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Laminaria japonica hydrolysate promotes fucoxanthin accumulation in *Phaeodactylum tricornutum*

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Abstract

Fucoxanthin (Fx) has gained a growing attention due to the remarkable biological activities. The limited biomass of was the restrictive factor for Fx production in *Phaeodactylum tricornutum*. In this study, *Laminaria japonica* hydrolysate (LPH) with a low addition proportion of 1.5 ml/L, was proved to promote fucoxanthin accumulation and cell growth simultaneously. Fx topped at 27.9 mg/L after 10-d cultivation in the LPH group, with a biomass of 1.59 g/L and a Fx content of 17.55 mg/g. Three key plant hormones in LPH were screened responsible for promoting fucoxanthin accumulation. Transcriptomic analysis and qRT-PCR results showed that genes related to Fx formation were generally up- regulated. The study demonstrated that LPH addition was a feasible and efficient strategy to enhance production of fucoxanthin, facilitating the scale-up production of Fx in autotrophic culture.

Keywords: Fucoxanthin, *Phaeodactylum tricornutum*, Autotrophic culture, *Laminaria japonica*, Plant hormones,

Introduction

Due to the remarkable biological activities, especially the obvious effects against Alzheimer's disease and diabetics, fucoxanthin (Fx) has gained a growing attention (Fu et al., 2015; Xiang et al., 2017). Global Fx production is expanding rapidly, with a considerable annual growth rate of 5.3% (Joel, 2016). Current commercial Fx was extracted from brown seaweeds (Verma et al., 2017). Although newly developed extraction methods have improved the yield of bioactive compounds from alga, commercial Fx extraction was still evaluated with low purity and high cost, caused by the low Fx content of brown seaweeds (Cajnko et al., 2019; Eleršek et al., 2020; Novak et al., 2020; Verma et al., 2017).

Microalgaes have been proved to be alternative source of Fx, including *Mallomonas* sp., *Tisochrysis lutea*, and *Phaeodactylum tricornutum*, with the Fx contents more than 100 folds of brown seaweeds (Gao et al., 2020; Petrushkina et al., 2017; McClure et al., 2018). Marine diatom *Phaeodactylum tricornutum*, has been widely studied to accumulate Fx, for higher growth rate and higher Fx content (10 mg/g to 60 mg/g) (Yan et al., 2020; McClure et al., 2018). Artificial photobioreactors has been developed for large-scale culture of *P. tricornutum* (Yan et al., 2020; McClure et al., 2018).

The limited biomass of *P. tricornutum* in artificial photobioreactors was the restrictive factor for improving Fx production (Yan et al., 2020; McClure et al., 2018). Mixotrophic culture of *P. tricornutum* strains significantly enhance the growth rate and increase the biomass, based on the ability of this microalgae for assimilating organic

carbon and nitrogen source. However, the photosynthesis activity was reduced considerably and Fx content was reduced to 5 mg/g-7 mg/g (Ceron-Garcia et al., 2013; Patel et al., 2019). In mixotrophic culture, it was considered not feasible to improve Fx content and biomass simultaneously. Therefore, it is important to explore active additives for achieving higher biomass of *P. tricornutum* in autotrophic culture.

In this study, *Laminaria japonica* hydrolysate (LPH) was proved to promote fucoxanthin accumulation and cell growth simultaneously, with a low addition proportion of 1.5 ml/L. Key plant hormones in LPH responsible for promoting fucoxanthin accumulation were screened. qRT-PCR showed that genes related to Fx biosynthesis were generally up- regulated.

2 Material and Methods

2.1 Autotrophic culture conditions

P. tricornutum X1 strain was isolated from coastal waters of Qingdao and stored in liquid nitrogen. Activated X1 strain was cultivated in f/2 medium as seed culture (Gao, et al., 2020). The seed cultures were incubated at 23 °C with 16L:8D photoperiod and a light intensity of 2000 lux, until cell density achieved 1.5×10^7. Then the seed culture was switched to 500-mL flasks containing 300-mL f/2 medium to produce Fx at 23 °C, with 16L:8D photoperiod and a light intensity of 5500 lux. The culture proceeded for a total period of 10 d.

2.2 Addition of active additives in autotrophic culture

The fresh *L. japonica* was dissolved in distilled water at a ratio of 1:1(w/v). 0.5 % (w/w) Celluclast 1.5L (Novozyme A/S, Denmark) was added and incubated at 40 °C for 4 h. Then, 1% (w/w) alginate lyase liquid of AlyL1 was added and incubated at 40 °C and pH 7.0 for 4 h (Li et al., 2019). The reaction mixture was centrifuged at 8000×g to get the supernatant, LPH liquid. LPH with different dosages (0.75-3.0 ml/L) were immediately added after the seed culture was switched. The culture proceeded for 10 d, and 50 ml broth was taken and centrifuged at 8000 × g and freeze-dried to get the cells every day. The biomass was monitored using weighing method and Fx content was detected as below.

