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Fluorescence-activated cell sorting-mediated directed evolution of *Wickerhamomyces ciferrii* for enhanced production of tetraacetyl phytosphingosine

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Abstract−Ceramides are a major lipid class known to play an essential role in maintaining skin function. Thus, efforts have been made to produce ceramides and ceramide precursors in large quantities for industrial applications. The yeast *Wickerhamomyces ciferrii*, a natural producer of the ceramide precursor tetraacetyl phytosphingosine (TAPS), has been isolated and engineered through various mutagenesis approaches aiming to enhance TAPS production. Herein, a high-throughput screening platform for isolating *W. ciferrii* mutants with improved TAPS production is described. A fluorescence-mediated reporter system that allows initial quantification of TAPS content in yeast cells based on BODIPY staining was developed. The optimal concentration of BODIPY for monitoring intracellular TAPS levels in *W. ciferrii* was 400 μg/L, as shown by a linear correlation between the actual TAPS levels and mean fluorescence intensities. Fluorescence-activated cell sorting was used for isolating high TAPS-producing strains from an ethyl methanesulfonate-induced mutant library. After several rounds of sorting, mutants exhibiting a high-TAPS phenotype were isolated, and the M40 strain with the highest TAPS titer was chosen for large-scale cultivation. The influence of different carbon sources for optimizing TAPS production was also evaluated using the M40 strain. A maximum production yield of 5.114 g/L of ceramide precursors, including TAPS and triacetyl phytosphingosine, was achieved with the supplementation of molasses. This novel platform enables rapid screening of high TAPS-producing strains using the common dye BODIPY and can be easily extended for the development of mutants with high productivity of ceramide precursors in yeast and other microorganisms.

Keywords: Tetraacetyl Phytosphingosine (TAPS), *Wickerhamomyces ciferrii*, Fluorescence-activated Cell Sorting (FACS), BODIPY 505/515, Molasses

INTRODUCTION

Ceramides are epidermal lipids that are crucial for a proper skin barrier function [1]. Alterations in ceramides level have been associated with several skin diseases, such as atopic dermatitis, psoriasis, and ichthyosis [2-4]. Therefore, the cosmetic industry has been using ceramides and ceramide derivatives in their hair and skincare products with the goal of exogenously supplying ceramides to improve skin function [5]. Ceramides used in cosmetic applications are typically synthetic and obtained by acylation of sphingosine, phytosphingosine, or dihydrosphingosine (also named sphinganine) [6]. Several approaches have been attempted to chemically synthesize sphingosines and ceramides using chlorobenzene, serine, or L-glyceric and D-tartaric acids [7-9]. However, these methods are costly and time consuming, and thus not suitable for industrial ceramide production [10]. In 1960s, the yeast *Wickerhamomyces ciferrii*, formerly known as *Pichia ciferrii*, *Hansenula ciferrii*, and *Endomycopsis ciferrii*, was found to produce tetraacetyl phytosphingosine (TAPS), a fully acetylated form of phytosphingosine [11-14]. As it is secreted to the medium, this precursor can be extracted and converted into a range of commercial ceramides. Therefore, *W. ciferrii* is an attractive microorganism for the industrial production of ceramides.

Large-scale fermentation of TAPS using *W. ciferrii* (F-60-10 mating type strain) was first described in 1962, with a maximum TAPS titer of 0.485 g/L [13]. In the past decades, efforts have been made to improve TAPS productivity by developing *W. ciferrii* mutant cells. For instance, the *W. ciferrii* mutant strain CSS.L4.O.L2.L1.S2 produces up to 2 g/L of TAPS compared with the maximum concentration of 0.284 g/L obtained using the parental strain [15]. This mutant was created by combining gene deletion (for *PcSHM1*, *PcSHM2*, *PcCHA1*, *PcLCB4*, and *PcORM12*) and overexpression (for *PcLCB1* and *PcLCB2*) strategies [15]. Although reverse genetic engineering has the potential to improve production of desired compounds, it sometimes can be costly and requires knowledge of the metabolic and genetic landscape of the microorganism. In yeasts, random mutagenesis and adaptive laboratory evolution have been

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widely employed for strain improvement with low cost and technical requirements [16]. Random mutagenesis can be achieved by physical or chemical treatment of yeast cells, followed by screening of mutants with enhanced production of the desired metabolites. Since random mutagenesis usually results in a high number of mutants, the use of high-throughput screening methods, such as fluorescence-activated cell sorting (FACS), can make a significant contribution toward strain selection [16].

