

DNA FINGERPRINTING OF SPONGE AAPTOS SP.

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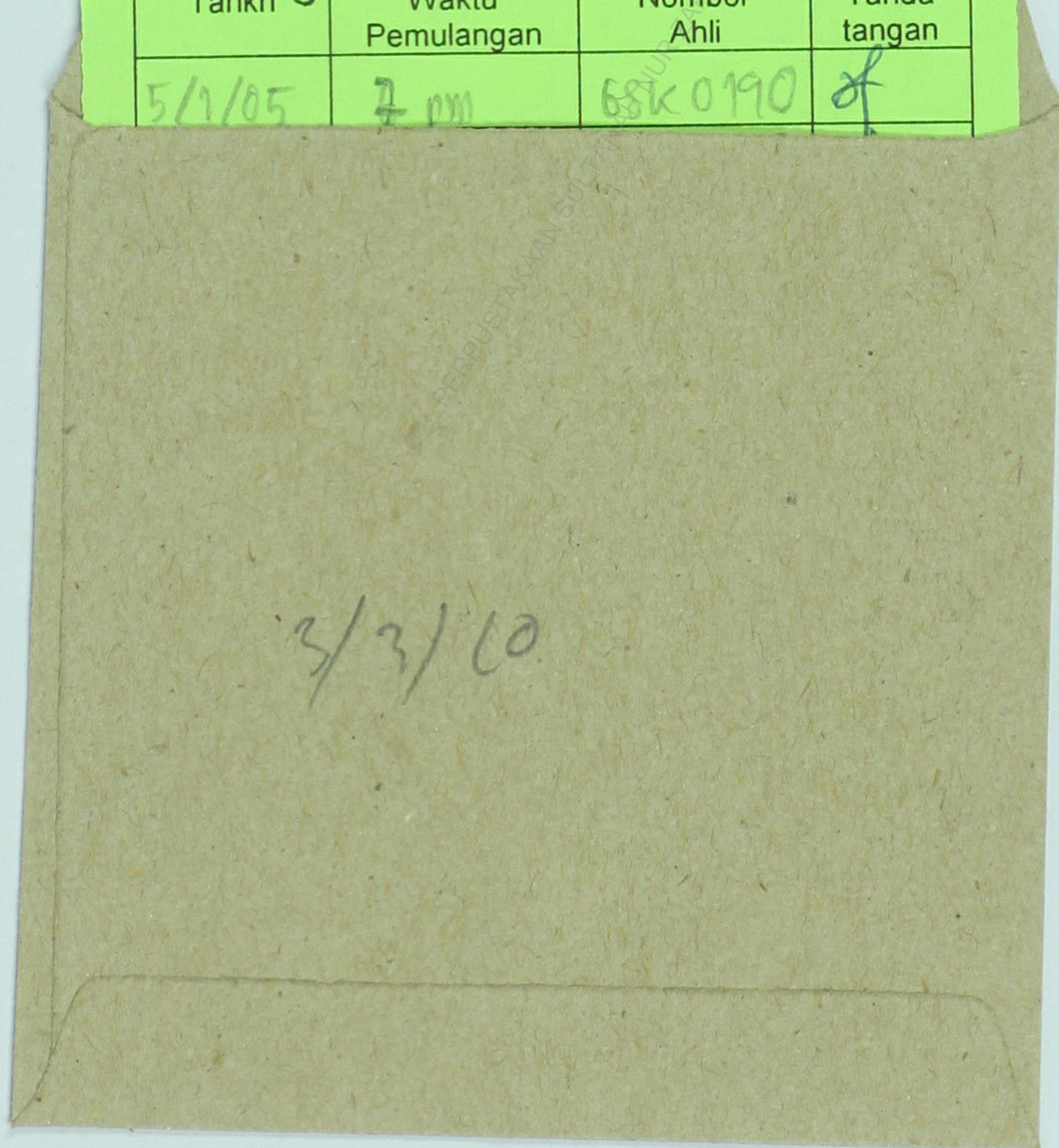
DNA fingerprinting of sponge Aaptos sp / Chew Mei Yem.



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Judul <b>DNA fingerprinting of sponge</b>		<i>[scribble]</i>	
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# DNA FINGERPRINTING OF SPONGE *Aaptos* sp.

