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Molecular Cloning, Bioinformatic Analysis, and Expression Control of DXS Gene in *Isochrysis zhanjiangensis*

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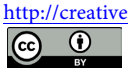


Abstract

Fucoxanthin, a compound with anti-cancer, antioxidant, anti-diabetic, and anti-obesity properties, can be produced in *Isochrysis zhanjiangensis*, a microalga. A key rate-limiting enzyme in MEP pathway for fucoxanthin biosynthesis is 1-deoxy-D-xylulose-5-phosphate synthase (IzDXS). This study aims to investigate the characteristics of IzDXS gene and protein and provide methods to enhance fucoxanthin yield considering its wide potential applications. Bioinformatic analysis, phylogenic analysis, and expression control experiments were conducted based on the sequence of IzDXS gene. Bioinformatic analyses revealed significant motifs and a new potential phosphorylation site, S154, in IzDXS. Phylogenetic analysis suggested a reevaluation of *I. zhanjiangensis*'s classification. Expression control results identified glycine as the most effective elicitor that increased fucoxanthin yield by 55.2 ± 4.1%, highlighting its unique role in enhancing IzDXS gene expression. Other elicitors increased IzDXS mRNA levels but not fucoxanthin yield, indicating complex interactions among transcriptional and post-translational processes affecting fucoxanthin synthesis. This study provides foundational data on DXS and fucoxanthin in *I. zhanjiangensis*, supporting further research into their biosynthesis and regulation.

Subject Areas

Molecular Biology

Keywords

Isochrysis zhanjiangensis, Fucoxanthin, Bioinformatic Characteristics, Elicitors, Transcript-Level Expression

1. Introduction

Fucoxanthin is a chemical that has a wide range of medicinal uses. Various researches have found that fucoxanthin has positive effects on our body including anti-cancer [1]-[5], anti-oxidation [6]-[9], anti-obesity and anti-diabetic [4] [10] [11] whilst having little side effects at a concentration of 2000 mg/kg single dose or 1000 mg/kg 30-day repeated dose on rodents and human skins cells [12]. Therefore, it has a promising perspective in becoming a vital component in medicines and foods. The main sources of fucoxanthin available nowadays include brown macroalgae such as *Laminaria japonica, Eisenia bicyclis,* and *Undaria pinnatifida* due to their presence in some traditional diets. Unfortunately, these macroalgae produce little and limited content of fucoxanthin [13]-[17], which would be unsatisfactory considering the demand for fucoxanthin. On the other hand, microalgae including *Phaeodactylum tricornutum, Chaetoceros gracilis*, and *Nitzschia sp.* considerably increase the content of fucoxanthin [18]. Among microalgae, *Isochrysis aff. galbana* has a high fucoxanthin yield (18.23 ± 0.54 mg/g dried weight content) [19]. Therefore, *Isochrysis zhanjiangensis*, being closely related to *I. aff. galbana*, is selected to investigate if it could lead to yield even more fucoxanthin compared to *I. aff. galbana*.

I. zhanjiangensis is fast-growing, has cellulose-free cell walls, and produces many polyunsaturated fatty acids [20] and carotenoids. Another preponderance of *I. zhanjiangensis* is its good heat tolerance compared to many other algae that produce fucoxanthin, with an optimum growing temperature of about 25˚C and being adaptable below 30˚C [21]. This allows *I. zhanjiangensis* to be bred in more tropical regions thus expanding its economic value when fucoxanthin synthesis is put into application.

Unfortunately, natural fucoxanthin yield in *I. zhanjiangensis* is insufficient, and using elicitors to alter the gene expression of enzymes in fucoxanthin biosynthetic pathway is required. Within the MEP pathway upstream of fucoxanthin synthesis, the enzyme of the key rate-limiting step is 1-deoxy-D-xylulose-5-phosphate synthase (DXS) [22], making DXS a possible important enzyme to be overexpressed to increase fucoxanthin yield. Yields of many secondary metabolites are improved significantly by overexpression of DXS, including abietane diterpenes in *Salvia sclarea* [23], zeaxanthin and antherxanthin in *Chlamydomonas reinhardtii* [24], steviol glycosides in *Stevia rebaudiana* [25]. DXS has good responsiveness to elicitors at transcript levels [26], so it is possible to use similar elicitors on *I. zhanjiangensis* to control DXS mRNA expression to manipulate fucoxanthin accumulation.

