

Short communication

Effects of temperature on the activity of *Congregibacter litoralis* KT 71 β -lactamase in the hydrolysis of 4-nitrophenyl dodecanoate

ABSTRACT

The effects of temperature on the activities of *Congregibacter litoralis* KT71 β -lactamase (CLL) was determined by varying the assay temperature from 30 °C to 70 °C. The enzyme's residual activity was also determined by incubating the assay mixture which excluded the substrate for one hour at 70 °C before addition of the substrate to start the reaction. The enzyme activity was measured using a spectrophotometer at a wavelength of 405 nm. The hydrolysis of 4-nitrophenyl dodecanoate to yield 4-nitrophenol was monitored by reading the absorbance at 25 minutes after the addition of substrate to the pre incubated assay mixture. The assay was done in three replicates. The initial velocity was determined and this was compared with *Thermomyces lanuginosus* Lipase (TLL) which served as a reference enzyme. Results show that increase in temperature from 30 °C to 70 °C proportionately decreased the activity of *Congregibacter litoralis* KT71 β -lactamase. Similarly, *Thermomyces Lanuginosus* Lipase showed a progressively decreasing activity as the temperature increased within the same range. Results from the assay on the residual activity at 70 °C shows that both CLL and TLL has residual activities of 63 % and 48 % respectively. This suggests that reactions involving these enzymes at 70 °C can occur without the enzyme completely inactivated. Results also shows that CLL is more thermally stable than TLL at 70 °C. Considering the vast importance of thermally stable lipases and their industrial applications, there is need to study the kinetics of this enzyme to give an understanding of how the enzyme system works. Hence findings from this study is of great significance

[Keywords: β -lactamase, *Congregibacter litoralis* KT71, Residual activity]

Introduction

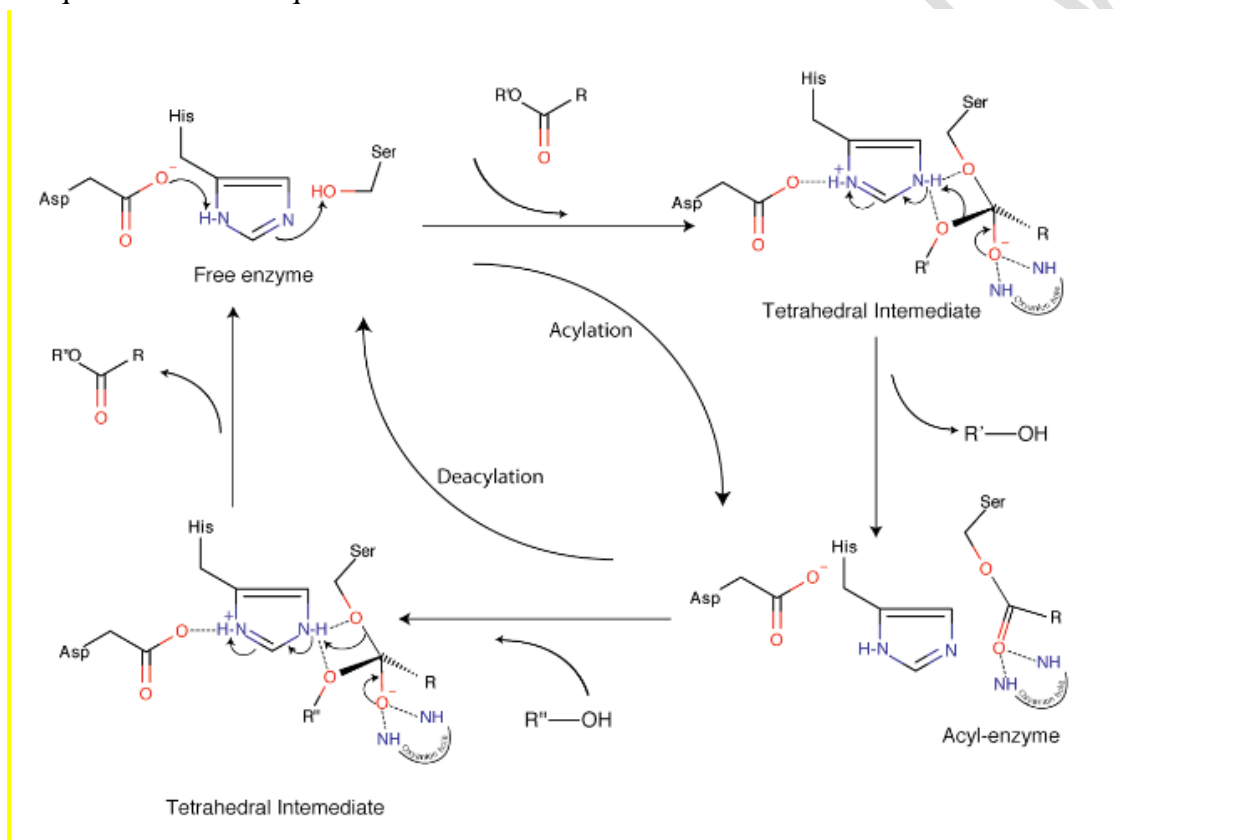
The marine ecosystem is subjected to a temperature range of 4 °C to 400 °C with high hydrostatic pressure [1] These extreme conditions confer on microorganisms novel microbial cellular metabolic processes. This makes them possess a unique enzyme system which includes increased salt tolerance and cold adaptivity which are key industrial requirements [2]. Enzymes

