

**A STUDY ON THE PATHOGENICITY OF
Elizabethkingia meningoseptica IN TREATED
AFRICAN CATFISH WITH *Excoecaria agallocha*
VIA METABOLOMICS PROFILING**

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DEDICATION

This thesis is dedicated to:

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A STUDY ON THE PATHOGENICITY OF *Elizabethkingia meningoseptica* IN TREATED AFRICAN CATFISH WITH *Excoecaria agallocha* VIA METABOLOMICS PROFILING

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June 2013

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Elizabethkingia meningoseptica is one of the most significant bacterial species for human infections. At present, no data is available on *E. meningoseptica* infection in diseased freshwater fish. This study was carried out to investigate *E. meningoseptica* infection in freshwater cultured African catfish. The bacterium was identified based on its morphological, biochemical, physiological properties and commercial kits such as BBL Crystal™ Enteric Non Fermenter kit, API 20E and API ZYM System. Following that, the selected bacteria (K1 and S1) were further confirmed based on their 16S rRNA gene sequences. Experimental infection of *E.meningoseptica* on juvenile catfish was carried out *via* intraperitoneal (i.p.) route and immersion. The Lethal Dose (LD₅₀) was 4x10⁵ CFUmL⁻¹. Nowadays, the occurrence of antibiotic resistance towards bacterial pathogens in aquaculture has been reported worldwide. This study is geared to seek new sources of natural antimicrobial agent from mangrove plants. Milky mangrove, *Excoecaria agallocha* methanolic leaf extracts

was chosen in the study. The minimum inhibitory concentration (MIC) of *E. agallocha* was 3.12 mg/ml, and the minimum bactericidal concentration (MBC) was 6.25 mg/ml. The effect of *E. agallocha* on blood parameters and tissues of infected African catfish (*Clarias gariepinus*) with *E. meningoseptica* was also conducted. The experimental fish were fed with crude extract-treated feed at 10, 20, 30, 40 and 50 mg kg⁻¹ body weight, respectively and one group was given flumequine at 25 mg kg⁻¹. These groups were injected with *E. meningoseptica* at dose levels of 4 x10⁵ CFU mL⁻¹. The best effective concentration of *E. agallocha* was 50 mg kg⁻¹; whereby the percentage of albumin, globulin, total serum protein, total leukocyte count and monocytes were significantly increased. On the other hand, the highest lysozyme activity was recorded in group fed with 50 mg kg⁻¹ in weeks 2, 3 and 4 with values of 1114.3 units/ml, 1114 units/ml and 1103.7 units/ml respectively, in contrast to the control and other treated group. Survival percentage was significantly higher ($P < 0.05$) in fish fed with 50 mg kg⁻¹ of *E. agallocha* methanolic extracts. The group fed with 50 mg kg⁻¹ showed slight changes in the kidney such as irregular cellular structures, cell shape and glomerular congestion while the liver showed marginal channel dilation and slight degeneration of epithelial cells in contrast with the antibiotic group. The group fed with 10 mg kg⁻¹ illustrated the most severe cellular morphological changes in contrast to other groups. They exhibited severe cellular and cytoplasmic vacuolation and degeneration, central vein destruction, and hepatocyte necrosis. Metabolic response of African catfish to two *E. meningoseptica* strains (K1, β -haemolytic) and (S1, γ -haemolytic) was investigated *via* LC/MS-QTOF. Metabolic profilings were done on the serum, kidney and skin of non infected and infected fish to determine the elevated levels of the metabolites. Bacterial

metabolic profiling was compared between β -haemolytic and γ -haemolytic bacteria. Analyses of individual metabolite shows significant difference in the level of α -amyrin in serum of catfish infected by *E.meningoseptica* (isolate S1). 11-Dehydrocorticosterone was up-regulated in the skin group compared to the kidney group. Corticosteroids were up-regulated in both kidney samples and skin samples due to stress induced and inflammation of infection compared to the non-infected control. Homosalate metabolite was down regulated in the skin group as a result of skin damage by bacteria infection compared to the kidney group. Higher level of tacrine was observed in the bacteria isolated from kidney. Analyses of individual metabolite shows significant difference in the level of α -amyrin in the serum of catfish infected by *E.meningoseptica* isolated from skin.

