The Egyptian Journal of Hospital Medicine (January 2025) Vol. 98, Page 311- 321

Production and Optimization of Thermoalkalo-lipase by *Parageobacillus thermoglucosidasius*, SO1 Using Agro-Industrial Wastes

Nesreen H. Ibrahim[1], Nagwa M. Sidkey[1], Mohamed A. Abd El-Rahman[2], Mai M. Elhateir[1*]

[1]Botany and Microbiology Department, Faculty of Science, Al-Azhar University (Girls Branch), Cairo, Egypt

[2]Botany and Microbiology Department, Faculty of Science, Al-Azhar University (Boys Branch), Cairo, Egypt

***Corresponding author:** Mai Mohamady El Hateir, Tel: 01011483393, E-mail: Maimohamady2915.el@azhar.edu.eg

ABSTRACT

Background: Bioremediation of industrial and environmental wastes using thermoalkalistable lipase-producing bacteria offers a sustainable solution for waste degradation and potential applications for various industries.

Objective: This study aimed to isolate and optimize the production of lipase from thermoalkalophilic bacterial strains, making them ideal for industrial processes. **Materials and Methods:** Agro-industrial waste samples *viz.* (water hyacinth, lubricant oil waste, frying oil, used sesame oil, and dried chicken skin) were used for isolation of lipase bacterial producers. These samples were enriched under alkaline and elevated temperature conditions (65ºC and pH10). Lipase production was optimized using one factor at a time approach by testing various physical and nutritional factors including temperature, time, pH, inoculum size, additional carbon sources, nitrogen sources, vitamins, metals, and amino acids.

Results: Fourteen isolates were obtained and screened for lipase productivity, the most potent isolate was selected and identified genetically based on 16srRNA as *Parageobacillus thermoglucosidasius*, SO1 with 100% ident to 100% query. The optimization was effective leading to 151.69% increase of lipase productivity (237.93±0.53U/ml) by *Parageobacillus thermoglucosidasius*, SO1 at the optimum conditions 70 ^{O}C, pH 9.5 for 2 days with 8% inoculum size and 2% sesame oil with the addition of 1% sucrose, 0.76% DL-Tyrosine, 200ppm $MnSO_4$ and 200ppm folic acid.

Conclusion: The study demonstrates that optimizing process significantly enhanced lipase production, making the selected bacterial strain suitable for different industrial applications as detergent industry, where thermostable and alkali-tolerant enzymes are in high demand.

Keywords agricultural wastes, Thermoalkalophilic bacteria, Lipase production, *Parageobacillus thermoglucosidasius*, optimization.

INTRODUCTION

Bioremediation using thermoalkalo-bacteria is a promising strategy for managing industrial and environmental waste, particularly in degrading fats, oils, and greases, which are prevalent in many industrial effluents [(1)]. Thermoalkalo-lipases, which can function under extreme conditions of temperature and pH, are particularly useful in numerous applications. These enzymes can effectively break down lipid-rich waste, reducing environmental pollution while enhancing the efficiency of waste treatment processes. Their role in converting hazardous compounds into environmentally benign products highlights their importance in sustainable waste management [(2)].

The production of thermoalkalo-lipase involves selecting extremophilic microorganisms capable of thriving under harsh environmental conditions. These organisms, often isolated from hot springs or oil-contaminated soils, are genetically modified or optimized through fermentation to produce high yields of stable enzymes [(3,4)]. Advances in recombinant DNA technology and protein engineering have facilitated the production of these enzymes on a commercial scale, making them accessible for large-scale industrial applications [(5)]. The optimization of production parameters, such as temperature, pH, and substrate concentration, further enhances the stability and activity of these enzymes in industrial processes [(6,7)].

Thermoalkalo-lipases are highly valued in different vital industries specially the detergent industry due to their ability to hydrolyze fats and oils effectively, even in alkaline and high-temperature conditions. These enzymes improve detergent formulations by enhancing the removal of lipid-based stains at lower washing temperatures, contributing to energy savings and better cleaning performance [(8)]. The enzyme's resilience in detergent environments, which are typically harsh due to surfactants and other chemical additives, ensures that it remains active during the entire wash cycle, improving the overall efficiency of the product [(9)].

Furthermore, using biodegradable lipases aligns with the growing demand for eco-friendly, sustainable consumer products in today's market [(10)]. The present study reinforces the idea which explores lipases efficiency in decomposing lipid substrates when conditions are optimized. **Paladhi *et al*. [(11)]** and **Kanmani *et al*. [(2)]** investigated the ecological function of microbial lipases, highlighting their potential for biodegradation. Both studies concur that when microbial lipases are generated under suitable conditions, they can act as effective agents for biodegradation and bioremediation, thereby underscoring the environmental usefulness of the enzymes from the thermoalkalophilic bacteria examined in these researches.

The future prospects of extremozymes in detergent applications were examined by several studies as **Al-**

Received: 08/08/2024
Accepted: 08/10/2024

Ghanayem [12] and **Sharma *et al*.** [13] who specifically pointed out the value of enzymes such as thermostable lipases in high-temperature washing conditions.

The aims of the current study are to isolate bacterial strains capable of producing thermoalkalo-lipase from agro-industrial waste, optimizing environmental and nutritional factors to enhance lipase productivity, and suggesting a marvelous tool with a potential efficiency in many industrial applications, particularly in the detergent industry.

MATERIALS AND METHODS

1. Isolation and primary screening of thermoalkalophilic lipase producing bacteria

Different samples *viz*. (water hyacinth, lubricant oil waste, frying oil, used sesame oil, and dried chicken skin) were used for isolation. Water hyacinth and chicken skin were dried at 70^{O}C and grounded before usage, while other sources were used as they are. As a pre-reinforcement step 1 g of each sample was inoculated in an enrichment media (nutrient broth) supplemented with tributyrin (2% v/v), soybean oil, waste cooking oil, and tributyrin oil emulsion, separately. All inoculated flasks were incubated at 65°C for 2 days and pH 10. Following incubation, 0.1 ml of each broth culture was further inoculated on 0.2% (v/v) tributyrin oil emulsion agar plates to screen for lipase-producing bacteria and incubated at the same conditions [14].

