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Electrophoretic studies on muscle myogens of some penaeid prawns

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Abstract

The muscle myogen patterns of some penaeid prawns studied by electrophoresis in cellulose acetate membrane are presented. Each pattern was species specific and appears to be independent of sex. The results suggest that muscle myogen patterns could be useful in taxonomic studies of penaeid prawns. Specimens preserved in phenoxyethanol were compared with fresh specimens. Preservation resulted in some losses of protein zones and, in cases where sufficient zones are preserved, the method could be used to advantage for solving taxonomic problems.

Introduction

Genetic information coded into deoxyribosenucleic acid (DNA) molecules are translated into the structure of proteins (CRICK, 1963; NIRENBERG et al., 1963; OCHOA, 1963), and the application of separation and structural studies of proteins to taxonomic studies is the simplest indirect approach for obtaining phylogenetic information.

The usefulness of electrophoretic myogen patterns as applied to taxonomic problems (TSUYUKI et al., 1965 a, 1968) and, in particular, to species identification (THOMPSON, 1960) is receiving increasing attention. TSUYUKI et al. (1965a) demonstrated the high degree of species specificity and constancy of muscle myogen patterns and their relative independence from sexual, physiological and ecological factors, in their study of anadromous, marine and a few fresh-water fish species.

According to TSUYUKI et al. (1965b), there is a group of fish muscle proteins, soluble in low ionic strength salt solution, which is useful for taxonomic characterisation. Various other workers have also shown that the muscle myogen patterns reveal a high degree of species specificity when separated by starch-gel electrophoresis (THOMPSON, 1960; GILES, 1962; TSUYUKI and ROBERTS, 1963).

These studies on fish muscle myogen, though highly successful as diagnostic characterisation for the identification of fishes have, as far as we are aware, not been applied to the studies of penaeid prawns. It is also the purpose of this study to define the applicability of protein specificity for purposes of species identification, and to discuss the value of this method as a complement to existing taxonomic information based on morphometric characters.

Materials and methods

Prawn specimens used in this investigation were collected from commercial otter trawlers and transported alive to the laboratory in fibre glass tanks (90 × 60 × 60 cm). Fresh sea water was added periodically en route. The animals were killed by severing the thorax from the abdomen, and the abdominal muscle was kept in deep freeze at about − 30 °C until use. In the case of preserved specimens, the animal was killed on board the boat and the muscle was preserved by immersion in a 2% phenoxyethanol solution according to the method of NAKANISHI et al. (1969). On arrival at the laboratory, the preserved specimens were deep frozen (− 30 °C) and stored until use.

The muscle, ground with fine sand in a pre-cooled mortar, was extracted with Tris-HCl buffer pH 7.4 (containing 7 gm NaCl/l). Extracted muscle myogen was examined electrophoretically using the Beckman microzone electrophoresis system (Beckman Instruments, Inc., Fullerton, California). Approximately 0.8 µl of sample was applied to the microzone cellulose acetate membrane using the microzone sample applicator. Separation was carried out at 300 V for $^1/_2$ h with barbital buffer (pH 8.6; ionic strength 0.075). After separation, the strips were stained in Ponceau-S stain (0.2 g Ponceau-S stain, 3 g trichloroacetic acid, and 3 g sulfosalicylic acid, all in 100 ml distilled water) for 10 min, and then rinsed with 5% acetic acid until no further stain could be removed. The electrophorograms were then photographed.

Identification of the prawns studied was based on the work of HALL (1962).

Results and discussion

Studies on fresh material

Certain basic generic and specific differences are evident in the muscle myogen patterns of the prawns studied (Fig. 1 A). Details of these differences are

* Mr. LIM unfortunately passed away in November 1969. Requests for reprints should be directed to Dr. S. S. LEE.

