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# Fucoxanthin Isolated from *Undaria pinnatifida* Can Interact with *Escherichia coli* and *lactobacilli* in the Intestine and Inhibit the Growth of Pathogenic Bacteria

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**Abstract** Fucoxanthin is a xanthophyll-type carotenoid that provides many benefits to human health. However, the mechanism by which fucoxanthin interacts with microbes and inhibits pathogenic bacteria is unknown. In this study, we investigated the effects of fucoxanthin isolated from the edible seaweed *Undaria pinnatifida* on pathogenic bacteria *Escherichia coli* and *lactobacilli* both *in vitro* and *in vivo*. Fucoxanthin strongly inhibited the growth of Gram-positive pathogenic bacteria but was less effective against Gram-negative bacteria. Fucoxanthin extracted from the crude mixture had a recovery rate of 93.38% and a purity of 82.70%, which were higher than those of fucoxanthin extracted using a previous method. Fucoxanthin also promoted the growth of intestinal microbes in mice. Fucoxanthinol, a metabolite of fucoxanthin, was generated in the culture media. Fucoxanthin can be deacetylated into fucoxanthinol not only by conventional digestive enzymes in the digestive tract, but also by *E. coli* and *lactobacilli* in the intestine. These results indicate that fucoxanthin interacts with and influences *E. coli* and *lactobacilli* in the intestine. Therefore, fucoxanthin isolated from *Undaria pinnatifida* possibly can be applied in human health maintenance.

**Key words** fucoxanthin; *Undaria pinnatifida*; pathogenic bacteria; antibacterial

## 1 Introduction

Fucoxanthin is a major marine carotenoid generally found in brown macroalgae and microalgae (Jaswir *et al*., 2012; Kim *et al*., 2012; Habeebullah *et al.*, 2018). When compared with other carotenoids, the structure of fucoxanthin is different from those of β-carotene and astaxanthin, but similar to those of neoxanthin, dinoxanthin, and peridinin (Komba *et al.*, 2018) (Fig.1). Fucoxanthin includes a polyene chain conjugated to a carbonyl group, an epoxide group, and an unusual allene motif that is also found in about 40 natural carotenoids (Hu *et al.*, 2010; Miyashita *et al.*, 2011). This special structural feature may lead to the antioxidant, anti-inflammatory, and anticancer activities of fucoxanthin (Sachindra *et al.*, 2007; Miyashita *et al.*, 2011; Zorofchian *et al.*, 2014). Some carotenoids demonstrating strong antimicrobial activity can be used as edible antibacterial agents (Milani *et al.*, 2016). For instance, astaxanthin exhibits high antimicrobial activities against tested pathogen species, including *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphyloccocus aureus* (Ushakumari *et al.*, 2013). Xanthin pigment at a concentration of 400 μg $mL^{-1}$ can lyse pathogenic bacterial cells (Sanjay *et al.*, 2009). Carotenoid pigments can also strongly inhibit *Escherichia coli* and exert good antimicrobial activity against *S. aureus*, *Streptococcus faecalis*, and *B. subtilis* (Manimala *et al.*, 2014). However, the antimicrobial activity of fucoxanthin has not been reported.

The human intestine contains trillions of non-pathogenic symbiotic microbes (Guarner *et al.*, 2003; Rakoff-Nahoum *et al.*, 2004). Intestinal microbes serve as an important 'microbial organ' that is involved in diverse functions related to immunity, nutrition absorption, and metabolism (Lievin *et al.*, 2000; Cho *et al.*, 2012; Wu *et al.*, 2017). Changes in the community of intestinal microbes due to aging and various environmental factors may influence the health status of their host. In addition, intestinal microbes survive by relying on their interactions with the environment, including the central nervous system, endocrine system, and immune system (Colpitts *et al.*, 2017; Velmurugan *et al.*, 2017; Yissachar *et al.*, 2017; Cheng *et al.*, 2017). Similar to fucoxanthin, curcumin and other curcuminoids are all carotenoids. In addition to their normal reductive metabolism, curcuminoids can also be biotransformed by intestinal microbes (Burapan *et al.*, 2017). Moreover, a fiber-free diet supplemented with *Undaria pinnatifida* is associated with the changes of microbial activity (Gudielurbano *et al.*, 2002). Further studies are

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needed to confirm the interaction between intestinal microbes and carotenoids. Therefore, this study aimed to determine whether intestinal flora interacts with fucoxanthin.

