ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2022; 27(1): 3215-3224
doi: 10.25083/rbl/27.1/3215-3224

Received for publication, January, 06, 2021
Accepted, February, 14, 2022

Original paper

Characterization of a lipolytic strain of Pseudomonas stutzeri SN-3 and production of triacylglycerol hydrolase with concomitant biodegradation of palm oil

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Abstract Lipases are triacylglycerol hydrolases (3.1.1.1) that under aqueous condition catalyzes the hydrolysis of triglycerides. Lipases are the ubiquitous enzymes with their applications ranging from food industry to cosmetics, pharmaceuticals and bioremediation purposes. The present research involves 16S rDNA sequencing of a lipase producing strain isolated indigenously. The strain was identified as a novel *Pseudomonas stutzeri* SN-3, the gene sequence of which was deposited in GenBank with accession number MH639065. The research design also includes the exploration of alternative fermentation conditions for maximum production of triacylglycerol hydrolase from the novel strain. Different physical and chemical parameters were studied which includes temperature, pH, fermentation time course, nitrogen sources, carbon sources, phosphate sources, different salts and their concentrations for getting optimal yield of triacylglycerol hydrolase. Utilization of olive oil for production of lipases is a conventional approach, which is quite costly for commercial applications; therefore, palm oil was incorporated in cultivation medium as an alternative cheap substrate for triacylglycerol hydrolase production. Enzyme yield from *Pseudomonas stutzeri* SN-3 was optimized by using 1 gm% palm oil and 4 gm% yeast extract as carbon and nitrogen sources respectively in the presence of 2 gm% $CaCl_2$ as enzyme stabilizer and 0.01% KH_2PO_4 as bacterial growth promoter. The maximum enzyme production was observed after 48 hours of fermentation with medium pH 7 at 37 ^{0}C. Conclusively, we had a novel *Pseudomonas stutzeri* SN-3 specie and a cost-effective and eco-friendly medium for commercial production of triacylglycerol hydrolase.

Keywords *Pseudomonas stutzeri*, Biocatalysis, Biodegradation, Lipase, Optimization

To cite this article: AHMAD S, RIAZ A, SYED MN. Characterization of a lipolytic strain of *Pseudomonas stutzeri* SN-3 and production of triacylglycerol hydrolase with concomitant biodegradation of palm oil. *Rom Biotechnol Lett*. 2022; 27(1): 3215-3224. DOI: 10.25083/rbl/27.1/3215-3224.

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Introduction

The Triacylglycerol hydrolases or lipases (E.C. 3.1.1.1) are the enzymes which catalyze the degradation of triglycerides into diacylglycerol, monoacylglycerol, fatty acids and glycerol (Jaeger *et al.,* 1999). They bear the tremendous potential of catalyzing the reverse reaction i.e. esterification along with acidolysis and alcoholysis (Stergiou *et al.,* 2013). The water interface favors hydrolytic reaction by lipases whereas in the oil interface, lipases catalyze the esterification. Thus, the action of lipases is medium dependent (Gupta *et al.,* 2004). Lipases exhibit both condition and catalytic promiscuity (Busto *et al.,* 2010; Wu *et al.,* 2010 ; Kapoor and Gupta, 2012). This is one of the reasons of tremendous applications of lipases in various industries. The regular substrates of lipases are glycerol esters. Lipases are highly specific in their action being stereo, regio and enantioselective (Jaeger and Eggert, 2002; Anobom *et al.*, 2014). Lipases are produced by a variety of fauna and flora; from microbes to higher organisms and plants. Among all these sources, microbial lipases are the most utilized and studied. Bacterial lipases play an imperative role in commercial sectors due to the ease of production (Salihu & Alam, 2012). Many bacterial genera produces potential triacylglycerol hydrolases but the *Pseudomonas* lipases are more economic, highly stable and provide a wide range of specificity (Lee *et al.*, 2015). Related to the eminent characteristics of lipases, they are equally important in the industries (Woittiez *et al.*, 2017). The applications of lipases are growing rapidly in organic synthesis, pharmaceuticals, biofuel production, cleaners and degreasing formulations, flavor development, food modification, the production of fine chemicals, paper making, manufacturing of cosmetics, and in oleochemical industry. Triacylglycerol hydrolases are equally important for bioremediation processes from oil spill to the degradation of plastics (Salihu & Alam, 2012).

