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HISTOCHEMICAL STUDIES ON DIGENETIC TREMATODE PARASITE, *ORIENTOCREADIUM STRIATUSAE* N.SP. FROM *CHANNA STRIATUS* (BLOCH, 1793)

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Abstract

Phosphatases have been localized by histochemical techniques in various organ of piscian digenetic trematode parasite, *Orientocreadium striatusae* n.sp. Alkaline and acid phosphatases were present in almost all structure viz., suckers, testes, ovary, uterus, seminal receptacles, egg shell and vitellaria.

Keywords: *Orientocreadium striatusae* n.sp., Alkaline and acid phosphatase, *Channa striatus*

Introduction

In 1878, Kuhne used the word enzyme to indicate the catalysis taking place in the biological systems. In human body uses thousand of enzymes to carryout a myriad of biochemical process. Enzymes are the catalyst biological origin which accelerates the various cellular reactions. Enzymes are the hydrolytic enzyme and are responsible for the breakdown of phosphate esters, can be divided into three type, mono, di and triphosphatases. Phosphatase plays an important role, regulating metabolic processes with alkaline phosphatase taking part in active transport through cellular membranes and acid phosphatase (isosome marker) indirectly providing information regarding intercell digestion process.

Acid and alkaline phosphatases in parasites have been much investigated. Work published before 1949 on phosphatases was reviewed by [3]. In recent years, these enzymes have been studied in parasites such as *Acanthocephala* 23 species [4], *Ancylostoma caninum* [18], *Ancylostoma duodenale,* Marzullo et al. [15], *Ascaridia galli* [16], *Ascaris lumbricoides* [25]; Chowdhury et al. [5], *Cysticercus tenuicollis* [9], *Dipylidium caninum* and *Fasciola hepatica* [24], *Haemonchus contortus*, nine species of helminths were studied [17], *Hymenolepis nana* [10], *Moniezia expansa* [9, 24], *Schistosoma mansoni* [8, 20], *Taenia pisiformi* [9, 24], *Taenia saginata* [7], and *Wuchereria bancrofti*,Chowdhury et al. [6].

In present study, the acid and alkaline phosphatase enzymes reactions found in the different organ of *Orientocreadium striatusae* n.sp.from *Channa striatus* (Bloch, 1793).

Materials and Methods

The freshwater fish, *Channa striatus* (Bloch, 1793) were collected from different places of Marathwada region of Maharashtra state. Fishes were brought to the laboratory and dissected or cut opened. The intestine, liver, stomach, gill cavity, heart, spleen etc. was examined carefully for the parasite. The parasites collected were kept in saline water, washed well in distilled water; the identical worms were separated and processed for taxonomical study. The trematode parasites were collected from intestine. Gomori's (1946) Cobalt nitrate method for alkaline phosphatase and acid phosphatase use of Gomori's (1950) Lead nitrate method was used. The trematode parasites were preserved in chilled Acetone for short time. The trematode parasites were fixed in chilled Acetone for 24-48 hours with 2-3 changes. Then passed through Chloroform for 1 hours, embedded in paraffin wax having a melting point not higher than 56 ^{O}C and blocks were prepared.

Section were cut at 6-8 µ, deparaffinized in xylene, hydrate to water through 100%, 80%, 60%, 40%, 20% alcoholic grades, rinsing with several dips in water. For Alkaline phosphatase the sections were transferred or placed in incubating mixture such as, 10 ml of 3% Sodium β- glycerophosphate, 10 ml of 2% Sodium diethyl barbiturate, 20ml of 2% Calcium chloride, 05 ml of Distilled water, keep at 37 ^{O}C. for 30-60 minutes. This stock solution ready mix to make incubation mixture at the time of use, wash well in distilled water, treat with 2% aqueous Cobalt nitrate for 3-5 minutes, rinsed in distilled water and transferred to 2% aqueous Yellow ammonium sulphide for 1-2 minutes, washed in distilled water.

For Acid phosphatase method, the section were transferred or placed in incubating mixture such as, 30

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ml of 0.01M Sodium β- glycerophosphate, 30 ml of 0.05 M Acetate buffer (pH 5.0), 10ml of 0.004 M Lead nitrate. Lead nitrate was dissolved in the buffer β-glycerophosphate was added. The final P^H of the incubation was maintained at 5.0 at 37 ^OC. for 30 minutes, washed well in distilled water and immersed in 2% aqueous Yellow ammonium sulphide for 2 minutes, rinsed in distilled water, then the section were dehydrated in (20%, 40%, 60%, 80%, 100%) alcohol grades, cleared in xylene and mounted in glycerin jelly. Observed under Microscope for the alkaline and acid phosphatase reaction in various structures which are stained black or brownish in colour.

