ABSTRACT

In shrimp-pond farming, effective water quality management is critical for shrimp growth and survival as well as to reduce the impact of shrimp farming on the environment. Therefore, this study was conducted with the objective of developing indigenous bacterial formulation, which can effectively treat shrimp pond effluents thus improving the water quality in shrimp farming. To ascertain possible bacterial species to be used in the formulation, water samples were collected from two shrimp farms in Muara Tebas and Santubong, Kuching, Sarawak, Malaysia. Eleven water samples were collected from these two shrimp farms. Microorganisms in the water samples with the necessary characteristics such as active proteolytic, cellulolytic and amylolytic were isolated. These eight species of microorganisms were then identified based on biochemical test, API 20NE and API 20E kit, followed by Polymerase Chain Reaction (PCR) technique. The main species that had been identified as potential degraders of pond effluents belonged to the *Bacillus* sp.. From the primary screening on solid media, eight active isolates were chosen based on the size of the diameter of the clear zone surrounding the colonies in the plate-screening medium. A time course study was performed for 72 hours for each of the potential isolates in liquid media to analyze their growth curve and enzymes production with respect to time. These isolates were individually further grown in shaked flask cultures to determine the pattern of enzyme production, protein content and bacterial growth. All of the monocultures (eight isolates) were then combined in minimal media for 24 hours at 37^{o}C to determine the pattern of growth and enzymes production when in combination of 1 : 1, 1 : 2, 2 : 1, 1 : 3, 3 : 1, 3 : 2 and 2 : 3. From the results obtained from biculture combination, the best combination of bacteria was then tested for their ability to grow and respond in a triculture system. 1% of *Bacillus megaterium* (S120): *Bacillus cereus* strain 3 (S65): *Enterococcus casseliflavus* (S11) was selected as the best triculture combination because they produced the highest enzymes of cellulase, amylase and protease. Therefore, the formulation consists of three bacterial species identified as *Bacillus megaterium*, *Bacillus cereus* and *Enterococcus casseliflavus*. The bacterial formulation was then tested on shrimp pond water from two different aquaculture farms and UNIMAS's pond water. Chemical and biological parameters in the water were analyzed for seven days except for UNIMAS pond where the treatment was extended to fourteen days. There were no significant differences ($P > 0.05$) in water quality parameters such as orthophosphate, total phosphorus, bacterial count, temperature and pH for samples from the shrimp pond water. However, ammonia-nitrogen, nitrite, nitrate, turbidity, suspended solid, chemical oxygen demand (COD), sulfide and biochemical oxygen demand (BOD) differed significantly ($P < 0.05$). Meanwhile for water samples from UNIMAS pond, nitrate, bacterial count, temperature and pH showed no significant differences ($P > 0.05$). Other parameters such as ammonia-nitrogen, nitrite, orthophosphate, total phosphorus, turbidity, suspended solid, chemical oxygen demand (COD), sulfide and biochemical oxygen demand (BOD) differed significantly ($P < 0.05$) compared to control experiment. The ability to reduce more than 50% of eight water quality parameters in this study clearly showed that the indigenous bacterial inoculums were a good shrimp pond effluents degrader.