LPH addition brought about concentration increase of carbohydrates and plant hormones. Every single component with the concentration equal in 1.5 ml/L LPH was added in the culture separately as above. The components included 12.8 mg/L glucose, 22.8 mg/L mannitol, 44.9 mg/L alginate oligosaccharides, 0.063 mg/L abscisic acid (ABA), 0.71 mg/L gibberellins 3 (GA3), 0.042 mg/L indole-3-carboxaldehyde (ICA), 0.37 mg/L indole-3-acetic acid (IAA), and 1.26 mg/L salicylic acid (SA), respectively. Samples cultivated in original f/2 medium was set as control. The cultures proceeded for 10 d, biomass and Fx content were monitored every day.

2.3 Fx measurement

P. tricornutum biomass was extracted by 10 mL ethanol using ULTRA-TURRAX Tube Drive (IKA, Germany) for 10 min, with a rotate speed of 4000 rpm. The supernatant was pelleted by centrifugation and concentrated to 4 mL using Rotational-Vacuum-

Concentrator (Chrisrt, Germany) at 65 ºC. The Fx solutions were filtered using 0.22 μm filtration membrane and run in a high-performance liquid chromatography (HPLC) system (Waters, USA). The stationary phase for HPLC analysis was chosen as a C18 column (Kinetex C18, 5 μm, 100 Å 150 × 4.6 mm). The mobile phase was acetonitrile: ultra-pure water =85:15 (Gao, et al., 2020).

2.4 Transcriptomic analysis and qRT-PCR

Strain X1 was cultured in f/2 medium with or without 1.5 ml/L LPH for 4 d, the cells were collected by centrifugation. Total RNA was isolated using TRIzol reagents and reversed transcribed into cDNA. The cDNA was sent for transcriptome sequencing (Novogene, China). Meanwhile, strain X1 was cultured in f/2 mediums containing 0.042 mg/L ICA, 0.37 mg/L IAA, or 1.26 mg/L SA, respectively. qRT-PCR was carried out in triplicates with the SYBR PCR master mix (Applied Biosystems). Relative transcriptional level of the genes in mediums containing LPH or plant hormones were analyzed by caculating the CT values. Samples cultivated in original f/2 medium was set as control.

3 Results and discussion

3.1 Effect of LPH addition on Fx production

Laminaria japonica biomass with huge annual production, has been developed as crop fertilizer and plant regulator (Gao et al., 2017). LPH with different dosages (0.75-3.0 ml/L) were immediately added to detected the effects on biomass and Fx production. As shown in Fig. 1a, with 1.5 mL/L LPH added in the culture, biomass was increased to 0.67 g/L after 4-d culture. The corresponding Fx production was 22.19 mg/L, nearly twice

of that in the control. With more LPH added, no increase of growth and Fx accumulation was observed. The results indicated that LPH can significantly stimulate growth and Fx accumulation; and 1.5 ml/L was chosen as the optimal addition proportion of LPH.Then, time courses of biomass and Fx accumulation in LPH group and control group were monitored. As shown in Fig. 1b, Fx of the both groups accumulated significantly in the first 4 days, and the accumulating rates slowed quickly from the 6th day to 10th day. However, the biomass increased steadily during the whole culture period. Fx topped at 27.9 mg/L after 10 d of cultivation in the LPH group, with a biomass of 1.59 g/L and a Fx content of 17.55 mg/g.

Different strategies were adapted to promote Fx production in *P. tricornutum*. Using 0.5% CO_2 as carbon source, Fx production in *P. tricornutum* CCAP1055/1 was increased to 26.79 mg/L by optimizing light intensity, with a biomass of lower than 0.2 g/L (Conceicao et al., 2020). In other study, by combining favorable conditions including light intensity, media composition, and CO_2 concentration, higher fucoxanthin content of 42.8 mg/g was achieved, with a biomass of 0.37 g/L (McClure et al., 2018). LED light shift was also used to improve fucoxanthin production in mixotrophic culture of *P. tricornutum* (Yan et al., 2020). Compared with the previous strategies, LPH addition strategy leads to higher and more efficient production of fucoxanthin. Coupled with the characteristic of low cost and no extra equipment introduced, LPH addition was proved a feasible and efficient strategy to enhance fucoxanthin production.

