Screening for Extremophilic Lipase Producing Bacteria: Partial Purification and Characterization of Thermo-Halophilic, Solvent Tolerant Lipase from Bacillus sp.

Cathbert Nomwesigwa*, Nehad Noby, Sherif Hammad, and Ahmed Abdel-Mawgood

***Abstract*—The introduced study aims to isolate potent lipase producing strain from extreme habitats. The aim is extended to characterize the produced enzyme with respect to the effect of salt, temperature, pH and different organic solvents. Tween 80 plate method was used for discriminating lipase producing strains in the isolation step. Lipase activity of the selected isolates was confirmed through Rhodamine B plate assay. The enzyme of the most potent isolate was partially purified using 80% ammonium sulphate, quantified, and characterized using the synthetic substrate, para nitrophenyl laurate (pNP-C12). Twenty-three lipase-producing isolates were screened from different soil samples in Wadi El-Natrun, Egypt. Among the tested strains, a thermo- halo-tolerant bacterial isolate identified as Bacillus sp. was the most potent in terms of lipase production under high temperature and high salt concentration. The enzyme displayed its optimal activity at 60 ºC and 2 M NaCl. Moreover, it showed a robust stability in 4M NaCl up to 2 h incubation time. Non-polar solvents have significantly raised the enzyme activity by 300% and 350% for ethyl acetate and n-hexane, respectively. The obtained data introduces useful insights for applying lipases in chemical catalysis and food processing fields.**

***Index Terms*—Lipase, thermo-tolerant bacteria, halophilic bacteria, partial purification, enzyme activity, enzyme stability**

I. Introduction

Lipases are triglyceride hydrolases (EC 3.1.1.3) with α/β-hydrolase fold, capable of hydrolyzing fats and oils into diglycerides, monoglycerides, fatty acids and glycerol. They are classified under serine hydrolases and do not require cofactors [1].

Lipases are very appealing industrial catalysts due to their chemo-regio- and enantioselectivity, as well as their efficiency in catalyzing several reactions, including acidolysis, esterification, alcoholysis, and transesterification reactions, as well as their ability to work in both aqueous and non-aqueous solvent systems [2].

Owing to their unique properties, lipases have been applied in food processing industries, for speeding up cheese ripening and lipolysis of fat, butter and cream [3]. Lipases are also used in cosmetics and personal care products to synthesize isopropyl myristate, which is used as a palliative in maintenance products such as dermal and sun-tan ointments [4]. Moreover, lipases are applied in the detergent industry to replace hazardous chemicals, which cause severe environmental problems [5].

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The lipase enzyme has been acquired from several sources, like plants, animals, fungi, and bacteria [6]. Despite the mentioned sources, bacterial lipases have been preferred due to their greater stability, ease of manipulating their genes, simple nutrition requirements, and shorter duration of their generation. Moreover, extremophilic microorganisms constitute an important source of durable lipases in adverse conditions [7]. Recently, lipolytic enzymes isolated from extremophilic microorganisms have attracted great attention. Such enzymes show robust stability during reactions under harsh conditions, including extreme temperatures, high salt concentrations, alkaline or acidic conditions, and elevated concentrations of organic solvents [7]. Halotolerant lipases are the best choice for catalyzing reactions with minimal water content, since salts minimize the water content [8]. Similarly, organic solvent stable lipases allow one to perform the catalysis at minimum water content, facilitating the solubility of hydrophobic substrates and reducing microbial contamination [9]. Among extremophilic properties, thermal stability is one of the most attractive traits in biocatalysts. Conducting biotechnological processes at high temperatures offers many advantages, including higher reaction rates due to a decrease in viscosity and increased solubility of substrates. Many industrial processes in which lipases are applied function at temperatures exceeding 45 ºC, like detergent formulation [10] and biodiesel production [11]. The enzymes, thus, need to exhibit an optimum temperature of around 50 ºC. Many thermophilic lipases have been reported in the literature [12–14]. However, the continuous demand for new enzymatic traits has sparked the interest in discovering novel lipases with unique catalytic efficiencies.

