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RESEARCH ARTICLE

A COMPARATIVE STUDY OF ESTERASE ISOZYME INHIBITION (CLASSIFICATION OF ESTERASES) STUDY IN GILL TISSUE OF *HETEROPNEUSTES FOSSILIS* AND *CHANNA PUNCTATUS.*

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ABSTRACT

In the gill tissue of Heteropneustes fossilis and Channapuntatus, three distinct esterase zones were identified, exhibiting Rm values of 0.60 for Est-1, 0.40 for Est-2, and 0.30 for Est-3. The esterase zones Est-1 (0.60) and Est-2 (0.40) in Heteropneustes fossilis were classified as ER (Esterase resistant to inhibitors) esterases. In contrast, Est-3 (0.30) was only inhibited by paraoxon, while pCMB and eserine had no effect, leading to its classification as a CE (Carboxylic Esterase). The ESe esterase, defined as Est-1 (0.60), was uniquely inhibited by eserine, with no inhibition observed from paraxine or pCMB. Est-2 (0.40) showed no response to any of the three inhibitors, confirming its classification as an ER esterase. Only paraoxon and eserine were able to inhibit Est-3 (0.30), although it remained unaffected by other inhibitors.

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INTRODUCTION

The current investigation aimed to explore the tissue-specific esterase banding patterns in various tissues of H. fossilis. The electrophoretic analysis of esterase from different tissues revealed species-specific variations, which can be effectively utilized for the identification of fish species. This electrophoretic profiling indicates that esterase patterns are unique to each species, thereby serving as a reliable method for fish species identification, as noted by Shengming *et al*. (1988). The significance of tissue-specific proteins and enzymes lies in their ability to aid in species recognition and the establishment of taxonomic relationships across numerous animal groups, as highlighted by Whitt (1987). Enzymes play a crucial role in identifying the differentiation stages of specific tissues during organismal development. A notable feature of these enzymes is the considerable divergence in heterogeneity and the presence of multiple enzyme forms in homologous tissues across different species, even among closely related ones, as discussed by Holmes and Whitt (1970) and Laksmipathi and Sujatha (1991). Extensive electrophoretic studies conducted on various animal tissues have demonstrated that enzymes exist in multimolecular forms and fulfill diverse functions, as reported by Market and Moller (1959).

Esterase Inhibition Study: Esterases are categorized based on their susceptibility to various inhibitors. In this study, esterase activity was examined to identify the tissue-specific and species-specific variations present in different tissues of H. fossilis. The primary objective was to ascertain the distribution of esterases across various tissues in this species. Comparisons of esterase activity were conducted among the tissues of H. fossilis, focusing on the electrophoretic mobility of distinct zones and their sensitivity to three specific inhibitors: Paraoxon, an organophosphate; Physostigmine, a carbamate; and Parachloromercuribenzoate, which inhibits thiol groups.

Esterases represent a diverse group of enzymes responsible for hydrolyzing carboxylic acid esters and are found in multiple forms. The esterase patterns identified in this investigation were classified according to their sensitivity to the aforementioned inhibitors: Paraoxon, Eserine, and pCMB (parachloro mercuric benzoate). Notably, Paraoxon demonstrated effectiveness comparable to DFP (Diisopropylphosphorofluoridate) in inhibiting carboxylesterases, as reported by Metcalf *et al*. (1972), Lakshmipathi and Reddy (1989), and Raju and Venkaiah (2014). Eserine was utilized to detect cholinesterases, which exhibited sensitivity to both organophosphates and carbamates, as noted in studies by Augustinson (1960),

Holmes *et al*. (1968), Hart and Cook (1976), and Lakshmipathi and Reddy (1989 & 1990).

RESULTS

Gill: Three distinct zones of esterase activity were identified in the gill tissue of Heteropneustes fossilis, exhibiting Rm values of 0.60 for Est-1, 0.40 for Est-2, and 0.30 for Est-3. The first two zones, Est-1 and Est-2, demonstrated resistance to all three inhibitors tested, leading to their classification as ER (Esterase resistant to inhibitors). In contrast, the third zone, Est-3, with an Rm value of 0.30, was specifically inhibited by paraoxon, while remaining unaffected by both Eserine and pCMB, thus categorizing it as CE (Carboxylic Esterase).

Table I. Tissue specific Distribution of esterase in Gill of *H. fossilis*

Gill	Est-1	Est-2	Est-3
Control	+++	++	++
Paraoxon	+	+	-
Eserine	+++	++	++
Pcmb	+++	+	+
Classification	ER	ER	CE

CE= Carboxylesterases
ER= Esterases resistant to inhibitors

GILL: Three distinct zones of esterase activity were identified in the gill tissue of Channa punctatus, exhibiting Rm values of 0.60 for Est-1, 0.40 for Est-2, and 0.30 for Est-3. The Est-1 zone, with an Rm value of 0.60, was specifically inhibited by Eserine, while it showed no inhibition from paraoxon or pCMB, leading to its classification as ESe esterase. In contrast, Est-2, with an Rm value of 0.40, demonstrated resistance to all three inhibitors, categorizing it as an ER (Esterase Resistant to inhibitors) esterase. The Est-3 zone, characterized by an Rm value of 0.30, was inhibited solely by paraoxon and Eserine, but not by pCMB, resulting in its classification as Che (Choline Esterase).