Application of the enzymes produced by living organisms for the disintegration of organic substances is known as biodegradation. Triacylglycerol hydrolases being the hydrolases of triglycerides contribute significantly in cleaning the oil based wastes via biodegradation. Biodegradation of oil is not only fruitful for the environment but also for the assessment of eco friendliness of the oil. Palm oil is the major vegetable oil produced in the world (Woittiez *et al.*, 2017). Palm oil encompasses 66.83 million metric tons or 34% of the total vegetable oil production across the globe and since 2015 its domestic utilization has climbed from 702 million metric tons to 75.098 million metric tons (Shahbandeh, 2019). The increased concentration of palm oil in waste water is an outcome of this raised level of its utilization globally (Saranya *et al.*, 2014). Lipid rich waste water is harmful for the living organisms and its proper handling is essential to create a safe living environment (Gombert *et al.*, 1999). Concerning this, use of bioresources such as enzymes for lipid hydrolysis is the most appropriate approach. Enzymatic hydrolysis of lipid rich waste water is preferred because of their ecofriendly and highly specific nature. By revealing the new and economic sources for large-scale enzyme preparations, mass production would become easy and inexpensive to meet the industrial demands of microbial lipases. The present research was therefore designed to optimize the fermentation parameters for maximum triacylglycerol hydrolase production from the isolated strain.

Materials and Methods

Chemicals

All analytical grade chemicals used in this study were procured from Sigma-Aldrich USA.

Bacterial strain

Bacterial culture used in this research was obtained from Enzyme Technology Unit, Department of Biochemistry, University of Karachi, Pakistan. The strain was previously isolated and identified as *Pseudomonas* specie SN-3 on morphological and biochemical characteristics. The strain was found to be gram negative, non-spore former, motile, acidophilic, halotolerant (Ahmad & Syed, 2019).

Molecular Identification

The bacterial strain was identified by using 16S rDNA sequencing. It is the most widely used technique for genotypic identification of bacterial strains (Drancourt *et al*., 2000). The molecular identification includes separation of genomic DNA, augmentation of 16S rDNA gene and its sequencing. EZ-10 Spin Column Genomic DNA Kit (Bacterial Samples) was used to separate genomic DNA. Isolation of the genomicDNA was tracked by using 1% agarose gel electrophoresis. 16S rDNA gene was amplified in the isolated genomic DNA using primers. Primer FD1 (10µM) 5'-AGAGTTTGATCCTGGCTCAG-3' and Primer RS16 (10µM) 5'-TACGGCTACCTTGTTACGACTT-3' were used as forward and reverse primer respectively.

Commercially available kit was use to purify amplified gene and then the purified product was subjected to Sanger's sequencing.

Culture maintenance

The strain revived primarily in nutrient broth and continually grown on nutrient broth complemented with olive oil (1%) at 37ºC for 24 hours. The strain was preserve at 4ºC in an olive oil (1%) agar medium and subculture routinely.

Inoculum preparation

For preparation of inoculum, nutrient broth was accompanied with palm oil (1% v/v), yeast extract (4% w/v) and $CaCl_2$ (2% w/v) and seed culture (10% v/v) was prepared by inoculating a 100 µL of preserved culture into it. For inoculum preparation, this culture then incubated at 37ºC for 24 hours and then used in cultivation.

Enzyme Production Medium

Fermentation was conducted in 100 ml Erlenmeyer flask. The basic fermentation medium consisted of 1% tryptone, 1.0 % olive oil, 0.05% $CaCl_2$, 0.05% $MgSO_4$ and 0.001% K_2HPO_4. (Syed *et al.*, 2010). A 24hr old 10% v/v inoculum was shifted into the basic cultivation medium and incubated for 48hrs at 37ºC. Subsequently the bacterial cells were pallet at 10,000x g at 0ºC for 10 minutes. The clear supernatant was pooled and utilized as the source of crude enzyme

Estimation of biomass

Growth of *Pseudomonas* strain estimated with the help of the absorbance at 600nm (Mobarak-Qamsari *et al.*, 2011).

Enzyme Assay

The total triacylglycerol hydrolase activity was monitored spectrophotometrically by using *p*-nitrophenyl-palmitate as chromogenic substrate as documented by Pencreac'h and Baratti (Pencreac'h and Baratti, 1996). The reaction mixture comprises of 1 mL of 40mM *p*-nitrophenylpalmitate (dissolved in n-hexane and 50mM phosphate buffer of pH 7.0) and 0.5 mL of CFF. After 15 minutes, Enzyme activity was ceased by adding 1 mL of 5% NaOH and the obtained product was read at 410 nm. One enzyme unit equals to the amount of enzyme that liberate one micromole of *p*-nitrophenol per mL under activity analysis conditions.

