Food Physics 2 (2025) 100041

Contents lists available at ScienceDirect

Food Physics

journal homepage: www.keaipublishing.com/en/journals/food-physics/

A low frequency magnetic field regulated the synthesis of carotenoids in *Rhodotorula mucilaginosa* by influencing iron metabolism

Menglin Ju[a], Jialan Zhang[b], Li Li[a,c], Yingbao Liu[a], Tong Gu[a], Mengxiang Gao[a,c,*]

[a] College of Life Science, Yangtze University, Jingzhou, Hubei 434025, China
[b] College of Animal and Technology Science, Yangtze University, Jingzhou, Hubei 434025, China
[c] Institute of Food Science and Technology, Yangtze University, Jingzhou, Hubei 434025, China

ARTICLE INFO

Keywords:
Rhodotorula mucilaginosa
Carotenoids
Low frequency magnetic field
Iron homeostasis

ABSTRACT

The purpose of this study is to investigate the effect of low frequency magnetic fields (LFMF) on carotenoid synthesis and iron metabolism in *Rhodotorula mucilaginosa* and to explore potential connections between the two processes. Various LFMF intensities were employed to assess carotenoid production, intracellular iron content, and the expression levels of genes related to carotenoid synthesis and iron metabolism in *R. mucilaginosa*. The results show that a LFMF could promote carotenoid synthesis, resulting in varying increases in intracellular iron content. Specifically, the expression of the iron metabolism-related gene transferrin receptor *(TFR)* was upregulated, while the expression of ferroportin *1 (FPN1)* was downregulated. Meanwhile, the expressions of the carotenoid synthesis-related genes geranylgeranyl diphosphate synthases (*GGPPS)* and phytoene synthase (*PSY)* displayed varying degrees of upregulation at different time points. Further, the *TFR* and *FPN1* gene knockout strains of *R. mucilaginosa* were subjected to treatment with a 3.5-mT LFMF, the results of which showed that an LFMF is capable of influencing the iron metabolism levels and subsequently regulating carotenoid synthesis in *R. mucilaginosa*.

1. Introduction

Carotenoids are a group of fat-soluble biological pigments known for their vibrant colors, ranging from yellow and orange to red and even deep red. These compounds have been used for thousands of years due to their beautiful colors and potent coloring capabilities [1]. Carotenoids are predominantly composed of a C40 backbone structure, which, through diverse chemical modifications, gives rise to a variety of carotenoids with different functional properties [2]. For example, beta-carotene has shown effectiveness in preventing liver cancer, improving the salt tolerance of basil, and serving as a beneficial ingredient for biological control [3–5]. Meanwhile, astaxanthin, known as the “king of Vitamin E,” has shown potential in enhancing reproductive health and protecting the kidney and nervous system [6,7], and lutein has been recognized for its role in protecting the optic nerve and preventing age-related macular degeneration [8].

* Corresponding author at: College of Life Science, Yangtze University, Jingzhou, Hubei 434025, China.
E-mail address: mgao@yangtzeu.edu.cn (M. Gao).

https://doi.org/10.1016/j.foodp.2024.100041
Received 8 August 2024; Received in revised form 10 October 2024; Accepted 12 October 2024
Available online 15 October 2024

Natural carotenoids are derived from numerous origins, including plants, animals, microorganisms, and others. Such microorganisms as *Rhodotorula mucilaginosa* and similar species are excellent sources for obtaining carotenoids through microbial fermentation [9]. When extracting carotenoids from these microorganisms, compared to plants and animals, several advantages were evident, including shorter production cycles, freedom from seasonal environmental limitations, high production efficiency, and the ability to control growth conditions effectively. Carotenoids produced by *R. mucilaginosa* can be employed in various sectors, such as food coloring, medical and health products, and beauty care goods, offering a wide range of market applications. As such, the exploration of methods and understanding the mechanisms used to increase the carotenoid yield in *R. mucilaginosa* hold practical and theoretical significance.

Currently, methods to increase carotenoid yield mainly involve inducing stress, optimizing the fermentation medium's composition, adjusting culture conditions, co-culturing with other strains, regulating gene expression, and strain mutagenesis, among other approaches [10]. Within this spectrum of methods, physical and chemical factors, such as magnetic fields [11], light exposure [12], zinc supplementation [13], iron availability [14], lead acetate [15], and other forms of elemental supplementation have been shown to promote carotenoid synthesis. The influence of external magnetic fields (MFs), environmental MFs, and MFs within biological systems on tissues or cells and their life processes is collectively referred to as magnetic biological effects. A variety of interactions between MFs and organisms can occur, impacting biological processes. For instance, electromagnetic fields have been shown to affect the growth and metabolism of microorganisms significantly [16]. More specifically, exposure to a 100-mT MF for 48 h led to notable increases in both vitamin K2 concentration and biomass in *Flavobacterium sp.* [17]. Similarly, exposure to a 1-mT MF resulted in a significant enhancement in citric acid yield and cellulase activity in *Aspergillus Niger* [18]. Hence, exposure to a MF could potentially serve as a method for enhancing carotenoid production in *R. mucilaginosa*.

Studies have shown that iron plays an important role in regulating the growth and photosynthetic pigment biosynthesis in siderophore-producing *Rhodopseudomonas palustris* [19]. Cells use iron for a variety of metabolic activities, but an excess of iron can be harmful. Thus, maintaining iron homeostasis is crucial to the growth and metabolism of microorganisms. Key components involved in iron transport and regulation include the high-affinity Fe^{3+} transporter permease (FTR1), which works alongside ferroxidase (FET3) to establish a high-affinity iron uptake system [20–23]. In iron-deficient conditions, the accumulation of the transcription factor Aft1 (activator of ferrous transport) in the nucleus triggered the expressions of FTR1 and FET3 [24,25]. Meanwhile, iron-reducing metalloreductase proteins FRE1 and FRE2 were associated with high-affinity uptake systems for iron and copper ions in *Saccharomyces cerevisiae* [26]. Iron in the periplasmic space could undergo reduction to generate ferrous ions, which are subsequently transported and absorbed by bivalent metal transporters, such as Zrt- and Irt-related protein (ZIP), before being taken up by high-affinity penetrase like FTR1, following reoxidation [27]. *S. cerevisiae* contains homologs of bacterial iron–sulfur cluster (Isc) assembly protein A, Isa1p, and Isa2p, which were involved in the formation or repair of iron–sulfur clusters [28]. Deficiencies in IscA proteins might induce an iron-starvation response, which, combined with the inhibition of glutathione biosynthesis, could lead to cellular ferroptosis [29]. Further, SirR functions as an iron-regulated repressor protein in *Staphylococcus epidermidis*, whereas transferrin receptor 1 (TFR1), a type II transmembrane glycoprotein, is bound to transferrin and plays a critical role in cellular iron uptake by interacting with iron-bound transferrin [30]. At the cellular level, iron regulatory proteins IRP1 and IRP2 play a key role in coordinating the regulated expression of iron uptake proteins, storage proteins, and ferroportin (FPN) [31].

