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# TISSUE AND SPECIES SPECIFIC ESTERASE BANDING PATTERNS OF *POMACEA PALUDOSA* AND *POMACEA CANALICULATA*

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## ABSTRACT

The electrophoretic banding patternof esterases were examined in six different tissues of *Pomacea paludosa* and *Pomacea canaliculata* by using different inhibitors viz: pCMB, Paraoxon, Physostigmine, EDTA and AgNo3. Same esterolytic active zones were observed in Ctenidia and Mantle of both species, the maximum number of bands exhibiting in Ctenidia of *P.paludosa* and *P.canaliculata*. Species specific differences were observed in some of the regions. Striking substrate – specific reactions were not observed, but based on sensitivity to inhibitors, the tissue esterase of both species classified in to CE, ER, AcE, ArE, AcE and Esdp esterases.

**KEYWORDS: Electrophoresis, inhibitor, Physostigmine, esterolytic, dextranase enzyme,**

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# INTRODUCTION

Esterases are a group of hydrolytic enzymes occurring in multiple forms with broad substrate specificity. Heterogenicity of esterase from several species of molluscans has been demonstrated by employing electrophoretic techniques and they are shown to be tissue specific (Hart and Coock 1976). These enzymes are ubiquitous in nature but their exact physiological significance is not known. Recent studies on esterase from several species implicate them to be involved in the regulation of their activity Gagnaire *et al.,* (2008) reported the Cholinesterase activities as potential biomarkers Characterization in two freshwater snails. Kumar *et al.,* (2012) studied the "Enzyme activity in the nervous tissue of *Lymnae acuminata* fed to different bait formulations. The present investigation deals with the characterization of the tissue esterase and observed the species specific patterns.

# MATERIALS AND METHODS

Snails were collected from ponds (tanks) located within the radius of 80 kms from Kakatiya University Campus by netting with the help of local fishermen. They were immediately brought to the laboratory in water in plastic buckets and acclimatized to laboratory conditions for about a week in aquaria. They were fed on natural plakton collected from their natural habitats. Snails were immobilized and the tissues were dissected out of animals. Six tissues were selected for the study Ctenidia, Hepatopancreas, Intestine, Foot, and Tentacles. The dissected tissues individuals were pooled, weighed to the nearest milligram and were homogenized in 0.01M Tris-Hcl buffer (pH 7.5) containing 0.9% of NaCl. The concentration of tissue homogenates varied from tissue to tissue. i) Ctenidia5% ii) Hepatopancreas – 2%, iii) Intestine-2%, iv) Mantle- 10%, v) Foot-30 %, vii) Tentacles-5%. The tissues after homogenization were placed in ice-jacketed centrifuge tubes. The extracts were centrifuged at 2,000 rpm for 10 minutes in a clinical centrifuge at room temperature. The supernatants were mixed with equal volumes of 20% sucrose solution containing 0.05% bromophenol blue as the tracking dye. An aliquot of 0.1ml of this mixture was used for loading the sample on to the gel for electrophoretic separation of esterase pattern.

**Table 1.1**
***Tissue specific distribution of esterase zones in Pomacea paludosa***

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Tissues / Rm values | .60 | .58 | .50 | .46 | .36 | .33 | .30 | .28 |
| Ctenidia | | ++ CE | | | +++ AcE | +++ ER | | ++ CE |
| Hepato pancreas | ++ CE | +++ ER | | | +++ ER | | ++ CE | |
| Intestine | | | | ++ ArE | | ++ Esdp | ++ ArE | |
| Mantle | | | ++ CE | | ++ CE | ++ ER | | |
| Foot | | | | | ++ AcE | | | ++ Esdp |
| Tentacles | | | | ++ CE | | | ++ CE | |