Chemicals capable of inducing or inhibiting secondary metabolite production were selected for expression control investigation. Methyl jasmonate (MeJA) upregulates genes including DXS in Italian Riesling grape, causing monoterpenes such as linalool and α-terpineol to accumulate [27]. MeJA increases the expression of DXS in *Ginkgo biloba* to enhance the yield of ginkgolide [26]. With the induction of MeJA, the expression level of DXS in *Tripterygium wilfordii* is raised by

about 26.2 times and the content of triptolide increases more than 3 times [28]. In addition to MeJA being functional in terrestrial plants, MeJA has been tested in algae similar to *I. zhanjiangensis, Dunaliella salina*, for its ability to increase *β*-carotene biosynthesis by 33.85%, which is a chemical highly resembling fucoxanthin and is also present in *I. zhanjiangensis* as a precursor of fucoxanthin [29]. In *Haematococcus pluvialis,* 800 μmol/L MeJA increases DXS mRNA by 2.162-fold and results in an 123.24% increase in astaxanthin content synthesized [30]. Photosynthetic induction factor (PIF) promotes the efficiency of photosynthesis and favors the bioaccumulation of fucoxanthin in *P. tricornutum*, increasing its content by 44.2% [31]. Glycine (GLY) has long been documented as a carbon and nitrogen source that can be readily used by plants. It is known that glycine promotes the accumulation of a range of secondary metabolites including glycosylated flavonoids [32]. Therefore it may act as a non-DXS-specific elicitor. 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was selected as a photosynthesis inhibitor to observe the effect of impeding photosynthesis on the production of fucoxanthin. *P. tricornutum* treated with DCMU produces fucoxanthin 26.98% less than the control group, and examination of genes downstream of DXS all show significant decrease in expression [33]. Acetylsalicylic acid (ASA), reported as a plant hormone involved in inducing abiotic stress tolerance [34], increases DXS mRNA level and accumulation of ginkgolide in *G. biloba* at a dose of 100 mg/L [26]. Naphthylacetic acid (NAA) is an effective plant growth-stimulating hormone that promotes the overall well condition of algae. Elicitors used in our research include MeJA, DCMU, PIF, GLY, ASA, and NAA for identification of the most effective elicitor in promoting fucoxanthin biosynthesis and deepening our understanding of DXS gene and DXS-related metabolic pathway in *I. zhanjiangensis*.

2. Materials and Methods

2.1. Incubation of *I. zhanjiangensis*

Isochrysis zhanjiangensis cultures were grown in nutrient solutions prepared with Crystal Sea, sodium chloride, sodium nitrate, potassium dihydrogen phosphate, iron (III) citrate, and vitamins B_1 and B_{12} at the School of Marine Science, Ningbo University. The cultures were maintained in conical flasks at 20 - 25˚C, with a light intensity of 6000 - 9000 lux and a 12:12 hour light: dark cycle.

2.2. Obtaining IzDXS Sequence

Samples were collected at the exponential growth phase, RNA was extracted using an E.Z.N.A. Plant RNA Kit, and cDNA was synthesized for sequencing. The IzDXS gene was identified and sequenced using specific primers (F: 5'-ATGAAACTGTTCTATGCAGCG-3', R: 5'-TTAAGCCTGTTCTGAAACTTG-3').

2.3. Bioinformatic Analysis

The IzDXS gene was analyzed for evolutionary relationships using NCBI BLAST and MEGA11.0 software to construct a phylogenetic tree. Protein characteristics

including isoelectric point, hydropathicity, and transmembrane tendencies were analyzed using ProtParam, ProtScale, and NetPhos 3.1. A 3D structure of the IzDXS protein was modeled using SWISS model (Table 1).

Table 1. Lists of organisms and their protein ID of DXS protein for construction of phylogenic tree.

Latin name	Protein ID
Artemisia annua	AAD56390.2
Arabidopsis thaliana	CAA74713.1
Solanum lycopersicum	AAD38941.1
Elaeis guineensis	AAS99588.1
Andrographis paniculata	AUG90532.1
Ginkgo biloba	AAS89341.1
Catharanthus roseus	CAA09804.2
Tagetes erecta	AAG10432.1
Salvia miltiorrhiza	ACQ66107.1
Mentha piperita	AAC33513.1
Chloropicon primus	UPR03764.1
Micractinium conductrix	PSC74684.1
Chlorella sorokiniana	PRW20607.1
Dunaliella salina	ACT21080.1
Chlamydomonas reinhardtii	CAA07554.1
Chromochloris zofingiensis	QUS47432.1
Raphidocelis subcapitata	GBF98923.1
Chondrus crispus	XP 005716785.1
Gracilariopsis chorda	PXF46994.1
Porphyridium purpureum	KAA8496827.1
Chrysochromulina tobinii	KOO20858.1
Emiliania huxleyi	XP 005788358.1
Pavlovales sp.	KAJ1635009.1
Isochrysis zhanjiangensis	no data
Phaeodactylum tricornutum	XP 002176386.1
Fragilariopsis cylindrus	OEU19211.1
Nitzschia inconspicua	KAG7370710.1

