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ORIGINAL PAPER

Enhancement of Polyunsaturated Fatty Acid Production by Selenium Treatment in Polyunsaturated Fatty Acid-Producing Fungus

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Abstract The effect of selenium (Se) treatment on the content of intracellular polyunsaturated fatty acids (PUFA) was investigated. Selenium treatment could effectively increase the yields of arachidonic acid (AA) and linoleic acid (LA). When AA-producing *Diasporangium jonesianum* recombinant strain D-A1 was cultured in a medium containing 90 μg mL^{-1} Se, the growth of *D. jonesianum* recombinant strain D-A1 was constrained, but the content of AA in the total fatty acids reached 9.3%, 1.7-fold higher than the control. The content of LA was also increased from 25.9 to 37.7%. Under various culture conditions, Se treatment (90 μg mL^{-1}) was generally beneficial to the yields of LA and AA. However, there was no significant increase in the contents of eicosapentaenoic acid (EPA) and γ-linolenic acid. Therefore, the use of Se is advantageous for regulating the content of intracellular PUFA in *D. jonesianum* recombinant strain D-A1, and could be good enhancer in the yields of AA and LA.

Keywords Selenium · Polyunsaturated fatty acid · Linoleic acid · Arachidonic acid

Introduction

Polyunsaturated fatty acids (PUFA) are important nutrients, because not only they are essential constituents of cell membranes or precursors for eicosanoids, but also they have their roles in gene expression in liver and brain [1]. Linoleic acid (LA, 18:2n-6), which cannot be synthesized in mammals, is essential precursors for n-6 series of PUFA. The intake of LA in European and North American countries increased greatly in the second half of the 20th century, following the introduction and marketing of cooking oils and margarines [2]. Arachidonic acid (AA, 5, 8,11,14-*cis*-eicosatetraenoic acid), which is produced from LA through a series of desaturation and elongation steps, is an essential fatty acid in human nutrition and a biogenetic precursor of the biologically active prostaglandins and leukotrienes [3].

Increasing interest has been generated in microbial production of lipid containing polyunsaturated fatty acids for the past decade. As a result, some screening procedures were developed to obtain PUFA producing strains. In our laboratory, *Diasporangium jonesianum*, an oleaginous fungus, had been screened and identified as a promising producer of polyunsaturated fatty acids. To increase AA productivity, we then further developed a new strain, *D. jonesianum* recombinant strain D-A1, by using genome shuffling [4].

To enhance the nutritional value of *D. jonesianum* recombinant strain D-A1, we added selenium (Se) to the culture medium. Increasing scientific evidence indicates that Se is a trace element that has many beneficial effects for human beings and many other forms of life [5]. It is generally believed that the organic Se compounds are better and safer in ingestion than inorganic Se. In recent years, Se supplementation using microorganisms has received much attention.

Our previous studies established that *D. jonesianum* recombinant strain D-A1 can biologically transform inorganic Se into organic Se. We found that the best content of additional Se in mycelia was 90 μg Se per mL of fermentation culture medium [6]. The stress of Se to the

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microorganism may be expressed in a number of ways, among which alterations in the cellular levels of reactive oxygen species (ROS) would be happened [7]. It is known that environmental stress can increase levels of ROS, such as the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), which disturb the steady-state balance of pro-oxidants and antioxidants, causing oxidative stress [8]. Dan and Ephraim [9] reported that proteins could also be involved in the protection of microorganism against Se stress. So far, there is no direct investigation on Se supplementation to polyunsaturated fatty acids (PUFA). Therefore we believe that it is important to understand whether Se stress could affect the content of PUFA. The objective of this study was to understand the effects of Se stress on fatty acid composition and growth parameters of *D. jonesianum* recombinant strain DA-1.

Materials and Methods

Strains, Medium and Stress Treatments

Diasporangium jonesianum recombinant strain D-A1 was maintained at 4 °C on potato dextrose agar (per liter) potato extract 200 g, glucose 20 g, agar 20 g) sub-cultured every month, and preserved by this laboratory. Inoculum was prepared by an overnight culture at 25 °C with 150 rpm shaking speed in 25 mL medium (per liter) potato starch (200 g as basis), glucose (50 g) and corn chip (∅1.2–1.3 cm) (50 g) as carbon source, soybean flour (not defatted) (10 g) as nitrogen source, K_2HPO_4 (0.5 g), $MgSO_4(7H_2O)$ (0.5 g), NaCl (0.5 g), Mn^{2+} (8.5×10^{-2} g), and Cu^{2+} (0.6×10^{-2} g) as inorganic salt, pH 6.5 contained in 250-mL Erlenmeyer conical flasks. The same medium was used in the initial fermentation. The mycelia were cultured in 50 mL of medium in 250-mL Erlenmeyer flasks for 5 days. For the treated medium, 90 μg mL^{-1} Na_2SeO_3 was added to the culture medium. The medium without Se addition was considered the blank control. The factors evaluated in this research included Se concentration (80, 90, 100, and 110 μg mL^{-1}), carbon source (40, 60, 80, and 100 g L^{-1}), temperature (18, 22, 25, and 28 °C), pH (4.5, 5.5, 6.5, 7.5, and 8.5) and shaking speed (180, 210, 250, and 280 rpm). The growth of mycelia was evaluated using the dry mycelia weight after suction filtration, washing with distilled water and drying at 60 °C for 24 h.

Fatty acid Methyl Ester (FAME) Preparation and Analysis

FAME preparation were conducted according to modified methods of Riley et al. [10] and Darbre [11]. Fresh wet mycelium (0.1 g) was added to 6 mL of methanol:sulfuric acid (10:1 v/v), vigorously mixed for 2 min and paced in a water bath at 55 °C for 4 h. The reaction mixture was cooled to room temperature and mixed vigorously with cyclohexane (6 mL) and allowed to stand until phase separation. The upper layer was collected, treated with anhydrous sodium sulfate to remove water and immediately analyzed.

FAME were determined by a Hewlett-Packard Model HPSF-1890 gas chromatograph (Hewlett-Packard Shanghai Ltd., Shanghai, PR China) with a flame-ionization detector (240 °C) and a split injector (240 °C). The column used was an HP-INNOWax (Crosslinked polyethylene Glycol) capillary column (30 m × 0.25 mm × 0.25 mm, Agilent Technologies, Inc., USA). The flow speed of Hydrogen gas was 26.8 mL/min, the flow speed of Nitrogen gas (carrier gas) was 4 mL/min, split was 22:1, and the flow speed of air was 354.2 mL/min.

