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Esterase and Lipase Activities of New Isolated Thermophilic *Bacillus* Strains HUTB 20 under Different Temperatures and in the Presence of various Carbon Sources

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The activities of esterase and lipase by thermophilic *Bacillus* strain HUTB 20 were monitored during incubation at 53°C, 25°C and after 53°C to 25°C shift in growth temperature. Activities of both enzymes were increased after 27 hours growth but were undetectable or reduced from 0 hour up to 3 hours. The highest activities for both enzymes were at 53°C in the presence of olive oil. The highest enzymes activities obtained in sonicated cells followed by homogenized cells and then cell culture supernatant. At 25°C, esterase activity was higher than the activity of lipase in the presence of Tween 80 and Tween 20. However, lipase was in high activity than esterase in the presence of olive oil at 25°C. The activity of both enzymes was low after cold shock in the presence of various carbon sources. The increased activity of a cell associated esterase and extracellular lipolytic enzymes and production of an extracellular polysaccharide suggested that these enzymes are required for growth at low and high temperatures.

Key words: Esterase, Lipase activies, Thermophilic *Bacillus* strains, Temperatures.

The interest in microorganisms that inhabit extreme environments has emerged because of their potential uses in industry and biotechnology. Enzymes isolated from thermophilic microorganisms are characterized by higher catalytic efficiencies and greater stability at low temperatures (Xu *et al*., 2003; Fujiwara *et al.*, 1996). Enhanced excretion of extracellular enzymes at low temperatures (D'Auria et al., 2000; Purcarea *et al*., 1994), in addition to inactivation at only moderately high temperatures (Collins *et al*., 2003; Giver *et al*., 1998) have also been observed.

Bacterial lipases and esterases enzymes have potential industrial applications such as production of flavor compounds in foods and beverages, the conversion of low-value triglycerides into more confectionary fats, detergents, and in the synthesis of drugs (Kontkanen *et al*., 2004; Harwood, 1989; McKay, 1993; Margolin, 1993). Enzymes isolated from thermophilic microorganisms are used in these industries and extend new uses in biotechnology and bioremediation (Barbaro *et al.*, 2001).

Lipases and esterases enzymes cleave ester linkages, allowing microorganisms to metabolize carboxylic esters (Kok *et al*., 1993). Moreover, esterases hydrolyze water-soluble and emulsified esters with short fatty acid chains, while lipases degrade emulsified water-insoluble substrates with long-chain fatty acids (Gilbert *et al*., 1991; Maheshwari *et al*., 2000).

Strain HUTB 20 was isolated from Ma'in hot spring, Jordan, and was identified as belonging to the genus *Bacillus*. The growth temperature range for this bacterium extends from at least 25°C

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up to 63°C (Hazem and Manar, 2003). The bacteria will be evaluated as a source of thermostable esterase and lipase.

MATERIALS AND METHODS

Bacterium, media and culture conditions

The isolation and identification of thermotolerant *Bacillus* strain HUTB 20 has been described (Hazem and Manar, 2003). It was grown in Basal Salts Medium (BSM) (Furukawa et al., 1983) with acetate (0.1% w/v), Tween 80 (0.2% v/v), or refined olive oil (0.2% v/v) as the sole source of carbon. Acetate and Tween 80 were added to BSM before sterilization. Olive oil was added to sterile BSM. Growth was determined spectrophotometrically by monitoring turbidity at 650 nm.

Cold shock conditions

Cultures were grown at 53°C until mid-exponential growth reached. Then 25 ml aliquots of the cultures were aseptically transferred to sterile 250 ml Erlenmeyer flasks cooled to 25°C. The cultures maintained at the lower temperature, samples aseptically removed at different times following the decrease in temperature and enzyme assays conducted. Results were compared to enzyme activities measured for cells grown at different temperatures from 10°C up to 73°C to mid-exponential growth (mid exponential control samples) and to cultures grown at 53°C and 25°C. For all cold shock experiments, the data points at 0 h represent the data observed for the 53°C mid-exponential control samples. All experiments conducted in triplicate.

Esterase and lipase activity measurements

Strain HUTB 20 was grown in 50 ml of BSM containing acetate (for esterase and activity), Tween80 and Tween20 (for esterase and lipase activity), or olive oil (for esterase and lipase activity) as sole sources of carbon. During growth at choosed temperature, 5 ml of culture removed at times corresponding too early-, mid- and late exponential growth, and early-stationary phase. Cold-shocked samples removed at different times after cold shocks. Samples compared to mid-exponential control samples grown at different temperatures from 10°C up to 73°C. Extracellular enzyme activity monitored in the culture supernatant that will be removed following centrifugation of the culture at 12 000 x g for 15 min at 25°C. The resulting cell pellet washed twice and then the cells will be suspended in 20 mM sterile HEPES buffer (pH 7.0). The cell suspension homogenized for 5 min (1 min on/off intervals). This suspension will be centrifuged at 12000 x g for 5 min to remove unbroken cells and cellular debris. Cell associated enzyme activities in the resulting supernatant was monitored. All experiments conducted in triplicate.

