STUDIES ON THE ELECTROPHORETIC PATTERNS OF FISH MUSCLE MYOGENS

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Electrophoretic patterns of the muscle myogens of prawns and some common Indian food fishes of marine and fresh water origin were obtained on polyacrylamide gel. It was observed that the characteristic band pattern which was species specific and was not altered by storage of fish in ice, could be employed as a means of identification of fish species

Introduction

Electrophoretic technique has been applied in recent years as a tool for the identification of fish species. Usually either the water soluble proteins (myogens) or blood proteins of the fish are subjected to electrophoresis and the developed electropherograms used for purposes of comparison. Earlier workers have employed different media like filter paper, starch gel, cellulose acetate and agar gel to obtain the electropherograms. Nikkila and Linko (1955) studied the electrophoretic patterns of proteins from ten species of fish on paper and Connell (1953) compared the electrophoretic patterns of the sarcoplasmic proteins of twenty species of fish using free boundary electrophoresis. Starch gel electrophoresis of the muscle myogens has been employed by Thompson (1960) and Tsuyuki *et al* (1962, 1965a, 1965b) for the identification of species of fish of Atlantic and Pacific waters of Canada and U. S. A. Manwell *et al* (1963) and Tsuyuki and Gadd (1963) used starch gel electrophoresis of haemoglobins for the same purpose. Thompson (1968) attempted electrophoretic separation of esterases of the tissue for identification of species in fish and some warm blooded animals.

Considering the many advantages of polyacrylamide gel, this medium seems to be well recognised for studying the electrophoretic patterns of muscle proteins. Disc electrophoresis in polyacrylamide gel (Ornstein and Davies 1964) has since been developed and used very widely by Payne (1963), Mancusso (1964), Thompson (1967) and Mackie (1969). Cowie (1968) on the other hand employed the slab polyacrylamide gel electrophoresis technique of Akroyd (1967) for separation

of muscle myogens in relation to fish taxonomy. The versatility of the method has been illustrated by Mackie (1968) who applied the technique to cooked fish also by a modified extraction procedure. The present paper reports observations made on the disc electrophoretic patterns of muscle myogens of certain Indian fishes and shell fishes and their usefulness in species identification The application of the technique as extended to ice stored fish has also been discussed.

Experimental

Fish species used for the study

Prawns (*Metapenaeus dobsoni*), oil sardine (*Sardinella longiceps*), mackerel (*Rastrelliger kanagurta*), lactarius (*Lactarius lactarius*) and mullet (*Mugil dussumieri*) were obtained in the fresh condition from the local market and the fresh water fishes, barbus (*Barbus carnaticus*) and tilapia (*Tilapia mosambica*), procured from the fresh water reservoir at Malampuzha.

Prawns (*M. dobsoni*) were stored in crushed ice and the sampling for the muscle extract carried out at intervals of five days.

Preparation of muscle extracts:

Fish muscle was extracted with chilled glass distilled water (1:1 W/V) in a waring blendor for two minutes. The homogenate was centrifuged at 5000 rpm for 30 minutes at O°C and the supernatant used for electrophoresis.

Electrophoresis of the extract was carried out in 7.5% gel according to the procedure of Ornstein and Davies (1964). Bromophenol blue was used as the marker dye. The buffer employed in the electrode compartments was Tris-glycine buffer (28 8 g glycine and 6.0 g of Tris (hydroxy methyl) aminomethane in 1 litre water, pH adjusted to 8.6). Acrylamide, Methylene Bis Acrylamide, Tetramethyl ethylene diamine (TEMED) and other chemicals used were of analar quality supplied by BDH (England).

Electrophoresis was completed in 40 minutes with 6.6 mA current per gel tube at 150-200 V. After the run gels were removed, carefully derimmed and taken out. They were then stained in 0.1% solution of Amido black IOB in 7% acetic acid for 30 minutes. Excess dye was removed by repeated washing with 7% acetic acid. The developed gels were kept in 7% acetic acid and photographs taken.

Results and Discussion:

Fig 1 shows the electrophoretic patterns given by muscle myogens of four species of marine fish (oil sardine, mackerel, lactarius and mullet), two species of fresh water fish (barbus and tilapia) and shell fish viz; prawn (*M. dobsoni*). The electrophoretic patterns obtained with muscle myogens of prawns, held in ice storage for 0, 5, 10 and 15 days are shown in Fig 2. It has been shown by Robertson *et al* (1967) that identification of species by electrophoresis of muscle proteins is unlikely to be invalidated by the nutritional status of the fish. Although blood proteins might undergo marked change on transformation of the animal from juvenile to adult form, the muscle myogens do not undergo any change during transformation (Uthe and Tsuyuki 1967). Slight variations in the intensities of some bands of the electropherograms were noted to occur in fish of same species when held in ice, as has been illustrated by samples of *M. dobsoni* kept in ice (Fig 2). The basic pattern, however, remained unchanged even after prolonged storage of the prawns in ice. One of the bands had become too feeble to be easily located in the band pattern in the case of sample held in ice for 15 days. The decrease in

Fig 1. Electrophoretic patterns of muscle myogens of different species of fish and shell fish.
A — Tilapia
B — Barbus
C — Mullet
D — Lactarius
E — Mackerel
F — Oil sardine
G — Prawns *(M. dobsoni)*

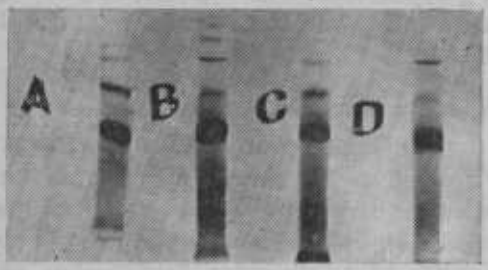

Fig 2. Changes in electrophoretic patterns of muscle myogens of prawns *(M. dobsoni)* on storage in ice.
A — 0 day in ice
B — 5 days in ice
C — 10 days in ice
D — 15 days in ice

the intensity of the electrophoretic bands is attributable to decrease in amounts of sarcoplasmic proteins during prolonged ice storage. Maruyama and Suzuki (1968) working with horse mackerel observed that the sarcoplasmic proteins extractable with 0.005 M KCl gradually decreased during ice storage.

The fact that electrophoretic band pattern is characteristic of the species and is almost remaining unaltered as a result of ice storage shows that the technique could be employed reliably in the field of fish taxonomy.

Further work on the effect of processing variables and storage conditions on the band patterns of myogens of fish and shell fish is in progress and will be reported in a subsequent communication.

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