



Studies on the extremo-lipase produced by the halotolerant *Oceanobacillus iheyensis* strain QCS

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Abstract

In the current work, different studies were carried out on the lipase enzyme produced by the halotolerant *Oceanobacillus iheyensis* strain QCS, with an expectation to be an important candidate in the industrial applications. Lipase of strain QCS was halo-alkali-thermo-detergent-solvent stable. Maximum production of lipase was obtained after 72 h of incubation, at 40°C and pH 8 and 9 in a medium containing 25 % (w/v) NaCl and 1 % (v/v) olive oil as a lipid substrate. This lipase was partially purified, highest lipase activity was obtained in 80% ammonium sulfate saturation, and in fraction eight of the Sephadex G-200 gel filtration chromatography. Lipase displayed wide spectrum of activity within a broad range of conditions including salinity, temperature and pH, it was optimally active at 25 % (w/v) NaCl, 40°C and pH 8 and 9, respectively. The effect of many metal ions, detergents and organic solvents on the activity of lipase was evaluated. Interestingly, lipase was able to retain the majority of its activity in the presence of Ni²⁺, Mg²⁺, Oxi and Fairy detergents, ethyl acetate, dimethyl formamide and toluene, respectively. Overall, as the lipase from *O. iheyensis* strain QCS has a number of interesting properties especially its stability at extreme conditions; it could be used as a potential promising candidate for detergents industry, and as a biocatalyst in low water enzymatic processes.

Keywords: Lipase, *Oceanobacillus iheyensis*, Extreme conditions, Halotolerant

1. Introduction

Among the most important widely distributed extremophilic microorganisms are the halophilic bacteria that can survive in hypersaline environments ([Aljohny, 2015](#); [Mohammadipanah et al., 2015](#)). Previous studies of [Kushner, \(1985\)](#); [Kushner and Kamekura, \(1988\)](#) documented that based on NaCl requirements, the extremely halotolerant bacteria can

tolerate a broad range of NaCl concentrations i.e. 0-32 (% , w/v). Later, [DasSarma and Arora, \(2001\)](#) added that these microorganisms can preserve their cell structure and function in high saline conditions. According to [Oren, \(2010\)](#), the halophilic bacteria are recognized as producers of polysaccharides, polyhydroxyalkanoate, carotenoid pigments,

compatible solutes and hydrolytic enzymes of great industrial importance. [De Lourdes Moreno et al., \(2013\)](#) added that the diversity of halophilic bacteria could produce several hydrolytic enzymes including; protease, lipase, amylase, cellulase, xylanase and DNase that have many applications in the different fields of industry. These enzymes are not only stable at high salt concentrations but also can efficiently function under extreme conditions [\(Kumar et al., 2012\)](#). A recent study conducted by [Karray et al., \(2018\)](#) added that the capacity of the halophilic bacteria to make cost-effective fermentation with minimum sterile precautions through their hydrolytic enzymes; is an interesting research topic. Due to their stability, high activity in presence of organic solvents and their broad substrate range, the lipases produced by halophilic bacteria are used in various biotechnological applications including; waste treatment, textile, food as well as detergents industries [\(De Lourdes Moreno et al., 2013; Schreck and Grunden 2014\)](#). The objectives of the present study were to optimize, purify and describe the extracellular lipase produced by the extremely halotolerant *Oceanobacillus iheyensis* strain QCS, with the expectation that such lipase will be an important candidate in the industrial applications.

2. Material and methods

2.1. Bacterial strain

The extremely halotolerant *Oceanobacillus iheyensis* strain QCS was isolated from the beach sands collected from El Quseir region (26°06'14"N 34°16'52"E), Red sea, Egypt, during July, 2018. It has been identified using biochemical tests and 16S rRNA gene sequence analysis, the data was deposited in NCBI GenBank as MT573497. This strain showed strong ability to produce an extracellular lipase, thus it was selected for this study.

2.2. Fermentation and crude enzyme preparation

Fermentation was carried out in 1000 ml Erlenmeyer flasks containing 200 ml minimal salt medium (150 NaCl, 2 KCl, 5 MgSO₄.7H₂O, 1 yeast

extract g/l) supplemented with 1 % (v/v) olive oil, and the pH was adjusted to 7. Flasks were inoculated with 2 ml of an overnight grown culture of strain QCS (1×10⁷ cells/ml), and then incubated at 37°C, 150 rpm for 72 h. The cell free supernatant was obtained by centrifugation at 5000 rpm and 4°C for 25 min., and used as a crude enzyme [\(Kiran et al., 2014\)](#).

2.3. Estimation of lipase activity

The lipase activity was estimated spectrophotometrically according to [Zehra and Metin \(2015\)](#). Briefly, 1 ml crude enzyme was added to the mixture of 8 ml Tris HCl buffer (50 mM, pH 8.0), and 1 ml *p*-nitrophenyl-palmitate (pNPP) (10 mM in ethanol). The reaction was allowed to proceed for 1 h at 37°C. The reaction was stopped using 1 ml trichloroacetic acid (0.6 N), and then the absorbance was measured at 410 nm. Lipase activity was evaluated in IU/ ml using the lipase standard curve. One unit of lipase activity was defined as the amount of an enzyme (ml) that hydrolyzed pNPP and released 1 μ mol of *p*-nitrophenol per min., under the standard assay conditions.

2.4. Optimization of conditions for maximum enzyme production

The effect of different parameters on the maximum production of lipase by strain QCS was studied using one variable at a time approach. The studied parameters were NaCl concentration (0- 32%, w/v), temperature (25-50°C) within an interval of 5°C, pH (4-10) within an interval of 1, and the incubation period (0-144 h) within an interval of 24 h.

