

**IDENTIFICATION AND ASSESSMENT OF PIGMENTED
BACTERIAL ISOLATES FROM SEA CUCUMBERS FOR
ANTIBACTERIAL ACTIVITY**

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UNIVERSITI SAINS ISLAM MALAYSIA

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ANTIBACTERIAL ACTIVITY**

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Thesis submitted in fulfilment for the degree of
MASTER OF SCIENCE

UNIVERSITI SAINS ISLAM MALAYSIA

February 2022

AUTHOR DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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ACKNOWLEDGEMENTS

Alhamdulillah, all praises and gratitude always uttered to Allah SWT for His blessing in completing this thesis. Firstly, I would like to express my appreciation to my supervisor, Dr Maryam Binti Mohamed Rehan for bringing me onto this research project and her constant advice and guidance throughout this project. Thank you for always putting up with me. Without her support, this thesis would not have been possible.

I owe much to my colleagues Dr. Kamarul Rahim Bin Kamarudin, Mr. Mohd Zulhafiz Bin Che Zahri, Ms Nabilah Binti Ahmad Alhadi and Ms Noor Shahida Binti Ishak for their time, support, recommendations and patience throughout my research. Thank you for being great lab partners and friends. I must also acknowledge laboratory technicians in the Department of Food Technology, Ms. Sarina Irma Binti Saidon, Mr. Muhammad Izzat B. Redzuan, and Mrs. Siti Nabilah Bt Mohd Rusly for their assistance.

A very special thank you goes to my beloved parents, Muhammad Lutfi Bin Ahmad and Junaidah Baharuddin for continuous support and belief in me. Last, but most certainly not least, I thank my husband Muhammad Zaki Bin Mohd. Zainuldin and our daughter, Husna Humaira' Binti Muhammad Zaki, for their endless love.

ABSTRACT

Sea cucumber is one of the most commercialized seafood products in Asia, including Malaysia, and its consumption has recently increased with recognition of its health beneficial values. This marine invertebrate is surrounded by microbial communities that may provide defence against pathogens and, in turn, the symbiotic relationship could be a source of bioactive compounds. Many research on marine invertebrate-associated bacteria have been carried out, yet limited studies have documented on bacteria from various body parts of sea cucumbers in Malaysia seawaters, specifically on pigmented bacteria. In this study, eight sea cucumbers were collected from Pangkor Island, Perak, and Manukan Island, Sabah, and the specimens were identified as two *H. leucospilota*, three *H. atra*, and one each of *H. edulis*, *H. hilla* and *B. vitiensis*. A total of 115 bacterial samples were isolated from various body parts of the sea cucumbers, surrounding sediments and seawaters, and 58 bacterial strains were successfully identified using 16S rRNA gene amplification, DNA sequencing and phylogenetic analysis. Bacteria from the genera *Vibrio* and *Bacillus* are the most affiliated with sea cucumber from Pangkor Island and Manukan Island, respectively. Other bacterial strains belong to the genera *Acinetobacteria*, *Staphylococcus*, *Pseudomonas*, *Paracoccus*, *Microbacterium*, *Brevundinomas*, *Exiguobacterium* and *Stenotrophomonas*. Phylogenetic analysis revealed that isolates were assigned to three primary cluster consisting of *Proteobacteria*, *Firmicutes* and *Actinobacteria* phylum. Bacterial isolates that showed pigmentation were selected for UV-Vis spectrophotometry analysis and antibacterial screening using disk diffusion method. The absorbance spectra of crude methanolic extract of *Pseudomonas* sp. PMCC1 suggested potential phenazine-based pigments, while those from *Staphylococcus* sp. PMDP, *Microbacterium* sp. PMCT1 and *Acinetobacteria* sp. PMDS1 resembling carotenoid pigments. However, more studies are needed to confirm these pigments. Four pigmented bacterial strains exhibited antibacterial activity against at least one pathogenic bacterium, with *Pseudomonas* sp. PMCC1 showed inhibition to all five tested bacteria, ranging from 13 to 22 mm. This study suggests that the bacteria isolated from sea cucumbers may have great potential in the discovery of useful bioactive compounds. Therefore, further studies are needed to explore this vast pool of bacterial isolates, especially the pigment-producing bacteria and to determine their biotechnological potentiality.