Optimization of Fermentation Parameters

The standard media was optimized for maximum lipase induction at the optimum pH 7.0 and temperature 37ºC by replacing the specific constituents of the production medium. Physical and chemical parameters for enriched yield of lipase were optimized. Physical parameters include temperature, time course and pH of fermentation medium. Whereas under the route of chemical parameters; different carbon sources, nitrogen sources, salts and various concentrations of the selected nutrients were optimized. At each step after fermentation, CFF was collected and evaluated for lipase production via the enzyme assay and total protein.

Chemical Parameters

Carbon Source and its concentration

Fermentation medium was tested for enhanced turnout of lipase with diverse carbon sources which were olive oil, almond oil, palm oil, glycerol, fructose and lactose. Optimization was studied at pH 7.0 and at 37ºC. These parameters studied individually at the concentration of 1%. The most efficient carbon source with highest lipase titer was then further optimized at different concentrations ranging from 1%-6%

Nitrogen Source and its Concentration

Various nitrogen sources which include ammonium chloride, urea, yeast extract, peptone, ammonium phosphate, and tryptone were incorporated in the cultivation medium for greater lipase formation (pH 7.0, 37^0C). These parameters studied individually at the concentration of 1%. Different concentrations (1%-6%) of most efficient nitrogen source for lipase production were further varied for selection of optimum one.

Metal ions and its concentrations

Enriched lipase turnout was tested with different salts such as $FeCl_3$, MgCl, NaCl, $MgSO_4$, KCl and $CaCl_2$. The assessment was taken individually with each salt and the most effective salt was studied at different concentrations (1%-6%).

Phosphate source and its concentrations

Suitable phosphate source was selected for enhanced lipase induction by incorporating various phosphate compounds includingKH_2PO_4, NaH_2PO_4, K_2HPO_4 and Na_2HPO_4. The selected source was further observed on varying concentration (0.01% to 0.06%).

Physical Parameters

Effect of cultivation time on lipase production

Time effect was observed by incubating the fermentation medium for varying time periods such as 3, 6, 24, 48 and 72 hrs.

Effect of pH on lipase induction

Media was studied for enriched lipase induction with the change in pH (from 3-10)

Effect of Temperature on lipase production

Different temperatures were also tested for maximum lipase yield. The fermentation media was incubated at diverse temperatures ranging from 30^0C-70^0C.

Results and discussion

Molecular Identification

The 16S rDNA of *Pseudomonas stutzeri* was found to consist of 598 base pairs (Fig.1). The obtained gene sequence was deposited to Genbank with the accession number MH639065 (*Pseudomonas stutzeri* SN-3). The gene sequence was further explored through BLAST analysis for determination of specie origin (https://blast.ncbi.nlm.nih.gov/Blast.cgi.). The gene sequence of *Pseudomonas stutzeri* SN-3 was aligned with selected twentyfour gene sequences of data base for the development of phylogenetic tree.

Optimization of Fermentation

Triacylglycerol hydrolases are extracellular enzymes. They are strongly influenced by composition and conditions of the growth medium. Optimization of these elements affects microbial growth, which ultimately enhances the enzyme induction. The chemical and physical factors of the cultivation medium not only play an important role in cost reduction of overall process but also govern the proper applicability of enzyme.

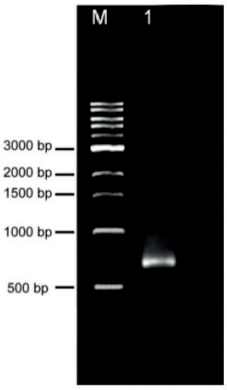


Figure 1. Molecular weight determination of amplified 16S rDNA. Lane M represents DNA ladder and Lane 1 denotes amplified PCR product

Chemical Parameters

Carbon Source and its concentration

Outcomes concerning with the effect of various carbon sources on the production of triacylglycerol hydrolase are depicted in Fig.3. It showed that all the carbon sources used, influence triacylglycerol hydrolase production varyingly but maximum triacylglycerol hydrolase production was attained when the medium was supplemented with palm oil. Highest triacylglycerol hydrolase production (82.09 ± 0.565 U) and cell growth (1± 0.05 mg/mL) was obtained when the palm oil used as carbon source. However, high cell mass was also observed when the cultivation medium was enriched with fructose and lactose. Outcomes of varying concentrations of palm oil on triacylglycerol hydrolase titer showed a sharp decline in enzyme yield with every rise in percent concentration of palm oil in the cultivation medium (Fig. 3B). The cell mass also observed to follow the same decline with increasing concentration of palm oil in the production medium.

