



Effect of culture conditions on growth, fatty acid composition and DHA/EPA ratio of *Phaeodactylum tricornutum*

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ABSTRACT

The effect of growth phase and environmental factors on growth, fatty acid composition and DHA/EPA ratio of *Phaeodactylum tricornutum* Bohlin was studied. Microalgae were grown in laboratory batch cultures in f/2 medium. Cultures were grown at different salinities (15, 20, 28, and 35 ppt), nitrogen (N) concentrations (1.24, 12.35, 24.70 and 49.40 mg L⁻¹), light intensities (50, 100 and 150 μmol m⁻² s⁻¹; 14:10 h light:dark cycle) and temperatures (15, 20 and 25 °C), and sampled at different points of the respective growth phases (inoculum, early-linear, middle-linear and late-linear phases). Samples were analyzed for biomass weight, fatty acid composition and total fatty acid content (TFAC). The main fatty acids in all culture conditions were C14:0 (5.25%–6.04%), C16:0 (13.96%–14.78%), C16:1n-7 (19.09%–35.73%), C18:1n-9 (5.56%–9.01%) and eicosapentaenoic acid (EPA, 22.81%–30.72%). The percentage of polyunsaturated fatty acids (PUFAs) was reduced while that of monounsaturated fatty acids (MUFAs) and TFACs increased with culture time. Salinity had no serious effect on fatty acid composition, however, a significant decline of TFAC was observed at the lowest salinity ($p < 0.05$). Significant ($p < 0.05$) increases of the relative contents of SFAs and MUFAs and a decrease of PUFAs were observed under N-limitation condition. The percentage of docosahexaenoic acid (DHA) was significantly enhanced with increasing light intensity ($p < 0.05$), while that of DHA, EPA and PUFA decreased significantly with increasing temperature ($p < 0.05$). DHA/EPA ratio tended to rise initially and fall later with increasing growth time, and reached the highest level with the lowest salinity, and the lowest temperature and initial N concentration, revealing a possible cell response to the stress brought from the unfavorable conditions. In conclusion, this study demonstrates the variation of growth, fatty acid composition and DHA/EPA ratio with growth phase and environmental factors in *P. tricornutum*, benefiting the production of PUFA-rich microalgae, with a DHA/EPA ratio optimal for aquaculture live food.

Statement of relevance

We conducted detailed analysis on the effect of the culture conditions on growth, fatty acid composition and DHA/EPA ratio in *Phaeodactylum tricornutum*, a widely used microalga for aquaculture feedstuff in China. We found that the environmental stress conditions increased DHA/EPA ratio. The research will benefit the production of PUFA-rich and DHA/EPA-ratio-optimal microalgae for aquaculture feedstuff.

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1. Introduction

Microalgae provide essential polyunsaturated fatty acids (PUFAs), in particular, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for the larvae of aquaculture animals (Borowitzka, 1997; Duerr et al., 1998; Guedes and Maccata, 2012). Recently, it has been also reported that moderate DHA/EPA ratio is vital on the egg and larval quality (Henrotte et al., 2010), nonspecific immunity and disease resistance

(Zuo et al., 2012), and n-3 LC-PUFA retention (Codabaccus et al., 2012). Since microalgae are the initial food for larvae, their DHA and EPA content and the DHA/EPA ratio are important for the development of aquaculture animals. However, the fatty acid profile and content of microalgae vary depending on culture conditions (Richmond and Hu, 2013). Therefore, it is important to find out the effect of these environmental factors on DHA and EPA content and DHA/EPA ratio of microalgae for the production of aquaculture feedstuff.

The main environmental factors reported to influence the fatty acid composition of microalgae include: 1) growth phase (Brown et al., 1996; Liang and Mai, 2005; Liang et al., 2006); 2) nutrient source (Terry et al., 1985; Flynn et al., 1992; Lourenço et al., 2002); 3) salinity

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(Renaud and Parry, 1994; Xu and Beardall, 1997); 4) light (Liang et al., 2001; Carvalho and Malcata, 2005; Guedes et al., 2010); and 5) temperature (Henderson and Mackinlay, 1989; Zhu et al., 1997; Renaud et al., 2002). Although these studies demonstrated the relations between fatty acid composition and culture conditions, most of these relations are species-specific. For example, PUFA and EPA have been reported that some diatoms show a rapid decline with culture age (Liang and Mai, 2005), however, the relative proportions of EPA and DHA have been shown to increase slightly with culture age in other microalgae (Hallegraeff et al., 1991; Shamsudin, 1992). Additionally, more information is needed on the relation between culture conditions and the variations of the DHA/EPA ratio. In this work, we conducted a detailed analysis on the effect of culture conditions on growth, fatty acid composition and DHA/EPA ratio in *P. tricornutum*, a microalga widely used as live food in Chinese aquaculture (Shi et al., 2008), to screen what factors have the maximal effect on growth, fatty acid composition and DHA/EPA ratio for aquaculture purposes.