Keywords: Sea cucumber, Bacteria, Pigment, 16S rRNA gene, Antibacterial activity

ABSTRAK

Timun laut adalah salah satu produk makanan laut yang banyak dikomersialkan di Asia, termasuk Malaysia, dan baru-baru ini penggunaannya meningkat kerana mengandungi nilai kesihatan yang telah diakui. Invertebrat laut ini dikelilingi oleh komuniti mikrob yang boleh memberi pertahanan daripada patogen dan sebagai pulangnya, hubungan simbiotik ini berpotensi menjadi sumber sebatian bioaktif. Banyak kajian mengenai bakteria yang berkaitan dengan invertebrate laut telah dijalankan, namun terdapat kajian yang terhad tentang bakteria yang berkait dengan pelbagai bahagian badan timun laut di perairan Malaysia, terutamanya bakteria berpigmen. Dalam kajian ini, lapan timun laut telah dikumpul dari Pulau Pangkor, Perak dan Pulau Manukan, Sabah, dan spesimen tersebut telah dikenal pasti sebagai dua *H. leucospilota*, tiga *H. atra*, dan setiap satu *H. edulis*, *H. hilla* and *B. vitiensis*. Sejumlah 115 sampel bakteria telah diasingkan dari pelbagai bahagian badan timun laut, persekitaran enapan dan air laut, dan 58 strain bakteria telah dikenal pasti menggunakan urutan gen 16S ribosom RNA (rRNA) dan analisis filogenetik. Bakteria dari genera *Vibrio* dan *Bacillus* adalah yang paling banyak berkait dengan timun laut dari Pulau Pangkor dan Pulau Manukan, masing-masing. Strain bakteria yang lain terdiri daripada genera *Acinetobacteria*, *Staphylococcus*, *Pseudomonas*, *Paracoccus*, *Microbacterium*, *Brevundinomas*, *Exiguobacterium* dan *Stenotrophomonas*. Analisis filogenetik mendedahkan isolat terbahagi kepada tiga kumpulan utama yang terdiri dari filum *Proteobacteria*, *Firmicutes* dan *Actinobacteria*. Bakteria yang diasingkan dan menunjukkan pigmentasi telah dipilih bagi menjalani analisis spektrofotometri UV-Vis dan antibakteria menggunakan kaedah penyebaran cakera. Spektra penyerapan untuk ekstrak mentah metanol bagi *Pseudomonas* sp. PMCC1 mencadangkan potensi pigmen adalah berdasarkan phenazin, manakala dari *Staphylococcus* sp. PMDP, *Microbacterium* sp. PMCT1 and *Acinetobacteria* sp. PMDS1 menyerupai pigmen karotenoid. Tetapi kajian yang lebih diperlukan untuk mengesahkan pigmen tersebut. Empat strain bakteria berpigmen mempamerkan aktiviti antibakteria terhadap sekurang-kurangnya satu bakteria patogen, dengan *Pseudomonas* sp. PMCC1 menunjukkan perencatan terhadap semua lima bakteria patogen, diameter antara 13 hingga 22 mm. Kajian ini mencadangkan bahawa bakteria berkait dengan timun laut ini berkemungkinan mempunyai potensi besar dalam penemuan sebatian bioaktif yang berguna. Oleh itu, kajian lanjut diperlukan bagi mendalami sumber bakteria, terutamanya yang menghasilkan pigmen, serta untuk memastikan potensi bioteknologi yang ada.