The effect of different substrates on enzyme production was evaluated using several lipid sources such as: tributyrin, tween 60, tween 80, olive oil and sunflower oil at a concentration of 1% (v/v). The fermentation media were inoculated and then incubated at 37°C and 150 rpm for 72 h. The optical density (OD) was measured at 600 nm, and lipase production (IU/ ml) was estimated as mentioned above.

2.5. Partial enzyme purification

2.5.1. Ammonium sulfate fractionation

The crude supernatant was precipitated with different concentrations of ammonium sulfate i.e. 20, 40, 60, 80 and 100% (w/v) according to the method of [Higa and Cazzulo, \(1973\)](#). The mixture was stirred well for 2 h, and then centrifuged at 6000 rpm for 20 min.

2.5.2. Gel filtration chromatography

According to [Hasan *et al.*, \(2007\)](#), the enzyme obtained from ammonium sulfate precipitation was dialyzed against 0.2 M boric acid- borax buffer (pH 7.0) and stirred gently overnight. The recovered concentrated enzyme was eluted through Pharmacia gel filtration column (3×10 cm) packed with Sephadex G-200 (particle size 200 μ, Sigma), using with 0.2 M boric acid- borax buffer (pH 7.0). Fractions of 2 ml were collected every 15 min. and both of the enzyme activity and protein content were determined for each separate fraction.

2.5.3. Determination of the protein content

The protein content (mg/ ml) of each fraction was determined according to [Lowry *et al.*, \(1951\)](#), using bovine serum albumin (BSA) standard curve.

2.6. Effects of different environmental factors on lipase activity

2.6.1. Effect of NaCl, temperature and pH

To determine the effects of NaCl, temperature and pH on the activity of lipase, the enzyme reaction was carried out for 3 h at different NaCl concentrations (0-32 %), temperatures from (10- 50°C) within an interval of 5°C, and different pH values that ranged from 5-12.

2.6.2. Effects of metal ions

The effect of different metal ions i.e., Zn²⁺, Ni²⁺, Sn²⁺, Co²⁺ and Cd²⁺ on the lipase activity was studied at different concentrations (0-300 mg\ l) within an interval of 50 mg\ l, in reference to [Mukesh kumar *et*](#)

[al., \(2012\)](#). The enzyme was incubated with the metal ions for 3 h at 37°C. The enzyme activity was measured using the pNPP standard method, and was calculated in IU/ ml compared to the activity of the control (without additions).

2.6.3. Effects of detergents

Some commercial detergents such as Oxi, Persil, Ariel, Pril and Fairy obtained from different supermarkets at Aswan governorate, were evaluated for their effects on lipase activity, according to the method of [Mukesh kumar *et al.*, \(2012\)](#).

Briefly, 1 ml of the detergent solution at a concentration of 0.7 % was incubated with 1 ml of the enzyme solution for 3 h at 37°C. The mixture without detergent was used as control. The lipase activity was measured and calculated using pNPP standard method, considering the control activity as 100 %.

2.6.4. Effects of different organic solvents

The effects of different organic solvents such as; ethanol, methanol, acetone, toluene, ethyl acetate and dimethyl formamide on lipase activity was estimated following the method of [Uttatree *et al.*, \(2010\)](#).

The same volumes of lipase solution and organic solvents were mixed individually in test tubes covered with rubber stopper, and then stored at 37°C and 150 rpm for 3 h. The lipase activity was estimated in reference to the activity of the non-treated control.

2.7. Statistical analysis

Statistically significant differences ($p > 0.05$) between the obtained data were determined using one-way analysis of variance (ANOVA) from Minitab statistical program (version 12).

3. Results and Discussion

Lipases are extracellular hydrolytic enzymes of considerable industrial potential. Due to their broad significance, the study of lipases is of great interest in many previous studies ([Alberghina *et al.*, 1991](#);

[Bornscheuer, 2000](#)). Microorganisms are valuable resources of commercially useful lipases ([Sharma et al., 2001](#)). The current study deals with optimization and partial characterization of extracellular lipase recovered from the extremely halotolerant *Oceanobacillus iheyensis* strain QCS (MT573497) that was isolated from the beach sands at El Quseir region, Red sea, Egypt.

A previous study conducted by [Bas and Boyaci, \(2007\)](#) highlighted that cost-savings are important concepts for any industry scale, thus optimization of the fermentation conditions is of great significance, to improve the efficiency of the process without increasing its cost. In the present study some parameters were evaluated for their effects on lipase production by *O. iheyensis* strain QCS including; different NaCl concentrations, temperatures, pH values, incubation periods, in addition to the use of several lipid sources as substrates.

Growth and lipase production by strain QCS are significantly ($F=34.45$, $p < 0.05$) affected by NaCl concentration. Lipase production occurred within a wide range of NaCl concentrations (0-32 %). Currently, the optimum growth and enzyme production are detected at 25 % (w/v) NaCl as shown in Fig. (1). On the other hand, during the early study of [Khunt et al., \(2012\)](#), *Halomonas salina* Ku-10 showed maximum growth and lipase production at 15 and 10% (w/v) NaCl, respectively. Moreover, [Samaei-Nouroozi et al., \(2015\)](#) found that the optimum NaCl concentrations for maximum growth and lipase production by *Alkalibacillus salilacus* strain SR-079 were recorded at 18 % , 12 % NaCl, respectively.

Temperature has a great effect on both growth and enzyme production by the halophilic bacteria ([Moreno et al., 2013](#)). In the current study, it is observed that temperature has significant effect on the growth and lipase production by strain QCS ($F=91.76$, $p < 0.05$). Lipase production occurred within a temperature range of 25-45°C, while the maximum growth and production occurred at 40°C, as demonstrated in Fig. (2). This is in accordance with the previous findings of

[Samaei-Nouroozi et al., \(2015\)](#), who reported that 40°C was the optimum temperature for maximum growth and lipase production by *Alkalibacillus salilacus* strain SR-079. Similarly, in a recent study of [Mazhar et al., \(2017\)](#), maximum production of lipase by *Bacillus subtilis* PCSIRNL-39 was recorded at 45°C.