TAPS is a type of sphingolipid with a long-chain sphingoid base, generally a C_{18} compound, in which all the functional groups of the base are esterified with acetic acid [12]. Since neutral lipids, such as triacylglycerides, also consist of long-chain (16-20 carbon) fatty acids, it is reasonable that BODIPY 505/515 (4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene), the most common fluorescent dye for lipids, can be used as a stain for TAPS in yeast cells. In addition, BODIPY staining does not compromise cell viability; thus, cells can be used to initiate new cultures after being subjected to FACS [17,18].

This study describes a novel strategy to improve TAPS production by *W. ciferrii* using FACS-based screening of a randomly mutated yeast library (Fig. 1). After selection of a valuable mutant, fed-batch fermentations were performed, and different feeding solutions were compared to determine the optimal condition for maximum TAPS biosynthesis. Hence, this study presents a *W. ciferrii* mutant strain with highly enhanced ceramide precursor biosynthesis potential. Furthermore, it presents for the first time a high-throughput screening platform for mutants with high TAPS levels using BODIPY staining.

MATERIALS AND METHODS

1. Yeast Strains and Culture Conditions

The yeast *Wickerhamomyces ciferrii* strain (NRRL Y-1031; ATCC 14091) was obtained from the Korean Collection for Type Cultures and used as the parental strain. Seed cultures were maintained in YM medium composed of 3.0 g/L of yeast extract, 3.0 g/L of malt extract, 5.0 g/L of peptone, and 10.0 g/L of dextrose, whereas TAPS medium [15] supplemented with 30 g/L D-Glucose (Sigma-Aldrich, St. Louis, MO, USA) or 30 g/L molasses (Wholesome, Sugar Land, TX, USA) was used for TAPS production. The molasses used in this study is blackstrap molasses made from sugar cane with about 50% content of fermentable sugars and a total of 70% carbohydrates according to the manufacturer. The initial pH of the medium was adjusted to 5.4. For flask-scale experiment in Fig. 2, the cells were cultivated in a 250 mL baffled Erlenmeyer flask with a working volume of 50 mL at 30 °C and agitation of 200 rpm. For Fig. 3,

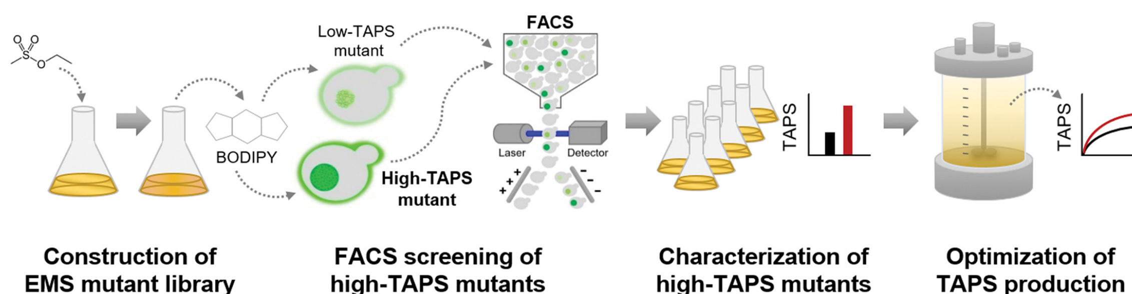

Fig. 1. Schematic illustration of the study approach. ***Wickerhamomyces ciferrii*** **cells were treated with ethyl methanesulfonate (EMS) to generate the mutant library, followed by staining with BODIPY 505/515, and then subjected to multiple rounds of fluorescence-activated cell sorting (FACS). Tetraacetyl phytosphingosine (TAPS) content of selected clones was analyzed and fed-batch cultivations were performed and optimized with the mutant exhibiting the highest TAPS titer.**