Kata kunci: Timun laut, Bakteria, Gen 16S rRNA, Pigmen, Aktiviti antibakteria

خلاصة

خيار البحر هو احدى من المنتجات التجارية بطلب كبير في منطقة آسيا بما فيها ماليزيا، وقد ارتفع الطلب لخيار البحر مؤخراً بفضل الاعتراف على المنافع الصحية باستهلاك خيار البحر. هذه الالفقارية البحرية محاطة بمجتمعات ميكروبية التي توفر دفاع ضد مسببات المرض، وفي المقابل، يمكن هذه علاقات تكافلية أن تكون مصدر المركبات الحيوية النشطة. وقد انجز العديد من الدراسات حول بكتيريا في سطح المحيطات على سبيل المثال الإسفنج، وقناذ البحر، وأصداف البحر، ورقبات، والأعشاب البحرية ولأسف لم يتم توثيق سوى القليل جداً عن البكتيريا متعلقة بخيار البحر في مياه البحر بماليزيا. في هذه الدراسة، تجمع عينات المياه، والرواسب، وثمانية خيار البحر من جزيرة بنكور بفريق، وجزيرة منوكن بصباح ووصف مجتمعات البكتيريا بتركيز إلى البكتيريا المنتجة بالصبغة. وقد حددت مجتمعات البكتيريا باستخدام قاعدة تسلسل المكتبة الجينات رنا الريبوسومي "16 إس"، وتحليل التطوري. اختيرت السلالات البكتيرية التي تظهر الصبغة من أوسط البكتيريا المعزول لتجربة المضادة للبكتيريا باستعمال طريقة الانتشار القرسي. وتم تعيين ما مجموعة 58 سلالات بكتيرية ووجدت أن ضمة (*Vibrio*) وعصية (*Bacillus*) تكون نوع أكثر منتسبة بخيار البحر في جزيرة بنكور وجزيرة منوكن على التوالي. بقية السلالات البكتيرية تتكون من ثمانية أجناس: *Acinetobacter*، *Staphylococcus*، *Pseudomonas*، *Paracoccus*، *Microbacterium*، *Brevundinomas*، *Exiguobacterium*، *Stenotrophomonas*. عين التحليل التطوري أن جاءت العزلات من ثلاث مجموعات الرئيسية وهي المتقلبات (*Proteobacteria*) ومتينات الجدار والأكتينوبكتيريا (*Actinobacteria*) شعبة. وتشير هذه النتائج إلى أن الاختلافات في أنواع بكتيريا بين مواعين لأخذ العينات ترجع إلى الاختلافات البيئية والموئل ثم، اختيارات سبع مستعمر المختلفة من البكتيريا لتجربة مضادة للبكتيريا بالنظر إلى استطاعة في إنتاج صبغات الملونة. وواحد فقط من بين هذه البكتيريا المصبوغة

(PMCC1) تظهر نشاط مضادات البكتيريا ضد كل خمسة البكتيريا الممرضة تتراوح بين 13، 22 ملليمتر. ويتم تحديد السلالة البكتيرية على أنها الزاءفة الزنجارية *Pseudomonas aeruginosa* نظرا لوجود العديد من العزلات تظهر النشاط ألثبيطي، فهذا يوحي بأن قد تكون لهذه البكتيريا من خيار البحر إمكانيات كبيرة في كشف المركبات الحيوية النشطة المفيدة. لذلك، هناك حاجة الى مزيد من الدراسات لاكتشاف مخزون هائل من البكتيريا المنتجة بالصبغة وإن تحدد الامكانيات في التكنولوجيا الحيوية.

الكلمات الرئيسية : خيار البحر، بكتيريا، رنا الريبوسومي 16 'إس'، النشطة المضادة للبكتيريا

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LIST OF ABBREVIATIONS

blastn	Basic Local Alignment Search Tool program for nucleotide
bp	base pair
COI	cytochrome c oxidase I
DNA	deoxyribonucleic acid
kb	kilobase
LB	Luria-Bertani
mtDNA	mitochondrial DNA
NA	nutrient agar
NaOH	sodium hydroxide
nm	nanometer
PCR	polymerase chain reaction
rpm	revolutions per minute
rRNA	ribosomal ribonucleic acid
v/v	volume per volume
w/v	weight by volume