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The Use of Isozyme Electrophoresis for Identifying Species of the Larvae of Penaeid Prawn*

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Abstract Isozyme electrophoresis was used to identify the larvae of 7 species of penaeid prawn: *Penaeus merguiensis*, *P. penicillatus*, *P. orientalis*, *P. monodon*, *P. japonicus*, *Metapenaeus ensis* and *M. affinis* taken from the artificial nursery plants. The 7 species were examined for esterase in two development stages (mysis and megalopa substage). A key to enzyme spectra of esterase, in the 7 species of penaeid prawn at larval stage, was suggested. The major factors affecting the electrophoretic identification system when being applied were discussed.

Keywords isozyme, electrophoresis, larvae, enzyme spectrum
Cassification number Q 959.223, Q 558.9

Since the 1970s, overfishing of prawns has almost exhausted their resource in China. In order to rationally manage and conserve the prawn resource in the Chinese coastal zone, attention has been focused on the prawn resource monitor, evaluation and propagation in the offshore area. It is noticed that one of the major obstacles is the difficulty in identifying the species of prawn at the larval development stages. For penaeid prawn, although the taxonomy of the adult stage is relatively well known and good keys are available[1], the taxonomy of earlier stages is poorly documents. Available keys to the identification of postlarvae and juveniles are either restricted in species and geographic coverage or limited to the level of species groups. In an attempt to remedy this situation, Rothlisberg et al[2] and Heales et al[3] used numerical taxonomy to identify the larval and postlarval stages respectively through discriminatory analysis of known specimens. Although this technique works satisfactorily for planktonic larvae and postlarvae, it does not yield reliable results for benthic postlarval specimens. To resolve this key problem, this paper tries to study and compare the enzyme spectrum characteristics of different species of prawn in different larval stages by using isozyme electrophoresis. It is aimed at identifying the species of individual prawn larvae and postlarvae, and at streamlining the existing techniques for rapid identification of large numbers of larvae and postlarvae.

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1 Materials and Methods

The larval samples for isozyme analysis were collected from the artificial nursery plants in Shenzhen and Zhuhai cities. They were second mysis, P_{2-5} (postlarva, mean body length 4.0~6.0 mm) for *P. orientalis*, *P. penicillatus and P. merguiensis*, and second mysis, P_{2-5} and P_{9-10} (mean body length 7.5~11.0 mm) for *P. monodon*, *Metapenaeus ensis* and *M. affinis*, and only P_{10-12} (mean body length 10.0~12.0 mm) for *P. japonicus*. The larval samples collected were immersed in liquid nitrogen (-196℃) for preservation during transportation from the plant to the laboratory. The larvae analyzed were about 15 mg wet samples, and collected at the same developmental stage. They were being identified carefully under a dissecting microscope and then transferred to a micro-homogenizer prior to the addition of 1.0 mL 20% sucrose solution and triturated in ice-water bath. The homogenates were centrifuged at 13 000~15 000 g for 10 min and most of the cell debris was separated from the supernatant at a temperature below 10℃. Approximately 50 or 100 μL of the supernatant was then used for continuous polyacrylamide gel electrophoresis. Details of electrophoresis were described by Li[4]. Following the electrophoresis, gels were stained for esterase[5] and incubated until bands appeared, usually 30 to 60 min later, washed with distilled water and fixed in 7% acetic acid.

2 Results

2.1 Esterase isozyme changes in mysis stage

Considerable isozyme changes occur in 5 species of prawns in the mysis stages (Fig. 1). In all of them, there is a similar enzyme band near the negative electrode. Three kinds of esterase are present in *P. orientalis* and *P. monodon*. Amongst them, enzyme band E_3 is similar, E_2 of *P. orientalis* is somewhat similar to E_1 of *P. monodon*, but the former is the narrow band grade Ⅰ and the latter is the narrow band grade Ⅲ. Four enzyme bands appear in *P. penicillatus* and *M. affinis*, five bands are found in *P. monodon*, *P. penicillatus* and *M. ensis*. It is apparent that each species has a unique enzyme spectrum characteristics and the differences among these species are highly remarkable.

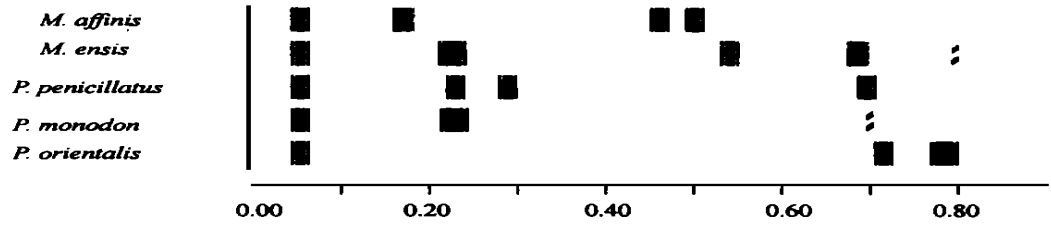

Fig. 1 Electrophoretograms of esterase in 5 species of penaeid prawns in the second mysis stage (Values below ordinate are relatively mobile)

2.2 Esterase isozyme changes in postlarval stages

The esterase of postlarvae of 7 species in P_{2-5} stage, except *P. japonicus* in P_{10-12} stage, is studied (Fig. 2). Three to six enzyme bands are exhibited; a similar band near the negative electrode is present in all 7 species. Three enzyme bands, all narrow band grade Ⅰ, are obtained in *P. penicillatus*. Four enzyme bands are observed in *M. ensis*.