BY:

CHEW MEI YEN

PERPUSTAKAAN SULTANAH NUR ZAHIRAH

This project is submitted in partial fulfillment of  
the requirements for the  
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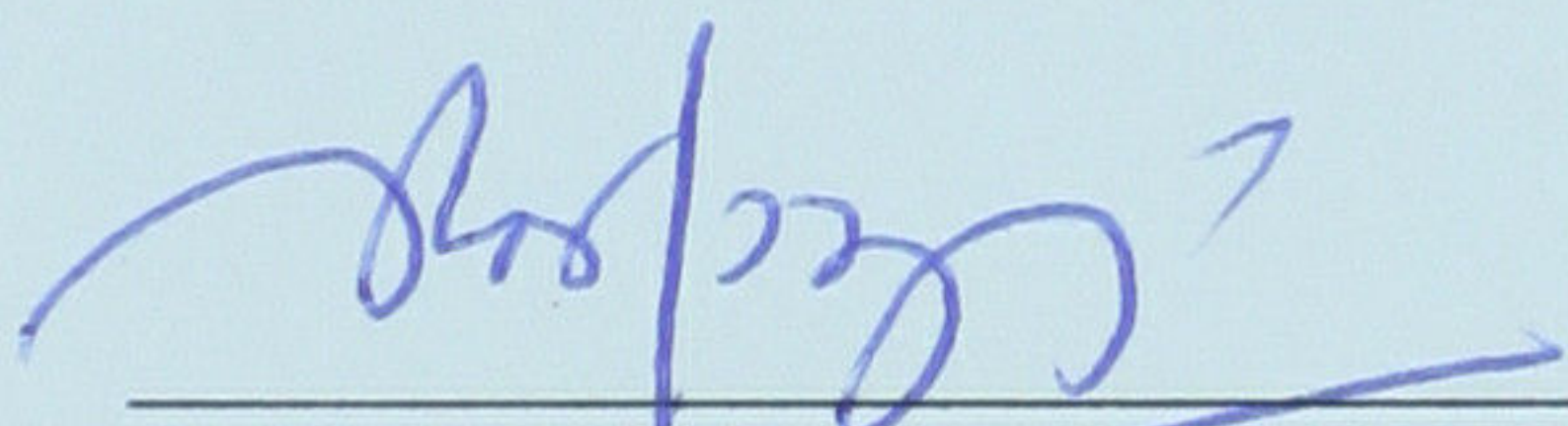


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APPROVAL AND CERTIFICATION FORM

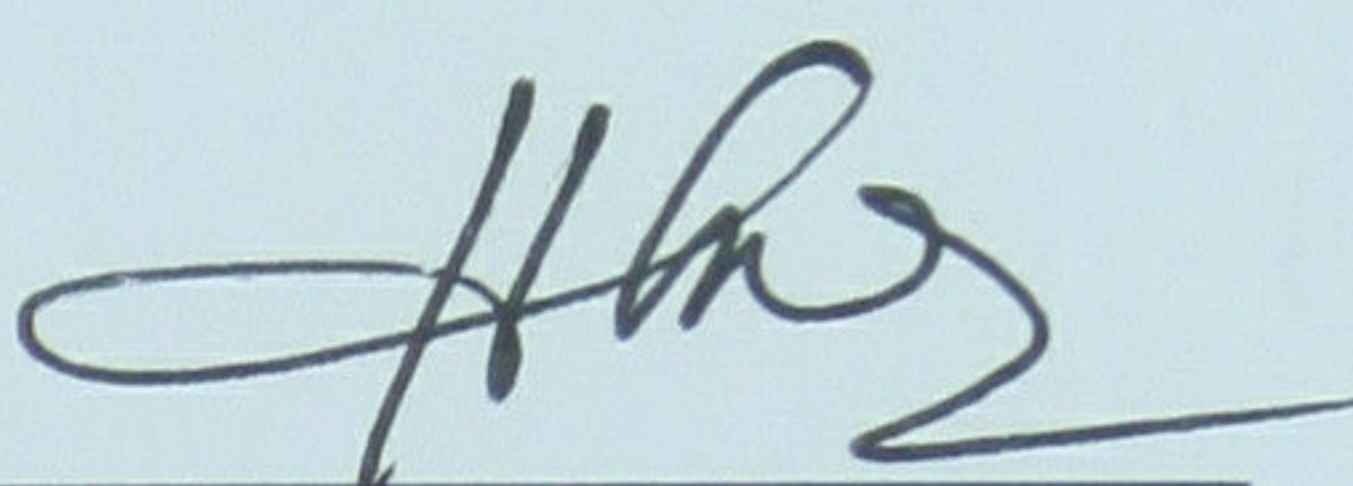
I certify that the report of this final year project entitled ' DNA Fingerprinting of sponge, *Aaptos* sp. by CHEW MEI YEN, matric no. UK4429 have been read and all the alteration and correction recommended by the examiners has been done. This thesis submitted to Department of Biological Sciences, have been accepted as fulfillment of requirement for degree of Sarjana Muda Science in Biological Science, Faculty Science and Technology, University College of Science and Technology Malaysia (KUSTEM).

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You Made Me Fly  
by J. Samm

I longed for acceptance  
You welcomed me with open arms

I longed to be heard  
You turned to listen

I felt worthless  
You treasured me

I thought I was incapable  
You gave me a chance to prove what I can do

I was without talent  
You called me gifted

I was trapped in a shell I built for myself  
You set me free

I never knew I could do anything that counts  
You believed in me

I thought I was limp  
You made me fly

And so I did  
And I soared high.