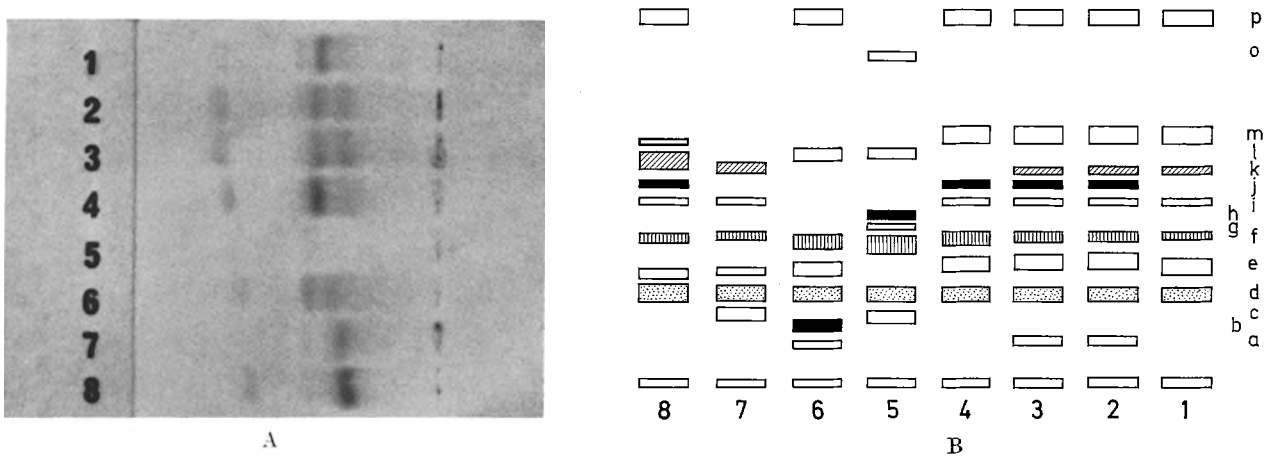
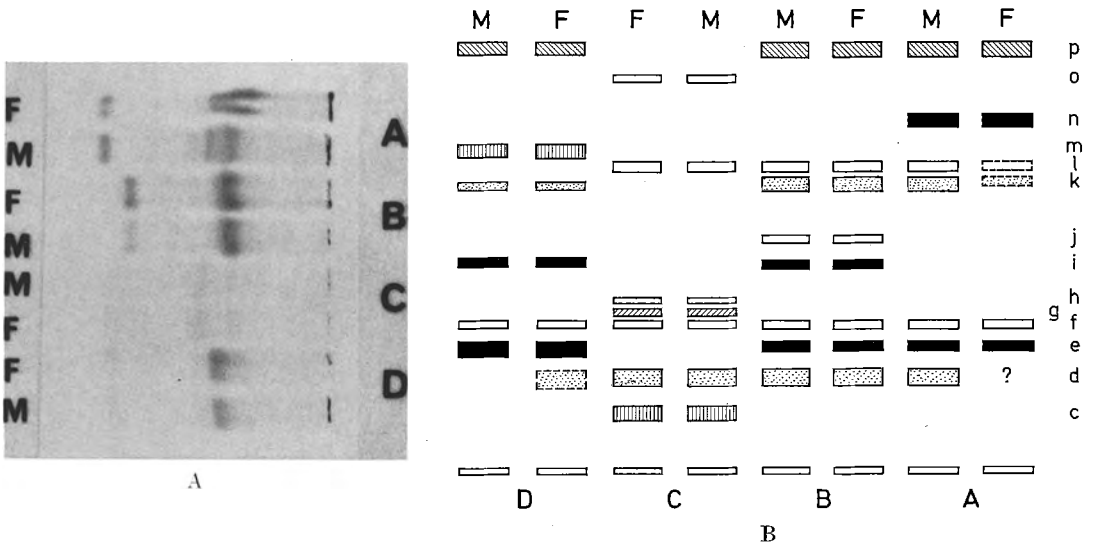