Esterase activity

Esterase and lipase were distinguished from each other based on their ability to hydrolyze different fatty acids. Often, these enzymes have overlapping substrate specificities that cause difficulty in identification of the enzyme. For the purposes of this study, esterases operationally distinguished from lipases based on the ability of the cell culture supernatant, cellsonicated and cell homogenated to hydrolyze either Á-nitrophenyl acetate (esterase) or Á-nitrophenyl palmitate (lipase).

Esterase activity measured as described by Kok *et al.* (1993). A substrate assay solution prepared by adding 100 µl of Á-nitrophenyl acetate (PNPA) stock solution (36 mg dissolved in 1 ml methanol) to 20 ml of 20 mM-HEPES buffer (pH 7.0) yielding a final substrate concentration of 1 mM. The reaction started by adding 200 µl of culture supernatant, sonicated cells or homogenized cell to 1.8 ml of substrate assay solution (final volume of 2 ml) the reaction monitored spectrophometrically by measuring the change in absorbance at 410 nm over 20 min at room temperature. Relative enzyme activity will be calculated from the rate of ρ-nitrophenol formation. An increase in absorbance of 0.100/ min= 1 Unit of enzyme activity.

Lipase activity

Lipase activity measured as described by Kok *et al.* (1993). The substrate assay solution prepared by adding 2.0 ml of ρ-nitrophenol palmitate (PNPP) stock solution (37.5 mg in 2 ml propanol) to 50 ml of buffer containing 50 mM Tris/ HCl (pH 8.0) and 0.1% Triton X-100, yielding a final substrate concentration of 2 mM. The reaction started by adding 200 µl of cell culture supernatant, sonicated cells or cell homogenated to 1.8 ml of substrate assay solution (final volume of 2.0 ml). Activity calculated as described for esterase activity.

RESULTS

Bacteria growth at different temperature and in the presence of various carbon sources

The growth of thermophilic *Bacillus* strains HUTB 20 at different temperatures and in different media is shown in Table 1. At 25°C, the bacteria showed growth anaerobically but not aerobically; the same result was obtained during the growth in nutrient broth. The Basal Salt broth (BSB) showed bacterial growth aerobically at both used temperatures 25°C and 53°C. BSB was used for further tests.

Effects of olive oil on esterase activity

Figure 1a-c shows esterase activity when cells grown with olive oil during the different incubation treatments. At 25°C, extracellular esterase activity was higher than activity measured in cell culture supernatant and homogenized cells at all times assayed. Extracellular activity increased over time from 0.0 U/ml at 2 hrs up to 22.3 U/ml at 48 hrs (Fig. 1a). Esterase activity was not detected in cell cultures supernatant until early-maximum stationary phase (24 hrs-1.9 U/ml).

At 53°C after 27 hrs of growth, extracellular esterase activity measured for sonicated cells start to increase, eventually reaching levels higher than those observed for cell culture supernatant and homogenized cells (23.8 U/ml at 30 hrs up to 26.0 U/ml at 48 hrs). The measurement is also higher than activity measured for cells grown at 25°C. The enzymatic activity measured in the cell culture supernatant from 1.8 U/ml at 5 ht up to 7.2 U/ml at 48 hr but this was lower than the activity measured in sonicated cells and homogenized cells grown at 53°C (Fig. 1b).

Trends were observed following cold shock (Fig. 1c). Esterase activity measured and remained constant over time from 1 hr up to 3 hrs culture supernatant, the enzymatic activity remained undetected up to 24 hrs but by 27 hrs activity measured at 1.9 U/ml. Otherwise, esterase

Table 1. Bacterial growth characteristics after incubation 24 hours at different temperatures and conditions.

Conditions	Temperatures	
	53°C	25°C
Bacterial growth in BSM agar after 24 hrs incubation:		
Aerobically	Circular, Light yellow and size 2-3 mm	No growth
Anaerobically	Circular, light yellow, orange color in centre and size 1-2 mm	Circular, light yellow, orange color in centre and size 1-2 mm
Bacterial growth in broth after 24 hrs incubation:		
Nutrient broth (NB)	Turbidity	No growth
Basal Salt broth (BSB)	Turbidity	Turbidity

Table 2. Effect of various carbon sources on the bacterial growth absorbance of tested bacteria after incubation for 24 hours at different temperatures

Various Carbon Sources	Temperatures	
	53°C	25°C
Bacterial colony growth (CFU/ml) using BSB and dilution 10^{-6}:		
None	3.1×10^7	5.0×10^7
Tween 20	2.4×10^7	0.8×10^7
Tween 80	2.0×10^7	1.1×10^7
Acetate	1.5×10^7	0.6×10^7
Olive oil	1.8×10^7	1.3×10^7

activity of homogenized cells was undetectable up to 5 hrs but by 24 hrs, the activity measured at 1.2 U/ml.