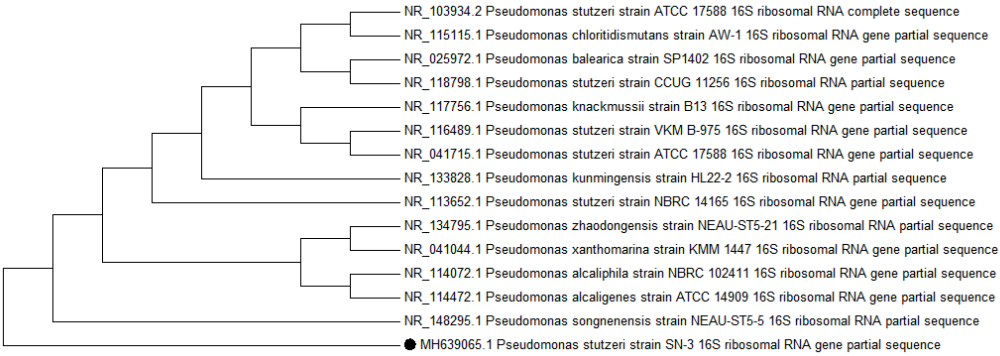


Figure 2. Phylogenetic tree by maximum likelihood method exhibiting the 16S rDNA sequence similarity against the available 16S rDNA sequences in Genbank database.

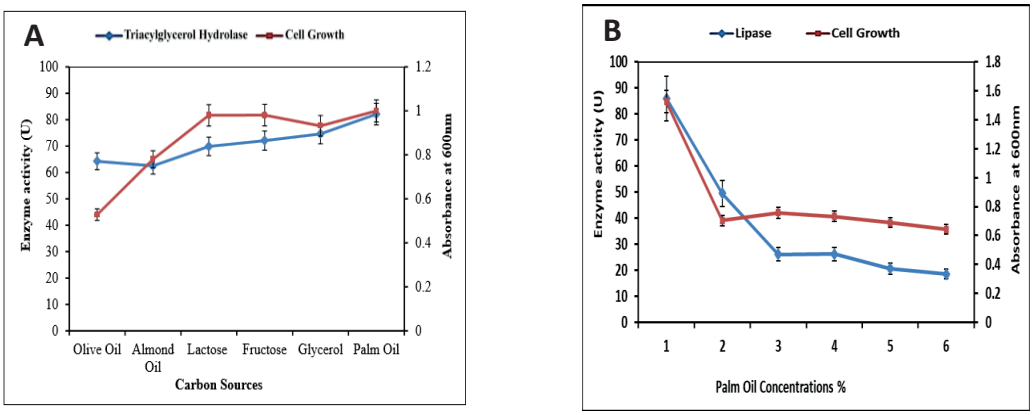


Figure 3. Effect of different carbons sources (A) and concentration of appropriate carbon source (B) on induction of lipase and bacterial growth. (Values are expressed as Mean ± S.E, n=3).

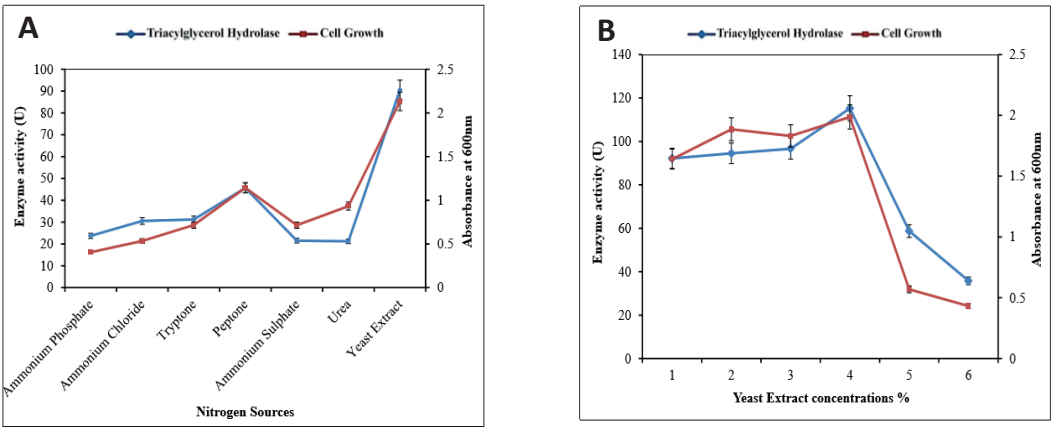


Figure 4. Effect of different nitrogen sources (A) and concentration of appropriate nitrogen source (B) on lipase induction and bacterial growth. (Values are expressed as Mean + S.E, n=3).

