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Selection of reference genes for transcript profiling of *Sargassum polycystum* by quantitative real-time polymerase chain reaction

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SUMMARY

Sargassum species are one of the major alginate-producing seaweed species in Asian countries. Alginate is widely used in food, feed, pharmaceutical and medical industries as thickening and stabilizing agents. To establish a set of consistently expressed genes as reference genes for quantitative real-time polymerase chain reaction (qRT-PCR) studies of *Sargassum polycystum* (Fucales, Ochrophyta) in samples collected at two distinct time points from the field, four candidate reference genes, namely ribosomal protein L3 (*RPL3*), ribosomal protein S15 (*RPS15*), alpha-tubulin (*α-TUB*) and eukaryotic translation elongation factor 1 alpha (*TEF1α*), were analyzed using geNorm and NormFinder. The results showed that *RPL3*, *α-TUB* and *TEF1α* were the most stable genes using both programs, whereas *RPS15* gene was shown to be the least stable. Identification of stably expressed reference genes is crucial for qRT-PCR studies to allow accurate quantification of target gene expression levels. In addition, the expression of key enzyme in the final step of alginate biosynthesis pathway mannuronan C5 epimerase-SP01411 (MC5E-SP01411) and mannuronan C5 epimerase-SP02271 (MC5E-SP02271) were differentially expressed in the seaweeds collected at two distinct time points from the field. To our knowledge, this is the first report on validation of reference genes for any *Sargassum* species. Our data provide a basis for the selection of reference genes for future biological research in related studies.

Key words: brown algae, mannuronan C5-epimerase (MC5E), normalization, qRT-PCR, reference genes.

INTRODUCTION

Sargassum C. Agardh (Fucales, Ochrophyta), is a brown seaweed, one of the key raw materials in alginate production. Alginate is widely used as stabilizing and thickening agents in pharmaceutical (Lee & Mooney 2012), textile (Heliopoulos *et al*. 2013) and food industries (Comaposada *et al*. 2015). In Malaysia, *Sargassum* is abundant and 39 species have been identified (Phang *et al*. 2008). *Sargassum* spp. living in intertidal habitats are regularly exposed to recurring, abiotic stresses, including changes in salinity, temperature, pH, irradiance, etc. (Yeong & Wong 2013).

Quantitative real-time polymerase chain reaction (qRT-PCR) is a common approach to quantify gene expression (Derveaux *et al*. 2010). Expression studies by qRT-PCR require normalization of genes of interest against reference genes (Zhuang *et al*. 2015; Harrison *et al*. 2016). The reference genes should consistently express under chosen experimental conditions to allow accurate gene expression studies (Zhang *et al*. 2017). A few programs, including geNorm (Vandesompele *et al*. 2002) and NormFinder (Andersen *et al*. 2004), are widely used to identify the best reference genes for normalization under designated experimental conditions. Several stable reference genes have been identified in red algae *Pyropia yezoensis* (Ueda) M. S. Hwang & H. G. Choi (Kong *et al*. 2015, then as *Porphyra yezoensis* Ueda; Wu *et al*. 2013), *Chondrus crispus* Stackhouse (Kowalczyk *et al*. 2014), and *Bostrychia moritziana* (Sonder ex Kützing) J. Agardh (Shim *et al*. 2016); green algae *Nannochloropsis* D. J. Hibberd (Cao *et al*. 2012), *Ulva linza* Linnaeus (Dong *et al*. 2012) and *Chlamydomonas* Ehrenberg (Liu *et al*. 2012). As for brown algae, reference genes have only been identified for *Ectocarpus siliculosus* (Dillwyn) Lyngbye (Le Bail *et al*. 2008). However, stable reference genes have been identified in a few species that are closely related to brown algae, such as diatom (Siaut *et al*. 2007) and dinoflagellates (Kamikawa *et al*. 2007; Chong *et al*. 2017).

In this study, four candidate reference genes were selected from the expressed sequence tag (EST) dataset of field-grown *Sargassum polycystum* C. Agardh. We further tested the expression stability of these candidate reference genes in eight individuals of *S. polycystum*, four each from two sampling time points (coinciding with the rainy and dry seasons) from the field. To our knowledge, this is the first report on validation of reference genes in *Sargassum* species.

MATERIALS AND METHODS

Algal material collection and preparation

Teluk Kemang, Port Dickson, located at the coast of the Southwest Peninsular of Malaysia was subjected to maximum

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