## ABSTRACT

Sponges are rich in bioactive compounds and natural products including alkaloids. Alkaloids found in the genus *Aaptos* shows potent antiviral activity, suggesting that they may be selectively targeted to inhibit virus replication. Application of DNA fingerprinting was used to identify DNA-based genetic polymorphism for determining the genetic variability and degree of polymorphism of *Aaptos* sp. In this study, samples from the *Aaptos* sponges were subjected to three methods for optimization. The results showed that fresh samples and frozen samples gave no bands when run on 0.8% agarose gel while samples preserved in alcohol showed the degraded DNA. Three protocols had been chosen to extract genomic DNA from sample *Aaptos* sp. but only modified GES method gave the expected result. The given purity of genomic was in the range of 1.516-1.702 ( $OD_{260}/OD_{280}$ ) and quantity of DNA was in the range of 302.5-1657.5 ng/ $\mu$ l. Random amplified polymorphic DNAs (RAPDs) were used to discriminate among eight individual *Aaptos* sp. with a total of 10-mer oligonucleotide primers. It yielded 43 polymorphic RAPD from three selected primers (OPA-07, OPA-08 dan OPA-10) that produced clear and reproductive bands. Two populations were compared to discriminate all individuals based on band sharing, even those not distinguished on the basis of morphological and phenotypic traits. The mean similarity index of the *Aaptos* sp. was  $0.43 \pm 0.11$  in the range of 0.16-0.67 while the genetic distance obtained was 0.33-0.67. The overall polymorphism was high among *Aaptos* sp., (92.97%) and the productive RAPD marker was in the range of 280-3200 bp. The constructed dendrogram differentiated the eight isolates into one main cluster and a single isolate. Genetic variability was high as no same genotypes occurred in this study. Analysis on more *Aaptos* sp. sample and primers should be performed in further studies to more accurately determine the degree of polymorphism.



## ABSTRAK

Span didapati kaya dengan sebatian bioaktif dan bahan semulajadi yang mempunyai kepentingan dalam bidang farmaseutikal. Kajian yang lalu membuktikan alkaloid baru, yang dijumpai dalam *Aaptos* merupakan sebatian yang mempunyai potensi antivirus, kemungkinan berupaya menghalang replikasi virus. Penggunaan pencapjarian DNA dalam membezakan polimorfik berdasarkan genetik bertujuan memaparkan kepelbagaian genetik dan darjah polimorfik *Aaptos* sp. Dalam kajian ini, tiga kaedah diguna untuk mengekstrakkan genomik DNA bagi mendapatkan kaedah terbaik. Keputusan yang diperolehi menunjukkan genomik yang diekstrak dari sampel segar dan sampel yang tersimpan dalam keadaan sejuk tidak memberi jalur apabila dilarikan atas 0.8% agarose gel. Sampel yang disimpan dalam 70% alkohol pula memberi genomik yang tidak memuaskan. Beberapa kaedah telah dioptimasikan untuk menentukan kaedah terbaik dalam mengekstrak genomik DNA dari sponge *Aaptos* sp. tetapi hanya kaedah GES yang telah diubahsuaikan memberi keputusan yang dijangkakan. Genomik memberi ketulenan dalam julat 1.516-1.702 ( $OD_{260}/OD_{280}$ ) dan 302.5-1657.5 ng/ $\mu\text{l}$ . Pengenalan Random Amplifikasi Polimorfisme DNA (RAPD) untuk menunjukkan polimorfik lapan individu *Aaptos* sp. dengan menggunakan sepuluh 10-mer oligonukleotida primer. 43 polimorfisme RAPD iaitu jalur yang jelas dan reproduktif telah dihasilkan daripada tiga primer yang terpilih (OPA-07, OPA-08 dan OPA-10). Dua populasi dibandingkan untuk mengecamkan jalur genomik yang dihasilkan, di mana tidak dibezakan berdasarkan bentuk morfologi dan ciri-ciri fenotip. Purata penunjuk kesamaan antara individu *Aaptops* sp. adalah  $0.43 \pm 0.11$  dalam julat 0.16-0.67 manakala jarak genetik yang terdapat adalah 0.33-0.67. Secara keseluruhan, *Aaptos* sp. memberi peratus polimorfik yang tinggi iaitu 92.97% dan penanda RAPD yang productif di dalam julat 280-3200 bp. Dendogram yang dibina berdasarkan maklumat jalur polimorfik memisahkan semua individu kepada satu kelas besar dan satu kelas kecil. Perbezaan genetik adalah tinggi dan tiada genotip yang sama wujud dalam kajian ini. Pengajian yang lebih mendalam harus diadakan dengan memperbanyakkan sampel *Aaptos* sp. dari lokasi yang lain.