In this research, a thermophilic halotolerant lipase producing bacteria was isolated from a compost manure pit. The produced enzyme was investigated for its poly-extremophilicity under various conditions like pH, temperature, solvents and salinity.

II. Materials and Methods

A. Sample Collection

Soil samples were picked from a compost manure pit located on a farm in Wadi El-Natrun, Egypt. The temperature of the soil was recorded as 60 ºC then. The samples were aseptically transferred to the lab in sterile polyethylene bags in a cool box. Two more samples were collected from soil containing olive oil industrial waste and transported under the above mentioned conditions.

B. Screening for Lipolytic Bacteria

The collected soil samples were activated overnight in nutrient broth, pH7.0 at 50 ºC. The activated cultures were serially diluted in nutrient agar plates containing 1% of both tween 80 and $CaCl_2$ [15]. The plates were further incubated at 50 ºC for 48 hours. Colonies with white precipitation were picked up on fresh nutrient agar plates and subjected to higher temperatures of 58 ºC.

C. Screening for Halophilic Lipase Producing Strains

To sort out halophilic strains, all lipase- producing strains from the previous step were challenged for lipase production against different salt concentrations. The obtained strains were streaked on tween 80 nutrient agar medium supplemented with 1M, 2M, 3M, and 4M NaCl. The plates were incubated at 50 ºC for 48 h. Positive isolates were stored in 50% glycerol stock.

D. Testing Lipase Activity in Crude Enzyme Preparation

Fresh colonies of the selected isolates were activated over-night in nutrient broth at 50 ºC. The grown cultures were re-inoculated into 50 mL fresh medium, sterilized in 250 mL Erlenmeyer flask and supplemented with 1% olive oil to trigger the enzyme production. The flasks were incubated for 3 days at 50 ºC. At the end of the incubation period, the cultures were centrifuged under cooling at 7000 rpm for 15 minutes. The supernatant was separated and stored at 4 ºC. The prepared crude extracts were tested using the Rhodamine-olive oil agar plate method [15], where an equal volume of each extract was incubated on agar plates supplemented with 1% olive oil as a lipidic substrate and 2M NaCl. The plates were incubated for 24 h at 50 ºC, then checked with UV light for the fluorescence.

E. Identification of the Selected Strain

The most promising isolate was subjected to biochemical identification using the Biolog system (21124 Cabot Blvd., Hayward CA, USA), which estimates the metabolic fingerprint of the strains.

F. Crude Enzyme Preparation

The Crude enzyme of the selected isolate; S2-5, was prepared as mentioned previously (see 2.4 section).

1) Protein quantification

Protein content in the prepared extract was assessed according to Bradford method [16].

2) Lipase assay

The enzyme assay was assessed calorimetrically using pNP-Laurate (C12), by measuring the released Para nitrophenol at 410 nm [17]. The reactions were carried out in Tris-HCl buffer, 50mM, pH 8.0 at 50 ºC using an appropriate concentration of the crude extract. The spectrophotometric readings were then taken using EMCLAB-NANO-UV-visible spectrophotometer, Germany. One unit of enzymatic activity was defined as the amount of enzyme required to generate 1nmol of para-nitrophenol.

G. Partial Purification using Ammonium Sulphate Precipitation

The crude supernatant was precipitated using ammonium sulphate in a sequential way up to a concentration of 80%. The mixture was then kept at 4 ºC overnight, centrifuged and dialyzed against 50 mM tris HCl buffer, pH 8.0. The protein content and the activity were further determined in the concentrated enzyme.

H. Biochemical Characterization of Lipase Enzyme

1) Effect of pH on enzyme activity and stability

The enzyme activity was executed in different buffers providing a pH range (5.0-10.0) to assess the optimal pH value. Each of the following buffers was prepared as 50 mM; Citrate phosphate buffer (pH 3.0–5.0); phosphate buffer (pH 6.0-7.0); Tris-HCl buffer (PH 8.0-9.0); carbonate buffer (pH 10-11).

