Biochemical Systematics and Ecology 47 (2013) 132–138

Contents lists available at SciVerse ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Characterization of esterase patterns in hepatopancreas of three species of *Macrobrachium* (Palaemonidae)

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ARTICLE INFO

Article history:
Received 29 July 2011
Accepted 27 October 2012
Available online 21 December 2012

Keywords:
Macrobrachium
Esterase
Hepatopancreas
Polymorphism
Prawn
Crustacean

ABSTRACT

The genus *Macrobrachium* (Bate, 1868) belongs to the Palaemonidae family. These species are commonly found in lakes, floodplains and rivers in tropical and subtropical regions of South America. The Macrobrachium genus encompasses nearly 210 species of ecological and economic importance. In this study, three species of *Macrobrachium* (*Macrobrachium jelskii*, *Macrobrachium amazonicum* and *Macrobrachium brasiliense*) were studied in order to characterize the esterase patterns in the hepatopancreas, which were still unknown. Esterases are enzymes which catalyze the hydrolysis of esters. In the hepatopancreas, these enzymes play important roles in several metabolic processes involved in some functions of this organ, such as detoxification and digestion. Twelve esterase bands (EST1 to EST12) were detected in these species, and a comparison among them showed no qualitative differences in interspecific bands, or between males and females. Inhibitors were used to classify the esterase bands. The results indicated seven acetylesterases, two carboxylesterases, one arylesterase, and one cholinesterase. The EST11 band was not detected in these procedures because of its lower frequency. Statistical analyses showed no variability among the species, in either interspecific or intraspecific assays. These results support the hypothesis of a high evolutionary conservation of esterases in the hepatopancreas of these crustaceans. The data enabled us to assess the genetic structure of these species through the use of esterasic enzymes. It also contributes to our knowledge about the biology of these poorly studied species. Knowledge on the genetic structure of populations and species are essential when defining priorities for their management and conservation.

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1. Introduction

The species of *Macrobrachium* (Bate, 1868) are decapod crustaceans from the suborder Pleocyemata, infraorder Caridea, superfamily Palaemonoidea and family Palaemonidae. This genus includes several species of prawns which are widely distributed in lakes, reservoirs, floodplains, and rivers in tropical and subtropical regions of South America. Currently, there are 210 species distributed worldwide, 45 of which are registered in the Americas, including approximately 18 in Brazil (Melo, 2003; Maciel and Valenti, 2009).

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0305-1978/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bse.2012.10.006

Most prawns with economic importance belong to the genus *Macrobrachium*. They are widely exploited by artisan fisheries, and have a high value in aquaculture. Recently, intense research efforts have been directed toward developing commercial culture technology for this species (Moraes-Valenti and Valenti, 2007, 2010). Additionally, these species have been shown to play important ecological roles, as feeding supplies and predation (Magalhães, 2000; Magalhães et al., 2005). These ecological roles emphasize the importance of studies on the drastic environmental degradation caused by exotic species and human activities.

In São Paulo state, Brazil, *Macrobrachium jelskii* and *Macrobrachium amazonicum* were introduced on 1960's (Torloni et al., 1993) in the fish farming stations owned by CESP (São Paulo Energy Company) as part of the fish *Plagioscion squamosissimus* (Heckle, 1840) transplantation process from reservoirs in Brazilian northeastern. These two species most likely escaped from artificial lakes and spread to the Pardo River, Grande River basin, and afterward reaching to the Ilha Solteira and Jupiá Reservoirs in the upper Paraná river on the beginning of 1970 years.

In current sport fishing practices, *M. jelskii* and *M. amazonicum* are used like live bait and are sometimes released into the aquatic environment. Additionally, larvae, juveniles and other immature specimens of these crustaceans were likely transported in aquatic macrophyte roots which were used as shelter for fishes in containers brought from the Pantanal region to populate ponds and reservoirs used in aquaculture, thus increasing these crustaceans invasion (Magalhães, 2000, 2001).