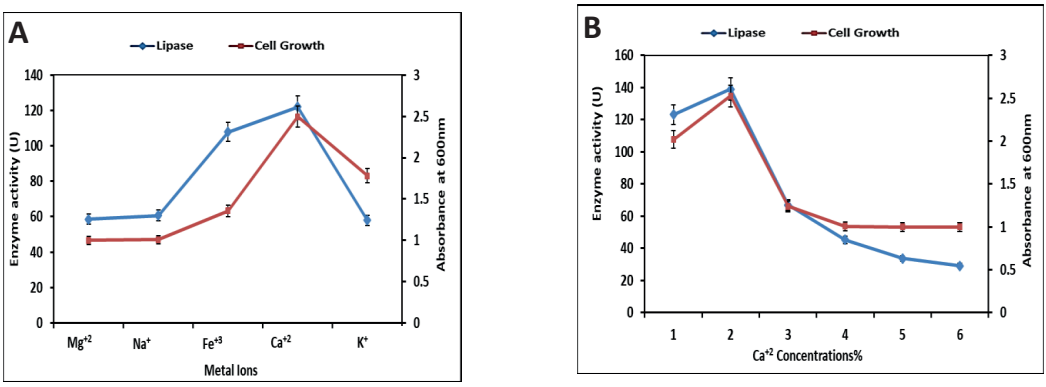


Figure 5. Influence of various metal ions (A) and concentration of appropriate metal ion i.e. Ca^{+2} (B) on induction of lipase and bacterial growth. (Values are expressed as Mean $\pm$ S.E, n=3).

Nitrogen Source and its Concentration

The effect of different nitrogen sources and the concentration of appropriate source on triacylglycerol hydrolase induction were evaluated as shown in Fig.4. Initially, various organic and inorganic nitrogen sources were analyzed and yeast extract was found to be the most preferred nitrogen source as reflected by the high titer of triacylglycerol hydrolase (90.52 ± 0.406 U) and highest cell mass. It was also observed that triacylglycerol hydrolase production by *Pseudomonas stutzeri* SN-3 indicate a direct relationship with the rise in yeast extract concentration up-to four percent and then decreases with five and six percent concentrations of yeast extract (Fig.4B). The isolated *Pseudomonas stutzeri* SN-3 was also observed to grow abundantly in the medium at 4% concentration of yeast extract.

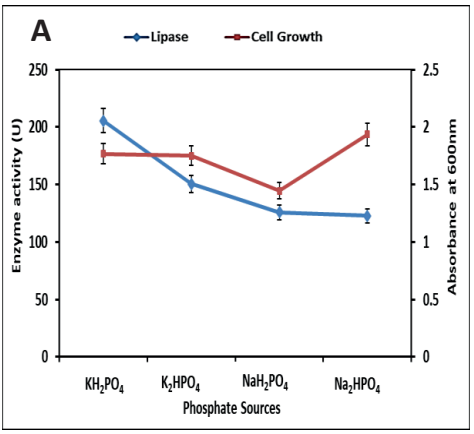
Metal ions and its concentrations

Data plotted in Fig.5 shows the effect of different metal ions on triacylglycerol hydrolase induction and bacterial growth. All the metal ions tested improved the triacylglycerol hydrolase induction distinctly however, $CaCl_2$ resulted in maximum secretion of triacylglycerol hydrolase (122.059 ± 1.341 U) at 2% concentration (Fig.5B). The parallel pattern of the cell growth was observed in metal ions and the different concentrations of Ca^{+2} ions.

