



Two DNA methyltransferase inhibitors (5-azacytidine and zebularine) affected the carotenoid accumulation in two green algae, *Chlamydomonas reinhardtii* and *Dunaliella bardawil*

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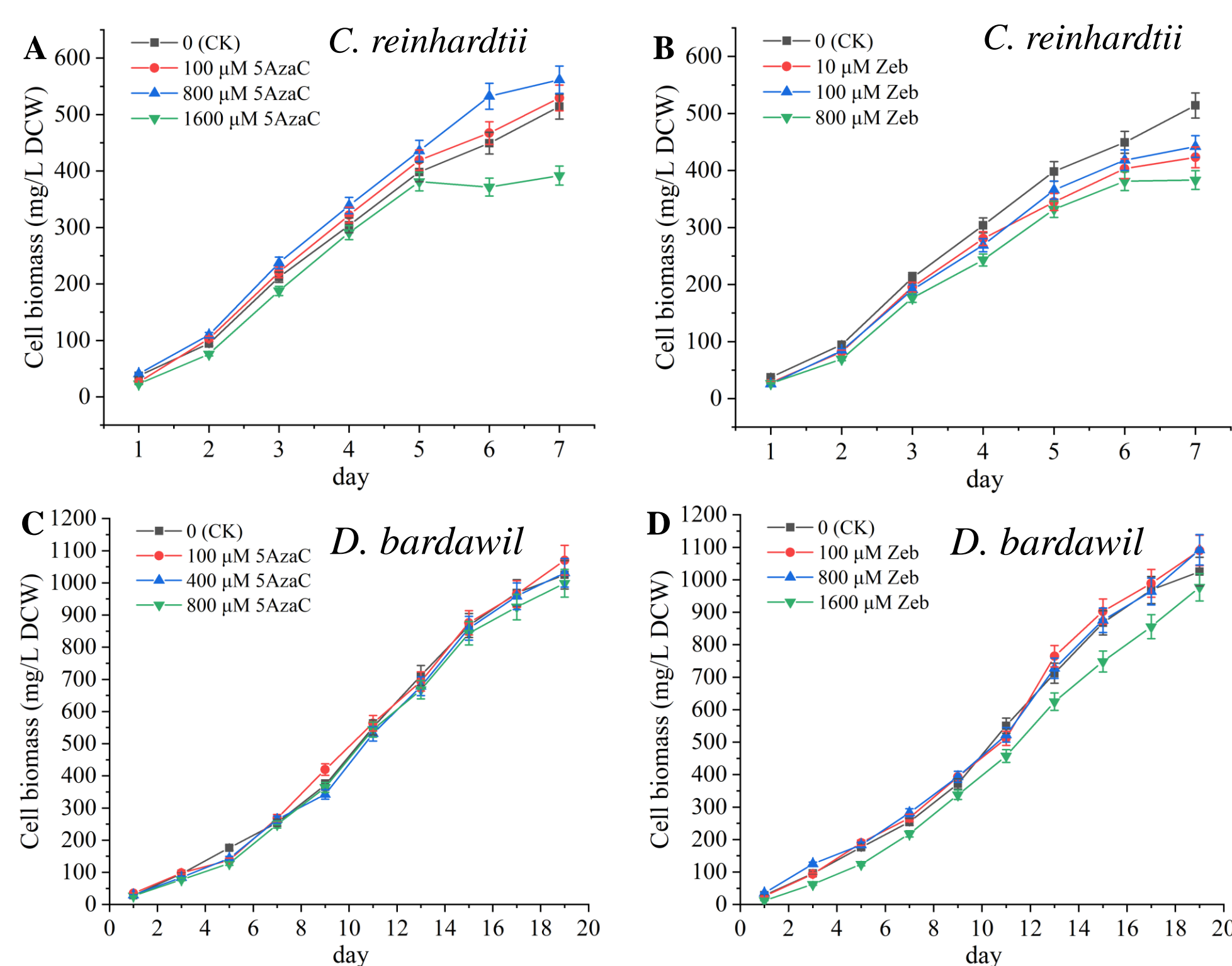
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1. Introduction

