UNIVERSITI PUTRA MALAYSIA

# PROTEIN PROFILING OF SEVERAL MALAYSIAN FRESHWATER FISH BY TWO-DIMENSIONAL ELECTROPHORESIS

NOOR AZLINA BINTI MASDOR.



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By

NOOR AZLINA BINTI MASDOR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

DECEMBER 2004

"Dedicated to my family, my dear husband who puts up with me and my pursuit of this project, and also to our daughter"

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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**Chairman : Ismail Bin Omar**

**Faculty : Science**

A proteomics approach using 2D-PAGE was initiated to investigate protein expression in fish organs especially hepatopancreas, with the aim of providing fish protein expression maps that are seriously lacking in the literature. Two dimensional electrophoresis maps of hepatopancreas from Jelawat (*Leptobarbus hoevenii*), Catfish (*Clarias batrachus*), Red tilapia (*Oreochromis mossambicus*), Patin (*Pangasius pangasius*) and Lampam Jawa (*Puntius gonionotus*); all Malaysian freshwater fish species were constructed. The soluble protein sample was analyzed by two dimensional electrophoresis, using immobilized pH gradient strip (pH 3-10) and 15% gel acrylamide. 200 protein spots from Red Tilapia and 44 spots from Catfish were observed. Compari sons of similarities in terms of protein spots in the fish species were made using ruler measurements and superimposing the electropherogram of one fish species with the other to confirm spots coordinate similarities. The maximum matching found in terms of pI and molecular weight coordinate is limited to a maximum of three fish species. Patin-Lampam Jawa-Jelawat and Lampam Jawa-Red tilapia-Jelawat

share the most number of spots at 8 spots. At the two-fish comparison level, the highest number of same spots was found in Red tilapia-Jelawat (33 spots) followed by Lampam Jawa-Jelawat (25 spots), Lampam Jawa-Red tilapia (20 spots), Patin-Lampam Jawa (17 spots), Patin-Red tilapia, Patin-Jelawat and Lampam Jawa-Catfish; all with 12 same spots.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**EKSPRESI PROTEIN IKAN AIR TAWAR DI MALAYSIA MENGGUNAKAN ELEKTROFORESIS DUA DIMENSI (2D-PAGE)**

Oleh

**NOOR AZLINA BINTI MASDOR**

**DISEMBER 2004**

**Pengerusi : Ismail Omar**

**Fakulti : Sains**

Satu pendekatan proteomik menggunakan teknik 2D-PAGE telah dijalankan untuk mengkaji ekspresi protein di dalam organ ikan terutamanya hepatopankreas dengan tujuan untuk membina peta ekspresi protein ikan. Peta ekspresi protein ikan air tawar Malaysia yang berjaya dibuat melibatkan lima spesis iaitu; Jelawat (*Leptobarbus hoevenii*), Keli (*Clarias batrachus*), Tilapia merah (Oreochromis *mossambicus*), Patin (*Pangasius pangasius*) dan Lampam Jawa (*Puntius gonionotus*). Sampel protein terlarut telah di analisa secara elektroforesis dua dimensi menggunakan lembaran kecerunan pH tersekat gerak (pH 3-10) dan gel akrilamida 15%. Sebanyak 200 bintik protein telah didapati dari ikan Tilapia merah dan bilangan yang paling sedikit adalah pada ikan Keli dengan jumlah bintik sebanyak 44. Perbandingan persamaan bintik protein di antara spesis ikan yang berbeza telah dijalankan dengan menggunakan pembaris dan juga menggunakan teknik pertindihan elektroferogram satu spesis ikan dengan spesis yang lain. Jumlah persamaan yang paling banyak terhad pada tiga spesis

ikan. Spesis-spesis ikan Patin-Lampam dan Jawa-Jelawat mempunyai persamaan yang paling banyak dengan 8 bintik protein yang sama. Pada tahap persamaan dua spesis ikan, persamaan yang paling banyak adalah pada Tilapia Merah-Jelawat dengan 33 bintik protein yang sama diikuti dengan Lampam Jawa-Jelawat (25 bintik protein), Lampam Jawa-Tilapia Merah (20 bintik protein), Patin-Lampam Jawa (17 bi ntik protein), Patin-Tilapia Merah, Patin-Jelawat dan Lampam Jawa-Keli; ketiga–tiganya dengan 12 bintik protein yang sama.