produced by microbe bound in sponges are likely to have unique biochemical and physiological characteristics making it possible to adapt to the unique ecosystem.

Extracellular lipases are highly relevant in many industrial applications such as: biosensors, leather, chemicals, pharmaceuticals, pesticides, foods, and cosmetics [3].

Lipases are also able to catalyze ester synthesis and transesterification in organic media containing minute concentration of water [3]

The Lipolytic enzymes (Esterases (EC 3.1.1.1) and the Lipases (EC 3.1.1.3) are one of the most important groups of enzymes. They carry out novel reactions and these reactions occur both in aqueous and non aqueous media.



Pic 1. Catalytic mechanism of lipases [4]

Several researches have been done on lipases which ranges from industrial applications and immobilization to biocatalytical properties of the pure enzymes [5].

There are current interests in lipases due to their applications in industries involved in biodiesel production, food flavoring, laundry applications, cosmetic production, and in pharmaceuticals [6]. Despite these potential applications, marine microbial lipase is under exploited.

Lipases possess a very wide range of catalytic properties. These properties are mostly strain-dependent. Lipases differ by their sizes, substrate specificities, stability profile, and activity in the presence of various modulators. They have previously been used in their crude extract form for the synthesis of chiral building blocks and enantiomeric compounds. Therefore catalytic properties and operational parameters such as like thermostability, among others are relevant because they define the enzyme application range and suitability.

It has been shown that the optimal activity of lipases obtained from conventional sources range from 30 °C to 60 °C [7], while lipases were obtained from extremophiles have optimal activity at 70 °C [7]. It has been also shown that there are quite a few advantages in using thermostable enzymes in industrial processes as compared to thermolabile enzymes. It is therefore important to characterize this novel enzyme *Congregibacter litoralis* KT71 β -lactamase based on temperature to determine if it is suitable to adapt them for use in reactions carried out at high temperatures. It is also important to verify if this novel lipase has the features characteristic of lipases from extremophiles hence kinetics under this temperature condition (70 °C) will be investigated.

The observed kinetics under varying temperatures can be compared with *Thermomyces Lanuginosus* Lipase (TLL) which is a well characterized enzyme and it is known to be thermostable. The observed temperature effects of these two lipases can then be compared and necessary inferences deduced.

Therefore, in this report, we explained the effects of temperature on the initial velocities and residual activity of *Congregibacter litoralis* KT71 β -lactamase.

Materials and Methods

Chemicals

Sodium dihydrogen phosphate, disodium hydrogen phosphate, 4-nitrophenyl dodecanoate (substrate) were of analytical grades and purchased from Sigma-Aldrich (Dorset, Poole, United Kingdom). All kinetic measurements were carried out using a UV-780 recording spectrophotometer.

Methods

Enzyme purification

The protein coding sequence (Genbank Accession number: EAQ98391.2) was codon-optimised for expression in *E. coli* strain BL21 (DE3) plyS and synthesized by GeneArt (Invitrogen). Protein expression and purification was via Nickel affinity chromatography.

The purified β -lactamase from *Congregibacter litoralis* used in this work was provided by Dr Femi Oloruniji (School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University).

