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Agrobacterium-mediated gene delivery and transient expression in the red macroalga *Chondrus crispus*

https://doi.org/10.1515/bot-2018-0028
Received 11 March, 2018; accepted 13 July, 2018; online first 12 September, 2018

Abstract: Molecular resources and transgenic studies in red algae are lagging behind those for green algae. The *Agrobacterium*-mediated gene-transfer method routinely used in plant transformation has not been fully utilised in the red algae, which, as an important source of phycocolloids, warrant more studies. In this regard, a stepwise methodology was developed for *Agrobacterium*-mediated transformation of the carrageenophyte *Chondrus crispus* using pCAMBIA 1301 and a construct featuring a codon-optimized beta-glucuronidase (*GUS*) reporter gene driven by the endogenous *Chondrus* actin promoter. The effects of several factors on transformation efficiency were investigated. An intimate association of *Chondrus* and bacterial cells was observed using scanning electron microscopy. *GUS* transient expression within *Chondrus* cortical and medullary cells with both expression cassettes testified to the amenability of *Chondrus* to *Agrobacterium*-mediated transformation. Darker staining, indicative of higher GUS activity, was observed with the *Chondrus*-specific construct, suggesting its superiority over the pCAMBIA 1301. Presence of acetosyringone, the wounding method and the type of co-cultivation medium significantly affected the transformation outcome and efficiency. The *Agrobacterium*-mediated transient expression presented here constitutes a first step towards tailoring a transformation strategy for *Chondrus*, which can serve to facilitate further transgenic studies in this important red alga.

Keywords: algal biotechnology; gene engineering; genetic transformation; transformed algae; transgenic algae.

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Introduction

The red macroalga, *Chondrus crispus* Stackhouse, found along the rocky shores of the North Western and North Eastern Atlantic, is an important commercial source of the phycocolloid carrageenan, which is used as a gelling, thickening and stabilizing agent in the food, cosmetic and phamaceutical industries (Guiseley 1989, Necas and Bartosikova 2013). According to the Food and Agricultural Organisation, 2000 tonnes of *Chondrus* were harvested in the year 2005. *Chondrus* was historically the only source of carrageenan and contributed to the approximately US$417 million per annum carrageenan market (Kraan 2012), highlighting its importance.

The greatest diversity in algae can be observed within the red algae or Rhodophyta (Yoon et al. 2004, Maggs et al. 2008, Verbruggen et al. 2010), many of which have not yet been adequately studied. The red algae deserve more attention for their potential – by virtue of their unique biosynthetic pathways, cell wall components and unique pigments absent in land plants (Collén et al. 2013, Brawley et al. 2017). They are a mine of novel bioactive compounds (Holdt and Kraan 2011) that can be harnessed by the biotechnological industry. Studying the red algae, an ancient eukaryote lineage and a potential sister group to green algae, can additionally increase our understanding of the evolution of other algae and land plants (Collén et al. 2013).

In order to tap into the above-mentioned economic potential of the red macroalgae, a molecular toolkit for red algae is desirable, but lacking. It is crucial to develop reliable transgene delivery techniques for analysing the expression, function and regulation of red algal endogenous genes which will, in time, allow the engineering of economically important traits in algae such as