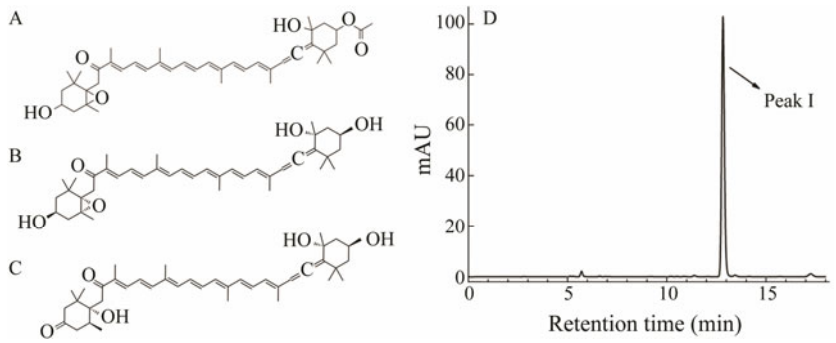


Fig.1 Fucoxanthin and its main metabolites. (A) Chemical structures of fucoxanthin; (B) chemical structures of fucoxanthinol; (C) chemical structures of amarouciaxanthin; (D) spectrum of fucoxanthin extraced from *Undaria pinnatifida*. The experiment was performed at a flow rate of 0.8 mL min$^{-1}$ (25℃) in a ZOR-BAX SB-C18 column (4.6 mm × 250 mm, 5 μm) placed in an HPLC system with an auto sampler, a column oven, and a variable wavelength detector.

In the human body, fucoxanthin can be metabolized into fucoxanthinol and amarouciaxanthin A (Sugawara *et al.*, 2002). In general, the former process occurs in the intestine under the action of digestive enzymes, such as lipase and cholesterol esterase (Asai *et al.*, 2004). Fucoxanthinol is then partially oxidized into amarouciaxanthin A in the liver. Fucoxanthinol preferentially accumulates in the liver and the heart, whereas amarouciaxanthin A accumulates in adipose tissues (Matsumoto *et al.*, 2010). Almost 80% of fucoxanthin metabolites accumulate in white adipose tissue, where subsequent fucoxanthin metabolism leads to the oxidation of fatty acids and heat release (Airanthi *et al.*, 2011). Fucoxanthin metabolism generates a large amount of energy, which is released as heat in fat tissues and thus stimulates thermogenesis (Hu *et al.*, 2012). This thermogenesis (*i.e.*, fat-burning) indicates that fucoxanthin and its metabolites exhibit anti-obesity activity. Fucoxanthin has known functions in the human body, but its digestion, absorption, and metabolism remain unclear to date. Fatty acid esters of carotenoids are believed to be hydrolyzed in the small intestine in humans because no esters have been detected in chylomicrons or serum (Wingerath *et al.*, 1995; Chen *et al.*, 2001; Gammone *et al.*, 2015). However, further studies are needed to explore the metabolic pathways of fucoxanthin in the gut. Therefore, in the present study, we determined whether intestinal microbes can utilize fucoxanthin and explored the effect of fucoxanthin on the community of intestinal microbes.

## 2 Materials and Methods

### 2.1 Materials

Dried *U. pinnatifida* was provided by Rongcheng Jiayi Aquatic Food Co., Ltd. (Weihai, China). The macroporous resins were acquired from Hailaien Chemical Company (Qingdao, China). The standard of all-*trans* fucoxanthin was acquired from Sigma Chemical Company (St. Louis, MO, USA). Solvents for HPLC, including acetonitrile and methyl tert-butyl ether (MTBE), were purchased from Merck (Darmstadt, Germany). The strains in the experiment were all provided by Qingdao Entry&Exit Inspection and Quarantine Bureau.