2) Effect of temperature on enzyme activity and stability

The optimum temperature was assessed over a wide range (30- 80) ºC. The thermal stability was tested by measuring the residual activity after incubating the enzyme at different temperatures (20-90) ºC over 2 h. Enzyme activity was considered 100% at 20 ºC incubation temperature, while readings at other temperatures were related to it.

I. Effect of Organic Solvents

The enzyme's durability in different organic solvents was tested. The enzyme was incubated in 50% of both polar solvents (Ethyl alcohol, methyl alcohol) and non-polar solvents (ethyl acetate, n-hexane) at 20 ºC for 2 h under agitation.

At the end of the incubation period, the biphasic system formed with non-polar solvents was separated via centrifugation. The assay was carried out using a suitable concentration of the enzyme as mentioned previously.

J. Effect of Salt on Enzyme Activity and Stability

Under the previously mentioned standard conditions, the enzyme assay was performed in (1 M, 2 M, 3 M, and 4 M) NaCl. The enzyme's stability was determined in 4 M NaCl for 2h incubation period at 20 ºC.

III. Results and Discussion

A. Screening for Thermo-Halo Tolerant Lipase Producing Isolate

Although there are hundreds of extremophilic isolates reported in the literature, discovering new strains would evolve novel enzymes with new characteristics. In this study, an extreme habitat known for its high temperature and high salt content was screened for extremophilic lipase producing strains.

Lipase activity has been discriminated on tween plates where the released fatty acids interact with the added $CaCl_2$ resulting in a white precipitate [15]. The initial screening conditions were tuned at 55 ºC and 3M NaCl to selectively isolate thermophilic-halotolerant isolates. The screening step has evolved into 23 lipase producing bacterial isolates. Amongst the obtained isolates, 3 of them, nominated as S2-5, S2-6, S2-7, had grown significantly up to 58 ºC, while only S2-5 grew well at 2 M NaCl indicating that this isolate could be a promising source for thermo- halophilic lipases (Table I).

TABLE I: A COMPARISON BETWEEN THE ISOLATED STRAINS WITH RESPECT TO THEIR HALOTOLERANCE AND MAXIMUM GROWTH TEMPERATURE

Isolate ID	Growth at 50ºC	Growth at 58°C	Growth at 2M NaCl/ 50°C	Lipase activity at 50°C*
S.8	-	-	-	-
soap 4	+	-	+	-
p 18	-	-	-	-
p 25	-	-	-	-
s1 .5	-	-	-	-
p-14	+	-	+	-
s1.6	-	-	-	+
s2.1	+	-	-	-
s1.11	+	-	-	+
soap 3	-	-	-	-
S1.17	+	-	-	+
B.9	+	-	+	+
s2.7	-	-	-	-
HS1-2	-	-	-	-
Hs2-5	+	-	-	-
S2-5	**+**	**+**	**+**	**+**
(S1-5)	+	-	-	+
S2-7	+	+	-	+
S2-6	+	+	-	-
3(S1-6)	-	-	-	-
3(S2-8)	+	-	-	-
3(S1-8)	+	-	-	+
3(S2-7)	+	-	-	-

The enzyme's optimal conditions might deviate from the preferred optimal growth conditions of the isolate. Therefore, the lipase activity of the 3 isolates was assessed on Rhodamine plate method under elevated temperature and high salt content, where the fluorescence intensity was an indication of the enzyme efficiency under the applied conditions. Among the 3 isolates, S2-5 was selected for further characterization.

B. Enzyme Production and Partial Purification

Ammonium sulphate was used to concentrate the crude extract. The salt concentration was raised gradually, until achieving 80% saturation. The specific activity of the dialyzed enzyme was 184 (U/mg).