At 53°C, esterase activity of all tested grown cells began constantly by 2 to 3 hrs (0.2 U/ml up to 1.0 U/ml). But the enzyme activity was higher of sonicated cells at 30 and 48 hrs (16.8 U/ml and 19.0 U/ml). Whereas, homogenized cells was 13 U/ml and 12.5 U/ml and cell culture supernatant was 6.8 U/ml and 7.4 U/ml).

Trends in esterase activity after cold shock obtained for Tween 80 grown cells were constant up to 27 hrs for all grown tested cells (0.9 U/ml at 2 hr, 1.0 U/ml at 3 hrs, 2.0 U/ml at 5 hrs, 3.0 U/ml at 24 hrs and 3.9 U/ml at 27 hrs). After of 27 hrs growth, esterase activity higher at 48 hrs in sonicated cells (8.0 U/ml) in comparison with homogenized cells (7.0 U/ml) and cell culture supernatant (5.8 U/ml).

Effects of Tween 20 on esterase activity

The esterase activity measured in all tested cells for Tween 20 grown cells was very low compared with those measured for olive oil and Tween 80 (Figure 3a-c). The activity of enzyme of cell culture supernatant and homogenized cells measured only at 30 hrs and 48 hrs (1.8 U/ml drop to 0.8 U/ml) and 3.2 U/ml drop to 2.9 U/ml). But the enzymatic activity of sonicated cells raised by 27 hrs up to 48 hrs (1.0 U/ml and 4.5 U/ml).

At 53°C, esterase activity measured for

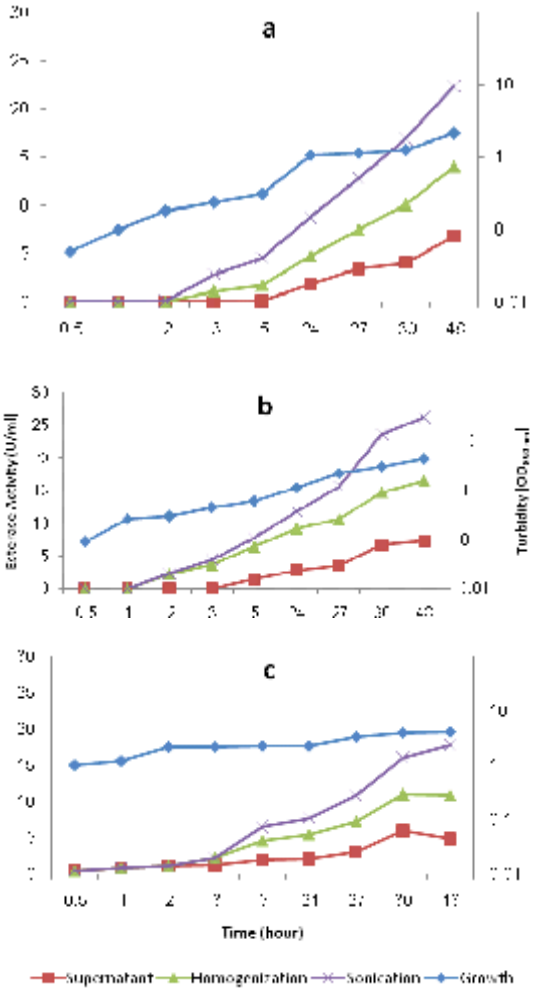

Fig 1. Esterase activity in culture supernatant, homogenized cells and cells sonicated. Cells were grown in olive oil and incubated at (a) 25°C, (b) 53°C or (c) Cold shock (following 53°C to 25°C).

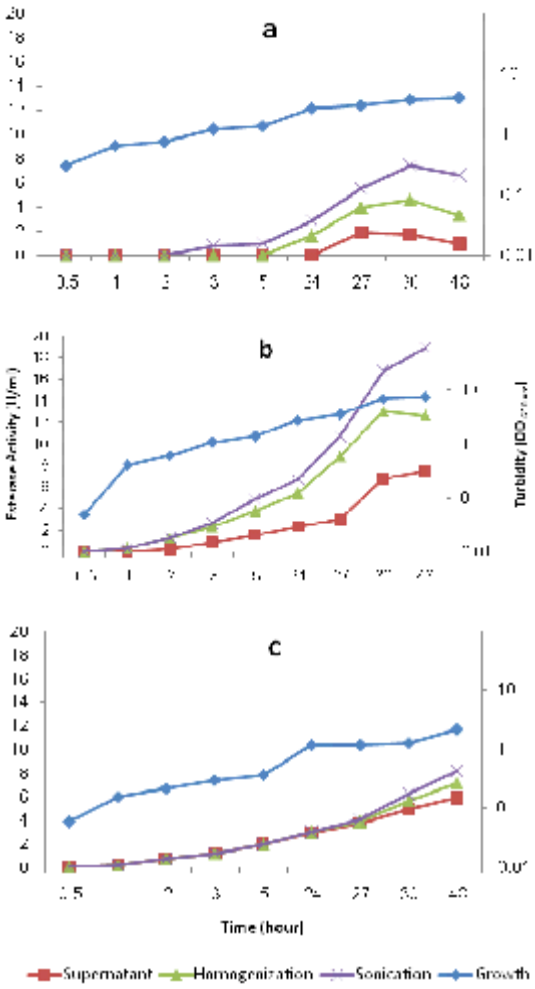

Fig 2. Esterase activity in culture supernatant, homogenized cells and cells sonicated. Cells were grown in Tween 80 and incubated at (a) 25°C, (b) 53°C or (c) Cold shock (following 53°C to 25°C).

sonicated cells and homogenized cells raised after 3 hrs grown cells in Tween 20, which was totally different from those grown in the presence of olive oil and Tween 80 (Figure 3b).

