

## **Original Research Article**

**Effects of some alkali and alkaline earth metal ions on the initial reaction rates of *Congregibacter litoralis* KT71  $\beta$ -lactamase hydrolysis of 4-nitrophenyl myristate.**

### **ABSTRACT**

The effects of some alkali metal ions ( $\text{Na}^+$  and  $\text{K}^+$ ) and alkaline earth metal ions ( $\text{Mg}^{2+}$  and  $\text{Ba}^{2+}$ ) on the initial reaction rates of *Congregibacter litoralis* KT71  $\beta$ -lactamase hydrolysis of 4-nitrophenyl myristate was investigated by varying the concentrations of the metal ions in the assay mixture which comprised of 100  $\mu\text{l}$  of standard enzyme solution, 200  $\mu\text{l}$  of varying concentration of metal ions, 500  $\mu\text{l}$  of 50 mM sodium phosphate buffer pH 7.5 and 200  $\mu\text{l}$  of 4-nitrophenyl myristate (substrate) which was added last to the assay mixture after an incubation time of 10 minutes at 44 °C. The enzyme activity was measured spectrophotometrically using a UV-780 recording spectrophotometer at a wavelength of 405 nm. The hydrolysis of 4-nitrophenyl myristate to yield 4-nitrophenol was monitored by reading the absorbance at 25 minutes. Results showed that the alkaline earth metal ions ( $\text{Ba}^{2+}$  and  $\text{Mg}^{2+}$ ) had higher enzyme activation effect than the alkali metal ions ( $\text{K}^+$  and  $\text{Na}^+$ ). Also, all metal ions except  $\text{Mg}^{2+}$  showed enzyme stimulatory effect at low concentrations (<2 mM) but inhibitory at higher ion concentrations (2 mM - 3 mM).  $\text{Mg}^{2+}$  caused a proportionate decrease in enzyme activity from its peak (when metal ion concentration was lowest). Results from this research is of great significance to the industrialist especially where the search for novel lipases with unique characteristics suitable for the industries are inevitable.

**KEYWORDS:**  $\beta$ -lactamase, 4-nitrophenyl myristate, initial reaction rates, Alkali/alkaline earth metal ions

### **INTRODUCTION**

Several industrially useful biocatalysts are found in the marine ecosystem. The marine ecosystems are subjected to extreme conditions such as temperatures ranging from 4 °C to 400 °C. This is also accompanied by high hydrostatic pressures [1]

Due to these extreme conditions, novel microbial cellular processes are conferred on these microorganisms present in the ecosystem. Consequently, they possess unique enzyme systems which includes increased salt tolerance and adaptivity to extreme conditions, both of which are key industrial requirements of enzyme characteristics. [2]

Marine sponges (phylum: *Porifera*) makes up one of the unique environmental niche, which inhabits several bacterial communities. Enzymes that are produced by microbe bound in sponges most likely have unique biochemical and physiological characteristics that makes it possible for them to adapt to the unique ecosystem. Metagenomic based approaches are now being used to access these novel enzymes [3]

One of the most important group of enzymes are the **Esterases and Lipases**. These enzymes are involved in novel reactions in both aqueous and non aqueous media. There are very strong interests in researches involving lipases because of their applications in the industries such as processes in biodiesel production, food flavoring, laundry, paper industries, cosmetic production, and in pharmaceutical industries. [4]. However, these marine microbial lipases still remain under exploited.

Generally, lipases differ from one another in several criteria such as sizes, substrate specificities and stability profile. They also differ in their activities in the presence of various modulators. Owing to these known features, and considering the importance of lipases in various industries, there is much interest in isolating novel enzymes from unique environmental niches.

Several metal ions found in environment or living organism are known to perform multiple functions in enzyme action. These functions include the modification of protein structure and the enhancement of the protein structural stability in a conformation required for its function.

Many enzymes are dependent on alkali and alkaline earth metal ions for their activity. The two alkali metal ion in this study which are commonly found in living system are  $\text{Na}^+$  and  $\text{K}^+$ , while the alkaline earth metal ions in this research are  $\text{Mg}^{2+}$  and  $\text{Ba}^{2+}$ .

In this study, the interactions of some of alkali and alkaline earth metals with *Congregibacter litoralis* KT71  $\beta$ -lactamase from the marine sponge *Haliclona Simulans* was investigated using 4-nitrophenyl myristate as substrate for hydrolysis

## **MATERIALS AND METHODS**

### **Chemicals**

Sodium chloride, Potassium chloride, Barium Chloride, Magnesium Chloride and 4-nitrophenyl myristate were all of analytical grade and purchased from were of analytical grade and purchased from Sigma-Aldrich (Dorset, Poole, United Kingdom).