Studies have shown that a static MF of 128 mT could influence the content of copper and zinc in the brain and spleen of mice [32]. Exposure of guinea pigs to an electromagnetic field has also been found to impact the transport of various metal ions, including copper, magnesium, calcium, and others, while imbalances in metal elements could potentially lead to physiological toxicity, including oxidative stress [33]. These studies indicate that MFs could influence microbial metabolism and metal ion homeostasis. However, the exact mechanism by which MFs affect the metal homeostasis of yeast cells and subsequently impact microbial metabolism remains unclear. Further research is necessary to explore these mechanisms and to clarify the interactions among MFs, metal ion homeostasis, and microbial metabolism.

As a result, this study primarily investigated the potential mechanism by which a low frequency magnetic field (LFMF) affected carotenoid synthesis of *R. mucilaginosa.* This was achieved by analyzing the effects of MF treatment on growth, carotenoid metabolism, intracellular iron content, and the expression levels of genes related to iron and carotenoid metabolism.

2. Materials and methods

2.1. Strains and culture conditions

R. mucilaginosa was sourced from the Microbiology Laboratory of the College of Life Sciences, Yangtze University, and stored on an inclined test tube in the yeast peptone dextrose (YPD) agar medium. The preserved *R. mucilaginosa* was then inoculated onto a YPD agar plate and incubated at 28 ℃ for 2 d. A single colony from the plate was inoculated into the YPD medium and cultured at 28 ℃, 200 rpm for 24 h. Subsequently, 1 % (v/v) of the *R. mucilaginosa* strain was inoculated into the YPD medium and cultured at 28 ℃, 200 rpm for 24 h to obtain secondary seeds.

2.2. LFMF treatment of R. mucilaginosa

Five percent (v/v) of the secondary seeds was inoculated into the YPD medium and exposed to the alternating LFMF (frequency 50 Hz) treatments at intensities of 3.0, 3.5, and 4.0 mT at 28 ˚C for 12 h each. Following the treatments, the cultures were further incubated at 28 ˚C and 200 rpm for 36 h to measure the metabolite content.

In addition, 5 % (v/v) of the secondary seeds was inoculated into the YPD medium and exposed to 3.5-mT alternating LFMF treatments at 28 ˚C for 12 h. The cells were harvested at the 12, 24, and 48 h of liquid fermentation, and quantitative real-time PCR was conducted to measure the expressions of genes related to iron metabolism and carotenoid synthesis in *R. mucilaginosa*.

2.3. Construction of TFR and FPN1 gene knockout strains

2.3.1. Vector construction for target gene knockout

The genomic DNA of *R. mucilaginosa* was extracted following the protocol described in reference [34]. Following extraction, the concentration and purity of the DNA were assessed using the Quawell Q5000 ultra-micro ultraviolet spectrophotometer (Quawell, USA) and agarose gel electrophoresis.

A gene knockout vector was designed using CRISPR/Cas9 technology, which involved incorporating the pGAP-Cas9-Hyg vector and the target gene sequence. The constructed plasmid pUC57-scaffold was used for gRNA cloning and expression, which contained *E. coli* genetic elements (pMB1 origin, kanamycin resistance), a gRNA expression cassette with the SNR52 promoter, the target gene's gRNA sequence, and the gRNA scaffold. In addition, the donor DNA was constructed by linking a left homologous arm, neomycin resistance marker, and right homologous arm. For gene knockout, the plasmid pGAP-Cas9-Hyg-SgRNA-Neo was constructed by integrating a pUC57-scaffold cassette and donor DNA into pGAP-Cas9-Hyg (Supplementary Fig S1). The primers used to construct and verify the *TFR* and *FPN1* gene knockout vectors are shown in Table S1 (Supplementary Table S1).

2.3.2. Preparation of competent cells of R. mucilaginosa

A modified method based on [35] was utilized to prepare *R. mucilaginosa* electro-competent cells. First, single colonies of *R. mucilaginosa* were inoculated into 5 mL of a YPD medium and cultured at 28˚C, 200 rpm until the optical density (OD) reached 0.2. Subsequently, the cells were transferred to 50 mL of the YPD medium and cultured until the OD value reached a range of 0.7–1.5. The cells were then harvested by centrifugation at 4 ˚C and 3000 $\times g$ for 5 min, and the pellet obtained was washed with 5 mL of ice-cold distilled H_2O. The washed cells were resuspended in a TMLSD buffer (comprising 10 mM Tris-HCl buffer [pH 7.5], 1 mM $MgCl_2$, 100 mM lithium acetate, 270 mM sucrose, and 5 mM dithiothreitol). This cell suspension was incubated at 25 ˚C for 1 h and subsequently washed and resuspended. After incubation, the cells were harvested, washed twice with TMS buffer (containing 10 mM Tris-HCl buffer [pH 7.5], 1 mM $MgCl_2$, and 270 mM sucrose). Finally, the electro-competent cells were resuspended in TMS buffer and stored at −80 ˚C for future use.

2.3.3. Screening and identification of gene deletion strains

The transformation process involved mixing 3 μg of plasmid with 100 μl of competent cells, followed by incubation on ice for 15 min. The DNA–cell mixtures were electroporated at 0.6 kV in MicroPulser Electroporation Cuvettes (Bio-Rad, USA) with a 0.1-cm gap. Following electroporation, the cells were resuspended in 1 mL of ice-cold 1 M sorbitol and incubated on ice for 15 min. Subsequently, the cells were placed in an incubator shaker at 30 ˚C, 150 rpm for 2 h. After incubation, the cells were collected by centrifugation at 4 ˚C and 3000 $\times g$ for 5 min and resuspended in 1 mL of the YPD medium. The cell suspension was then incubated at 30 ˚C, 150 rpm for 2 h again. Finally, the cell suspension was spread onto YPD plates containing Kanamycin (G418) and incubated at 28 ˚C for 3–5 d for screening and further growth.

After obtaining the transformants, they were sub-cultured for three generations to ensure the selection of stable transformants. Subsequently, the transformants were verified by PCR. Each transformant was subjected to PCR using gene-specific primer pairs (as listed in Supplementary Table S2). Following the PCR confirmation, the transformants with positive identification were cloned into the pUC19 vector for sequencing. The sequences obtained from the transformants were aligned with the wild-type (WT) sequence to compare and identify the mutants.