Enzyme Assay

The effects of temperature on the activities of *Congregibacter litoralis* KT71 β -lactamase (CLL) was determined by varying the assay temperature from 30 °C to 70 °C. The enzyme's residual activity was also determined by incubating the assay mixture for one hour at temperatures of 44 °C (control) and at 70 °C before the addition of the substrate. The hydrolysis of 4-nitrophenyl dodecanoate to yield 4-nitrophenol was monitored by reading the absorbance at the 25th minute after the addition of the substrate at a wavelength of 405 nm. The initial velocity was thereafter calculated.

The enzymatic activity at 44 °C was taken as reference (100%) for the calculation of residual (%) activity for the enzymes.

$$\text{Residual activity (\%)} = \left(\frac{Y - X}{Y} \right) \times 100$$

Where "X" is the enzyme activity at assay temperature, and "Y" is enzyme activity at 44 °C

Results and Discussion

Results from figure 1 show that at low temperatures (30 °C – 44 °C), TLL had a higher enzyme activity than CLL. But at temperatures higher than 44 °C, CLL was more thermally stable than TLL. The enzyme activity of CLL decreased as temperature increased from 30 °C to 70 °C. A sharp loss of activity was observed at temperature above 50 °C. TLL also showed reduced activity as temperature increased from 30 °C to 70 °C. The activity of TLL was higher than CLL at temperatures between 30 °C and 44 °C. Higher temperatures lead to reduced enzyme activity which was comparatively lower than CLL within temperature range higher than 44 °C

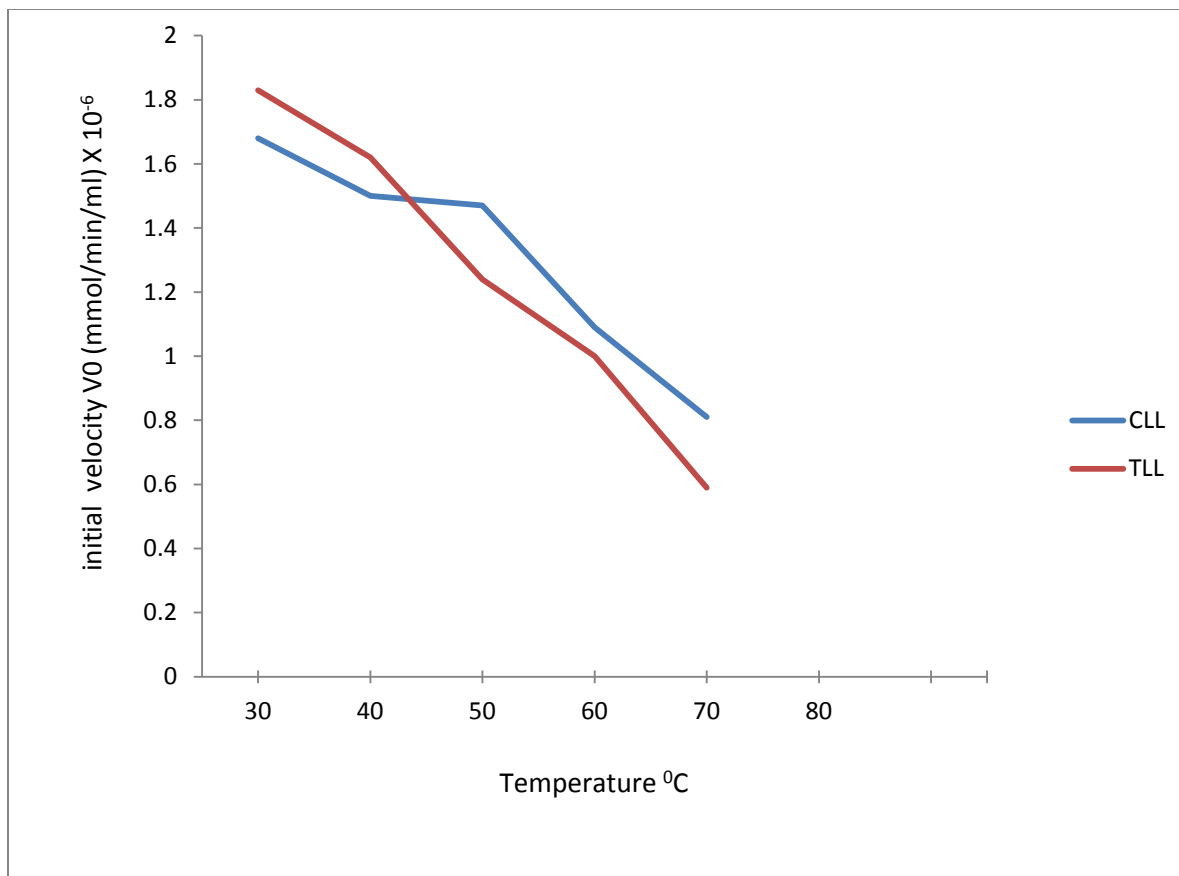


Figure 1: Effect of varying temperatures on the activities of *Congregibacter litoralis* KT71 β -lactamase and *Thermomyces Lanuginosus* lipase