### **Methods**

#### **Enzyme purification**

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

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

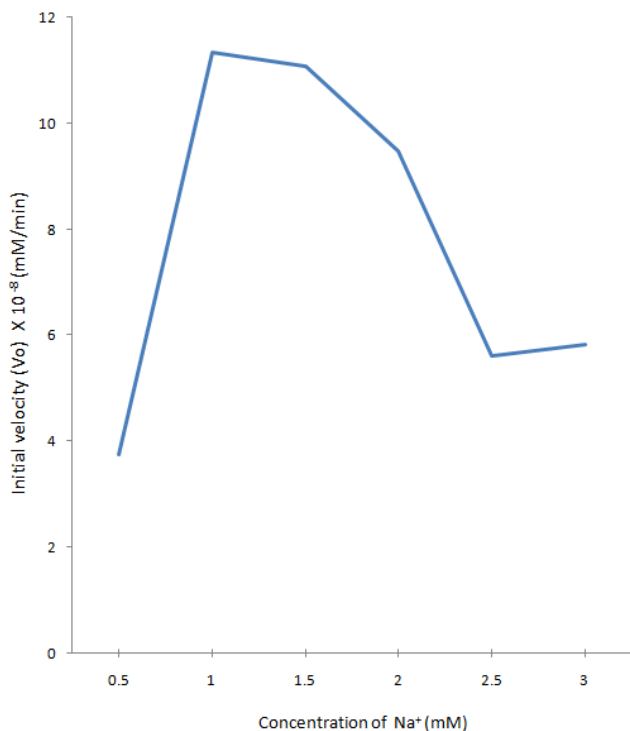
## Enzyme Assay

The effects of some alkali metal ions ( $\text{Na}^+$  and  $\text{K}^+$ ) and alkaline earth metal ions ( $\text{Mg}^{2+}$  and  $\text{Ba}^{2+}$ ) on the initial reaction rates of *Congregibacter litoralis* KT71  $\beta$ -lactamase hydrolysis of 4-nitrophenyl myristate was investigated by varying the concentrations of the metal ions in the assay mixture which comprised of 100  $\mu\text{l}$  of standard enzyme solution, 200  $\mu\text{l}$  of varying concentration of metal ions, 500  $\mu\text{l}$  of 50 mM sodium phosphate buffer pH 7.5 and 200  $\mu\text{l}$  of 4-nitrophenyl myristate (substrate) which was added last to the assay mixture after an incubation time of 10 minutes at 44  $^{\circ}\text{C}$ . The enzyme activity was measured spectrophotometrically using a UV-780 recording spectrophotometer at a wavelength of 405 nm (Selvin *et al* 2012).

## RESULTS AND DISCUSSION

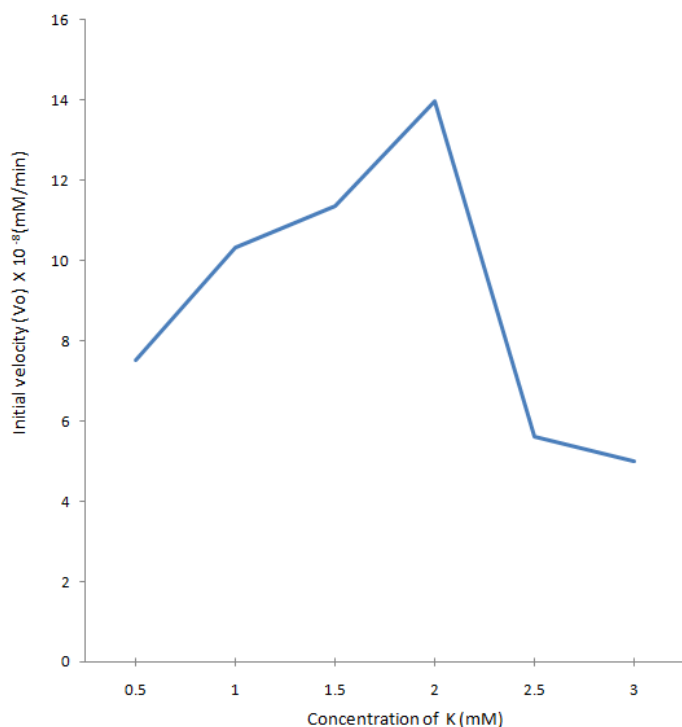
It has been shown that some enzymes have a catalytic preference for  $\text{K}^+$  [5] Like  $\text{K}^+$  channel proteins, they only allow  $\text{K}^+$  but not  $\text{Na}^+$  to pass through the plasma membrane.