DNA methylation especially cytosine methylation (5mC) catalyzed by cytosine-5 DNA methyltransferases (DNMT) plays an important role in cell growth and secondary metabolism. Much research has been done about treatment with DNA methyltransferase inhibitors on plants, whereas the effects of DNA methyltransferase inhibitors especially zebularine on microalgae are less reported. In this study, we investigated the effects of two DNA methyltransferase inhibitors (5-azacytidine [5AzaC] and zebularine [Zeb]) on cell growth, chlorophyll content, carotenoid accumulation, global 5-methylcytosine content and gene expression pattern in *Chlamydomonas reinhardtii* and *Dunaliella bardawil*.

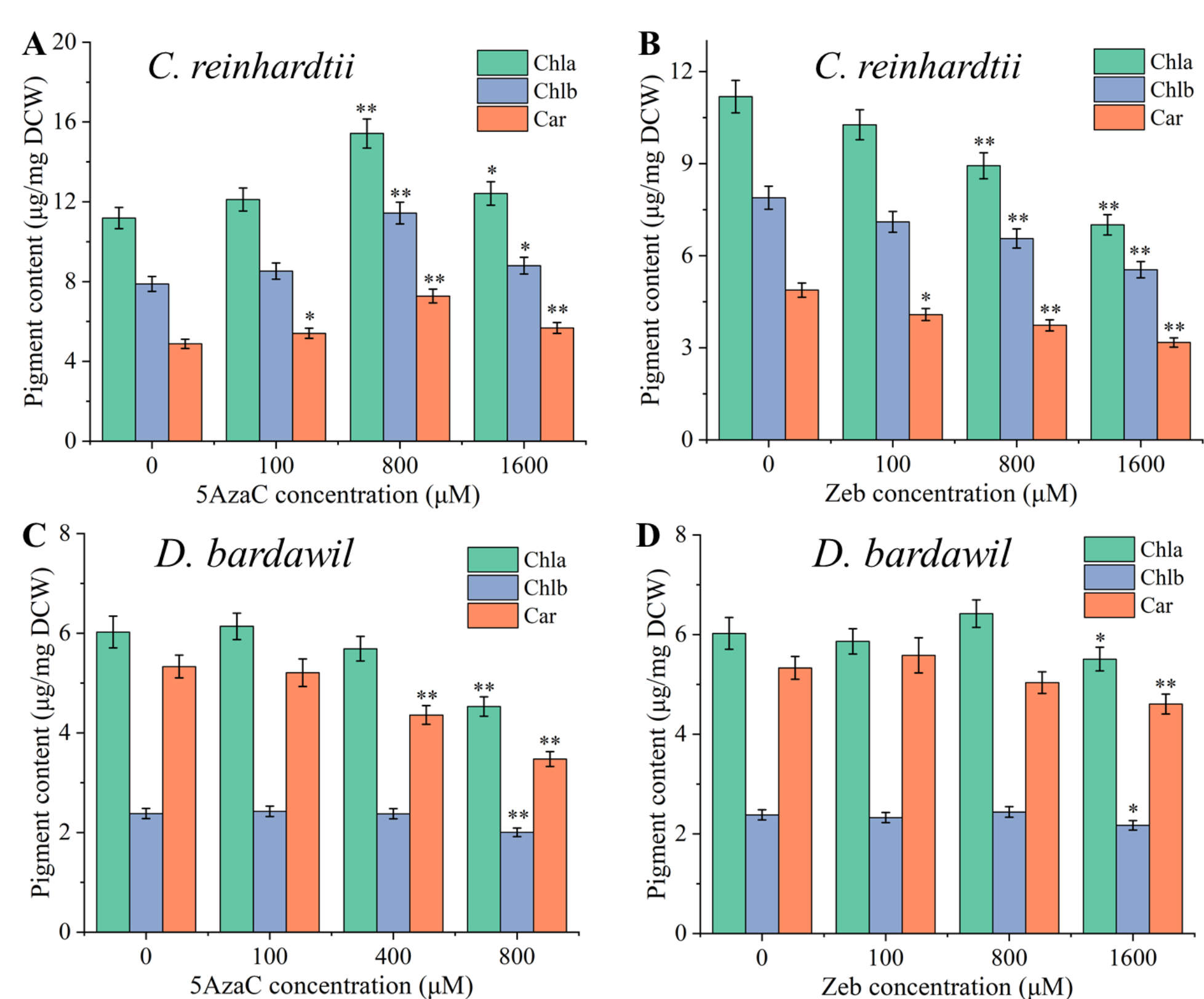
2. Results and Discussion

2.1 5AzaC and Zeb affected cell growth



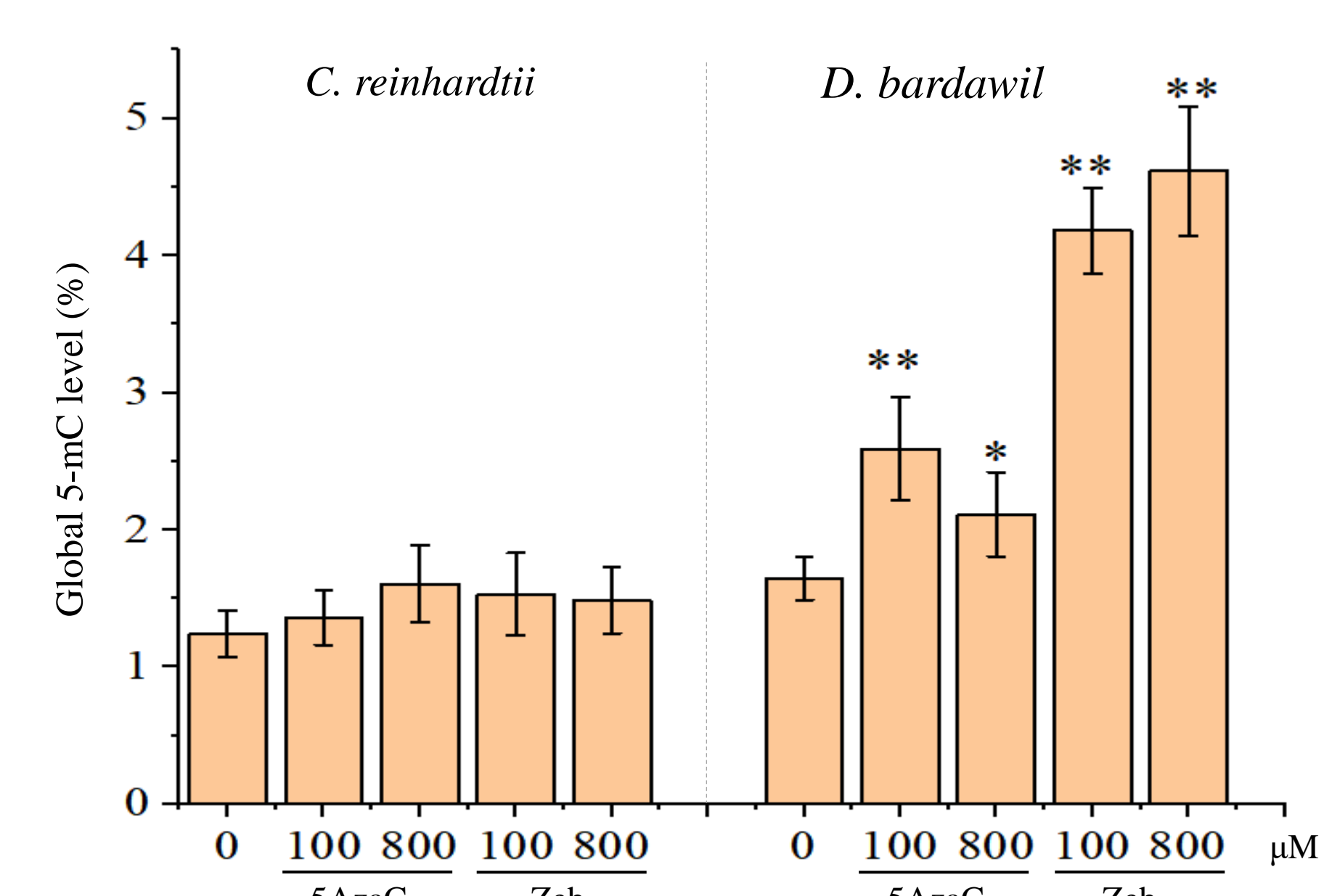
- 800 μM 5-azacytidine accelerated the cell growth of *C. reinhardtii*, but suppressed the cell growth of *D. bardawil*.
- *C. reinhardtii* and *D. bardawil* exposed to zebularine showed delayed growth.