The hydrographic basins of São Paulo have been suffering several anthropogenic interventions that drastically changed the aquatic biota, such as artificial dams, a lack of riparian vegetation, and contamination from agriculture, industries and organic wastes (Magalhães et al., 2005; Maciel and Valenti, 2009). These interventions may lead to siltation, increase in suspended material and changes in the physical and chemical parameters of these aquatic environments. The introduction of exotic species in a previously altered environment may favor the exotic species over the native ones, either by means of predation or as a competition result.

Some studies have shown that actions meant to protect endemic or native species in natural areas that are recovering altered environments play a fundamental role in biodiversity maintenance in the ecosystem and offer a better understanding of the biotic and abiotic interaction processes in the area (Tundisi et al., 1999; Cajaraville et al., 2000). Despite the knowledge of this problem, there are few studies on the genetic structure of the species of *Macrobrachium*, the genetic alterations caused by their introduction into a new environment, or the environmental contamination caused by human influence.

The goal of this study is characterizing the esterase isozymes from the hepatopancreas of three species of *Macrobrachium*: *M. amazonicum*, *M. jelskii*, and *Macrobrachium brasiliense*, in order to classify the esterase polymorphism in this organ. Esterases are enzymes that catalyze the hydrolysis of esters. They are involved in important physiological processes, including digestion, reproduction, developmental processes, and the detoxification and tolerance of many xenobiotics (Castiglioni-Ruiz et al., 1997; Sousa-Polezzi and Bicudo, 2005; Vioque-Fernández et al., 2007; Frasco et al., 2010).

The results presented in this study are pioneer and can be used in other studies involving the detection of esterasic biomarkers, which play an important role in predicting environmental water quality.

The hepatopancreas is analogous to the liver, pancreas and intestine of vertebrates, moreover it plays important roles in several metabolic processes in crustaceans in which esterase activity is essential for these physiological reactions (Wu et al., 2008; Frasco et al., 2006, 2010). Given the importance of this organ and the paucity of knowledge about it, this paper answers some basic issues which can support a better understanding about these prawns physiology.

2. Materials and methods

2.1. Sample sources

The samples of *M. amazonicum* and *M. jelskii* were collected at the Barra Mansa Dam, in the city of Mendonça in São Paulo state, Brazil (21°14′27″S; 49°56′28″W). The *M. brasiliense* samples were obtained from the Talhadinho Stream, in Talhados, São Paulo (20°47′07″S; 49°20′35″W) (Fig. 1). The collected specimens were identified and sexed according to Melo (2003), and their hepatopancreases were then excised and frozen at −80 °C.

2.2. Isozyme analysis

In this study, 750 *M. jelskii* specimens (45 male and 705 female), 650 *M. amazonicum* specimens (30 male and 620 female) and also 650 *M. brasiliense* specimens (30 male and 620 female) were analyzed.

Esterase patterns were analyzed in 10% polyacrylamide gels (size 0.20 × 0.15 m) according to the methods described by Castiglioni-Ruiz et al. (1997). Hepatopancreas samples were individually homogenized at 0 °C in 35 μL of a buffer solution (1.5 M Tris–HCl, pH 8.8, plus 10% glycerol), for each sample preparation. Then, 10 μL of the supernatant were undergone to electrophoresis for 5 h at ~25 °C, and at a constant of 200 V. The running buffer used was 0.1 M tris-glycine at pH 8.3.

Esterases were identified after the gels were pre-incubated for 1 h at room temperature (~25 °C), in 50 mL of 0.1 M sodium phosphate, pH 6.2. The esterases were then put in a staining reaction containing both α-naphthyl and β-naphthyl acetate (30 mg and 15 mg, respectively), 60 mg of fast blue and 5 mL of N-propanol in 50 mL of a sodium phosphate solution. The esterases staining were performed in the dark for 1 h with the solution described above. The gels were dried at room temperature using gelatin and cellophane, as described by Ceron et al. (1992).

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