The pH value of the medium plays an important role in enzyme production and activity. [Gupta et al., \(2003\)](#) reported that the change in pH of the medium affects the enzyme stability. A previous research work of [Friedrich et al., \(1989\)](#) highlighted that the pH values serve as a valuable indicator of the initiation and end of the enzyme synthesis. In the present study, the growth and lipase production are significantly ($F=56.04$, $p < 0.05$) affected by the change in pH value. Maximum growth and enzyme production are achieved at alkali pH 8 and 9 (Fig. 3). Similar results have been reported in the case of lipases from halophilic *Halomonas salina* Ku-10 and *Alkalibacillus salilacus* SR-079, recorded in the previous studies of [Khunt et al., \(2012\)](#); [Samaei-Nouroozi et al., \(2015\)](#), respectively. On the other hand, [Mazhar et al., \(2017\)](#) stated that lipase was optimally produced from *Bacillus subtilis* PCSIRNL-39 at pH 7.

The effect of incubation period on lipase production by strain QCS was evaluated by estimating the enzyme production (IU/ ml) every 24 h, during a period of 144 h. It is observed that the incubation period has significant ($F= 20.27$, $p < 0.05$) role on enzyme production. Lipase production reached its maximum level after 72 h of incubation, after that it is declined (Fig. 4).

This may be attributed to depletion of nutrients, changes in media pH and the accumulation of toxic end products. This agreed with the findings of [Samaei-Nouroozi et al., \(2015\)](#) who stated that the maximum production of lipase from *Alkalibacillus salilacus* SR-079 was achieved after 72 h of incubation period, and then the production began to decrease rapidly with further incubation.

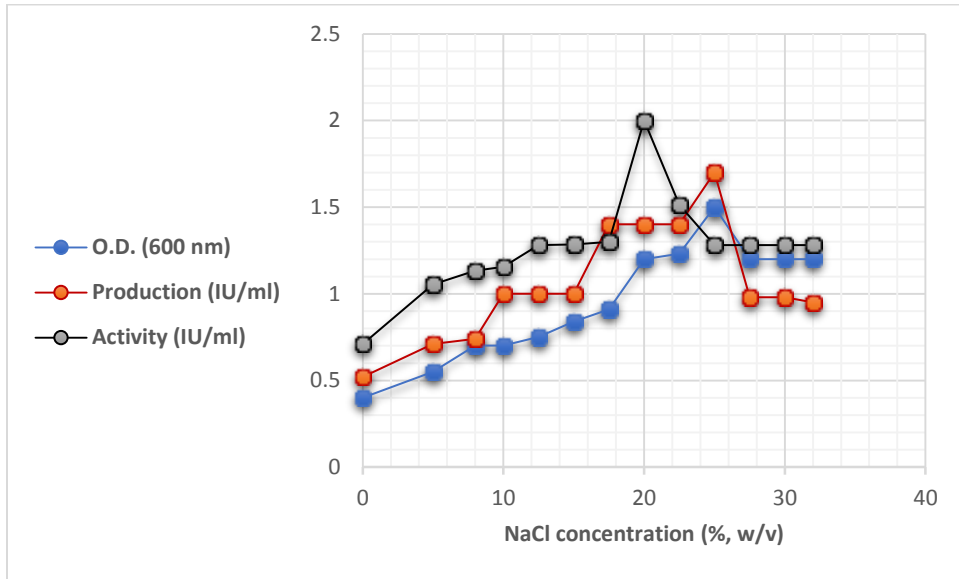


Fig. 1. Effects of different NaCl concentrations on optical density (OD), production and activity of lipase enzyme

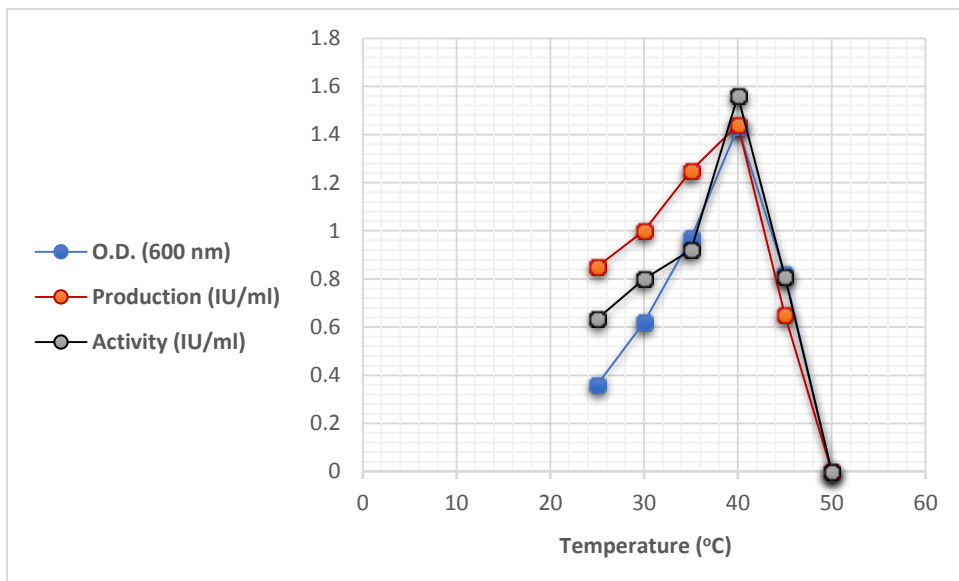


Fig. 2. Effects of different temperatures (°C) on optical density (OD), production and activity of lipase enzyme

