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**Outline of Research Result**

# Elucidation of triglyceride accumulation mechanism in an oleaginous yeast

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## Research aims

Fats (triglycerides, TGs) are essential for life and are used in both food and industrial applications. TGs are normally extracted from a variety of oil seed plants and animal sources. However, the Japanese self-sufficiency ratio for fats is only 13%, making it crucial to secure fat resources to prepare for the increasing global demand for fat materials.

The extraction of TGs from oleaginous yeasts such as *Yarrowia* and *Cryptococcus* is a well-established alternative for the manufacture of TG without relying on oil seed plants. However, these yeasts have not yet been used in food; therefore, there is a concern regarding the safety of such usage. Furthermore, because fatty acids (FAs) constituting TGs of such oleaginous yeasts include an abundance of saturated FAs, they are unsuitable for use as biodiesel fuel in cold districts, such as Hokkaido.

Certain yeasts of the genus *Kluyveromyces* are composed of edible ingredients and their whole genome sequences have been decoded, which renders them ideal cellular models to elucidate mechanisms underlying TG accumulation. In the present study, as part of our efforts to develop a technology for efficient TG production for both food and fuel applications, we analyzed effects of varied genetic functions and culture conditions on TG production.

## Methods

Based on the genome sequence of the yeast *Saccharomyces cerevisiae*, 11 genes encoding enzymes involved in the synthetic pathway from dihydroxyacetone phosphate (DHAP) to TG were amplified via polymerase chain reaction using the genomic DNA of *K. lactis*. After linkage to an expression vector, as described in previous reports,[1] each amplified gene was incorporated into the genomic DNA of *K. lactis*. The TG content of each gene overexpression strain was analyzed in accordance with previously described methods.[2]

To discover novel yeasts of the genus *Kluyveromyces* that accumulate TG in higher concentrations compared to *K. lactis*, we examined the distribution of yeasts in raw milk. Using sweet sorghum juice as a culture medium, the TG content in *K. marxianus* specimens isolated from raw milk was analyzed.

## Results

While the 11 gene overexpression strains did not exhibit any significant differences in cell weight compared with the parental strain, three of the overexpression strains tended to contain increased amounts of TG (Fig. 1). Of all the strains, the *DGA1* overexpression strain had the highest TG content, producing 1.4 times the volume of TG produced by the parental strain. In addition, the TG production of *LPP1* and *ARE* overexpression strains was 1.3 times greater than that of the parental strain. *DGA1*, *LPP1*, and *ARE* are all involved in the synthetic pathway downstream of phosphatidic acid (PA), an intermediate substance of TG synthesis. PA, which is also used in the synthesis of cell membrane phospholipids (PLs), is reported to increase TG yield and decrease PL yield in *SNF2*-deficient *S. cerevisiae*.[3] Thus, it can be predicted that in *K. lactis*, the overexpression of genes downstream of PA synthesis will reduce the activities of the PL synthetic pathway and, consequently, increase TG production.

A *K. marxianus* strain isolated from raw milk produced TG more efficiently than its type strain or *K. lactis*. An initial pH of the culture medium between 5.0 and 6.0 yielded the greatest amount of TGs, which was estimated to reach 4.1 g/L. The constituent FAs of TGs produced by the iso-

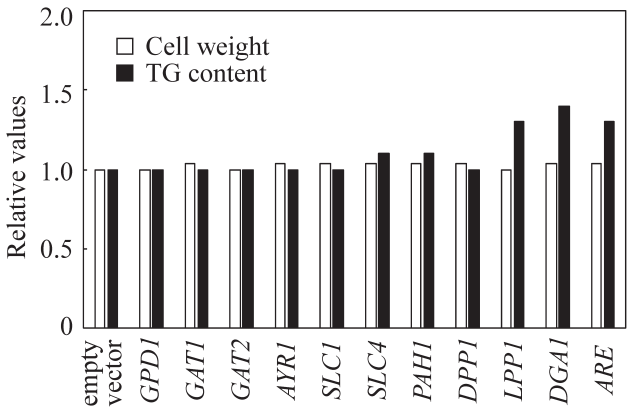


Fig. 1. Effects of overexpression of the 11 genes on the growth and TG content of *K. lactis*. *GPD1*, glycerol-3-phosphate (G3P) dehydrogenase; *GAT1* and *GAT2*, G3P acyltransferases; *AYR1*, 1-acyl DHAP reductase; *SLC1* and *SLC4*, 1-acyl G3P acyltransferases; *PAH1*, *DPP1* and *LPP1*, phosphatidate phosphatases; *DGA1* and *ARE*, diacylglycerol acyltransferases.

Table 1. Fuel properties of biodiesel from K. marxianus.

| Property | SG (g/cm$^3$) | KV (mm$^2$/s) | FP (°C) | CN | IV |
|---|---|---|---|---|---|
| *K. marxianus* (isolate) | 0.88 | 4.9 | 162 | 59 | 97 |
| Biodiesel standards (EU and Japan) | 0.86–0.90 | 3.5–5.0 | ≥120 | ≥51 | ≤120 |

SG, specific gravity; KV, kinematic viscosity; FP, flash point; CN, cetane number; IV, iodine value.

lated strain are all commonly found in edible oils. Moreover, the FA composition of TGs produced by the isolated strain conformed to all biodiesel standards that can be estimated from FA composition, such as specific gravity (Table 1).

## Conclusion

The results of the present study revealed that in the yeast *K. lactis*, TG production was improved by overexpression of genes encoding enzymes downstream of PA in the synthetic pathway from DHAP to TG. In the yeast *Y. lipolytica*, TG production was accelerated through the overexpression of genes encoding enzymes in the synthetic pathways of FAs and disruption of genes encoding enzymes responsible for TG degradation.[4] Therefore, it can be inferred that altering functions of such genes in *K. lactis* also leads to the amelioration of TG production.

Additionally, these results indicate that *K. marxianus* isolated from raw milk excels in producing TG, which can be used in food and as fuel. If the genes in the TG synthetic pathway are altered in this isolated strain, it may be possible to further increase TG yield.

## References

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