The temperature program was as follows: initial temperature 170 °C and held for 2.5 min, then increased to 190 °C at 10 °C/min and held for 2 min at 190 °C, then increased at 5 °C/min to 210 °C and held for 4 min at 210 °C, and then increased at 10 °C/min to 220 °C and finally held for 7.5 min. Quantitative data are obtained by integrating of peak areas with a Hewlett-Packard model HP 3295 integrator. Identification of fatty acid methyl esters was carried out using external and internal standards [12].

Index of unsaturation of fatty acids (IUFA) = 16:1 (mol%) + 18:1 (mol%) + 18:2 (mol%) × 2 + 18:3 (γ-) (mol%) × 3 + 20:3 (mol%) × 3 + 20:4 (mol%) × 4 + 20:5 (mol%) × 5 + others (mol%) [13].

Statistical Analysis

All determinations were carried out in triplicate, and data were subjected to analysis of variance (ANOVA). ANOVA analysis and principal component analysis (PCA) were performed using SPSS (Statistical Program for Social Sciences). Significant differences between means were determined by Student–Newman–Keuls. Means were considered significantly different at $P < 0.05$.

Results and Discussion

Response of Growth Characteristics

Figure 1 shows the growth profile of *D. jonesianum* recombinant strain D-A1 at 25 °C in the media containing Se and the control. Decreased growth rate of cells was found in the medium containing 90 μg mL^{-1} Se. The highest biomass accumulation occurred at day 5, when the Se content in the treated group reached 6.5 g L^{-1}, 49.5%

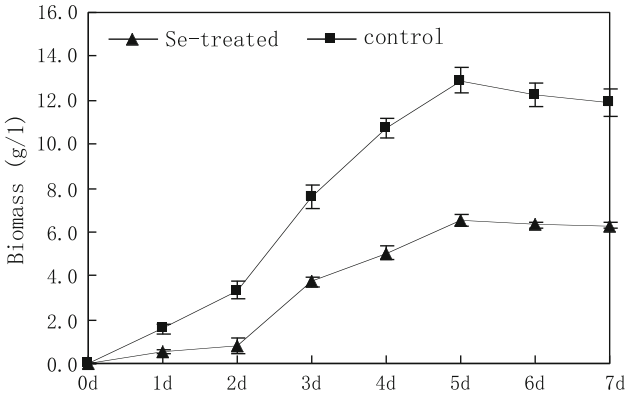

Fig. 1 Growth characteristics of *D. jonesianum* recombinant strain D-A1 in media with or without Se

lower than that of the control. Both treated and control samples stopped growing after day 5 due to insufficient energy and carbon sources. The growth rates lower than the control might be due to the stress of Se [14]. Se can be involved in the normal biochemical processes of the cell, but can be toxic in excess [15]. Although our previous studies showed that *D. jonesianum* recombinant strain D-A1 had a high tolerance to Se, uptake of Se in excess of requirements might have caused some negative or even toxic physiological and biochemical effects [16], resulting in inhibition in growth. The mechanism by which Se affects the growth of organisms is still unknown.

Effects of Se Treatment on Fatty Acid Composition of *Diasporangium jonesianum* Recombinant Strain D-A1

Diasporangium jonesianum recombinant strain D-A1 was rich in PUFA (Table 1). Table 2 shows the fatty acid composition of *D. jonesianum* recombinant strain D-A1 grown in the medium containing different levels of Se. The contents of LA (18:2) and AA (20:4) increased markedly with Se addition at all the tested concentrations (80, 90, 100, 110 μg mL^{-1}). The highest LA (18:2) content (37.7%, mol%) was obtained from cell medium containing 90 μg mL^{-1} Se. This linoleic acid level was 1.4-fold of that of the control. The Se treatment at 90 μg mL^{-1} apparently enhanced the content of AA 1.7-fold, and IUFA by 1.2-fold, as compared to the control. Meanwhile, the production levels of myristic acid (14:0), hexadecenoic acids (16:1), and octadecenoic (18:1) decreased with Se addition to the medium, whereas the level of other fatty acids such as EPA and γ-linolenic acid changed only slightly with the Se treatments.

We further studied how different culture conditions, such as temperature, shaking speed, pH, and carbon sources content affect the composition and yields of fatty acids at Se treatment of 90 μg Se per mL. The PCA was also used to assess the factors which were mostly correlated to the change of fatty acids under Se stress conditions.

There were apparent differences in the fatty acid compositions between Se-treated strains and non-treated cells (Tables 3, 4, 5, 6, 7, 8, 9, 10). The biomass of *D. jonesianum* recombinant strain D-A1 with different culture conditions under Se stress was much lower than the control (Tables 7, 8, 9, 10). The accumulation of total fatty acids was also affected by Se treatment. Most fatty acids were lower than the control. However, the LA (18:2) and AA yields were much higher than the non-treated ones. Compared to the control, the LA (18:2) content in Se-treated cells was 1.4-fold higher when culture temperature was at 18 °C, 1.6-fold at 210 rpm shaking speed, 2.1-fold when the carbon sources content was 40 g L^{-1} and 1.5-fold when pH was 6.5. Similarly, the amount of arachidonic acid in Se-treated cells was 1.7-fold higher than the control at 25 °C, 1.6-fold at 150 rpm, 1.7-fold when the carbon sources content was 100 g L^{-1}, and 1.8-fold when pH was 4.5. Both LA and AA contents were enhanced significantly by Se stress. The yields of LA and AA in Se-treated cells were also higher than the control with different culture conditions (Tables 7, 8, 9, 10). There was no significant increase in the contents of eicosapentaenoic acid (EPA) and γ-linolenic acid, whereas the levels of hexadecenoic acids (16:1), octadecenoic (18:1) and myristic acid (14:0) were decreased. The IUFA of Se-treated strains were much higher than those in the control, indicating that Se may improve the accumulation of PUFA. With Se treatment, the contribution rates of the top two principal components, carbon source and temperature, were 77.3 and 13.1% while the cumulative contribution rate was 90.4%. Compared to other culture conditions, carbon content had the largest positive correlation with the effect of Se treatment on the fatty acids, while temperature was the second factor (Tables 11, 12).