The isolates, which exhibited the highest clear zones on the agar plates were selected and purified using quadrate streaking technique on 0.2% (v/v) tributyrin oil emulsion agar plates under the same conditions used for isolation and kept for further investigations [14].

2. Quantitative assay of lipase by the selected bacterial isolates

The isolates exhibited the largest clear zones on the screening step that were selected and purified were then inoculated into nutrient broth medium containing 2% of the same waste source for isolation and incubated at the same conditions. After the incubation period, the cell free filtrate was prepared for quantitative analysis. Spectrophotometric analysis was used to calculate the lipase activity using para-nitrophenyl laurate (pNPL) as the substrate that was acquired from Sigma-Aldrich (St Louis, MO, USA). The reaction mixture was prepared by adding 2.5 mL of 420 µM pNPL to 2.5 mL of phosphate buffer (0.1 M, pH 7) and 1 mL of filtrate. The mixture was incubated for 30 minutes at 65°C following a 3-minute incubation period. After that, 1 mL of a cold ethanol:acetone (1:1) combination was added to stop the reaction. The mixture was allowed to stand at room temperature for 20 minutes. Then, 0.2 mL of the assay mixture was put into a 96-well microplate and read at 410 nm using a Synergy MX Microplate Reader (Biotek, Winooski, VT, USA) [14].

3. Optimization of lipase production

Lipase production was optimized by testing various environmental and nutritional factors using one factor at a time approach, including incubation time (4-72 hrs.), incubation temperature (0-80°C), pH (7-12), inoculum size (2–16% v/v), substrate concentration (0.25-4%), additional carbon sources (Glucose, Fructose Lactose, Maltose, Sucrose, Cellulose, and Pectin), additional nitrogen sources ($NaNO_3$, $(NH4)_2SO_4$, peptone, Tryptone, Beef extract, and Yeast extract), vitamins (B1, B2, B6, B12, Vitamin C and folic Acid at different concentrations (50, 100, 200 ppm)), metals ($ZnSO_4$, $CoSO_4$, $FeSO_4$, $CaSO_4$, $MnSO_4$, $MgSO_4$, Na_2SO_4 at different concentrations (50, 100, 200 ppm)) and amino acids (DL-glycine, DL-Valine, Proline, DL-Alanine, DL-Tyrosine, Glutamic acid, and Iso-Leucine) using one factor at a time approach. The lipase productivity was measured using quantitative method described previously [14].

4. Molecular analysis of the most potent selected isolates

4.1. Sample collection and DNA extraction

The selected bacterial strain used in this study, was identified genetically based on 16s rRNA. First, Genomic DNA was extracted from bacterial cultures, following the manufacturer's instructions or standard protocols for bacterial DNA extraction.

4.2. PCR amplification of 16S rRNA gene

The 16S rRNA gene was targeted for amplification to assess the phylogenetic relationships between the bacterial strains. PCR amplification was performed using the universal bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') [15].

The thermocycling conditions included an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 90 seconds, with a final extension step at 72°C for 10 minutes [15]. The resulting sequences were checked for quality. Any low-quality reads were trimmed or excluded from the analysis.

The final sequences were stored in FASTA format and processed further for alignment and phylogenetic analysis. The length of the sequence (1367 nucleotides) suggests it represents a near-complete 16S rRNA gene, which is ideal for taxonomic and phylogenetic analyses[16]. The sequences were aligned using the ClustalW algorithm implemented in MEGA X software. The alignment was manually inspected to ensure accurate homology between the nucleotide sequences across all taxa [16].

4.3. Tree construction

Phylogenetic trees were constructed using the Neighbor-Joining method in MEGA X. The evolutionary distances were computed using the Kimura 2-parameter

model, and bootstrap analysis was performed with 1000 replicates to assess the reliability of the tree nodes [16].

The sequences obtained from this study were compared with reference sequences from the NCBI GenBank database. The resulting phylogenetic trees were visualized using FigTree software and interpreted based on clustering patterns and branch support values. Species closely related to the unknown query sequences were highlighted for further taxonomic investigation.

RESULTS

Fourteen bacterial isolates were obtained from different samples *viz*. (water hyacinth, lubricant oil waste, frying oil, used sesame oil, and dried chicken skin) on an enrichment media (nutrient broth) supplemented with tributyrin as a carbon source (2%, v/v), soybean oil, waste cooking oil (WCO)and tributyrin oil emulsion, separately. All inoculated flasks were incubated at 65°C for 2 days, following by inoculation on 0.2% (v/v) tributyrin oil emulsion agar plates. All the isolates were purified using quadrate streaking technique on 0.2% (v/v) tributyrin oil emulsion agar plates and re-inoculated on 0.2% (v/v) tributyrin oil emulsion agar plates for accurate and confirmative qualitative lipase determination by measuring the clear zone, table (**1**) and figure (**1**).

Table (1): Isolation of thermoalkalo-lipases-producing bacteria

Isolate code	Source of isolation	Diameter of clear zone (mm)
WH1	Water Hyacinth	17
WH2		15
WH3		13
LOW1	Lubricant Oil Waste	18
LOW2		11
LOW3		16
LOW4		20
FO1	Frying Oil	12
FO2		10
SO1	Used Sesame Oil	22
SO2		19
SO3		18.5
CW1	dried chicken skin	9
CW2		9.8

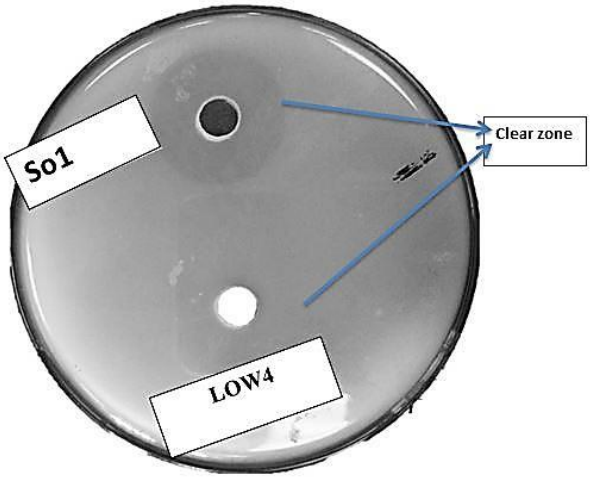

Figure (1): Qualitative assay of lipase by the most potent isolates. Both isolates SO1 and LOW1 have shown the greatest clear zone in diameter (22 and 20 mm, respectively) on 0.2% (v/v) tributyrin oil emulsion agar plates indicate their potential for maximum lipase production ability among the 14 obtained bacterial isolates.