Table 2. Tissue specific Distribution of esterase in Gill tissue of *Channa puntatus*

Liver	Est-1	Est-2	Est-3
Control	++	+++	++
Paraoxon	+	++	-
Eserine	-	++	-
Pcmb	+	+	+
Classification	Ese	ER	ChE

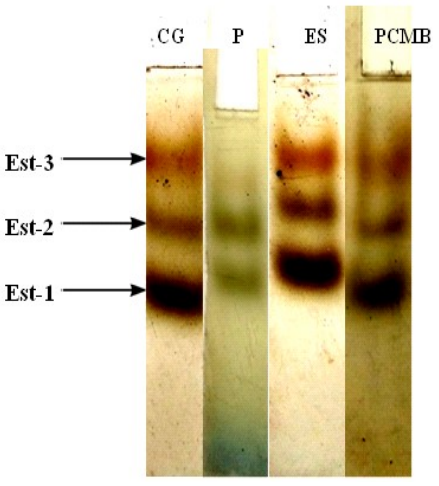

Fig.1. Electrophoretic patterns of esterases inhibition in gill tissue of *H. fossilis*

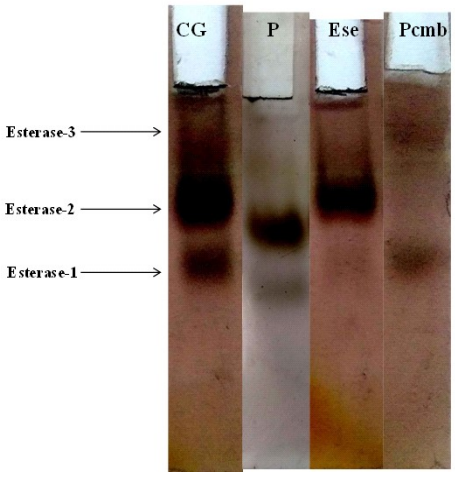

Fig. 2. Electrophoretic patterns of esterases inhibition in Gill tissue of Channa puntatus

CG= Control gill tissue
P=Gill tissue in the presence of paraoxon
ES= Gill tissue in the presence of Esarine
PCMB= Gill tissue in the presence of Pcmb

DISCUSSION

Thomson *et al*. (1983 & 1989) identified two distinct categories of esterases: one being a general non-specific esterase that facilitates the hydrolysis of P-nitrophenyl acetate, while the other specifically targets tyrosine esters. The physiological roles of these enzymes remain largely unknown. The classification of esterases into various groups has been based on their substrate specificities and sensitivity to inhibition. Typically, esterases that are inhibited by organophosphate compounds are categorized as carboxyl esterases, as noted by Holmes and Master (1967), Haritos and Salamastrakis (1982), and Reddy *et al*. (1989). Holmes *et al*. (1968) utilized substrate specificity and inhibitor sensitivity to classify the electrophoretically separated esterases present in the tissues of various vertebrates.

Previous studies have indicated that both vertebrate and invertebrate esterases display a significant degree of polymorphism. Similar inhibition patterns have been observed in the esterases of fish, as well as in other organisms such as crustaceans, insects, mollusks, and amphibians, as reported by Bheem Rao (2018), Swapna Ravinder Reddy (2015, 2017), Venkaiah *et al*. (2013), and Pranavi *et al*. (2012). However, the investigations surrounding esterases remain ambiguous. Notably, bufodienoloids, which are found in the skin and glandular secretions of toads, exist in multiple conjugate forms.

Dicarboxylic acid esters, including arginyl-dicarboxylic esters, have been studied extensively (Schmiada and Wanbara, 1979). The observed inhibition patterns indicate that these esterase enzymes exhibit sensitivity to organophosphate (OP) compounds such as Paraoxon and Physostigmine, categorizing them as Carboxyl esterases (Reddy and Pathi, 1988). These enzymes play a crucial role in metabolic processes related to insecticidal resistance and the detoxification of allelochemicals, as previously documented.

Esterases are capable of undergoing post-translational modifications and forming hybrid polymers, with their band patterns displaying significant variability under different electrophoretic conditions (Gopalakrishnan *et al*., 1997). Consequently, studies on substrate specificity are essential for the characterization and genetic interpretation of esterase zymograms, as well as for the application of inhibitor techniques. Arylesterases were found to be inhibited solely by pCMB, while enzymes exhibiting mixed inhibition were categorized as Esdp esterases, which are inhibited by both paraoxon and pCMB, and CHsp esterases, which are choline-like esterases inhibited by all three inhibitors. Additionally, Ese esterases were specifically inhibited by Eserine (Hart & Cook, 1976; Haritos & Salamastrakis, 1982; Lakshmipathi & Reddy, 1989). In 1968, Holmes *et al*. conducted comparative studies on the electrophoretic patterns of esterases across various vertebrate tissues, including that of a catfish. The substrate specificity and inhibitor sensitivity of these enzymes were utilized by Holmes *et al*. to classify the electrophoretically separated esterases found in the tissues of several vertebrates. Prior research has also documented the tissue and species-specific distribution of esterases in two catfish species and a toad (Venkaiah & Lakshmipathi, 2006). Furthermore, the esterase patterns in the muscle and brain of Channiforms and Perchiforms have been reported (Rajaiah *et al*., 2010), and non-specific esterase isozymes, following electrophoresis, have been employed to identify the species of Anabas and Clarius (Ramaseshaiah and Dutt, 1984; Begu).

CONCLUSION

Esterases play a crucial role in the biotransformation and detoxification of pesticides. Their significance extends to various biotechnological applications, including serving as antidotes for poisoning and facilitating the bioremediation of organophosphate sensors. In the current study, CE esterases, ChE esterases, and ER esterases have been identified in the gill tissue of Channa punctatus, while ER esterases and ChE esterases are also present in the gill tissue of Heteropneustes fossilis.

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