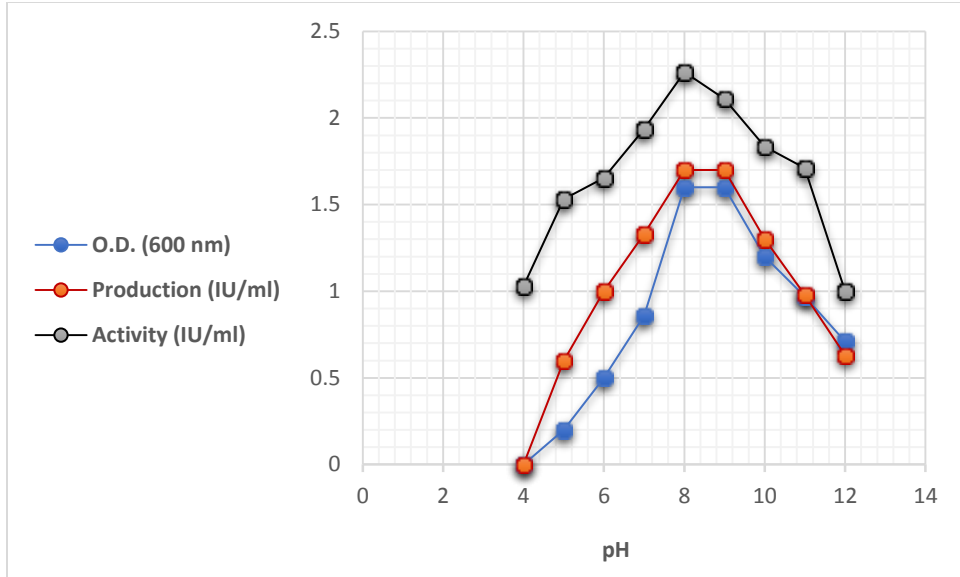


Fig. 3. Effects of different pH values on optical density (OD), production and activity of lipase enzyme

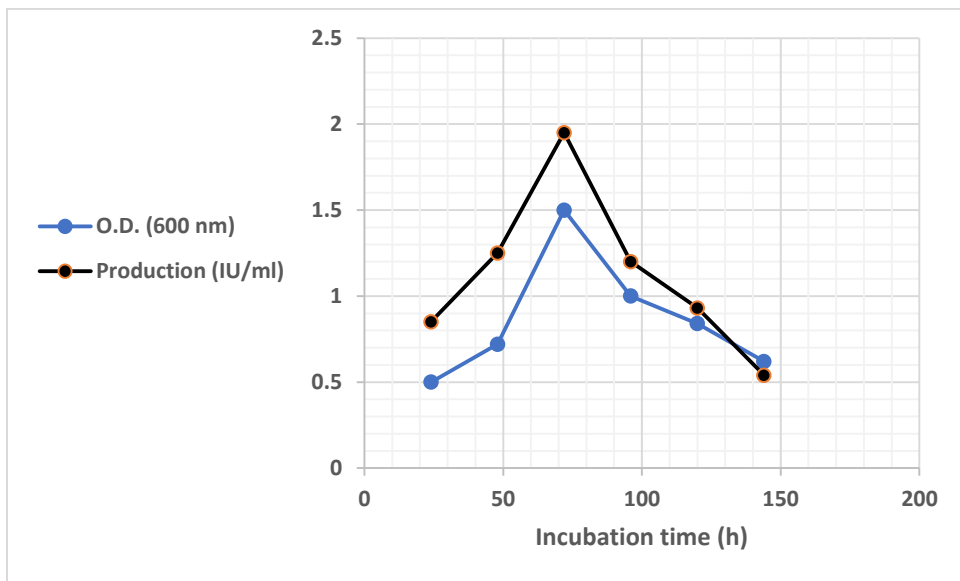


Fig. 4. Effects of different incubation periods (h) on optical density (OD) and lipase production

One of the major factors that affect lipase production by strain QCS is the type of lipids. According to [Samaei-Nouroozi et al., \(2015\)](#), the stimulatory effects of the lipid sources on lipase production mostly depend on the physiological and biochemical pathways of the bacterium. Results of the current study recorded that olive oil is the best lipid source that induced the maximum production of lipase, followed by sunflower oil as demonstrated in Fig. (5). Similar findings of lipase production by various microorganisms were reported by previous studies of; [Ahmed et al., \(2010\)](#); [Mehta et al., \(2012\)](#); [Balan et al., \(2013\)](#); [Samaei-Nouroozi et al., \(2015\)](#). Results of [Jaiswal et al., \(2017\)](#) work demonstrated that maximum lipase production by *Proteus mirabilis* and *B. coagulans* was obtained when sunflower oil was

used as a lipid substrate. Furthermore, during the recent study of [Furini et al., \(2018\)](#), maximum production of lipase by *Acinetobacter baylyi* was achieved on using olive oil, grape seed oil and canola oil as lipid substrates.

Under the optimum production conditions, lipase is produced from strain QCS and was subjected to purification using ammonium sulfate precipitation followed by Sephadex G-200 gel filtration chromatography. The protein content (mg/ ml) and enzyme activity (IU/ ml) for the collected fractions are determined (Fig. 6). Amongst the 15 fractions recovered, fraction number eight exhibited the highest specific enzyme activity (Fig. 6).