Phosphate source and its concentrations

Data presented in figure 6 (A) displayed that all the phosphate sources added to the cultivation medium affect bacterial cell division and triacylglycerol hydrolase titer. Maximum triacylglycerol hydrolase production (205.67 ± 5.283U) with a subsequent highest cell biomass was found when medium was supplemented with KH_2PO_4. Yield of triacylglycerol hydrolase was shown to drop significantly, when the fermentation medium was incorporated with K_2HPO_4, NaH_2PO_4, and Na_2HPO_4.The Na_2HPO_4 existence in growth medium displayed uppermost cell growth; however

the triacylglycerol hydrolase titer was found to drop comparatively. Various Concentrations of KH_2PO_4 supplementation were also explored to efficiently formulate the cultivation medium for enhanced triacylglycerol hydrolase induction. Obtained results were plotted in figure 6B. It was found that 0.01% $KH_2PO_4$was suitable for *Pseudomonas stutzeri* SN-3 to produce maximum triacylglycerol hydrolase. Further increase in $KH_2PO_4$was found to be inhibitory for both cell proliferation and triacylglycerol hydrolase induction.



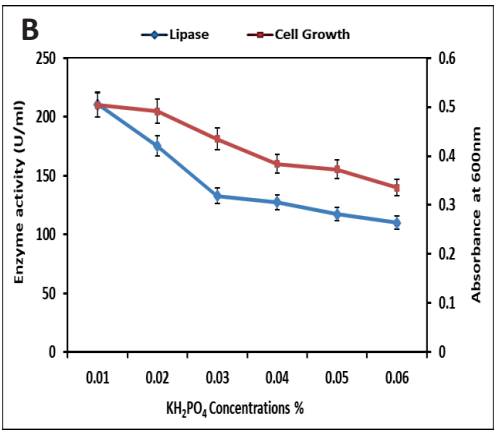


Figure 6. Effect of different phosphate sources on cell proliferation and lipase production by *Pseudomonas stutzeri* SN-3. (Values are expressed as Mean + SD, n=3).

Fermentation Optimization with Physical Parameters

Effect of cultivation time on lipase production

Outcomes of cultivation time exhibited that utmost 48hours required by *Pseudomonas stutzeri* SN-3 to produce high triacylglycerol hydrolase titer (Fig. 7). The graph of cell growth displayed that the appearance of triacylglycerol hydrolase titer into the growth medium was initiated in exponential phase and then gradually increases, reached to its upper limit at the commencement of stationary phase (Fig. 7). A gradual decline in triacylglycerol hydrolase titer was obtained after 48 hours of fermentation.

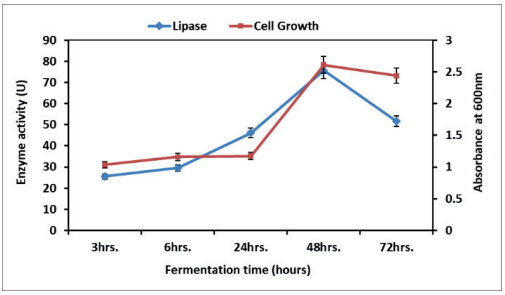


Figure 7. Time course optimization for maximum induction of lipase and bacterial growth. (Values are expressed as Mean ± S.E, n=3).

Effect of pH on lipase induction

Concerning triacylglycerol hydrolase induction in present research, pH 7.0 was found to be the most appropriate pH as it showed highest triacylglycerol hydrolase titer and highest cell mass (Fig 8).

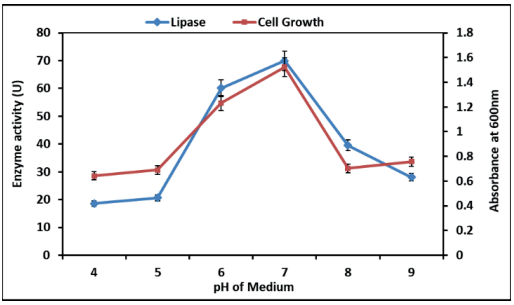


Figure 8. Effect of pH on induction of lipase and bacterial growth. (Values are expressed as Mean ± S.E, n=3).

Effect of Temperature on lipase production

Temperature was found to be the inducer of triacylglycerol hydrolase production as the enzyme yield affects with all the temperatures tested. Results interpret 40ºC as the suitable temperature for *Pseudomonas stutzeri* SN-3 as it was observed to grow adequately and produced maximum triacylglycerol hydrolase at 40ºC.

Triacylglycerol hydrolasess are the most versatile enzymes used in the industries. Their promiscuous nature makes them the most suitable candidate for use in various industries. Due to the increasing demand of microbial triacylglycerol hydrolasess in industrial site, this study was therefore conducted to isolate potential triacylglycerol hydrolase producing bacteria and to evaluate the effect of different physico-chemical parameters for hyper production of triacylglycerol hydrolase.

