



UNIVERSITI PUTRA MALAYSIA

**PARTIAL PURIFICATION AND CHARACTERISATION OF ALKALINE
PHOSPHATASE FROM HEPATOPANCREAS AND INTERTINE OF
RED TILAPIA, (TILAPIA MOSSAMBICA)**

VANITHA MARIAPPAN.

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ALKALINE PHOSPHATASE FROM HEPATOPANCREAS AND
INTESTINE OF RED TILAPIA, (*Tilapia mossambica*)**

By

VANITHA MARIAPPAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfillment of the Requirement for the
Degree of Master of Science**

July 2005



DEDICATION

Specially dedicated to the memory of my dear aiyah, late Mr. **MARIAPPAN ARUNASALAM** who left me & my siblings 15 years ago and to my beloved amma **BABY MARIAPPAN** who have been standing strong all her life just for us.....

Aiyah even if you are gone for long,

Your presence I feel within me,

But you will always be in my memory,

As sweet as it can be,

All my life forever & ever...

**HARD IS LIFE,
FOR HE WHO DESIRE DEATH,
BUT LIVES ON,
FOR THE SAKE OF HIS LOVED ONE!!!**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirement for the degree of Master of Science

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Alkaline phosphatase (EC 3.1.3.1) is a metalloenzyme, which catalyze nonspecific hydrolysis of phosphate monoesters. Partial purification was conducted on alkaline phosphatase (ALP) extracted from hepatopancreas and intestine of red tilapia, (*Tilapia mossambica*) using two main steps – ammonium sulphate precipitation and ion exchange chromatography on DEAE – 52. Samples from the ion-exchange step were analysed for ALP activities and characterised by SDS-PAGE. SDS-PAGE analysis showed 2 identical bands and was found to have molecular weight of 68, 000 Da (hepatopancreas ALP) and 180, 500 Da (intestinal ALP) subunits. Overall, purification fold obtained from the final step are 1.8 and 21.9 for hepatopancreas and intestinal



respectively, with recovery of only 0.22% from hepatopancreas and 0.01% from intestine. The specific activity of the enzyme was $1.72 \times 10^{-2} \mu\text{mol min}^{-1} \text{mg}^{-1}$ and $2.93 \times 10^{-1} \mu\text{mol min}^{-1} \text{mg}^{-1}$ from hepatopancreas and intestine respectively. The ALP from hepatopancreas remained stable at temperatures up to 50°C , and ALP from intestine enzyme had an optimum temperature of 60°C . The optimum pH for both hepatopancreas and intestine ALP of *Tilapia mossambica* is pH 10. The positive monovalent alkali metal ions (Li^+ , Na^+ and K^+) have no effect on the ALP enzyme activity. However, the positive divalent alkali metal ions (Mg^{2+} and Ca^{2+}) activate the enzyme activities. Heavy metal ions (Zn^{2+} , Cu^{2+} , Cd^{2+} and Hg^{2+}) were found to inhibit the enzyme activity.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENULENAN SEPARA DAN PENCIRIAN ENZIM ALKALINE FOSFATASE DARI HEPATOPANKREAS DAN USUS IKAN TILAPIA MERAH (*Tilapia mossambica*)

Oleh

VANITHA MARIAPPAN

Julai 2005

Pengerusi: Profesor Nor Aripin Shamaan, PhD

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Alkaline fosfatase (EC 3.1.3.1) adalah metalloenzim yang hidrolisis secara takspesifik fosfat monoester. Penulenan separa telah dijalankan ke atas alkaline fosfatase (ALP) dari hepatopankreas dan usus ikan tilapia merah (*Tilapia mossambica*) dengan menggunakan dua kaedah utama iaitu pemendakan amonium sulfat dan kromatografi penukaran ion DEAE - 52. Sampel dari penukaran ion DEAE yang mengandungi aktiviti ALP dianalisis dan dicirikan melalui kaedah elektroforesis SDS. Analisis SDS-PAGE telah menunjukkan 2 jalur yang serupa dan berat molekulnya adalah 68, 000 Da (ALP hepatopankreas) dan 180, 500 Da (ALP usus) bagi subunitnya. Secara keseluruhannya, faktor penulenan yang diperolehi daripada kaedah terakhir ialah 1.8