### 2.2 Fucoxanthin Extraction

Fucoxanthin was extracted from *U. pinnatifida* as described by Zhu *et al*. (2016) with slight modifications. All dried samples were ground into fine powder and then passed through 200 μm sieves. Seaweed powder (100 g) was mixed with 75% (v/v) ethanol with an overhead stirrer for 0.5 h at room temperature. The mixture was kept for 11.5 h at 40℃ to allow the powder to precipitate, and then the supernatant was decanted. The precipitated powder was extracted two more times with the same volume of 75% ethanol. Then all the supernatant was pooled together. All procedures were carried out in the dark to avoid light-induced degradation. Fucoxanthin was purified from the crude extracts using macroporous resins (Qingdao, China). In brief, the resins were activated by being immersed in 75% ethanol for 24 h. Thereafter, the resin was packed into a glass column and equilibrated with 75% ethanol. Then, six column void volumes of the extracts were loaded on the column at a flow rate of 0.5 BV h$^{-1}$ (BV = bed volumes) and eluted with absolute ethanol. Fucoxanthin was collected, concentrated in vacuo, and then stored at −20℃.

### 2.3 HPLC Analysis of Fucoxanthin

HPLC conditions were set as previously reported with slight modifications (Turnbaugh *et al.*, 2006). Fucoxanthin was quantitated with an external standard using an HPLC system (1100, Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a column oven and a variable wavelength detector. A ZORBAX Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 μm, Agilent Technologies Inc., Palo Alto, CA, USA) was used at 25℃ with a detection wavelength of 450 nm. The elution was set up at a flow rate of 0.8 mL min$^{-1}$ with linear gradients of solvents A (acetonitrile/water 80/20, v/v) and B (MTBE). The solvent gradient was started with 100% A. Then, B was

linearly increased from 0 to 10% in 8 min and held constant for 5 min before returning to the initial condition in 3 min. Finally, the solvent was equilibrated with 100% A for 4 min.

### 2.4 Antibacterial Activity

The antibacterial activity of fucoxanthin was measured by well diffusion with slight modifications (Zhu *et al*., 2016). Five human pathogens were selected in this study, including *Enterococcus* sp., *S. aureus*, *S. faecalis*, *B. subtilis*, and *P. aeruginosa*. All bacteria were grown in nutrient broth at 37℃ for 18–24 h with a load of $10^8$–$10^9$ CFU $mL^{-1}$. Aliquots (100 μL) of the cultures were spread on nutrient agar plates. A pre-experiment was carried out to select the appropriate concentration. Three wells with 6 mm width and 5 mm depth were created in the agar and respectively filled with 100 μL of 1 mg $mL^{-1}$ chloramphenicol (positive control), dehydrated alcohol (negative control), and 4.25 mg $mL^{-1}$ fucoxanthin. Three plates were prepared for each bacterium. The plates were incubated at 37℃ for 24 h. At the end of the incubation period, we measured the diameter of the inhibition zone to evaluate the susceptibility of the microorganism.

The effect of fucoxanthin on intestinal microbes was determined. Forty 8-week-old male C57BL/6 mice were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All mice were caged individually, had free access to feed and water, and were raised at 22℃ in a 12 h:12 h light:dark cycle. The diet was prepared in accordance with the recommendations of the American Institute of Nutrition (AIN-93G). Mouse feces were collected after 30 min and placed on ice. The feces were diluted 1:10 (w/v) and broken up in sterile ice-cold phosphate buffered saline (PBS) (0.1 mol $L^{-1}$, pH 7.0). The mixture was centrifuged at 5000×*g* for 5 min to obtain a bacterial suspension. A preliminary experiment was carried out to find the optimal concentration of fucoxanthin. The bacterial suspension was inoculated in brain heart infusion broth in anaerobic culture with concentrations of 0.025 and 0.1 mg $mL^{-1}$ fucoxanthin. After 48 h of incubation at 37℃, 1.0 mL of each culture solution was mixed with ethyl acetate in a 1:1 ratio to extract the metabolites. The extraction was repeated three times, and the extracts were pooled together. The metabolites were analyzed and characterized by HPLC and LC-DAD-APCI- MS/MS. The culture solution was diluted by $1.0\times10^{-4}$, $1.0\times10^{-5}$, and $1.0\times10^{-6}$, respectively. Each dilution was inoculated on MRS and MC agar, and the plates were incubated at 37℃. After 24 h, the colonies were counted. The numbers of *E. coli* and *lactobacilli* were then calculated.

### 2.5 Statistical Analysis

Statistical analysis was performed by SPSS software version 18.0 (SPSS, Inc., Chicago, IL, USA). ANOVA and Duncan tests were used to test differences between the means. The difference was considered significant when $P<0.05$. All experiments were conducted in triplicate, and the results were reported as the mean ± SD (standard deviation).