In this study, selenium treatment could enhance the levels of unsaturated fatty acids of the n-6 series, especially

Table 1 Lipid composition of *D. jonesianum* recombinant strain D-A1

Lipid composition	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:3	20:4	20:5	Others
Content (mol%)	9.9	19.8	3.6	2.9	21.7	25.2	3.0	4.5	5.6	1.6	2.2

14:0 (myristic acid); 16:0 (palmitic acid); 16:1 (hexadecenoic acids); 18:0 (stearic acid); 18:1 (octadecenoic); 18:2 (linoleic acid); 18:3(γ-) (γ-linolenic acid); 20:3 (eicosatrienoic acid); 20:4 (arachidonic acid); 20:5 (eicosapentaenoic acid)

Table 2 Effects of Se on fatty acid composition from cultured *D. jonesianum* recombinant strain D-A1

Fatty acid (mol%)	Selenium (μg mL^{-1})				
	0	80	90	100	110
14:0	10.3 ± 0.8^{e}	6.5 ± 0.0^{ab}	6.1 ± 0.0^{a}	7.1 ± 0.3^{c}	6.7 ± 0.1^{ab}
16:0	20.3 ± 0.6^{a}	17.7 ± 0.7^{a}	19.6 ± 0.6^{a}	19.9 ± 2.0^{a}	20.0 ± 1.2^{a}
16:1	3.4 ± 0.1^{c}	1.1 ± 0.0^{a}	1.2 ± 0.1^{a}	1.9 ± 0.1^{b}	2.0 ± 0.0^{b}
18:0	2.8 ± 0.1^{c}	2.2 ± 0.0^{a}	2.6 ± 0.0^{b}	2.8 ± 0.0^{c}	2.6 ± 0.0^{b}
18:1	20.9 ± 1.3^{b}	18.6 ± 1.4^{a}	16.2 ± 0.4^{a}	16.4 ± 1.4^{a}	17.8 ± 1.4^{a}
18:2	26.0 ± 1.2^{a}	31.4 ± 1.0^{b}	37.7 ± 0.7^{e}	35.5 ± 1.1^{d}	33.3 ± 1.0^{c}
18:3	3.1 ± 0.0^{e}	2.9 ± 0.0^{d}	2.8 ± 0.0^{c}	2.0 ± 0.0^{b}	1.9 ± 0.0^{a}
20:3	4.4 ± 0.1^{d}	3.8 ± 0.0^{c}	3.2 ± 0.0^{a}	3.6 ± 0.0^{b}	4.4 ± 0.1^{d}
20:4	5.5 ± 0.0^{a}	9.2 ± 0.1^{c}	9.3 ± 0.2^{c}	7.3 ± 0.2^{b}	7.2 ± 0.1^{b}
20:5	1.6 ± 0.0^{d}	1.2 ± 0.1^{b}	1.0 ± 0.0^{a}	1.9 ± 0.0^{e}	1.4 ± 0.0^{c}
Others	1.9	5.4	0.2	1.8	2.6
IUFA	128.8	145.3	153.5	144.6	141.2

Diasporangium jonesianum recombinant strain D-A1 was cultured at 25 °C with 150 rpm shaking speed in the medium (per liter) 200 g potato starch as basis, 50 g glucose and 50 g corn chip (∅1.2–1.3 cm) as carbon source, 10 g soybean flour (not defatted) as nitrogen source, 0.5 g K_2HPO_4, 0.5 g $MgSO_4(7H_2O)$, 0.5 g NaCl, 8.5×10^{-2} g Mn^{2+}, and 0.6×10^{-2} g Cu^{2+} as inorganic salt, pH 6.5. The fatty acid contents are given as means ± SD of triplicate samples. Within the same column, values followed by different letters (a, b, c) differ significantly at $P < 0.05$

Table 3 The effect of culture temperature on fatty acid composition from cultured *D. jonesianum* recombinant strain D-A1, with or without Se

Fatty acid (mol%)	Temperature (°C)							
	18		22		25		28	
	Se	C	Se	C	Se	C	Se	C
14:0	4.0 ± 0.1^{a}	8.6 ± 0.2^{d}	3.8 ± 0.1^{a}	7.6 ± 0.4^{c}	5.1 ± 0.0^{b}	9.7 ± 0.2^{e}	5.2 ± 0.1^{b}	10.2 ± 0.2^{f}
16:0	16.1 ± 0.6^{a}	18.5 ± 0.2^{ab}	17.7 ± 0.2^{ab}	21.4 ± 2.2^{c}	18.3 ± 1.1^{ab}	20.6 ± 1.8^{bc}	18.6 ± 0.8^{ab}	20.5 ± 0.2^{bc}
16:1	1.4 ± 0.1^{b}	3.9 ± 0.1^{f}	1.1 ± 0.0^{a}	3.1 ± 0.1^{d}	1.1 ± 0.0^{a}	3.3 ± 0.0^{e}	1.0 ± 0.0^{a}	2.2 ± 0.0^{c}
18:0	2.6 ± 0.1^{c}	2.4 ± 0.0^{b}	2.6 ± 0.1^{c}	1.9 ± 0.1^{a}	2.5 ± 0.0^{bc}	2.6 ± 0.0^{c}	2.5 ± 0.0^{c}	3.0 ± 0.0^{d}
18:1	18.6 ± 0.8^{ab}	20.4 ± 0.3^{c}	17.9 ± 0.2^{a}	19.5 ± 0.6^{bc}	17.6 ± 0.6^{a}	22.0 ± 1.6^{d}	17.3 ± 0.3^{a}	22.9 ± 0.4^{d}
18:2	41.5 ± 0.2^{d}	29.0 ± 0.2^{a}	40.9 ± 0.7^{d}	31.5 ± 2.1^{b}	38.2 ± 2.1^{c}	27.1 ± 0.6^{a}	38.4 ± 0.4^{c}	26.8 ± 0.1^{a}
18:3	2.9 ± 0.0^{d}	2.6 ± 0.0^{c}	2.6 ± 0.0^{e}	3.2 ± 0.1^{c}	3.3 ± 0.0^{e}	2.3 ± 0.0^{a}	3.4 ± 0.0^{f}	2.4 ± 0.0^{b}
20:3	2.5 ± 0.1^{a}	3.9 ± 0.1^{e}	3.2 ± 0.0^{c}	3.7 ± 0.1^{d}	2.7 ± 0.0^{b}	5.0 ± 0.1^{f}	3.9 ± 0.0^{e}	5.5 ± 0.0^{g}
20:4	7.7 ± 0.1^{a}	7.4 ± 0.1^{b}	8.4 ± 0.5^{b}	6.0 ± 0.1^{ab}	8.4 ± 0.5^{b}	5.0 ± 0.0^{a}	8.0 ± 0.5^{b}	5.2 ± 0.0^{a}
20:5	2.8 ± 0.0^{e}	2.2 ± 0.1^{d}	1.5 ± 0.1^{c}	1.6 ± 0.0^{c}	1.3 ± 0.0^{b}	1.3 ± 0.0^{b}	1.1 ± 0.0^{a}	1.2 ± 0.0^{a}
Others	0.1	1.1	0.3	0.7	1.5	1.2	0.6	0.3
IUFA	163.8	142.3	159.6	137.9	153.0	127.5	154.6	128.7