The trends in esterase activity after cold shock obtained for Tween 20 grown cells (Figure 3c) were constant from 5 hrs up to 48 hrs (0.1 U/ml up to 3.0 U/ml).

Effects of acetate in esterase activity

At 25°C, cell associated esterase activity was undetectable up to 27 hrs, but at 30 and 48 hrs was detectable and remained constant between 1.0 and 3.0 U/ml (Figure 4a). Esterase activity at 53°C in acetate grown cells was lower than activity measured in olive oil and Tween 80 but similar to measured activity in Tween 20 (Figure 4b). Changes in esterase activity following cold shock were similar to those associated for cell grown in olive oil, Tween 80 and Tween 20 (Figure 4c). A small decrease in cell associated esterase activity was observed up to 3 hrs after cold shock followed by a steady increase in sonicated cells up to 8.2 U/ml at 48 hrs and in homogenized cells up to 4.2 U/ml at 48 hrs. Extracellular esterase activity of cell culture supernatant appeared to be unaffected by cold shock remaining steady between 1 and 2 U/ml after cold shock (Figure 4c).

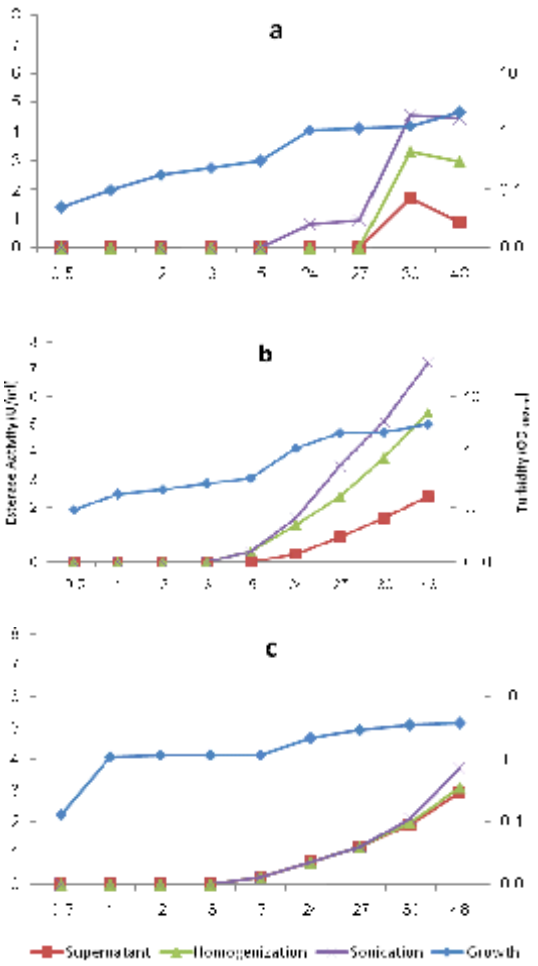

Fig 3. Esterase activity in culture supernatant, homogenized cells and cells sonicated. Cells were grown in Tween 20 and incubated at (a) 25°C, (b) 53°C or (c) Cold shock (following 53°C to 25°C).

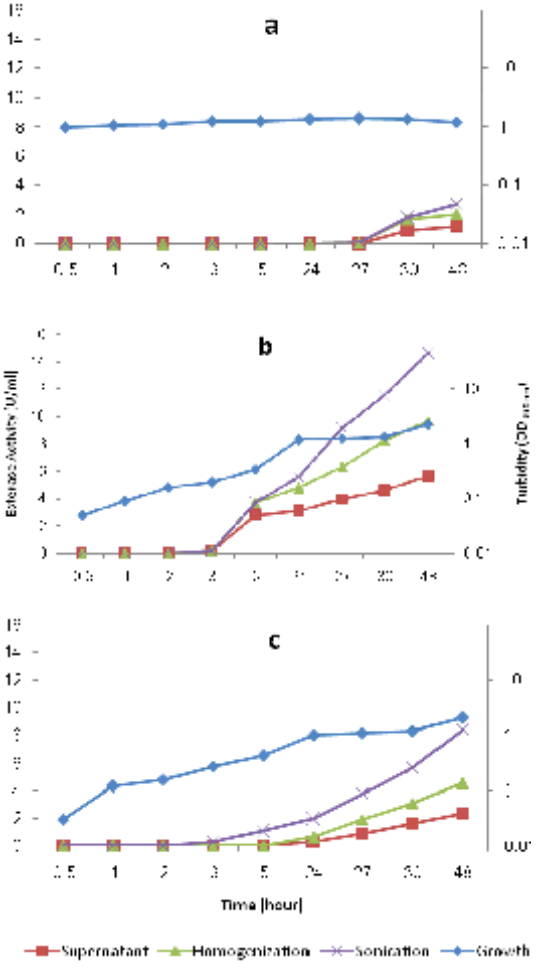

Fig 4. Esterase activity in culture supernatant, homogenized cells and cells sonicated. Cells were grown in acetate and incubated at (a) 25°C, (b) 53°C or (c) Cold shock (following 53°C to 25°C).