2.4. Treatment with Different Elicitors

Post-incubation, cultures were treated with various chemical elicitors. IzDXS mRNA levels were quantified using qPCR, and fucoxanthin content was determined spectrophotometrically based on optical density. 120 hours after the start of

incubation, the algae suspension was separated into 7 groups, each composed of 3 conical flasks of algae for repeats. 6 groups were treated with 10.0 mmol/L MeJA, 20 μg/L PIF, 1.6 g/L GLY, 0.2 mg/L DCMU, 10.0 mg/L ASA and 0.3 mg/L NAA respectively. A control group was set up without the addition of any chemicals.

2.5. Quantitative Measurement of IzDXS mRNA Level

24 h after treatment, RNA of IzDXS in each algae sample was extracted and reversely transcribed along with beta-actin gene which was taken as the reference housekeeping gene (Beta-actin F: 5'-TTCCGCTGCCCAGAGGCCCTCTT-3', R: 5'-CGGATGTCAACATCGCACTTCATG-3'). RNA total mass of each sample before reverse transcription is calibrated to be identical to ensure the relative quantity of IzDXS cDNA in samples is comparable after RT-PCR and later in quantitative fluorescent PCR (qPCR). qPCR was carried out with primers FDXS: 5'-ATTTGTTGCCATCTACTCAACC-3' and RDXS: 5'-GACCATCATTTCCGACAACACC-3'.

2.6. Determining the Content of Fucoxanthin in the Sample of *I. zhanjiangensis*

144 h after elicitor treatment, three 5 mL samples were taken from each group for fucoxanthin content measurement. The A_{445} and A_{680} OD values of samples were obtained by UV-5200 METASH spectrophotometer using cuvettes with a width of 1 cm. Formulae below were used to calculate fucoxanthin content:

Using the formula derived from the growth curve of *I. zhanjiangensis* in the same laboratory environment, the cell density was approximated to follow the formula:

$$Cd = 167.7 \times A_{680} + 1.569 \tag{1}$$

where Cd is the cell density (unit: 10^5 cells per mL), A_{680} is optical density absorbance (OD value) of light at a wavelength of 680 nm.

Number of cells is calculated by:

$$Cn = V_1 \times Cd \tag{2}$$

where Cn is number of cells (unit: 10^5 cells) and V_1 is volume of sample solution (unit: mL).

(3) Fucoxanthin content is given by:

$$Fx = \frac{1000 \times A_{445} \times N \times V_1}{A' \times Cn \times 100} \tag{3}$$

where Fx is content of fucoxanthin produced (unit: mg per 10^5 cells), A_{445} is OD value at 445 nm wavelength light, N is the dilution multiple of samples, A' is the theoretical absorbance of 445 nm light of the standard 1 g/L fucoxanthin solution in 1 cm wide cuvette, with a numerical value of 1600.

2.7. Statistical Analysis

Analysis of variance (ANOVA) was applied using SPSSAU statistical analyzer to

show the significance of the difference in fucoxanthin contents between treatment groups. Standard deviations of IzDXS expression level and fucoxanthin content were output from Microsoft Excel 2016.

3. Results

3.1. Cloning and Sequence Analysis of the DXS Gene and Fundamental Biochemical Properties of the DXS Protein in *I. zhanjiangensis*.

The IzDXS gene from *I. zhanjiangensis* spans 2202 bp, encoding 733 amino acids (Figure 1). It shows high similarity to sequences from *Nitzschia inconspicua* (88% positive, 79% identity), *P. tricornutum* (87% positive, 78% identity), and *Seminavis robusta* (86% positive, 78% identity), confirming it as the IzDXS ORF. The molecular formula of IzDXS is $C_{3532}H_{5598}N_{976}O_{1059}S_{31}$ with a molecular weight of 79673.07. It has an isoelectric point of 6.28 and an instability index of 43.19, classifying it as unstable. The protein is predominantly hydrophilic (GRAVY score −0.152), with the most hydrophilic point at S154 (hydropathicity score −2.856). S154 also shows the highest phosphorylation potential (score = 0.998). The secondary structure comprises 39.02% random coil, 37.93% alpha helix, 15.14% extended strand, and 7.91% beta-turn.