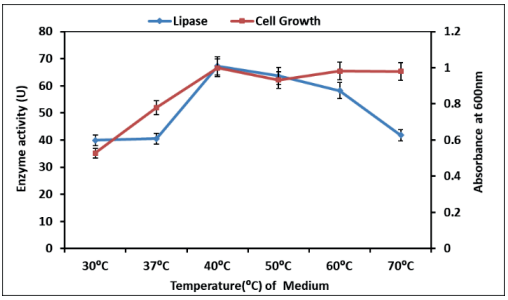


Figure 9. Effect of temperature on induction of lipase and bacterial growth. (Values are expressed as Mean ± S.E, n=3).

Molecular identification of the previously isolated *Pseudomonas* strain encompasses the exploration of 16S rDNA sequence. The obtained dendrogram presented in figure 2 showed that isolated *Pseudomonas stutzeri* SN-3 originated from other strains of *Pseudomonas stutzeri* and form an exclusive array with *Pseudomonas songnenensis* which has its origin from other *Pseudomonas* species. Based on these findings, the isolated *Pseudomonas stutzeri* SN-3 is placed in genus *Pseudomonas Sensustricto*, *Protobacteria* phyla and class *Gammaproteobacteria* (Parwata *et al.*, 2014; Lalucat *et al.*, 2006).

Carbon has always been the chief considerable component for the expression of triacylglycerol hydrolase activity due to the inducible nature of lipases (Marina *et al.*, 1998; Fabiszewska *et al.*, 2015). Consequently lipases are generally produced in the presence of lipid sources such as oil, hydrolysable esters like glycerol (Gupta *et al.*, 2004) and other carbon sources which include sugars (Ghanem *et al.*, 2000; Rashid *et al.*, 2001). Therefore, the effects of various carbon sources on production of triacylglycerol hydrolase have been included in this research design.

According to the available literature, palm oil industrial residues shown promising potential to induce triacylglycerol hydrolase production by *Aspergillus niger* (Charles and James, 2011). Palm oil mill effluent (POME) was found to be a significant factor in induction of triacylglycerol hydrolases from fungal species (Ibegbulam-Njoku and Achi, 2014). Palm agro-industrial waste has reported to enhance lipase production from the nonpathogenic yeast *Yarrowia lipolytica* (Fraga *et al*., 2021). The *Bacillus pumilus* lipase produced with palm oil as substrate (Saranya *et al.*, 2014). The current research exhibited comparable outcomes with Saranya *et al* (Saranya, 2014) validating the efficiency of palm oil in lipase synthesis by *Pseudomonas* sp. In addition to palm oil, fructose and glycerol were also observed to be the appropriate substrates for lipase induction. The high cell growth may be due to the inclination of microbes towards simple sugars for cell division and growth (Mazmira *et al.*, 2012).

Formerly olive oil was an extensively used source for lipase production (Amin *et al.*, 2014; Erick *et al.*, 2016). However, in present research, palm oil has shown promising potential for lipase production besides olive oil. Hence it can be predicted that palm oil is a comparatively new bioresource to enhance triacylglycerol hydrolase induction in *Pseudomonas.* At the same time the isolated *Pseudomonas stutzeri* SN-3 can be utilized for bioremediation of palm oil contaminated soil and water.

Organic nitrogen sources were found to increase lipase production by *Pseudomonas sp*. (Mobarak-Qamsari *et al.*, 2011). However, concerning to the cell growth, other organic nitrogen sources also exhibited positive results. These sources used as active ingredients during cell multiplication and therefore they are preferable over their inorganic counterparts (Djekrif-Dakhmouche *et al.,* 2006).

According to present research findings, triacylglycerol hydrolase induction has been greatly enhanced in presence of 4% yeast extract and the cell growth also perfectly coincides with this result. Traditionally, enriched triacylglycerol hydrolase production was obtained by using both organic and inorganic nitrogen sources. Yeast extract has proven to be the best source for maximum lipase induction in *Bacillus* sp. (Laachari *et al.*, 2014) in *Pseudomonas aeruginosa* UKHL1 (Patel *et al.,* 2020). Yeast extract supports greater cell yield as it comprises of the essential amino acids, peptides and water soluble vitamins necessary for microbial growth (Mobarak-Qamsari *et al.*, 2011; Peighamy-Ashnaei *et al.*, 2006). Therefore, the present results are valuable addition in evidences to the previous studies.