Fig. 1. (A) Electrophorogram of muscle myogen of *Metapenaeus mutatus 1*, *Parapeneopsis hungerfordi 2* and *3*, *Parapeneopsis hardwickii 4*, *Metapeneopsis stridulans 5*, *Metapenaeopsis barbata 6*, *Penaeus monodon 7*, and *Penaeus semisulcatus 8*. *Metapenaeus mutatus* is used as a standard for comparison with the other species; (B) diagrammatic representation of myogen patterns in (A). Protein zones are named alphabetically (a) to (p) in respect of their distance from the origin, using *M. mutatus* as a standard reference. Faint zones not reproducable in the photograph, but visible in the original electrophorogram, are included in this and subsequent diagrams



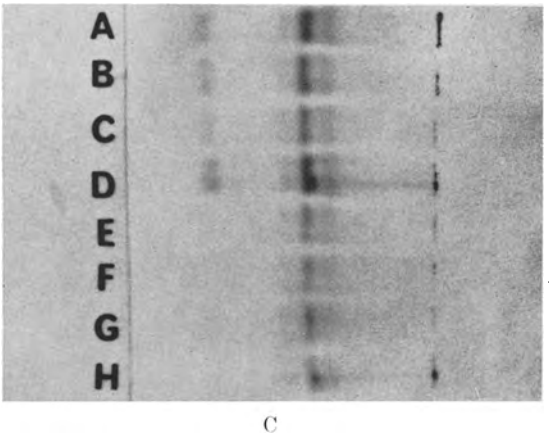


Fig. 2. (A) Electrophorogram of muscle myogen of female *F* and male *M* specimens. *Metapenaeus ensis A*, *Penaeus semisulcatus B*, *Metapenaeopsis stridulans C*, and *Metapenaeus mutatus D*. Streaking has caused some interference in the protein zones of *M. ensis* (female). No sexual differences however can be observed in the patterns of the other species; (B) diagrammatic representation of patterns in (A); (C) Electrophorogram of the muscle myogen of male *Metapenaeus mutatus*, *A* – *H*

Table 1. *Summary of muscle myogen patterns based on Fig. 1 B*

Species	Maximum number of muscle protein zones																Comparison with *M. mutatus*			
																	No. of common zones	Type of differences		
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p		Addition of zones	Deletion of zones	No. of zones differing
Metapenaeus mutatus	+	−	−	+	+	+	−	−	+	−	+	−	+	−	−	+	7	0	0	0
Parapeneopsis hungerfordi	+	−	−	+	+	+	−	−	+	+	+	−	+	−	−	+	7	2	0	2
Parapeneopsis hardwickii	−	−	−	+	+	+	−	−	+	+	−	−	+	−	−	+	6	1	1	2
Metapenaeopsis stridulans	−	−	+	+	+	−	+	+	−	−	−	+	−	−	+	−	2	5	5	10
Metapenaeopsis barbata	+	+	−	+	+	+	−	−	−	−	−	+	−	−	−	+	4	3	3	6
Penaeus monodon	+	−	+	+	+	+	−	−	+	−	+	−	−	−	−	−	5	1	2	3
Penaeus semisulcatus	−	−	−	+	−	+	−	−	+	+	+	+	−	−	−	+	6	2	1	3

+ Indicates presence and − absence of protein zones in the positions designated by a to p.

presented diagrammatically for easy comparison (Fig. 1 B) and in a tabulated form (Table 1).

Overall similarities are evident, particularly in the d, e and f protein zones (Table 1, Fig. 1 B) of the different genera and species. A comparison of the two species of *Parapeneopsis*, *P. hungerfordi* and *P. hardwickii*, and of *Metapenaeus mutatus* and also *Penaeus semisulcatus*, shows very minor differences, indicating that these species may be closely related. This is also reflected in Fig. 1 B, where the number of differences and similarities in protein zones of these prawns are compared with that for *Metapenaeus mutatus*. The myogen patterns of *Metapenaeopsis stridulans* and *Metapenaeopsis barbata*, on the other hand, reveal wide differences when compared with that of *M. mutatus*.

Generic relationships among the species are demonstrated by the overall similarities and differences in their protein myogen patterns. This is not unexpected, since protein syntheses in living organisms are controlled by fundamental genetic systems, and their expressions lead to structural differentiation, the product(s) of which is (are) a measure of genetic differences that could be employed to provide additional data for systematic studies.

