dried test tubes. Number and date the tubes, and transfer the needle-sized bacterial colonies into the tubes. Place the tubes in a constant temperature shaker at 37°C and continuously culture at 200 rpm for 48 hours. Take a clean culture dish, record the number and date, pour in 15–20 mL of culture medium, and let it solidify. Dip the test strain with a needle tip and place 5

different positions on each plate. After sealing with sealing film, place it upside down in a constant temperature incubator at 37℃ for cultivation. After 48 hours of cultivation, take out the samples and confirm the growth of the strains in each plate. Observe whether there are obvious hydrolysis zones around the colonies and record the corresponding data. By comparing the ratio of the radius of the transparent hydrolysis zone to the radius of the colonies in different plates, determine the optimal carbon source for the experimental strain to produce lipase. The above experiment consists of five biological replicates, each containing three technical replicates, and the data obtained is the average of the above replicates.

2.3. The impact of different temperatures on enzyme production by bacterial strains

Prepare a fermentation medium with glucose as the carbon source, sterilize it, and place it on a clean bench. The formula of fermentation medium, which was described in the reference [22], includes 1.5 g of trypton, 0.1 g of MgSO4·7H2O, 0.1g of K2HPO4, 1 mL of triglyceride butyrate emulsion, 1.5 g of agar powder, 1 g of the most suitable carbon source, 100 mL. Take a clean culture dish and record the number and date. Study the effects of five temperatures, including 28℃, 31℃, 34℃, 37℃, and 41℃, on enzyme production. Pour 15–20 mL of culture medium into each petri dish, solidify, and sterilize. Dip the needle tip-sized bacteria into each plate and place them at 5 different positions. Seal with sealing film and incubate upside down at different temperatures. After 48 hours of cultivation, remove and confirm the growth of the strains in each plate. Observe whether there are obvious transparent circles around the inoculated colonies and record the corresponding data. By comparing the ratio of the radius of different transparent hydrolysis circles to the radius of the colonies, determine the optimal temperature for the strains to produce lipase. The above experiment consists of five biological replicates, each containing three technical replicates, and the data obtained is the average of the above replicates.

2.4. Gram staining of bacterial strain

The Gram staining was conducted according to the procedure described in reference [23]. A small amount of the bacterial strain was transferred to a sterile water droplet on a glass slide, and the droplet was mixed and allowed to air-dry at room temperature. Then, an appropriate amount of ammonium oxalate crystal violet stain was added for one minute. The stain was poured off and rinsed with water until colorless. Next, the cells were stained with iodine solution for one minute, rinsed with water, and decolorized with 95% ethanol for 30 seconds. The cells were rinsed with water again and counterstained with safranin for one minute, rinsed with water, and air-dried. The sample was prepared and observed under an optical microscope, and the color and morphology of the cells were recorded.

2.5. 16S rDNA sequencing of bacterial strain zjy1-5

The partial DNA sequence for the 16S rRNA gene of strain zjy1-5 was amplified by PCR using the universal primers 27F: 5’-AGAGTTTGATCCTGGCTCAG-3’ and 1492R: 5’-GGTTACCTTGTTACGA CTT-3’ [24]. The amplification was conducted using T100 thermocycler (BIO-RAD) with the following cycling parameters: 5 min at 94℃, followed by 30 cycles of 30s at 94℃, 30s at 50℃, 90s at 72℃ with final extension for 10 min at 72℃. The amplified product was purified by AxyPrepTM PCR Cleanup Kit (Axygen Biosciences), then was ligated into the pMD18-T vector (TaKaRa Co.) and was sequenced by Beijing Tsingke Biotechnology Company Limited. Homologous analysis of nucleic acid sequence was performed online with GenBank BLAST website.

3. Results

3.1. Screening of lipase-producing strains

One lipase-producing strain zjy1-5 was screened from 15 bacterial strains by culturing on a medium plate containing glycerol triacetate emulsion. A clear hydrolysis circle appeared around the colonies on the plate (see **Figure 1**), indicating that strain zjy1-5 was selected as the material for subsequent enzyme production characteristic analysis experiments.