The assay mixtures comprised of 100 μ l of standard enzyme solution (0.0005nM), 200 μ l of distilled water, 500 μ l of 50 mM sodium phosphate buffer pH 7.5 and 200 μ l of 0.025 mM 4-nitrophenyl dodecanoate (substrate) which was added last to the assay mixture after an incubation time of 10 minutes at varying temperatures (30 °C to 80 °C).

Figure 2 shows the effect of pre incubating the enzyme at 70 °C on the enzyme's residual activity. The results show that CLL had a higher residual activity than TLL at 70 °C. At temperatures as high as 70 °C, both CLL and TLL retained 63 % and 48 % of their activities respectively. This is of great significance in understanding the kinetics of the enzyme. Results from this study therefore shows that both CLL and TLL could be useful at reactions operating on temperatures as high as 70 °C.

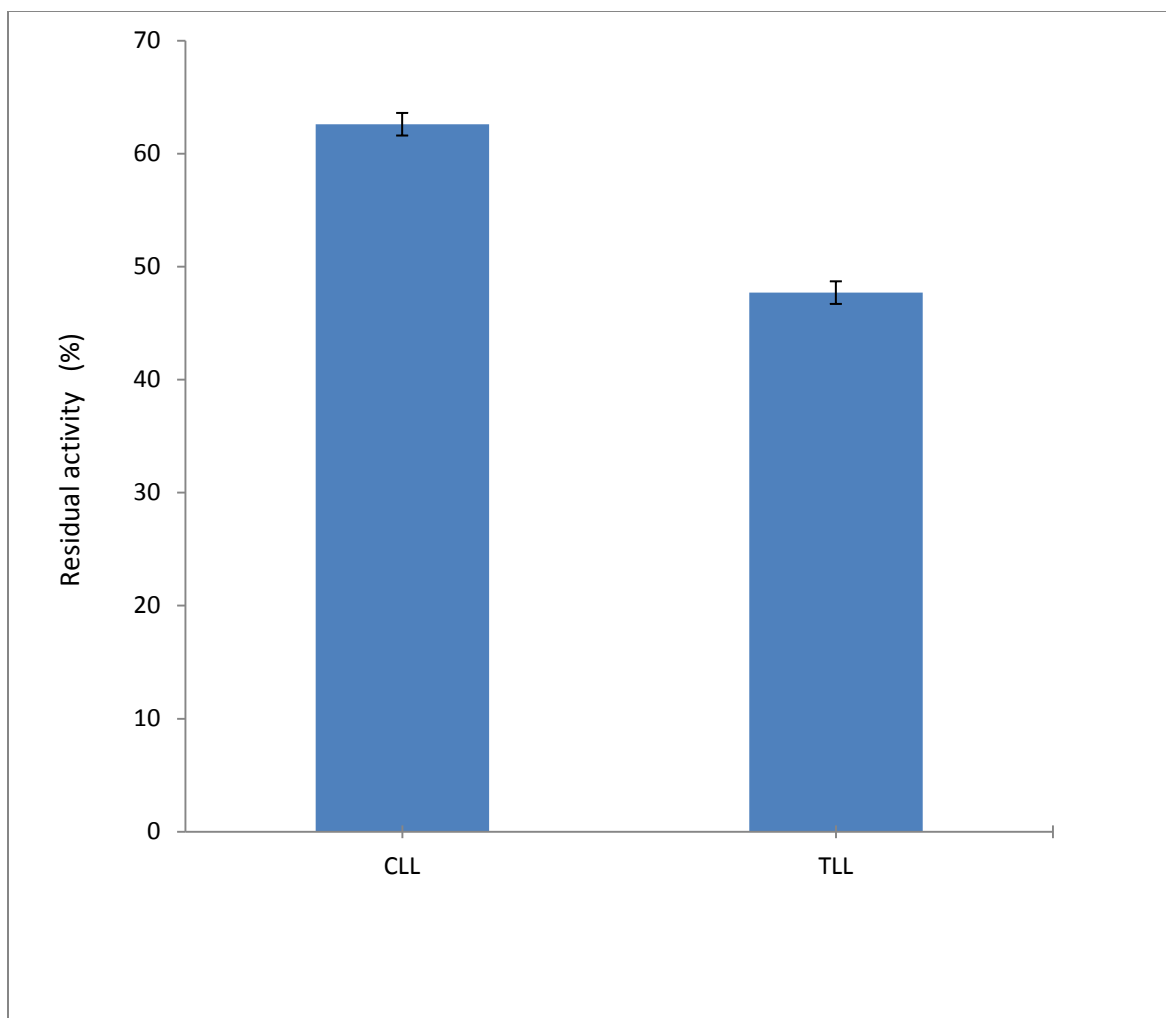


Figure 2: Residual activity of CLL and TLL at 70 °C.

The enzymatic activity at 44 °C was taken as reference (100%) for the calculation of residual (%) activity for the enzymes.

The assay mixtures comprised of 100 µl of standard enzyme solution(0.0005 nM), 200 µl of distilled water, 500 µl of 50 mM sodium phosphate buffer pH 7.5 and 200 µl of 0.025 mM 4-nitrophenyl dodecanoate (substrate) which was added last to the assay mixture after an incubation time of one hour at temperatures of 70 °C .

The molecular basis with respect to the effect of temperature on enzyme activity has been well elucidated and presents an equilibrium model which provides a quantitative explanation of enzyme thermal behaviour under reaction conditions. This is by introducing an inactive (but not denatured) intermediate that is in rapid equilibrium with the active form of the enzyme [8]

Increasing the temperature of the assay mixture increased the internal energy of the reaction molecules as well as the energy involved in chemical bonding of the molecules and nonbonding interactions, some of this heat energy been converted into chemical potential energy. High chemical potential energy can denature the weak bonds responsible for the three dimensional shape of the active proteins, hence the reduced activity observed with increase in temperature for both CLL and TLL. This could lead to a thermal denaturation of the protein and thus reduce or inactivate the protein.