## 3 Results

### 3.1 Identification of Fucoxanthin in Extracts by HPLC

Fucoxanthin extracted from dried seaweed *U. pinnatifida* was eluted with a retention time of 12.8 min and an extraction yield of 3.37 mg $g^{-1}$. The recovery rate of fucoxanthin from the crude mixture was 93.38%. The purity of fucoxanthin is 82.70%, which is higher than that obtained using the method reported by Xia *et al.* (2013). Fucoxanthin was present in both the crude extracts and the purified products. The macroporous resins effectively separated fucoxanthin from other impurities and demonstrated high extraction efficiency (Fig.2).

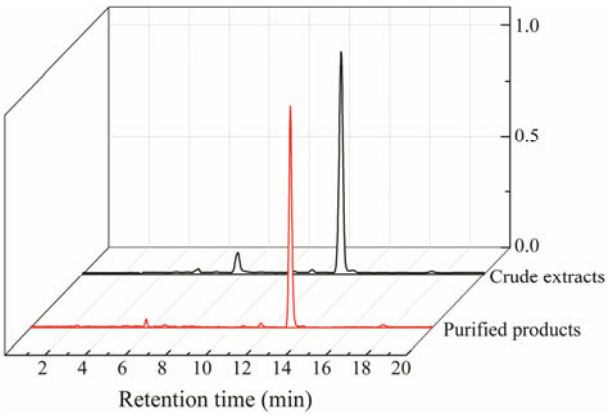


Fig.2 Identification of fucoxanthin in extracts by HPLC.

### 3.2 Antibacterial Activity of Fucoxanthin

Fucoxanthin strongly inhibited *Enterococcus* sp., *S. aureus*, *S. faecalis*, and *B. subtilis* that all belong to Gram-positive bacteria; however, it only weakly inhibited *P. aeruginosa*, a Gram-negative bacterium, suggesting that it is mainly against the Gram-positive bacteria (Table 1).

Table 1 Diameters (mm) of the inhibition zone by fucoxanthin on Gram-positive and Gram-negative bacteria

| Activity | Pathogens | Fucoxanthin (4.25 mg $mL^{-1}$) | Control | |
|---|---|---|---|---|
| | | | PC | NC |
| Gram-positive | *Enterococcus* sp. | $12.66\pm0.38^{a}$ | $11.64\pm0.32^{a}$ | $7.24\pm0.29^{b}$ |
| | *S. aureus* | $21.80\pm0.89^{a}$ | $7.56\pm0.29^{b}$ | $7.45\pm0.17^{b}$ |
| | *S. faecalis* | $25.24\pm1.44^{a}$ | $26.13\pm0.78^{a}$ | $6.00\pm0.01^{b}$ |
| | *B. subtilis* | $25.49\pm1.21^{a}$ | $18.37\pm0.91^{b}$ | $6.00\pm0.01^{c}$ |
| Gram-negative | *P. aeruginosa* | $9.50\pm0.74^{b}$ | $16.07\pm0.66^{a}$ | $6.00\pm0.01^{c}$ |

Notes: PC and NC indicate positive control (chloramphenicol) and negative control (dehydrated alcohol), respectively. The superscripts a, b, and c in the same column indicate significant differences ($P<0.01$) between experimental groups.

Moreover, the inhibitory effect of fucoxanthin on *Enterococcus* sp. and *S. faecalis* was similar to that of the positive control (chloramphenicol). However, fucoxanthin was significantly more effective than chloramphenicol on resisting *S. aureus* and *B. subtilis*.

Effect of fucoxanthin on intestinal microbes was also determined. In this stage, analysis of the culture medium by HPLC and LC-DAD-APCI-MS/MS showed that new compounds were produced, indicating that fucoxanthin was metabolized by intestinal microbes (Fig.3). Peak I was eluted at a retention time of 7.05 min, and Peaks II, III, IV, and V were respectively eluted at retention times of 11.95, 12.48, 15.71, and 17.55 min. Peak II represents fucoxanthin that was not hydrolyzed by intestinal microbes, and Peaks III, IV, and V were identified as isomers of fucoxanthin based on our previous work. Then, Peak I was analyzed using LC-DAD-APCI-MS/MS. All identifications were further verified by analyzing the MS/MS fragmentation of all the assigned [M+H]+parent ions. During APCI MS, most carotenoids undergo fragmentation, which helps to characterize their structures (Breemen *et al.*, 2012). The MS spectra revealed a product with m/z 307 and exhibited a daughter ion with m/z 599, which corresponded to the loss of OH-on C3', [M+H−18]+(Fig.3). Another fragment, m/z 581 ([M+H−18−18]+), corresponded to the loss of an OH- on C5. A third ion (m/z 267, [M+H−18−18−314] +) indicated that the dehydrated molecule underwent a bond cleavage between C15 and C15'. The last ion (m/z 211, [M+H−18−18−314−56] +) was attributed to a bond cleavage between carbons 11' and12'. Accordingly, the product was identified as fucoxanthinol, a metabolite of fucoxanthin (Fig.3).