Sexual independence of the protein myogen patterns of the 4 genera studied is clearly shown by the uniformity of the protein zones of the male and female specimens (Fig. 2 A and B, Table 2), while intraspecific uniformity of the muscle myogen is demonstrated by the constancy of their mobility in the electrophorogram of *Metapenaeus mutatus* (Fig. 2C).

The muscle myogen pattern of each species of prawn examined is distinctly different, exhibits a high degree of constancy, and is relatively independent of sex. It would, therefore, appear that these patterns are "fingerprints" for identification purposes, and that this method can be extremely useful for identifying unknown samples of freshly frozen prawn flesh when other means of identification are not possible. As regards the effect of environmental and physiological factors on these muscle myogen patterns, it would be expected that they would behave in the same manner as other living organisms. Uthe et al. (1966), working on the muscle myogen of lampreys, emphasized the relative independence of muscle myogen patterns from the physiological state of the animal.

Studies on preserved material

The use of electrophoresis as a bio-physical method for systematic studies often requires fresh or freshly frozen materials. This is sometimes difficult, or impossible, under certain field conditions. Formalin and other preservatives, often used for preserving animal tissues, result in the denaturation of proteins. A method which can preserve protein without the use of refrigeration would hence be of great convenience to

Table 2. *Summary of muscle myogen patterns based on Fig. 2 B*

Species	Sex	Maximum number of muscle protein zones															
		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p
Metapenaeus ensis	F	–	–	–	?	+	+	–	–	–	–	+	+	–	+	–	+
	M	–	–	–	+	+	+	–	–	–	–	+	+	–	+	–	+
Penaeus semisulcatus	F	–	–	–	+	+	+	–	–	+	+	+	+	–	–	–	+
	M	–	–	–	+	+	+	–	–	+	+	+	+	–	–	–	+
Metapenaeopsis stridulans	M	–	–	+	+	–	+	+	+	–	–	–	+	–	–	+	–
	F	–	–	+	+	–	+	+	+	–	–	–	+	–	–	+	–
Metapenaeus mutatus	F	–	–	–	+	+	+	–	–	+	–	+	–	+	–	–	+
	M	–	–	–	–	+	+	–	–	+	–	+	–	+	–	–	+