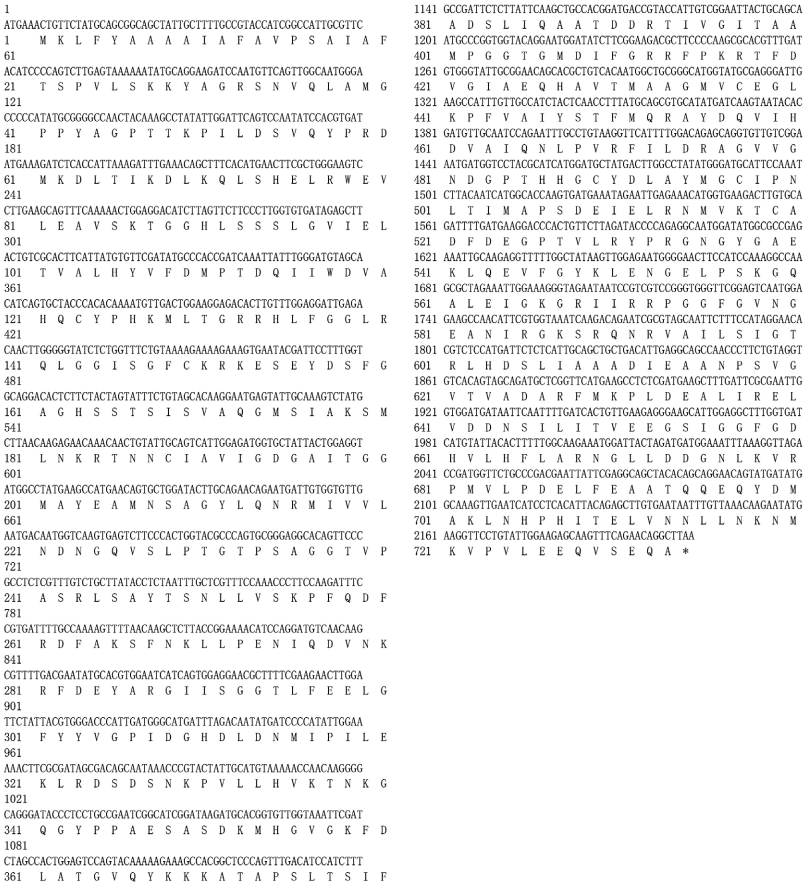
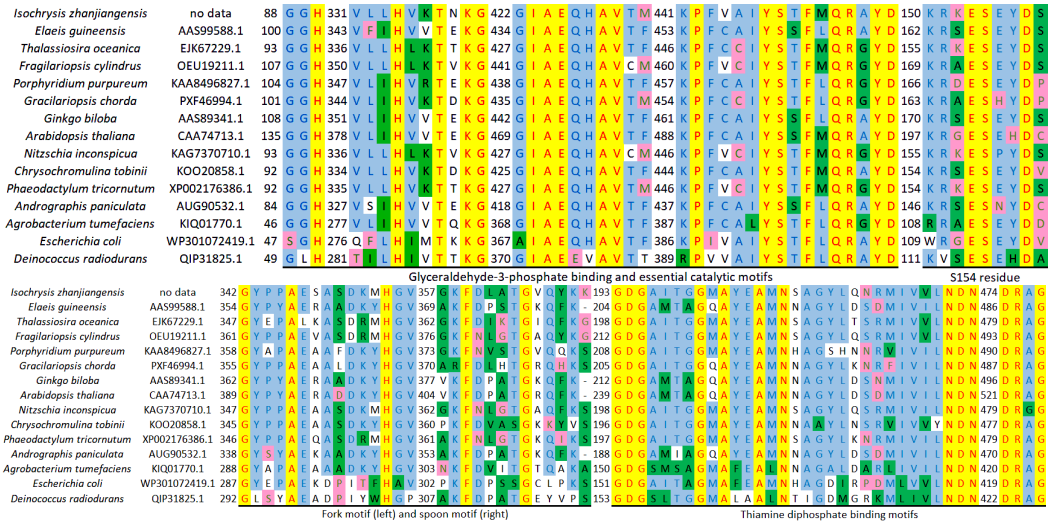


Figure 1. Sequence of IzDXS open reading frame and translated amino acid sequence.

3.2. Tertiary Structure and Functional Motifs of DXS in *I. zhanjiangensis*

IzDXS contains functional motifs for substrate and cofactor binding and catalytic activity (Figure 2). Notable motifs include the thiamine diphosphate binding site (G193-N223) [26] [35] [36] [37], glyceraldehyde-3-phosphate substrate binding motifs (G88-H90, V331-G340, K441-D456) [22] [36], and a pyrimidine ring binding motif (D474-G477) [22] [38]. The protein also features catalytically active sites such as H354, which aids in conformational changes during the catalytic cycle, and "spoon and fork" motifs (G357-K369 and G342-V356) that position catalytic residues near reactants [39].

