Population Structure and Triploidy in Baung M*ystus nemurus* (C&V)*

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Introduction

Mystus nemurus is rapidly becoming one of the most popular and important fresh water food fish species for aquaculture in the country. The wild and hatchery samples of M*ystus nemurus* (C&V), from seven populations in Malaysia namely: Pulau Pinang, Kelantan, Selangor, Terengganu, Melaka, and Johor in the north, northeast, central and south of West Malaysia and from Sarawak in East Malaysia. The samples were analysed using starch gel electrophoresis and RAPD-PCR fingerprinting technique. Sample populations of *M. nemurus* from north, northeast, central and south Thailand were also analysed for the purpose of comparison of variation. Genetic improvement in cultured fish using chromosome manipulation such as gynogenesis, androgenesis and triploid have been developed for the last decade. Triploidization is a genetic tool in avoiding deleterious influences of sexual maturation on growth, meat quality and disease resistance. Diseases in aquaculture are mainly a result of combined interacting stress factors associated with environmental changes and poor management practices. These two main stress factors could be predispose conditions highly conducive for both existing and opportunistic pathogens to infect and slowly killing the fish. One of the most common bacterial pathogen associated with freshwater aquaculture is by *Aeromonas hydrophila.* Fish infected by *A. hydrophila* normally develops fin rot, ulcer, skin lesion and dropsy. Susceptibility of diploid and triploid *M. nemurus* to challenge by *Aeromonas hydrophila* was performed.

Materials and Methods

Tissue samples from muscles, liver, heart and kidney were used to examine genetic variation from thirteen enzyme systems and a sarcoplasmic protein, whilst whole blood and muscle tissue were used for RAPD-PCR fingerprinting technique. Two min post-fertilisation, the eggs of *M. nemurus* were subjected to cold (0°C, 2°C, 5°C and 7°C) and warm (35°C, 38°C, 40oC and 42°C) water temperature shocks for duration of 2, 5, 7 and 10 min for cold and 0.5, 1.0, 1.5 and 2.0 min for warm shock. Erythrocyte nuclei size measurement, chromosome count, and gonad development and growth performance verified triploid. *Aeromonas hydrophila* was allowed to infect three-month-old triploid and diploid *M. nemurus*. The colony concentrations of *A. hydrophila* used in this study ranged from 7.6 x 10^1 CFU/ml to7.6 x 10^{10} CFU/ml. The fish were injected intramuscularly with 0.2ml/fish dilution of *A. hydrophila* concentrations. Observations were made at 24hrs interval for one-week duration.

Results and Discussion

Out of 25 loci examined 16 (64%) were found to be polymorphic (at P= 0.95) (Siraj *et al.*, 1998). Gene frequencies of this *M. nemurus* populations were largely in Hardy-Wienberg equilibrium with high genetic variability and observed heterozygosities ranged from 0.004 to 0.104. Whilst, for population from Thailand out of the 23 loci examined (65.22%) fifteen were polymorphic at the 0.95 levels (Leesanga *et al.*, 2000). Observed heterozygosities ranged from 0.041 to 0.111, with an average of 0.068±0.028. Genetic distances ranged from 0.005 to 0.164. Five arbitrary primers chosen to amplify products, which showed 28 polymorphic loci in Thai populations and 142 polymorphic markers, were detected in the Malaysian populations. The results of RAPD analysis provide similar conclusions as far as the population clustering is concerned to the isozyme analysis. Triploid appeared in fertilised eggs subjected to cold shock (0°C, 2°C and 7°C at 5, 7 and 10 min duration and 5°C at all duration tested) and only at 38 °C at 2-min duration for warm shock. Cold shock at 10 min duration produced 100% triploid whilst warm shock at 38°C produced 59% triploid at 2 min duration exposure. Triploid was verified by erythrocyte nucleus size measurement, which was found to be significantly ($P<0.05$) larger than the diploid. The chromosome number of normal diploid *M. nemurus* analysed was found to be 54 and 81 for the triploid. Mean total length from week 1-7 did not show any significant difference ($P>0.05$) between diploid and triploid *M. nemurus* and so was at 6 -month of growth. Histological analysis of the gonads showed that there was a marked disruption in gonad development with undeveloped testes and ovary in the triploid, which lead to its sterility. This has been revealed by earlier findings in *Clarias batrachus* (Siraj *et al.*, 1992; 1993). Infection at 7.6 x 10^{10} CFU/ml concentration caused 100% mortality to both the triploid and diploid individuals. No mortality was observed in both the diploid and triploid at 7.6 x 10^7 CFU/ml concentration; however, fin rot was noted on the dorsal and caudal region.