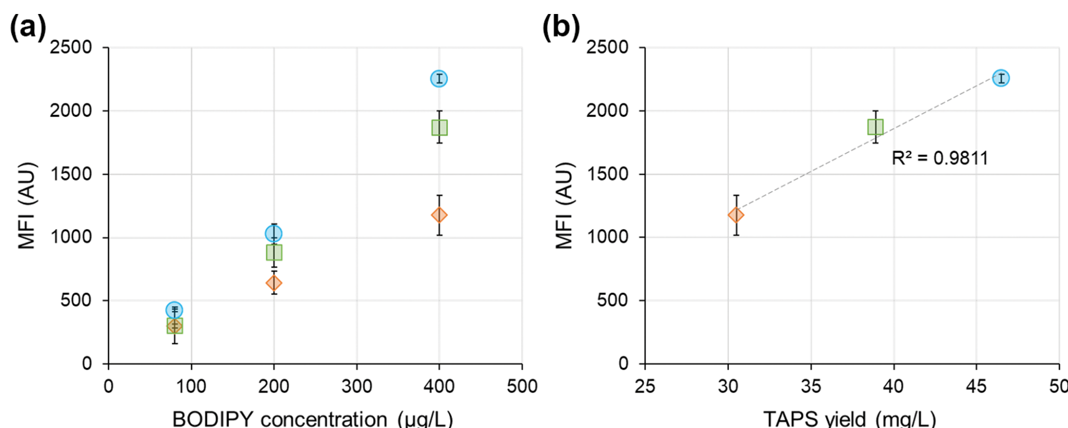

Fig. 2. Demonstration of the BODIPY-based fluorescent reporter system. (a) Optimization of BODIPY concentration for detecting tetraacetyl phytosphingosine (TAPS) levels in *W. ciferrii*. (b) Mean fluorescence intensities (MFIs) after BODIPY staining (400 μg/L) plotted according to TAPS titer. *W. ciferrii* cells were cultivated in TAPS media containing 10, 20 and 30 g/L of glucose (◇, □ and ○, respectively).