2.3.4. Phenotypic observation of mutant strains

The secondary seed solution of both the WT and mutant strain were appropriately diluted and spread onto YPD plates, which were then incubated at 28 ˚C, allowing *R. mucilaginosa* to form distinct single colonies on YPD plates. Daily observation of colony growth was conducted, and any alterations in colony color and size were noted and recorded for further analysis.

2.4. Metabolite determination

2.4.1. Determination of biomass and carotenoids

Biomass was quantified using the dry weight method [36], where cells were separated by centrifugation and washed twice with distilled water. Subsequently, the collected cells were freeze-dried until a constant weight was achieved.

Further, the cell wall of *R. mucilaginosa* was disrupted using an acid-heat method, followed by carotenoid extraction using acetone. The carotenoid content was then quantified using a spectrophotometer according to the previously described method [37].

2.4.2. Determination of intracellular iron content

The intracellular iron content of *R. mucilaginosa* was quantified using the phenanthroline method. In brief, the cells were collected through centrifugation and then subjected to freeze drying. Subsequently, the dried cells were carbonized and ashed at 550 ℃ for 5–6 h. Following this, acid water [pH 4.6] was added to dissolve the ashed material, creating a solution. The analysis of Fe(II) ions in solution was performed by complexing them with 1,10-phenanthroline (phen) to form the $[Fe(phen)_3]^{2+}$ complex, which was then

quantified using visible spectrophotometry following the methodology described by Crispin and Varey [38]. In addition, the concentrations of Fe(III) ions were determined by first reducing them to Fe(II) using hydroxylamine hydrochloride, followed by complexation with phen, as previously described. In the analysis process, it was necessary to consider and correct for any potential interference from other components present in the solution at the analysis wavelength.

2.5. Quantitative reverse transcription PCR (qRT-PCR)

Total RNA was extracted from *R. mucilaginosa* cells using a Trizol reagent (Vazyme Biotech Co., Ltd., Nanjing, China) and quantified using the QUAWELL Q5000 spectrophotometer (Quawell Technology, Inc., United States). Meanwhile reverse transcription was performed with the HiScript II Q RT SuperMix for qPCR (Vazyme Biotech Co., Ltd., Nanjing, China) following the manufacturer's instructions. The expression levels of genes related to iron metabolism and carotenoid synthesis were analyzed using ChamQ Universal SYBR qPCR Master Mix kit (Vazyme Biotech Co., Ltd., Nanjing, China) with the SLAN Fluorescence Detection System (SLAN, Shanghai, China). The cDNA obtained was used as a template, and β-*actin* was used as the reference gene. The primer sequences used for qRT-PCR are provided in Supplementary Table S2.

2.6. Data analysis

The mean value was calculated from three replicates, and a one-way analysis of variance was conducted using the Prism 7.0 program (GraphPad Software Inc., La Jolla, CA, USA). Significance was determined at $p < 0.05$.

3. Results

3.1. Effects of the LFMF on the growth, carotenoid production, and intracellular iron content of R. mucilaginosa

In the MF intensity range of 3.0–4.0 mT, there was no significant effect on *R. mucilaginosa* growth ($p > 0.05$), but significant promotion of both the production capacity of carotenoids ($p < 0.05$) and the intracellular iron content ($p < 0.05$) was observed. The most pronounced promotional effect was detected at an MF intensity of 3.5 mT (Fig. 1). These results indicate that MF treatment could elevate the iron content in cells and enhance carotenoid production to a certain level in *R. mucilaginosa*.

3.2. Effects of LFMF on the expressions of genes related to iron metabolism and carotenoid synthesis in R. mucilaginosa

The expression levels of genes related to iron metabolism (*TFR*, *FPN1*) and carotenoid synthesis (3-hydroxy-3-methylglutaryl-CoA reductase *HMGR*, geranylgeranyl diphosphate synthases *GGPPS*, phytoene synthase *PSY*) were evaluated at 12, 24, and 48 h

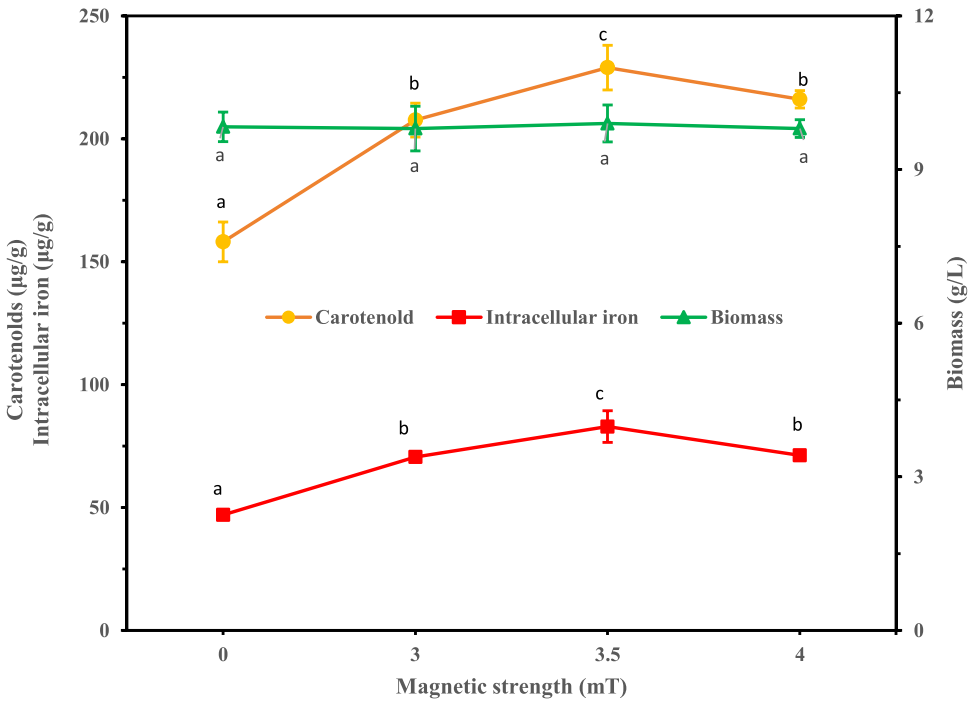

Fig. 1. Effects of LFMF on growth, carotenoid production, and intracellular iron content in *R. mucilaginosa*.

