The JSFS 85th Anniversary-Commemorative International Symposium
"Fisheries Science for Future Generations"
Symposium Proceedings, No. 07009

Original article

Difference in fatty acid composition of *Rhodomonas* sp. at each growth phase

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Keywords: *Rhodomonas* sp.; Microalgae; Fatty acids; Eicosapentaenoic acid; Docosahexaenoic acid.

Received: 18 July 2017 / Accepted: 4 September 2017

Introduction

Highly unsaturated fatty acids (HUFAs) such as Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) are essential for the growth and survival of marine animals [1]. *Rhodomonas* sp. is used in this study because of its high level contents of HUFAs [2]. For this reason, in the case of this species, it is expected to be used for the purpose of strengthening nutrition. Although microalgae are said to have an influence on the composition of algal bodies by culture conditions and harvest time [3], its effect varies from species to species. Even in this species, although it has been confirmed that the nutritional value is high by the component analysis test and feeding test, it is yet to be studied how the culture environment and the harvest time affect the nutritional value. If the content of n-3 HUFAs in microalgae is increased which is important for marine animals in aquaculture it is considered that the value of nutrition can be improved from a nutritional aspect by effectively examining the culture conditions and harvest time. Fatty acid composition in microalgal cells vary by changes in the culture environment. In this study, *Rhodomonas* sp. was cultured at different growth phases (log-phase of growth, transitional phase, stationary phase), incubation temperatures (16, 20, 24°C) and photon flux densities (PFD: 50, 200, 550 µmol m^{-2} s^{-1}).

Materials and methods

Growth phases
In this study, in order to set the incubation period of the cells to be analyzed, a growth curve was drawn firstly. Cultivation was carried out using four 500 ml flat bottom flasks in an incubator in which the internal temperature was 20°C and the PFD was 50 µmol m^{-2} s^{-1} (white LED, 24 h continuous illumination). An artificial seawater (Live Sea Salt, made by DELPHIS) whose salinity was adjusted to 28 psu was sterilized in an autoclave at 121°C for 15 minutes, and a nutrient salt of Guillard 2F culture solution was added. In addition, aeration by stirring was performed at 900 ml min^{-1} to equalize the culture solution. Cells were fixed with 1% phosphate buffered formalin solution on a daily basis, and the number of cells was counted using an optical microscope and Toma hemocytometer. Culturing and counting continued until the stationary phase (where cells did not increase), and the results of three trials were averaged to obtain the following growth curves. It was decided on the 7th day ($\mu = 0.14$) after inoculation as log-phase of growth, on the 19th day ($\mu = 0.04$) as transitional phase, on the 31st day ($\mu = 0.01$) as stationary phase and cells were collected at each phase. For the calculation of the specific growth rate (μ), the following formula was used.

$$\mu=\ln(mt_1/mt_2)/(t_2-t_1);\ t_2 > t_1$$

*mt_1 is the cell density at day t_1, mt_2 is the cell density at day t_2.

Cultivation temperature
Except for changing temperature and PFD, culturing was carried out using the same method as the '*growth phase*' experiment. Collection was carried out in the log-phase of growth, cultured PFD was 200 µmol m^{-2} s^{-1}, and temperature was 16°C, 20°C and 24°C, respectively.

Photon flux density (PFD)
Except for changing the PFD, using the same method as the '*growth phase*'. Collection was carried out in the log-phase of growth, and the PFD was 50, 200, 550 µmol m^{-2} s^{-1}, respectively.

Results and discussion

As the culture period increased with differences in growth phases, there was a decrease in fatty acids with long carbon chains and an increase in fatty acids with short carbon chains (Table 1). It was found that HUFAs such as EPA and DHA reached their highest peaks in the log-phase of growth (10.2%, 6.2%) and then

decreased as the culture period progressed (Table 1).

Table 1. Fatty acid composition of laboratory batch cultures of *Rhodomonas* sp. grown at different growth phase

Fatty acid	Percentage (area%)		
	Log-phase of growth	Transitional phase	Stationary phase
Palmitic acid (C16:0)	9.3	8.4	15
Oleic acid (C18:1)	10.5	8.2	15
Linoleic acid (C18:2n-6)	16.5	22.6	23.6
α-Linolenic acid (C18:3n-3)	13.6	6.9	4.7
Arachidonic acid (C20:4n-6)	2.8	2.9	ND
EPA (C20:5n-3)	10.2	7	3.2
DHA (C22:6n-3)	6.2	5.3	3.3

* ND, not detected.