Diasporangium jonesianum recombinant strain D-A1 was cultured at 150 rpm shaking speed with Se concentration as 90 μg mL^{-1} in the medium (per liter) 200 g potato starch as basis, 50 g glucose and 50 g corn chip (∅1.2–1.3 cm) as carbon source, 10 g soybean flour (not defatted) as nitrogen source, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$ ($7H_2O$), 0.5 g NaCl, 8.5×10^{-2} g Mn^{2+}, and 0.6×10^{-2} g Cu^{2+} as inorganic salt, pH 6.5. Se represents the strain treated by Se. C represents the control. The fatty acid contents are given as means ± SD of triplicate samples. Within the same column, values followed by different letters (a, b, c) differ significantly at $P < 0.05$

long chain fatty acids, such as 18:2(n-6) and 20:4(n-6). These results are partially in agreement with a study on human breast milk, which claimed that the content of polyunsaturated fatty acids especially LA could be significantly increased by Se-enriched diets, while saturated fatty acids content decreased [17].

Czauderna et al. [18] found that Se could increase the content of conjugated linoleic acid in rat muscle. Hock et al. [19] reported that Se treatment tended to increase polyunsaturated fatty acids of the n-3 series in heart and aorta, 22:5(n-3) and 20:5(n-3), respectively. The latter of these may serve as the precursor of PGI_3, a vasodilator and

Table 4 The effect of shaking speed on fatty acid composition from cultured *D. jonesianum* recombinant strain D-A1, with or without Se

Fatty acid (mol%)	Shaking speed (rpm)							
	120		150		180		210	
	Se	C	Se	C	Se	C	Se	C
14:0	4.6 ± 0.2^{bc}	8.6 ± 0.1^{c}	5.2 ± 0.1^{c}	10.1 ± 0.3^{d}	3.7 ± 0.1^{a}	9.6 ± 0.3^{d}	4.1 ± 0.2^{ab}	10.0 ± 1.0^{d}
16:0	18.7 ± 1.2^{a}	17.8 ± 1.2^{a}	19.0 ± 0.7^{a}	20.2 ± 1.7^{a}	19.1 ± 0.3^{a}	25.5 ± 2.9^{a}	20.8 ± 1.6^{b}	21.8 ± 0.8^{a}
16:1	1.0 ± 0.0^{a}	2.6 ± 0.0^{d}	1.0 ± 0.0^{a}	3.5 ± 0.1^{e}	1.0 ± 0.0^{a}	1.2 ± 0.0^{b}	1.0 ± 0.0^{a}	2.5 ± 0.0^{c}
18:0	2.7 ± 0.0^{e}	2.5 ± 0.03^{c}	2.5 ± 0.0^{c}	2.6 ± 0.0^{d}	2.3 ± 0.0^{b}	1.0 ± 0.0^{a}	2.4 ± 0.0^{b}	3.0 ± 0.1^{f}
18:1	17.2 ± 1.3^{ab}	22.1 ± 1.1^{d}	17.4 ± 1.4^{ab}	22.2 ± 1.2^{d}	18.2 ± 1.1^{c}	16.6 ± 0.8^{ab}	15.1 ± 0.5^{a}	21.8 ± 1.3^{d}
18:2	40.1 ± 1.8^{bc}	28.8 ± 1.5^{a}	39.4 ± 1.4^{bc}	26.9 ± 2.0^{a}	43.8 ± 4.1^{bc}	35.8 ± 4.8^{b}	38.6 ± 1.3^{bc}	23.5 ± 2.5^{a}
18:3	2.8 ± 0.0^{d}	3.4 ± 0.1^{f}	3.2 ± 0.0^{e}	1.9 ± 0.0^{a}	2.8 ± 0.0^{d}	2.3 ± 0.0^{b}	3.4 ± 0.0^{f}	2.5 ± 0.1^{c}
20:3	3.9 ± 0.0^{e}	3.1 ± 0.2^{d}	2.5 ± 0.0^{c}	4.9 ± 0.1^{g}	1.9 ± 0.0^{a}	2.1 ± 0.0^{b}	4.7 ± 0.1^{f}	5.5 ± 0.1^{a}
20:4	7.6 ± 0.1^{e}	7.1 ± 0.7^{e}	8.5 ± 0.4^{f}	5.2 ± 0.1^{b}	5.9 ± 0.2^{c}	4.6 ± 0.0^{a}	8.3 ± 0.1^{f}	6.4 ± 0.1^{d}
20:5	1.4 ± 0.0^{b}	1.6 ± 0.0^{c}	1.3 ± 0.0^{b}	2.0 ± 0.0^{d}	1.1 ± 0.0^{a}	1.1 ± 0.0^{a}	1.4 ± 0.0^{b}	2.3 ± 0.0^{e}
Others	0.1	2.6	0.1	0.6	0.4	0.3	0.2	0.6
IUFA	155.5	138.0	154.7	130.6	149.5	126.4	157.7	132.5

Diasporangium jonesianum recombinant strain D-A1 was cultured at 25 °C with Se concentration as 90 μg mL^{-1} in the medium (per liter) 200 g potato starch as basis, 50 g glucose and 50 g corn chip (∅1.2–1.3 cm) as carbon source, 10 g soybean flour (not defatted) as nitrogen source, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$ ($7H_2O$), 0.5 g NaCl, 8.5×10^{-2} g Mn^{2+}, and 0.6×10^{-2} g Cu^{2+} as inorganic salt, pH 6.5. Se represents the strain treated by Se. C represents the control. The fatty acid contents are given as means ± SD of triplicate samples. Within the same column, values followed by different letters (a, b, c) differ significantly at $P < 0.05$