2.2 5AzaC and Zeb affected pigment accumulation



- 800 μM 5-azacytidine enhanced the photosynthetic pigments of *C. reinhardtii*, whereas 800 μM zebularine treatment reduced the carotenoid contents of *C. reinhardtii*.
- 800 μM 5-azacytidine suppressed the pigment accumulation of *D. bardawil*.

2.3 Analysis of global 5-methylcytosine level of algal samples

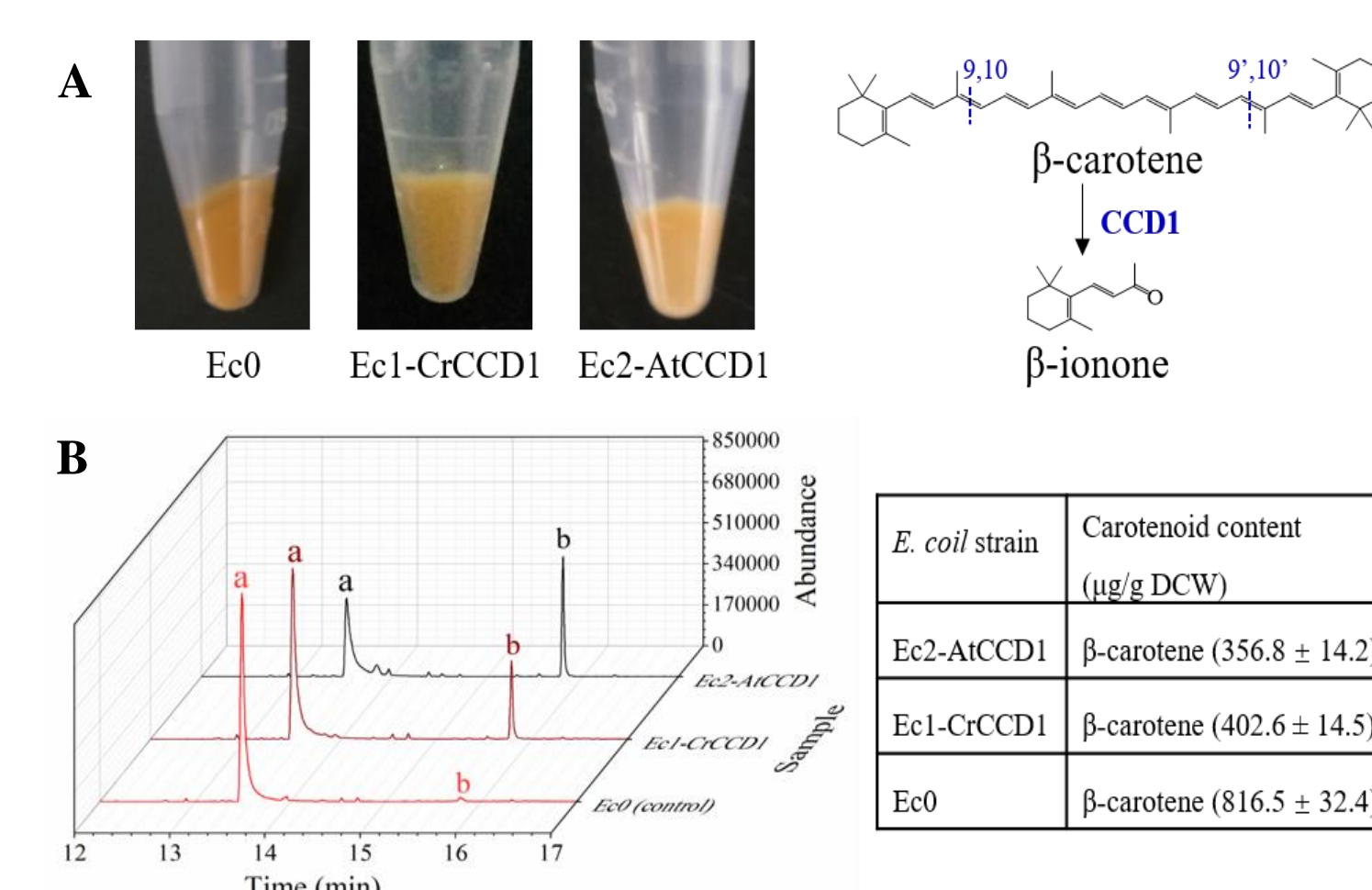


- The 5-azacytidine- and zebularine-treated algal samples showed unexpected hypermethylation.

2.4 Expression of specific genes in response to 5-azacytidine and zebularine in *C. reinhardtii*

Gene ID	Description	Log ₂ (Fold change)	GenBank accession number
Genes of isoprenoid and carotenoid metabolism in CrCK vs CrT1 (5AzaC)			
5719435	15-cis-phytoene desaturase (PDS)	1.35	XP_001693625.1
5723787	prolycopene isomerase (CRTISO)	1.43	XP_001698231.1
5729265	lycopene beta-cyclase (LcyB)	2.85	XP_001703727.1
5724272	beta-carotene 3-hydroxylase (BCH)	1.76	XP_001698698.1
5724445	Carotenoid beta-ring hydroxylase (CYP97A)	2.08	XP_001698892.1
5725398	zeaxanthin epoxidase (ZEP)	2.52	XP_001699847.1
