Genetic modification of the diatom *Phaeodactylum tricornutum* for enhanced production of the carotenoid fucoxanthin

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Microalgae have a diverse and interesting set of uses, such as dietary supplements, lipids, biopolymers, pigments, biofertilizers, and biofuels, with high commercial value and industrial application. In this study we concentrated on *Phaeodactylum tricornutum*, a marine diatom, which has been widely studied due to its potential for fucoxanthin production. Fucoxanthin is one of the main pigments and a major component in the Fucoxanthin-Chlorophyll Protein (FCP) complex of *P. triconrutum*. Fucoxanthin is a carotenoid that can be used as a dietary supplement for its high antioxidant, antinflammatory, antitumor and antiobesity qualitites. Due to its great biotechnological potential but rather low content in algal biomass, improvement of the strain is needed to enhance production in algal biomass. The aim of this work is improvement the fucoxanthin production in *Phaeodactylum tricornutum* by directed mutagenesis.

We performed the Crispr/Cas9 modification system to knock-out genes involved in chlorophyll c biosynthesis. The knock outs were introduced into the target organism *P. tricornutum* by bacterial conjugation. We expect that cells can compensate for the loss of chlorophyll c with the overproduction of fucoxanthin.

Another approach was the overproduction of genes involved in fucoxanthin biosynthesis. We selected five genes (zeta-carotene desaturase, phytoene synthase, phytoene desaturase 1, phytoene desaturase 2, and bacterial-like phytoene desaturase / zeta-carotene desaturase) in the early stage of fucoxanthin biosynthesis. The biolistic assay was carried out to introduce target genes into *P. tricornutum* cultures. We expect that expression of these genes under the fcpA promotor will lead to enhanced production of fucoxanthin.

Part of this work is also modification of the overproduction system by replacing promoters with new, stronger promoters. Endogenous promoters for constitutive and efficient expression of transgenes were obtained from non-coding regions upstream of a transcription initiation site of genes Phatr3_EG01275 and Phatr3_EG01278. The strength of these promoters were checked by expression of green fluorescence protein. Preliminary experiments showed much stronger GFP production compared to fcpA promoter.

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