Table 5 The effect of carbon sources content on fatty acid composition from cultured *D. jonesianum* recombinant strain D-A1, with or without Se

Fatty acid (mol%)	Carbon sources content (g/L)							
	40		60		80		100	
	Se	C	Se	C	Se	C	Se	C
14:0	4.6 ± 0.1^{b}	10.9 ± 0.8^{d}	4.5 ± 0.1^{b}	11.4 ± 0.4^{d}	3.3 ± 0.0^{a}	11.7 ± 0.9^{d}	5.2 ± 0.2^{b}	9.8 ± 0.0^{c}
16:0	18.6 ± 0.8^{ab}	20.4 ± 1.6^{ab}	19.4 ± 1.4^{ab}	21.8 ± 0.8^{b}	18.3 ± 1.4^{a}	20.0 ± 1.0^{ab}	18.8 ± 1.5^{ab}	20.7 ± 0.9^{ab}
16:1	0.9 ± 0.0^{a}	3.5 ± 0.0^{d}	1.0 ± 0.0^{a}	3.4 ± 0.1^{d}	1.1 ± 0.0^{b}	2.3 ± 0.2^{c}	1.2 ± 0.0^{b}	3.4 ± 0.1^{d}
18:0	2.3 ± 0.0^{a}	4.2 ± 0.2^{d}	2.9 ± 0.1^{c}	2.8 ± 0.1^{bc}	2.7 ± 0.0^{bc}	2.7 ± 0.1^{bc}	2.6 ± 0.1^{b}	2.6 ± 0.1^{b}
18:1	15.5 ± 0.5^{a}	24.0 ± 1.7^{b}	17.0 ± 0.9^{a}	25.2 ± 1.5^{b}	17.7 ± 1.3^{a}	24.1 ± 2.3^{b}	17.0 ± 1.1^{a}	22.1 ± 2.0^{b}
18:2	42.5 ± 1.3^{c}	19.9 ± 1.4^{a}	40.1 ± 1.8^{c}	19.6 ± 1.9^{a}	43.3 ± 3.1^{c}	21.2 ± 0.9^{a}	38.9 ± 1.7^{c}	26.9 ± 2.1^{b}
18:3	2.8 ± 0.1^{c}	1.9 ± 0.0^{a}	3.5 ± 0.0^{e}	2.0 ± 0.0^{a}	2.3 ± 0.0^{b}	2.3 ± 0.1^{a}	3.1 ± 0.1^{d}	1.9 ± 0.0^{b}
20:3	5.7 ± 0.1^{d}	7.3 ± 0.1^{f}	2.8 ± 0.0^{b}	5.1 ± 0.1^{c}	2.1 ± 0.0^{a}	6.2 ± 0.4^{e}	2.7 ± 0.0^{b}	4.9 ± 0.1^{c}
20:4	6.1 ± 0.3^{cd}	5.7 ± 0.1^{b}	7.2 ± 0.1^{e}	5.9 ± 0.1^{bc}	8.0 ± 0.2^{f}	6.3 ± 0.2^{d}	8.5 ± 0.1^{g}	5.1 ± 0.1^{a}
20:5	1.0 ± 0.0^{a}	2.2 ± 0.03^{d}	1.2 ± 0.0^{b}	2.6 ± 0.0^{e}	1.1 ± 0.0^{ab}	3.1 ± 0.2^{f}	1.1 ± 0.0^{ab}	1.8 ± 0.0^{c}
Others	0.2	0.1	0.4	0.2	0.1	0.2	0.8	0.9
IUFA	156.1	128.5	151.8	125.9	156.1	134.7	153.1	128.7

Diasporangium jonesianum recombinant strain D-A1 was cultured at 25 °C with 150 rpm shaking speed in the medium (per liter) 200 g potato starch as basis, 50 g glucose and 50 g corn chip (∅1.2–1.3 cm) as carbon source, 10 g soybean flour (not defatted) as nitrogen source, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$ ($7H_2O$), 0.5 g NaCl, 8.5×10^{-2} g Mn^{2+}, and 0.6×10^{-2} g Cu^{2+} as inorganic salt, pH 6.5, with Se concentration as 90 μg mL^{-1}. Se represents the strain treated by Se. C represents the control. The fatty acid contents are given as means ± SD of triplicate samples. Within the same column, values followed by different letters (a, b, c) differ significantly at $P < 0.05$

a platelet antiaggregant. Many studies have indicated that the improvement of PUFA with Se treatment was associated with the role of this element in biological processes, including antioxidant function, thyroid hormone metabolism, and redox balance [20]. As the important component of iodothyronine deiodinases, Se was likely to participate in the synthesis of coenzyme A and Q, had an impact on the oxidative phosphorylation and enhanced the activity of α-ketoglutaric acid oxidase which could promote the decarboxylation of pyruvic acid. Accordingly, Se might

Table 6 The effect of pH value on fatty acid composition from cultured *D. jonesianum* recombinant strain D-A1, with or without Se