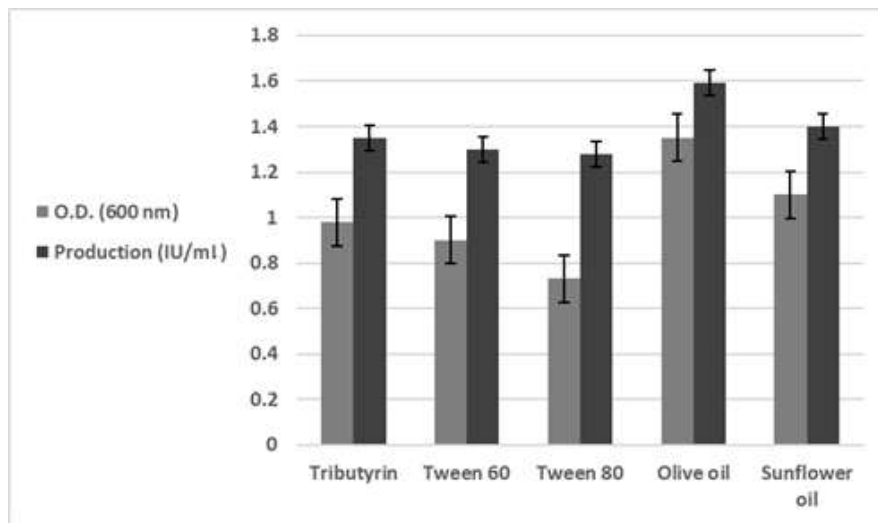


Fig. 5. Effects of different lipid substrates on optical density (OD) and lipase production. Values are means \pm standard errors (SEs)

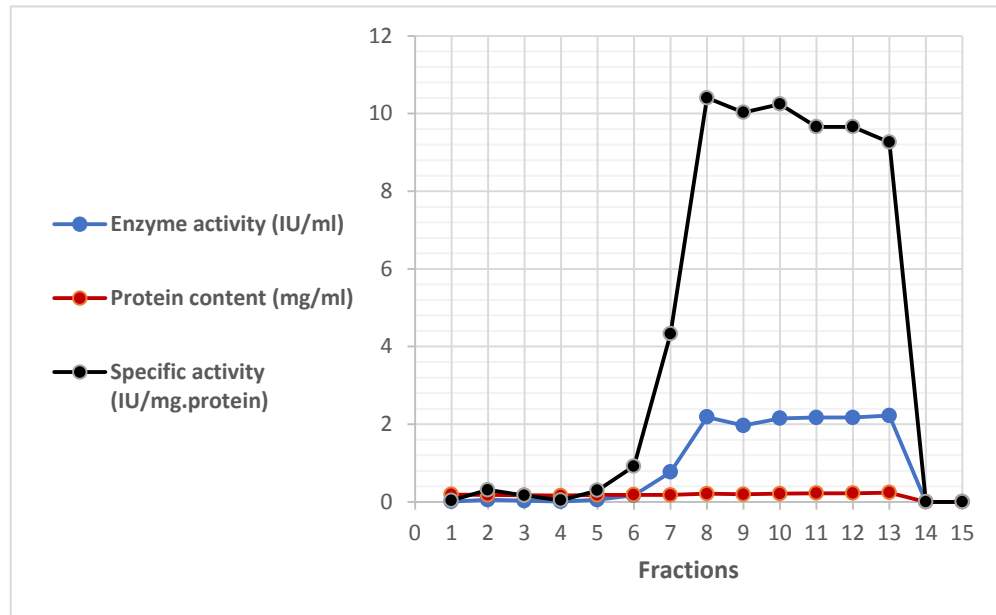


Fig. 6. Sephadex G-200 filtration chromatography of lipase enzyme produced by strain QCS. Fraction number eight exhibited the highest specific activity

Currently, NaCl concentration had significant ($F=33.56$, $p < 0.05$) effect on the lipase activity. Maximum activity was obtained at 22.5 % (w/v) NaCl (Fig.1). In general, halophilic enzymes required the presence of NaCl for optimal activity, as reported by [Mevarech et al., \(2000\)](#). This property is important from the biotechnological point of view.

Activity of the lipase was detected between 25-45°C, while the maximum activity was achieved at 40°C (Fig. 2). This makes its application in the industrial processes that require high temperatures possible ([Coronado et al., 2000](#)). In accordance with the current findings, lipase from the halophilic *Alkalibacillus salilacus* was optimally active at 40°C ([Samaei-Nouroozi et al., 2015](#)).

The pH is a significant ($F=46.82$, $p < 0.05$) parameter that effected the activity of lipase from strain QCS. The present lipase exhibited activity within a wide range of pH from 4 to 12, while the maximum activity was recorded at pH 8 (Fig. 3). This

agreed with the previous results of [Samaei-Nouroozi et al., \(2015\)](#) who reported that pH 8 was the optimum pH for maximum lipase activity produced by the halophilic *Alkalibacillus salilacus*.

In the current study, the response of lipase to the metal ions is variable (Fig. 7). Metal ions such as Ni^{+2} and Mg^{2+} are found to enhance the enzyme activity, while Zn^{2+} , Co^{2+} and Cd^{2+} ions suppressed the enzyme activity. The same results were reported for other lipases produced by halophilic bacteria ([Kiran et al., 2014](#); [Samaei-Nouroozi et al., 2015](#)).

Since the last two decades, the biotechnological applications of lipases have been significantly increased ([Jaeger and Eggert, 2002](#)). The enzyme-based detergents have better desirable properties compared to the synthetic detergents such as; better cleaning, activity and stability at low washing temperatures, as well as being ecofriendly ([Kumar et al., 1998](#)). Lipases are used as detergents for laundry and automatic dishwashing machines, to degrade the

fatty stains such as fats, butter and sauces. Accordingly, [Hasan et al., \(2010\)](#) demonstrated that lipases that are stable in the presence of harsher detergent formulation ingredients are of great interest in the detergents industry. For this purpose, the effects of some available commercial detergents on the activity and stability of the current lipase was studied, to exploit the enzyme in the detergents industry. Interestingly, compared to the control (without detergent), the activity of the present lipase is slightly

affected by the tested detergents. Lipase showed excellent stability and activity in the presence of detergents i.e. Oxi followed by Fairy, Persil, Ariel and Pril after 3 h of incubation at 37°C (Fig. 8). In accordance with the current findings, [Hemlata et al., \(2016\)](#) reported that the thermostable alkaline lipase from *B. sonorensis* 4R retained its activity and stability in the presence of different detergents such as; Ariel, Surf excel, Tide and Rin.

