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FACULTY OF AGROTECHNOLOGY AND FOOD SCIENCE
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MOLECULAR CHARACTERIZATION OF *Vibrio cholerae* ISOLATED FROM OYSTER (*Crassostrea iredalei*) CULTURE AT GONG BATU, SETIU, TERENGGANU

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This project report is submitted in partial fulfillment of the requirement of the degree of Bachelor of Science in Agrotechnology (Aquaculture)

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ABSTRACT

Ten isolates of *Vibrio cholerae* were identified from 40 oyster (*Crassostrea iredalei*) samples. The presumptive isolates of *V. cholerae* were recovered in alkaline peptone water (APW), followed by selective plating on thiosulphate citrate bile salt sucrose (TCBS) agar and identification by conventional biochemical tests. DNA fingerprinting were obtained by randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) using $(GTG)_5$ primer. Dendrogram generated by NTSYSpc version 2.1 was useful to establish genetic distance among the *V. cholerae* isolates. The results of RAPD fingerprinting separated the ten *V. cholerae* isolates into two main clusters, whereby the first cluster consisted nine isolates and the other cluster consisted only one isolate. High genetic polymorphisms were observed among these bacteria isolates. Antibiotic susceptibility to ampicillin, furazolidone, kanamycin, nalidixic acid, sulphamethoxazole and tetracycline was performed by Kirby-Bauer disc diffusion method. Four out of ten isolates were resistant to one or two antibiotics tested. *Vibrio cholerae* in present study were highly resistant to kanamycin. On the other hand, patterns of plasmid profile were discovered, where sizes of these plasmid were marked as 3.7 MDa, 4.8 MDa and 3.4-, 4.8- and 35.3 MDa. However, the relatedness of the antibiotics resistance in conjunction with the presence of resistant genes in the plasmids was found to be weak. The above approaches are indeed useful to ensure safe consumption of cultured oyster. Thus, food poisoning due to the consumption of contaminated raw oyster can be avoided.

ABSTRAK

Daripada jumlah empat puluh biji tiram (*Crassostrea iredalei*), sepuluh isolat *Vibrio cholerae* dapat dikesan. Isolat yang disyaki sebagai *V. cholerae* dipulihkan dalam alkaline peptone water (APW), diikuti pemiringan pilihan pada agar thiosulphate citrate bile salt sucrose (TCBS) sebelum dibuat kenalpasti identitinya melalui siri ujian biokimia convensional. Cap jari DNA diperolehi secara amplifikasi rawak DNA polimorfik - tindak balas berantai polymerase (RAPD-PCR) dengan menggunakan primer $(GTG)_5$. Dendrogram yang dihasilkan daripada program NTSYSpc versi 2.1 adalah berguna untuk menghubungkaitkan jarak genetik antara isolat-isolat *V. cholerae*. Sepuluh isolat *V. cholerae* itu terbahagi kepada dua kelompok utama, di mana kelompok pertama mempunyai sembilan isolat, manakala kelompok yang satu lagi hanya mempunyai satu isolat. Isolat-isolat ini menunjukkan sifat polimorfik genetik yang tinggi. Kajian tahap pendedahan kepada antibiotic-antibiotik ampisilin, furazolidon, kanamisin, asid nalidisik, sulfamethoxazol dan tetrasiklin pula dilakukan dengan cara serapan disk Kirby-Bauer. Empat daripada sepuluh isolat itu menunjukkan rintangan terhadap satu atau dua antibiotik. Dalam kajian ini, *V. cholerae* mempamerkan tahap rintangan yang tinggi terhadap kanamisin. Pada masa yang sama, corak profil plasmid yang dikesan menunjukkan bahawa plasmid-plasmid itu bersaiz 3.7 MDa, 4.8 MDa serta 3.4-, 4.8- dan 35.3 MDa. Walau bagaimanapun, tiada hubungan rapat yang membuktikan bahawa rintangan terhadap antibiotik adalah disebabkan oleh kehadiran gen rintangan pada plasmid. Pendekatan seperti yang disebut di atas sememangnya berguna dalam aspek memastikan keselamatan pemakanan tiram ternakan. Dengan demikian, keracunan makanan akibat makan tiram mentah yang terjangkit dapat dielakkan.