They are one broad band grade Ⅰ, one narrow band grade Ⅲ and two narrow bands grade Ⅰ. Five enzyme bands are found in *P. orientalis* and *P. monodon*, of which one is broad band grade Ⅰ and the remainder narrow bands grade Ⅰ. Six enzyme bands are in existence in *P. merguiensis* and *M. affinis*. In the former, esterase E_5 is broad band grade Ⅰ and the remainder are narrow bands grade Ⅰ. In the latter, esterase E_4 is narrow band grade Ⅲ, E_5 is narrow band grade Ⅱ, E_1, E_2, E_3 and E_6 are narrow bands grade Ⅰ. For *P. japonicus* in P_{10-12} stage, six kinds of esterase are revealed. Except enzyme band E_5 is narrow band grade Ⅱ, all are narrow bands grade Ⅰ. Although the postlarvae of different species are in the same developmental stage, their enzyme spectra are varied. It indicates there are remarkable differences among the species.

In accordance with the relative mobility of esterase isozyme, the enzyme bands in Fig. 2 can be divided into four dense regions, namely, region A (0.00~ 0.10, relative mobility, Rm.), B(0.10~ 0.30), C(0.31~ 0.60), and D(0.61~ 0.85). Observations show that the enzyme spectra among 5 species of *Penaeus* were similar to a certain degree. The esterase for *P. orientalis*, *P. monodon* and *P. merguiensis* are distributed in regions A, C, and D, whereas, the bands in region D are found overlapping for *P. penicillatus* and *P. japonicus*, the esterase are distributed only in regions A and C. there are considerable differences in the case of the enzyme spectra between *Metapenaeus* and *Penaeus*. It is distributed in four dense regions for *Metapenaeus*. This not only indicates that these are differences in esterase, but also reveals their relationship in evolution.

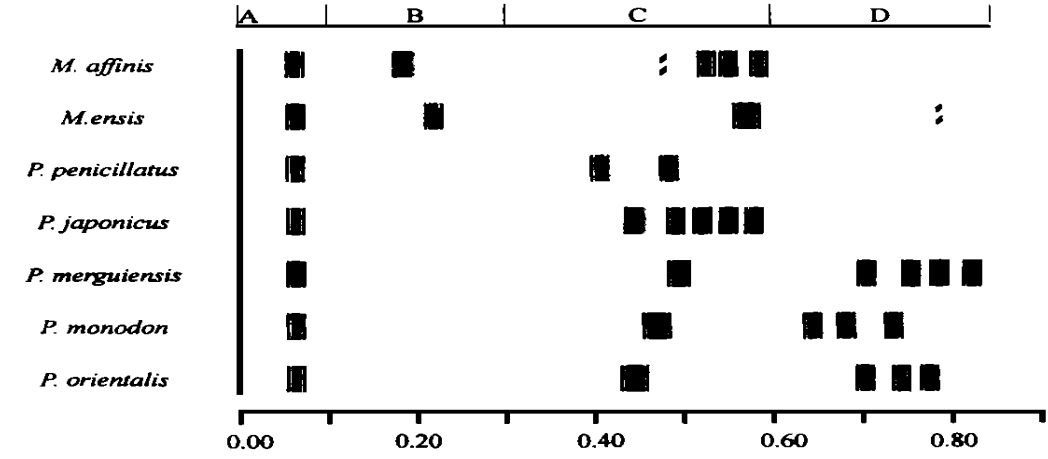

Fig. 2　Electrophoretograms of esterase in the postlarval stage of 7 species of penaeid prawns (Values below ordinate are relatively mobile)

To ascertain the changes of isozyme during its development, the esterase in 3 species have been examined in P_{2-5} and P_{9-10} stages. The differences displayed on the electrophoretograms are plainly visible. Six kinds of esterase are present between P_{2-5} and P_{9-10} stage, but the patterns are different for the 3 species (Fig. 3). For *P. monodon*, esterase 3 and 6 are always present, while esterase 1 and 5, found in P_{2-5} stage, disappear in P_{9-10} stage, and esterase 4 appears abruptly in P_{9-10} stages. For *M. ensis* in P_{2-5} stage, only 4 esterase bands, but in P_{9-10} stage, additional isozyme bands 1 and 3 appear. For *M. affinis*, isozyme bands are varied as the same trend as the two species stated above. Six