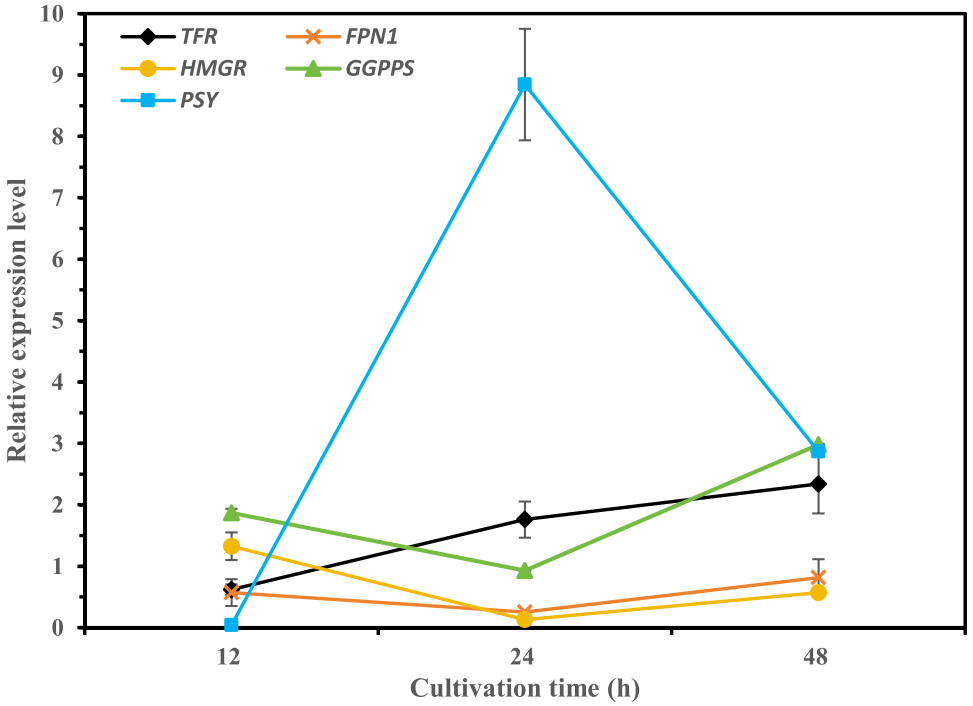

Fig. 2. Effects of LFMF on gene expression related to iron metabolism and carotenoids synthesis.

following treatment with a 3.5-mT LFMF (Fig. 2). At 12 h of LFMF treatment, there was a significant up-regulation in the expression levels of the *HMGR* and *GGPPS* genes, while by 24 h, the expression levels of the *TFR* and *PSY* genes exhibited significant up-regulation, while the *FPN1* gene was notably down-regulated. At 48 h, the *TFR* gene expression remained significantly up-regulated, and the expression levels of the *GGPPS* and *PSY* genes also increased. These results suggest that LFMF treatment could enhance iron uptake into cells and stimulate carotenoid synthesis.

3.3. Growth, carotenoid production, and intracellular iron content of the TFR and FPN1 gene knockout strains of R. mucilaginosa

Strains with knockout mutations in the iron metabolism-related genes *TFR* and *FPN1* (ΔRm*TFR* and ΔRm*FPN1*) were generated using the CRISPR/Cas9 method in *R. mucilaginosa*, where their deletion influenced the strains' growth and carotenoid production. Compared to the WT strain, both the ΔRm*TFR* and ΔRm*FPN1* strains exhibited smaller colonies, and the colony color of ΔRm*FPN1* appeared lighter than that of WT, while the colony color of strain ΔRm*TFR* appeared darker (Fig. 3.). These observations suggest that deleting the *TFR* gene enhances carotenoid accumulation in *R. mucilaginosa*, whereas deletion of the *FPN1* gene inhibited carotenoid accumulation.

Deletion of the *TFR* and *FPN1* genes had distinct effects on the growth, carotenoid production, and intracellular iron content of *R. mucilaginosa* (Fig. 4). Compared to the WT strain, the biomass of ΔRm*TFR* and ΔRm*FPN1* showed a significant decrease ($p < 0.05$), whereas the intracellular iron content of ΔRm*TFR* exhibited no significant change ($p > 0.05$) and the carotenoid yield showed a significant increase ($p < 0.05$). In contrast, the intracellular iron content of ΔRm*FPN1* was significantly increased ($p < 0.05$), whereas the carotenoid yield was significantly decreased ($p < 0.05$).

3.4. Effects of LFMF on growth, carotenoid production, and intracellular iron content of ΔRmTFR and ΔRmFPN1 strains

The effects of a LFMF on the two mutant strains differed. In comparison to the WT strain, ΔRm*TFR* exhibited a significant decrease in biomass content ($p < 0.05$), along with a significant increase in carotenoid production and intracellular iron ($p < 0.05$) (Fig. 5). Meanwhile, LFMF treatment did not induce significant changes in the biomass, carotenoid yield, and intracellular iron content of ΔRm*TFR* ($p > 0.05$). Notably, in ΔRm*TFR* exposed to a LFMF, there was a significant increase in the carotenoid concentration ($p < 0.05$), while the intracellular iron content showed a significant decrease ($p < 0.05$) when compared to the WT strain treated with the LFMF.

Compared to the WT strain, ΔRm*FPN1* exhibited a significant decrease in biomass ($p < 0.05$) and a significant increase in intracellular iron content ($p < 0.05$). Following LFMF treatment, there were no significant changes observed in the biomass and intracellular iron content of ΔRm*FPN1* ($p > 0.05$), while the carotenoid yield showed a significant increase ($p < 0.05$). When comparing ΔRm*FPN1* to the WT strain treated with the LFMF, the carotenoid synthesis concentration in ΔRm*FPN1* did not vary

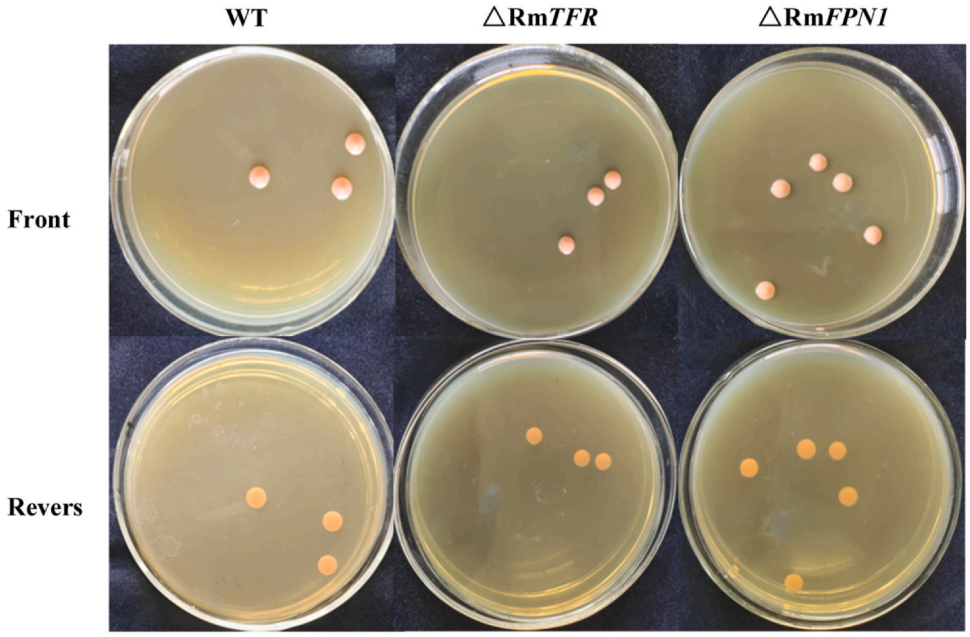

Fig. 3. The growth of Δ*RmTFR* and Δ*RmFPN1 R. mucilaginosa*.