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I certify that an Examination Committee met on 23rd December 2004 to conduct the final examination of Noor Azlina binti Masdor on her Master of Science thesis entitled "Protein Profiling of Several Malaysian Freshwater Fish by Two-Dimensional Gel Electrophoresis" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Johari Ramli, PhD**
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

**Mohd. Noor Abd. Wahab, PhD**
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Mohd. Puad Abdullah, PhD**
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Musalmah Mazlan, PhD**
Associate Professor
Faculty of Medicine
Universiti Kebangsaan Malaysia
(External Examiner)

**GULAM RUSUL RAHMAT ALI, PhD**
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

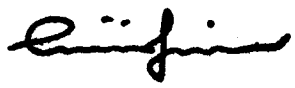
Date: 22 APR 2005

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee are as follows:

**ISMAIL OMAR**
Faculty of Science
Universiti Putra Malaysia
(Chairman)

**NOR ARIPIN SHAMAAN, Phd**
Associate Professor
Faculty of Science and Environmental Studies,
Universiti Putra Malaysia
(Member)

**MOHD. ARIF SYED, Phd**
Associate Professor
Faculty of Science and Environmental Studies,
Universiti Putra Malaysia
(Member)



**AINI IDERIS, Phd**
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date : 12 MAY 2005

## DECLARATION

I hereby declared that the thesis is based on my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

**NOOR AZLINA BINTI MASDOR**

Date: 14 June 2004

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| | |
|---|---|
| APS | Ammonium persulfate |
| ASB14 | Aminosulfobetaine-14 |
| BSA | Bovine serum albumin |
| Cs A | Cyclosporine A |
| $^{o}C$ | Degree Centigrade |
| CBB | Coomassie brilliant blue |
| CHD | Coronary heart disease |
| CHAPS | 3-[(3 Cholamidopropyl) dimethylamino]-1-propanesulfonate |
| cm | Centimeters |
| Da | Dalton |
| DHA | Docosahexanoic acid |
| DNA | Deoxyribonucleic Acid |
| DTE | Dithioerythritol |
| DTT | Dithiothreitol |
| EFA | Essential fatty acids |
| EPA | Eicosapentaenoic acids |
| g | Gram |
| HCl | Hydrochloric acid |
| IPG | Immobilized pH Gradient |

| | |
|---|---|
| IEF | Isoelectric Focusing |
| IU | International Unit |
| kDa | [illegible] |
| kg | Kilogram |
| L | Litres |
| LAB | Lactic acid bacteria |
| µg | Microgrammes |
| µl | Microlitres |
| mA | Miliamperes |
| MALDI-TOF | Matrix-Assisted-Laser Desorption-Time-of-Flight |
| mg | Miligrammes |
| ml | Mililitres |
| mm | Milimetres |
| mM | Milimolar |
| mRNA | messenger RNA |
| Mwt | Molecular Weight |
| min | Minutes |
| M | Molar |
| ng | Nanogramme |
| nm | Nanometer |
| NEPHGE | Nonequilibrium pH gradient electrophoresis |

| | |
|---|---|
| NP-40 | Nonidet P-40 |
| ω-3 | Omega-3 |
| ω-6 | Omega-6 |
| PAP | Prostatic acid phosphatase |
| % | Percentage |
| pI | Isoelectric potential |
| pH | Potential hydrogen |
| PMSF | Phenylmethylsulphonylfluoride |
| RNA | Ribonucleic acid |
| SDS-PAGE | Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis |
| SB 3-19 | Sulfobetaines with Mono Propane Sulfonate |
| SCC | Squamous Cell Carcinoma |
| SDS | Sodium Dodecyl Sulfate |
| Triton-X | Octyl Phenoxypolyethoxyethanol |
| TBP | Tributylphosphines |
| TCA | Trichloro acetic acid |
| tRNA | Transfer RNA |
| USDA | United State Department of Agriculture |
| V | Voltage |
| w/v | Weight/volume |

| | |
|---|---|
| v/v | Volume/volume |
| 1D-PAGE | One-dimensional Polyacrylamide Gel Electrophoresis |
| 2D-PAGE | Two-dimensional Polyacrylamide Gel Electrophoresis |

# CHAPTER 1

## PROTEIN EXPRESSION IN SEVERAL FRESHWATER FISH LIVER BY TWO-DIMENSIONAL ELECTROPHORESIS

## INTRODUCTION

Fish and aquaculture products supply an important amount of animal proteins as well as valuable nutrition to the diets human diet. In addition, fishing is a source of income for millions of people worldwide (Jayashree and Arunachalam, 2000).

