Algal Research 20 (2016) 61–69

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

A β-carotene ketolase gene (*bkt1*) promoter regulated by sodium acetate and light in a model green microalga *Chlamydomonas reinhardtii*

Chaogang Wang [a,b], Xiaocong Peng [a], Jiangxin Wang [a,c], Anping Lei [a,b], Hui Li [a,b], Zhangli Hu [a,b,*]

[a] Guangdong Technology Research Center for Marine Algal Bioengineering, College of Life Sciences, Shenzhen University, Shenzhen 518060, People's Republic of China
[b] Guangdong Key Laboratory of Plant Epigenetics, College of Life Sciences, Shenzhen University, Shenzhen 518060, People's Republic of China
[c] Nanshan District Key Lab for Biopolymers and Safety Evaluation, Shenzhen University, Shenzhen 518060, People's Republic of China

ARTICLE INFO

Article history:
Received 21 March 2016
Received in revised form 1 August 2016
Accepted 20 September 2016
Available online xxxx

Keywords:
Haematococcus pluvialis
Chlamydomonas reinhardtii
bkt1 promoter
Transgenic algae
Transcriptional regulation
Regulatory mechanism

ABSTRACT

Many microalgae could accumulate high level of astaxanthin, especially a green microalga *Haematococcus pluvialis*. β-carotene ketolase gene (*bkt*) is a key enzyme for astaxanthin biosynthesis in *Haematococcus pluvialis*, and astaxanthin accumulation could be induced effectively by abiotic stresses such as high concentration of sodium acetate and high light. In *Haematococcus* there are 3 *bkt* gene copies, among them, the transcription level of *bkt1* was obviously correlated with high light and sodium acetate. Thus, the function of *bkt1* promoter played a key role in understanding the regulatory mechanisms of astaxanthin biosynthesis. A TATA-box and *cis*-acting elements associated with high light and stress-related responses were detected in *bkt1* promoter in previously studies. However, detailed information about *cis*-acting regulatory elements of this important promoter is still unavailable since a reliable genetic manipulation technique is not available yet. To identify the functions of *cis*-elements in *bkt1* promoter, 8 constructs contained different length of *bkt1* promoter were successfully introduced into a model microalga *Chlamydomonas reinhardtii* and different transformants were obtained with zeomycin resistant selection. Exposed to high light or sodium acetate, differentially increased *ble* transcripts and protein production in transformants indicated that these *cis*-elements respond to high light and sodium acetate. Moreover, we found that TATA-box in *bkt1* promoter is essential for the promoter function and *ble* expression was correlated with *cis*-acting elements in *bkt1* promoter. Our results shed lights on regulation mechanisms of the key genes from *Haematococcus* for astaxanthin biosynthesis in *Chlamydomonas reinhardtii*.

1. Introduction

Haematococcus pluvialis is a eukaryotic unicellular green microalga belonging to Chlorophyta Chlorophyceae. It attracts a lot of attention due to its ability to accumulate the highest amount of astaxanthin under high light and salt stress conditions [1,2]. However, the study for regulation mechanisms behind this is still on its early stage, even though with some efforts. For instance, a number of studies show that β-carotene ketolase (BKT) is a key enzyme for astaxanthin biosynthesis in *H. pluvialis* [3,4]. Under stress conditions, such as high light, sodium acetate or nitrogen deficiency, *bkt* transcriptional levels increased up to 40 fold [5,6], highly correlated with the accumulation of astaxanthin under these stresses [7,8]. Further, Huang et al. [9] found three *bkt* genes (*bkt1*, *bkt2* and *bkt3*) in the genome of *H. pluvialis*. Their transcripts respond differentially under the same stress conditions, indicating the possibility of different transcriptional regulation mechanisms. Thus, it would be interesting to investigate the *bkts* transcriptional regulation mechanisms and to compare the functions of different *bkt* promoters. However, the sequences of *bkt* promoters have not been identified and a reliable genetic transformation system has not established in *H. pluvialis* yet. Qin cloned the partial sequence of β-C-4-oxygenase gene promoter, but failed to validate its responsive elements and functions [10]. Recently two segments upstream of the 5′ end of *bkt1* gene have been isolated by genomic walking technology, and putative regulatory elements responding to light and salinity, such as DRE, ABRE C-repeat/DRE, G-box and MBS have been recognized [11]. However, the TATA-box core sequence in the promoter has not been identified [11]. The fragment of 306 bp upstream of the 5′ end of the *bkt2* transcript showed the promoter activity [12], successfully driving the transient expression of β-galactosidase gene (*lacZ*) in *H. pluvialis* [13]. However, this system was not suitable for stable gene transformation and expression in *Haematococcus*. As a breakthrough on *bkt1* promoter research, Wang et al. [14] had isolated the sequence of *bkt1* promoter from the genomic