3.2. Effects of different carbon source culture media on lipase production by strain zjy1-5

Glucose, sucrose, maltose, starch, and corn flour were used as the sole carbon source of the fermentation medium. The ratio of the measured transparent circle radius to the colony radius is shown in **Table 1**. When glucose is selected as the sole carbon source, the ratio of the radius of the transparent circle around the colony of strain zjy1-5 to the colony radius is the highest, and the lipase enzyme activity is the highest. Therefore, glucose is the most suitable carbon source for zjy1-5 enzyme production medium.

3.3. The effect of different temperatures on lipase production by strain zjy1-5

Study the effects of five temperatures, including 28℃, 31℃, 34℃, 37℃, and 41℃, on enzyme production. After 48 hours of constant temperature cultivation, the ratio of the transparent circle radius to the colony radius measured is shown in **Table 2**. When cultured at a constant temperature of 37℃, the ratio of the radius of the transparent circle around the colony of strain zjy1-5 to the colony radius is the highest, and the lipase enzyme activity is the highest. Therefore, 37℃ is the optimal temperature for enzyme cultivation process.

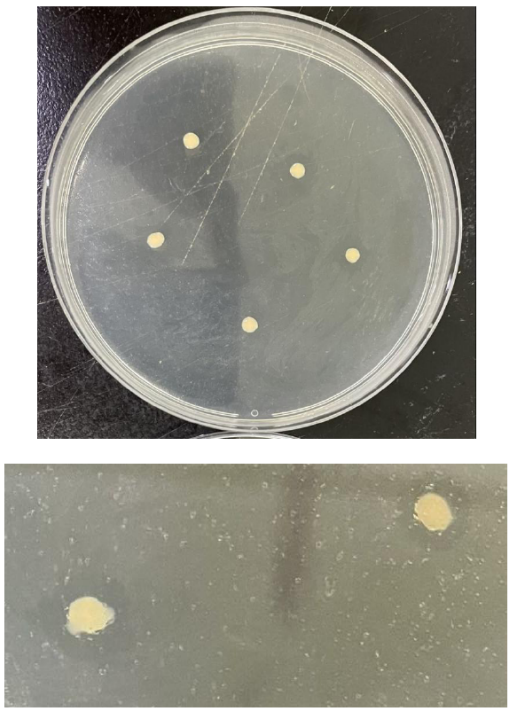

Figure 1. Colony morphology of strain zjy1-5 and its hydrolysis circle on an ester-containing plate (top) and local magnified image (bottom)

Table 1. Enzyme production capacity of strain zjy1-5 under different carbon sources

Types of carbon sources	Ratio of transparent circle radius to colony radius (including variance)
glucose	2.13 ± 0.04
sucrose	1.81 ± 0.05
maltose	1.86 ± 0.03
starch	1.89 ± 0.06
corn flour	1.71 ± 0.02

Table 2. Enzyme production capacity of strain zjy1-5 at different cultivation temperatures

Culture temperature	Ratio of transparent circle radius to colony radius (including variance)
28℃	1.78 ± 0.03
31℃	1.95 ± 0.04
34℃	2.08 ± 0.02
37℃	2.21 ± 0.03
41℃	2.13 ± 0.05

3.4. Characterization of bacterial strain zjy1-5

The Gram staining characteristics of strain zjy1-5 are shown in **Figure 2**. After culturing LB agar plates for a period of time, the colonies turned red and had a smooth surface (data not shown). Under a 100x magnification of an optical microscope, zjy1-5 bacterial cells appeared as short rod-shaped cells, and Gram staining showed that the cells were purple, indicating that strain zjy1-5 is a Gram-positive bacterium. The genome of the zjy1-5 strain was extracted and subjected to 16S rDNA gene sequencing analysis. The obtained sequences were compared for homology using the NCBI website BLAST. It was found that the zjy1-5 strain had the highest similarity in 16S rDNA sequence with *Rhodococcus rhodochrous*, a member of the *Rhodococcus* genus (see **Figure 3**). Therefore, the zjy1-5 strain was identified as *Rhodococcus rhodochrous*, a member of the *Rhodococcus* genus.