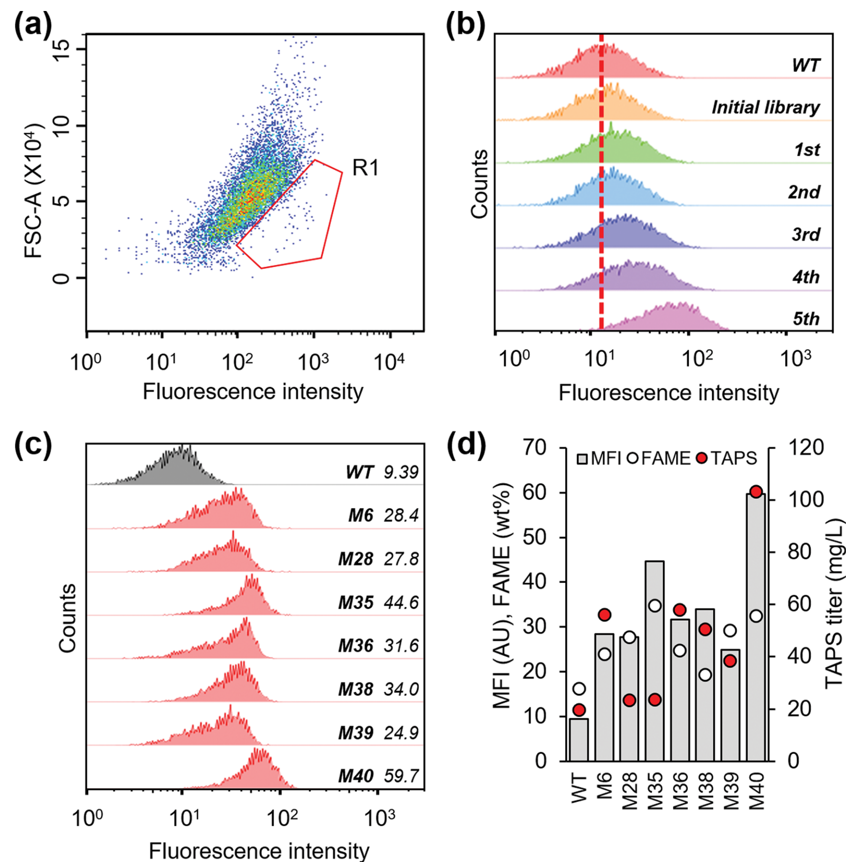

Fig. 3. FACS-based screening of high-TAPS *W. ciferrii* mutants. (a) Gating strategy to select the cell population (about 2%) with high fluorescence intensity. (b) Fluorescence enrichment during the repeated rounds of screening. The red-dashed line indicates the mean fluorescence intensity of wild-type (WT) strain. (c) BODIPY fluorescence in WT and individual clones. MFI values are presented, respectively, next to the strain name. (d) Comparison of MFI (bars), total FAME (wt%, ○) and TAPS titers (mg/L, ●). Abbreviations: FACS, fluorescence-activated cell sorting; FAME, fatty acid methyl ester; MFI, mean fluorescence intensity; TAPS, tetraacetyl phytosphingosine.

the cells were cultivated in a 250 mL T-flask (SPL, Pocheon, Republic of Korea) with the same conditions mentioned above.

2. EMS-based Random Mutagenesis

To generate a mutant library of *W. ciferrii*, cells were grown for 24 h at 30 °C on YM medium. A total of 10^8 cells for each vial were harvested, washed twice with 0.1 M NaH_2PO_4 (pH 7.0) and suspended in 1.7 mL of the same buffer. For cell suspension, EMS was added to the final concentration of 3% (v/v) [19-21]. The samples were incubated for 1 h at 30 °C with weak agitation. After treatment, 8 mL of 5% sodium thiosulfate solution was added to neutralize EMS for 10 min on ice, and pelleted cells were washed and suspended in liquid YM medium for spreading on YM agar plates. The next day, the cells were collected using a scrapper and placed in an appropriate volume of liquid YM medium.

3. FACS Analysis and Screening

A total of 10^7 cells were harvested and washed with 1 mL of 1× phosphate-buffered saline (PBS) (pH 7.4). Washed cells were resuspended in 1 mL of 1× PBS and incubated with different concentrations (80, 200, or 400 μg/L) of BODIPY 505/515 (Sigma-Aldrich, St. Louis, MO, USA) for 30 min at room temperature [22]. After staining, the cells were washed twice with 1 mL of 1× PBS and it was used for subsequent analysis and sorting by using BD FACSAria III cell sorter (BD Biosciences, Franklin Lakes NJ, US). BODIPY was excited with a 488 nm argon laser and emitted fluorescence signals were detected in the fluorescence channel 1 (FL1) centered at 530-540 nm. For cell sorting, the flow rate was maintained under 3,000 events per second, and approximately 100,000 cells exhibiting high fluorescence (~2%) were sorted for every round of screening.

4. HPLC Analysis

For TAPS quantification, cellular TAPS was extracted from the culture solution using acetone with vigorous vibration for 30 min at 40 °C. Analysis was conducted as previously described [15]. Briefly, TAPS quantification was performed using an HPLC system (1260 Infinity, Agilent, Santa Clara, CA, USA) equipped with a diode array detector using a reverse-phase column ZORBAX SB-C8 (4.6×150 mm, 3.5 μm, Agilent). The column temperature was set at 40 °C and an isocratic mobile phase comprising 81.5% (v/v) methanol, 18.45% (v/v) water, and 0.05% (v/v) trifluoroacetic acid was used with a flow rate of 1.2 mL·min^{-1}. Absorbance at 200 nm was monitored for 10 min per sample and the TAPS content was quantified by substituting the area value of the TAPS peak of each sample into the calibration curve obtained by analyzing TAPS standard material (DOOSAN, Seoul, Republic of Korea) at concentrations of 100, 250, 500, and 1,000 ppm.