Figure 1 shows the effect of sodium ion on the activity of *Congregibacter litoralis* KT71  $\beta$ -lactamase hydrolysis of 4-nitrophenyl myristate. Results show that increasing the concentration of sodium ion from 0.5 mM increased the activity of the enzyme up to 1 mM. Further increase in concentration of sodium ion up to 3 mM resulted in a decrease in enzyme activity.



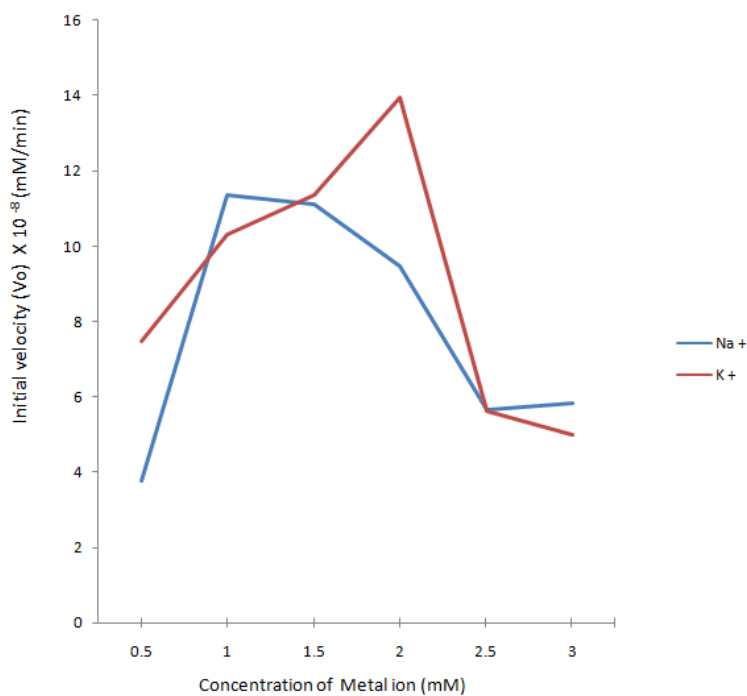
**Figure 1: Effect of Sodium ion on the activity of *Congregibacter litoralis* KT71  $\beta$ -lactamase activity in the hydrolysis of 4-nitrophenyl myristate**

Figure 2 shows the effect of potassium ion on the activity of *Congregibacter litoralis* KT71  $\beta$ -lactamase activity in the hydrolysis of 4-nitrophenyl myristate. Result show that increasing the concentration of potassium from 0.5 mM to 2 mM steadily increased the activity of the enzyme. Further increased in concentration of potassium ion up to 3 mM led to a decrease in enzyme activity.



**Figure 2: Effect of potassium ion on the activity of *Congregibacter litoralis*KT71  $\beta$ -lactamase activity in the hydrolysis of 4-nitrophenyl myristate**

A comparative study of the effects of the two alkali metal ions on the initial reaction rates of the enzyme (figure 3) showed that K<sup>+</sup> ions caused a steady increase in enzyme reaction rates which peaked at a velocity of 13.95 X 10<sup>-8</sup>(mM/min) when the K<sup>+</sup> concentration was 2 mM unlike Na<sup>+</sup> which peaked at 11.35 X 10<sup>-8</sup> (mM/min) at a Na<sup>+</sup> concentration of 1 mM. At high alkali metal ion concentration (3 mM), though a decrease in initial reaction rate was observed, K<sup>+</sup> had a higher initial reaction rate than Na<sup>+</sup> (as shown in figures 1 and 2) .