(hepatopankreas) dan 21.9 (usus) dengan pemulihan hanya 0.22% dari hepatopankreas dan 0.01% dari usus. Aktiviti spesifik enzim ialah $1.72 \times 10^{-2} \text{ U mg}^{-1}$ bagi hepatopankreas dan $2.93 \times 10^{-1} \text{ U mg}^{-1}$ bagi usus. ALP hepatopankreas adalah stabil pada suhu sehingga 50°C dan ALP usus pula mempunyai suhu optimum 60°C . pH optimum bagi kedua-dua hepatopankreas dan usus *Tilapia mossambica* adalah pH 10. Ion monovalen logam alkali positif (Li^+ , Na^+ and K^+) tidak memberikan sebarang kesan kepada aktiviti ALP. Bagaimana pun, ion divalen logam alkali positif (Mg^{2+} and Ca^{2+}) mengaktifkan aktiviti enzim. Ion logam berat pula (Zn^{2+} , Cu^{2+} , Cd^{2+} and Hg^{2+}) menghalang tindak balas enzim.



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I certify that an Examination Committee met on 20th July 2005 to conduct the final examination of Vanitha Mariappan on her Master of Science thesis entitled “Partial Purification and Characterisation of Alkaline Phosphatase from Hepatopancreas and Intestine of Red Tilapia (*Tilapia mossambica*)” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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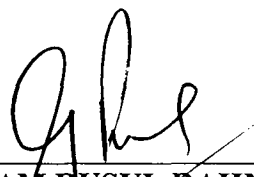
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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations that have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any degree at Universiti Putra Malaysia or other institutions.



VANITHA MARIAPPAN

Date: 12/8/05

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LIST OF ABBREVIATION

α	- alpha
β	- beta
$^{\circ}\text{C}$	- degree Celsius
%	- percentage
Abs	- absorbance
ALP	- alkaline phosphatase
APS	- ammonium persulphate
BSA	- bovine serum albumin
cm	- centimetre
Da	- Dalton
DEAE	- diethylaminoethylcellulose
IEF	- isoelectric focusing
kD/kDa	- kilodalton
kg	- kilogram
mA	- mili Amp
mg	- miligram
ml	- mililiter
min	- minute



mins	- minutes
MW	- molecular weight
NaOH	- sodium hydroxide
$(\text{NH}_4)_2\text{SO}_4$	- ammonium sulfate
nm	- nanometer
pH	- (-) log concentration of H^+
<i>p</i> -NPP	- para nitrophenyl phosphate
<i>p</i> -NP	- para nitrophenol
SDS	- sodium dodecyl sulphate
U	- unit
μmol	- micromole
μg	- microgram
X g	- gravity
w/v	- weight/volume
w/w	- weight/weight

CHAPTER 1

INTRODUCTION

The existence of enzymes has been known for well over a century. Some of the earliest studies were performed in 1853 by the Swedish chemist Jon Jacob Berzelius who termed their chemical action catalytic. It was not ever since James B. Sumner of Cornell University purified and crystallizes the enzyme urease from the jack bean (EC 3.5.1.5) for the first time in the year 1926 as mentioned in Dixon and Webb, (1979); biochemists have successfully purified perhaps, thousands of enzymes thus far.

Alkaline phosphatase, ALP, (EC 3.1.3.1) is found in abundance in nature. ALP is a hydrolase and catalyzes the hydrolysis of various bonds. It has found in wide application especially in molecular biology, medical, and industries. It is a hydrolytic enzyme which catalyses the cleavage of a chemical bond with the addition of water (McComb *et al.*, 1979). ALP is in a group of enzyme acting on ester bonds and these esterases are subdivided into those acting on phosphoric monoester hydrolases, the phosphatases under an alkaline condition (McComb *et al.*, 1979).