5726439	abscisic acid 8'-hydroxylase	1.15	XP_001701069.1
Genes of isoprenoid and carotenoid metabolism in CrCK vs CrT2 (Zeb)			
5719579	1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR)	-1.30	XP_001693958.1
5724440	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase (MCT)	-2.62	XP_001698942.1
5728730	geranylgeranyl diphosphate synthase (GGPS)	-1.69	XP_001703169.1
5716430	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MECS)	1.36	XP_001690985.1
5716593	geranyl diphosphate synthase (GPS)	2.33	XP_001691069.1
5719435	15-cis-phytoene desaturase (PDS)	1.91	XP_001693625.1
5723787	prolycopene isomerase (CRTISO)	1.67	XP_001698231.1
5729265	lycopene beta-cyclase (LcyB)	2.75	XP_001703727.1
5724272	beta-carotene 3-hydroxylase (BCH)	3.86	XP_001698698.1
5724445	Carotenoid beta-ring hydroxylase (CYP97A)	2.80	XP_001698892.1
5725398	zeaxanthin epoxidase (ZEP)	3.56	XP_001699847.1
5721657	carlactone synthase, carotenoid cleavage dioxygenase (CCD8)	4.45	XP_001696046.1
5721655	carotenoid 9,10(9',10')-cleavage dioxygenase 1 (CCD1)	4.98	XP_001695565.1

