UNIVERSITI PUTRA MALAYSIA

APPLICATIONS OF DNA MICROSATELITE MARKERS INTILAPIA CULTURE

SUBHA BHASSU

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APPLICATIONS OF DNA MICROSATELITE MARKERS IN TILAPIA CULTURE

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of Requirement for the Degree of Doctor of Philosophy

September 2002

To my parents (Amma and Papa).....

Who believed that the most priceless gift one can give to their children is a good education.

To my dearest Rajeev....

Who believed I could achieve my goals.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

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September 2002

Chairman: Profesor Datin Dr. Khatijah Yusoff

Faculty : Science and Environmental Studies

In Malaysia, the role of aquaculture in fish production is anticipated to increase as marine fish catches have already exceeded the maximum sustainable yield. In order to address this issue, aquaculture is being developed on a commercial scale. Fish farming in Malaysia is focused on providing the fish grower with the best fingerlings, with uniformity and fast growth rates. There is great necessity for genetic evaluation, monitoring of stocks and application of appropriate breeding approaches if tilapia production is to continue to meet market demands. With the aid of microsatellite markers, stock integrity and genetic variability can be examined.

Three major experiments were carried out; a population genetic study on eight populations of tilapia, quantitative genetics study on two populations of *O. niloticus*, Taiwan A and B across two generations and heritability study on the Taiwan A population.

The primary focus of the population genetic study was to determine the relationships among eight populations which was used for breeding and to determine the viability of these populations. Microsatellite markers were used to determine the relationships among tilapia populations. The technique was optimized by varying parameters including the amount of template DNA, different thermal cyclers and others. The results showed that microsatellite markers are good markers for tilapia genetic studies.

For the population genetic study, the mean allele number and the mean heterozygosity level for the 40 loci were 43 and 0.5420 respectively. The F_{ST} value of 0.2401 among the populations suggested a moderate amount of genetic differentiation among the tilapia populations studied. Low heterozygosity levels suggest low effective population sizes, which may result in high levels of inbreeding. Most loci showed a deficiency in heterozygosity, which may be a sign of inbreeding. Most sampled populations showed significant deviations from Hardy-Weinberg equilibrium, which could result from mutation, migration or selection. This outcome could also be due to the small sample sizes examined and the high number of alleles present at individual loci.

Based on 11 loci, different genetic distance measures were applied to test the difference in the topology of the five populations examined. The topology and correlation values varied using two models, IAM and SMM. The genetic distances used in this study was selected to resolve relationships among the sampled populations as an

aid for breeding and not to look in detail at the underlying causes of differentiation, either due to genetic drift or mutation or both. The genetic distance values (Nei, 1978) among the populations ranged from 1.7 to 3.5. The phenogram showed that the Taiwan A and B populations grouped together with *O. mossambicus* as an isolated clade. *UNH 112* can be used to differentiate between *O. niloticus* and *O. mossambicus* populations as alleles for this locus can only be detected in the *O. niloticus* stocks.

In the quantitative genetic study, Taiwan A displayed the highest mean weight in both generations. Both populations in generation one showed significant correlations ($P<0.05$) between growth and multi locus heterozygosity. The correlation value is 0.582 for Taiwan A and 0.415 for Taiwan B. Significant positive correlations were only detected in generation 1; could be due to the higher number of individuals sampled and the high levels of variation detected in generation 1 compared to generation 2.. The final study was focused on obtaining a heritability estimate for growth. However, the value obtained was negative, probably due to the low number of individuals and families used in this study.

The three experiments conducted on the Malaysian tilapia stocks will give breeders better insights and abilities to manage their tilapia breeding programs. Specifically, they can now see the value of maintaining stocks with high genetic variabilities under proper environmental conditions and the applications of good hatchery practices in the breeding systems that they use.

Abstrak tesis yang dikemukakan kepada Senate Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

APLIKASI TEKINK MIKROSATELLITE DALAM KULTUR TILAPIA.

Oleh

SUBHA BHASSU

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Pengerusi: Profesor Datin Dr. Khatijah Yusoff

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Di Malaysia, peranan akuakultur dalam pengeluaran ikan dijangka akan meningkat memandangkan penangkapan ikan laut telab melebihi tahap optimum. Justeru, akuakultur diperkembangkan ketahap komersial. Perternakan ikan di Malaysia bertumpu kepada penghasilan anak ikan yang baik, keseragaman dan kadar pertumbuhan yang cepat. Oleh itu, penilaian genetik, pengawasan stok dan aplikasi pendekatan pembiakan yang sesuai diperlukan jika penghasilan ikan tilapia diperlukan untuk menampung permintaan pasaran. Dengan kehadiran penanda mikrosatelit, integriti stok dan kepelbagaian genetik boleh ditentukan.