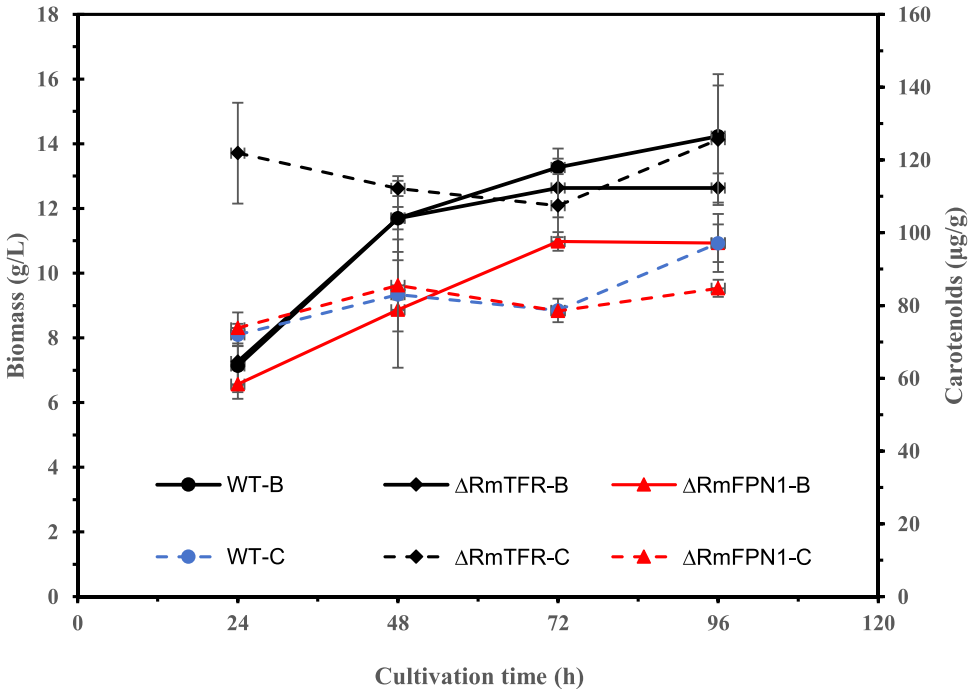

Fig. 4. Effects of the deletion of the *TFR* and *FPN1* genes on growth, carotenoid production, and intracellular iron content.

significantly ($p > 0.05$), the biomass of ΔRm*FPN1* was significantly decreased ($p < 0.05$), and the intracellular iron content was significantly increased ($p < 0.05$).

These results indicate that TFR could inhibit cellular iron uptake, while FPN1 facilitates iron efflux from cells, preventing excessive iron accumulation within cells. The deletion of the *FPN1* gene led to a notable increase in intracellular iron content, as the absence of FPN1 inhibited iron export, significantly raising intracellular iron levels, while the deletion of the *TFR* or *FPN1* genes significantly reduced the cell's responsiveness to iron in the presence of LFMF. Compared to the WT strain, both ΔRm*TFR* and ΔRm*FPN1* exhibited notably increased intracellular iron content, with ΔRm*FPN1* showing significantly higher iron content compared to the WT strain. Following LFMF treatment, the intracellular iron content of ΔRm*TFR* and ΔRm*FPN1* did not show significant

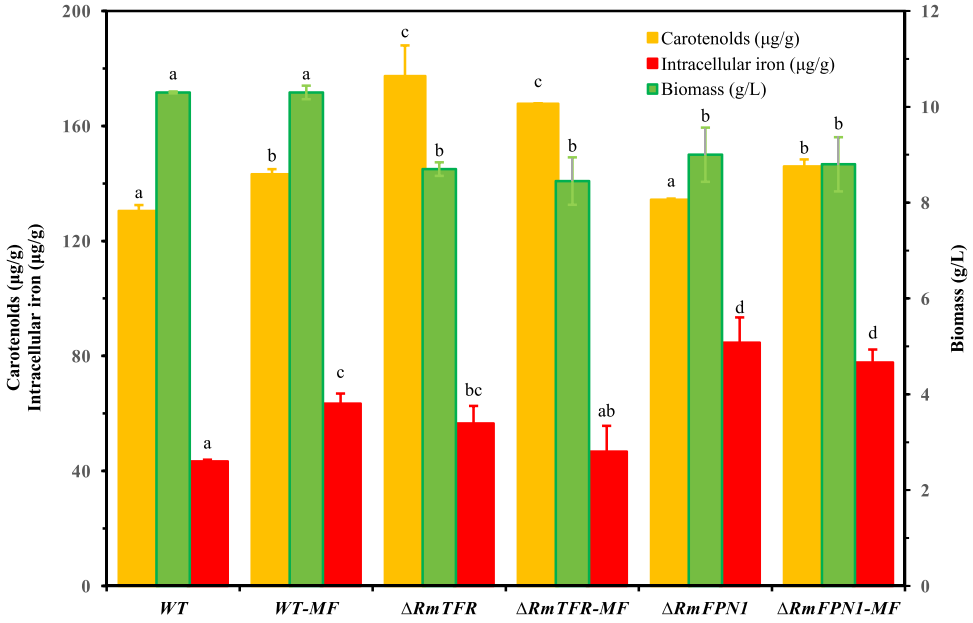

Fig. 5. Effects of LFMF on growth, carotenoid production, and intracellular iron content of Δ*RmTFR* and Δ*RmFPN1* strains.

differences compared to untreated samples. However, the carotenoid content in ΔRm*FPN1* increased when compared to untreated conditions. These observations suggest that the iron sensitivity of the ΔRm*FPN1* mutant might be diminished, enhancing the cell's tolerance to iron and surpassing the intracellular iron levels of the WT strain, thereby promoting carotenoid synthesis.