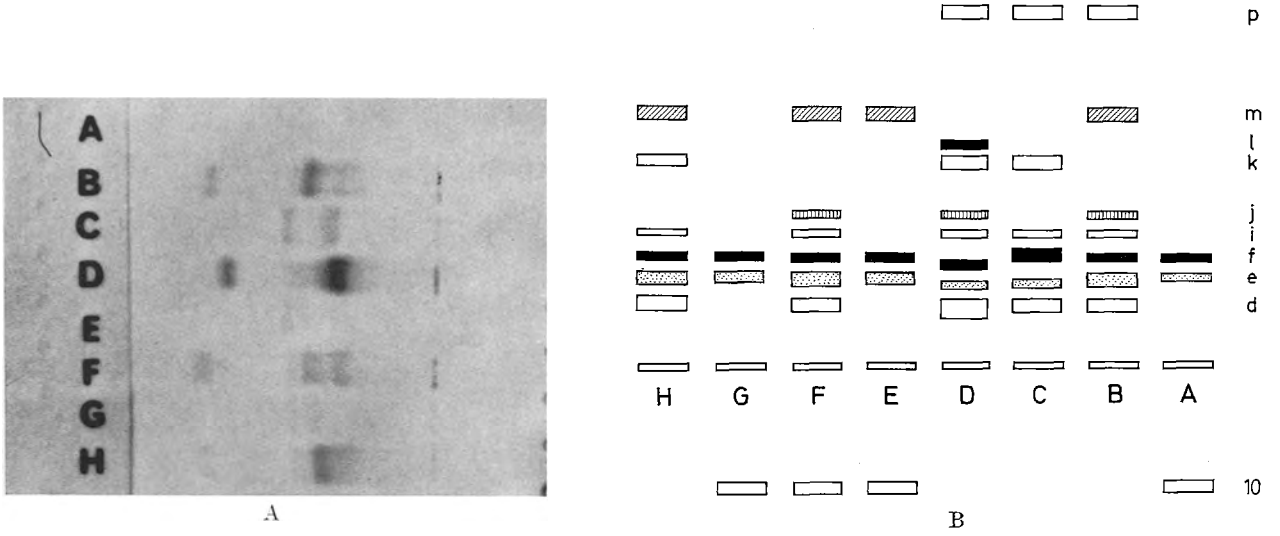


Fig. 3. (A) Electrophorogram comparing muscle myogen of freshly frozen specimens and that of specimens preserved in 2% 2-phenoxyethanol for about 12 h on board, prior to deep-freeze storage in the laboratory. *Parapeneopsis hardwickii* *A* – P, *B*; *Penaeus semisulcatus* *C* – P, *D*; *Parapeneopsis affinis* *E* – P, *F*; *Metapenaeus mutatus* *G* – P, *H*. Preserved samples – P; (B) diagrammatic representation of patterns in (A)

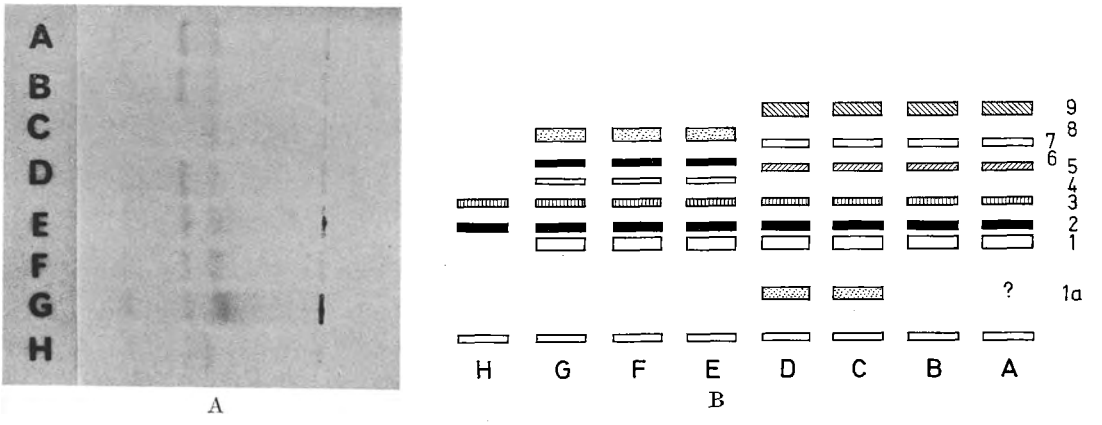


Fig. 4. (A) Electrophorogram of muscle myogen preserved in 2% 2-phenoxyethanol. *Metapenaeus brevicornis*, males *A* and *B*, females *C* and *D*; *Penaeus merguiensis*, males *E*, *F* and *G*; *Metapenaeus mutatus*, male *H*. Species specificity and sexual independence of the electrophoretic patterns are maintained. *M. mutatus*, however, has lost most of its proteins through leakage into the preservative; (B) Diagrammatic representation of patterns in (A). The protein zones are not constructed with reference to *M. mutatus* (which has lost some of its protein zones) as the standard, and therefore do not correspond to those for the other 3 figures

Table 3. *Summary of muscle myogen patterns based on Fig. 3 B. Samples treated with 2% 2-phenoxyethanol are indicated by P; those freshly frozen by F*

Species		Maximum number of muscle protein zones															Number of zones lost between treated and untreated specimens
		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	
Parapeneopsis hardwickii	P	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	5
	F	−	−	−	+	+	+	−	−	+	+	+	−	+	−	−	+	
Penaeus semisulcatus	P	−	−	−	+	+	+	−	−	+	−	+	−	−	−	−	+	2
	F	−	−	−	+	+	+	−	−	+	+	+	−	−	−	−	+	
Parapeneopsis affinis	P	−	−	−	−	+	+	−	−	−	+	+	+	+	−	−	−	3
	F	−	−	−	+	+	+	−	−	+	−	−	−	+	−	−	−	
Metapenaeus mutatus	P	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	4
	F	−	−	−	+	+	+	−	−	+	−	+	−	+	−	−	−[a]	