C. Effect of Temperature and pH

The effect of temperature on enzyme activity is illustrated in Fig. 1. The enzyme displayed its optimal activity at 60 ºC followed by 70 ºC (Fig. 1 a), while the activity was dropped sharply at 80 ºC with 40% activity left. Similarly, other thermophilic lipases from Geobacillus sp. [18] and Bacillus sp. displayed their optimal activity at the same range [19].

Thermal stability was measured in the (20 ºC -90 ºC) range. The enzyme demonstrated a full stability for 2 h at 60 ºC (Fig. 1b). However, at 70 ºC there was a noticeable decline in the activity, where the residual activity recorded 60% and 40% for 1 h and 2 hrs, respectively. On the other hand, crude lipase isolated from a thermophilic isolate; Geobacillus sp. TW1 had lost around 60% of its activity at 70 ºC after 15 minutes [20]. The variation in thermal stability could be explained by the ratio of flexible residues and their distribution around the active site [21]. Incubating the enzyme at lower temperature range (30-50 ºC) has significantly enhanced the enzyme activity, where the residual activity raised to 160% after incubating the enzyme at 40 ºC for 1 h, then declined to 140% at 50 ºC.

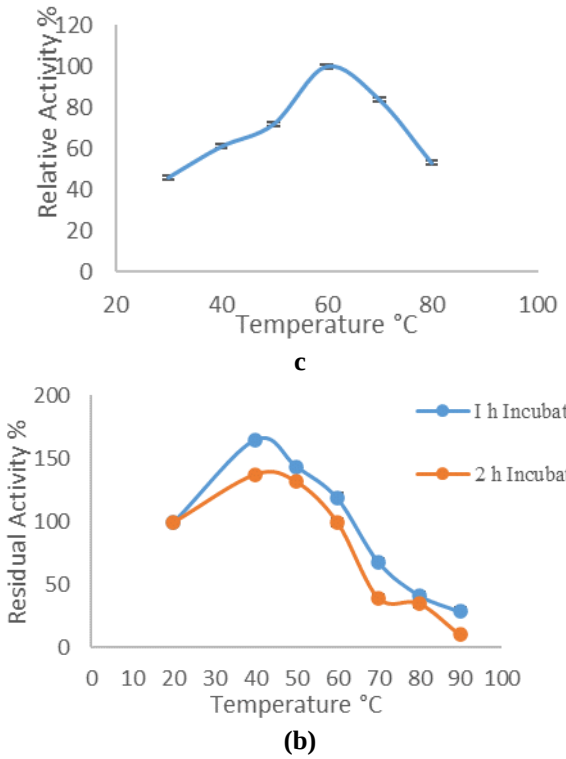


Fig. 1. Effect of Temperature on the enzyme activity. **(a)**: Estimating the optimal temperature of the enzyme. The lipolytic activity was tested over a temperature range (30 ºC -80 ºC). The reaction was performed in 50mM tris HCl, pH 8. The activity at 60 ºC was set as 100%. **(b)**: Enzyme thermal stability along 1 h and 2h incubation time. Enzyme incubation at 20 ºC was set as 100%. The enzyme assay was performed at 60ºC in pH 8.0. 184 U/mg was used to direct all enzyme tests. The average of three experimental readings is used, with SE (standard error) bars.

The enzyme displayed its optimal activity in the near alkaline region (pH 8.0). While the activity dropped significantly at pH 9 &10, achieving 40% residual activity (Fig. 2). A lipase enzyme, LipEH166, isolated from a metagenomic library has shown a similar pattern with maximal activity at pH 8.0 [22].