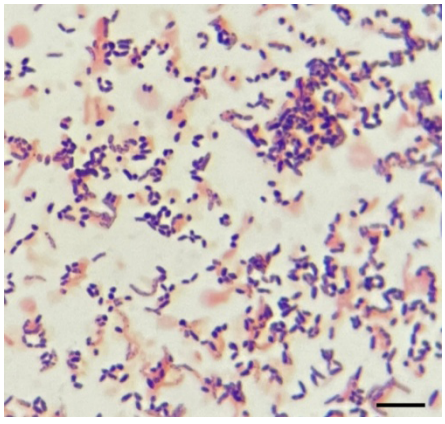

Figure 2. Cell morphology of strain zjy1-5 after Gram staining (bar = 10 μm)

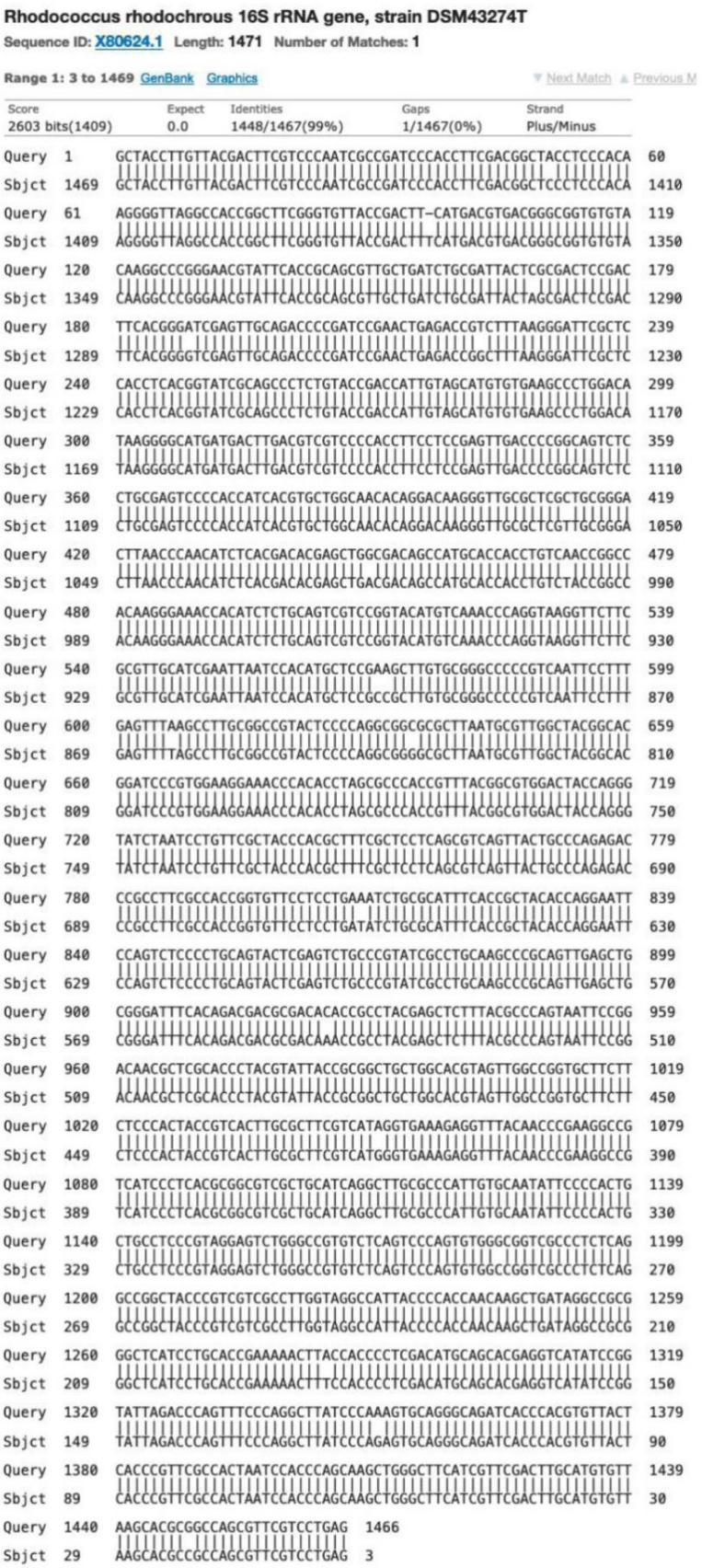

Figure 3. Alignment results of 16S rDNA sequence of strain zjy1-5 with Rhodococcus rhodochrous sequence

