

Studies of Metabolic Engineering of Useful Lipid-producing Microorganisms

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Research aims

Polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA), can serve not only as structural components of membrane phospholipids but also as precursors of the eicosanoids of signaling molecules in mammals. Therefore, studies on PUFA production are important for the medical and pharmaceutical fields. *Mortierella alpina* 1S-4, belonging to Zygomycetes, was found as the best producer of lipids containing more than 40% of AA in the total fatty acids. A host-vector system of this fungus to enhance the PUFA productivity was established. In this study, I have improved the fatty acid composition and the lipid productivity by means of overexpression of an enzyme gene involved in PUFA biosynthesis.

Methods

Transformation of the *M. alpina* 1S-4 *ura5*⁻ strain with the expression vectors was performed with a PDS-1000/He Particle Delivery System (Bio-Rad Laboratory Inc., Hercules, CA) as described previously.¹

Results

1) Construction of an improved transformation system for expression of a target gene

The first trial involved expression of the enhanced green fluorescent protein (EGFP) gene in the *ura5*⁻ strain (uracil auxotroph) derived from *M. alpina* 1S-4 by use of a homologous *ura5* gene-containing vector (pDura5). The EGFP gene was placed between the same promoter and terminator as used in pDura5, resulting in construction of an EGFP-expression vector, pDura5GFP. Transformation was successfully performed, and six stable transformants were selected from the 45 isolates. All the stable ones were determined to be transformed through vector insertion into the rDNA locus. Under microscopic fluorescent observation, a strong fluorescence was detected only in spores of the transformants, not in those of the control strain (*ura5*⁻ strain). On the other hand, we could not observe the difference of fluorescent intensity of the fungal filaments between the transformant and the control strain, because of the strong self-fluorescence of the filaments of this fungus.

2) Improvement of fatty acid composition and AA productivity²

GLELOp has an elongation activity of γ -linolenic acid

(GLA) to dihomo- γ -linolenic acid (DGLA) and involved in AA biosynthesis in *M. alpina* 1S-4. A transformation vector, pDura5GLELO, was constructed and used for transformation of the *ura5*⁻ strain to express the GLELO gene. Out of the 36 transformants obtained, 9 were determined to be stable transformants with pDura5GLELO. The transformant #3 selected from the stable transformants exhibited the different fatty acid composition from that of the host cells: GLA, which serves as a substrate for GLELOp, was decreased and AA was increased. For further investigation, time course experiments were performed with transformant #3 and the host cells (Fig. 1).

The accumulation of AA continued after 4 days in both strains, regardless of the culture conditions. In the case of 5% glucose in the medium, AA production remained low in comparison with that with 2% glucose. Although this observation was in accordance with results previously reported, the AA productivity (mg/ml of culture broth) was proportional to the glucose concentration. In the case of 2% glucose (Fig. 1A and B), the AA content increased linearly

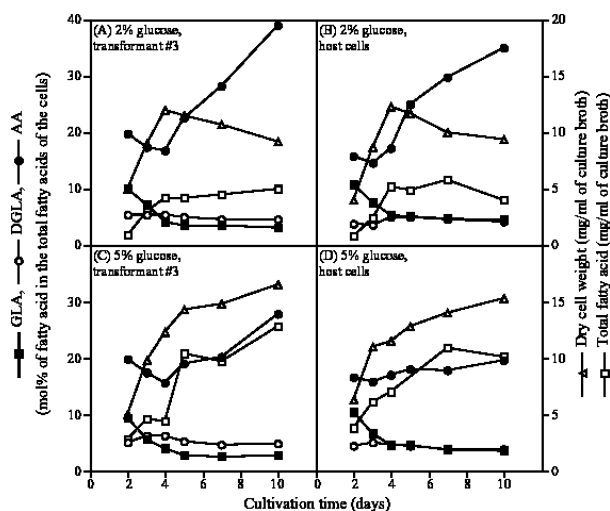


Fig. 1. Time courses of changes in the mycelial fatty acid content in the GLELO-overexpressing *M. alpina* 1S-4 transformant and the host cells. All cultivations were started by inoculation of the respective spores and performed with reciprocal shaking (120 strokes/min) at 28°C. The culture conditions were as follows: (A, B) GY medium containing 2% glucose. (C, D) GY medium containing 5% glucose. All media also contained 1% yeast extract and 0.05 mg/ml of uracil. The strains used were as follows: (A, C) transformant. (B, D) host cells. Fatty acid analysis was performed with total biomass obtained from each cultivation. The values are the means of triplicate experiments. GLA, γ -linolenic acid; DGLA, dihomo- γ -linolenic acid; AA, arachidonic acid.

after 4 days in the transformant but not in the host cells. The final content of AA in the transformant was higher than that in the host cells. On the other hand, in the case of 5% glucose (Fig. 1C and D), differences clearly appeared: the AA content of transformant #3 increased more greatly than that of the host cells after 5 days. The GLA content of the transformant cells remained lower than that of the host cells, especially after 4 days. The DGLA content increased at the beginning of cultivation and then decreased concomitantly with the increase in the AA content. The AA content of transformant #3 increased by 8.3% at the end point. As a further analysis, real-time quantitative PCR (RTQ-PCR) showed that the quantity of GLELO RNA in transformant #3 was 7.4-fold higher than that of the host strain on 4 days. Therefore, the results obtained for stable transformant #3 must be directly due to GLELO gene expression.

Conclusion

We succeeded in expression of target genes such as EGFP or GLELO genes in *M. alpina* 1S-4 *ura5⁻* strain. Overexpression of GLELO gene led to enhance AA production in the transformant. Furthermore, GLELOp was shown to serve as the rate-limiting step in AA biosynthesis.

Reference

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2. Takeno S., Sakuradani E., and Shimizu S. (2004) Creation and application of host-vector system in oleaginous microorganism *Mortierella alpina* 1S-4. *Japan Appl. Microbiol. Soc.* **2**, 82–87. <http://www.kuicr.kyoto-u.ac.jp/labos/bm2/oubikyo/2004120107.pdf>