3.2 Screening key plant hormones in LPH

Fx accumulation and growth were maintained stable, with addition of glucose, mannitol, or alginate oligosaccharides. Thus, the carbohydrates were not the driving force to promote Fx accumulation. As shown in Fig.2a, all the five plant hormones can efficiently stimulate the growth of *P. tricornutum* X1. However, only addition of IAA, ICA, and SA can significantly promote Fx accumulation. The Fx production in culture with IAA, ICA, or SA addition, were 20.9 mg/L, 17.8 mg/L, and 16.7 mg/L, respectively. The productions were all more than 50% higher than that in the control. The results indicated that IAA, ICA, and SA were the key plant hormones in LPH, responsible for promoting fucoxanthin accumulation. The effect of LPH addition on biomass and Fx production was the result of synergistic effect of the key plant hormones.

Recently, plant hormones have been proved a powerful method to enhance accumulation of the carotenoids and unsaturated fatty acids in various microalgae (Zhao et al., 2019). By adding 7.8 mg/L IAA and 10 mg/L indole propionic acid, the maximum astaxanthin content of 13.1 mg/g and production of 89.9 mg/L were obtained in *Chromochloris zofingiensis* (Chen et al., 2020). In addition, by treatment with 70-250 μmol/L SA and 10 μmol/L methyl jasmonate, total carotenoids were significant improved in *Dunaliella salina* and *Tetraselmis suecica* (Ahmed et al., 2015). In this study, plant hormones were found to promote fucoxanthin accumulation for the first time.

3.3 Transcriptional analysis of key genes in Fx synthesis pathway

Fx biosynthesis was involved with several pathways, including MEP pathway, IPP pathway and final Fx formation (Bertrand, 2010). Transcriptomic analysis showed that

up-regulated genes were obviously enriched in several pathways, including photosynthesis, carbon fixation, porphyrin and chlorophyll metabolism, glycolysis, and carbon metabolism in the LPH group. These up-regulated pathways construct the most parts of Fx synthesis pathway. To further verify this, the transcriptional level of genes in Fx synthesis pathway were further verified by qRT-PCR.

As show in Fig.2b, Fx biosynthesis pathway was generally clear (Dambek et al., 2012). The rate-limiting steps of Fx biosynthesis were catalyzed by DXS and PSY (Eilers et al., 2016). In the LPH group, the transcriptional level of *DXS* and *PSY* were 1.23 folds and 8.40 folds to those in the control group, suggesting that LPH induced more carbon flux redirected to pigments formation. The other genes of Fx biosynthesis were also generally up-regulated (Fig.2b). It was noteworthy that the transcriptional level of *ZEP1*, *ZEP2*, and *ZEP3* were all very significantly upregulated by adding LPH or the key plant hormones (Fig.2b, Fig.2c). Among these genes, *ZEP3* was the most sensitive one (Fig.2b, Fig.2c). In the LPH group, the transcriptional level of *ZEP3* was 12.23 folds higher, while the transcriptional level of the same gene were 7.14 folds, 7.00 folds, and 3.22 folds higher in the three plant hormone groups, respectively. In the previous study, the transcriptional level of *ZEP1*, *ZEP2*, and *ZEP3* were also up-regulated by blue light (Yan et al., 2020). The results indicated these three genes can be important regulation targets to respond to environment factors. In the LPH and plant hormone groups, only *VDE* was down-regulated, demonstrating that VDE may not play a positive role in Fx synthesis.

4. Conclusion

In this study, LPH was proved to promote fucoxanthin accumulation and growth simultaneously, with a low addition proportion of 1.5 ml/L. Fx in LPH group was nearly twice of that in the control. Coupled with the low cost and simple prepared method, LPH addition can be a feasible and efficient strategy to enhance production of fucoxanthin, facilitating the scale-up production of Fx in autotrophic culture.

CRediT authorship contribution statement

Zhi-Peng Wang: Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Pei-Kang Wang:** Investigation, Formal analysis, Data curation. **Yan Ma:** Writing - review & editing. **Jia-Xue Lin:** Investigation, Software. **Cheng-Long Wang**: Investigation, Visualization. **Yu-Xiang Zhao:** Visualization**. Xin-Yue Zhang:** Software. **Bei-Chen Huang:** Methodology. **Shou-Geng Zhao:** Software. **Lei Gao:** Methodology. **Jing Jiang:** Methodology. **Hai-Ying Wang:** Funding acquisition. **Wei Chen:** Supervision, Writing - review & editing.

Conflict of interests

The authors declare that they have no competing interests.

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Figure Captions

Fig.1 (a) Effect of LPH on fucoxanthin accumulation and growth in *P. tricornutum* X1. (b) Time course of fucoxanthin production and biomass of strain X1 in the medium with or without 1.5 ml/L LPH. Data are given as means ± standard deviation, n = 3.