Besides macromolecules, micromolecules (minerals) are also necessary for proliferation and growth of microorganisms. Therefore, in this study, the effect of various metal ions was examined on isolated strain for triacylglycerol hydrolase production. These minerals were found to augment triacylglycerol hydrolase activity by modifying the structure and removal of fatty acids from the reaction site. Therefore, enhance the induction of triacylglycerol hydrolase by providing a suitable environment to it (Kumar and Valsa, 2007). Various studies showed that triacylglycerol hydrolase yield improve significantly by varying the concentrations of $CaCl_2$ (Patel *et al*., 2020; Bokhari *et al.*, 2013; Kumar *et al.*, 2012).

Phosphorous compounds also play crucial role in bacterial multiplication and development. They serve as regulatory and structural sources in bacterial cultures. In excess amount, they may render the phosphate metabolism and thereby reduces bacterial cell growth (Kulakovskaya, 2014). Therefore various phosphate compounds (KH_2PO_4, NaH_2PO_4, K_2HPO_4, and Na_2HPO_4) were included into the fermentation medium to document their effects on cell division of *Pseudomonas stutzeri* SN-3 and triacylglycerol hydrolase titer. In present research, KH_2PO_4 was found to stimulates triacylglycerol hydrolase production. The same phosphate source that is KH_2PO_4 was reported as microbial

stimulant for yield enhancement of lipid degrading enzyme (). In combination to soy meal, KH_2PO_4 was also found to prefer by *Botryosphaeriaribis* EC-01 to produce lipase proficiently (Barbosa *et al.*, 2011). Fungal preference of KH_2PO_4 has also been reported such as in *Trichodermaviride* (Osman *et al.*, 2012). A lipid hydrolyzing enzyme from *Aspergillusniger* was also found to produce in higher amounts when 0.2% KH_2PO_4 was incorporated in the growth medium (Pokorny *et al.*, 1994).

After optimizing chemical parameters, effect of various physical parameters on enzyme production was also observed. The decline in triacylglycerol hydrolase titer may be due to presence of other extracellular metabolites that are responsible to change the pH of enzyme production medium (Saranya *et al.*, 2014). The rise in pH and level of proteases results in low triacylglycerol hydrolase production (Gupta *et al.*, 2004). All these observation are in accordance with previous studies that also reported maximum lipase production in 48hours by different bacterial species (Bokhari *et al.*, 2013; Esteban-Torres *et al.*, 2015; Paul *et al.*, 2015 and Deyaa *et al.*, 2016).

The pH of the production medium plays a critical role in maintenance of microbial physiological functions and movement of nutrient and mineral sources through cellular membrane. Consequently, optimum pH ultimately enhances the enzyme production. The optimal pH of fermentation medium was found to be 7.0 reflecting the preference of *Pseudomonas stutzeri* towards neutral pH (Lalucat *et al.*, 2006). Inclination of bacterial lipases for pH 7.0 has been evident from the literature (Paul *et al.*, 2015; Deyaa *et al.*, 2016).

Optimum temperature of fermentation medium was found to be 40^0C. The same temperature was observed for lipase production by an *Actinomycete* (Patel *et.al.*, 2021). The results of effect of temperature on triacylglycerol hydrolase production reflects that the isolated *Pseudomonas stutzeri* SN-3 is mesophilic in nature. Many lipase producing bacterial strains were reported to cultivate in temperature ranges 25-40^0C (Fatima *et.al.*, 2021). As the high cell growth associated with increased enzyme yield therefore many bacterial strains exhibited maximum lipase production at 37^0C including *lactobacillus plantarum* and g*eobacillus thermoleovorans* DA2 (Esteban-Torres *et al.*, 2015; Paul *et al.*, 2015; Kanimozhi *et al.*, 2011).

Conclusion

Triacylglycerol hydrolases are crucial enzymes for the growing field of Biotechnology. Through present research, a novel lipase producing strain was identified molecularly as *Pseudomonas stutzeri* SN-3. The sequence of 16S rDNA was deposited in the Genbank [MH639065]. Further, an economical fermentation medium was designed for maximum lipase yield. The property of *Pseudomonas stutzeri* SN-3 to degrade palm oil as carbon source efficiently by the action of triacylglycerol hydrolase made it a useful bioresource for management of palm oil industrial effluents and palm oil containing wastewater. Future research on purification and characterization studies of *Pseudomonas* SN-3 triacylglycerol hydrolase may reveal its possible forthcoming applications.

Acknowledgement

The authors are grateful to University of Karachi for funding this research.

Conflict of interest

There are no conflicts of interest.

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