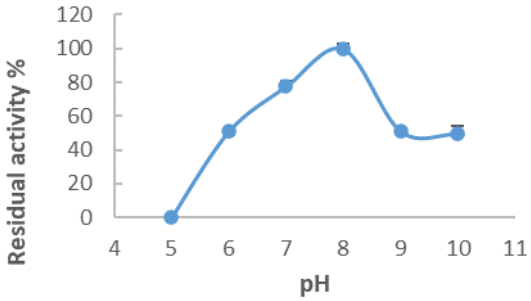
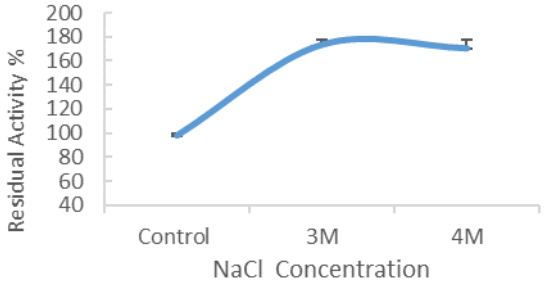


Fig. 2. Effect of pH on the enzyme activity. The activity was tested at 60 °C over a pH range (5-10). 184 U/mg was used to direct all enzyme tests. The average of three experimental readings is used, with SE (standard error) bars.

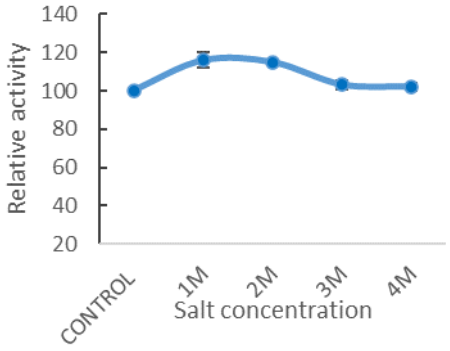
D. Effect of Salt Concentration on the Enzyme Activity

Halophilic lipases are highly demanded in in food

industries, for instance; food ripening and fermentation under high salt concentrations [23]. Compared to other reported halophilic lipases, the enzyme had showed strong tolerance to high-salt content. Increasing salt concentration is accompanied by an enhancement in enzyme activity, achieving 110% relative activity up to 2 M NaCl, while the activity had declined to 100% at higher concentrations (Fig. 3a). The obtained results confirm the halophilicity of the enzyme as reported by Mevarech *et al.*, 2000, that halophilic enzymes require 1 to 4 M salt for maximum activity [24]. This could be because the halophilic amino acid moiety improves the enzyme's thermodynamic stability, thereby indirectly stimulating its activity [25]. This also explains the relatively constant activity after incubating the enzyme in 3M and 4M sodium chloride (Fig. 3b).



(a)



(b)

Fig. 3. Effect of different NaCl concentrations on the enzyme activity and stability. A: Enzyme stability in 3&4M NaCl. The enzyme was incubated for 2h and the residual activity was measured at 60 oC in tris-HCl pH8.0.the activity of untreated sample was set as 100%. B. Estimating the optimal enzyme activity at different NaCl concentrations. The assay was executed at 60°C in tris-HCl pH 8.8. 184 U/mg was used to direct all enzyme tests. The average of three experimental readings is used, with SE (standard error) bars.

E. Effect of Organic Solvents

Organic solvents stable lipases are of great importance in several applications, for instance: pharmaceutical industries and chemical synthesis fields. The effect of solvents is attributed to water stripping from the enzyme surrounding layer, which differs according to the log *p*-values of the solvents.

Where the effect of polar solvents (low log *p*-value) is more drastically than the non-polar ones (high log *p*-value). The obtained results (Table II) have confirmed the theory, where 50% concentration of ethanol and methanol have reduced the activity into half of its value, while the same concentration of ethyl acetate and n-hexane have enhanced the activity 2.5 and 3.5-fold, respectively.

TABLE II: EFFECT OF ORGANIC SOLVENTS ON THE ENZYME STABILITY

Reagent	*Log *p*	[b]Relative activity (%) after 2h
[a]Control	-	100 ±0.5
Methanol	−0.764	67.5 ±3.4
Ethanol	−0.310	84 ±2
Ethyl acetate	0.71	268 ±0.5
n-hexane	3.900	348 ±5.9

*The activity was tested against PNP-laurate using a suitable concentration of the crude extract.
a: The activity of untreated sample was set 100%.
b: The average of three experimental readings is used, with SE (standard error) bars relative to the control.

