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Molecular cloning and characterization of GDP-mannose-3′,5′-epimerase from *Gracilaria changii*

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Abstract GDP-mannose-3′,5′-epimerase (GME) is an enzyme involved in the biosynthesis of GDP-L-galactose which is a building unit of agar and cell wall polysaccharides. GME catalyzes the formation of GDP-β-L-galactose and GDP-L-gulose from GDP-mannose. In this study, the gene and transcript encoding GME from the red alga *Gracilaria changii* (GcGME) were cloned. The structural gene sequence of *GcGME* is devoid of an intron. The *cis*-acting regulatory element involved in light response is the most abundant element at the 5′-flanking region of *GcGME*. The open reading frame of GcGME consists of 1,053 nucleotides with 351 amino acids. This cDNA was cloned into pET32a expression vector for recombinant protein production in *Escherichia coli*. High yield of soluble recombinant GcGME (55 kDa) was expressed upon isopropyl β-D-1-thiogalactopyranoside induction. The enzyme activity of recombinant GcGME was detected using thin layer chromatography and high-performance liquid chromatography. The transcript abundance of GcGME was the highest in *G. changii* and the lowest in *Gracilaria salicornia* corresponding to their agar contents. The characterization of GcGME from *G. changii* is important to facilitate the understanding of its role in agar production of this seaweed.

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Introduction

Agar is a commercial algal galactan produced by marine algae. Its backbone consists of a linear chain of galactose β-D-galactopyranose residues and α-L-galactopyranose residues that are arranged in an alternating order (Anderson et al. 1965; Usov 1992). The galactose residues in agar are modified by sulfate (Araki and Hirase 1960; Duckworth and Yaphe 1971; Izumi 1973; Craigie 1990). Meanwhile, agar also has been reported to be pyruvated and methylated in varying degrees (Hirase 1957; Duckworth et al. 1971; Craigie 1990). Agar is found as cell wall polysaccharide in rhodophytes. *Gracilaria* species are one of the major sources of agar. Seaweeds belonging to this species contribute approximately 65 % of the raw materials used for agar production in the world (McHugh 2003).

The D-galactose component of agar is synthesized via glucose-1-phosphate, UDP-D-glucose, and UDP-D-galactose (Manley and Burns 1991) (Fig. 1). Galactose-1-phosphate uridylyltransferase (GALT, E.C. 2.7.7.12) catalyzes the formation of glucose-1-phosphate and UDP-galactose from galactose-1-phosphate and UDP-glucose (Mayes 1976). The molecular cloning of *GALT* gene has been reported in two rhodophytes, *Gracilaria gracilis* (Lluisma and Ragan 1998) and *Gracilariopsis lemaneiformis* (Li et al. 2010), while the transcript encoding GcGALT has been cloned and characterized in *Gracilaria changii* (Siow et al. 2012).

The L-galactose component of agar is produced from GDP-L-galactose (Manley and Burns 1991) (Fig. 1). In this pathway, GDP-mannose pyrophosphorylase (E.C. 2.7.7.22)