The results in table (1) indicated that the highest lipase producing bacterial were SO1 and LOW1 which represented by the greatest clear zone in diameter on 0.2% (v/v) tributyrin oil emulsion agar plates (22 and 20 mm), and giving rise on quantitative assay 82.47 and 53.01 U/ml, respectively), the bacterial isolate (SO1), which exhibited the greatest lipase productivity (82.47 U/ml) was subjected to optimization for maximizing lipase production.

The isolate SO1 was identified genetically based on 16s rRNA as *Parageobacillus thermoglucosidasius* strain ATCC 43742 with ident 100% to query 100% and accession number NR 112058.1. The phylogenetic tree in figure (2) represents the evolutionary relationships among the most selected bacterial strain and various species from the genera *Geobacillus* and *Parageobacillus*, inferred from 16S rRNA gene sequences. The selected isolated was named as *Parageobacillus thermoglucosidasius*, SO1.

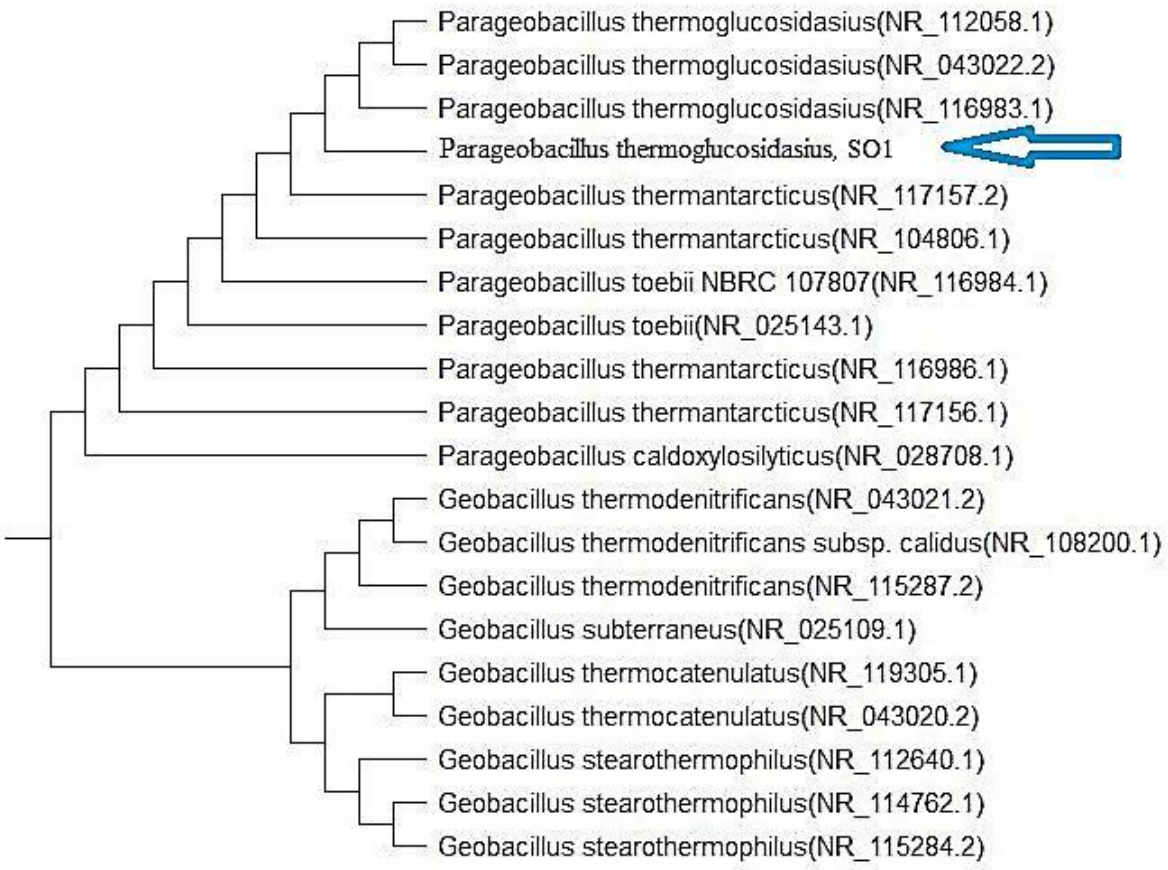

Figure (2): Phylogenetic tree of *Parageobacillus thermoglucosidasius*, SO1 and relation to other *Geobacillus* and *Parageobacillus* species based on 16S rRNA gene sequences. It represented that the selected strain is 100% identical to *Parageobacillus thermoglucosidasius* strain ATCC 43742 with query 100%.

The optimization of lipase production by *Parageobacillus thermoglucosidasius*, SO1 was done using one factor at a time approach. Figure (3) illustrate the effect of different factors (Temperature, pH, time and inoculum size) on lipase productivity. The effect of different incubation times was studied across a range of 4-72 hours, maximum productivity (94.53 U/ml) was observed at 32 hrs. In addition, different incubation temperatures (0-80 ^{O}C) effect on lipase productivity was studied. The results revealed that it increases with temperature rise up to 70°C reaching maximum productivity (107.52 ±0.21 U/ml) then decreases. This data suggests that 70°C is the optimum temperature for lipase production by *Parageobacillus thermoglucosidasius*, SO1, while above or below 70°C, the effectiveness of lipase productivity was reduced. Also, the effect of different pHs on lipase productivity was monitored which revealed that the optimum pH is 9.5 with maximum productivity of 183.72 ± 0.35 U/ml, while the optimum inoculum size is 8% among the tested range of 2-16% with maximum productivity of 186.08 ±0.64 U/ml. Substrate concentration (sesame oil) was another studied factor that influenced lipase productivity by *Parageobacillus thermoglucosidasius*, SO1 giving the maximum productivity (186.36 ± 1.24) at 2%.