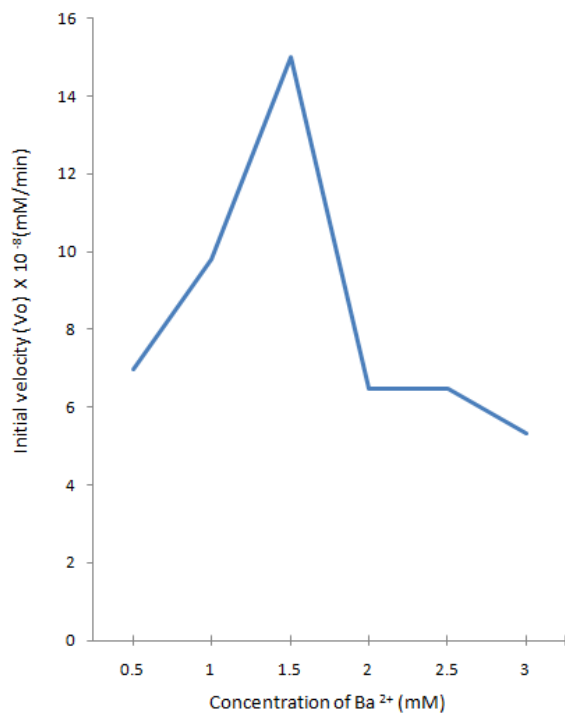


**Figure 3: A comparative effect of sodium and potassium ion on the activity of *Congregibacter litoralis* KT71  $\beta$ -lactamase activity in the hydrolysis of 4-nitrophenyl myristate.**

This finding is similar to that observed by previous research [6] in a study on the ion specific effects of sodium and potassium on the catalytic activity of HIV-1 protease. In their study, it was observed that the initial reaction rate of peptide substrate hydrolysis increases with salt concentration more dramatically in potassium than in sodium chloride solutions. It can be inferred that the active site of the  $\beta$ -lactamase was more responsive to catalysis with substrate in the presence of  $K^+$  than  $Na^+$ .

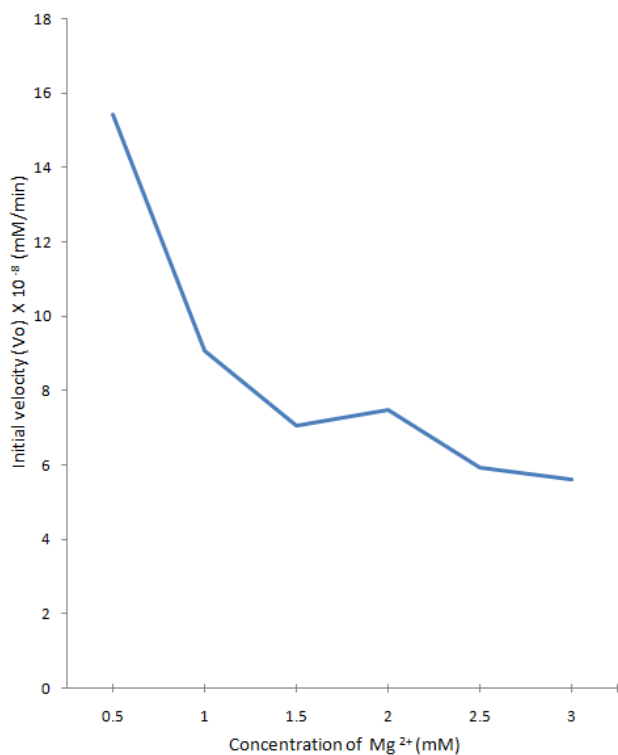
Generally, for the alkali metal ion studied, low concentrations stimulated enzyme activity while high concentrations were inhibitory. It can be concluded that the alkali metal ions ( $Na^+$  and  $K^+$ ) have both stimulatory and inhibitory effect on *Congregibacter litoralis* KT71  $\beta$ -lactamase depending on the concentrations of the metal ions.

Figure 4 shows the effect of barium ion on the activity of *Congregibacter litoralis* KT71  $\beta$ -lactamase activity in the hydrolysis of 4-nitrophenyl myristate. Results show that increasing the concentration of barium ion from 0.5 mM to 1.5 mM increased the activity of the enzyme. Further increase in the concentration of Barium resulted in the decrease in activity of the enzyme up to 3 mM. This result is similar to that observed in using the alkali metal ions. Results from barium ion showed that it resulted in the highest activity of the  $\beta$ -lactamase compared to other metal ions in this study. This suggests a very high activating effect of barium ion.



**Figure 4: Effect of Barium ion on the activity of *Congregibacter litoralis*KT71  $\beta$ -lactamase activity in the hydrolysis of 4-nitrophenyl myristate**

Figure 5 shows the effect of Magnesium ion on the activity of *Congregibacter litoralis* KT71  $\beta$ -lactamase activity in the hydrolysis of 4-nitrophenyl myristate. Results show that increasing the concentration of Magnesium ion from 0.5 mM to 3 mM resulted in a proportionate decrease in the activity of the enzyme. However at very low concentration of Mg<sup>2+</sup> ion, the initial **reaction rate** was comparable to that of barium ion. Mg<sup>2+</sup> has been shown to have inhibitory effect at high concentrations [7] in a research on the regulatory effect of divalent cations on rat liver alkaline phosphatase activity. In the research, Mg<sup>2+</sup> acted both as an activator (optimal concentration) and inhibitor (at higher concentration).