Effects of olive oil in lipase activity

At 25°C, lipase activity in all tested cells remained relatively constant over time (0.5 U/ml at 2 hrs to 4.9 U/ml at 27 hrs) (Figure 5a). After 30 hrs of growth, lipase activity at 48 hrs measured in sonicated cells up to 10 U/ml, homogenized cells up to 8.2 U/ml (Figure 5a).

At 53°C, measured trends in lipase activity were similar to those observed at 25°C (Figure 5b). After cold shock, lipase activity in cell culture supernatant decreased from 2.9 U/ml at 30 min to 0.0 U/ ml at 2 hrs, in sonicated cells from 4.5 U/ml at 30 min to 1.8 U/ml at 2 hrs and in homogenized cells from 3.8 U/ml at 30 min to 1.8 U/ml at 2 hrs. However, by 27 hrs post cold shock, activity increased from undetected to 2.0-6.9 U/ml (Figure 5b).

Effects of Tween 80 in lipase activity

At 25°C, lipase activity measured was higher than observed in olive oil (Figure 6a). At 53°C, lipase activity was found to be similar to those observed in olive oil (Figure 6b). A small decrease in cell associated lipase activity was observed up to 3 hrs after cold shock followed by a steady increase in sonicated cells up to 4.0 U/ml at 48 hrs and in homogenized cells up to 4.0 U/ml at 48 hrs (Figure 6c).

Effects of Tween 20 in lipase activity

At 25°C, cell associated lipase activity was undetectable up to 30 hrs, but at 48 hrs was

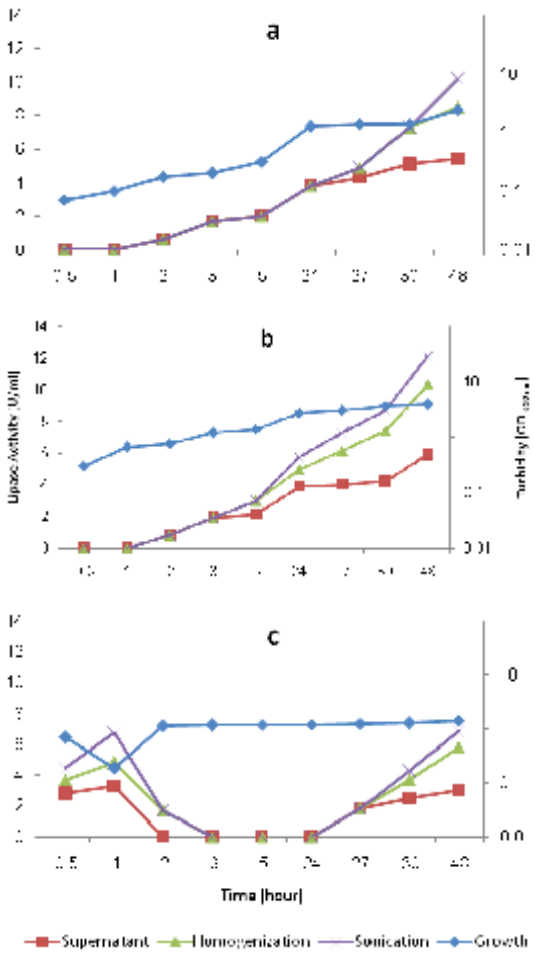

Fig 5. Lipase activity in culture supernatant, homogenized cells and cells sonicated. Cells were grown in olive oil and incubated at (a) 25°C, (b) 53°C or (c) Cold shock (following 53°C to 25°C).