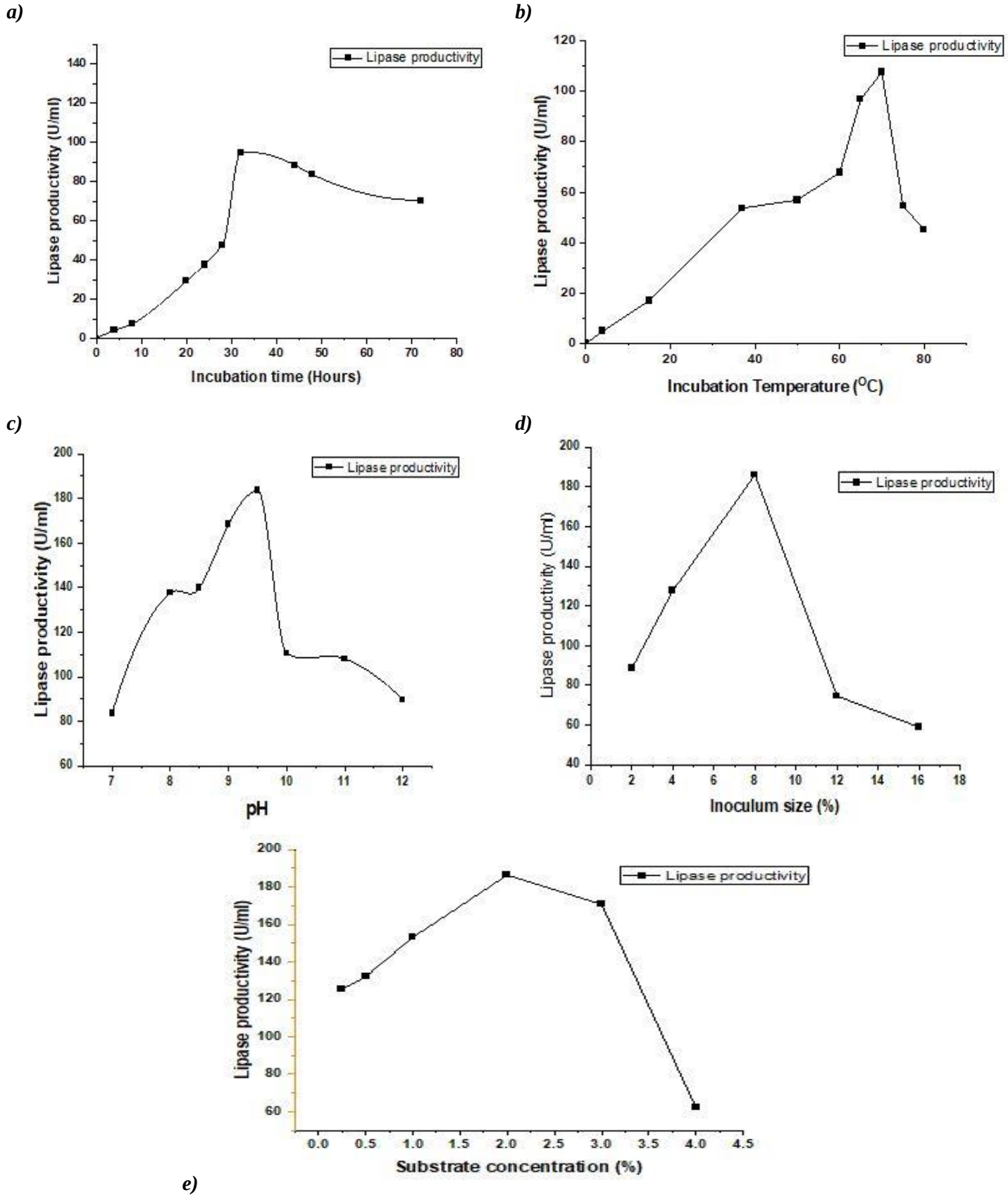

Figure (3): the effect of several physiological factors on lipase productivity by *Parageobacillus thermoglucosidasius*, SO1 a) incubation time; b) incubation temperature; c) pH; d) inoculum size and Substrate concentration (sesame oil). The graph revealed that the optimum conditions are at 70 ^{O}C, pH 9.5 for 2 days with 8% inoculum size and 2% sesame oil.

Also, the effect of several nutritional factors on lipase productivity by *Parageobacillus thermoglucosidasius*, was explored *viz.* additional carbon sources (glucose, fructose, lactose, maltose, sucrose, cellulose, and pectin), additional nitrogen sources ($NaNO_3$, $(NH4)_2SO_4$, peptone, tryptone, beef extract, and yeast extract), amino acids (DL-glycine, DL-valine, proline, DL-alanine, DL-tyrosine, glutamic acid and iso-Leucine), metals ($ZnSO_4$, $CoSO_4$, $FeSO_4$, $CaSO_4$, $MnSO_4$, $MgSO_4$, Na_2SO_4 at different concentrations (50, 100, 200 ppm)) and vitamins (B1, B2, B6, B12, vitamin C & folic Acid at different concentrations (50, 100, 200 ppm)).

The results in figure (4) indicated that the addition of sucrose enhanced lipase productivity up to 208.66 ±0.77 U/ml, while other sources inhibited the productivity compared to the control in which no additional carbon source was added. This step of sucrose addition was fixed following testing other parameters. Concerning nitrogen sources addition, all experimented sources caused slight inhibition of lipase productivity.

None of the nitrogen sources was selected as no increasing in lipase productivity occurred. For amino acids addition analysis, it was found that DL-tyrosine exhibited slight increase in lipase productivity (210.03 ±0.46 U/ml) compared to the control 208.71 ±0.20Uml so, it was kept as an enhancement factor in the following experiments.

Concerning the effect of various metallic ions at different concentrations on lipase productivity, it showed a wide range of influence depending on the type of metal ion and its concentration. Among the metallic ions tested, $MnSO_4$ at 200 ppm exhibited the highest boost in lipase productivity (225.92 ± 0.85) with 7.3% increase comparing to control (without added metallic ions), followed by $CoSO_4$ at 50 ppm with a productivity of 217.66 ± 0.51U/ml, indicating 3.4% increase, further increasing in concentration caused declining of productivity.