Tiga eksperimen utama telah dijalankan iaitu; kajian populasi ke atas lapan populasi ikan tilapia, kajian genetik kualitatif yang melibatkan dua populasi ikan tilapia, Taiwan A dan Taiwan B (2 generasi) dan kajian heritabiliti terhadap populasi Taiwan A.

Fokus utama dalam kajian genetik populasi adalah untuk menentukan hubungan antara lapan populasi yang digunakan untuk pembiakan dan menentukan keviabelan populasi

ini. Penanda mikrosatelit digunakan dalam kajian ini untuk menentukan hubungan antara populasi tilapia. Teknik ini dioptimumkan dengan merubah parameter seperti jumlah templat DNA, kitaran haba yang berbeza dan sebagainya. Keputusan menunjukkan bahawa penanda mikrosatelit adalah penanda yang baik untuk kajian genetik tilapia.

Dalam kajian genetik populasi, nombor min alel, dan nombor min heterozigositi untuk 40 lokus adalah 43 dan 0.5420 masing-masing. Nilai Fst antara populasi adalah 0.2401. Ini menunjukkan perbezaan genetik yang sederhana dalam ikan tilapia yang dikaji. Tahap heteozigositi yang rendah pula menujukkan keberkesanan saiz populasi yang rendah, ini yang mengakibatkan tahap pembiakan sebaka yang tinggi. Kebanyakkan lokus menunjukkan heterozigositi yang rendah, ini wujudnya pembiakan sebaka. Kebanyakkan populasi menunjukkan penyimpangan yang jelas daripada keseimbangan Hardy-Weinberg. Ini mungkin disebabkan oleh mutasi, migrasi atau pemilihan dan juga disebabkan oleh saiz sampel kajian yang rendah dan bilangan alel yang tinggi dalam lokus individu.

Berdasarkan 11 lokus, jarak genetik yang berlainan digunakan untuk mengiji perbezaan topologi antara lima populasi yang dikaji. Nilai topologi dan korelasi berbeza mengikut dua model, IAM dan SMM. Jarak genetik yang digunakan dalam kajian telah dipilih untuk mengatasi masalah hubungan antara populasi yang disampel sebagai rujukan pembiakan dan bukan untuk mengkaji secara terperinci kesan pembezaan, yang diakibatkan sama ada daripada hanyutan genetik atau mutasi atau kedua-duanya.

Nilai jarak genetik (Nei, 1978) antara populasi menjulat daripada 1.7 ke 3.5. Fenogram menunjukkan bahawa populasi Taiwan A dan Taiwan B dikumpul bersama dengan O. mossambicus sebagai populasi terasing. UNH 112 boleh digunakan untuk membezakan antara populasi O. niloticus dan O. Mossambicus kerana alel untuklokus ini hanya dapat dikesan dalam stok O.niloticus.

Dalm kajian genetik kualitatif, Taiwan A telah mempamerkan min berat yang paling tinggi dalam kedua-dua generasi. Kedua-dua populasi dalam generasi 1 telah menunjukkan korelasi yang signifikan ($P< 0.05$) antara pertumbuhan dan heterozigositi lokus majmuk. Nilai korelasi adalab 0.582 untuk Taiwan A dan 0.415 untuk Taiwan B. Korelasi positif yang signifikan hanya dapat dikesan dalam generasi 1. Ini mungkin disebabkan oleh bilangan individu yang disampel dan variasi yang tinggi dalm generasi 1 berbanding generasi 2. Kajian terakhir adalah untuk memperoleh anggaran heritabiliti bagi pertumbuhan. Walau bagaimanapun nilai yang diperolehi adalah negatif, ini mungkin disebabkan oleh bilangan individu dan famili yang digunakan adalah rendah dalam kajian ini.

Ketiga-tiga eksperimen yang dijalankan terhadap stok ikan tilapia Malaysia akan membantu perternak mengurus program pembiakan dengan lebih baik. Khususnya, mereka dapat melihat kepentingan nilai dalam mengekalkan sto yang mempunyai kepelbagaian genetik yang tinggi di bawah keadaan persekitaran serta penggunaan amalan penetasan yang baik bagi sistem pembiakan yang digunakan.

I certify that an Examination Committee met on 12th September 2002 to conduct the final examination of Subha Bhassu on her Doctor of Philosophy thesis entitled "Applications of DNA Microsatellite Markers in Tilapia Culture" in accordance with University 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:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosopy. The member of the Supervisory Committee are as follows:

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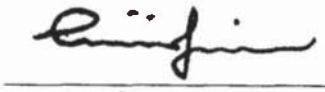

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DECLARATION

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

SUBHA BHASSU

Date:

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During the course of my project, I thank God for showering me with kindness, affection, love, patience and perseverance.

