CHARACTERIZATION OF *Aeromonas hydrophila* ISOLATED FROM DISEASED FISH AND THE POTENTIAL OF SODIUM ALGINATE AS A CARRIER FOR ORAL VACCINE

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To my parents and family,
Supervisor and all of my friends,
Without whom none of my success would be possible

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Bacterial diseases in aquaculture are considered to be a major cause of fish mortalities. Disease due to *Aeromonas hydrophila* is one of the most common bacterial diseases in aquaculture. This bacterium causes hemorrhagic septicaemia, abdominal dropsy and ulcers. Effective prevention is necessary to overcome the outbreak of bacterial disease. Thus this study was conducted to isolate, identify and characterize *A. hydrophila* from diseased fish. The virulence and pathogenicity of the isolates were evaluated for vaccine candidate selection. The *in vitro* suitability of sodium alginate as coating agent for oral formalin killed *A. hydrophila* vaccine was evaluated. *A. hydrophila* were isolated, identified and characterized using conventional biochemical tests. Two representative isolates were subjected to 16S rRNA gene sequencing and analysed for identity confirmation. Genetic characterization was conducted using RAPD-PCR. Virulence was characterized by hemolysis, proteolysis, lipolysis, biofilm formation, motility, antimicrobial sensitivity and growth characteristics. Pathogenicity test was conducted by injecting tilapia intraperitoneally with bacterial concentrations of 10^3, 10^4, 10^5, 10^6, 10^7 and

10^8 CFU/ml to determine median lethal dose (LD_{50}) and histopathological changes. *In vitro* evaluation was conducted by several tests such as morphology, mean diameter, size distribution, *A. hydrophila* vaccine incorporation efficiency and particle stability in water and different pH. A total of ten strains of *A. hydrophila* were isolated and they are all Gram negative, indole and oxidase positive, and resistant to vibriostatic agent 0/129. Analysis of the 16S rRNA gene sequence was confirmed as *A. hydrophila* using NCBI BLAST analysis and EzTaxon e-server. RAPD-PCR showed genetic coefficient ranging from 0.15 to 1.0. All strains were positive in all virulence tests except for slime production test where only 30% show positive results.

The multiple antibiotic resistances (MAR) index values were between 0.18 and 0.36. The generation time was 1.8 per hour. The median lethal dose was $10^{7.68}LD_{50}$/ml. Sodium alginate beads produced by simultaneous method showed smooth surface, smaller particle size, higher vaccine incorporation efficiency and stability compared to beads produced using sequential method. MC2 bead from simultaneous method was significantly ($p < 0.05$) better in terms of vaccine incorporation efficiency and particle stability. However, further improvement of bead production is necessary to maximize antigen entrapment for better immunological response

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PENCIRIAN *Aeromonas hydrophila* TERPENCIL DARIPADA IKAN BERPENYAKIT DAN POTENSI NATRIUM ALGINAT SEBAGAI PEMBAWA VAKSIN

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Penyakit yang disebabkan oleh bakteria adalah dianggap sebagai punca utama kematian ikan dalam industri akuakultur. Penyakit yang disebabkan oleh bakteria *Aeromonas hydrophila* merupakan salah satu jangkitan bakteria yang biasa dihadapi. Bakteria ini menyebabkan pendarahan kronik, busung perut dan ulser. Langkah pencegahan yang berkesan perlu diambil bagi mengatasi wabak penyakit bakteria ini. Oleh yang demikian, kajian ini telah dijalankan untuk memencil, mengenal pasti dan menciri *A. hydrophila* daripada ikan berpenyakit. Tahap bahaya dan kepatogenan setiap pencilan bakteria dinilai bagi pemilihan dalam penghasilan vaksin. Kajian *in vitro* dilakukan bagi mengetahui dan menilai kesesuaian natrium alginat sebagai agen salutan untuk vaksin *A. hydrophila* yang dimatikan mengunakan formalin. Sebanyak 10 strain *A. hydrophila* telah dipencilkan, dikenalpasti dan dicirikan menggunakan ujian biokimia konvensional. Penjujukan dan analisis gen 16S rRNA telah dilakukan untuk pengenalpastian terhadap 2 strain bakteria yang terpilih. Pencirian genetik telah dijalankan menggunakan kaedah RAPD-PCR. Tahap bahaya bakteria dicirikan melalui jenis hemolisis, proteolisis, lipolisis, pembentukan biofilem, kebolehan bakteria bergerak, kepekaan agen antimikrob dan ciri-ciri

pertumbuhan. Ujian tahap bahaya bakteria telah dijalankan dengan menyuntik ikan tilapia dengan kepekatan bakteria 10^3, 10^4, 10^5, $10^{6,}$ 10^7 dan 10^8 CFU/ml secara peritoneal dalaman bagi menentukan tahap dos median membunuh (LD50) dan perubahan histopatologi. Penilaian *in vitro* telah dijalankan melalui beberapa ujian iaitu ujian morfologi, diameter, taburan saiz, tahap keberkesanan vaksin *A. hydrophila* serta kestabilan zarah di dalam air dan pH berbeza. Kesemua sepuluh pencilan *A. hydrophila* adalah Gram negatif, positif dalam ujian indol dan oksidase, dan tahan kepada agen vibriostatik 0/129. Analisis jujukan gen 16S rRNA telah dikenalpasti sebagai *A. hydrophila* oleh menggunakan analisis NCBI BLAST dan EzTaxon e-server. RAPD-PCR menunjukkan nilai pekali genetik antara 0.15 dan 1.0. Semua pencilan menunjukkan keputusan yang positif dalam semua ujian tahap bahaya kecuali ujian pengeluaran lendir yang mana hanya 30% menunjukkan hasil yang positif.

Nilai indeks bagi kepelbagaian rintangan terhadap antibiotik (MAR) adalah di antara 0.18 dan 0.36. Masa pertumbuhan adalah 1.8 per jam. Dos median kematian ikan ialah $10^{7.68}$ LD50/ml. Manik natrium alginat yang dihasilkan melalui kaedah serentak menunjukkan permukaan yang licin, saiz zarah yang lebih kecil, kecekapan penubuhan vaksin yang lebih tinggi dan lebih stabil berbanding dengan manik yang dihasilkan menggunakan kaedah berjujukan. Manik MC2 dari kaedah serentak adalah ketara ($p < 0.05$) lebih baik dari segi kecekapan pengambilan vaksin dan kestabilan. Walau bagaimanapun, penambahbaikan dalam pengeluaran manik adalah perlu bagi memaksimumkan perangkap antigen untuk tindak balas imunologi yang baik.