[a] The P protein zone is normally very faint and was not visible here.

biochemical taxonomists. According to NAKANISHI et al. (1969), tissue samples collected in the field may be stored in 2-phenoxyethanol for later biochemical analysis. They found that the surviving proteins appeared to be identical with those of fresh tissues in physical, catalytic and immunological properties. An attempt was therefore made to investigate the feasibility of this method of preservation when applied to electrophoretic analysis of prawn specimens for phylogenetic relationships.

The fresh muscle myogen of 3 genera (4 species) of penaeid prawns was compared with that of the preserved material (Fig. 3 A and B). Preservation in 2-phenoxyethanol resulted in some loss of protein zones of the muscle myogen patterns in all samples studied. Certain species experienced greater losses of protein zones than others; in the case of preserved *Penaeus semisulcatus*, only 2 protein zone deletions were observed. The deletion of protein zones observed in the electrophorograms is considered to be due to protein leakage from the tissues into the surrounding phenoxyethanol-containing fluid. Slight leakage of a myogen protein, which occurs only in small traces, would hence result in complete absence of that particular protein zone. NAKANISHI et al. (1969) have also reported that proteins are more sensitive to thermal denaturation in the presence of phenoxyethanol and, presumably, this may account for some of the losses observed. (Preserved materials were exposed to about 31 °C on board the trawler prior to deep-freeze storage in the laboratory).

Four specimens of *Metapenaeus brevicornis* (two males and two females), 3 specimens of *Penaeus merguiensis* (all males) and a single specimen of *Metapenaeus mutatus* are compared in Fig. 4 A and B. It appears that, to a large extent, there is constancy of muscle myogen patterns when preserved materials are used. A comparison of the myogen patterns of preserved prawns suggests that there is species specificity, and that preserved specimens may be employed for taxonomic purposes provided that the conditions of preservation are uniform and that sufficient protein zones are preserved. This confirms, in part, the results of NAKANISHI et al. (1969). However, it must be emphasized that further work is required to evaluate the use of such preserved specimens for systematic studies.

It is premature to discuss these findings in great detail, since we hope to return to them shortly in a wider context. Some features, nevertheless, are apparent. The results presented suggest that the cellulose acetate electrophoretic method is a suitable tool for providing a biochemical basis for identifying prawns, and that an analysis of a single specimen gives reliable results. This method is particularly useful when specimens for identification are presented without certain appendages due to damage or other reasons or when these appendages are not well developed.

Furthermore, the method requires only small samples, and can be applied to frozen shelled prawns when identification by morphometric characteristics becomes impossible, and hence, can be of use in solving some of the problems in food quality control and other aspects of food technology. Further work is required to evaluate whether this technique is applicable to the identification of juvenile prawns, as present morphometric methods are difficult and unreliable.

It must be pointed out that many of the faint zones to which references are made in (B) Figs. 1—4 and Tables 1—3 were frequently unavoidably lost upon photographic reproduction. However, all the zones considered were visible in the electrophorograms when viewed by transmitted light. Other means of presentation of the pattern are currently being considered and tried.

Summary

1. The muscle myogens of 11 species of penaeid prawns belonging to 4 genera have been separated by cellulose acetate electrophoresis.

2. Sexual independence of the myogen patterns is shown by the uniformity of the protein zones of the male and female specimens studied.

3. The myogen patterns of each species examined exhibits a high degree of constancy.

4. These results suggest that muscle myogens could be used to complement morphometric characters in taxonomic studies of penaeid prawns.

5. Studies on materials preserved in phenoxyethanol show that some protein zones are lost during preservation. This method is, however, applicable in cases where sufficient protein zones are retained.

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