MOLECULAR TAXONOMIC STUDIES OF *Gracilaria changii* FROM VARIOUS LOCATIONS USING THE RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) TECHNIQUE

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ABSTRACT

Malaysia which has abundant *Gracilaria changii* (Xia et Abbott) Abbott, Zhang et Xia has the potential to become a producer of good food grade agar. However, for effective utilisation of this agarophytic seaweed, Bird and Van der Meer (1993) emphasised the importance of taxonomic studies in ensuring the correct identification and recognition of this seaweed.

Molecular biology has been applied in the approach to study the phylogenetic relationship of the *Gracilaria* species. In this study, *Gracilaria changii* samples were collected from six geographical locations, namely, Phuket & Takbai (Thailand) and Ban Merbok, Morib, Carey Island & Sungai Pulai (Malaysia). Although these samples were identified as *Gracilaria changii*, some morphological differences were observed such as thallus appearance, diameter of thallus and the reproductive stage. The objective of this study is to assess the use of the Random Amplified Polymorphic DNA (RAPD) in characterising these samples. Interspecies relationship between selected *Gracilaria* species collected from Morib such as *Gracilaria changii*, *Gracilaria edulis*, and *Gracilaria salicornia* were also studied while *Sargassum oligocystum* was used as an outgroup.

In this study, two DNA extraction protocols were carried out: the cetyltrimethylammonium (CTAB) and phenol combination adapted from Sinnappah (1994) and a modification of this protocol (with additional steps of

isolating from the residue and a reextraction step). The modified protocol was found to produce higher purity of DNA with better yield.

Of the 20 primers screened, four primers: OPA3 (AGTCAGCCAC), OPA10 (GTGATCGCAG), OPA11 (CAATCGCCGT) and OPA13 (CAGCACCCAC) were selected to generate polymorphic band patterns for all the samples under an optimised temperature. The RAPD data analysis was carried out using the Gelcompar Software 4.1 which calculated the matrix of similarities based on the Dice coefficient (S_D) and dendrograms were generated using the unweighted pair group method using arithmetic averages (UPGMA). All the four primers showed that there were great intrapopulational variations among the *Gracilaria changii* as well as interpopulational variations. Overall, these primers were unable to form a relationship at the geographical scales studied as there is high intrapopulational variation. There is a possibility of developing individual fingerprints with these primers. However, for larger geographical distance, primer OPA13 can separate the Malaysian *Gracilaria changii* from the *Gracilaria changii* collected from Thailand.

In the interspecies study, OPA13 can separate *Gracilaria changii* from both *Gracilaria salicornia* and *Gracilaria edulis* while OPA11 showed that there is a possibility of hybridisation between *Gracilaria changii* and *Gracilaria salicornia* or between *Gracilaria changii* and *Gracilaria edulis*. Both OPA11 and OPA13 separate *Gracilaria* samples from *Sargasssum* samples.

ABSTRAK

Malaysia dengan sumber *Gracilaria changii* (Xia et Abbott) Abbott, Zhang et Xia mempunyai keupayaan menjadi pengeluar agar gred pemakanan. Walau bagaimanapun, untuk penggunaan berkesan rumpair laut 'agarophytic', Bird dan Van der Meer (1993) menekan kepentingan kajian taksonomi untuk memastikan ketepatan dalam mengenalpastikan rumpairlaut ini.

Biologi molekul telah digunakan untuk mengkaji perhubungan filogenetik *Gracilaria* spesis. Dalam kajian ini, sampel-sampel *Gracilaria changii* dikumpul dari enam kawasan geografi iaitu Phuket & Takbai (Negara Thai) dan Ban Merbok, Morib, Pulau Carey & Sungai Pulai (Malaysia). Walaupun sampel-sampel ini dikenalpasti sebagai *Gracilaria changii*, terdapat perbezaan morfologi seperti rupabentuk tallus, diameter tallus dan tahap pembiakan. Objektif kajian ini adalah mengkaji penggunaan 'Random Amplified Polymorphic DNA' (RAPD) dalam mengenalpasti sampel-sampel ini. Perhubungan interspesis antara pilihan *Gracilaria* spesis yang dikumpul dari Morib seperti *Gracilaria changii*, *Gracilaria edulis* and *Gracilaria salicornia* juga dikaji dan *Sargassum oligocystum* diguna sebagai bandingan (outgroup).

Dalam kajian ini, dua protokol pengekstrakan DNA telah dijalankan: kombinasi cetyltrimethylammonium (CTAB) dan fenol diaplikasi dari Sinnappah (1994) dan modifikasi protokol ini (dengan penambahan langkah – langkah pengekstrakan dari sisa dan pengulangan pengekstrakan). Protokol

yang dimodifikasi itu menghasilkan DNA yang mempunyai ketulenan yang tinggi dengan hasil yang lebih baik.

Antara 20 primer yang dikaji, empat primer: OPA3 (AGTCAGCCAC), OPA10 (GTGATCGCAG), OPA11 (CAATCGCCGT) dan OPA13 (CAGCACCCAC) dipilih untuk menghasilkan corak jalur yang berlainan untuk kesemua sampel-sampel pada suhu yang dioptimakan. Data RAPD analisis dijalankan dengan menggunakan 'Gelcompar Software 4.1' di mana matriks persamaan dikira berdasarkan 'Dice coefficient' (S_D) dan dendrogram-dendrogram dihasilkan dengan menggunakan 'unweighted pair group method using arithmetic averages' (UPGMA). Kesemua empat primer-primer ini menunjukkan perbezaan intrapopulasi yang besar antara *Gracilaria changii* selain daripada perbezaan interpopulasi. Primer-primer ini tidak dapat membentukkan hubungan pada skala geografi yang dikaji. Terdapat kemungkinan besar untuk menghasilkan 'fingerprints' untuk individu sampel dengan primer-primer ini. Walau bagaimanapun, untuk jarak geografi yang lebih besar, primer OPA13 boleh memisahkan *Gracilaria changii* dari Thailand daripada *Gracilaria changii* dari Malaysia.

Dalam kajian interspesis, OPA13 memisahkan *Gracilaria changii* daripada kedua-dua *Gracilaria salicornia* dan *Gracilaria edulis* sementara OPA11 menunjukkan kemungkinan penghibridisasian antara *Gracilaria changii* dan *Gracilaria salicornia* serta antara *Gracilaria changii* dan *Gracilaria edulis*. Kedua-dua OPA11 dan OPA13 memisahkan sampel-sampel *Gracilaria* daripada sampel-sampel *Sargassum*.

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