IV. CONCLUSION

The introduced study showed the poly-extremophilicity of a lipase enzyme produced from a thermo-halotolerant Bacillus strain. The enzyme activity was enhanced significantly in 4M NaCl and at elevated temperature range (60°C-70°C). The activity was significantly raised in presence of 50% of non-polar solvents. These characteristics are suitable for application in several biotechnological and industrial synthetic reactions like esterification and inter-esterification reactions.

CONFLICT OF INTEREST

This work has no conflict of interest.

AUTHOR CONTRIBUTIONS

Cathbert Nomwesigwa conducted laboratory experiments under the supervision of Ahmed Abdel-mawgood. Then, Nehad Noby guided experimental set up and Sherif Hammad provided chemicals and reagents.

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REFERENCES

[1] A. Castilla, S. R. Giordano, and G. Irazoqui, "Extremophilic lipases and esterase: Characteristics and industrial applications," *Microbial Extremozymes*, Elsevier, pp. 207-222, 2022.
[2] S. Wu, R. Snajdrova, J. C. Moore, K. Baldenius, and U. T. Bornscheuer, "Biocatalysis: Enzymatic synthesis for industrial applications," *Angewandte Chemie International Edition*, vol. 60, no. 1, pp. 88-119, 2021.
[3] C. A. Salgado, C. I. A. dos Santos, M. C. D. Vanetti, "Microbial lipases: Propitious biocatalysts for the food industry," *Food Bioscience*, vol. 45, p. 101509, 2021.
[4] R. R. de Sousa, M. C. C. Pinto, E. C. G. Aguieiras, E. P. Cipolatti, E. A. Manoel, A. S. A. da Silva, J. C. Pinto, D. M. G. Freire, and V. S. Ferreira-Leitão, "Comparative performance and reusability studies of lipases on syntheses of octyl esters with an economic approach," *Bioprocess and Biosystems Engineering*, vol. 45, no. 1, pp. 131-145, 2022.
[5] A. R. Ismail, H. Kashtoh, and K.-H. Baek, "Temperature-resistant and solvent-tolerant lipases as industrial biocatalysts: Biotechnological