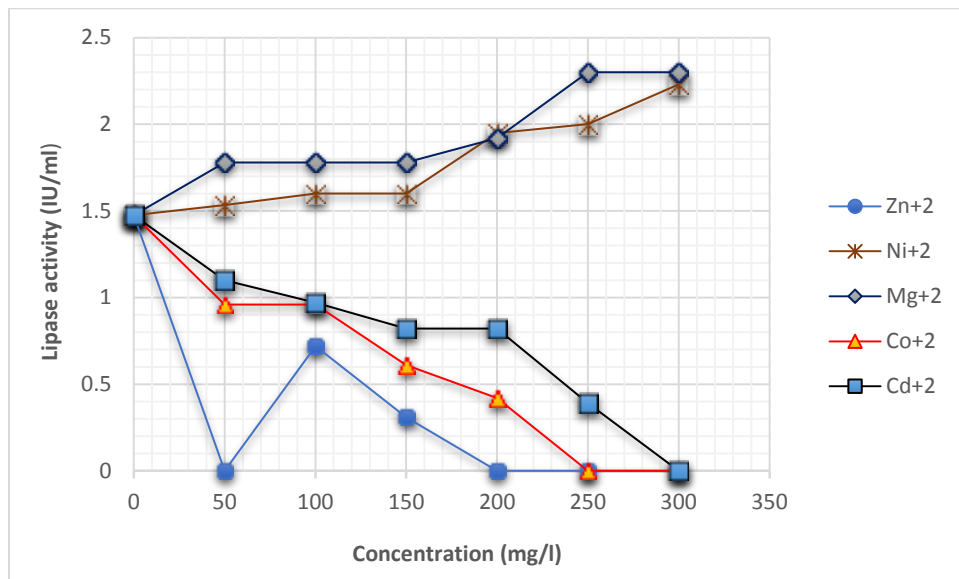


Fig. 7. Effects of different metal ions on lipase activity

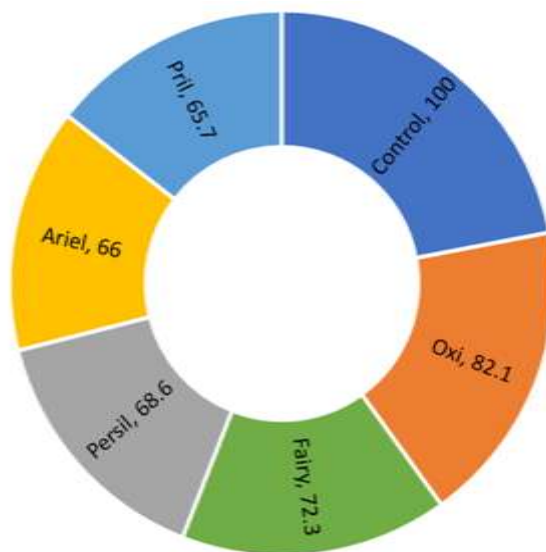


Fig. 8. A sunburst diagram shows the effects of different commercial detergents on lipase activity. Values are the residual activity (%) compared to the control (without detergent, 100 %)

A recent study conducted by [Kumar *et al.*, \(2016\)](#) reported that the stability of the halophilic enzymes towards organic solvents makes them potential catalytic agents effective for non-aqueous enzymology. Consequently, the organic solvent-tolerant halophilic lipases could be of great interest in the industrial processes, as they catalyze reactions under high salt concentrations and in presence of hydrophobic organic solvents, as revealed by [Ahmed *et al.*, \(2010\)](#); [Mo *et al.*, \(2016\)](#). Therefore, the effects of various organic solvents on the activity of the lipase were currently investigated. Compared to the control (without solvent), the lipase enzyme retained about 98.8, 97, 94.6 and 60.5 % of its activity in the presence of ethyl acetate, dimethyl formamide, toluene and methanol, respectively.

The activity and stability of lipase in the presence of organic solvents indicated that these

solvents maintained the enzyme in an open conformation, i.e. they did not cover the active site of the enzyme, and kept its flexible conformation ([Klibanov, 2001](#); [Jiewei *et al.*, 2014](#); [Mo *et al.*, 2016](#)). On the other hand, in the presence of acetone the lipase lost more than half of its activity, whereas, ethanol severely suppressed the activity of the enzyme (Fig. 9).

These results are in agreement with the previous findings of [Jiewei *et al.*, \(2014\)](#) who stated that lipase from *Oceanobacillus* strain PT-11 was highly denatured in the presence of the hydrophilic solvents such as acetone and ethanol. Several organic solvent-tolerant bacterial lipases were detected in the previous research works of [Ahmed *et al.*, \(2010\)](#); [Uttaree *et al.*, \(2010\)](#); [Li *et al.*, \(2014\)](#); [Mo *et al.*, \(2016\)](#).