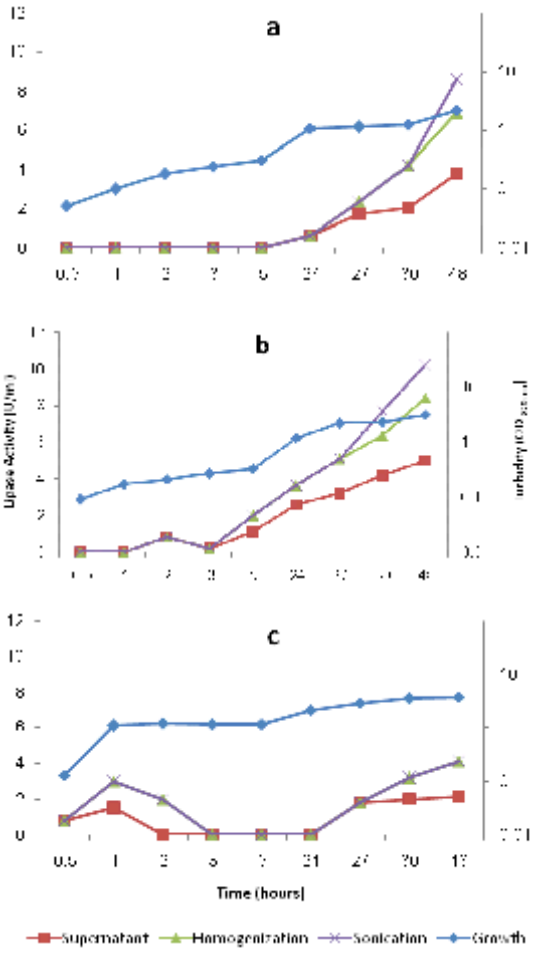

Fig 6. Lipase activity in culture supernatant, homogenized cells and cells sonicated. Cells were grown in Tween 80 and incubated at (a) 25°C, (b) 53°C or (c) Cold shock (following 53°C to 25°C).

detectable and remained constant between 1.1 and 2.8 U/ml (Figure 7a). Lipase activity at 53°C in Tween 20 grown cells was slightly lower than activity measured in olive oil and Tween 80 (Figure 7b). Changes in lipase activity following cold shock were similar to those associated for cell grown in olive oil and Tween 80 (Figure 7c). After 30 hrs growth, extracellular lipase activity of cell culture supernatant appeared to be unaffected by cold shock remaining steady between 0.6 and 1.2 U/ml after cold shock (Figure 7c). Whereas, for sonicated cells was between 1.5 U/ml at 30 hrs and 3.8 U/ml at 48 hrs, and for homogenized cells was between 1.5 U/ml at 30 hrs and 2.2 U/ml at 48 hrs after cold shock.

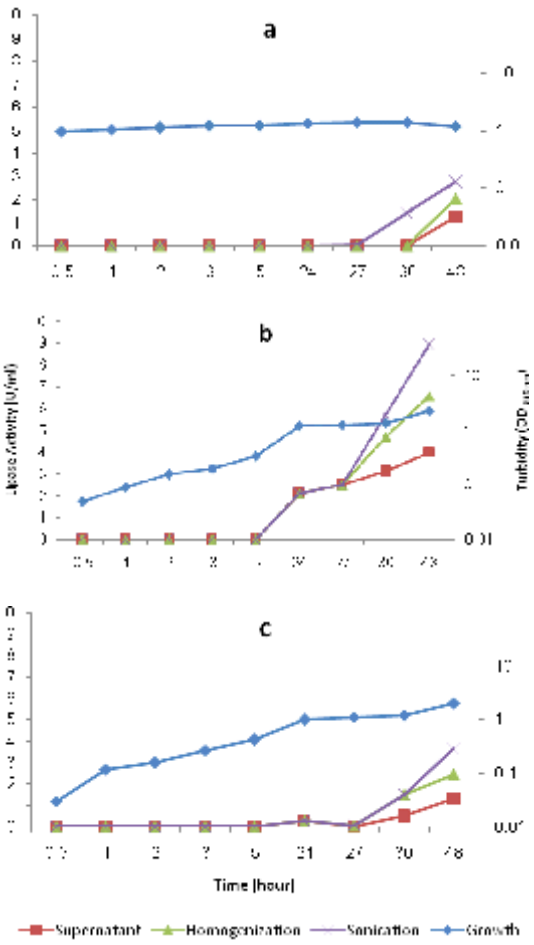

Fig 7. Lipase activity in culture supernatant, homogenized cells and cells sonicated. Cells were grown in Tween 20 and incubated at (a) 25°C, (b) 53°C or (c) Cold shock (following 53°C to 25°C).

DISCUSSION

There are two main functions of carbon compounds in the living systems, i.e. to provide raw materials for the structure and energy production. Each microorganism requires a different carbon source to produce lipase and esterase at their maximum level. The influence of different carbon sources on lipase and esterase synthesis was therefore investigated by their addition to the growth medium. To select the most potent source for lipase and esterase production a variety of carbohydrates were used.

In the present study it was found that maximum esterase and lipase activity were obtained when olive oil is used as a source of a carbon source followed by Tween 80, Tween 20 and acetate at low and high temperatures. This result is in agreement with previous works. Lee *et al* (1999) used olive oil to isolate thermophilic *Bacillus thermoleovorans* ID-1 which grew rapidly at 65°C and its lipase activity attained a maximum value of 520 U/L during the late exponential growth phase. The isolate ID-1 could grow on a variety of lipidic substrates such as triglycerides and Tween 20.Sugihara *et al.* (1991) reported lipase production from *Bacillus* species in the presence of 1% olive oil in the culture medium. Little enzyme activity was observed in the absence of olive oil. Similarly, a thermophile *Bacillus* strain A30-1 produced maximal levels of thermostable alkaline lipase when olive oil was used as carbon source (Wang *et al.*, 1995).

