

UNIVERSITI MALAYA

ORIGINAL LITERARY WORK DECLARATION

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**EVALUATION OF MICROBIOLOGICAL QUALITY AND SAFETY IN
KUALA SEPETANG ESTUARIES (PERAK, MALAYSIA) USING
CULTIVATION-DEPENDENT METHOD**

Field of Study: Environmental Biotechnology

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ABSTRACT

Estuaries are crucial coastal and marine habitats of various aquaculture organisms. However, uncontrolled development has caused contamination in many coastal areas all over the world that will eventually bring negative effects to aquaculture industry and public health. Bacteriological study based on culturing methods is appropriate not only for routine monitoring in laboratories, but also for making inferences on the physiological and metabolic properties of the organisms. Therefore the applicability of CHROMagar™ Orientation agar was evaluated in microbiological survey of estuarine waters and sediments as well as to detect and isolate human-associated bacterial pathogens in the surface waters and sediments along Kuala Sepetang estuary. Genomic diversity of the isolated *E. coli* strains based on CHROMagar™ Orientation agar was determined from Kuala Sepetang estuary. Water and sediment samples were collected from Kuala Sepetang estuary. The total culturable bacteria (TCB) was determined by direct-plating on both nutrient agar and CHROMagar™ Orientation. Nutrient agar, in all cases, yielded a higher TCB count than CHROMagar™ Orientation. The cultivable bacteria on CHROMagar™ Orientation could be characterized into four major color morphotypes: purple, green, blue and colorless. The green morphotype was found to be more dominance in the sediment of upper estuary while the colorless morphotype dominated the bottom sediment of lower estuary. Identification of representative isolates of green morphotype (n=107) using Biolog gen III and biochemical assays showed that green morphotype comprised of *Enterococcus* sp., *Exiguobacterium* sp., *Paenibacillus* sp., *Stenotrophomonas* sp. and *Staphylococcus scirius*. The colorless morphotype comprised of *Pseudomonas* sp., and *Acinetobacter* sp. Direct-plating on CHROMagar™ Orientation yielded low recovery of *Enterobacteriaceae*, specifically *E. coli*, *Klebsiella* and *Enterobacter*, which could be due to cell stress and injury while these enteric

bacteria were exposed to the unfavourable environment. Detection and isolation of these bacteria were achieved by using direct plating method or by incorporating pre-enrichment before plating onto CHROMagar™ Orientation. A wide range of could identify, specifically disease-associated bacteria, such as *E. coli*, *Pseudomonas* sp., *Serratia* sp., *Enterococcus* sp., *Staphylococcus* sp., *Klebsiella* sp. and *Enterobacter* sp. REP-PCR genotyping of *E. coli* isolates (n=53) revealed high genetic diversity along the estuaries. The REP-PCR profiles were grouped into clusters: I, II, III, IV, V, VI, VII, VIII and IX. Cluster III was major cluster consisting of 41% (n=22/53) of total isolates. Generally, since water is streaming from upstream to downstream of estuary and due to geographical location of the water which is in interconnected in Kuala Sepetang estuary most of the isolates clustered together, even from different Rivers (Kuala Selinsing and Kuala Sangga Besar River). In conclusion, CHROMagar™ Orientation was found to be a useful medium in the estuary study as it allows simultaneous isolation and identification of waterborne bacterial pathogens within two to three days. Kuala Sepetang estuary, as important area for one of the major estuary supporting numerous aquacultural activities, was found to be heavily contaminated with various types of waterborne bacterial pathogens.

ABSTRAK

Muara adalah habitat pantai dan laut yang penting kepada pelbagai organisma akuakultur. Tetapi, pembangunan yang tidak terkawal telah menyebabkan pencemaran di kawasan pantai di seluruh dunia, yang akan mengakibatkan kesan-kesan negatif kepada industri akuakultur dan kesihatan awam. Kajian bakteria berpandukan kepada kaedah kultur adalah sesuai bukan sahaja dalam pemantauan rutin dalam makmal, tetapi juga dalam membuat pentafsiran ke atas sifat-sifat fisiologi dan metabolik organisma. Tujuan utama kajian ini adalah, untuk menilai kesesuaian agar CHROMagar™ Orientation dalam kajian mikrobiologi air dan sedimen muara; untuk mengesan dan memencil patogen bakteria manusia dalam air permukaan dan sedimen muara di sepanjang Kuala Sepetang dan, untuk menentukan diversiti genom *E. coli* yang dipencil dari muara Kuala Sepetang. Sampel air dan sedimen telah diambil dari stesen yang berlokasi muara Kuala Sepetang. “Total culturable bacteria” (TCB) telah ditentukan dengan inokulasi-terus pada kedua-dua agar nutrien dan CHROMagar™ Orientation dalam semua kes, menghasilkan kiraan TCB yang lebih tinggi daripada CHROMagar™ Orientation. Bakteria yang boleh dikultur pada CHROMagar™ Orientation boleh digolongkan kepada empat morfotaip warna: ungu, hijau, biru dan tiada warna. Morfotaip hijau telah didapati lebih dominan dalam sedimen dari hulu muara manakala morfotaip tiada warna mendominasi sedimen di hilir muara. Identifikasi wakil-wakil pencilan dari morfotaip hijau (n=107) dengan Biolog gen III dan ujian biokimia menunjukkan bahawa morfotaip hijau terdiri daripada *Enterococcus* sp., *Exiguobacterium* sp., *Paenibacillus* sp., *Stenotrophomonas* sp. and *Staphylococcus scirius*. Morfotaip tiada warna terdiri daripada *Pseudomonas* sp., dan *Acinetobacter* sp. Inokulasi-terus pada CHROMagar™ Orientation telah menghasilkan pemencilan Enterobacteriaceae, terutamanya *E. coli*, *Klebsiella* dan *Enterobacter* yang rendah, yang mungkin disebabkan oleh tekanan dan pencederaan sel semasa bakteria-bakteria enterik