2. Materials and methods

2.1. Strain and culture

P. tricornutum Bohlin was obtained from the Microalgae Culture Center (MACC), Ocean University of China. Cultures were grown in 500-mL flasks with f/2 medium (Guillard and Ryther, 1962), and shaken at 20 ± 1 °C and 100 rpm under illumination from LED lamps with an irradiance of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ on a 14:10 h light:dark cycle. Salinity and pH of the medium was adjusted to 28 and 8.0, respectively before sterilization. Cultures in the late-exponential growth phase were centrifuged at 4000 g for 5 min and resuspended into the fresh medium for inoculums. Bacterial contamination was checked by inoculating f/2 medium plus 1.5% yeast extract with 1 mL of the culture.

2.2. Salinity, nitrogen, light and temperature

Single factorial experiments were conducted in 500 mL glass flasks with 300 mL of f/2 medium and mean initial cellular concentration of 10^5 mL^{-1} . All cultures were sampled on day 7 for biomass and fatty acids analysis. Four salinities (15, 20, 28, and 35 ppt) were used, diluting seawater (28 ppt) with distilled water to 15 and 20 ppt, or adding natural sea salt to 35 ppt. Sodium nitrate was used as nitrogen source in f/2 medium, resulting in different initial N concentrations of 1.24, 12.35, 24.70 and 49.40 mg L^{-1} , respectively corresponding to 10, 100, 200 and 400% the strength of f/2 medium. Light was provided by LED-arrays with irradiances of 50, 100 and $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ on a 14:10 h light:dark cycle. Finally, cultures were kept at different temperatures of 15, 20 and 25 °C. All treatments were performed in triplicate. Light and temperature treatments were carried out in three incubators with manual shaking four times a day for 2 min each time. Culture conditions of other treatments were the same as the stock culture.

2.3. Growth phase

Cultures were sampled at every day for cell density determination with a hemocytometer (Qiujiang, Shanghai, China). Cultures under normal growth conditions (salinity of 28 ppt, N concentration of 12.35 mg L^{-1} , irradiance of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ and temperature of 20 °C) were sampled on days 0, 3, 7 and 16 for fatty acid analysis, respectively. Growth rates were calculated according to Kratz and Myers (1955).

2.4. Biomass analysis

Cell growth was determined using optical density (OD) readings at 680 nm with a UV-2010 (Hitachi, Japan) spectrophotometer in a 1 cm-

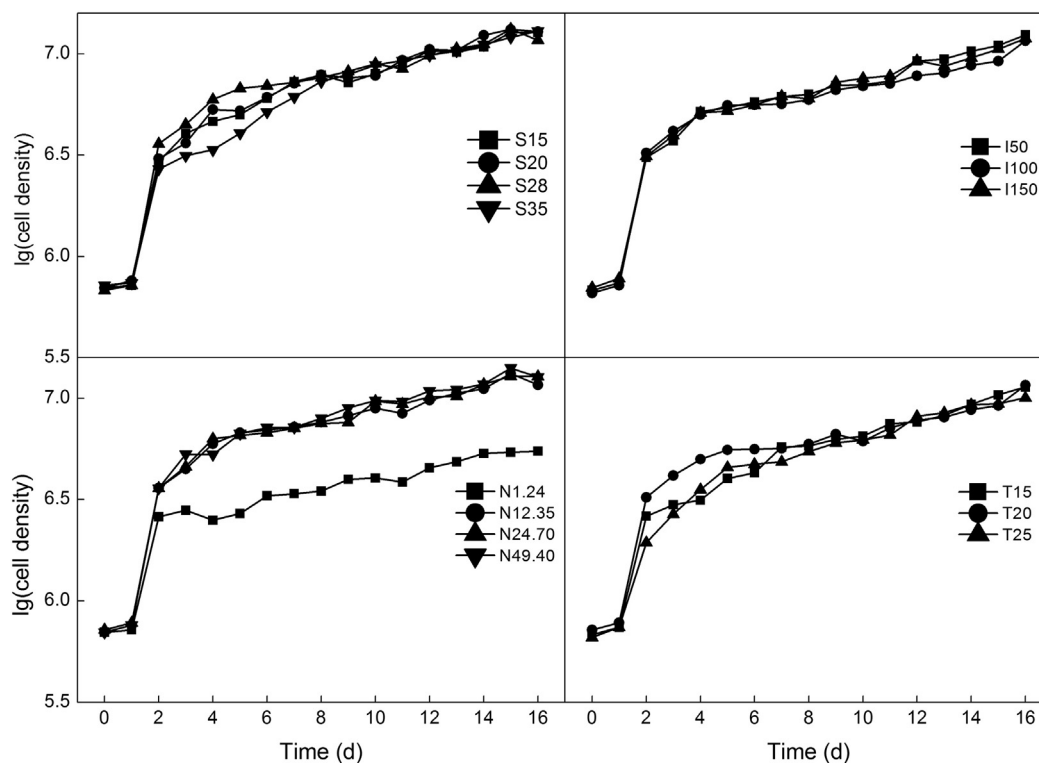


Fig. 1. Growth curves of *Phaeodactylum tricornutum* grown at different conditions. The units for salinity (S), irradiance (I), initial nitrogen concentration (N) and temperature (T) are ppt, $\mu\text{mol m}^{-2} \text{s}^{-1}$, mg L^{-1} and °C, respectively. Cell density is cell numbers per milliliter.

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