Fatty acid (mol%)	pH									
	4.5		5.5		6.5		7.5		8.5	
	Se	C	Se	C	Se	C	Se	C	Se	C
14:0	7.4 ± 0.1^{c}	8.5 ± 0.5^{d}	6.5 ± 0.0^{b}	8.1 ± 0.4^{cd}	5.4 ± 0.1^{a}	10.4 ± 0.9^{e}	7.9 ± 0.1^{cd}	8.4 ± 0.3^{d}	5.1 ± 0.3^{a}	8.3 ± 0.2^{d}
16:0	20.8 ± 1.7^{ab}	22.3 ± 2.0^{ab}	20.0 ± 1.1^{a}	23.7 ± 1.2^{bc}	19.7 ± 0.6^{a}	20.7 ± 1.5^{ab}	22.2 ± 0.7^{ab}	25.8 ± 0.7^{c}	22.0 ± 1.5^{ab}	25.7 ± 1.5^{c}
16:1	3.8 ± 0.2^{h}	3.2 ± 0.2^{g}	1.0 ± 0.0^{c}	1.2 ± 0.0^{de}	1.1 ± 0.0^{cd}	3.1 ± 0.0^{g}	0.7 ± 0.0^{b}	2.8 ± 0.1^{f}	0.5 ± 0.0^{a}	1.4 ± 0.1^{e}
18:0	0.2 ± 0.0^{a}	1.1 ± 0.0^{b}	1.4 ± 0.1^{c}	2.2 ± 0.0^{d}	2.1 ± 0.0^{d}	2.5 ± 0.1^{e}	1.4 ± 0.0^{c}	3.1 ± 0.3^{f}	1.2 ± 0.0^{bc}	2.6 ± 0.0^{e}
18:1	12.1 ± 0.2^{a}	19.0 ± 1.6^{cde}	15.8 ± 1.7^{bc}	17.5 ± 1.5^{cd}	17.1 ± 0.2^{cd}	21.8 ± 1.6^{e}	13.4 ± 1.4^{ab}	19.7 ± 1.3^{dc}	13.7 ± 2.4^{ab}	17.4 ± 1.3^{cd}
18:2	35.1 ± 1.4^{b}	28.0 ± 1.8^{a}	35.3 ± 1.6^{b}	29.5 ± 2.2^{a}	38.3 ± 1.6^{b}	26.4 ± 1.4^{a}	34.7 ± 2.2^{b}	28.3 ± 0.6^{a}	36.5 ± 2.6^{b}	30.5 ± 1.0^{a}
18:3	4.2 ± 0.1^{e}	2.4 ± 0.1^{d}	3.7 ± 0.1^{d}	2.3 ± 0.0^{b}	3.1 ± 0.1^{c}	1.9 ± 0.1^{a}	4.6 ± 0.1^{f}	2.0 ± 0.1^{a}	3.6 ± 0.0^{d}	1.9 ± 0.2^{a}
20:3	2.1 ± 0.0^{b}	3.8 ± 0.2^{g}	2.5 ± 0.1^{c}	2.7 ± 0.0^{d}	2.4 ± 0.0^{c}	4.2 ± 0.1^{h}	1.4 ± 0.0^{a}	2.0 ± 0.1^{b}	3.6 ± 0.1^{f}	3.3 ± 0.1^{e}
20:4	10.8 ± 0.1^{f}	5.9 ± 0.3^{c}	8.2 ± 0.1^{d}	4.2 ± 0.4^{a}	8.3 ± 0.0^{d}	5.1 ± 0.1^{b}	9.0 ± 0.3^{e}	6.1 ± 0.3^{c}	10.7 ± 0.8^{f}	7.7 ± 0.0^{d}
20:5	2.1 ± 0.1^{d}	2.3 ± 0.1^{e}	1.0 ± 0.0^{a}	2.7 ± 0.1^{f}	1.1 ± 0.0^{a}	1.4 ± 0.0^{bc}	2.6 ± 0.1^{f}	1.5 ± 0.1^{c}	2.8 ± 0.2^{f}	1.2 ± 0.0^{b}
Others	1.6	3.6	4.7	6.0	1.5	2.5	1.9	0.2	0.4	0.1
IUFA	158.2	131.5	143.8	123.0	149.7	123.5	151.0	123.1	165.5	132.2

Diasporangium jonesianum recombinant strain D-A1 was cultured at 25 °C with 150 rpm shaking speed in the medium (per liter) 200 g potato starch as basis, 50 g glucose and 50 g corn chip (∅1.2–1.3 cm) as carbon source, 10 g soybean flour (not defatted) as nitrogen source, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$ ($7H_2O$), 0.5 g NaCl, 8.5×10^{-2} g Mn^{2+}, and 0.6×10^{-2} g Cu^{2+} as inorganic salt, with Se concentration as 90 μg mL^{-1}. Se represents the strain treated by Se. C represents the control. The fatty acid contents are given as means ± SD of triplicate samples. Within the same column, values followed by different letters (a, b, c) differ significantly at $P < 0.05$

Table 7 The effect of culture temperatures on fatty acid yields from cultured *D. jonesianum* recombinant strain D-A1, with or without Se

Indexes		Temperature (°C)							
		18		22		25		28	
		Se	C	Se	C	Se	C	Se	C
Biomass (g/L)		2.9 ± 0.1^{a}	3.6 ± 0.2^{b}	3.0 ± 0.1^{a}	3.8 ± 0.3^{bc}	3.5 ± 0.1^{b}	4.3 ± 0.4^{c}	2.5 ± 0.0^{a}	3.6 ± 0.4^{b}
Fatty acid yield (mg/L)		542.6 ± 27.7^{a}	669.5 ± 23.7^{abc}	605.9 ± 38.3^{ab}	758.6 ± 80.1^{cd}	777.6 ± 28.9^{cd}	828.5 ± 76.3^{d}	537.5 ± 20.7^{a}	715.6 ± 104.5^{bcd}
18:2	mg/L	225.2 ± 11.3^{ab}	194.0 ± 7.8^{a}	247.7 ± 14.3^{b}	239.3 ± 33.8^{ab}	297.3 ± 17.4^{c}	224.1 ± 17.7^{ab}	206.1 ± 7.0^{ab}	191.6 ± 27.7^{a}
	mg/g	76.6 ± 1.8^{c}	53.4 ± 1.0^{a}	83.1 ± 3.3^{d}	62.0 ± 3.9^{b}	85.7 ± 5.8^{d}	52.5 ± 0.8^{a}	83.1 ± 1.9^{d}	53.6 ± 2.1^{a}
20:4	mg/L	41.7 ± 2.8^{ab}	49.7 ± 2.6^{b}	51.0 ± 1.8^{b}	45.4 ± 5.4^{ab}	65.5 ± 5.8^{c}	41.0 ± 3.8^{ab}	42.7 ± 1.8^{ab}	37.0 ± 5.6^{a}
	mg/g	14.2 ± 0.5^{c}	13.7 ± 0.2^{c}	17.1 ± 0.5^{d}	11.8 ± 0.4^{b}	18.9 ± 2.1^{d}	9.6 ± 0.4^{a}	17.2 ± 0.8^{d}	10.3 ± 0.4^{ab}

18:2 (linoleic acid); 20:4 (arachidonic acid). Se represents the strain treated by Se. C represents the control. The fatty acid contents are given as means ± SD of triplicate samples. Within the same column, values followed by different letters (a, b, c) differ significantly at $P < 0.05$. *Diasporangium jonesianum* recombinant strain D-A1 was cultured at 150 rpm shaking speed with Se concentration as 90 μg mL^{-1} in the medium (per liter): 200 g potato starch as basis, 50 g glucose and 50 g corn chip (∅1.2–1.3 cm) as carbon source, 10 g soybean flour (not defatted) as nitrogen source, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$ ($7H_2O$), NaCl 0.5 g, 8.5×10^{-2} g Mn^{2+}, and 0.6×10^{-2} g Cu^{2+} as inorganic salt