4. Discussion

Iron, in its Fe^{2+}/Fe^{3+} forms, plays a pivotal role in catalyzing diverse biochemical reactions by undergoing valence changes and electron transfers, serving as a core component of cytochrome in the oxidative phosphorylation process of cellular respiration, providing essential energy for vital activities [39]. However, iron's biochemical properties also enable the transfer of electrons to oxygen or hydrogen peroxide, generating highly toxic oxygen free radicals that have the potential to damage cell membrane lipids and proteins, ultimately exerting toxic effects on cells [40,41]. To maintain balance and prevent iron deficiency or overload, iron availability was tightly regulated at both the cellular and systemic levels. Iron played a crucial role in carotenoid synthesis, having served as a cofactor in numerous carotenoid synthases and having played a key role in regulating enzyme activity and metabolic pathways [42,43]. Adequate iron levels could facilitate carotenoid synthesis, ultimately leading to increased carotenoid production in organisms [44]. The findings of this study demonstrate that LFMF treatment effectively enhanced carotenoid production in *R. mucilaginosa* without compromising growth ability. The intracellular iron content increased at varying degrees under different MF intensities. Previous research has shown that a 3.0-mT MF could boost carotenoid production in *Phaffia rhodozyma* by 59.4 %, accompanied by a significant increase in biomass [11]. Conversely, the carotenoid production of the halophilic bacterial strain SC8 was significantly enhanced by extreme LFMF treatment, without a corresponding increase in biomass [45]. In addition, studies have reported that exposure to an electromagnetic field could influence the transport of metal ions, such as Cu, Mg, and Ca, potentially leading to physiological toxicity, including oxidative stress [33]. Therefore, the MF treatment of *R. mucilaginosa* affected iron metabolism, consequently influencing carotenoid biosynthesis.

The expression levels of the *TFR* and *FPN1* genes exhibited an up-regulated and down-regulated trend, respectively, after 24 h of MF treatment. Notably, the TFR and FPN1 proteins were involved in iron input and output, respectively [30,31], suggesting that LFMF treatment of *R. mucilaginosa* could enhance the uptake of iron ions while inhibiting their expulsion to some extent, resulting in an overall increase in intracellular iron levels. However, the excessive presence of iron ions could potentially induce oxidative damage and toxic effects on cells [20]. Carotenoids, being antioxidants, have the potential to counteract the toxic effects of reactive oxygen species (ROS) [46] and to improve cellular membrane function [47], thereby mitigating oxidative damage and physiological toxicity arising from heightened intracellular iron content.

Studies have demonstrated that mutations in genes involved in iron export and iron reserve functions, such as *DR1440* and *DRA0258* in *Deinococcus radiodurans*, could enhance the sensitivity of cells to ferrous ions [48,49]. In this study, it was observed that the deletion of the *TFR* and *FPN1* genes significantly affected the growth of *R. mucilaginosa*, with distinct outcomes for each mutation. The absence of *TFR* resulted in the inhibition of *R. mucilaginosa* growth, while markedly boosting carotenoid production, suggesting that the lack of *TFR* restricted the iron uptake capacity of *R. mucilaginosa* while making the cells more sensitive to iron, leading to the exacerbation of oxidative damage in mutant ΔRm*TFR*, even when the cellular iron content remained consistent. In contrast, the deletion of *FPN1* suppressed the growth and did not affect the carotenoid production of ΔRm*FPN1*. Concurrently, intracellular iron

content significantly increased, aligning with the iron efflux function of *FPN1* [30,31]. This underscored that excessive iron levels could strongly hinder carotenoid synthesis and even impede *R. mucilaginosa* growth. Therefore, TFR and FPN1 play important roles in iron metabolism and could influence carotenoid synthesis in *R. mucilaginosa.*

5. Conclusion

The findings of this study suggest that an appropriate LFMF intensity could enhance carotenoid yield without adversely affecting *R. mucilaginosa* growth. In addition, the impact of an LFMF on carotenoid synthesis was linked to intracellular iron content and the expression levels of the *TFR* and *FPN1* genes. Deletion of the *TFR* and *FPN1* genes could significantly disrupt the growth and carotenoid synthesis of *R. mucilaginosa*, with distinct effects observed for each gene. Loss of *TFR* inhibited the growth of *R. mucilaginosa*, while markedly increasing carotenoid production and intracellular iron content. Conversely, the growth of ΔRm*FPN1* was impeded, leading to a significant increase in intracellular iron content. In summary, LFMF promoted the inward absorption of iron ions and inhibited iron ion excretion, resulting in an elevated iron ion concentration within the cell. This, in turn, stimulated carotenoid synthesis by *R. mucilaginosa*. The study has provided initial insights into the mechanism by which LFMFs impact carotenoid synthesis through the regulation of iron metabolism. These findings offer valuable insights for applications and practical considerations related to carotenoid production.

CRediT authorship contribution statement

Menglin Ju: Writing – original draft, Validation, Conceptualization. **Jialan Zhang:** Writing – review & editing, Conceptualization. **Li Li:** Writing – review & editing, Conceptualization. **Yingbao Liu:** Writing – review & editing, Methodology. **Tong Gu:** Writing – review & editing, Methodology. **Mengxiang Gao:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of Competing Interest

Mengxiang Gao is an editorial board member for Food Physics and was not involved in the editorial review or the decision to publish this article. All other authors declare that there are no competing interests.

Acknowledgements

This study was funded by National Natural Science Foundation of China (32372472).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foodp.2024.100041.