Also, $FeSO_4$ enhanced productivity at very slight manner, reaching around 210.19 ± 0.74U/ml at 50 ppm then decreased on higher concentrations. In contrast, other metallic ions such as ZnSO4**,** CaSO4, Na_2SO_4 and $MgSO_4$ at all tested concentrations (50–200 ppm) drastically reduced lipase activity, with productivity falling particularly with $MgSO_4$.

The data revealed that $MnSO_4$ at 200 ppm was the most applicable inducer for lipase productivity by *Parageobacillus thermoglucosidasius*, SO1. In case of the tested vitamins, folic acid at 200 ppm exhibits the highest productivity of 237.92 ± 0.53 U/ml at 200 ppm with 8.1% increase; other tested vitamins have a negative impact on lipase productivity with the strong negative effect with B12 addition. Accordingly, folic acid at 200 ppm is the most favorable for enhancing lipase production.

The overall optimization process led to remarkable increase of lipase productivity up to 151.17% (237.92± 0.53 U/ml) which manifest the effectiveness of the protocol used for maximizing lipase production by *Parageobacillus thermoglucosidasius*, SO1 at the optimum conditions 70 ^{O}C, pH 9.5 for 2 days with 8% inoculum size and 2% sesame oil with the addition of 1% sucrose, 0.76% DL- Tyrosine, 200 ppm $MnSO_4$ and 200 ppm folic acid.

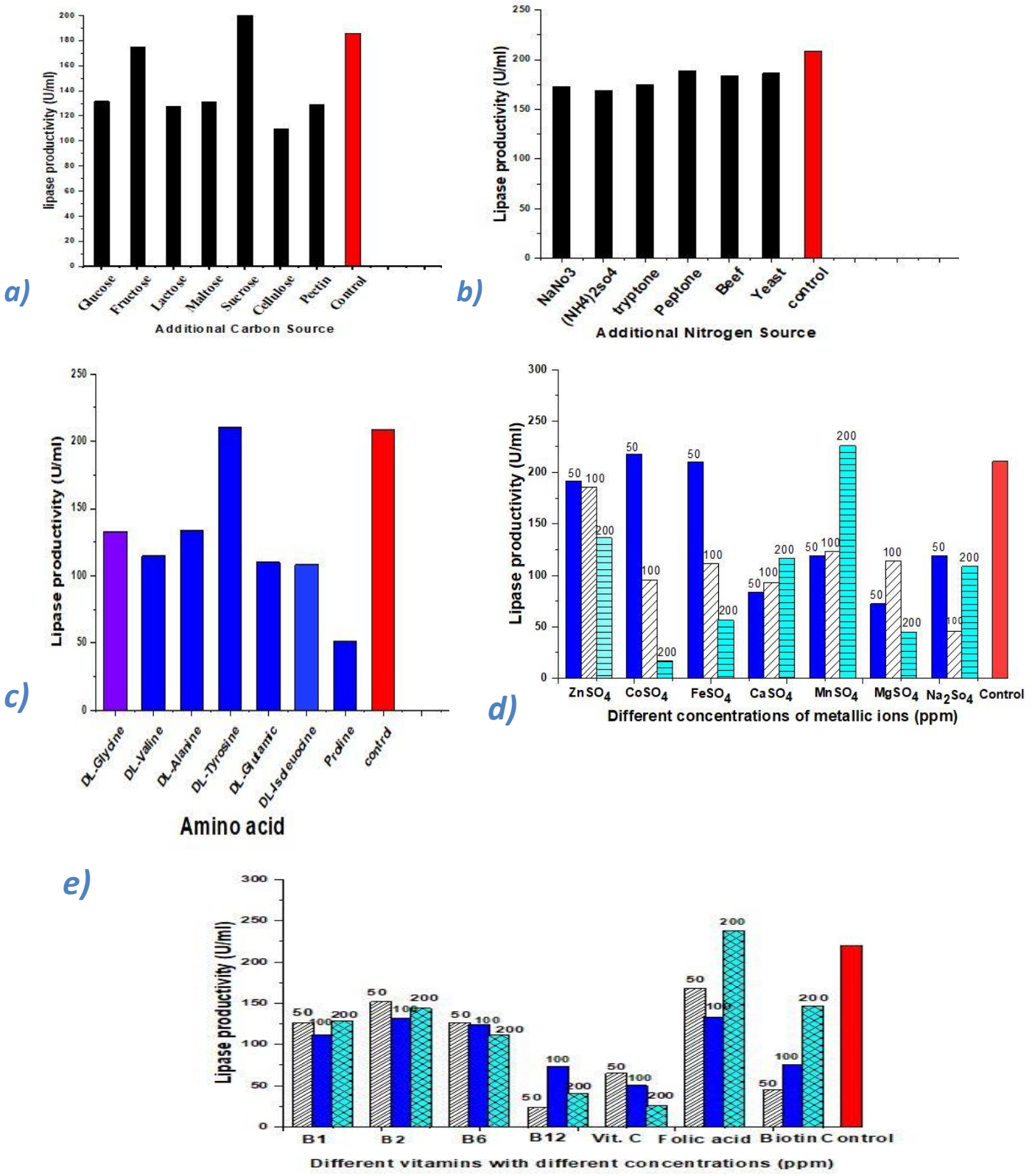

Figure 4: the effect of several nutritional factors on lipase productivity by *Parageobacillus thermoglucosidasius*, SO1 a) Additional carbon sources; b) Additional nitrogen sources; c) Amino acids; d) Metals and e) Vitamins. The results included in the figure illustrated that sucrose, dl- tyrosine, folic acid at 200 ppm and Mn^{+2} at 200 ppm are the positive inducers for lipase production.

DISCUSSION

The results indicated that the highest lipase producing bacterial isolates were SO1 and LOW1 obtained from Used Sesame Oil and Lubricant Oil Waste, respectively, which represented by the greatest clear zone in diameter on 0.2% (v/v) tributyrin oil emulsion agar plates (22 and 20 mm), and giving rise on quantitative assay 82.47 and 53.01 U/ml, respectively. These results was in agreement with other researches as **Amin *et al.*** [17] who used five different agro-industrial wastes (canola oilseed cake, peanut shells, wheat bran, rice bran and sunflower hulls) for lipase production by *A. melleus* and **Szymczak *et al.*** [18] who reported microbial lipase production using straw, bran, oil cakes and industrial effluents. Agro-industrial wastes considered as a rich source for effective lipase production strategy along with evolving a solution for the problem of Agricultural waste over accumulation which represents a significant issue that is progressively escalating worldwide as a result of diverse human activities [19-22].