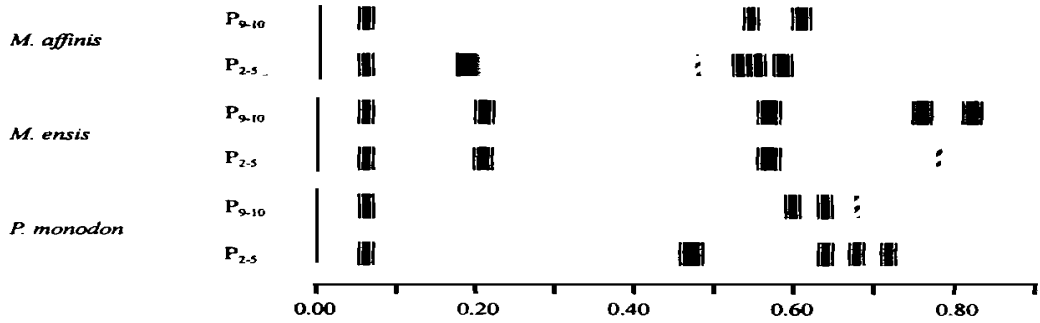

Fig. 3 The comparison of enzyme spectra of esterase in postlarval stage of 3 species of prawns (Values below ordinate are relatively mobile)

kinds of esterase are present in P_{2-5} stage, but esterase 3, 4 and 5 disappear suddenly and no new bands appear in P_{9-10} stage. All this indicates that changes of isozyme occur in penaeid prawns.

3 Discussion

It is of great importance to identify the species of penaeid prawns in the larval and postlarval stages using the ecological theory concerning the dynamics of marine populations. In recent years, many scientists have been trying to probe into valid methods for resolving this key problem. Now, it is encouraging that quite a few relatively effective methods have been put into practice. Besides the numerical taxonomy[2], Lavery et al[6] developed allozyme electrophoresis to identify the postlarvae of two species of tiger prawns, *P. esculentus* and *P. semisulcatus*. His studies indicated that glucose 6 phosphate isomerase (GPI) was the only enzyme locus examined which could be used successfully in identifying the species of postlarval tiger prawns. This technique is a great improvement in the accuracy, efficiency and cost of identification.

Since Market and Mullor first advanced the concept of "isozyme" during 1957~1959, with the improvement of the methods of isozyme analysis and isolation, isozyme isolation has become an important technique for studying biological phenomena at the molecular level and it has been widely applied. Much information has been obtained on aquatic animals through isozyme analysis[4,7,8]. These studies showed that the isozyme spectrum characteristics revealed remarkable species-specificity, tissue-specificity and development-specificity. The present study proves that isozyme electrophoresis is also an effective technique for identifying the species of prawns at larval stage. However, it must be pointed out that the following conditions must be met when using this technique for identifying the species between prawns. Firstly, synchronized comparison between different development stages is necessary because of the changes of isozymes occurred in penaeid prawns during the period of development[4]. Secondly, it is extremely difficult to identify the species of prawns at the nauplius and protozoa stages, because individuals at these stages are too small to have their isozyme change examined by taking a single larva as a sample. Only after the prawns develop to the Mysis stage, when their average body lengths range from 4 to 5 mm, is it possible to analyze their isozyme changes, using a single individual as a sample. Besides,

the difficulty in identifying prawns at the larval and postlarval stages is the central problem in assessing and monitoring the larval resources of prawns and in carrying out the prawn′s recruitment project. For these reasons, the larval stages from Mysis to megalopa substages and the postlarval or the earlier juvenile stage is chosen for identifying the species of larvae.

The esterase isozyme spectra of the larval stages of the 7 species of prawns obtained through this study can be used for reference in monitoring and assessing the larvae resources of prawns. It is worth noticing, however, that these results were derived from the larvae collected from the nursery plants, they need to be tested and supplemented in the course of practice in order to be used as practical keys to the identification of the larvae of the main economic prawns in the waters along the shores.

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同工酶电泳法鉴定对虾幼体种类的研究

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摘 要 用同工酶电泳法鉴定了南海几种主要养殖对虾幼体发育阶段的种类.通过对 7种对虾 2个不同发育阶段幼体 (糠虾幼体、仔虾 P_{2-5}及 P_{10-12}期幼体)酯酶同工酶的分析表明, 其酶谱特征显示出明显的种间特异性. 文中提出了这 7种对虾幼体的同工酶酶谱特征检索图, 并就该酶谱检索图在对虾幼体资源监测、评估应用时的具体分析方法及其应用范围进行了较为详细的说明和讨论.

关键词 同工酶, 电泳, 对虾幼体, 酶谱
分类号 Q 959.223, Q 558.9

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