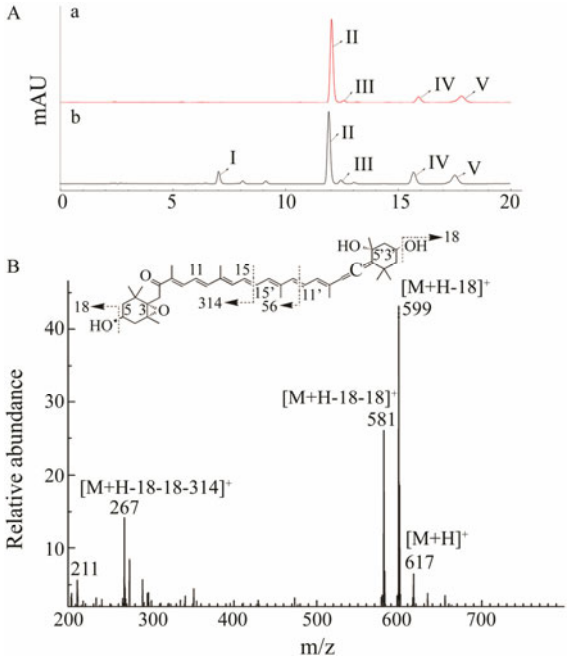


Fig.3 Gut flora can degrade fucoxanthin into fucoxanthinol. (A) HPLC chromatograms of extracts from *Undaria pinnatifida* (a) and the culture solution (b). (B) MS/MS spectra of Peak I in (A).

The effect of fucoxanthin on intestinal microbes was investigated by the plate count method. The quantities of *E. coli* and *lactobacilli* were calculated. As shown in Fig.4, treatment with fucoxanthin at a low concentration (0.025 mg mL$^{-1}$) promoted the growth of *lactobacilli* and *E. coli* in each group. Treatment with fucoxanthin at a high concentration (0.1 mg mL$^{-1}$) promoted the growth of *lactobacilli* but inhibited the growth of *E. coli* in each group.

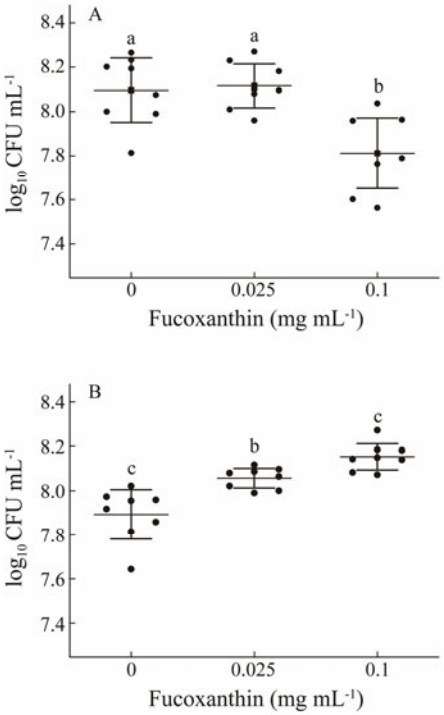


Fig.4 Effect of fucoxanthin on intestinal microbes *E. coli* (A) and *lactobacilli* (B). ANOVA and Duncan tests were used to determine the differences between the means of intestinal microbial growth. Statistical significance was considered at $P<0.05$, which was marked with an asterisk.