**Table 1.2**
***Tissue specific distribution of esterase zones in Pomacea canaliculata***

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Tissues / Rmvalues | .76 | .58 | .50 | .43 | .33 | .30 |
| Ctenidia | ++ Esdp | | | ++ Esdp | +++ AcE | +++ AcE |
| Hepato pancreas | ++ ArE | | | ++ ArE | +++ ER | |
| Intestine | ++ CE | ++ ChE | ++ ChE | | ++ CE | |
| Mantle | | ++ ArE | | ++ ArE | +++ CE | |
| Foot | | | ++ Esdp | ++ ArE | +++ ArE | |
| Tentacles | | | ++ CE | | ++ AcE | |

**Figure-1**
***Tissue specific distribution of esterase zones in Pomacea paludosa***

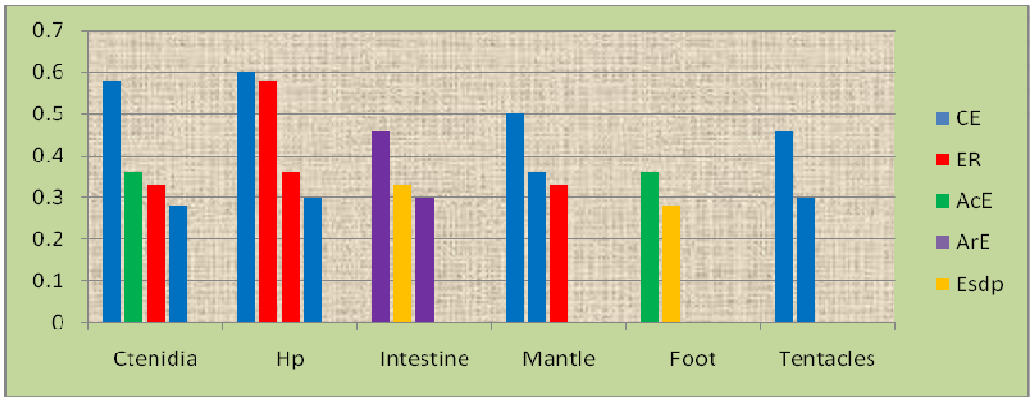
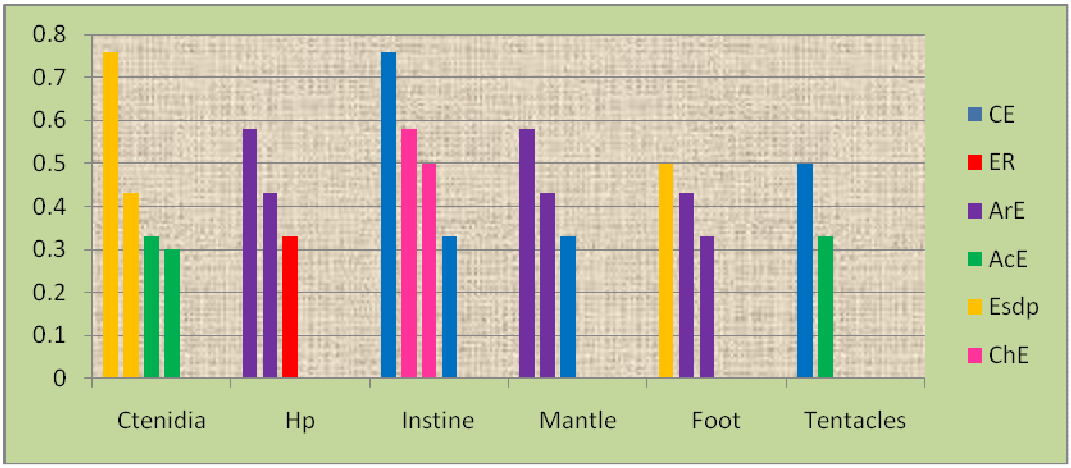
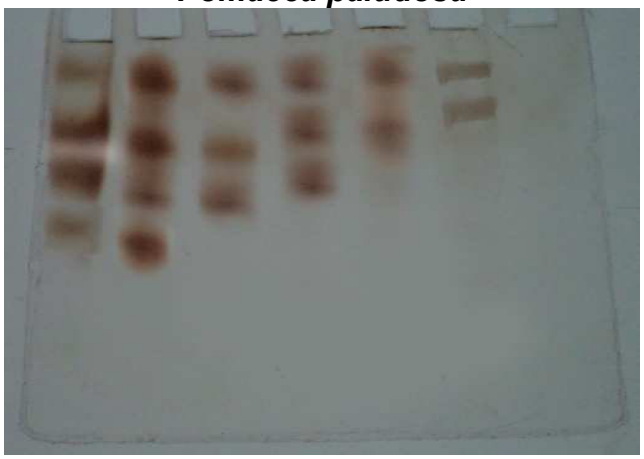