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KAJIAN PATOGENISITI *Elizabeth meningoseptica* TERHADAP IKAN KELI AFRIKA YANG DIRAWAT DENGAN *Excoecaria agallocha* MELALUI PROFIL METABOLOMIK

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Elizabethkingia meningoseptica adalah antara spesis bakteria yang paling penting kepada jangkitan manusia. Setakat ini, tiada data menunjukkan jangkitan *E. meningoseptica* kepada ikan air tawar yang berpenyakit. Kajian ini dijalankan untuk menyiasat jangkitan *E. meningoseptica* terhadap ikan keli Afrika air tawar. Bacteria ini dikenalpasti berdasarkan sifat-sifat morfologi, biokimia, fisiologi dan penggunaan kit komersial iaitu BBL Crystal™ Enteric/Non Fermenter kit, API 20E dan API ZYM System. Berdasarkan pengenalpastian awal, bakteria terpilih (K1 dan S1) disahkan melalui turutan gen 16S rRNA. Kajian jangkitan *E. meningoseptica* terhadap keli juvenil dijalankan melalui suntikan intraperitoneum dan rendaman. Dos kematian (LD₅₀) adalah 4×10^5 CFU/mL⁻¹. Kini, berlakunya rintangan antibiotik terhadap patogen bakteria dalam akuakultur telah dilaporkan di seluruh dunia, dan kajian ini bertumpu untuk mendapatkan sumber baru bagi ejen antimikrob semulajadi daripada tumbuh-tumbuhan bakau. Ekstrak daun dari pokok Buta Buta,

Excoecaria agallocha menggunakan metanol dipilih dalam kajian ini. Nilai kepekatan perencat minimum (MIC) *E. agallocha* adalah 3.12 mg/ml, dan nilai kepekatan bakterisid minimum (MBC) adalah 6.25 mg/ml. Kesan – kesan *E. agallocha* pada parameter darah dan tisu ikankeliAfrika (*Clarias gariepinus*) yang dijangkiti dengan *E.meningoseptica* juga dijalankan. Ikan ujikaji diberi makan ekstrak mentah yang dirawat berkepekatan 10, 20, 30, 40 dan 50mg kg⁻¹ mengikut berat badan dan satu kumpulan lain diberi antibiotik flumequine pada kepekatan 25 mg Kg⁻¹. Ikan di dalam kumpulan ini disuntik dengan *E. meningoseptica* pada 4x10⁵ CFUml⁻¹. Kepekatan yang paling berkesan ditunjukkan oleh kepekatan 50mg kg⁻¹, dimana peratusan albumin, globulin, jumlah serum protein, jumlah bilangan leukosit dan monosit meningkat secara ketara. Sebaliknya, aktiviti lysozym tertinggi telah dicatatkan oleh kumpulan dalam kepekatan 50mg kg⁻¹dalam minggu2, 3 dan 4 dengan nilai masing-masing adalah 1114.3unit/ml, 1114 unit/ml dan 1103.7unit/ml, berbanding dengan kumpulan kawalan dan rawatan. Peratusan ikan hidup adalah lebih tinggi (P<0.05) ditunjukkan oleh ikan yang diberi 50mg kg⁻¹ ekstrak methanolik *E. agallocha* manakala kumpulan ekstrak 10mg kg⁻¹ digambarkan perubahan morfologi selular yang paling teruk di kalangan semua kumpulan. Kumpulan ekstrak 50mg kg⁻¹ menunjukkan sedikit perubahan pada ginjal dimana ia ditunjukkan pada struktur sel, bentuk sel yang tidak sekata dan kesesakan glomerular, sementara hati pula menunjukkan pengembangan saluran marginal dan sedikit kemerosotan pada sel epithelium berbeza dengan kumpulan antibiotik. Mereka menunjukkan pengvakuolan dan kemerosotan sel dan sitoplasma, kerosakan vena sentral, dan nekrosis hepatosit. Reaksi metabolik ikan keli Afrika terhadap dua strain *E. meningoseptica* (K1, β-hemolitik) dan (S1, γ-hemolitik) dikaji dengan

menggunakan LC/MS- QTOF. Profil metabolik dijalankan kepada serum, ginjal dan kulit ikan yang dijangkiti dan tidak dijangkiti untuk melihat peningkatan paras metabolik. Profil metabolik dibandingkan antara bakteric β -hemolitik and γ -hemolitik. Analisa terhadap setiap metabolit menunjukkan perbezaan paras α -amyrin di dalam serum ikan keli yang dijangkiti dengan *E. meningoseptica* (isolat S1). 11-Dehydrocorticosterone adalah terkawal di paras atas dalam kumpulan kulit berbanding ginjal. Corticosteroids terkawal di paras atas pada kedua-dua sampel ginjal dan kulit disebabkan oleh tekanan dan keradangan daripada jangkitan berbanding dengan kumpulan kawalan yang tidak dijangkiti. Metabolit homosalate adalah terkawal pada paras bawah dalam kumpulan kulit sebagai tindak balas daripada kerosakan kulit yang disebabkan oleh jangkitan bakteria berbanding dengan kumpulan ginjal. Paras tertinggi tacrine telah diisolat daripada ginjal. Perbezaan paras α -amyrin pada kulit turut dilihat di dalam serum keli yang dijangkiti dengan *E.meningoseptica*.