## 4 Discussion and Conclusions

Intestinal microbes have various physiological functions. In this study, we showed that fucoxanthin interacts with intestinal microbes. Some studies suggested that fucoxanthin can be hydrolyzed to fucoxanthinol in the intestine by digestive enzymes such as lipase and cholesterol esterase (Sugawara *et al.*, 2002). In addition, fucoxanthinol can be detectable in human plasma after a daily intake of fucoxanthin for 1 week (Asai *et al.*, 2008). Intestinal microbes can degrade fucoxanthin into fucoxanthinol (Fig.5), which may provide a new mechanism for the deacetylation of fucoxanthin. In general, digestive enzymes in the gut can hydrolyze fucoxanthin into fucoxanthinol, or gut flora can degrade fucoxanthin into fucoxanthinol. Similarly, fucoxanthin can promote the growth of gut flora. Intestinal microbes promote nutrient digestion and absorption from the diet by producing different enzymes that assist in the digestion of some indigestible nutritive substances, such as xylan, cellulose, and digestion-resistant starch (Turnbaugh *et al.*, 2006; Mallett *et al.*, 2010; Wang *et al.*, 2011). This finding indicates that the intesti-

nal microbes and nutritive compositions are interrelated and interact with each other.

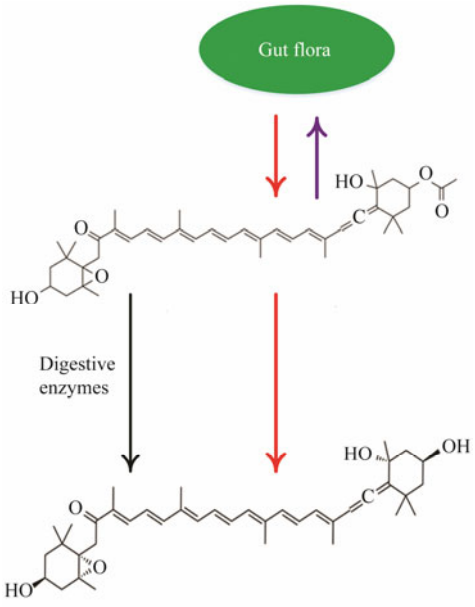


Fig.5 Model of cross-interactions between fucoxanthin and intestinal microbes.

In this study, we obtained fucoxanthin with higher purity than those obtained using other extraction methods inpreviousstudies. We found that the inhibitory effect of fucoxanthin on *Enterococcus* sp. and *S. faecalis* was similar to that of the positive control (chloramphenicol). However, fucoxanthin was significantly more effective than chloramphenicol against *S. aureus* and *B. subtilis*. Although carotenoids have potential antimicrobial activities, plant carotenoids cannot inhibit the growth of Gram-negative bacteria (Milani *et al.*, 2016; Galasso *et al.*, 2017). This selective antibacterial activity may be due to several factors, including charge density, the structure of lipopolysaccharides, and different lipid composition of the cytoplasmic membrane in Gram-negative and Gram-positive bacteria (Devine *et al.*, 2002). This finding indicates that Gram-negative bacteria have stronger defenses or more protective mechanisms than Gram-positive bacteria. Fucoxanthin can be hydrolyzed by intestinal microbes into fucoxanthinol.

Fucoxanthin is a nutritive compound that can be metabolized by intestinal microbes. In addition, intestinal microbes can hydrolyze fucoxanthin into fucoxanthinol, which demonstrates a new mechanism of fucoxanthin metabolism in the intestinal tract.

*Lactobacilli* are beneficial bacteria normally present in the digestive tract (Wang *et al.*, 2017). They can effectively resist the invasion of harmful bacteria to maintain intestinal health. *Lactobacilli* are often used as probiotics because of their preventive and therapeutic effects against disease conditions, such as allergy and inflammation (Johansson *et al.*, 2011). MRS and MC agar have been used to screen *E. coli* and *lactobacilli* separately. In addition, fucoxanthin can promote the growth of intestinal microbes at low concentration since the number of *E. coli* and *lactobacillus* increased when treated with a low concentration of fucoxanthin. On the other hand, high concentrations of fucoxanthin advance the growth of *lactobacillu* and restrain the development of *E. coli* (Fig.4). In this way, different concentrations of fucoxanthin can have distinctive impacts on the development of intestinal microbes, demonstrating that the concentration of fucoxanthin is the key factor. In general, these results demonstrate that fucoxanthin regulates the growth of intestinal microbes and maintains the health of the intestine, which agrees with a previous report (Sarmientorubiano *et al.*, 2007).

In summary, fucoxanthin's interaction with intestinal microbes may increase its bioavailability. Further studies are needed to determine the mechanisms of interaction between fucoxanthin and intestinal microbes.

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