Abbreviations: bkt, β-carotene ketolase gene; *RBCS2*, ribulose bisphosphate carboxylase small chain 2; *hsp70A*, heat shock protein hsp70; TAP, tris-acetate-phosphate; SD, standard deviation; TSS, transcription start site; BR1 promoter, *bkt1-RBCS2* fusion promoter; DIG, digoxin; HL, intensive light; Ac, sodium acetate; LIP, light-inducible protein gene; L1, 20 μmol · m^{-2} · s^{-1}; L2, 40 μmol · m^{-2} · s^{-1}; L3, 80 μmol · m^{-2} · s^{-1}.

* Corresponding author.
E-mail address: huzl@szu.edu.cn (Z. Hu).

http://dx.doi.org/10.1016/j.algal.2016.09.020
2211-9264/

Table 1
List of primer sets used in PCR.

Primer name	Oligonucleotide sequence	Product (bp)
PrBle1	5′-GGCCAAGCTGACCAGCGCCGTTC-3′	464
PrBle2	5′-CTCCCGCCCCCACGGCTGCTC-3′	
Act-1	5′-GCGGAGTCGGAGGTTAGGT-3′	852
Act-2	5′-GGCGGCGATGTTTAGATG-3′	
pcrto1	5′-GTTGGATCCGGCACAGTAGTG-3′	1986, *5′bkt*1-2
pcrto2	5′-CAAGATATCTCGTCTAGCTGTGC-3′	
pcrto5	5′-CAGCTAGATGACTGCCTCAC-3′	200, *5′bkt*1-0.2
pcrto6	5′-TCGTCTAGCTGTGCTATGGC-3′	

library of *H. pluvialis*. This provides an opportunity to study the function of *bkt1* promoter and help us understand about the regulatory mechanisms of *bkts* and thus astaxanthin biosynthesis in *H. pluvialis*.

The green microalga *Chlamydomonas reinhardtii* has being used as a model in the research areas such as photosynthesis, flagellar motility and chloroplast function [15]. The transformation methods such as "glass beads" [16] and "micro-particle bombardment" [17] are well established and applied often in gene manipulation of *Chlamydomonas*. The gene of *ble* encoding phleomycin-binding protein has been widely used as a selection marker in this case [18]. The *RBCS2* [19] and *hsp70A* promoters [20] has been proved effective in *Chlamydomonas reinhardtii*. Since both *H. pluvialis* and *C. reinhardtii* belong to Chlorophyceae and share similar morphology and genetic background, we can take advantages of established genetic transformation technology in *C. reinhardtii* to study *H. pluvialis bkts* promoter characteristics.

In the present study, *C. reinhardtii* was employed to elucidate the regulation mechanisms of *bkt1* gene expression through the promoter and to characterize the function of different segments of this promoter. *In silico* analysis of *bkt1* promoter predicted a TATA-box and many *cis*-acting elements associated with light and high salinity stresses. The *bkt1* truncated promoter mutants with different 5′ deletion fragments were obtained by PCR and fused with *ble* gene. And then those truncated promoter constructs were introduced into *C. reinhardtii* with the "glass-bead" method and transformants were selected and verified through zeomycin resistance and PCR technique. Analysis of *bkt1* promoter supported the notion that it could be activated by high light and sodium acetate.

2. Materials and methods

2.1. Algal strains and plasmids

Chlamydomonas reinhardtii strain CC-849 was purchased from the Chlamydomonas Center in the U.S.A. and maintained in College of Life Sciences at Shenzhen University in the medium of tris-acetate-phosphate (TAP) [21], under temperature of 22 °C with continuous light at 20 μmol · m^{-2} · s^{-1}.