Result and Discussion

The detailed taxonomic observations shows that, the worm are belonging to the genus *Orientocreadium* with its, new species *Orientocreadium striatusae* n.sp.

The observation of the longitudinal section of the whole parasite and anterior region of the *Orientocreadium striatusae* n.sp. reveals that, the intense alkaline phosphatase enzyme reaction in the vitellaria, sucker whereas no reaction in musculature and cirrus pouch (Fig-1 A&B).

In the posterior region of the *Orientocreadium striatusae* n.sp., it is found that, the intense alkaline phosphatase enzyme reaction in the vitellaria, uterus; weak reaction in the ovary, caeca and moderate in the testes (Fig.-1 C)

The acid phosphatase enzyme reaction seen in the whole and anterior region of the *Orientocreadium striatusae* n.sp. was indicated that, the maximum acid phosphatase enzyme reaction in the vitellaria; small reaction in the sucker and no reaction in the musculature (Fig.-2 A&B). In posterior region, it is found that, the intense acid phosphatase enzyme reaction in the vitellaria, uterus, moderate reaction in the ovary, testes and weak activity in seminal receptacles(Fig.-2C).

Table-1: Staining reaction of alkaline and acid phosphatase reaction in *Orientocreadium striatusae* n.sp.

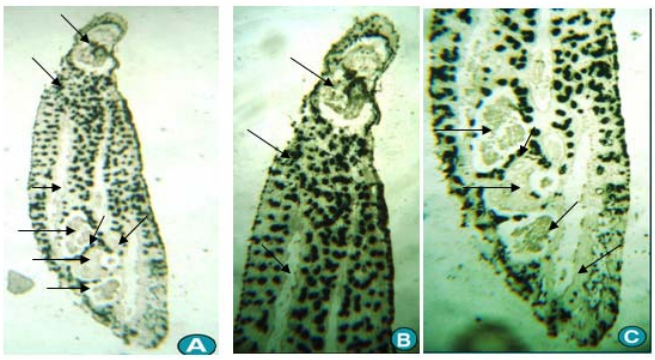
Sr. No.	Character	Alkaline phosphatase	Acid phosphatase
1	Sucker	+++	+
2	Caeca	+	-
3	Testes	++	++
4	Seminal receptacles	-	+
5	Ovary	+	++
6	Uterus	+++	+++
7	Vitellaria	+++	+++

(+++): Intense enzyme reaction; (++): Moderate enzyme reaction; (+): Small enzyme reaction; (-): None

The present studies show the distribution of the alkaline and acid phosphatase enzyme reaction in the trematode parasite. This result are similar and discussed with [13] who reported higher activity of alkaline phosphatase than acid phosphatase in intestinal trematode, *Ganeo tigrinum* only one peak for acid and alkaline phosphatase activity was noted in *P. egretti*, which with most of the previous finding [22,14].

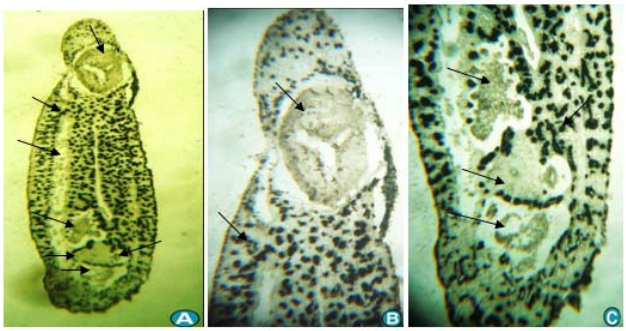
The vitellaria and reproductive system, including the gonads were also shown to have very strong reactions for both acid and alkaline phosphatases. These findings are reported in *Fasciola hepatica*, *Schistosoma mansoni* and many other trematodes [2, 19,1,21,23].

Fig.1): Showing Alkaline phosphatase enzyme reaction in the whole, anterior and posterior region of *Orientocreadium striatusae* n.sp.



Whole parasite **Anterior region** **Posterior region**

Fig. 2): Showing Acid phosphatase enzyme reaction in the whole, anterior and posterior region of *Orientocreadium striatusae* n.sp.



Whole parasite **Anterior region** **Posterior region**

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