Currently, research to improve the yield of fish has turned into looking at protein expression profiles or maps using the technique of 2D-PAGE. A significant and growing application of 2D-PAGE is "proteome analysis." Proteome analysis is "the analysis of the PROTEin complement expressed by a genOME" (Wilkins *et al.*, 1995). Proteomic studies of fish organs especially the hepatopancreas can help to identify proteins and enzymes that are responsible for increasing meat yield, the commercially important fish vitamins and unsaturated fatty acids, as well as, treatment of fish diseases.

To date there is lack of data on protein expression profiles of fish hepatopancreas and other organs. The major theme of this thesis is to provide protein expression profiles or maps of tropical fish

hepatopancreas. These maps will be very useful for the studies of protein maps under different diet and environmental conditions in order improve all aspects of the fish industry as well as generating new knowledge.

The objectives of this study are firstly is to perform initial or preliminary experiments on extraction and separation of fish hepatopancreas from five common local fresh water fish. Secondly is to provide fish hepatopancreas protein profile expression map to serve as database for a reference source in future research on fish protein.

# CHAPTER 2

# LITERATURE REVIEW

## Importance of fish

Fish is a food source with excellent nutritional value, providing high quality protein and a wide variety of vitamins and minerals, including vitamins A and D, phosphorus, magnesium, selenium and iodine. Even in small quantities, fish can have significant positive impact in improving the quality of dietary protein by complementing the essential amino acids that are often present in low quantities in vegetable based diets. Table 1 shows the nutrition information for catfish (*Clarias batrachus*). However, these values usually vary from one species to another (Gebhardt and Thomas, 2002)

Fish is one of the few good dietary sources of essential fatty acids (EFA). EFAs are polyunsaturated `good' fats. They are essential because the body does not synthesize them naturally. Some freshwater fish are good source of fatty acids in the form of docosahexanoic (DHA) and eicosapentaenoic acids (EPA) as well as ω-3 (omega-3) and ω-6 (omega-6) fatty acids (Loorgeril *et al.*, 1999).

**Table 1**: Nutrition information on catfish (*Clarias batrachus*) source: Gebhardt and Thomas (2002).

| Nutrient | Units | 1 fillet/143g |
|---|---|---|
| Energy | Kcal | 217.360 |
| Protein | g | 26.770 |
| Total lipid | g | 11.469 |
| Calcium, Ca | mg | 12.870 |
| Magnesium, Mg | mg | 37.180 |
| Phosphorus, P | mg | 350.350 |
| Potassium, K | mg | 459.030 |
| Sodium, Na | mg | 114.400 |
| Niacin | mg | 3.594 |
| Folate, total | µg | 10.010 |
| Vitamin A, IU | IU | 71.500 |
| Retinol | µg | 21.450 |
| Fatty acids, total saturated | g | 2.558 |
| Fatty acids, total monounsaturated | g | 5.942 |
| Fatty acids, total polyunsaturated | g | 1.991 |

During the past decade, researchers have sought clues to explain the benefits of fish oil. Fish oils in fatty fish are the richest source of a type of fat that is vital to normal brain development in unborn babies and infants. Besides this, fish oil can also reduce incidences or severity of asthma, psoriasis, gastrointestinal diseases and coronary heart disease (CHD). The notion that eating fish may reduce the risk of CHD apparently originated from reports on the small population of native Eskimos in artic Greenland with the lowest incidence of CHD (Loorgeril *et al.*, 1999).

**Freshwater fish industry in Malaysia**

Aquaculture is not a method to solve the problems faced by the fishing industry. It acts as a supplementary aid. The definition of aquaculture is the rearing of fresh, salty and brackish fish or aquatic organisms under controlled conditions. In addition, aquaculture encompasses all aspects of fishing activity such as loading, processing and marketing of fish. Its main aims are;

1. to increase the quality and quantity of aquatic organisms
2. to utilize maximally available lands and resources for aquaculture

Aquaculture in Malaysia began and quickly developed in the late 50s. These days, freshwater fish are reared mostly in Perak, Terengganu