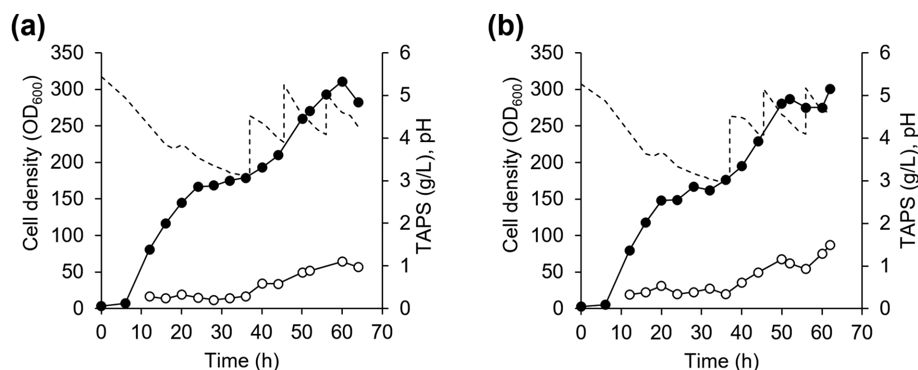

Fig. 4. Growth curves of wild-type and isolated mutant (M40) in fed-batch cultivation. Cell densities (OD_{600}, ●), tetraacetyl phytosphingosine (TAPS) titers (g/L, ○), and pH profiles (dashed lines) of (a) wild-type *W. ciferrii* and (b) M40 strain.

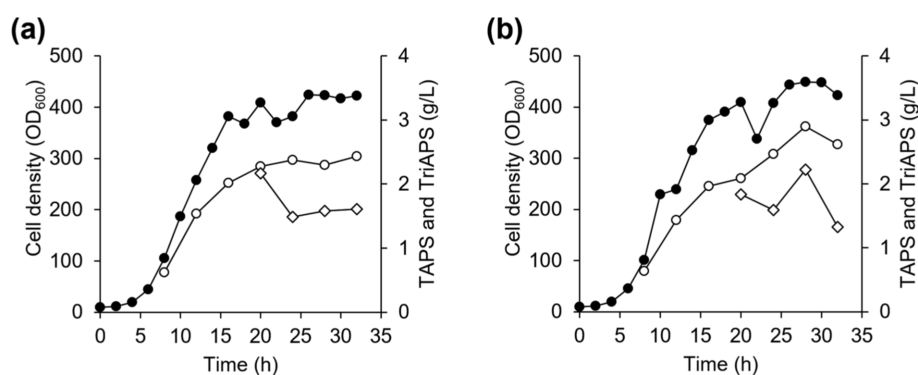

Fig. 5. Growth curves of M40 strain with different carbon sources. Cell density (OD_{600}, ●), and tetraacetyl phytosphingosine (TAPS) and triacetyl phytosphingosine (TriAPS) titers (g/L, ○ and ◇, respectively) with (a) glucose feeding and (b) glucose+molasses feeding.

5. FAME Analysis

FAME content was analyzed using a modified Folch's method [22,23]. Briefly, 10 mg of lyophilized yeast cells were treated with 10 mL of chloroform-methanol (2 : 1, v/v) and vortexed at maximum speed for 10 min. Then, heptadecanoic acid (C17:0) was added to the samples as an internal standard to a final concentration of 500 ppm, and 1 mL of methanol and 300 µL of sulfuric acid were added in order. The samples were boiled at 100 °C for 20 min for transesterification. After cooling the samples, 1 mL of deionized water was added and vortexed for 5 min. Next, the samples were centrifuged at 4,000 rpm for 10 min, and the lower layer of the organic phase was collected and filtered using a 0.20-µm RC-membrane syringe filter (Sartorius Stedim Biotech, Göttingen, Germany). FAMEs were detected via gas chromatograph (HP 6890, Agilent) with an HP-INNOWax polyethylene glycol column (HP 19091 N-213, Agilent) and a flame ionization detector. The temperature of the oven was increased from 50 to 250 °C, at 15 °C per min.