PEMBANGUNAN INOKULUM BAKTERIA UNTUK RAWATAN EFLUEN INDUSTRI PENTERNAKAN UDANG

ABSTRAK

Pengurusan kualiti air yang berkesan adalah amat kritikal bagi ladang penternakan udang yang intensif. Kesan penternakan udang terhadap alam sekitar juga tidak boleh di pandang ringan. Oleh itu, kajian ini dijalankan bagi membangunkan formulasi bakteria natif, yang efektif dalam proses rawatan kumbahan daripada kolam udang. Ini sekaligus akan memperbaiki mutu kualiti air kolam udang terbabit. Spesis-spesis ini telah dipencilkan daripada dua ladang penternakan udang yang terdapat di Muara Tebas dan Santubong, Kuching, Sarawak, Malaysia. Di ladang-ladang tersebut, sebelas sampel air daripada kolam-kolam yang berlainan diperolehi. Mikoorganisma di dalam sampel air yang mempunyai ciri-ciri penting seperti aktif proteolitik, selulolitik dan amilolitik diasingkan. Kesemua lapan spesis bakteria yang berpotensi ini tadi, kemudiannya dikenalpasti berdasarkan penggunaan kit API 20E dan API 20NE, ujian biokimia dan seterusnya menggunakan teknik tindakbalas rantai polimerase. Spesis utama yang berjaya dikenalpasti berpotensi menguraikan bahan kumbahan kolam udang tergolong dalam spesis Bacillus sp.. Melalui ujian awal pada media pepejal, lapan pencilan yang aktif telah dipilih berdasarkan diameter zon lutsinar yang mengelilingi koloni-koloni bakteria di dalam piring medium ujikaji. Satu kajian yang berterusan selama 72 jam telah dijalankan di dalam media cecair bagi setiap pencilan yang berpotensi supaya pola pertumbuhan dan pengeluaran enzim pencilan terbabit dapat dianalisis. Pencilan-pencilan ini tadi kemudiannya dibiakkan secara individu di dalam kelalang kultur yang bergetar bagi menentukan corak pengeluaran enzim, kandungan protein dan pertumbuhan bakteria. Kesemua monokultur (lapan pencilan) ini seterusnya digabungkan di dalam media yang minimum selama 24 jam pada suhu 37^oC bagi *menentukan corak pertumbuhan dan pengeluaran enzim apabila di dalam kombinasi 1 : 1, 1 : 2, 2 : 1, 1 : 3, 3 : 1, 3 : 2 dan 2 : 3. Kombinasi bikultur yang terbaik akan dipilih untuk dibiakkan di dalam sistem trikultur bagi melihat kebolehan mereka bertindakbalas dan membiak. 1% Bacillus megaterium (S120): Bacillus cereus strain 3 (S65) : Enterococcus casseliflavus (S11) telah dipilih sebagai kombinasi trikultur yang terbaik di atas kebolehan mereka menghasilkan enzim selulase, amilase dan protease yang tertinggi. Oleh itu, formulasi ini terdiri daripada tiga spesis bakteria yang dikenalpasti sebagai Bacillus megaterium, Bacillus cereus dan Enterococcus casseliflavus. Formulasi bakteria ini kemudiannya diuji pada air kolam udang daripada dua ladang berbeza dan juga pada air kolam yang terdapat di UNIMAS. Parameter biologi dan kimia di dalam air kolam kemudian dianalisis selama tujuh hari kecuali bagi air kolam di UNIMAS, di mana proses rawatan diteruskan selama empat belas hari. Hasil kajian mendapati, tiada perbezaan yang signifikan* ($P > 0.05$) *bagi parameter kualiti air kolam udang seperti ortofosfat, jumlah fosforus, bilangan bakteria, suhu dan pH. Bagaimanapun, bagi ammonia-nitrogen, nitrit, nitrat, kekeruhan air, pepejal terlarut, keperluan oksigen kimia, sulfida dan keperluan oksigen biokimia menunjukkan perbezaan yang signifikan* ($P < 0.05$)*. Sementara bagi sampel air daripada kolam UNIMAS, nilai nitrat, bilangan bakteria, suhu dan pH tidak menunjukkan perbezaan signifikan* ($P > 0.05$)*. Parameter lain seperti ammonia-nitrogen, nitrit, orthofosfat, jumlah fosforus, kekeruhan air, pepejal terlarut, keperluan oksigen kimia, sulfide dan keperluan oksigen biokimia menunjukkan perbezaan yang signifikan* ($P < 0.05$) *jika dibandingkan dengan eksperimen kawalan. Kebolehan untuk mengurangkan lebih daripada 50% daripada lapan parameter kualiti air di dalam kajian ini, jelas menunjukkan keberkesanan inokulum bakteria natif di dalam proses penguraian bahan kumbahan daripada kolam udang.*