Molecular Characterisation of Two *Sargassum* species (Phaeophyta) Using the Random Amplified Polymorphic DNA (RAPD) Technique

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Ng Seok Min

Institute of Postgraduate Studies and Research
University of Malaya
Kuala Lumpur
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ABSTRACT

Sargassum species are known to be sources of many useful products. However, confusions exist in the classification of *Sargassum* because the thalli are highly differentiated. Therefore, according to Bird and van der Meer (1993), taxonomic studies are important to ensure that algae of interest are correctly identified and recognised. Molecular studies of *Sargassum* can help in resolving phylogenetic and evolutionary relationships in the genus. In this study, two *Sargassum* species (*S. binderi* Sonder and *S. oligocystum* Montagne) collected from Teluk Kemang and Cape Rachado in Port Dickson, Negeri Sembilan which were alike in morphology except for the vesicles and receptacles, were characterised using Random Amplified Polymorphism DNA - Polymerase Chain Reaction (RAPD-PCR). The genomic DNA of both species were isolated from the leaves and young shoots using a modified CTAB method (Doyle and Doyle, 1990). The DNA was then successfully amplified by five random primers, that is, OPA13, OPK7, OPK9, OPN6 and OPN16 with optimised RAPD-PCR conditions. The polymorphisms generated by these five primers were analysed using the coefficient of similarity (F value) (Nei and Li, 1979) and phylogenetic trees were generated by UPGMA method (Sokal and Michener, 1958) of the computer programme NTSYS (Kianian, 1993). All five primers gave similarity greater than 72% for intraspecific relationship within *S. bindri* and within *S. oligocystum*. When compared interspecifically, the percentage of similarity generated clearly showed that the two species from Malaysia are only distantly related throughout all the five primers. Therefore, *S. binderi* and *S. oligocystum* are not

recommend as synonyms as reported by Womersley and Bailey (1970) but remain separate as proposed by Ajisaka (1998). However, OPN16 is not a very good primer to separate out samples of *S. binderi* or *S. oligocystum* intraspecifically. Therefore, it is not advisable to use it as a probe for DNA fingerprinting. To develop primers for rapid screening of these two *Sargassum* species, OPK9 has the potential to be used as a molecular probe based on the convincing results obtained for this study.

ABSTRAK

Spesis-spesis *Sargassum* merupakan satu punca bagi pelbagai produk yang berguna dalam kehidupan harian. Akan tetapi, kekeliruan wujud bagi mengklasifikasikan *Sargassum* disebabkan perebezaan tangkainya. Justeru, Bird dan van der Meer (1993) menyatakan bahawa kajian taksonomi adalah sangat penting untuk memastikan alga yang penting ini dikenalpasti dengan tepat. Kajian molekular *Sargassum* dapat menyelesaikan masalah-masalah filogenetik dan evolusi pada tahap 'genus'. Dalam kajian ini, dua spesis *Sargassum* (*S. binderi* Sonder and *S. oligocystum* Montagne) yang kelihatan seakan-akan sama dari segi morfologinya kecuali pundi udara dan struktur persenyawaannya, telah dikutip dari Teluk Kemang dan Cape Rachado, Port Dickson, Negeri Sembilan, dan dikenalpasti dengan teknik 'Random Amplified Polymorphism DNA - Polymerase Chain Reaction' (RAPD-PCR). DNA genomik kedua-dua spesis ini diekstrakkan dari bahagain pucuk dan daunnya dengan menggunakan cara modifikasi CTAB (Doyle dan Doyle, 1990). DNA kedua-dua spesis ini telah berjaya diamplifikasikan oleh lima primer, iaitu OPA13, OPK7, OPK9, OPN6 dan OPN16 dengan keadaan RAPD-PCR yang telah dioptimasikan terlebih dahulu. Kemudian, hasil-hasil RAPD-PCR ini dianalisis dengan menggunakan koefisi kesamaan (Nilai F) (Nei dan Li, 1979) dan juga pokok-pokok filogenetik dihasilkan dengan cara UPGMA (Sokal dan Michemer, 1958) dengan program berkomputer NTSYS (Kianian, 1993). Kesemua primer memberi peratusan kesamaan melebihi 72% bagi kajian perhubungan intra-spesis apabila dibandingkan di antara *S. binderi* dan juga *S. oligocystum* masing-masing. Akan tetapi, apabila dibandingkan

perhubungan inter-spesis di antara kedua-dua spesis ini, didapati bahawa *S. binderi* hanya mempunyai perhubungan yang sedikit dengan *S. oligocystum* bagi kelima-lima primer yang digunakan. Maka, *S. binderi* dan *S. oligocystum* bukanlah sinonim seperti yang dilaporkan oleh Womersley dan Bailey (1970) tetapi ia seperti yang dicadangkan oleh Ajisaka (1998). Dari kajian ini, didapati bahawa OPN16 bukanlah satu primer yang baik untuk mengesahkan perhubungan intra-spesis bagi *S. binderi* atau *S. oligocystum*. Jadi, OPN16 tidak sesuai dijadikan proba untuk 'DNA fingerprinting". Untuk menghasilkan primer-primer bagi mengasingkan kedua-dua sampel *Sargassum* ini dengan cepat, OPK9 mempunyai potensi sebagai proba molekular berdasarkan keputusan eksperimen yang menyakinkan ini.

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