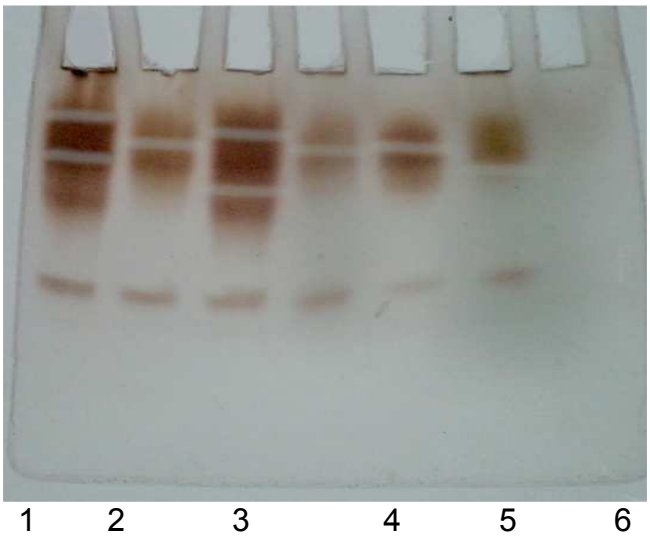

**Figure-2**
***Tissue specific distribution of esterase zones in Pomacea canaliculata***



***Pomacea paludosa***




***Pomacea canaliculata***




***1 = Ctenidia 2= Hepatopancreas 3= Intestine 4= Mantle 5=Foot 6=Tentacles***

## *1.3 Species specific distribution of esterase in ctenidia of two snails*

| Rm Values | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| | .76 | .58 | .50 | .36 | .33 | .30 |
| *1) Pomacea paludosa* | | ++ CE | | +++ AcE | +++ ER | |
| *2)Pomaceacanaliculata* | ++ Esdp | | | | +++ AcE | +++ AcE |

## *1.4 Species specific distribution of esterase in Hepatopancreas of two snails*

| Rm Values | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| | .76 | .60 | .58 | .43 | .36 | .33 | .30 |
| *1)Pomacea paludosa* | | ++ CE | +++ ER | | +++ ER | | ++ CE |
| *2)Pomacea canaliculata* | ++ ArE | | | ++ ArE | | +++ ER | |

### *1.5 Species specific distribution of esterase in Intestine of two snails*

| Rm Values | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| | .76 | .58 | .50 | .46 | .33 | .30 |
| *1)Pomacea paludosa* | | | | ++ ArE | ++ Esdp | ++ ArE |
| *2)Pomacea canaliculata* | ++ CE | ++ ChE | ++ ChE | | ++ CE | |

### *1.6 Species specific distribution of esterase in Mantle of two snails*

| Rm Values | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| | .58 | .50 | .48 | .43 | .36 | .33 | .25 |
| *1) Pomacea paludosa* | | ++ CE | | | ++ CE | ++ ER | |
| *2)Pomacea canaliculata* | ++ ArE | | | ++ ArE | | +++ CE | |

### *1.7 Species specific distribution of esterases in Foot of two snails*

| Rm Values | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| | .60 | .55 | .50 | .48 | .43 | .36 | .33 | .28 |
| *1) Pomacea paludosa* | | | | | | ++ AcE | | ++ Esdp |
| *2)Pomacea canaliculata* | | | ++ Esdp | | ++ ArE | | +++ ArE | |

