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Production of Sphingoid Bases in Yeasts

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Sphingolipids are integral membrane components found in eukaryotic cells. Sphingolipids have structural functions in maintaining cell membrane integrity, and they act as anchors to proteins. In addition, metabolites of sphingolipids such as ceramide, sphingosine, and sphigosine 1-phosphate have been demonstrated to be involved as bioeffector molecules and second messengers in cell growth, differentiation, cell senescence, apoptosis, and stress responses. Phytosphingosines found in yeasts have attracted a great attention as a specialty ingredient for the moisture retention and protection of the skin in the cosmetic industry. The yeast *Pichia ciferrii* has been known to produce large quantities of sphingoid bases which are secreted into extracellular medium as partially acetylated bases, mostly tetraacetyl phytosphigosine (TAPS), a precursor of sphingolipid. The TAPS productions in wild type *P. ciferrii*, however, are not satisfactory for commercial uses.

For the manipulation of the genes required for the improved production of sphingolipids by metabolic engineering, we first constructed an integrative genetic transformation system for *P. ciferrii* using the mutagenized ribosomal protein L41 gene as dominant selection marker against an antibiotic, cycloheximide, and the rDNA fragment for multi-copy chromosomal gene integration.

Synthesis of the long chain base component of sphingolipids begins with the condensation of serine and palmitoyl-CoA to yield 3-ketosphinganine by serine palmitoyltransferase (SPT). To increase the metabolic flux to TAPS, the *LCB2* gene encoding a subunit of SPT was cloned from *P. ciferrii* and overexpressed under the control of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) promoter of *P. ciferrii*. The expression of LCB2 gene was further optimized by recruitment of 5'-UTR of LCB2 gene.

For production of sphinganine (dihydrosphingosine) in yeast, we constructed *Saccharomyces cerevisiae* strains defective in sphinganine kinases (LCB4, LCB5) and sphingaine hydroxylase (SYR2) genes to block the conversion of sphinganine into pphosphorylated form (sphinganine 1-phosphate) and the hydroxylated form (phytosphingosine), respectively. Accumulation of sphinganine in the engineered yeast strain was confirmed by HPLC analysis. Sphingosine is produced by hydrolysis of ceramide by ceramidase in animal and rarely present in yeast, and sphingosine has chemical structure identical to phytosphingosine except a 4-trans double bond in sphingosine in place of hydroxyl group in phytosphingosine. Since sphingaine hydroxylase belongs to diiron motif-containing hydroxylase/desaturae family, possibility of product specificity change of the hydroxylase into desaturase was explored.