The activity of lipase at 25°C and after cold shock decreased compared with the activity of the enzyme at 53°C (Figure 5, 6 and 7). The lipase activity was very high in sonicated cells compared with homogenized cells and cell culture supernatant. The decrease in lipase activity in homogenized cells and cell culture supernatant, and increase that measured in sonicated cells suggests that either production of lipase or secretion of lipase, or a combination of production and secretion, was reduced by low temperatures.

At 53°C and 25°C, lipase activity was higher in the presence of olive oil in comparison with the results obtained for Tween 80 and Tween 20. The effect of additional oleic acid associated with olive oil grown cells may affect lipase production at the molecular level when cells are

incubated at low and high temperatures. The increase in production and activity of extracellular lipase activity will have a greater impact on cells grown with olive oil relative to cells grown with either Tween 80 or Tween 20. In contrast, Tween 80 has been shown to increase the lipolytic activity of other microorganisms such as *Rhodosporidium babjevae* BD19 (Lukaszewicz *et al.*, 2013), *Rhizopus delemar* (Espinosa *et al*, 1990) and *Bacillus stearothermophilus* (Gowland *et al.*, 1987). However, Mates and Sudakevitz (1973) showed that the Tween substrate did not affect growth and lipase production was inhibited in case of *Staphylococcus aureus*. Kumar et al. (2005) showed that the lipase activity (pH 8.5 at 55ºC) for *Bacillus coagulans* BTS3 was higher in the presence of Tween 80 followed by Tween 20 and olive oil.

The extracellular esterase activity was undetectable at different temperatures from 0 hour up to 3 hours and sometimes to 5 hours. Inactivation of an extracellular esterase concurrent with induction of a cell associated esterase may be responsible for the observed response. These results suggested the cell-associated esterase might be adapted to function at lower and higher temperatures.

The esterase activity was lower in the presence of Tween 20 in comparison with the results obtained for olive oil. The differences in activity and site of action of esterase between olive oil grown cells and the other carbon sources suggests that cell-associated esterase is important for growth under all incubation conditions when olive oil is used as the sole carbon source.

Previous studies indicated that the lipase and esterase activities varied between *Bacillus* strains and also between varying parameters tested (Mohan *et al.*, 2008; Prasad and Sethi, 2013). The bacterial strain *Bacillus* sp. Strain HUTB 20 has been shown to exhibit both lipase and esterase activities on the basis of the above results using different cell extractions and using different carbon sources at high, low and cold shock temperatures.

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REFERENCES

1. Barbaro SE, Trevors JT and Inniss WE. Effects of low temperature, cold shcck and lipase activities and exopolysaccharide production by a psychotrophic *Acinetobacter* sp.. *Can. J. Microbiol*., 2001; **47**: 194-205.
2. Breuil, C. and Kushner, D.J. Lipase and esterase formation by psychrophilic and mesophilic *Acinetobacter* species. *Can. J. Microbiol*. 1975; **21**: 423-433.
3. Collins, T., Meuwis, M.A., Gerday, G., and Feller, G. Activity, stability and flexibility in glycosides adapted to extreme thermal environments. *J. Mol. Biol.* 2003; **328**: 419-428.
4. D'Auria, S., Herman, P., Lakowicz, J.R., Tamfani, F., Bertoli, E., Manco, G. and Rossi, M. The esterase from the thermophilic eubacterium *Bacillus acidocaldarius* structural dfunctional relationship and comparison with the esterase from the hyper thermophilic archaeon *Archaeoglobus fulgidus*. *Proteins*, 2000; **40**: 473-481.
5. Espinosa E, Sanchez S, Farres A. Nutritional factors affectinglipase production by *Rhizopus delemar* CDBBH313. *Biotechol. Lett.*, 1990; **12**: 209-214
6. Frukawa, K., Simon, J.R. and Chkrabarty, A. M. Common induction and regulation of biphenyl, xylene/toluene, and salicylate catabolism in *Pseudomonas paucimolbilis*. *J. Bacteriol*., 1983; **154**: 1356-1362.
7. Fujiwara, S., Lee, S., Haruki, M., Kanaya, S., Tokagi, M. and Imanaka, T. Unusual enzyme characterisctics of aspartyl tRNA synthetase from hyperthermophilic archeon *Pyrococcus* sp. KOD1. *FEBS Lett*. 1996; **394**: 66-70.
8. Gilbert, E.J., Drozd, J.W. and Jones, C.W. Physiological regulation and optimization of lipase activity in *Pseudomonas aeruginosa* EF2. *J. Gen. Microbiol.* 1991; **137**: 2215-2221.
9. Giver, L., Gershenson, A., Freshgard, P. and Arnold, F.H. Directed evolution of a thermostable esterase. *Proc. Natl. Acad. Sci.* USA 1998; **95**: 12809-12813.
10. Harwood, J. The sensitivity of lipases for industrial uses. *TIBS*. 1989; **14**: 125-126.
11. Gowland P, Kernick M and Sundaram TK. Thermophilic bacterial isolates producing lipase.