The isolate SO1 was identified genetically based on 16s rRNA as *Parageobacillus thermoglucosidasius* strain ATCC 43742 with ident 100% to query 100% and accession number NR 112058.1.

The *Geobacillus* genus, are known for efficient thermostable enzymes production, such as lipases and proteases. These enzymes have significant potential in industrial applications, such as in detergents, bioremediation, and biodiesel production, as demonstrated in other studies like **Ibrahim & Ma [6] and Rmili *et al.*** [9]. Also, **Al-Dhumri and Bayoumi** [5] reported the production of hyperthermostable alkaline lipase by *Bacillus stearothermophilus*.

The studies by **Al-Ghanayem** [12] **and Sharma *et al.*** [13] emphasized the application of extremozymes in detergents due to their stability in harsh conditions, such as high temperatures and alkaline ph.

The optimization of lipase production by *Parageobacillus thermoglucosidasius*, SO1 was done using one factor at a time approach. The results demonstrated that various factors significantly influence lipase production from thermoalkalophilic bacterial strains. The optimal incubation period for lipase productivity was found to be 32 hours, with maximum productivity (94.53±7.55 U/ml). Also, Temperature played a crucial role, lipase productivity increased with increasing temperature up to 70^{O}C reaching a peak at 107.52±0.21 U/ml, beyond which productivity decreased due to enzyme denaturation, and denaturation of some medium components which reduced productivity [21]. This was in agreement with **Abol-Fotouh *et al.*** [24] who reported maximum lipase productivity (488 U/ml) at 60 °C by *Geobacillus stearothermophilus* FMR12 using utilized fish wastes at 20%. Also, **Ibrahim and Ma** [6] investigated the industrial uses of thermostable enzymes and illustrated that extremophilic microorganisms are exceptionally effective at producing enzymes, such as lipases, which operate best in high-temperature and alkaline environments. Their study backs up the current results, in which lipase activity was highest at 70°C and pH 9.5, showcasing the resilience of these enzymes for industrial applications. However, at 55 °C, *Bacillus sonorensis* (KKUMS14), which was isolated from hot springs, demonstrated the highest lipase productivity [25].

Herein, the pH range of 9.5 was identified as optimal, with the highest productivity of 183.72 ± 0.35 U/ml, indicating that the enzyme functions best in slightly alkaline conditions, the reduced enzyme productivity blow or above pH 9.5 may be due to production of byproducts at non optimal pH and depletion of nutrients in the cultivation medium [23,26]. This result is in agreement with **Kowsalya *et al.*** [27] who reported lipase maximum productivity at alkaline conditions (pH 8) by *Aeromonas media* VBC8 and **Sharma *et al.*** [28] at pH 9 by *Bacillus methylotrophicus* PS3 above which reduction occur due to the proteinaceous enzyme nature, while **Pharm *et al.*** [29] reported lower pH nearly at 6.

Also, the generation of hyperthermostable alkaline lipase was the core of **Al-Dhumri and Bayoumi** [4] research by *Bacillus stearothermophilus* sourced from oil-contaminated soil, noting that the enzyme exhibited peak activity at 55°C and pH 8.0, whereas the optimal temperature in this study is 70°C. Both investigations emphasize the capability of these bacteria to thrive and generate lipases under extreme alkaline and high temperature conditions.

Furthermore, the results of this study are consistent with a number of other studies that look into the synthesis, refinement, and uses of thermo-alkali-stable enzymes such as lipases from extremophilic microbes. For instance, **El-Ghonemy *et al.*** [30] highlighted the importance of statistical optimization for optimizing enzyme synthesis when they isolated a thermo-alkali-stable lipase from *Aspergillus niger*. Similar to the current study, their investigation revealed that alkaline and high temperatures were the ideal circumstances for lipase activity.

Moreover, **Zheng *et al.*** [31] isolated a thermo-alkali-stable lipase from oil-contaminated soil using a metagenomic approach. In accordance with the results of this study, their lipase demonstrated excellent activity in alkaline and hot circumstances. The industrial potential of these enzymes is under the extreme condition was reported.

In the current study, the inoculum size was also a significant factor, with the highest lipase production observed 8% among the tested range of 2- 16% with maximum productivity of 186.08±0.64 U/ml. Both lower and bigger inoculum sizes resulted in decreased

productivity, this situation varies from isolate to another and may be caused by an insufficient amount of inoculum in balance with the nutrition, aeration, and other parameters affecting the production [32]. In contrast, **Yasin *et al.*** [33] reported 5% (v/v) of inoculum being the optimum for lipase production by *Exiguobaterium* sp. strain AMBL-20^T, while, **Abdel Aziz *et al*** [34], **Kowsalya *et al.*** [27] reported 1% inoculum size.

Substrate concentration (sesame oil) was another key factor that influenced lipase productivity by *Parageobacillus thermoglucosidasius,* SO1 giving the maximum productivity (186.36±1.24) at 2%. Earlier studies revealed that olive and sunflower oils are preferable substrates for lipase production [33-35], while **Abol-Fotouh *et al.*** [24] reported utilized fish wastes at a concentration of 20% (w/v). other substrates were used including fish liver oil by *Aeromonas media* VBC8 which exhibited highest lipase productivity (89 U/mL) followed by peanut oil, while sesame oil exhibited less productivity [27].

Furthermore, media components and supplements such as carbon and nitrogen sources, amino acids, metallic ions, and vitamins had a profound impact on lipase production. Sucrose was the most effective additional carbon source, yielding productivity levels of 208.66±0.77 U/ml, while other sources inhibited the productivity compared to the control. All experimented inorganic and organic additional nitrogen sources caused slight inhibition of lipase productivity. This variability contributed to the behavior of the enzyme producing microorganism. It may be due to that carbon may result in increasing N compound uptake and transport changing the behavior of microorganism concerning lipase production **resemebling Abdel Aziz *et al.*** [35] findings which reported peptone as an inducer for lipase production comparing to our findings, it was the least additional nitrogen source that caused slight inhibition.