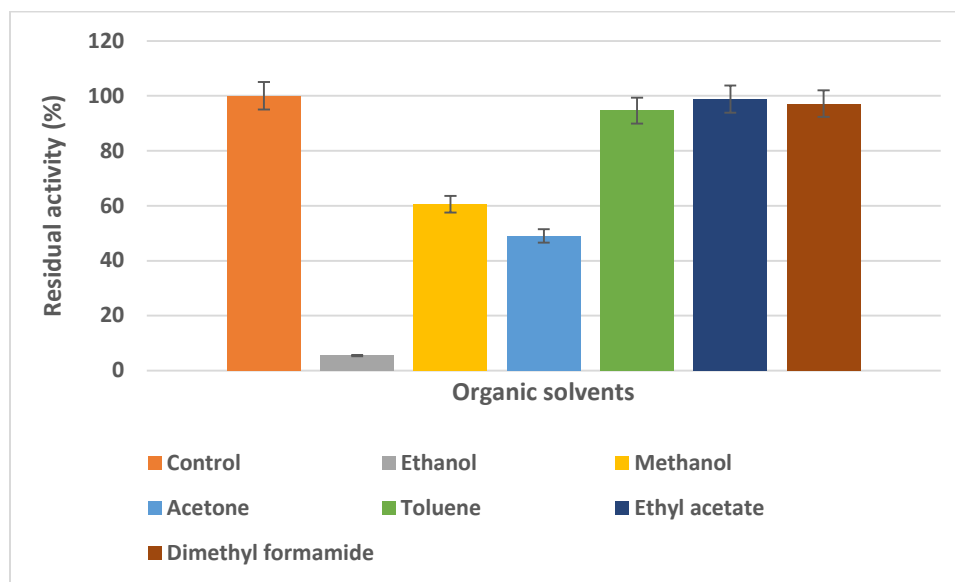


Fig. 9. Effects of different organic solvents on lipase activity. Values are the residual activity (%) compared to the control (without organic solvent, 100 %). Values are means \pm standard errors (SEs)

Conclusion

In the current study, the extremely halotolerant *Oceanobacillus iheyensis* strain QCS produces an extracellular lipase that revealed significant activity and stability at extreme conditions such as; salinity, temperature, pH, metal ions, detergents and organic solvents. Accordingly, this halophilic lipase could be potentially useful for practical applications, acting as a biocatalyst in several reactions carried out at low water activity systems, and as a laundry detergent ingredient.

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Ethical Approval

Non-applicable.

4. References

- Ahmed, E.H.; Raghavendra, T. and Madamwar, D. (2010).** An alkaline lipase from organic solvent tolerant *Acinetobacter* sp. EH28: application for ethyl caprylate synthesis. *Bioresource Technology*. 101: 3628-3634.
- Alberghina, L.; Schmid, R.D. and Verger, R. (1991).** Lipases: structure, mechanism and genetic engineering Weinheim: VCH. 16: 425-428.

- Aljohny, B.O. (2015).** Halophilic Bacterium- A Review of New Studies. *Biosciences Biotechnology Research Asia*. 12(3): 2061-2069.
- Balan, A.; Ibrahim, D. and Abdul-Rahim, R. (2013).** Organic-solvent and surfactant tolerant thermostable lipase, isolated from a thermophilic bacterium, *Geobacillus thermodenitrificans* IBRL-nra. *Advanced Studies in Biology*. 5: 389-401.
- Bas, D. and Boyaci, I.H. (2007).** Modeling and Optimization, I: Usability of response surface methodology. *Journal of Food Engineering*. 78: 836-845.
- Bornscheuer, U.T. (2000).** Enzymes in lipid modification. Wiley-VCH, Weinheim. 235-262.
- Coronado, M-J.; Vargas, C.; Hofemeister, J.; Ventosa, A. and Nieto, J.J. (2000).** Production and biochemical characterization of an α -amylase from the moderately halophile *Halomonas meridiana*. *FEMS Microbiology Letters*. 183: 67-71.
- DasSarma, S. and Arora, P. (2001).** Halophiles. *Encyclopedia of Life Sciences*. 1: 1-9.
- De Lourdes Moreno, M.; Pérez, D.; García, M.T. and Mellado, E. (2013).** Halophilic bacteria as a source of novel hydrolytic enzymes. *Life*. 3: 38-51.
- Friedrich, J.; Cimerman, A. and Steiner, W. (1989).** Submerged production of pectinolytic enzymes by *Aspergillus niger*: effect of different aeration/agitation regimes. *Applied Microbiology and Biotechnology*. 31: 490-494.
- Furini, G.; Berger, J.S.; Campos, J.A.M.; Sand, S.T.V.D. and Germani, J.C. (2018).** Production of lipolytic enzymes by bacteria isolated from biological effluent treatment systems. *Annals of the Brazilian Academy of Sciences*. 90(3): 2955-2965.
- Gupta, R.; Gigras, P.; Mohapatra, H.; Goswami, V.K. and Chauhan, B. (2003).** Microbial α -amylases: a biotechnological perspective. *Process Biochemistry*. 38: 1599-1616.
- Hasan, F.; Shah, A.A.; Javed, S. and Hameed, A. (2010).** Enzymes used in detergents: Lipases. *African Journal of Biotechnology*. 9(31): 4836-4844.
- Hasan, F.; Shah, A. and Hameed, A. (2007).** Purification and characterization of a mesophilic lipase from *Bacillus subtilis* FH5 stable at high temperature and pH. *Acta Biologica Hungarica*. 58 (1): 115-132.
- Hemlata, B.; Uzma, Z. and Tukaram, K. (2016).** Substrate kinetics of thiol activated hyperthermostable alkaline lipase of *Bacillus sonorensis* 4R and its application in bio-detergent formulation. *Biocatalysis and Agricultural Biotechnology*. 8: 104-111.
- Higa, A.I. and Cazzulo, J.J. (1973).** On the fractionation of halophilic enzymes with ammonium sulfate. *Experientia*. 29 (9): 1081-1083.
- Jaeger, K.E. and Eggert, T. (2002).** Lipases for biotechnology. *Current Opinion in Biotechnology*. 13: 390-397.
- Jaiswal, A.; Preet, M. and Tripti, B. (2017).** Production and Optimization of Lipase Enzyme from Mesophiles and Thermophiles. *Journal of Microbial and Biochemical Technology*. 9(3): 126-131.
- Jiewei, T.; Zuchao, L.; Peng, Q.; Lei, W. and Yongqiang, T. (2014).** Purification and Characterization of a Cold-Adapted Lipase from *Oceanobacillus* strain PT-11. *PLOS ONE*. 9(7): e101343.
- Karray, F.; Ben Abdallah, M. and Kallel, N. (2018).** Extracellular hydrolytic enzymes produced by halophilic bacteria and archaea isolated from hypersaline lake. *Molecular Biology Reports*. 45: 1297-1309.
- Khunt, M.; Pandhi, N. and Rana, A. (2012).** Effect of Medium and Environmental Parameters on Lipase Production from *Halomonas salina* Ku-10. *Journal of Pharmacy Research*. 5 (7): 3844-3847.