Conclusions

There is a rapidly growing demand for thermostable lipases for different applications. This study has shown the effects of temperature on *Congregibacter litoralis* KT 71 β -lactamase in the hydrolysis of 4-nitrophenyl dodecanoate in order to characterize this novel enzyme if it is suitable to adapt it for use in reactions carried out at high temperatures. Results from this study shows that CLL had a residual activity of 63% at 70 °C suggesting that reactions involving these enzymes this temperature can occur without the enzyme completely inactivated thus making this enzyme potentially useful in reactive systems operating under this thermal conditions.

REFERENCES

1. Selvin, J, Kennedy, J, David P.H, Lejon, G, Seghal K, and Alan D.W. Isolation identification and biochemical characterization of a novel halo-tolerant lipase from the metagenome of the marine sponge *Haliclona simulans*. *Microbial Cell Factories*. 2012; 11:72.
2. Trincone, A. Marine Biocatalysts- Enzymatic Features and Applications. *Mar Drugs*. 2011; 9:478–499.
3. Pandey, A, Benjamin, S, Soccol, C.R. Nigam, P. Krieger, N. and Soccol, V.T. The real of microbial lipases in biotechnology. *Biotechnol. Appl. Biochem*. 1999; 29 : 119–131.
4. Casas-Godoy L., Duquesne S., Bordes F., Sandoval G., Marty A., Godoy L.C., Lipases: An Overview, In: Sandoval G., (Ed.). 2012. Lipases and Phospholipases, Humana Press. 3-30.
5. Pera, L.M, Romero, C.M, Baigori, M.D and Castro, G.R . Catalytic Properties of Lipase Extracts from *Aspergillus niger*. 2006; *Technol. Biotechnol*. 44 (2) 247–252
6. Jaeger, K.E. and Eggert, T. Lipases for biotechnology. *Curr. Opin. Biotechnol*. 2002; 13:390–397.

7. Demiorijan, D.C., F. Moris-Varas and C.S. Cassidy. Enzymes from extremophiles. *Curr. Opin. Chem. Biol.* 2001; 5: 144-151.

8. Daniel, R.M, Peterson, M.E, Danson, M.J, Price, N.C, Kelly, S.M, Monk, C.R, Weinberg, C.S, Oudshoorn, M.L. and Lee, C.K. The molecular basis of the effect of temperature on enzyme activity. *Biochem. J.* 2010; 425 (2) 353-360.

UNDER PEER REVIEW