Table 8 The effect of shaking speed on fatty acid yields from cultured *D. jonesianum* recombinant strain D-A1, with or without Se

Indexes		Shaking speed (rpm)							
		120		150		180		210	
		Se	C	Se	C	Se	C	Se	C
Biomass (g/L)		2.3 ± 0.0^{a}	3.3 ± 0.2^{d}	3.5 ± 0.1^{e}	4.2 ± 0.2^{f}	3.7 ± 0.1^{e}	3.6 ± 0.1^{e}	2.6 ± 0.0^{b}	3.1 ± 0.0^{c}
Fatty acid yield (mg/L)		424.1 ± 37.3^{a}	526.8 ± 33.5^{b}	796.5 ± 39.9^{d}	861.4 ± 48.9^{e}	886.5 ± 30.3^{e}	717.5 ± 25.1^{c}	523.9 ± 21.4^{b}	557.1 ± 26.9^{b}
18:2	mg/L	170.1 ± 20.0^{ab}	151.8 ± 16.9^{a}	314.0 ± 17.2^{e}	232.5 ± 28.5^{cd}	387.9 ± 28.0^{f}	256.1 ± 28.1^{d}	202.2 ± 1.6^{bc}	130.8 ± 11.8^{a}
	mg/g	72.6 ± 7.2^{c}	45.3 ± 3.5^{ab}	89.0 ± 4.1^{d}	55.3 ± 5.3^{b}	106.1 ± 8.4^{e}	70.4 ± 8.9^{c}	79.0 ± 1.0^{cd}	41.6 ± 3.5^{a}
20:4	mg/L	32.1 ± 2.6^{a}	37.4 ± 4.5^{a}	67.3 ± 5.0^{d}	45.0 ± 2.1^{b}	52.2 ± 0.9^{c}	33.2 ± 1.2^{a}	43.3 ± 1.3^{b}	35.9 ± 2.1^{a}
	mg/g	13.7 ± 0.9^{c}	11.2 ± 1.3^{b}	19.1 ± 1.2^{e}	10.7 ± 0.2^{b}	14.3 ± 0.4^{c}	9.1 ± 0.2^{a}	16.9 ± 0.3^{d}	11.4 ± 0.5^{b}

18:2 (linoleic acid); 20:4 (arachidonic acid). Se represents the strain treated by Se. C represents the control. The fatty acid contents are given as means ± SD of triplicate samples. Within the same column, values followed by different letters (a, b, c) differ significantly at $P < 0.05$. *Diasporangium jonesianum* recombinant strain D-A1 was cultured at 25 °C with Se concentration as 90 μg mL^{-1} in the medium (per liter): 200 g potato starch as basis, 50 g glucose and 50 g corn chip (∅1.2–1.3 cm) as carbon source, 10 g soybean flour (not defatted) as nitrogen source, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$ ($7H_2O$), 0.5 g NaCl, 8.5×10^{-2} g Mn^{2+}, and 0.6×10^{-2} g Cu^{2+} as inorganic salt

Table 9 The effect of carbon sources content on fatty acid yields from cultured *D. jonesianum* recombinant strain D-A1, with or without Se

Indexes		Carbon sources content(g/L)							
		40	60		80		100		
		Se	C	Se	C	Se	C	Se	C
Biomass (g/L)		2.3 ± 0.0^{a}	3.0 ± 0.0^{c}	2.7 ± 0.0^{b}	4.0 ± 0.1^{f}	3.3 ± 0.1^{d}	4.1 ± 0.0^{f}	3.5 ± 0.1^{e}	4.5 ± 0.2^{f}
Fatty acid yield (mg/L)		589.7 ± 25.1^{a}	577.2 ± 32.7^{a}	673.4 ± 23.2^{b}	765.6 ± 5.9^{c}	864.2 ± 52.5^{e}	816.6 ± 47.3^{cd}	808.1 ± 72.1^{cd}	873.2 ± 4.0^{e}
18:2	mg/L	250.5 ± 14.7^{c}	114.4 ± 6.5^{a}	270.5 ± 20.6^{c}	149.6 ± 14.1^{ab}	374.2 ± 40.7^{e}	172.8 ± 14.1^{b}	314.1 ± 25.5^{d}	234.7 ± 19.3^{c}
	mg/g	108.0 ± 8.0^{cd}	38.2 ± 1.5^{a}	99.2 ± 9.1^{bc}	37.6 ± 2.4^{a}	115.3 ± 14.4^{d}	42.6 ± 3.2^{a}	89.2 ± 6.5^{b}	51.9 ± 6.7^{a}
20:4	mg/L	35.9 ± 3.1^{a}	32.9 ± 1.8^{a}	48.6 ± 2.5^{b}	45.5 ± 1.1^{b}	69.0 ± 3.5^{c}	51.5 ± 1.1^{b}	68.6 ± 6.7^{c}	44.3 ± 1.3^{b}
	mg/g	15.5 ± 1.5^{c}	11.0 ± 0.6^{ab}	17.8 ± 1.2^{d}	11.4 ± 0.6^{ab}	21.3 ± 1.5^{e}	12.7 ± 0.1^{b}	19.5 ± 1.6^{de}	9.8 ± 0.7^{a}

18:2 (linoleic acid); 20:4 (arachidonic acid). Se represents the strain treated by Se. C represents the control. The fatty acid contents are given as means ± SD of triplicate samples. Within the same column, values followed by different letters (a, b, c) differ significantly at $P < 0.05$. *Diasporangium jonesianum* recombinant strain D-A1 was cultured at 25 °C with 150 rpm shaking speed in the medium (per liter): 200 g potato starch as basis, 50 g glucose and 50 g corn chip (∅1.2–1.3 cm) as carbon source, 10 g soybean flour (not defatted) as nitrogen source, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$ ($7H_2O$), 0.5 g NaCl, 8.5×10^{-2} g Mn^{2+}, and 0.6×10^{-2} g Cu^{2+} as inorganic salt, with Se concentration as 90 μg mL^{-1}

Table 10 The effect of pH value on fatty acid yields from cultured *D. jonesianum* recombinant strain D-A1, with or without Se