4. Discussion

This study analyzed the enzyme production characteristics of the experimental bacteria, involving different carbon sources and cultivation temperatures. Glucose serves as a preferred carbon source for many microorganisms due to its fundamental role in central carbon metabolism. As a monosaccharide, glucose enters the glycolytic pathway directly without requiring additional hydrolytic enzymes, enabling rapid ATP generation and production of metabolic precursors. The efficient assimilation of glucose provides ample acetyl-CoA pools that serve as essential precursors for both fatty acid biosynthesis and the synthesis of amino acids needed for enzyme production [25]. This metabolic advantage may explain why glucose outperformed complex carbon sources like starch, maltose, and corn flour in this study, as these require additional enzymatic steps for assimilation. In addition, the superior lipase production observed with glucose may be attributed to its role as a metabolic signal modulating enzyme biosynthesis. In several bacterial systems, glucose has been shown to influence the expression of extracellular enzymes through complex regulatory networks. For instance, studies with *Pseudomonas aeruginosa* strains have demonstrated significant lipase production when glucose served as the primary carbon source [26]. Similarly, research on *Candida rugosa* revealed that glucose can stimulate lipase secretion under certain cultivation conditions, although its effects appear to be strain-specific and dependent on cultivation parameters [27].

The optimal lipase production at 37°C suggests bacterial strain zjy1-5 is a mesophile, with temperature adaptation profiles similar to many well-characterized lipase-producing bacteria. This temperature likely represents an optimal balance between bacterial growth rate and enzyme stability. At temperatures below 37°C (28°C, 31°C, and 34°C in this study), reduced molecular kinetic energy may slow down metabolic reactions, membrane transport, and protein translation, thereby decreasing overall lipase production. Conversely, temperatures above 37°C (such as the 41°C condition tested) may induce thermal stress, leading to protein denaturation, impaired membrane integrity, and potential induction of heat-shock responses that divert cellular resources away from lipase production [28]. The 37°C optimum correlates well with the temperature profiles of many characterized lipases. For instance, lipase from *Candida* species typically exhibits optimal activity in the range of 35–37°C [27]. Similarly, a lipase from *Bacillus coagulans* BTS-3 showed optimal production of this enzyme at 37°C, which supported robust bacterial growth [29]. The temperature optimum for lipase production is intrinsically linked to the overall metabolic rate of the bacterium. At 37°C, the combined processes of transcription, translation, folding, and secretion likely operate at their most efficient coordination. Research on a psychrophilic *Geotrichum* lipase demonstrated that while the enzyme itself had low thermal stability, its production was optimal at temperatures supporting substantial biomass generation [30]. This parallels our observation that 37°C supported optimal lipase production, suggesting this temperature represents the ideal compromise between growth-associated production and enzyme stability.

In subsequent in-depth experiments, the temperature gradient range can be reduced to more accurately determine the optimal cultivation temperature for the experimental bacteria. According to current literature, the optimal carbon source for the culture medium not only includes a single carbon source, but may also be influenced by a composite carbon source [31–34]. When optimizing the enzyme production conditions of the experimental bacteria, it was found that using 3% olive oil plus 2% glucose as a composite carbon source in the culture medium was more favorable for the enzyme production of the experimental bacteria [35]. This paper only involves the selection of a single carbon source for optimal carbon source selection. Subsequent experiments can investigate whether different composite carbon sources have a synergistic effect on the lipase production ability of experimental bacteria, and compare the differences in the effects of single carbon sources and composite carbon sources on the lipase production ability of experimental bacteria. In addition, whether the length of cultivation

time, the selection of different single and composite nitrogen sources in the culture medium, the initial pH value of the culture medium, and the addition of different metal ions to the culture medium will have different effects on the ability of the experimental bacteria to produce lipase is something that can be further studied and explored.