FEMS Microbiol. Lett., 1987; **48**: 339-343.

12. Hazem, A. and Manar, O. Genetic polymorphism by RAPD-PCR and phenotypic characteristics of isolated thermotolerant *Bacillus* strains from hot spring sources. *New Microbio*. 1987; **26**: 249-256.
13. Kleman, G.L. and Strohi, W.R. Acetate metabolism by *Escherichia coli* in high cell density fermentation. *Appl. Environ. Microbiol*. 1994; **60**: 3952-3958.
14. Kok, R.G., Christoffels, V.M., Vosman, B., and Hellingwerf, K.J. Growth phase dependent expression of the lipolytic system of *Acinetobacter calcoaceticus* B413: Cloning of a gene encoding one of the esterases. *J. Gen. Microbiol*. 1993; **139**: 2329-2342.
15. Kok, R.G., VanThor, J.J., Nugteren-Roodzant, I.M., Vosman, B., and Hellingwerf, K.J. Characterization of lipase deficient mutants of *Acinetobacter calcoaceticus* B413: Identification of a periplasmic lipase chaperone essential for the production of extracellular lipase.. *J. Bactriol*. 1995; **177**: 3295-3307.
16. Kontkanen, H., Tenkanen, M., Fagerstrom, R. and Reinikainen, T. Characterization of steryl esterase activities in commercial lipase preparations. *J. Biotech.* 2004; **108**: 51-59.
17. Kouker, G. and Jaeger, K.E. Specific sensitive plate assay bacterial lipases. *Appl. Environ. Microbiol*. 1987; **53**: 211-213.
18. Kumar S., Kikon K., Upadhyay A., Kanwar S. and Gupta R. Production, purification and characterization of lipase from thermophilic and alkaliphilic *Bacillus coagulans* BTS-3. *Protein Expression and Purification*,2005; **41**: 38-44.
19. Lee D, Kok Y, Kim K, Kim B, Choi H, Suhartono MT and Pyun Y. Isolation and characterization of a thermophilic lipase from *Bacillus thermoleovorans* ID-1. *FEMS Microbiol. Lett.*, 1999; **179**: 393-400.
20. Lukaszewicz M., Jablonski S. and Krasowska A. Characterization of alkaline lipase from an arctic yeast strain Rhodosporidiumj babjevae BD19. 1st International Interdisciplinary Conference, AIIC 2013, 2013; 24-26 April, Azores, Portugal.
21. Maheshwari, R., Bharadwaj, G. and Bhat, M. K. Thermophilic fungi: their physiology and enzymes. *Microbiology and Molecular Biology Reviews*. 2000; **64**(3): 461-488.
22. Margolin, A.L. Enzymes in the synthesis of chiral drugs. *Enzyme Microb. Technol.* 1993; **15**: 266-280.
23. Mates A., and Sudakevitz D., Production of lipase by *Staphylococcus aureus* under various growth conditions. *J. Appl. Bact*., 1973; **36**: 219-226.
24. McKay, A.M. Microbial carboxylic ester hydrolases (E.C. 3.1.1) in food biotechnology. *Lett. Appl. Microbiol.* 1993; **16**: 1-6.
25. Mohan, S., A. Palavesam and Immanvel, G. Isolation and characterization of lipase-producing *Bacillus* strains from oil mill waste. *African. J. Biotechnol.* 2008; **7**: 2728-2735.
26. Prasad M. and Sethi R. Comparative studies on the production of lipase by Bacillus species under various growth parameters. *International Journal of Current Microbiology and Applied Sciences*, 2013; **2**: 179-185.
27. Purcarea, C., Erauso, G., Prieur, D. and Herve, G. The catalytic and regulatory properties of aspartate transcarbamoylase from *Pyrococcus abyssi*, a new deep-sea hyperthermophilic archaebacterium. *Microbiol.* 1994; **140**: 1967-1975.
28. Rosenberg, M. Roegner, V. and Becker, E.F. The quantificationof rate serum esterases by densitometry of acrylamide gels stained for enzyme activity. *Anal. Biochem*. 1975; **66**: 206-212.
29. Shabtai, Y. and Gutnick, D.L. Exocellular esterase and emulsan release from the cell surface of *Acinetobacter calcoaceticus*. *J. Bacteriol*. 1985; **161**: 1176-1181.
30. Sugihara A, Tani T, and Tominaga Y., Purification and characterization of a novel thermostable lipase from *Bacillus* sp.. *J. Biochem*., 1991; **109**: 211-216.
31. Wang Y, Srivastava KC, Shen GJ and Wang HY. Thermostable alkaline lipase from a newly isolated thermophilic Bacillus strain, A30-1 (ATCC53841). *J. Ferment. Bioeng*., 1995; **79**: 433-438.
32. Xu, Y. Feller, G., Gerday, C. and Glansdorff, N. Metabolic enzymes from psychrophilic bacteria: challenge of adaptation to low temperature in ornithine carbamoyl transferase from *Mortella abyssi*. *J. Bacter*. 2003; **185**: 2161-2168.