### *1.8 Species specific distribution of esterases in Tentacles of two snails*

| Rm Values | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| | .50 | .48 | .46 | .33 | .30 |
| *1) Pomacea paludosa* | | | ++ CE | | ++ CE |
| *2)Pomacea canaliculata* | ++ CE | | | ++ AcE | |

## RESULTS AND DISCUSSION

***Ctenidia***

Ctenidia consisting of six esterase active zones with Rm value .76, .58, .43, .36, .33 and .30. Among these one of the zone with Rm value .33 is present in Ctenidia of both species with strong activity, this zone is noticed as ER esterase in *P.paludosa* species but it is examined as AcE esterase in *P.canaliculata* species. The zones with Rm value .76 and .43 were both classified as Esdp esterase and remaining zones with Rm value .33 and .30 were both examined as AcE in *P.canaliculata* species .The zone with Rm value .58, .28 are moderate activity with CE esterase and another zone with Rmvalue .36 is noticed as AcE esterase with higher activity in *P. paludosa* species. Esdp esterase is not noticed in Ctenidia of *P.paludosa* species.

***Hepatopancreas***

Hepatopancreas of both snails exhibits six zones on the zymogram, with Rm value .76, .60, .58, .36, .33 and .30. Among these the zones with Rm value .76 and .43 were moderate activity with ArE esterase and another zone with Rm value .33 is noticed as ER esterase with strong activity in *P.canaliculata* species .On the other hand the zones with Rm

value .60, .30 were complete inhibited by Paraoxon and AgNo3 so this zones are considered as CE esterase and remaining zones with Rm value .58 and .36 are not inhibited by any inhibitors used. Hence it is noticed as ER esterase in both species.

***Intestine***

Intestine of *Pomacea paludosa* exhibits ArE and Esdp esterases, but in *P.canaliculata* noticed as CE and ChE esterase.

***Mantle***

Mantle contains five esterase active zones with Rm value .58, .50, .43, .36 and .33. The zone with Rm value .33 was found in both species, and this zone is noticed as ER esterase in *P. paludosa,* whereas the same band is considered as CE esterase in *P.canaliculata* species. The zones with Rm value .58 and .48 were both noticed as ArE esterase in *P.canaliculata* but ArE esterase are not noticed in Mantle of *P.paludosa* species. On the other hand ER esterases are found in mantle of *P. paludosa* but not found in *P.canaliculata.*

***Foot***

Foot contains five esterase zones in the both species the zones with Rm value .50, .43, .36, .33 and .28. Among these one of the zone with Rm value .36 is not inhibited by any inhibitors used but only showed the weak activity by pCMB and AgNo3, so it is noticed as AcE esterase and another zone with Rm value .28 is examined as moderate activity with Esdp in *P.paludosa*. The zones with Rm value .36 and .28 were found in only *P.paludosa* with AcE, Esdp esterases respectively. ArE esterases are not examined in *P.paludosa*, but it was noticed in *P.canaliculata*.

***Tentacles***

Tentacles consisting of only four zones with Rm value .50, .46, .33, .30. Among these the zone With Rm value .46 and .30 were found in only *P.paludosa* with CE esterase. On the other hand the zone with Rm value .50 and .33 were found in *P.canaliculata*, these zones are classified as CE, AcE esterases respectively.

Distribution of esterases observed in various tissues of the two snail species of *P.paludosa* and *P.cnaliculata* were presented in Tables 1.1, 1.2 and Figure 1&2 and species specific distribution of esterases were presented in Tables 1.3-1.8. The zone with Rm value .33 is found in all tissues of *P.canaliculata* species, but in *P.paludosa* it is found in Ctenidia, Intestine and Mantle only. ChE esterases are not noticed in *P.paludosa* species but it was found in *P.canaliculta* species only, where as CHsp esterases were not examined in both species. CE and ArE esterase are principal contributors of *P.paludosa* and *P. canaliculata* species respectively. In conclusion, the electrophoretic banding pattern of soluble esterases of *P.paludosa* and *P.canaliculata* shown to be species -specific and tissue specific differences. The basic profile of esterases can be useful in detecting genetic introgression and polymorphism of these *Pomacea* species.

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