This paper involves the comparison of lipase production ability under different factors and conditions, which is achieved by comparing the ratio of transparent circle radius to colony radius. Whether a lipase-producing strain can be put into large-scale industrial applications requires quantitative detection and analysis of its enzyme activity and the impact of the enzyme production environment on enzyme activity stability. According to current literature, in order to screen for advantageous strains with high lipase production, the experimental strains are subjected to initial screening and re-screening [36–39]. Re-screening is generally performed by measuring enzyme activity to select the more advantageous strains. The methods for measuring lipase activity usually include acid-base titration, spectrophotometry, immunoassay, and fluorescence assay [40–43]. Subsequently, the enzyme activity of the experimental strains can be quantitatively detected through the above methods, providing an experimental basis for further screening and cultivation of high-yield lipase strains for development and utilization.

5. Conclusion

This study used 15 strains of bacteria isolated from soil in the early stage of the laboratory as materials, and screened a lipase-producing strain, zjy1-5, using the transparent circle method on a tributyl glycerol plate, which reflects the enzyme activity by the ratio of the radius of the hydrolysis transparent circle generated around the colony in the plate to the radius of the colony. The effects of different carbon sources, including glucose, sucrose, maltose, corn flour, and soluble starch, and different temperatures including 28℃, 31℃, 34℃, 37℃, and 40℃ on the lipase activity of strain zjy1-5 were studied. The results showed that the optimal carbon source for the culture medium of the lipase-producing strain was glucose, and the optimal culture temperature was 37℃. Based on the analysis of 16S rDNA sequence and cell Gram staining results, zjy1-5 bacteria were identified as *Rhodococcus rhodochrous* of the *Rhodococcus* genus, which is a Gram-positive bacterium.

Funding

The 2025 Doctoral Science Foundation of Shanghai Jian Qiao University (BBYQ202524)

Disclosure statement

The authors declare no conflict of interest.

References

[1] Joseph B, Ramteke PW, Thomas G, 2008, Cold Active Microbial Lipases: Some Hot Issues and Recent Developments. Biotechnol Adv, 26(5): 457–470.

[2] Davis BG, Boyer V, 2001, Biocatalysis and Enzymes in Organic Synthesis. Nat Prod Rep, 18: 618–640.

[3] Wang L, Cai Y, Liao X, et al., 2008, Optimization of Fermentation Conditions for a Low-Temperature Lipase-Producing Strain. J Food Sci Biotechnol, 27(3): 114–119.

[4] Niu D, Zhang Y, 2002, Selection of an Alkaline Lipase-Producing Strains and Optimalization of Its Fermentation

Conditions. Food Ferment Ind, 29(5): 28–31.

[5] Li J, Shen W, Fan G, et al., 2018, Screening, Purification and Characterization of Lipase from Burkholderia pyrrocinia B1213. 3 Biotech, 8(9): 387.

[6] Ramani K, Chockalingam E, Sekaran G, 2010, Production of a Novel Extracellular Acidic Lipase from Pseudomonas gessardii Using Slaughterhouse Waste as a Substrate. J Ind Microbiol Biotechnol, 37(5): 531–535.

[7] Kantak JB, Bagade AV, Mahajan SA, et al., 2011, Isolation, Identification and Optimization of a New Extracellular Lipase-Producing Strain of Rhizopus sp. Appl Biochem Biotechnol, 164(7): 969–978.

[8] Rasol R, Rashidah AR, Nazuha RS, et al., 2014, Psychrotrophic Lipase Producers from Arctic Soil and Sediment Samples. Pol J Microbiol, 63(1): 75–82.

[9] Kiplimo D, Mugweru J, Kituyi S, et al., 2019, Diversity of Esterase and Lipase-Producing Haloalkaliphilic Bacteria from Lake Magadi in Kenya. J Basic Microbiol, 59(12): 1173–1184.