As shown in Figure 2, conserved sequences highlighted include histidines (H90, H354), the DRAG sequence, and sequences like GDG and NDN in the thiamine diphosphate binding motif, and YS-F-QR-YD in the glyceraldehyde-3-phosphate binding motif. Conversely, the spoon and fork motifs show less conservation.



Note: Red highlighted with yellow: Identical in all organisms tested. Blue highlighted with blue: Identical in most organisms. Black highlighted with green: Different amino acids with similar properties. No highlight: Without much consensus.

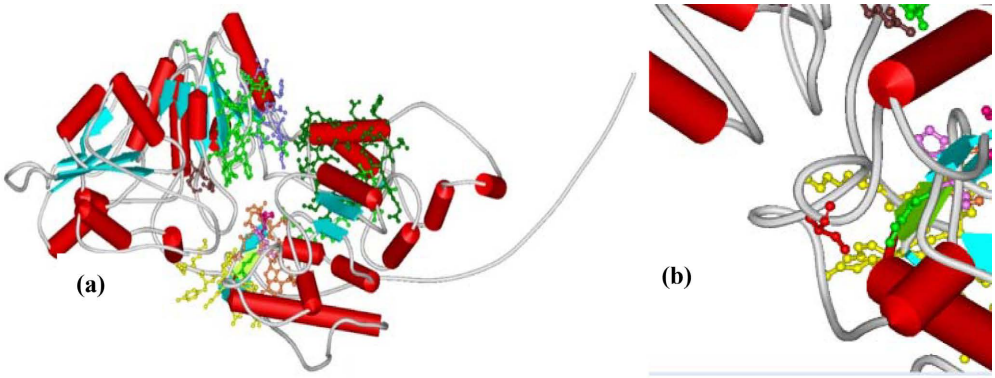
Figure 2. Comparison of DXS motifs between *I. zhanjiangensis* and other organisms.

Structurally, most motifs are centered around the enzyme's cleft, with substrate binding motifs located at the upper side and the spoon and fork motifs at the lower side. This arrangement helps clarify the relative positions and functions of the motifs (Figure 3(a)). In Figure 3(b), S154, a highly conserved sequence noted for its significant hydrophilicity and phosphorylation potential, is positioned near key functional motifs, with its potential role discussed subsequently.

3.3. Phylogenetic Analysis of DXS in *I. zhanjiangensis*

The phylogenic tree results indicated that 5 main groups of organisms were ex-

amined: Phylum *Tracheophyta, Chlorophyta*, *Rhodophyta*, *Haptophyta,* and *Bacillariophyta* (Figure 4). Under *Tracheophyta,* DXS is divided into DXS1 and DXS2, with *A.annua, A. thaliana, S. lycopersicum, E. guineensis, A. paniculata* and *G. biloba* having DXS1 sequence and *C. roseus, T. erecta, S. miltiorrhiza* and *M. piperita* having DXS2. *C. primus, M. conductrix, C. sorokiniana, D. salina, C. reinhardtii, C. zofingiensis, R. subcapitata*belong to *Chlorophyta.C. crispus, G. chorda, P. purpureum* belongs to *Rhodophyta. C. tobinii, E. huxleyi, Pavlovales sp.*, *I. zhanjiangensis* are grouped as *Haptophyta.P. tricornutum, F. cylindrus, N. inconspicua*belong to *Bacillariophyta. N. inconspicua* has the closest genetic relationship to *I. zhanjiangensis*, followed by *P. tricornutum* and *F. cylindrus*.



(a) (1) pink-H90 (2) magenta-H354 (3) orange-G342-V356 (4) yellow-G357-K369 (5) light green-G88-G89, V331-G340, K441-D456 (6) dark green G193-N223 (7) brown D474-G477 (8) purple G422-M431. (b) Position of S154 residue (red) relative to glyceraldehyde-3-phosphate binding motif G88-G89 (light green), H90 (pink) and spoon motif (yellow).

Figure 3. Predicted three-dimensional configuration of IzDXS.

