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**ISOLATION AND PURIFICATION OF POLYSACCHARIDE
FROM GRAM-NEGATIVE BACTERIA ASSOCIATED WITH
MARINE SPONGES, *Aaptos* sp.**

By

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JABATAN SAINS SAMUDERA
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PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:

The Isolation and Purification of Polysaccharide from Gram-Negative Bacteria associated With Marine Spnges *Aptos* sp. Oleh Nafifah Triana Bt Jamaluddin No. Matrik UK 9177 telah diperiksa dan semua pembedaan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Samudera sehagai memenuhi sebahagian daripada keperluan memperoleh Ijazah Sarjana Muda Sains (Biologi Marin) Fakulti Sains Dan Teknologi Malaysia

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ABBREVIATIONS

SSW	Sucrose sea water
HCL	Hydrogen Chloride
TFA	Trifluorocloric acid
NACL	Natrium chloride
EMB	Eosin Methylene-blue agar
XLD	Xylose Lysine Deoxycholate agar
SIM	Simmon-Indole-Motility
TCBS	Thiosulfate Citrate Bile Sucrose Agar
MR-VP	Methyl-red test. Voges-Proskauer Test.

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ABSTRACT

The objective of these studies is to isolate and purified polysaccharide from gram negative bacteria associated with marine sponges *Aaptos sp.* The studies are also to identify isolated bacterium and the chemical analysis of the purified polysaccharide. The sponges were taken from Pulau Bidong and the bacteria were isolated from the outer layer of the sponges. The targeted bacterium labeled as AO-1 for further analyses. The morphological characteristic, biochemical test and RapID™ NF Plus and ONE System identification kit were used to identify the bacterium. The bacterium is gram-negative, straight rod and identified as *Pseudomonas stutzeri*. The production of crude polysaccharide and acidic polysaccharide were 100 mg/l and 180mg/l polysaccharide respectively. The neutral sugars and amino sugars were determined using paper chromatography (PC) and high performance liquid chromatography (HPLC) with a universal evaporative light scattering detector. From PC results, it showed that isolated polysaccharide contain glucose, mannose, raffinose and glucosamine for both crude and acidic polysaccharide. For HPLC, the result for crude polysaccharide was glucose and mannose detected while for acidic polysaccharide hydrolyzed with 2 M TFA was glucose and mannose and raffinose, for hydrolyzed 2 M HCL was glucose and mannose.

ABSTRAK

Tujuan kajian ini adalah untuk pemencilan dan penentuan polisakarida daripada gram negatif bacteria yang diekstrak daripada span *Aptos* sp. Kajian ini juga untuk mengenalpasti bacteria yang diekstrak dan juga untuk menjalankan analisa kimia terhadap polisakarida yang telah dihasilkan. Span *Aptos* sp diambil daripada Pulau Bidong dan bacteria yang dipencilkan adalah dari lapisan luar span. Bacteria yang dipencilkan dilabel sebagai AO-1 untuk analisis yang selanjutnya. Ciri-ciri morfologi, ujian biokimia dan RapID™ NF Plus and ONE System identification kit digunakan untuk pengenalpastian bacteria. Melalui ujian-ujian tersebut, bacteria yang dikenalpasti ialah bacteria gram negatif yang berbentuk rod dan berwarna ungu yang dikenali sebagai *Pseudomonas stutzeri*. Crude polisakarida dan asid polisakarida yang dihasilkan ialah 100 mg/l dan 180mg/l masing-masing. Gula amino dan gula semulajadi daripada polisakarida yang dihasilkan dikenalpasti melalui kaedah HPLC dan PC. Bagi keputusan PC menunjukkan kedua-dua crude dan acid polisakarida mengandungi glukos, mannose, trehalos dan glicosamine. Keputusan yang diperolehi daripada HPLC ialah gula yang dihasilkan ialah glukos dan mannose bagi crude polisakarida manakala bagi asid polisakarida yang dihidolisis dengan 2M TFA adalah glukos, mannose dan trehalose bagi asid polisakarida yang dihidrolisis dengan 2 M HCL adalah glucose dan mannose