In a similar manner, **Basha** [1] examined the role of oil-degrading lipases and their applications in environmental contexts, emphasizing that effective lipase production necessitates precise regulation of substrate concentration and carbon sources. This study identified sucrose as the most advantageous carbon source for lipase production, supporting Basha's [1] assertion that specific carbon sources are critical for enhancing enzyme productivity. Furthermore, Basha's [1] emphasis on the use of lipases for environmental remediation aligns with the current study's focus on bioremediation, highlighting the wider ecological importance of lipases beyond their industrial uses.

Among the amino acids, DL-tyrosine promoted the highest lipase production (210.03±0.46 U/ml), while others amino acids reduced productivity significantly. Metallic ions like $MnSO_4$, $CoSO_4$ and $FeSO_4$ significantly enhanced lipase productivity up to 225.92±0.85, 217.66±0.51 and 210.19±0.74 U/ml at 200, 50, 50 ppm, respectively. Other metals as $ZnSO_4$, $CaSO_4$, $MgSO_4$ and Na_2SO_4 inhibited lipase productivity. Among the tested vitamins, only folic acid at 200 ppm greatly enhanced lipase productivity up to 237.93±0.53 U/ml. The later result may be due to the fact that vitamins are cofactor aiding in metabolism of amino acids or proteins which can affects enzyme production directly.

The optimum conditions of lipase production by *Parageobacillus thermoglucosidasius,* SO1 were summarized to be at 70^{O}C, pH 9.5 for 2 days incubation with 8% inoculum size and 2% subatrate concentration (sesame oil) with addition of 1% sucrose, 0.76% DL-Tyrosine, 200 ppm $MnSO_4$ and 200 ppm folic acid. The productivity was increased up to 151.17% (237.92±0.53 U/ml) which manifesting the importance and efficacy of the optimization process.

Another study by **Ilesanmi *et al.*** [14] reported the production of lipase by alkalophilic *Pseudomonas aeuriginosa* strain isolated from a mechanic's workshop soil sample exploring maximum lipase productivity (528.54 U/L) at 12 h incubation in media with olive oil and yeast extract were regarded as the best carbon and nitrogen source, respectively.

Comparing to other studies, different over all conditions was established. Accordingly, **Pharm *et al.*** [27] tested several bacterial strains isolated from forest soil around the plant roots. The study reported maximum lipase productivity at temperature of 35–40 °C and at pH 6, peaking at 480 U/mL and 420 U/mL, respectively, produced by *Lysinibacillus* PL33 and *Lysinibacillus* PL35. Also, Tween-80 and yeast extract were found to be the optimal carbon and nitrogen source, respectively. He added that, among the metal ions Ca^{+2}, Mg^{+2} and K^+ enhanced lipase productivity with Contrary to our findings.

In addition, **Yasin *et al.*** [33] looked at the synthesis of microbial lipase and its several uses, talking about variables that affect enzyme activity such as temperature, pH, and media components. The authors pointed out that improving lipase activity for industrial uses requires careful consideration of these parameters. Similar to the bioremediation and industrial detergent applications investigated in the current study, Sari *et al.* also emphasized the broad industrial uses of microbial lipases, spanning from waste treatment to biodiesel synthesis.
Their results revealed that optimum conditions of lipase production by *Exiguobaterium* sp. strain AMBL-20^T were at 2% Olive oil, 0.2% peptone at 25°C, pH 8, and 24 h.

CONCLUSION

In conclusion, this study's results complement previous findings by demonstrating that thermoalkalophilic bacteria are excellent producers of thermostable lipases. The optimization of various factors

was crucial for enhancing enzyme production, which aligns with the growing body of research on extremophilic lipases. The optimization proved successful, resulting in a 151.69% boost in lipase productivity (237.93 ± 0.53 U/ml) from *Parageobacillus thermoglucosidasius*, SO1, under optimal conditions of 70 °C, pH 9.5 for two days, using an 8% inoculum size and 2% sesame oil, along with the incorporation of 1% sucrose, 0.76% DL-Tyrosine, 200 ppm MnSO4, and 200 ppm folic acid. This work contributes to the understanding of how these enzymes can be applied in both industrial and environmental contexts, reinforcing their versatility and significance. The research shows that optimizing the process greatly improved lipase production, indicating that the chosen bacterial strain is well-suited for various industrial uses, particularly in the detergent sector, where there is a strong need for thermostable and alkali-resistant enzymes which require more investigation for monitoring the efficacy of the produced lipase in the urgent applications.

Conflicts of interest: None.
Funding: None.