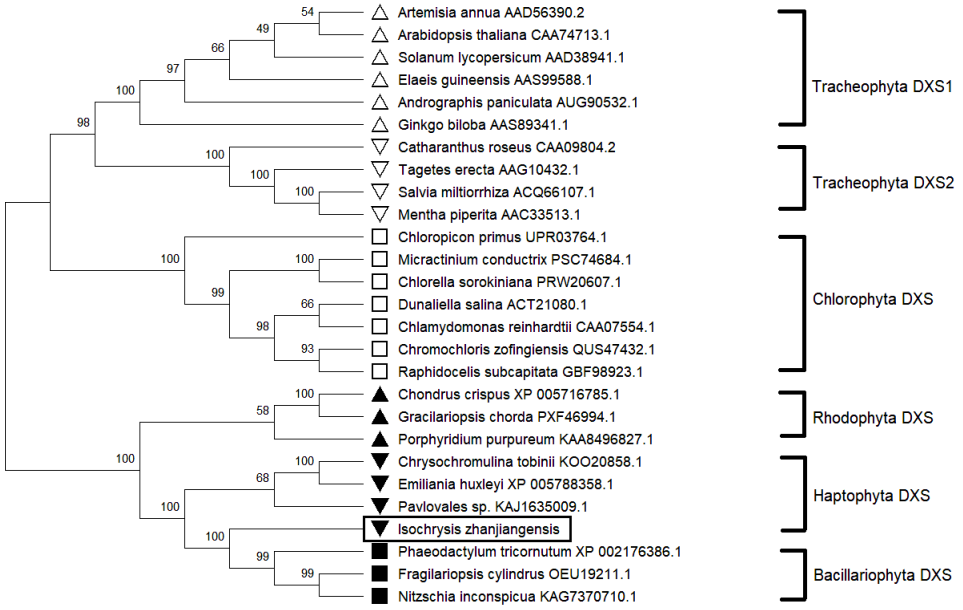


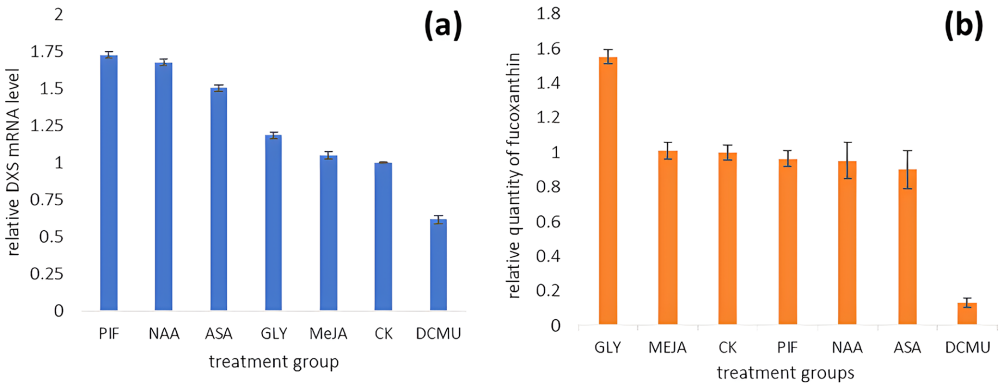
Figure 4. Phylogenic tree showing the evolutionary position of *I. zhanjiangensis* in various plants and algae.

3.4. Expression Control of IzDXS mRNA and Fucoxanthin Content

By qPCR, quantitative relationships of expressed DXS mRNA in different treatment groups were obtained (**Figure 5(a)**). The highest DXS mRNA level was induced by PIF, followed by NAA, ASA, GLY, MeJA, the control group, and DCMU. PIF group had a 72.7 ± 2.1% increase in DXS transcript level expression relative to the control group. NAA, ASA, GLY had an increase of 67.7 ± 2.3%, 50.5 ± 2.1%, and 18.5 ± 2.3% compared to the control group, respectively. The increase of MeJA group is only 4.7 ± 2.5%, though it is statistically significant ($p < 0.05$). In DCMU group, IzDXS mRNA level was only 61.6 ± 2.6% that of the control group. Overall, the best elicitor in increasing IzDXS transcription is PIF.

The fucoxanthin content, however, revealed a pattern different from that of the IzDXS mRNA level (**Figure 5(b)**). Fucoxanthin content was raised most significantly in glycine-treated group ($p < 0.01$). The fucoxanthin content is 155.2 ± 4.1% that of the control group ($5.176 * 10^{-5} \pm 1.38 * 10^{-6}$ mg per 10^5 cells). Other chemicals hypothesized as good elicitors did not reveal a significant increase in fucoxanthin content. MEJA, PIF, NAA, and ASA gave yields similar to that of the control group ($p > 0.05$). Fucoxanthin yield in MEJA group ($3.360 * 10^{-5} \pm 1.60 * 10^{-6}$ mg per 10^5 cells) was closest to that in control group ($3.336 * 10^{-5} \pm 1.42 * 10^{-6}$ mg per 10^5 cells) ($p > 0.05$). Similar to IzDXS mRNA level, DCMU group yielded $4.41 * 10^{-6} \pm 8.9 * 10^{-7}$ mg fucoxanthin per 10^5 cells, which is only 13.2 ± 2.7% of that in the control group. Overall, GLY is most effective in increasing fucoxanthin yield among selected elicitors.

