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A new approach to express transgenes in microalgae and its use to increase the flocculation ability of *Chlamydomonas reinhardtii*

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Abstract The choice of strong efficient promoters is a critical step in the development of efficient transformation systems for microalgae; however, the physiological and genetic diversity among microalgae groups makes very difficult to develop standard universal plasmids for a wide number of microalgal species as has been achieved for higher plants. Here, we propose a new approach to express transgenes in microalgae: cotransformation with two naked promoterless genes, a selectable antibiotic-resistant gene and a gene of our interest. These genes are randomly inserted into the nuclear genome, where their transcription relies on their adequate insertion in a region adjacent to an endogenous genomic promoter or in frame with a native gene. In a high percentage of the transformants obtained, both genes are, not only adequately incorporated in the nuclear genome, but also efficiently transcribed and translated. This transformation method is validated in the model microalga *Chlamydomonas reinhardtii* with the bleomycin-resistant gene from *Streptoalloteichus hindustanus* (*ShBLE*) as gene of interest, and it is employed to express a flocculin gene from *Saccharomyces bayanus* (*SbFLO5*), which is responsible for the flocculation process in yeasts. *Chlamydomonas reinhardtii* transformants exhibited self-flocculation abilities between 2- and 3.5-fold higher than the control untransformed strain. The successful cotransformation of *C. reinhardtii* with two promoterless genes opens doors for the establishment of a universal transformation system based on endogenous promoters, applicable to any microalgal species.

Keywords *APHVIII* · *Chlamydomonas reinhardtii* · Cotransformation · *FLO5* · Genetic transformation · Promoterless gene · Flocculation

Introduction

Microalgae are a heterogeneous group of photosynthetic microorganisms with high ecological importance and an enormous biotechnological potential (Enzing et al. 2014). The use of microalgae for the commercial production of carotenoids, polyunsaturated fatty acids (PUFAs), or other high added-value compounds is well established (Borowitzka 2013; Scaife et al. 2015), and in the last years, there has been an increasing interest in microalgae as a feedstock for the production of biofuels (Wijffels and Barbosa 2010; Vanthoor-Koopmans et al. 2013; Benemann 2013). This has made to increase the attention on genetic engineering of microalgae as a potential tool to aim the economically feasible production of bulk materials and to enhance the productivity of the high added-value compounds (León and Fernández 2007; Georgianna and Mayfield 2012; Scranton et al. 2015).

Genetic engineering represents, according to many researchers, the most promising strategy for the improvement of microalgae (Lee et al. 2008; Radakovits et al. 2010; Larkum et al. 2012; Scaife et al. 2015), but until recently, routine genetic manipulation has been limited to a few species (i.e., the classical model microalgae: *Chlamydomonas*

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