[10] Dahiya P, Arora P, Chaudhury A, et al., 2010, Characterization of an Extracellular Alkaline Lipase from Pseudomonas mendocina M-37. J Basic Microbiol, 50(5): 420–426.

[11] Li Z, Tang X, Huang W, et al., 2011, Rheology, Microstructure, and Baking Characteristics of Frozen Dough Containing Rhizopus chinensis Lipase and Transglutaminase. Cereal Chem, 88(6): 596–601.

[12] Cho SS, Park DJ, Simkhada JR, et al., 2012, A Neutral Lipase Applicable in Biodiesel Production from a Newly Isolated Streptomyces sp. CS326. Bioprocess Biosyst Eng, 35(1–2): 227–234.

[13] Cherif S, Mnif S, Hadrich F, et al., 2011, A Newly High Alkaline Lipase: An Ideal Choice for Application in Detergent Formulations. Lipids Health Dis, 10: 221.

[14] Rashid N, Shimada Y, Ezaki S, et al., 2001, Low-Temperature Lipase from Psychrotrophic Pseudomonas sp. Strain KB700A. Appl Environ Microbiol, 67(9): 4064–4069.

[15] Zhang B, Yang J, Shu H, et al., 2007, Screening, Identification of Lipase Production Bacteria and Optimization of the Lipase Production. Biotechnology, 17(1): 23–26.

[16] Guo W, Guo X, Yuan H, et al., 2013, Screening and Identification of Efficiently-Producing Fungi from Lipase and Optimization of Its Lipase-Producing Condition. Feed Ind, 34(4): 39–44.

[17] Mo Q, Liu A, Guo H, et al., 2016, A Novel Thermostable and Organic Solvent-Tolerant Lipase from Xanthomonas oryzae pv. oryzae YB103: Screening, Purification and Characterization. Extremophiles, 20(2): 157–165.

[18] Das A, Shivakumar S, Bhattacharya S, et al., 2016, Purification and Characterization of a Surfactant-Compatible Lipase from Aspergillus tamarii JGIF06 Exhibiting Energy-Efficient Removal of Oil Stains from Polycotton Fabric. 3 Biotech, 6(2): 1–8.

[19] Ayaz B, Ugur A, Boran R, 2015, Purification and Characterization of Organic Solvent-Tolerant Lipase from Streptomyces sp. OC119-7 for Biodiesel Production. Biocatal Agric Biotechnol, 4(1): 103–108.

[20] Ungcharoenwiwat P, H-kittikun A, 2015, Purification and Characterization of Lipase from Burkholderia sp. EQ3 Isolated from Wastewater from a Canned Fish Factory and Its Application for the Synthesis of Wax Esters. J Mol Catal B Enzym, 115: 96–104.

[21] Wen J, Shi B, Wu W, et al., 2008, Isolation and Identification of a Lipase-Producing Strain of Bacillus subtilis FS321 and Its Preliminary Optimization for Lipase Production. Food Ferment Ind, 34(2): 37–41.

[22] Shang H, 2009, Screening and Molecular Identification of Lipase-Producing Strain. J Hebei Agri Sci, 13(10): 50–52, 81.

[23] Gao H, Wu G, (eds), 2015, Laboratory Manual for Microbiology, Higher Education Press, Beijing, China, 29–30.

[24] Moreno C, Romero J, Espejo RT, 2002, Polymorphism in Repeated 16S rRNA Genes is a Common Property of Type Strains and Environmental Isolates of the Genus Vibrio. Microbiology (Reading), 148: 1233–1239.

[25] Huo Y, Danecka W, Farquhar I, et al., 2025, The Type of Carbon Source Not the Growth Rate It Supports Can Determine Diauxie in Saccharomyces cerevisiae. Commun Biol, 8: 325.

[26] Kantak JB, Bagade AV, Mahajan SA, et al., 2011, Isolation, Identification and Optimization of a New Extracellular Lipase-Producing Strain of Rhizopus sp. Appl Biochem Biotechnol, 164(7): 969–978.

[27] Dalmau E, Montesinos JL, Lotti M, et al., 2000, Effect of Different Carbon Sources on Lipase Production by Candida rugosa. Enzyme Microb Technol, 26(9–10): 657–663.