Indexes		pH									
		4.5		5.5		6.5		7.5		8.5	
		Se	C	Se	C	Se	C	Se	C	Se	C
Biomass (g/L)		2.34 ± 0.1^{a}	3.5 ± 0.5^{bc}	3.3 ± 0.0^{bc}	4.1 ± 0.2^{ef}	3.4 ± 0.1^{bc}	4.2 ± 0.2^{f}	3.8 ± 0.1^{cd}	4.0 ± 0.1^{ef}	3.2 ± 0.1^{b}	3.8 ± 0.1^{cd}
Fatty acid yield (mg/L)		404.1 ± 14.1^{a}	581.8 ± 55.8^{b}	768.4 ± 37.9^{cd}	838.0 ± 27.9^{d}	782.9 ± 33.8^{cd}	967.0 ± 44.0^{e}	757.0 ± 27.6^{cd}	820.9 ± 27.8^{d}	634.3 ± 62.8^{b}	700.6 ± 19.2^{c}
18:2	mg/L	141.6 ± 2.2^{a}	163.0 ± 17.8^{a}	271.4 ± 21.1^{cd}	247.5 ± 23.4^{bc}	299.7 ± 15.1^{d}	255.1 ± 21.2^{c}	262.7 ± 20.3^{c}	232.5 ± 3.2^{bc}	230.8 ± 15.3^{bc}	213.9 ± 12.4^{b}
	mg/g	60.6 ± 2.8^{b}	46.9 ± 4.4^{a}	81.5 ± 5.3^{d}	60.2 ± 3.0^{b}	87.1 ± 2.6^{d}	60.6 ± 2.3^{b}	70.0 ± 3.8^{c}	57.7 ± 0.9^{b}	71.3 ± 5.4^{c}	56.6 ± 3.4^{b}
20:4	mg/L	43.5 ± 1.8^{b}	34.1 ± 3.1^{a}	63.1 ± 2.5^{e}	34.8 ± 3.2^{a}	64.7 ± 2.4^{e}	49.7 ± 2.5^{bc}	68.4 ± 4.4^{e}	50.0 ± 4.1^{bc}	68.1 ± 9.3^{e}	54.0 ± 1.8^{d}
	mg/g	18.6 ± 1.1^{d}	9.8 ± 0.8^{a}	19.0 ± 0.6^{d}	8.5 ± 1.0^{a}	18.8 ± 0.7^{d}	11.8 ± 0.2^{b}	18.2 ± 0.9^{d}	12.4 ± 1.1^{bc}	21.0 ± 2.7^{d}	14.3 ± 0.4^{c}

18:2 (linoleic acid); 20:4 (arachidonic acid). Se represents the strain treated by Se. C represents the control. The fatty acid contents are given as means ± SD of triplicate samples. Within the same column, values followed by different letters (a, b, c) differ significantly at $P < 0.05$. *Diasporangium* sp. recombinant strain D-A1 was cultured at 25 °C with 150 rpm shaking speed in the medium (per liter): 200 g potato starch as basis, 50 g glucose and 50 g corn chip (∅1.2–1.3 cm) as carbon source, 10 g soybean flour (not defatted) as nitrogen source, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$ ($7H_2O$), 0.5 g NaCl, 8.5×10^{-2} g Mn^{2+}, and 0.6×10^{-2} g Cu^{2+} as inorganic salt, pH 6.5, with Se concentration as 90 μg mL^{-1}

Table 11 The character value and accumulate contribution rate of correlated matrix

Value	Eigenvalue	Contribution rate (%)	Cumulative contribution rate (%)
1	3.1	77.3	77.3
2	0.5	13.1	90.4
3	0.2	5.4	95.8
4	0.1	4.2	100.0

Table 12 The component value

Variable	Temperature	Shaking speed	Carbon sources content	pH
Component	0.908	0.879	0.937	0.786

play a role in the TCA cycle and respiratory electron transport chain that influence the metabolism of PUFA, particularly AA [21]. So far, this aspect has been studied inadequately. The objectives of the present investigation were to establish whether and how Se could influence the metabolism of PUFA.

As Se shares similar physicochemical properties to sulfur (S), Se might follow the same metabolic pathway as S and interact on the same enzyme system. Fatty acid desaturase contains hydrophobic domains between amino acids, which contained disulfide bridges. Therefore, Se may indiscriminately substitute S, and incorporate Se-amino acids into proteins [22]. Fordyce reported that in plants, Se is present in diverse organoselenium compounds including selenoamino acids (SeCys and SeMet) and selenoproteins [23]. The replacement of cysteine by SeCys and methionine by SeMet may alter protein stability and functional activity and is thought to account for Se toxicity [24]. The conversion of selenate to organoselenium compounds proceeds through adenosine 5′-phosphoselenate, selenite, and selenide. Selenide is then converted to SeCys, from which SeMet is synthesized via selenocystathionine and homoselenocysteine. Both SeCys and SeMet can be incorporated into proteins or methylated. For example, Semethylselenocysteine (SeMSeCys), γ-glutamyl-SeMSeCys and Semethylselenomethionine (SeMSeMet) are characteristic Se assimilation products of species in the genera Brassica [25–27].

In this study, n-6 series fatty acids (18:2, 20:4) were increased by the Se treatment while n-3 series fatty acids (18:3, 20:5) did not increase significantly. Cysteine and cystine were further analyzed, and the results showed that with Se treatment, cysteine and cystine content decreased 85.7 and 80.8%, respectively, compared to the control (data not shown), implying that Se might have replaced S in cysteine and cystine to form selenocysteine and selenocystine, respectively. However, there are some differences in the activity and biochemical characteristics between Se and S. Genes encoding enzymes involved in S and Se uptake and assimilation are differentially expressed during the metabolic pathway.

Conclusions

The selection of strains that are high in accumulating PUFAs is the key step for commercialized PUFA production using microorganisms. Our study indicated Se treatment can enhance the intracellular PUFA production in PUFA-producing fungi. Selenium treatment could effectively increase the yields of AA and LA with little effect on the content of EPA and γ-linolenic acid. With different culture conditions, carbon content was the first principal component that affected the content of fatty acids under Se stress condition. These findings can significantly contribute to the production of PUFA-related lipids in fungi. It was presumed that Se might replace the S in the protein which may cause the evident change of spatial structures and function of proteins by impacting the conformation of enzyme active sites of desaturase, which leads to the increase of AA and LA yields.

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