References

[1] G. Britton, Carotenoid research: History and new perspectives for chemistry in biological systems, BBA Mol. Cell Biol. L. 1865 (11) (2020) 158699, https://doi.org/10.1016/j.bbalip.2020.158699.
[2] A.J. Meléndez-Martínez, A.I. Mandić, F. Bantis, V. Böhm, G.I.A. Borge, M. Brnčić, ... N. O'Brien, A comprehensive review on carotenoids in foods and feeds: Status quo, applications, patents, and research needs, Crit. Rev. Food Sci. 62 (8) (2022) 1999–2049, https://doi.org/10.1080/10408398.2020.1867959.
[3] S. Youssef, Light and electron microscopic study of the effect of orlistat on the liver of Aadult male albino rats and the possible protective role of *β*-carotene, Forensic Med. Anat. Res. 06 (02) (2018) 20–36, https://doi.org/10.4236/fmar.2018.62003.
[4] H. Kahveci, N. Bilginer, E. Diraz-Yildirim, M. Kulak, E. Yazar, F. Kocacinar, S. Karaman, Priming with salicylic acid, β-carotene and tryptophan modulates growth, phenolics and essential oil components of *Ocimum basilicum L.* grown under salinity, Sci. Hortic. Amst. 281 (2021) 109964, https://doi.org/10.1016/j.scienta.2021.109964.
[5] Y.X. Sun, Y.N. Hao, T.X. Liu, A *β*-carotene-amended artificial diet increases larval survival and be applicable in mass rearing of *Harmonia axyridis*, Biol. Control 123 (2018) 105–110, https://doi.org/10.1016/j.biocontrol.2018.04.010.
[6] S.V. Singh, Y.M. Somagond, A. Deshpande, Astaxanthin-King of antioxidants as immune modulator and anti-inflammatory for enhancing productive performance and health of animals, Indian J. Dairy Sci. 74 (1) (2021) 1–7.
[7] R. Landon, V. Gueguen, H. Petite, D. Letourneur, G. Pavon-Djavid, F. Anagnostou, Impact of astaxanthin on diabetes pathogenesis and chronic complications, Mar. Drugs 18 (7) (2020) 357, https://doi.org/10.3390/md18070357.
[8] B.M. Steiner, D.J. McClements, G. Davidov-Pardo, Encapsulation systems for lutein: a review, Trends Food Sci. Tech. 82 (2018) 71–81, https://doi.org/10.1016/j.tifs.2018.10.003.
[9] T. Roukas, The role of oxidative stress on carotene production by *Blakeslea trisporain* submerged fermentation, Crit. Rev. Biotechnol. 36 (3) (2016) 424–433, https://doi.org/10.3109/07388551.2014.989424.
[10] S.S. Choi, G.D. Kim, Production of carotenoids by bacteria; carotenoid productivity and availability, J. Life Sci. 32 (5) (2022) 411–419, https://doi.org/10.5352/JLS.2022.32.5.411.
[11] P.G.P. da Silva, D.P. Júnior, L. Sala, J.F. de Medeiros Burkert, L.O. Santos, Magnetic field as a trigger of carotenoid production by *Phaffia rhodozyma*, Process Biochem 98 (2020) 131–138, https://doi.org/10.1016/j.procbio.2020.08.001.
[12] Z. Yi, Y. Su, P. Cherek, D.R. Nelson, J. Lin, O. Rolfsson, ... W. Fu, Combined artificial high-silicate medium and LED illumination promote carotenoid accumulation in the marine diatom *Phaeodactylum tricornutum*, Microb. Cell Fact. 18 (1) (2019) 1–11, https://doi.org/10.1186/s12934-019-1263-1.
[13] E. Breierová, M. Čertík, I. Márová, R. Vadkertiová, The effect of Zn (II) ions and reactive oxygen on the uptake of zinc and production of carotenoids by selected red yeasts, Chem. Biodivers. 15 (6) (2018) e1800069, https://doi.org/10.1002/cbdv.201800069.
[14] P. Zhou, W. Xie, A. Li, F. Wang, Z. Yao, Q. Bian, ... L. Ye, Alleviation of metabolic bottleneck by combinatorial engineering enhanced astaxanthin synthesis in *Saccharomyces cerevisiae*, Enzym. Microb. Tech. 100 (2017) 28–36, https://doi.org/10.1016/j.enzmictec.2017.02.006.

