Improvement of Stearidonic Acid Production in Oleaginous Saccharomyces cerevisiae

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Note

Rat Δ6 desaturase was introduced in “oleaginous” Saccharomyces cerevisiae genetically modified by the snf2 disruption, DGA1 overexpression, and LEU2 expression to improve stearidonic acid (18:4n-3 (Δ6, 9, 12, 15)) production. When this transformed yeast was cultured in the presence of 0.7 g/l α-linolenic acid for 7 d, 0.4 g of stearidonic acid was produced, mainly as a triacylglycerol and phospholipids, with a total lipid content of 15% production. When this transformed yeast was cultured in the same medium except for Tergitol and a fatty acid with 20% linolenic acid, 20–22% linoleic acid, and 0–2% α-linolenic acid, was added to the medium in the form of 44 mg of stearidonic acid in the 1 liter broth.

Key words: Δ6 desaturase; DGA1; lipid accumulation; polyunsaturated fatty acid; stearidonic acid

Polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA), icosapentaenoic acid (EPA), and arachidonic acid, play important roles in human health care.¹³ Humans cannot synthesize the essential fatty acids, linoleic acid and α-linolenic acid, which are metabolic precursors of PUFAs, and have to obtain them from their diet. The principal sources of PUFAs to date are marine products, particularly blue-backed fishes, salmons, and squids. In recent years, the demand for fish and shellfish as food and feed for aquaculture are increasing.¹⁴ Hence, we must establish alternative sources of PUFAs. Studies of the application of certain marine algae and fungi, which contain PUFAs, have shown that the microorganisms are an attractive source of PUFAs.¹³ Polyunsaturated fatty acid production in transgenic organisms with heterogeneous expression of PUFAs producing enzymes has also been extensively studied.¹⁵ In many cases, Saccharomyces cerevisiae has been used as a favorable transgenic host because of extensive genetic knowledge of it, but the lipid accumulation in S. cerevisiae is poor. To improve the low lipid content, we have recently established an oleaginous S. cerevisiae, which accumulates lipids in the cell by genetic modifications of the disruption of SNF2, overexpression of DGA1, and expression of LEU2.⁸ This modified yeast accumulates lipids mainly as triacylglycerol, with a total lipid content of 29% in nitrogen-limited medium. Stearidonic acid production was examined by introducing the rat Δ6 desaturase gene⁹ into this oleaginous yeast.

The wild-type strain of S. cerevisiae (BY4741: MATa leu2Δ0 his3Δ1 ura3Δ0 met15Δ0) and its snf2 disruptant (BY4741Δsnf2: MATa leu2Δ0 his3Δ1 ura3Δ0 met15Δ0 snf2::kanMX), purchased from Invitrogen (Carlsbad, CA, USA) were used. The pL1091-5 vector contains the 2-μm replication origin, an ADHI (alcohol dehydrogenase gene) promoter, and a selection marker, URA3, and pL1177-2 is a LEU2 version of pL1091-5.¹⁰ The entire ORFs of rat Δ6 desaturase (GenBank accession number AB021980)⁹ and yeast diacylglycerol acyltransferase (DGA1/YOR245C) were inserted into pL1091-5 and pL1177-2 respectively. The transforms were cultured at 30°C using a reciprocal shaker at 140 rpm in a synthetic medium containing 0.67% Bacto-nitrogen base without amino acids, 2% glucose, 20 μg/ml histidine, 20 μg/ml methionine, 2.5 g/l Tergitol NP-40, and 1 g/l fatty acid with 20 μg/ml uracil and 60 μg/ml leucine. Practical grade α-linolenic acid, purchased from Wako Pure Chemicals Industries (Osaka, Japan), which contained about 70–78% α-linolenic acid, 20–22% linoleic acid, and 0–2% γ-linolenic acid, was added to the medium in the form of an ethanol solution just before the inoculum. The seed, cultured in the same medium except for Tergitol and a fatty acid at 30°C for 2 d, was inoculated at 1 v/v% and cultured for 7 d. The dried cells were directly transmethylated for analysis of the fatty acids by gas chromatography, as described previously.¹¹ Total lipids were extracted by homogenizing the yeast cells with glass beads in chloroform/methanol (2:1, v/v). The resulting lipid extract was dissolved in chloroform for loading on a Silica gel 60 plate (Merck, Darmstadt, Germany). TLC was developed using a plate with hexane/diethyl ether/acetic acid (80:40:1, by volume) to about 18 cm above the origin. The TLC spots corresponding to the lipid classes were scraped off and then transmethylated for analysis of the fatty acids, as mentioned above.