**Figure 5: Effect of Magnesium ion on the activity of *Congregibacter litoralis* KT71  $\beta$ -lactamase activity in the hydrolysis of 4-nitrophenyl myristate**

Results from this research is of great significance to the industrialist especially where the search for novel lipases with unique characteristics suitable for the industries are inevitable.

## CONCLUSION

Researches has shown that commercially available enzymes are mostly derived from animals, plants and microorganisms. The greater component of industrial enzymes are of microbial origin [8] when compared to other enzymes derived from plant and animal origin. Enzymes from microbial origin are also more stable, covering a greater variety of catalytic activities. A very high proportion of enzymes currently in use for industrial processes are hydrolytic in action [9]. These include the lipases which is the enzyme of interest in this study. Lipases is the third most commercialized enzymes found in nature, after the proteases and carbohydrases [10]. The applications of lipases in the industries such as its use in biodiesels, food, nutraceuticals and detergents, bioremediation, agriculture, cosmetics and leather [11] makes the search for alternative cheap sources inevitable. In this study the effect of alkali and alkaline earth metal ions on the initial reaction rates of *Congregibacter litoralis* KT71  $\beta$ -lactamase hydrolysis of 4-nitrophenyl myristate was investigated with the aim of understanding how the enzyme works in the presence of some alkali and alkaline earth metal ions. Results from this study has shown that the alkaline earth metal ions (Ba<sup>2+</sup> and Mg<sup>2+</sup>) had higher enzyme activation effect than the alkali metal ions (K<sup>+</sup> and

Na+). Results from this research is of great significance to the industrialist especially where the search for novel lipases with unique characteristics suitable for the industries are inevitable.

## REFERENCES

1. **Selvin, J, Kennedy, J, David P.H, Lejon, G, Seghal K, and Alan D.W.** (2012). Isolation identification and biochemical characterization of a novel halo-tolerant lipase from the metagenome of the marine sponge *Haliclona simulans*. *Microbial Cell Factories*11:72.
2. **Trincone, A.** (2011). Marine Biocatalysts- Enzymatic Features and Applications. *Mar Drugs*, 9:478–499.
3. **Simon C. and Daniel R.** (2011) *Metagenomic analyses: past and future needs. Appl Environ Microbiol.* 77:1153–1161.
4. **Jaeger, K.E. and Eggert, T.** (2002). Lipases for biotechnology. *Curr. Opin. Biotechnol.* 13:390–397.
5. **Pokorna, J., Heyda, J. and Konvalinka, J.** (2013). Ion specific effects of alkali cations on the catalytic activity of HIV-1 protease. *Faraday Discuss.* (160): 359-370;
6. **Heyda, J.; Pokorna, J.; Vrbka, L.; Vacha, R.; Jagoda-Cwiklik, B.;Konvalinka, J.; Jungwirth, P.; Vondrasek, J.** (2009). Ion Specific Effects of Sodium and Potassium on the Catalytic Activity of HIV-1 Protease. *Phys. Chem. Chem. Phys.* 11(35): 7599–7604.
7. **Arise, R.O., Bolaji F.F, Jimoh, O.A., Adebayo, J.O, Olorunniji, F.J, and Malomo, S.O** (2005). Regulatory effect of divalent cations on rat liver alkaline phosphatase activity: How Mg<sup>2+</sup> activates (and inhibits) the hydrolysis of p-nitrophenylphosphate. *Biokemistri.* 17(2):129-136.
8. **Jemli S., Ayadi-Zouari D., Hlima H.B., Bejar S.**(2014). Biocatalysts: Application and engineering for industrial purposes. *Crit. Rev. Biotechnol.* 6:1–13.
9. **Prakash D., Nawani N., Prakash M., Bodas M., Mandal A., Khetmalas M., Kapadnis B.**(2013) Actinomycetes: A repertory of green catalysts with a potential revenue resource. *Biomed. Res. Int.* 2013:1–8.
10. **Hasan F., Shah A.A., Hameed A.** (2006). Industrial applications of microbial lipases. *Enzym. Microb. Technol.* 39:235–251.
11. **Andualema B., Gessesse A.** (2012) Microbial lipases and their industrial applications: Review. *Biotechnology.*11:100–118.