Research review paper

Advances in genetic engineering of marine algae

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ABSTRACT

Algae are a component of bait sources for animal aquaculture, and they produce abundant valuable compounds for the chemical industry and human health. With today's fast growing demand for algae biofuels and the profitable market for cosmetics and pharmaceuticals made from algal natural products, the genetic engineering of marine algae has been attracting increasing attention as a crucial systemic technology to address the challenge of the biomass feedstock supply for sustainable industrial applications and to modify the metabolic pathway for the more efficient production of high-value products. Nevertheless, to date, only a few marine algae species can be genetically manipulated. In this article, an updated account of the research progress in marine algal genomics is presented along with methods for transformation. In addition, vector construction and gene selection strategies are reviewed. Meanwhile, a review on the progress of bioreactor technologies for marine algae culture is also revisited.

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1. Introduction

Marine algae, including marine cyanobacteria, marine eukaryotic microalgae, and seaweed, are the oldest members of the plant kingdom, dating back three billion years and distributing from the polar region to tropical areas and from nutrient-rich coastal seas to oligotrophic open oceans. They are photoautotrophs unified primarily by their lack of roots, leaves, and other organs that characterize higher plants and their possession of Chlorophyll *a*. Marine algae range in size from microscopic individual cells of microalgae to huge plants that are greater than 30 m long and are called macroalgae *Macrocystis*. Marine algae are responsible for approximately 40%–50% of the photosynthesis that occurs on Earth each year (Falkowski et al., 1998). Marine photosynthesis is dominated by algae, including cyanobacteria (i.e., blue-green algae or cyanophyta) and eukaryotic taxa. Marine algae are a component of the bait sources for animal aquaculture and produce abundant valuable compounds for the chemical industry and human health, including oils (e.g., triglyceride), polysaccharides (e.g., algin, agar), pigments (e.g., phycobiliproteins, carotenoids), and also potential new pharmaceuticals (Apt and Behrens, 1999; Chisti, 2007, Lin et al., 2011, Witvrouw and DeClercq, 1997). Recently, a microbial platform that can simultaneously degrade, uptake, and metabolize alginate was established based on new discovered enzymes for alginate transport and metabolis, which enables bioethanol production directly from brown macroalgae via a consolidated process (Wargacki et al., 2012). The ancient cyanobateria are the last common ancestor of all oxygenic photosynthetic lineages, which have the closet evolutionary relationship with heliobacteria and other anaerobic photoautotrophs (Xiong et al., 2000), while photosynthetic eukaryotes acquired their photosynthetic properties from endosymbiosis with cyanobacteria (Gray, 1992; Reyes-Prieto and Bhattacharya, 2007). The green algae are primitive members of the kingdom Plantae from which land plants evolved approximately 500 million years ago (Parker et al., 2008, Wise, 2006). Due to the algal complex and the unique genetic and evolutionary scheme, the genetic engineering of algae must be considered to apply both of the methodologies from prokaryotic microorganisms and plants. Since the end of the 1970s, marine algal genetic engineering began being implemented in the model system cyanobacterial strain *Synechococcus* 7002, which can be transformed by exogenously adding homologous DNA carrying a selectable marker (Buzby et al., 1985; Matsunaga and Takeyama, 1995; Stevens and Porter, 1980). In the late 1980s, several eukaryotic marine microalgae and seaweeds were successfully transformed by different transformation methods, e.g., micro-injection in the marine macro-green alga *Acetabalaria* sp. (Neuhaus et al., 1986), plasmid vectors in the marine diatom *Cyclotella cryptica* (Dunahay et al., 1995), and gene gun or the biolistic method in the macro-red alga *Eucheuma* sp. (Kurtzman and Cheney, 1991) and brown alga *Laminaria japonica* (Qin et al., 1998). Entering the new century, the trend of the genetic engineering of marine algae has been to apply the transgenic marine algae as cell factories and marine bioreactors (León-Bañares et al., 2004; Qin et al., 2005; Zaslavskaia et al., 2001). To date, the most successful algal genetic transformation system is still the model system of the eukaryotic freshwater green alga *Chlamydomonas reinhardtii* (Grossman, 2000) whose nucleus and chloroplast transformations have reached promising commercial relevance

(Franklin and Mayfield, 2004, 2005). Although *C. reinhardtii* may not be a suitable applied species, the technologies established with this species have the potential for applications in other algal species (Hannon et al., 2010).

With today's rapidly growing demand and development for bio-energy from algae and the profitable market for cosmetics and pharmaceuticals from algal natural products, the genetic engineering of marine algae has been attracting an increasing amount of attention as a crucial systemic technology to overcome the biomass problem in industrial applications (John et al., 2011), to modify the metabolic pathway for high-value products (Schmidt et al., 2010), and to engineer the bio-bricks and design the artificial photoautotroph in the rising and promising field of synthetic biology (Heidorn et al., 2011, Muers, 2012). Nevertheless, to date, only a few marine algae species have been genetically manipulated successfully. In this article, an updated account of the research progress in marine algal genomics is presented as well as methods for transformation. In addition, vector construction and gene selection strategies are reviewed. Meanwhile, a review of the progress of bioreactor technologies for marine algae culture is also revisited.

2. Marine algal genomics

Genomes are fundamental for genetic manipulation and further genetic engineering, which not only provide the location and the distribution of metabolic pathways and enzymes but also aid in the identification of elements that can improve genetic engineering, including cis-acting elements, trans-acting factors, and other regulatory elements.

2.1. Cyanobacterial genomics

In evolutionary terms, chloroplasts are cyanobacteria (Allen et al., 2011). The emerging field of marine algal genomics first began with publications of the three genomes of the smallest known oxygen-evolving autotroph *Prochlorococcus* (Dufresne et al., 2003, Rocap et al., 2003). To date, over 20 cyanobacterial genomes have been released. Marine cyanobacteria possess several traits in their genome that are different from other algae. Examples of these differences include their out-membrane light harvesting antenna, a two-component signal transduction system, and their autotrophic metabolism. Many cyanobacteria have water-soluble, light-harvesting protein-pigment complex phycobilisomes, which can reach a width of 40 nm (Yi et al., 2005) and attach to the cytoplasmic surface of the thylakoid membrane. These pigments (chromophores) are phycobilins as opposed to chlorophylls and covalently bind to their apoproteins. In marine *Synechococcus* spp, the genes for the metabolism of phycobiliproteins are concentratedly distributed in several operons or gene clusters. The cyanobacteria can intimately attune to ambient light conditions with shifts in the levels of their phycobilisome composition, i.e., chromatic acclimation (Kehoe and Gutu, 2006), which can be achieved by the regulations of a two-component signal system (Gutu and Kehoe, 2012). However, in *Prochlorococcus*, most of the genes for phycobiliproteins disappear, and only a small set of genes for phycoerythrin Type III and their reductases is conserved in the genome, which suggests that the genes for phycobilierythrin are being lost through selection in the evolutionary process (Ting et al., 2001). Compared to the complicated environments