The fatty acid profiles of the transformants cultured with the exogenous fatty acids for 7 d are shown in Fig. 1. In the transformat expressing the Δ6 desaturase gene and DGA1, the exogenous α-linolenic acid was

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Abbreviations: DG, diacylglycerol; DHA, docosahexaenoic acid; EPA, icosapentaenoic acid; FFA, free fatty acid; MG, monoacylglycerol; PL, phospholipids; PUF, polyunsaturated fatty acid; SE, sterol ester; TG, triacylglycerol
GC/MS spectra to that of the authentic standard (methyl confirmed as stearidonic acid by the similarity of the 

The novel peak of the chromatogram of Fig. 1A was 

44 mg, was detected in the snf2 disruptant transformats, in the absence of DGA1 overexpression and LEU2 expression. The best production of stearidonic acid, 44 mg, was much greater than the 0.3 mg in the wild-type strain with DGA1 overexpression and LEU2 expression. This means that stearidonic acid production increased about 17-fold and the conversion ratio to stearidonic acid increased about 12-fold by using the oleaginous yeast constructed by snf2 disruption, DGA1 overexpression, and LEU2 expression. These results indicate that the use of this oleaginous yeast effectively improved PUFA production.

The profiles of the lipid classes in these transformed strains are shown in Fig. 2. The total lipids extracted from the cells were fractionated into triacylglycerols (TG), diacylglycerols (DG), monoacylglycerols (MG), and sterol esters (SE). Most of accumulated fatty acids were detected in the TG and FFA fractions. In the wild-type and snf2 disruptant transformats, in the absence of DGA1 overexpression and LEU2 expression, less than half of the added exogenous fatty acids accumulated, primarily in the FFA and secondarily in the TG, and less than half of them remained in the broth. On the other 

Table 1. Growth and Fatty Acid Compositions of the BY4741 Transformats with DGA1 and/or Rat Δ6 Desaturase Gene

<table>
<thead>
<tr>
<th>Strain vectors</th>
<th>Dry Cell Weight (g/l)</th>
<th>Total FA (mg/l)</th>
<th>16:0 (mg/l)</th>
<th>16:1 (mg/l)</th>
<th>18:0 (mg/l)</th>
<th>18:1 (mg/l)</th>
<th>18:2n-6 (mg/l)</th>
<th>18:3n-3 (mg/l)</th>
<th>18:4n-3 (mg/l)</th>
<th>Conversion ratio to 18:4n-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>BY4741 wild type</td>
<td>no vector</td>
<td>0.76</td>
<td>288</td>
<td>9</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>55</td>
<td>1</td>
<td>201</td>
</tr>
<tr>
<td></td>
<td>+pL1177-2/-, +pL1091-5/-</td>
<td>2.82</td>
<td>729</td>
<td>53</td>
<td>7</td>
<td>32</td>
<td>16</td>
<td>123</td>
<td>3</td>
<td>454</td>
</tr>
<tr>
<td></td>
<td>+pL1091-5/rD6d</td>
<td>0.92</td>
<td>351</td>
<td>10</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>69</td>
<td>2</td>
<td>242</td>
</tr>
<tr>
<td></td>
<td>+pL1177-2/-, +pL1091-5/-, +pL1091-5/rD6d</td>
<td>2.74</td>
<td>710</td>
<td>52</td>
<td>6</td>
<td>32</td>
<td>15</td>
<td>119</td>
<td>4</td>
<td>411</td>
</tr>
</tbody>
</table>

The fatty acid compositions are the mean values (n = 4–9) expressed as fatty acid amounts in the cells of a 1 liter broth of 7-d culture. rD6d, rat Δ6 desaturase gene; FA, fatty acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2n-6, linoleic acid; 18:3n-3, α-linolenic acid; 18:4n-3, stearidonic acid.
In conclusion, improvement of stearidonic acid production was achieved using an oleaginous yeast constructed by the snf2 disruption, DGA1 overexpression, and LEU2 expression. This PUFA production scheme using the oleaginous yeast appears attractive for improving PUFA production.

References