ini terdedah kepada persekitaran yang tidak sesuai. Pengesanan dan pemencilan bakteria-bakteria ini telah dicapai dengan menggunakan kaedah “direct plating” atau dengan menggabungkan suatu pre-pengayaan sebelum inokulasi pada CHROMagar™ Orientation. Pelbagai bakteria dapat dikenalpasti, terutamanya bakteria yang berkait dengan penyakit, contohnya *E. coli*, *Pseudomonas* sp., *Serratia* sp., *Enterococcus* sp., *Staphylococcus* sp., *Klebsiella* sp. dan *Enterobacter* sp.. Genotaip REP-PCR bagi pencilan-pencilan *E. coli* (n=53) menunjukkan bahawa diversiti genetik sepanjang muara adalah tinggi. Profil REP-PCR telah digolongkan kepada 9 kluster: I, II, III, IV, V, VI, VII, VIII dan IX. Kluster III adalah kelompok utama yang terdiri daripada 41% (n = 22/53) diasingkan keseluruhan. Secara umumnya, sejak air streaming dari hulu ke hilir muara dan disebabkan lokasi geografi air yang berada dalam saling di muara Kuala Sepetang kebanyakan pencilan dikumpulkan bersama, acara dari Sungai berbeza (Kuala Selinsing dan Kuala Sangga Besar Sungai). Sebagai kesimpulan, CHROMagar™ Orientation merupakan suatu medium yang berguna dalam kajian muara kerana ia membolehkan pemencilan dan identifikasi serentak patogen bawaan air dalam masa dua ke tiga hari. Muara Kuala Sepetang sebagai bidang penting untuk aktiviti akuakultur, telah didapati dicemari dengan pelbagai jenis patogen bakteria bawaan air.

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LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|--------------------|--------------------------------------|
| % | percentage |
| et al | Et alia |
| °C | Degree Celsius |
| Sp. | Species |
| & | And |
| LB | Luria-Bertani |
| SIM | Sulfur reduction-Indol-Motility test |
| MR | Methyl Red |
| VP | Voges-Proskaur |
| UV | Ultra Violet |
| DNA | Deoxyribonucleic Acid |
| PCR | Polymerase Chain Reaction |
| REP | Repetitive Extragenic Palindromic |
| dNTP | Deoxynucleotide triphosphate |
| ddH ₂ O | Double-distilled water |
| MgCl ₂ | Magnesium Chloride |
| NaCl | Sodium Chloride |
| TBE | Tris-borate EDTA |

| | |
|------------|---|
| <i>Taq</i> | <i>Thermus aquaticus</i> |
| Bp | Base pair |
| μl | microliter |
| ml | milliliter |
| μM | micromolar |
| mM | millimolar |
| pH | measurement of H ⁺ ions |
| mg | milligram |
| g | gram |
| h | hour/hours |
| min | minute/minutes |
| s | seconds |
| CRO | CROMagar Orientation |
| EMB | Eosin methylene blue |
| BPS | Phosphate buffered saline |
| PPT | Parts Per Thousand |
| FIB | Faecal Indicator Bacteria |
| DGGE | Denaturing Gradient Gel Electrophoresis |
| FISH | Fluorescence in Situ Hybridization |

| | |
|-------------------------------|--|
| MUG | 4-methylumbelliferone β -D-galactopyranoside |
| ONPG | o-Nitrophenyl- β -D-galactopyranosid |
| BST | Bacteria source tracking |
| BPW | Buffered Peptone Water |
| KOH | Potassium hydroxide |
| H ₂ O ₂ | Hydrogen peroxide |
| RPM | Revolutions per minute |
| X | time |
| U | unite |
| NA | nutrient agar |
| CFU | Colony-forming unit |
| Log | Logarithm |
| ORSA | oxacillin resistant <i>staphylococcus aureus</i> |
| MRSA | Methicillin-resistant <i>Staphylococcus aureus</i> |
| GPS | Global positioning system |
| TE | Tris-EDTA |

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