DNA FINGERPRINTING OF LOKAN, *Polyunesoda expansa* USING RAPD - PCR TECHNIQUE

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Adalah ini diakui dan disahkan bahawa laporan penyelidikan ilmiah tahun akhir bertajuk DNA Fingerprinting of Lokan, *Polymesoda expansa* Using RAPD-PCR Technique oleh Lee Sau Yee, no. matrik UK 4090 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi ijazah Sarjana Muda Sains – Pengurusan dan Pemuliharaan Biodiversiti, Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

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ABSTRACT

Lokan, *Polymesoda expansa* (*P.* expansa) formerly known as *Geloina coaxans* is a filter feeder and can be found in Southern Asia and Malaysia. Besides playing an important role as a bioindicator, it is also a food resource for the east coast of Peninsular Malaysia. Random Amplified Polymorphic DNA (RAPD) based on Polymerase Chain Reaction (RAPD-PCR) technique was used to amplify the genomic DNA of *P. expansa*. The aims of this study were to establish the DNA fingerprinting of *P. expansa*, to verify the genetic variability and to analyze the degree of polymorphisms on DNA level marker of *P. expansa*. The genomic DNA of 12 samples (six samples per population) was extracted from muscular tissue using Phenol-Chloroform Protocol. The purity of DNA ranged from 1.453-1.731, estimated from the ratio between the reading of absorbance at 260 nm and 280 nm (OD_{260}/OD_{280}) in UV-spectrophotometer. The purity of DNA can be observed through the single band formed on a 0.8% agarose gel stained with 0.5 µg/mL ethidium bromide. DNA quantifications ranged between 235.00-580.00 ng/µL. 40-50% of 20 primers with 60-70% GC content were able to amplify the fragments from each genomic DNA. The fragments of PCR products were observed when electrophoresised in 2.0% (w/v) agarose gel stained with 0.5 µg/mL ethidium bromide. The three primers selected (OPA 08, 09 and 12) generated a total of 51-55 scorable loci (bands) with 90.91-93.98% polymorphic loci. RAPD banding for Kg. Pulau Tok Aji and Kg. Laut populations ranged from 3-12 bands and 3-9 bands with 250-2200 bp and 240-2080 bp. The similarity index among individuals between populations was 0.263 $\pm$ 0.152 while the genetic distance value was 0.737 $\pm$ 0.152. From this study, a monomorphic band (650 bp in OPA 09) was found to be reliable as a diagnostic marker of *P. expansa*. Further studies on this species should be carried out in order to conserve and maintain the gene pool of *P. expansa* which have the commercial potential in aquaculture.

ABSTRAK

Lokan, *Polymesoda expansa* (*P. expansa*) pada mulanya dikenali sebagai *Geloina coaxans* merupakan organisma jenis pemakan hasil tapisan dan ditemui di benua Asia Selatan dan Malaysia. Selain daripada sebagai organisma penunjuk bio, ia juga merupakan sumber makanan bagi penduduk di pantai timur Semenanjung Malaysia. Teknik "Random Amplified Polymorphic DNA" (RAPD) yang berasaskan "Polymerase Chain Reaction" (RAPD-PCR) digunakan untuk mengamplifikasi genomik DNA *P. expansa*. Tujuan kajian ini dijalankan adalah untuk menerbitkan satu pencapjarian DNA untuk *P. expansa*, untuk mengenalpasti perubahan genetik dan untuk menganalisis tahap polimorfik berdasarkan penanda DNA *P. expansa*. Genomik DNA untuk 12 sampel (Enam sampel untuk satu populasi) diekstrak daripada tisu otot dengan menggunakan kaedah Fenol-Kloroform. Julat ketulenan DNA yang diperolehi daripada nisbah bacaan penyerapan pada 260 nm dan 280 nm (OD_{260}/OD_{280}) pada spektrofotometer ialah 1.453-1.731. Ketulenan DNA yang diperolehi juga boleh diperhatikan dengan pembentukan satu jalur tunggal pada 0.8% gel agaros yang telah diwarnai dengan 0.5 μg/mL etidium bromida. Julat kepekatan DNA ialah antara 235.00-580.00 ng/μL. Daripada 20 primer yang mengandungi 60-70% GC, hanya 40-50% dapat mengamplifikasi fragmen daripada setiap genomik DNA. Hasil fragmen daripada PCR dapat diperhatikan apabila di elektroforesiskan dalam 2.0% gel agaros yang diwarnai dengan 0.5 μg/mL etidium bromida. Sejumlah 51-55 jalur dicatatkan dengan 90.91-93.98% jalur polimorfik serta julat penjaluran RAPD antara 3-12 jalur dan 3-9 jalur dengan 250-2200 bp dan 240-2080 bp bagi populasi Kg. Pulau Tok Aji dan Kg. Laut diperolehi daripada tiga primer (OPA08, 09 dan 12) yang dipilih. Indek kesamarataan sesama individu antara populasi adalah 0.263 ± 0.152 dengan jarak genetik antara 0.737 ± 0.152. Satu jalur monomorfik iaitu 650 bp dalam OPA09 yang diperolehi daripada kajian ini dapat digunakan sebagai penanda diagnostik untuk *P. expansa*. Kajian yang lebih mendalam harus dijalankan untuk memelihara dan mengekalkan kolam genetik *P. expansa* yang mempunyai potensi untuk dikultur dalam bidang akuakultur.