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Chemicals

EDTA	Ethylenediamine tetraacetate
KCL	Potassium chloride
$MgCl_2$	Magnesium chloride
NACl	Sodium chloride
SDS	Sodium dodecyl sulphate
TBE	Tris-borate-EDTA buffer
Tris	Trisma base
TA	Taiwan A
TB	Taiwan B

Units

Bp	base pair
° C	Degree of celcius
h	hour
kb	kilobase
min	minute
mM	millimolar
ng	nanogram
nmole	nanomole
OD	optical density
pmole	picomole
s	second

CHAPTER 1

INTRODUCTION

Aquaculture is currently being viewed as a source of relief for human population that face protein shortage in their daily lives. This situation has arisen because many countries have limited resources of marine fishes due to excessive fishing. It is clear that the demand for food resources have grown over the years and, therefore, a balance must be struck to ensure that mankind can produce the required amount of protein to an optimal level while preserving environmental quality and integrity. Asia accounts for 80% of the world's aquaculture production (FAO, 1997), giving an impression that fish farming is only of real benefit to developing countries. This could be due to the lack of understanding of the potential benefits provided by aquaculture (Pillay, 1994).

Over the last few decades, aquaculture has made remarkable growth in the economic sector. For example, the most recent estimate of the global value of tilapia is US $3 billion, a figure which has almost trippled since 1984. According to FAO data, the annual world production of tilapia for 1989 was 366,000 tonnes and this had increased to 473, 477 tonnes in 1992 and 675,000 tonnes in 2000 (FAO, 2000). Demand for tilapia in the US is expected to increase to 400,000 tonnes with an estimated value of US $2 billion by the year of 2005. The most important species of tilapia in terms of

production by weight is *Oreochromis niloticus* (64%), followed by *O. mossambicus* (10%) and *O. aureus* (3.6%). The primary objective for aquaculture in any developing country is to provide an alternative source of protein in the diet of an increasing population (Pullin, 1993). This is different to the objectives in developed countries where the focus has been on output of high quality food products that meet certain dietary requirements (Barnabe, 1994).

In Malaysia, the role of aquaculture in fish production is anticipated to increase significantly as marine fish catches have already exceeded their maximum sustainable yield. Aquaculture is being developed on a commercial scale as a sustainable industry under the aquaculture action development plan by the Department of Fisheries which aims to meet the requirements of dietary protein by the year 2010 (NAP, 1992). Fish farming in Malaysia has focused on providing tilapia growers with the best fingerlings, of uniform quality and showing fast growth rates. This would enable farmers to market all the fish over a short period of time and then restock the ponds or tanks to grow the next batch of fish, thus maximizing the total weight of tilapia that can be grown per year with the limited space available. This requires low levels of management time, handling and pond construction cost. Faster growth rates will shorten the time between the farmer's investment and the time when the stocks begin to return income from the marketable fish. Good feed conversion of available feed resources is another major goal because feed is generally the highest single cost in culture.

Fish farming in Malaysia has focused on high selection intensities in order to provide the farmers with economic gains. In order to achieve this goals, breeding programs should aim to produce high survival of fries to fingerling and to market size, lower breeding cost, improved feed conversion ratio and predictability of growth for feed management and marketing. The fish also should have an appearance and presentation suitable for the intended market; that includes good color and shape, which in some markets make the difference between high or low price. They should have good tolerance to a range of water conditions, allowing the farmer to use the available water sources without spending extra cost on water treatment, and ease of breeding to ensure a reliable supply of fingerlings.

Recently, however, the tilapia industry in Malaysia has seen a reduction in growth rates, a familiar scenario to that is seen in the tilapia industry worldwide. Cultured stocks also suffer from widespread poor and variable fish performance and in some cases due to the presence of gene introgression from poor performing stocks (Pante and Macaranas, 1985). The relatively high fecundity of tilapia allows breeders to apply high selection intensity and to derive their grow-out stock from a small number of parents. This means a small number of individuals contribute to the genetic make-up of the subsequent generation. If done continuously, this may eventually lead to fixation of alleles, which could express advantageous traits, but also unfavourable traits causing inbreeding depression. Loss of genetic variability will also be evident (Silliman, 1975, Harvey, 1986,). High reproduction, on the other hand, leads to overcrowding resulting in large quantities of small sized fish (Herpher and Pruginin, 1982). In addition,