Fig. 2 (a) Effect of plant hormones on fucoxanthin accumulation and growth in *P. tricornutum* X1. Data are given as means ± standard deviation, n = 3. (b) Key enzymes of Fx formation in *P. tricornutum*. Figures in bold represent the changes of the transcriptional level in LPH group. (c) Effect of plant hormones on transcriptional level of the genes related to Fx formation. Data are given as means ± standard deviation, n = 3.

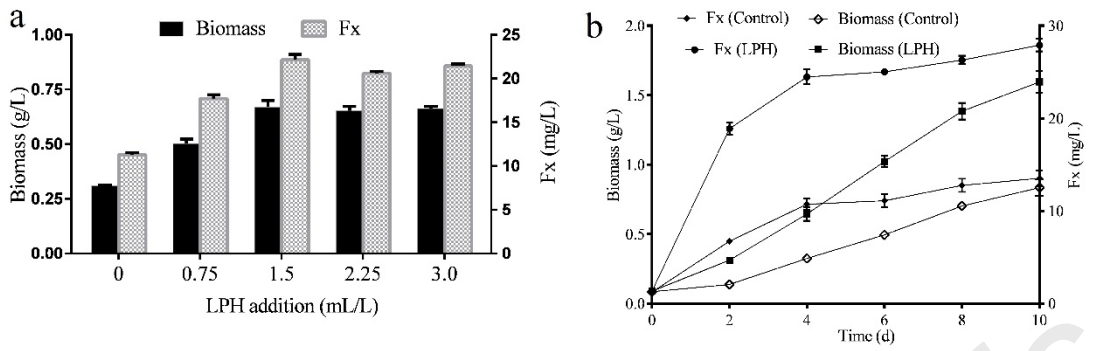

Fig.1

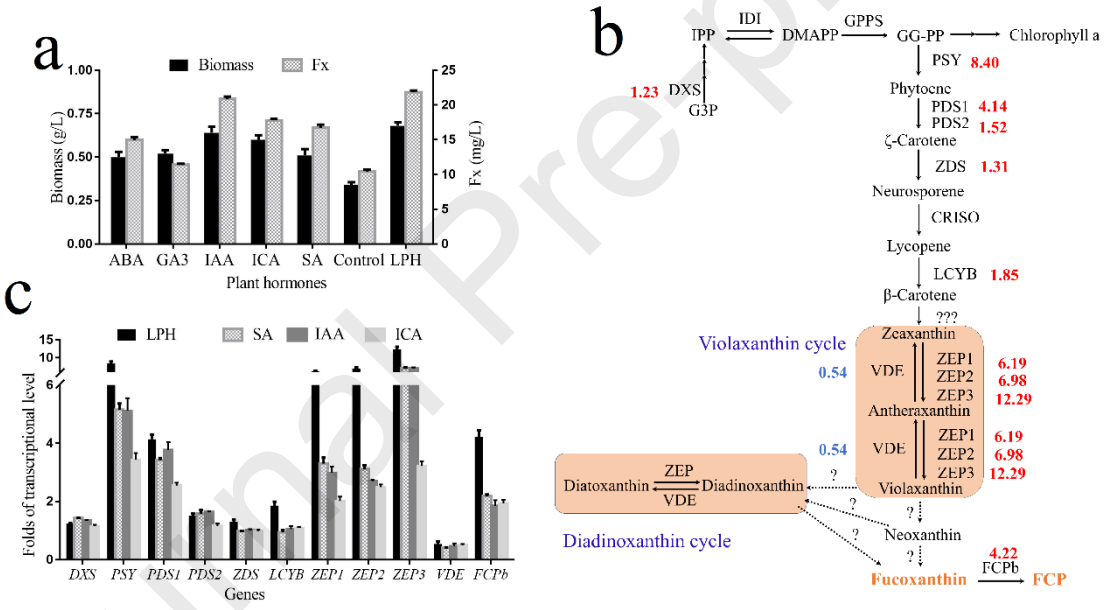

Fig.2

CRediT authorship contribution statement

Zhi-Peng Wang: Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Pei-Kang Wang:** Investigation, Formal analysis, Data curation. **Yan Ma:** Writing - review & editing. **Jia-Xue Lin:** Investigation, Software. **Cheng-Long Wang**: Investigation, Visualization. **Yu-Xiang Zhao:** Visualization**. Xin-**

Yue Zhang: Software. **Bei-Chen Huang:** Methodology. **Shou-Geng Zhao:** Software. **Lei Gao:** Methodology. **Jing Jiang:** Methodology. **Hai-Ying Wang:** Funding acquisition. **Wei Chen:** Supervision, Writing - review & editing.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

• LPH promote fucoxanthin accumulation and growth in *P. tricornutum* simultaneously.

• Plant hormones were found to promote fucoxanthin accumulation for the first time.

• Genes related to fucoxanthin formation were generally up-regulated.