REFERENCES

1. **Basha P (2021):** Oil degrading lipases and their role in environmental pollution. In: Recent developments in applied microbiology and biochemistry. *Academic Press*., 2: 269-277.
2. **Kanmani P, Aravind J, Kumaresan K (2015):** An insight into microbial lipases and their environmental facet. Int J Environ Sci Technol., 12:1147-1162.
3. **Khudhair S (2016):** Isolation and optimization of thermophilic lipase producing bacteria from soil contaminated with used engines oil. J Waist Sci Med., 8(4):66-74.
4. **Al-Dhumri S, Bayoumi R (2019):** Bacterial hyperthermostable alkaline lipase production by B. stearothermophilus isolated from oil polluted soil. Int J Advance Res Biolo Sci., 6(2): 166-184.
5. **Ghasemian A, Moradpour Z (2019):** Production of recombinant microbial thermostable lipases. In: New and Future Developments in Microbial Biotechnology and Bioengineering, Elsevier, 133-150.
6. **Ibrahim N, Ma K (2017):** Industrial applications of thermostable enzymes from extremophilic microorganisms. Curr Biochem Eng., 4(2): 75-98.
7. **Dahiya P, Panwar J, Kumar A (2024):** Enzyme Biotechnology for Environmental Sustainability. 1st ed. Academic Press, 1-50.
8. **Basotra N, Sharma G, Singh K *et al.* (2022):** Bioprospecting Extremophiles for Sustainable Biobased Industry. In *Extremophiles*. CRC Press, 83-109.
9. **Rmili F, Hadrich B, Chamkha M *et al.* (2022):** Optimization of an organic solvent-tolerant lipase production by *Staphylococcus capitis* SH6. Immobilization for biodiesel production and biodegradation of waste greases. Prep Biochem Biotechnol., 52(1): 108-122.
10. **Lap B, Debnath A, Singh GK *et al.* (2024):** Microbial Enzymes in Biodegradation of Organic Pollutants: Mechanisms and Applications. Microbes Based Approaches Manage Hazard Contaminants, Wiley, 12-19.
11. **Paladhi A, Joshi J, George A *et al.* (2022):** Lipase and lactic acid bacteria for biodegradation and bioremediation. In Microbes and Microbial Biotechnology for Green Remediation. Elsevier, 265-286.
12. **Al-Ghanayem A (2024):** Recent developments and future prospects of extremozymes in detergent applications. Afr J Microbiol Res., 18(7): 137-146.
13. **Sharma S, Kour S, Avatsingh A,Kumar N , Singh N (2024):** Microbial enzymes in laundry detergents: Recent advances, future prospects, and risk assessment. In: Enzyme Biotechnology for Environmental Sustainability. Academic Press, 13-31.
14. **Ilesanmi O, Adekunle A, Omolaiye J *et al.* (2020):** Isolation, optimization and molecular characterization of lipase producing bacteria from contaminated soil. Sci Afr., 8: e00279.
15. **Frank J, Reich C, Sharma S *et al.* (2008):** Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Appl Environ Microbiol., 74(8): 2461-2470.
16. **Awe O, Nyamari M, Mukanga L (2023):** Comparative study between molecular and genetic evolutionary analysis tools using African SARS-CoV-2 variants. Inform. Med. Unlocked, 36: 101143.
17. **Amin M, Bhatti H, Zuber M, Bhatti I *et al.* (2024):** Potential use of agricultural wastes for the production of lipase by *Aspergillus melleus* under solid state fermentation. JAPS,, 24(5): 1530-1437.
18. **Szymczak T, Cybulska J, Podleśny M *et al.* (2021):** Various perspectives on microbial lipase production using agri-food waste and renewable products. *Agriculture*, 11(6): 540.
19. **Costa T, Hermann K, Garcia-Roman M *et al.* (2017):** Lipase production by *Aspergillus niger* grown in different agro-industrial wastes by solid-state fermentation. Braz J Chem Eng., 34(2): 419-427.
20. **Singh A, Singh A (2022):** Microbial degradation and value addition to food and agriculture waste. *Curr Microbiol.*, 79(4):119.
21. **Machado B, Michelon M, Santos L *et al.* (2023):** Industrial Lipases Production Using Agri-Food Wastes Through Microbial Applications. In: Microbial Bioprocessing of Agri-food Wastes. 1st ed. CRC Press, 187-207.
22. **da S Pereira A, Souza C, Franson R *et al.* (2024):** From Agri-food Wastes to Enzyme Production: A Systematic Review with *Methodi Ordinatio*. *Waste and Biomass Valori.*, 15: 5843–5870.
23. **Ire F, Berebon D (2016):** Production and characterization of crude 1, 4-β-endoglucanase by *Pseudomonas aeruginosa* using corn (*Zea mays*) cobs and pawpaw (*Carica papaya*) fibres as substrates. *J adv biol biotechnol.*, 8(4): 1-16.
24. **Abol-Fotouh D, AlHagar O, Hassan M (2021):** Optimization, purification, and biochemical characterization of thermoalkaliphilic lipase from a novel

Geobacillus stearothermophilus FMR12 for detergent formulations. Int J Biol Macromol., 181: 125-135.

25. **Sulaiman A, Yasser S, Shekha A *et al*. (2018):** Hydrolytic enzyme production by thermophilic bacteria isolated from Saudi hot springs, Open Life Sci., 13(1): 470–480.
26. **Asad S, Tabassum A, Hameed A *et al.* (2015):** Determination of lytic enzyme activities of indigenous *Trichoderma* isolates from Pakistan. Braz J Microbiol., 46: 1053-1064.
27. **Kowsalya R, Saravanan K, Selvam K *et al*. (2024):** Enhanced lipase production and characterization from *Aeromonas media* VBC8: Applications in biodegradation of lubricating oil waste. Biocatal Agric Biotechnol., 62: 103423.
28. **Sharma P, Sharma N, Pathania S *et al*. (2017):** Purification and characterization of lipase by *Bacillus methylotrophicus* PS3 under submerged fermentation and its application in detergent industry. JGEB., 15(2): 369-377.
29. **Pham V, Kim J, Chang S *et al.* (2021):** Investigation of lipolytic-secreting bacteria from an artificially polluted soil using a modified culture method and optimization of their lipase production. Microorganisms, 9(12): 2590.
30. **El-Ghonemy D, Ali T, Hassanein N *et al.* (2021):** Thermo-alkali-stable lipase from a novel *Aspergillus niger*: statistical optimization, enzyme purification, immobilization and its application in biodiesel production. Preparative Biochemistry & Biotechnology, 51(3): 225-240.
31. **Zheng J, Liu C, Liu L *et al*. (2013):** Characterisation of a thermo-alkali-stable lipase from oil-contaminated soil using a metagenomic approach. Syst Appl Microbiol., 36(3): 197-204.
32. **Mould F, Kliem K, Morgan R *et al*. (2005):** In vitro microbial inoculum: A review of its function and properties. AFST., 124: 31-50.
33. **Yasin M, Ali Y, Ahmad K, Ghani A *et al*. (2021):** Alkaline lipase production by novel meso-tolerant psychrophilic *Exiguobacterium* sp. strain (AMBL-20) isolated from glacier of northeastern Pakistan. Arch Microbiol., 203:1309-1320.
34. **Abdel Aziz M, Elgammal E, Ghitas R (2020):** Comparative study on modeling by neural networks and response surface methodology for better prediction and optimization of fermentation parameters: Application on thermo-alkaline lipase production by *Nocardiopsis* sp. strain NRC/WN5. Biocatal Agric Biotechnol., 25:101619.
35. **Adetunji A, Olaniran A (2018):** Optimization of culture conditions for enhanced lipase production by an indigenous *Bacillus aryabhattai* SE3-PB using response surface methodology. Biotechnol Biotechnol Equip., 32 (6): 1514-1526.