Integrating results of IzDXS mRNA level and fucoxanthin yield (**Figure 6**), it is found that these two parameters did not correlate strongly with each other. Whereas the PIF group induced the highest IzDXS mRNA level, its fucoxanthin yield is lower than that of control group by 3.7% (approximately by $1.25 * 10^{-6}$ mg per 10^5 cells). On the other hand, glycine treatment resulted in the highest fucoxanthin yield but promoted only an 18.5 ± 2.3% increase in DXS mRNA level.



(a) (b)

Figure 5. (a) IzDXS mRNA content level and (b) quantity of fucoxanthin (right) relative to control group sorted in descending order from left to right. Error bar plotted as standard deviations (SD).

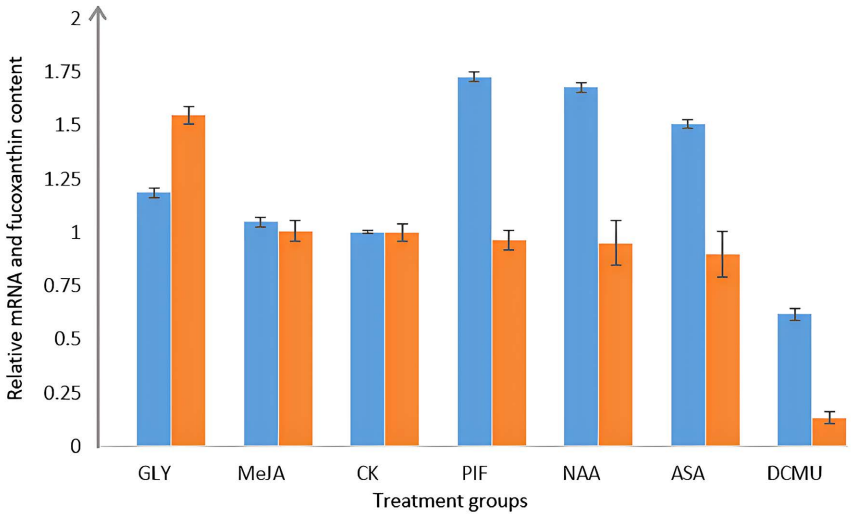


Figure 6. Comparison between relative IzDXS mRNA content (blue bar) and relative fucoxanthin content (orange bar) in different treatment groups.

4. Discussion

4.1. Conservativeness and Function of IzDXS Motifs

Our analysis of DXS sequences across 15 organisms including *I. zhanjiangensis* in **Figure 2** shows that substrate and cofactor binding motifs are more conserved than the spoon and fork motif. For instance, glyceraldehyde-3-phosphate binding motif K441-D456 has 50% (8 of 16) amino acids identical in all 15 species, whereas that is only 20% (3 of 15) in fork motif. This difference in extent of conservation is consistent with previous studies on roles of motifs—binding motifs precisely fits cofactors and substrates, whereas the spoon and fork motif, facilitates only conformational shifts, requiring less sequence precision [39] [40]. The exception is the highly conserved H354 residue, crucial for pre-decarboxylation processes and binding pyruvate-derived intermediates [39]. This consistency supported accurate motif positioning in our sequence analysis, and established regions of motifs along the whole amino acid sequence of IzDXS that could be of particular interest for further investigations in terms of their functions and importance in *I. zhanjiangensis*.

4.2. Serine154 Residue in *I. zhanjiangensis* DXS

S154 stands out for its lowest hydropathicity (−2.856) and highest phosphorylation potential (99.8%), suggesting a unique role in activating or inactivating the IzDXS enzyme. This residue does not correspond to known phosphorylation sites in related species, pointing to a potentially novel site in IzDXS. As shown in **Figure 3(b)**, located near key motifs on the enzyme's outer surface, S154 is ideally positioned for kinase accessibility, which could significantly affect enzyme activity upon phosphorylation. This discovery based on bioinformatic parameters could be of interest for altering the activity of IzDXS, in turn affecting the yield of fucoxanthin.