[15] H. Souahi, Impact of lead on the amount of chlorophyll and carotenoids in the leaves of *Triticum durum* and *T. aestivum*, *Hordeum vulgare* and *Avena sativa*, Biosyst. Divers. 29 (3) (2021) 207–210, https://doi.org/10.15421/012125.
[16] H. Torgomyan, A. Trchounian, Bactericidal effects of low-intensity extremely high frequency electromagnetic field: an overview with phenomenon, mechanisms, targets and consequences, Crit. Rev. Microbiol. 39 (1) (2013) 102–111, https://doi.org/10.3109/1040841X.2012.691461.
[17] H. Tang, P. Wang, H. Wang, Z. Fang, Q. Yang, W. Ni, ... Z. Zheng, Effect of static magnetic field on morphology and growth metabolism of *Flavobacterium* sp. m1-14, Bioproc. Biosyst. Eng. 42 (2019) 1923–1933, https://doi.org/10.1007/s00449-019-02186-7.
[18] M. Gao, J. Zhang, H. Feng, Extremely low frequency magnetic field effects on metabolite of *Aspergillus niger[J*], Bioelectromagnetics 32 (1) (2010) 73–78, https://doi.org/10.1002/bem.20619.
[19] B.H. Hu, C.G. Zhao, S.B. Yang, Influence of iron on siderophore and photosynthetic pigments biosynthesis by siderophore-producing *Rhodopseudomonas palustris* (in Chinese), Acta Microbiol. Sin. 54 (4) (2014) 408–416, https://doi.org/10.13343/j.cnki.wsxb.2014.04.007.
[20] R. Stearman, D.S. Yuan, Y. Yamaguchi-Iwai, R.D. Klausner, A. Dancis, A permease-oxidase complex involved in high-affinity iron uptake in yeast, Science 271 (5255) (1996) 1552–1557, https://doi.org/10.1126/science.271.5255.155.
[21] C. Askwith, D. Eide, A. Van Ho, P.S. Bernard, L. Li, S. Davis-Kaplan, ... J. Kaplan, The FET3 gene of *S. cerevisiae* encodes a multicopper oxidase required for ferrous iron uptake, Cell 76 (2) (1994) 403–410, https://doi.org/10.1016/0092-8674(94)90346-8.
[22] H.G. Sherman, C. Jovanovic, S. Stolnik, K. Baronian, A.J. Downard, F.J. Rawson, New perspectives on iron uptake in eukaryotes, Front. Mol. Biosci. 5 (11) (2018) 97, https://doi.org/10.3389/fmolb.2018.00097.
[23] E.Y. Kwok, S. Severance, D.J. Kosman, Evidence for iron channeling in the Fet3p-Ftr1p high-affinity iron uptake complex in the yeast plasma membrane, Biochemistry 45 (20) (2006) 6317–6327, https://doi.org/10.1021/bi052173c.
[24] Y. Yamaguchi-Iwai, R. Ueta, A. Fukunaka, R. Sasaki, Subcellular localization of Aft1 transcription factor responds to iron status in *Saccharomyces cerevisiae*, J. Biol. Chem. 277 (21) (2002) 18914–18918, https://doi.org/10.1074/jbc.M200949200.
[25] J. Schothorst, G.V. Zeebroeck, J.M. Thevelein, Identification of Ftr1 and Zrt1 as iron and zinc micronutrient transceptors for activation of the PKA pathway in *Saccharomyces cerevisiae*, Microb. Cell 4 (3) (2017) 74, https://doi.org/10.15698/mic2017.03.561.
[26] L.J. Martins, L.T. Jensen, J.R. Simon, G.L. Keller, D.R. Winge, metalloregulation of FRE1 and FRE2 homologs in *Saccharomyces cerevisiae*, J. Biol. Chem. 273 (37) (1998) 23716–23721, https://doi.org/10.1074/jbc.273.37.23716.
[27] R. Sutak, J.M. Camadro, E. Lesuisse, Iron uptake mechanisms in marine phytoplankton, Front. Microbiol. 11 (2020) 566691, https://doi.org/10.3389/fmicb.2020.566691.
[28] L.T. Jensen, V.C. Culotta, Role of *Saccharomyces cerevisiae ISA1* and *ISA2* in iron homeostasis, Mol. Cell. Biol. 20 (11) (2000) 3918–3927, https://doi.org/10.1128/MCB.20.11.3918-3927.2000.
[29] S.W. Alvarez, V.O. Sviderskiy, E.M. Terzi, T. Papagiannakopoulos, A.L. Moreira, S. Adams, ... R. Possemato, NFS1 undergoes positive selection in lung tumours and protects cells from ferroptosis, Nature 551 (7682) (2017) 639–643, https://doi.org/10.1038/nature24637.
[30] P.V. Candelaria, L.S. Leoh, M.L. Penichet, T.R. Daniels-Wells, Antibodies targeting the transferrin receptor 1 (TfR1) as direct anti-cancer agents, Front. Immunol. 12 (2021) 607692, https://doi.org/10.3389/fimmu.2021.607692.
[31] L.C. Kühn, Iron regulatory proteins and their role in controlling iron metabolism, Metallomics 7 (2) (2015) 232–243, https://doi.org/10.1039/c4mt00164h.
[32] S.R. De Luka, A.Ž. Ilić, S. Janković, D.M. Djordjevich, S. Ćirković, I.D. Milovanovich, A.M. Trbovich, Subchronic exposure to static magnetic field differently affects zinc and copper content in murine organs, Int. J. Radiat. Biol. 92 (3) (2016) 140–147, https://doi.org/10.3109/09553002.2016.1135266.
[33] O. Erdem, C. Akay, S.C. Cevher, A.G. Canseven, A. Aydın, N. Seyhan, Effects of intermittent and continuous magnetic fields on trace element levels in guinea pigs, Biol. Trace Elem. Res. 181 (2018) 265–271, https://doi.org/10.1007/s12011-017-1053-8.
[34] S. Banerjee, A. Sarkar, K.V.B. Rao, Extraction and characterization of carotenoid pigments with antioxidant and antibacterial potential from marine yeast *Rhodotorula* sp. KSB1, Int. Microbiol. (2024) 1–20, https://doi.org/10.1007/s10123-024-00529-3.
[35] S. Takahashi, H. Okada, K. Abe, Y. Kera, Genetic transformation of the yeast Rhodotorula gracilis ATCC 26217 by electroporation, Appl. Biochem. Microbiol. 50 (2014) 624–628, https://doi.org/10.1134/s0003683814110040.
[36] J. Zhang, D. Zeng, C. Xu, M. Gao, Effect of low-frequency magnetic field on formation of pigments of *Monascus purpureus*, Eur. Food Res. Technol. 240 (2015) 577–582, https://doi.org/10.1007/s00217-014-2358-x.
[37] M. Ju, J. Zhang, T. Mai, L. Li, T. Gu, Y. Liu, M. Gao, Co-culture of *Rhodotorula mucilaginosa* and *Monascus purpureus* increased the yield of carotenoids and *Monascus* pigments, LWT 183 (2023) 114949, https://doi.org/10.1016/j.lwt.2023.114949.
[38] D.J. Crispin, J.E. Varey, Iron release from spinach: effects of treatment on levels of iron (II) and iron (III) released in vitro, Food Chem. 76 (1) (2002) 117–123, https://doi.org/10.1016/S0308-8146(01)00290-4.
[39] K.A. Weber, L.A. Achenbach, J.D. Coates, Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction, Nat. Rev. Microbiol. 4 (10) (2006) 752–764, https://doi.org/10.1038/nrmicro1490.
[40] G. Papanikolaou, K. Pantopoulos, Iron metabolism and toxicity, Toxicol. Appl. Pharm. 202 (2) (2005) 199–211, https://doi.org/10.1016/j.taap.2004.06.021.
[41] R. Meneghini, Iron homeostasis, oxidative stress, and DNA damage, Free Radic. Bio. Med. 23 (5) (1997) 783–792, https://doi.org/10.1016/S0891-5849(97)00016-6.
[42] P.D. Fraser, P.M. Bramley, The biosynthesis and nutritional uses of carotenoids, Prog. Lipid Res. 43 (3) (2004) 228–265, https://doi.org/10.1016/j.plipres.2003.10.002.
[43] J. Beltrán, B. Kloss, J.P. Hosler, J. Geng, A. Liu, A. Modi, ... E.T. Wurtzel, Control of carotenoid biosynthesis through a heme-based cis-trans isomerase, Nat. Chem. Biol. 11 (8) (2015) 598–605, https://doi.org/10.1038/nchembio.1840.
[44] W. Xing, W.M. Huang, D.H. Li, Y.D. Liu, Effects of iron on growth, pigment content, photosystem II efficiency, and siderophores production of *Microcystis aeruginosa* and *Microcystis wesenbergii*, Curr. Microbiol. 55 (2007) 94–98, https://doi.org/10.1007/s00284-006-0470-2.
[45] S. Hassanzadeh, M. Haghi, E. Haliki, A.H. Uztan, Effect of 50 Hz extremely low magnetic field on total carotenoid production of SC8 isolated from Camalti saltern-Izmir Turkey, Eur. J. Biomed. 6 (1) (2019) 555–559.
[46] A.J. Young, G.L. Lowe, Carotenoids-antioxidant properties, Antioxidants 7 (2) (2018) 28, https://doi.org/10.3390/antiox7020028.
[47] A. Vershinin, Biological functions of carotenoids - diversity and evolution, Biofactors 10 (2-3) (1999) 99–104, https://doi.org/10.1002/biof.5520100203.
[48] S. Dai, Y. Jin, T. Li, Y. Weng, X. Xu, G. Zhang, ... Y. Hua, DR1440 is a potential iron efflux protein involved in maintenance of iron homeostasis and resistance of *Deinococcus radiodurans* to oxidative stress, PLOS One 13 (8) (2018) e0202287, https://doi.org/10.1371/journal.pone.0202287.
[49] S. Yang, M. Liu, L. Wang, Y. Hua, Antioxidant function of the iron binding protein DRA0258 in *Deinococcus radiodurans*, Wei Sheng wu xue bao = Acta Microbiol. Sin. 57 (1) (2017) 140–153, https://doi.org/10.13343/j.cnki.wsxb.20160340 (Chinese).