[28] Nguyen K, Lee CW, 2025, Catalytic His-Loop Flexibility Drives High Activity in Hyperthermophilic Esterase EstE1 While Preserving Structural Stability. Microbiol Spectr, 13(10): e0100325.

[29] Kumar S, Kikon K, Upadhyay A, et al., 2005, Production, Purification, and Characterization of Lipase from Thermophilic and Alkaliphilic Bacillus coagulans BTS-3. Protein Expr Purif, 41(1): 38–44.

[30] Qiao H, Zhang W, Guan W, et al., 2017, Enhanced Expression of Lipase I from Galactomyces geotrichum by Codon Optimisation in Pichia pastoris. Protein Expr Purif, 138: 34–45.

[31] Liu G, Hu S, Li L, et al., 2015, Purification and Characterization of a Lipase with High Thermostability and Polar Organic Solvent-Tolerance from Aspergillus niger AN0512. Lipids, 50(11): 1155–1163.

[32] Tripathi R, Singh J, Bharti RK, et al., 2014, Isolation, Purification and Characterization of Lipase from Microbacterium sp. and Its Application in Biodiesel Production. Energy Procedia, 54: 518–529.

[33] Bae JH, Kwon MH, Kim IH, et al., 2014, Purification and Characterization of a Cold-Active Lipase from Pichia lynferdii Y-7723: pH-Dependent Activity Deviation. Biotechnol Bioprocess Eng, 19(5): 851–885.

[34] Shu Z, Lin H, Shi S, et al., 2016, Cell-Bound Lipases from Burkholderia sp. ZYB002: Gene Sequence Analysis, Expression, Enzymatic Characterization, and 3D Structural Model. BMC Biotechnol, 16(1): 38.

[35] Miao C, Luo W, Lv P, et al., 2014, Screening, Identification of the Lipase-Producing Strains and Optimization of the Lipase Production. Acta Energiae Solaris Sinica, 35(9): 1708–1714.

[36] Bora L, 2014, Purification and Characterization of Highly Alkaline Lipase from Bacillus licheniformis MTCC 2465: A Study of Its Detergent Compatibility and Applicability. J Surfactants Deterg, 17(5): 889–898.

[37] Hong Y, Shi P, Lu H, et al., 2015, A Thermophilic β-Mannanase from Neosartorya fischeri P1 with Broad pH Stability and Significant Hydrolysis Ability of Various Mannan Polymers. Food Chem, 173(173): 283–289.

[38] Aulitto M, Fusco S, Limauro D, et al., 2019, Galactomannan Degradation by Thermophilic Enzymes: A Hot Topic for Biotechnological Applications. World J Microbiol Biotechnol, 35(2): 32.

[39] Sadaqat B, Dar MA, Sha C, et al., 2024, Thermophilic β-Mannanases from Bacteria: Production, Resources, Structural Features and Bioengineering Strategies. World J Microbiol Biotechnol, 40(4): 130.

[40] Chen X, Tian Z, Zhou H, et al., 2023, Enhanced Enzymatic Performance of β-Mannanase Immobilized on Calcium Alginate Beads for the Generation of Mannan Oligosaccharides. Foods, 12(16): 3089.

[41] Sun D, Li C, Cui P, et al., 2022, Reshaping the Binding Channel of a Novel GH113 Family β-Mannanase from Paenibacillus cineris (PcMan113) for Enhanced Activity. Bioresour Bioprocess, 9(1): 17.

[42] Ahirwar S, Soni H, Rawat HK, et al., 2016, Experimental Design of Response Surface Methodology Used for Utilisation of Palm Kernel Cake as Solid Substrate for Optimised Production of Fungal Mannanase. Mycology, 7(3): 143–153.

[43] Li YX, Wang NN, Yan QJ, et al., 2022, A Novel Neutral Thermophilic β-Mannanase from Malbranchea cinnamomea for Controllable Production of Partially Hydrolyzed Konjac Powder. Appl Microbiol Biotechnol, 106(5–6): 1919–1932.