

MOLECULAR CHARACTERIZATION OF
Citrobacter freundii, *Edwardsiella tarda* AND
Aeromonas hydrophila ISOLATED FROM RED
TILAPIA (*Oreochromis* sp.) FINGERLINGS
CULTURED AT TERENGGANU
AND SELANGOR

WENDY WEE

MASTER OF SCIENCE
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A special dedication to my
beloved parents and respected
supervisor & co-supervisor

WENDY WEE

**Thesis Submitted in Fulfillment of the Requirement
for the Degree of Master of Science
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Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirements for the degree of Master of Science

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WENDY WEE

June 2008

Chairperson : Associate Professor Hajjek Musa, Ph.D.

Member : Nur Azma Artifa

Faculty : Agrotechnology and Food Science

Red tilapia has been an important food fish that ranked on top of all freshwater aquaculture production in Malaysia. The prevalence of the pathogenicity of the *Chromobacterium freudenreichii*, *Edwardsiella ictaluri* and *Aeromonas hydrophila* was investigated. A total number of 45 isolates were obtained from red tilapia fingerlings farmed at Terengganu and Selangor. Presumptive isolation of *C. freudenreichii*, *E. ictaluri* and *A. hydrophila* has been made from moderate dark pink colony on Cetuxedim-trypan-Neovibrio (CIN) agar, dry clear transparent colony with black dot on Xyloac-Lysine-Dimethylolam (XLD) agar and moderate yellow colony on Gistman's Starch Phenol red (GSP) agar, respectively. Meanwhile, bacterial identification was accomplished by 52 biochemical and physiological tests by conventional method and BBL Crystal™ Enteric Non fermenter Identification Systems (ENF ID). The production of hemolysins which lysed on blood agar has been regarded as strong evidence of pathogenic potential in *A. hydrophila* isolated from Terengganu farm despite the majority of the isolates were lack of hemolytic activity. Molecular characterization of

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June 2008

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Red tilapia has been an important food fish that ranked on top of all freshwater aquaculture species in Malaysia. Nevertheless, productivity and sustainability of the red tilapia can be affected once diseases struck. In present study, phenotypic and genotypic characterization of fish disease causative agents has been focus on *Citrobacter freundii*, *Edwardsiella tarda* and *Aeromonas hydrophila*. A total number of 45 isolates were obtained from red tilapia fingerlings farmed at Terengganu and Selangor. Presumptive isolation of *C. freundii*, *E. tarda* and *A. hydrophila* has been made from moderate dark pink colony on Cefsulodin-Irgasan-Novobiocin (CIN) agar, tiny clear transparent colony with black dot on Xylose-Lysine-Deoxycholate (XLD) agar, and moderate yellow colony on Glutamate Starch Phenol red (GSP) agar, respectively. Meanwhile, bacterial identification was accomplished by 52 biochemical and physiological tests by conventional method and BBL Crystal™ Enteric/ Nonfermenter Identification Systems (E/NF ID). The production of hemolysins which lysed on blood agar has been regarded as strong evidence of pathogenic potential in *A. hydrophila* isolated from Terengganu farm despite that majority of the isolates were lack of hemolytic activity. Molecular characterization of

C. freundii, *E. tarda* and *A. hydrophila* isolates by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) with combination of universal M13, wild-type phage M13, and T3-T7 primers yielded polymorphic bands which ranged from 150 to 2700 bp, 200 to 3200 bp, and 220 to 4200 bp for respective species. Group separation was achieved at 0.32 Dice similarity coefficient while strains were distinguished at 0.94 Dice similarity coefficient. Genetic relatedness among species from same location has been high while genetic distance was great among species from Terengganu and Selangor. Such investigation on genetic relatedness of different geographic populations of a species could be an important tool in the study of disease vector movement. On the other hand, antibiotic susceptibility testing by disc diffusion method to seven broad spectrum antibiotics of ampicillin, furazolidone, kanamycin, nalidixic acid, sulphamethoxazole, chloramphenicol, and tetracycline exhibited 13 different antibiograms. All isolates were multi-resistant to more than two antibiotics and particularly isolates from Terengganu were resistant to furazolidone and sulphamethoxazole while isolates from Selangor were resistant to tetracycline. In addition, plasmid profiling by horizontal agarose gel electrophoresis detected only eight isolates that harboring low molecular weight plasmids with estimated size ranging from less than 2.7 to 16.6 kb. However, no association between the detection of plasmid and antibiotic resistance was established.