4.3. Evolutionary Relationship and Classification Status of *I. zhanjiangensis*

From the phylogenic tree, it is concluded that DXS amino acid sequences are useful in approximating the classification status of specific species, as interspecies differences in DXS sequence reflect their classification relationship in most cases. However, *I. zhanjiangensis* is shown to have a closer relationship to *Bacillariophyta* instead of *Haptophyta* by IzDXS amino acid sequence, which is contradictory with its current taxonomic status in Phylum *Haptophyta*. Since both *Haptophyta* and *Bacillariophyta* originated from Phylum *Chrysophyta* and were separated due to distinctive anatomical and morphological features[37], grouping *I. zhanjiangensis* into *Bacillariophyta* instead of *Haptophyta* may not be estranged. What remains as a point of discussion is that IzDXS amino acid sequence has a higher identity (79%) with *N. inconspicua*, a member of *Bacillariophyta* than that with *E. huxleyi* (65%) which belongs to the same order (*Isochrysidales*) as that of *I. zhanjiangensis*. It is suggested that further examination and comparison of 18S ribosomal RNA of *I. zhanjiangensis* with a wider range of algae from different phyla, especially those from Bacillariophyta, may help confirm the classification status of *I. zhanjiangensis*.

4.4. Analysis of IzDXS Expression and Fucoxanthin Production

Our qPCR analysis showed that 0.2 mg/L DCMU decreased IzDXS mRNA levels to 61.6% and fucoxanthin levels to 13% of the control, consistent with studies showing DCMU's inhibitory effect on photosynthesis and downstream gene expression. Conversely, photosynthetic enhancer PIF increased IzDXS mRNA 1.73 times, indicating a positive correlation between photosynthesis and DXS gene transcription, which influences the MEP pathway and fucoxanthin synthesis.

Our other results showed differences from what was previously documented. In past research, glycine has been widely used as a multifunctional nutrient that provides both carbon and nitrogen in a structure that can easily enter the metabolic pathway to be assimilated and utilized by organisms, such as *Penicillium janthinellum* [41] and *E. coli* [42]. Therefore, glycine was not documented as a specialized elicitor targeted for a pathway closely related to fucoxanthin production. Unexpectedly, from our result, glycine induces a nearly 50% increase in final fucoxanthin content by acting as a gene expression elicitor to stimulate IzDXS mRNA expression in the synthesis of fucoxanthin. It is speculated that glycine may enter the metabolic pathway and either be converted to or stimulate the production of, a form of elicitor that facilitates fucoxanthin production via regulating gene expression.

Treatments with PIF, NAA, and ASA significantly boosted IzDXS mRNA but not fucoxanthin yield (**Figure 6**), suggesting other regulatory or rate-limiting steps may be influencing fucoxanthin synthesis. This was not observed with MeJA treatment, which showed no increase in mRNA or fucoxanthin, contrast-

ing with previous studies where MeJA enhanced DXS mRNA and fucoxanthin levels significantly.

These unexpected differences are likely to be explained on closer inspection between factors that cause the correlation between IzDXS and mRNA content to hardly follow a specific trend. Two main reasons were considered as the presence of other rate-limiting steps or post-transcriptional and post-translational regulations. Since DXS is the rate-limiting step within MEP pathway[22], downstream fucoxanthin synthesis could have other rate-limiting enzymes. This would explain how glycine promoted the highest yield of fucoxanthin yet did not elicit the greatest quantity of IzDXS mRNA as glycine may have elicited the expression of multiple genes related to fucoxanthin synthesis, and particularly some genes downstream of IzDXS. Such an explanation is also consistent with the result of PIF group, in which the quantity of IzDXS is enhanced to the most significant extent but has an insignificant difference in fucoxanthin produced compared to the control group, perhaps owing to a bottleneck effect imposed by rate-limiting enzymes downstream.

4.5. Advantages, Limitations, and Outlook

Our study provides new and foundational data about the sequence, properties, and functions of IzDXS gene and protein, providing aspects of particular interest for further investigations. Nevertheless, it did not include further details on the mechanism of glycine as an elicitor and post-transcriptional and post-translational modifications of IzDXS. Future studies could focus on the effects of glycine on downstream genes in the fucoxanthin pathway to uncover how it significantly boosts fucoxanthin production and to determine if other rate-limiting enzymes play a role. Additionally, investigating post-transcriptional and post-translational modifications could provide insights into the intricate control mechanisms affecting the correlation between mRNA levels and fucoxanthin yield.

5. Conclusion

In this study, bioinformatic analysis revealed important motifs in IzDXS along with the discovery of a new possible phosphorylation site S154. Phylogenetic inconsistencies with current taxonomy suggested a reevaluation of status of *I. zhanjiangensis*. Expression analysis revealed that among elicitors tested, glycine most effectively increased fucoxanthin yield and upregulated IzDXS gene expression. Results of other elicitors indicate complex relationships between IzDXS mRNA levels and fucoxanthin production. Our research provides new insights into IzDXS gene and protein in terms of their bioinformatic properties, phylogenetics and expression regulations which lay the foundations for future research on IzDXS and fucoxanthin biosynthesis.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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