Conclusions

Dendrogram derived from the genetic distances based on the allele frequencies showed genetic similarity among populations of *M. nemurus* except for Terengganu population, indicating a substantial level of differentiation from the rest of the seven populations. The greatest genetic distance was found in the Chainat and Suratthani populations (0.164), a level indicative of subspecific differentiation in *M. nemurus* from Thailand. Successful results on the induction of triploids were established using cold and heat temperature shock treatments on fertilised eggs M. nemurus. The growth performance and disease resistance of the normal diploid was comparable to the triploid.

Benefits from the study

The information on genetics diversity study carried out on this species is important in determining the success of its breeding program. Successful results on the induction of triploid were established using cold and hot temperature shock treatments on fertilised eggs of *M. nemurus*. Triploidy leads to sterility, which is benificial to the growth of *M. nemurus*.

Literature cited in the text

Leesanga, S., Siraj, S.S., Daud, S.K., Sodsuk, P.K., Tan, S.G. and Sodsuk, S. 2000. Biochemical polymorphism in yellow catfish, *Mystus nemurus* (C&C), from Thailand. *Biochemical Genetics.* 38 (5/6): 1-9.

Siraj, S.S, Law, Y.K., Cheah, S.H. and Ang, K.J. 1992. Induced triploidy in walking catfish, *Clarias batrachus* by water temperature shock. *Malays. Appl. Biol.* 21(1) : 65-69

Siraj, S.S., Daud, S.K. and Ang, K.J. 1993. Growth performance and gonad development in triploid *Clarias batrachus* (Linnaeus). *Pertanika J. Trop. Agric. Sci.* 16(3): 167-171.

Siraj, S.S., Daud, S.K., Aruah, O. and Tan, S.G. 1998. Population genetic structure of baung, *Mystus nemurus* (C&V), in Malaysia. *Malys. Appl. Biol.* 27 (1&2): 77-82.

Project Publications in Refereed Journals

Christianus, A, Siraj, S.S., Harmin, S.A. and Nordin, W.M.W. 1998. Morphology and histology of the gonad of matured male river catfish, *Mystus nemurus* (C&V). *Malays. Appl. Biol.* Vol. 27.

Leesanga, S., Siraj, S.S., Daud, S.K., Sodsuk, P.K., Tan, S.G. and Sodsuk, S. 2000. Biochemical polymorphism in yellow catfish, *Mystus nemurus* (C&C), from Thailand. *Biochemical Genetics.* 38 (5/6): 1-9.

Siraj, S.S, Daud, S.K., Aruah, O. and Tan, S.G. 1998. Population genetic structure of baung, *Mystus nemurus* (C&V), in Malaysia. *Malys. Appl. Biol.* 27 (1&2): 77-82.

Siraj, S.S., Seki, S. and Taniguchi, N. 1998. DNA fingerprinting in a Malaysian strain of Javanese carp, *Puntius gonionotus* (Bleeker), detected by YNZ DNA probe. *Aquaculture Research.* 453-455.

Graduate Research

Abdul Rahman Ismail. 1998. Fish Genetics [M.Sc.]. Universiti Putra Malaysia.

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