Regarding temperature, fatty acids such as myristic acid and palmitic acid increased as temperature rised(Table 2). EPA and DHA reached maximum levels at 20°C (12.2%, 5.3%) (Table 2).

Table 2. Fatty acid composition of laboratory batch cultures of *Rhodomonas* sp. grown at different incubation temperature

Fattyacid	Percentage (area%)		
	16°C	20°C	24°C
Palmitic acid (C16:0)	12.9	15.0	19.4
Oleic acid (C18:1)	3.6	8.4	5.4
Linoleic acid (C18:2n-6)	11.5	10.7	5.4
α-Linolenic acid (C18:3n-3)	14.8	11.8	16.0
Arachidonic acid (C20:4n-6)	1.0	3.0	ND
EPA (C20:5n-3)	7.9	12.2	6.4
DHA (C22:6n-3)	4.9	5.3	4.2

With accordance to PFD, myristic acid and palmitic acid increased as light intensity became stronger, and EPA and DHA remarkably decreased when reaching a PFD of 550 μmol m^{-2} s^{-1} (Table 3). There was no significant change in HUFA amount at PFD, 50 μmol m^{-2} s^{-1} and 200 μmol m^{-2} s^{-1}, but the growth rate was lower at PFD, 50 μmol m^{-2} s^{-1} (Table 3, Fig. 1). For this reason, it is considered that a PFD of 200 μmol m^{-2} s^{-1} is best suited for producing *Rhodomonas* sp. as live feed for marine animals in aquaculture.

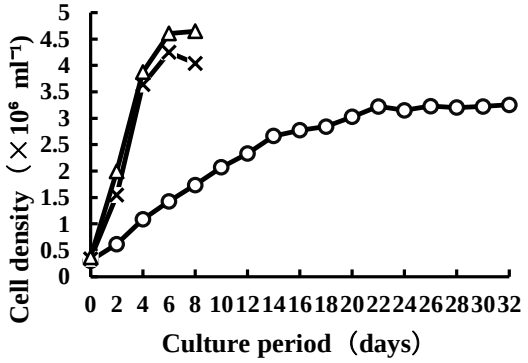

Fig. 1. Growth of laboratory batch cultures of *Rhodomonas* sp. cultured at different incubation PFD: △, 550 μmol m^{-2} s^{-1}; ×, 200 μmol m^{-2} s^{-1}; ○, 50 μmol m^{-2} s^{-1}

Table 3. Fatty acid composition of laboratory batch cultures of *Rhodomonas* sp. grown at different incubation PFD

Fatty acid	Percentage (area%)		
	50 μmol m^{-2} s^{-1}	200 μmol m^{-2} s^{-1}	550 μmol m^{-2} s^{-1}
Palmitoleic acid (C16:0)	9.3	15.0	15.6
Oleic acid (C18:1)	10.5	8.4	6.1
Linoleic acid (C18:2n-6)	16.5	10.7	9.2
α-Linolenic acid (C18:3n-3)	13.6	11.8	12.5
Arachidonic acid (C20:4n-6)	2.8	3.0	3.3
EPA (C20:5n-3)	10.2	12.2	6.2
DHA (C22:6n-3)	6.2	5.3	4.4

Conclusions

In this study, the composition of fatty acids greatly varies depending on culture conditions and culture period. Considering the growth rate and fatty acid composition, optimum temperature of 20°C and the PFD of 200 μmol m^{-2} s^{-1} is best for producing feed with high value. In the future experiments, research is to be conducted including the total lipid content, and it is also recommended to study the culture conditions and harvest time in a larger scale practical experiment.

Acknowledgements

I am very grateful for technical support given by H. Onishi.

References

1. Takeuchi T (1991) Kagaku to Seibutsu 29: 571–580
2. Yamamoto S, Okauchi M, Yoshimatsu T (2015) *Nippon Suisan Gakkaishi* 81: 973–978
3. Okauchi M, Zhou W-J, Zou W-H, Fukusho K, Kanazawa A (1990) *Nippon Suisan Gakkaishi* 56: 1293–1298