For stress treatments, cells in the liquid TAP media at the middle of the exponential stage were inoculated into the appropriate culture flasks with triplicates. Stress conditions were set up as follows: L1, L2, L3 at continuous light exposure at 20, 40 and 80 μmol · m^{-2} · s^{-1}, respectively. The stock sodium acetate solution (3 M) was added to

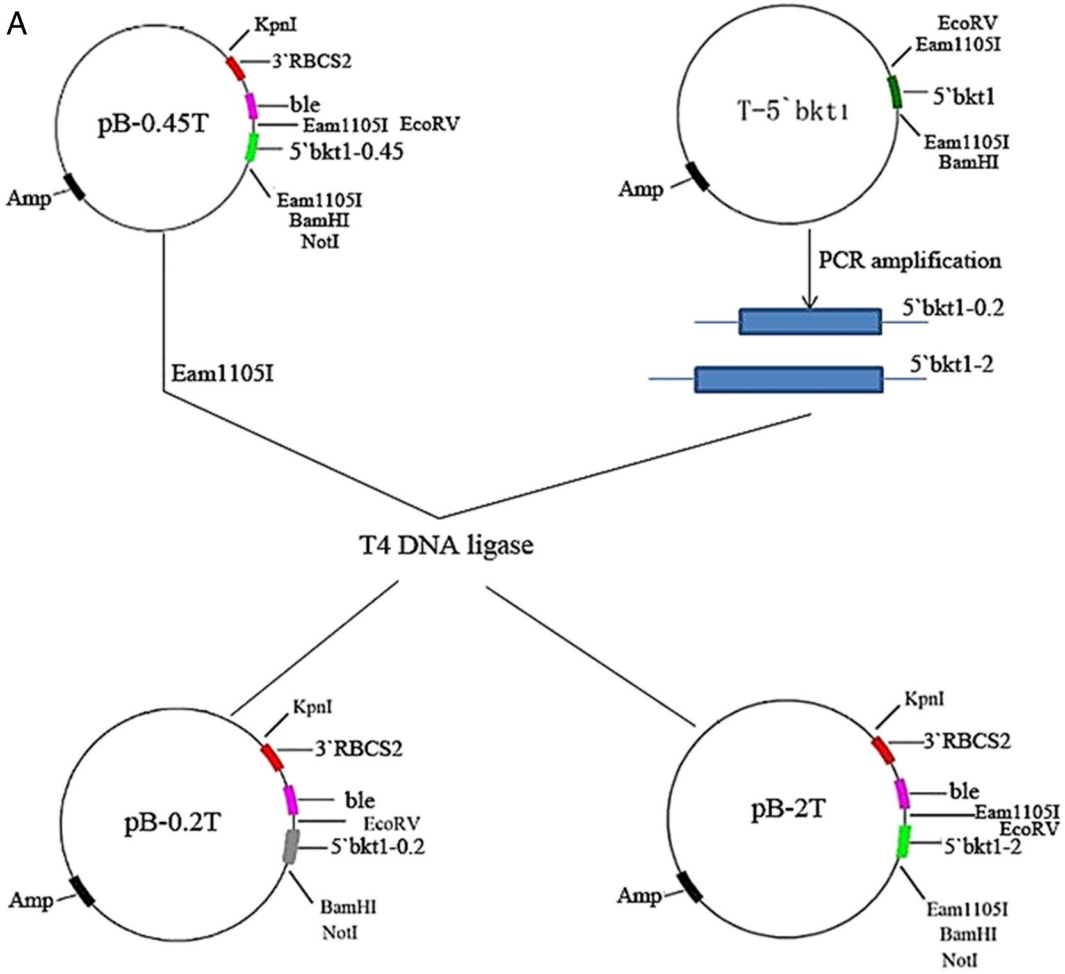

Fig. 1. Map of expression vectors. a) diagram showing the construction of pB-2 and T pB-0.2T. b) diagram showing the construction of pRB-2T, pRB-0.45T and pRB-0.2T.