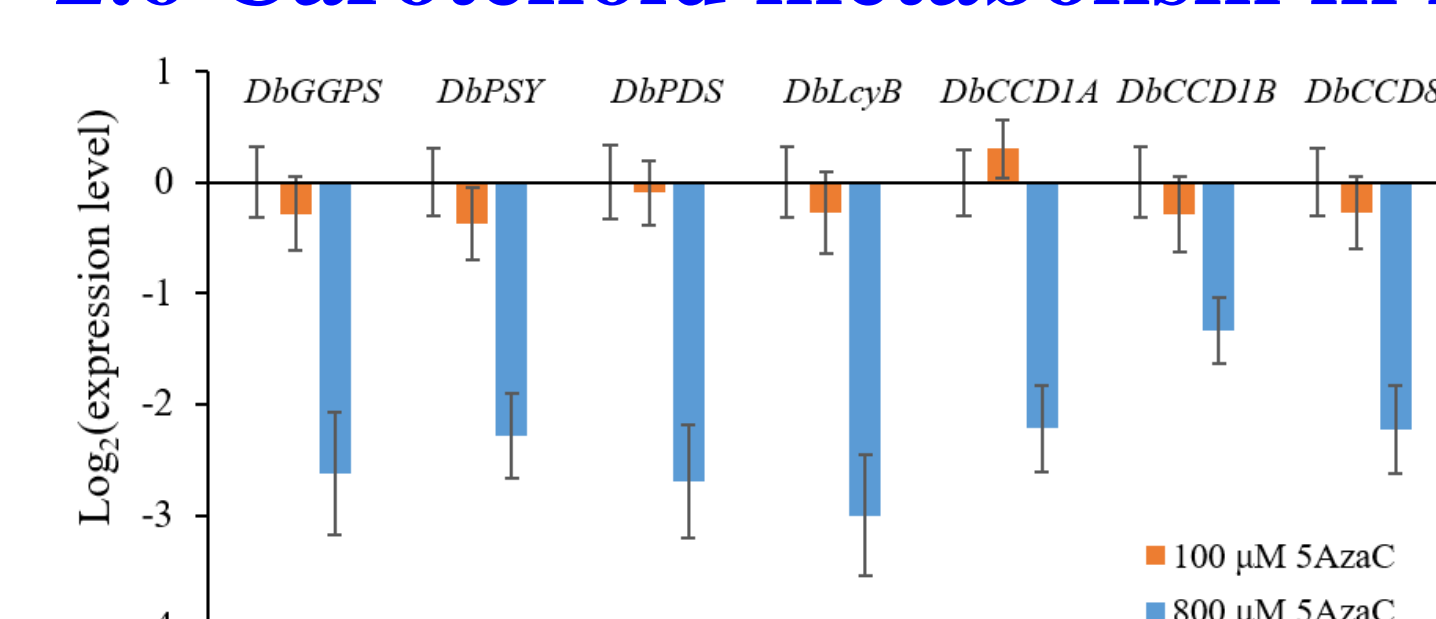
2.5 Functional identification of CrCCD1



- 800 μM 5-azacytidine enhanced the carotenoid accumulation of *C. reinhardtii*, which may be due to the up-regulation of quite a lot of carotenogenic genes, such as *CrPDS*, *CrCRTISO*, *CrLcyB*, *CrBCH*, and *CrCYP97A*.

- 800 μM zebularine repressed carotenoid accumulation of *C. reinhardtii*, which may be associated with the prominent up-regulation of carotenoid cleavage dioxygenase 1 (*CrCCD1*).

2.6 Carotenoid metabolism in response to 5-azacytidine in *D. bardawil*



- In *D. bardawil*, 800 μM 5-azacytidine repressed multiple genes including *DbGGPS*, *DbPSY*, *DbPDS*, and *DbLcyB* responsible for carotenoid metabolism, thus inhibiting the carotenoid accumulation.

Acknowledgements

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