- Kiran, G.S.; Lipton, A.N.; Kennedy, J.; Dobson, A.D.W. and Selvin, J. (2014).** A halotolerant thermostable lipase from the marine bacterium *Oceanobacillus* sp. PUMB02 with an ability to disrupt bacterial biofilms. *Bioengineered*. 5(5): 305-318.
- Klibanov, A.M. (2001).** Improving enzymes by using them in organic solvents. *Nature*. 409: 241-246.
- Kumar, A.; Dhar, K.; Kanwar, S.S. and Arora, P.K. (2016).** Lipase catalysis in organic solvents: advantages and applications. *Biological Procedures Online*. 18: 1-1.
- Kumar, S.; Karan, R.; Kapoor, S.; Singh, P.S. and Khare, S.K. (2012).** Screening and isolation of halophilic bacteria producing industrially important enzymes. *Brazilian Journal of Microbiology*. 43(4): 1595-1603.
- Kumar, C.G.; Malik, R.K. and Tiwari, M.P. (1998).** Novel enzyme-based detergents: An Indian perspective. *Current Science*. 75: 1312-1318.
- Kushner, D.J. and Kamekura, M. (1988).** Physiology of Halophilic Eubacteria. In: Rodriguez-Valera, F. (ed.). *Halophilic bacteria*, vol. 1. CRC Press, Inc. Boca Raton, Fla. pp. 109-138.
- Kushner, B.J. (1985).** The Halobacteriaceae. In: Woese and Wolfe (Eds.). *The Bacteria: A Treatise on Structure and Function*, vol. VIII, The Archaeobacteria. New York: Academic Press. pp. 171-214.
- Li, X.; Qian, P.; Wu, S.G. and Yu, H.Y. (2014).** Characterization of an organic solvent-tolerant lipase from *Idiomarina* sp. W33 and its application for biodiesel production using *Jatropha* oil. *Extremophiles*. 18: 171-178.
- Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951).** Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*. 193: 265-275.
- Mazhar, H.; Abbas, N.; Ali, S.; Sohail, A.; Hussain, Z. and Ali, S.S. (2017).** Optimized production of lipase from *Bacillus subtilis* PCSIRNL-39. *African Journal of Biotechnology*. 16(19): 1106-1115.
- Mehta, A.; Kumar, R. and Gupta, R. (2012).** Isolation of lipase producing thermophilic bacteria: optimization of production and reaction conditions for lipase from *Geobacillus* sp. *Acta Microbiologica et Immunologica Hungarica*. 59:435-450.
- Mevarech, M.; Frolov, F. and Gloss, L.M. (2000).** Halophilic enzymes: proteins with a grain of salt. *Biophysical Chemistry*. 86: 155-164.
- Mo, Q.; Liu, A.; Guo, H.; Zhang, Y. and Li, M. (2016).** A novel thermostable and organic solvent-tolerant lipase from *Xanthomonas oryzae* pv. *oryzae* YB103, screening, purification and characterization. *Extremophiles*. 20: 157-165.
- Mohammadipanah, F.; Hamed, J. and Dehghani, M. (2015).** Halophilic Bacteria: Potentials and Applications in Biotechnology. In: Maheshwari D., Saraf M. (eds) *Halophiles. Sustainable Development and Biodiversity*. Springer, Cham. 6: 444.
- Moreno, M.de-L.; Pérez, D.; García, M.T. and Mellado, E. (2013).** Halophilic Bacteria as a Source of Novel Hydrolytic Enzymes. *Life*. 3: 38-51.
- Mukesh kumar, D.J.; Rejitha, R.; Devika, S.; Balakumaran, M.D.; Immaculate, N.R.A. and Kalaichelvan, P.T. (2012).** Production, optimization and purification of lipase from *Bacillus* sp. MPTK 912 isolated from oil mill effluent. *Advances in Applied Science Research*. 3(2): 930-938.
- Oren, A. (2010).** Industrial and environmental applications of halophilic microorganisms. *Environmental Technology*. 31: 825-834.
- Samaei-Nouroozi, A.; Rezaei, S.; Khoshnevis, N.; Doosti, M.; Hajihoseini, R.; Khoshayand, M.R. and Faramarzi, M.A. (2015).** Medium-based optimization of an organic solvent-tolerant

extracellular lipase from the isolated halophilic *Alkalibacillus salilacus*. *Extremophiles*. 19: 933-947.

Schreck, S.D. and Grunden, A.M. (2014). Biotechnological applications of halophilic lipases and thioesterases. *Applied Microbiology and Biotechnology*. 98: 101-121.

Sharma, R.; Chistib, Y. and Banerjee, U.C. (2001). Production, purification, characterization, and applications of lipases. *Biotechnology Advances*. 19: 627-662.

Uttatree, S.; Winayanuwattikun, P. and Charoenpanich, J. (2010). Isolation and characterization of a novel thermophilic organic solvent stable lipase from *Acinetobacter baylyi*. *Applied Biochemistry and Biotechnology*. 162: 1362-1376.

Zehra, B.B. and Metin, K. (2015). Screening for industrially important enzymes from thermophilic bacteria; selection of lipase-producing microorganisms and optimization of culture conditions. *European Journal of Biotechnology and Bioscience*. 6: 43-48.