approaches and applications," *International Journal of Biological Macromolecules*, vol. 187, pp. 127-142, 2021.
[6] N. Li and M.-H. Zong, "Lipases from the genus Penicillium: production, purification, characterization and applications," *Journal of Molecular Catalysis B: Enzymatic*, vol. 66, no. 1-2, pp. 43-54, 2010.
[7] N. Basotra, G. Sharma, K. A. Singh, D. Sharma, R. Rai, and B. S. Chadha, "Bioprospecting extremophiles for sustainable biobased industry," *Extremophiles*, CRC Press, pp. 83-109, 2022.
[8] H. Demirci, G. Kurt-Gur, and E. Ordu, "Microbiota profiling and screening of the lipase active halotolerant yeasts of the olive brine," *World Journal of Microbiology and Biotechnology*, vol. 37, no. 2, pp. 1-13, 2021.
[9] P. J. M. Lima, R. M. da Silva, C. A. C. G. Neto, N. C. Gomes e Silva, J. E. D. S. Souza, Y. L. Nunes, and J. C. Sousa dos Santos, "An overview on the conversion of glycerol to value-added industrial products via chemical and biochemical routes," *Biotechnology and Applied Biochemistry*, 2021.
[10] D. Abol-Fotouh, O. E. AlHagar, and M. A. Hassan, "Optimization, purification, and biochemical characterization of thermoalkaliphilic lipase from a novel Geobacillus stearothermophilus FMR12 for detergent formulations," *International Journal of Biological Macromolecules*, vol. 181, pp. 125-135, 2021.
[11] J. Zhang, H. Chen, Z. Wang, H. Xu, W. Luo, J. Xu, and P. Lv, "Heat - induced overexpression of the thermophilic lipase from Bacillus thermocatenulatus in Escherichia coli by fermentation and its application in preparation biodiesel using rapeseed oil," *Biotechnology and Applied Biochemistry*, 2021.
[12] T. H. T. A. Hamid, N. A. Z. Abidin, and N. Hasan, "Isolation of thermophilic lipase producing bacterium from hot springs at the east coast of peninsular Malaysia," *Journal of Tropical Life Science*, vol. 11, no. 1, 2021.
[13] F. Nehal, M. Sahnoun, A. Dab, M. Sebaihia, S. Bejar, and B. Jaouadi, "Production optimization, characterization, and covalent immobilization of a thermophilic Serratia rubidaea lipase isolated from an Algerian oil waste," *Molecular Biology Reports*, vol. 46, no. 3, pp. pp. 3167-3181, 2019.
[14] J.-M. Park, C.-H. Kang, S.-M. Won, K.-H. Oh, and J.-H. Yoon, "Characterization of a novel moderately thermophilic solvent-tolerant esterase isolated from a compost metagenome library," *Frontiers in Microbiology*, vol. 10, p. 3069, 2020.
[15] S. Lanka and J. N. L. Latha, "A short review on various screening methods to isolate potential lipase producers: Lipases-the present and future enzymes of biotech industry," *Int. J. Biol. Chem*, vol. 9, no. 4, pp. 207-219, 2015.
[16] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," Analytical Biochemistry, vol. 72, no. 1-2, pp. 248-254, 1976.
[17] J. Ma, Y. Ma, W. Wei, and D.-Z. Wei, "In vivo functional expression of an extracellular Ca2+-independent Bacillus pumilus lipase in Bacillus subtilis WB800N," *Annals of Microbiology*, vol. 65, no. 4, pp. 1973-1983, 2015.
[18] A. Mehta, R. Kumar, and R. Gupta, "Isolation of lipase producing thermophilic bacteria: Optimization of production and reaction conditions for lipase from Geobacillus sp," *Acta Microbiologica et Immunologica Hungarica*, vol. 59, no. 4, pp. 435-450, 2012.
[19] L. Bora and M. Bora, "Optimization of extracellular thermophilic highly alkaline lipase from thermophilic bacillus sp isolated from hot spring of Arunachal Pradesh, India," *Braz J Microbiol*, vol. 43, no. 1, 30-42, 2012.
[20] H. Li and X. Zhang, "Characterization of thermostable lipase from thermophilic Geobacillus sp. TW1," *Protein Expr Purif*, vol. 42, no. 1, pp. 153-159, 2005.
[21] J. C. Marx, T. Collins, S. D'Amico, G. Feller, and C. Gerday, "Cold-adapted enzymes from marine Antarctic microorganisms," *Mar Biotechnol (NY)*, vol. 9, no. 3, pp. 293-304, 2007.
[22] E.-Y. Kim, K.-H. Oh, M.-H. Lee, C.-H. Kang, T.-K. Oh, and J.-H. Yoon, "Novel cold-adapted alkaline lipase from an intertidal flat metagenome and proposal for a new family of bacterial lipases," *Applied and Environmental Microbiology*, vol. 75, no. 1, pp. 257-260, 2009.
[23] M. Esteban-Torres, J. M. Mancheño, B. de las Rivas, and R. Muñoz, "Characterization of a halotolerant lipase from the lactic acid bacteria Lactobacillus plantarum useful in food fermentations," *LWT-Food Science and Technology*, vol. 60, no. 1, pp. 246-252, 2015.
[24] M. Mevarech, F. Frolow, and L. M. Gloss, "Halophilic enzymes: Proteins with a grain of salt," *Biophys Chem*, vol. 86, no. 2-3, pp. 155-164, 2000.
[25] G. Ortega, A. Laín, X. Tadeo, B. López-Méndez, D. Castano, and O. Millet, "Halophilic enzyme activation induced by salts," *Scientific Reports*, vol. 1, no. 1, pp. 1-6, 2011.

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