6. Fed-batch Cultivation

Fed-batch fermentation was performed in 5.6-L bioreactors (CNS, Seoul, Republic of Korea) with working volume of 2 L. Up to 1.8 L of fresh TAPS media was prepared, and 200 mL of flask-cultivated inoculum was added at the start of cultivation. The fermentation was performed at 30 °C with agitation speed of 200 rpm and an aeration rate of 4 L/min. The agitation speed was automatically increased up to 1,000 rpm to maintain the dissolved oxygen over than 40%. For the glucose or glucose+molasses regimes, 20 g/L of $MgSO_4 \cdot 7H_2O$ with 700 g/L glucose or 500 g/L glucose+100 g/L molasses were used as feeding solution, respectively. Fermentation experiments (Fig. 4) were performed with manual feeding upon carbon depletion and the residual glucose content was monitored using a glucose test strip (Self-Stik4, CHUNGDO PHARM, Chuncheon-si, Republic of Korea), whereas the experiments in Fig. 5 were carried out with pH stat strategy. For the pH stat, 50% (v/v) ammonia solution and feeding solutions were automatically added to maintain the pH at 5.4. Antifoam 204 (Sigma-Aldrich) was frequently added to relieve excessive foaming. Small amounts of samples were collected periodically and were used for cell density measurement (OD at 600 nm), TAPS, and TriAPS quantifications.

RESULTS

1. Development of a Fluorescence-based System for Monitoring TAPS Level in Yeast

In an attempt to isolate *W. ciferrii* strains with high TAPS production potential from an ethyl methane sulfonate (EMS)-induced mutant library, a high-throughput screening platform based on BODIPY staining and flow cytometry was developed. Flow cytometry is a powerful tool that enables screening of large mutant libraries for desired functions through fluorescence signals [24]. First, wild-type *W. ciferrii* cells were cultivated in TAPS media containing dif-

Table 1. Quantification of FAME content in *W. ciferrii* wild-type and mutant strains

Fatty acid (weight %)[a]	WT	M6	M28	M35	M36	M38	M39	M40
C14:0	5.4	4.6	3.8	3.7	4.0	3.3	2.7	2.7
C16:0	31.1	35.2	39.2	38.7	32.0	33.9	38.5	37.6
C16:1	30.1	31.3	33.4	32.8	32.2	32.6	34.2	34.0
C18:0	2.1	2.0	1.6	1.7	2.4	2.2	1.5	1.8
C18:1n9c	1.3	1.2	1.3	1.2	1.3	1.4	1.6	1.5
C18:1n9t	9.8	9.0	8.8	8.4	7.6	8.3	7.9	7.6
C20:3n3	4.3	4.0	3.1	3.1	4.3	4.0	2.9	3.2
C20:5n3	14.8	11.9	8.2	8.2	14.2	11.3	7.9	8.4
Total FAME content/DCW (% w/w)	16.3	24.0	27.8	34.9	24.6	19.4	29.4	32.5

Abbreviations: DCW, dried cell weight; FAME, fatty acid methyl esters; M, mutant; WT, wild-type.

ferent concentrations of glucose to vary the TAPS titer for the optimization of BODIPY staining conditions. The resulting TAPS titers at glucose concentrations of 10, 20 and 30 g/L were 30.9±0.6, 40.9±2.8 and 46.5±2.3 mg/L, respectively. It was consistent with the previous study that reported a greater accumulation of TAPS with increasing glucose concentration [13]. To further determine the best staining conditions, the wild-type cells obtained from the above experiment were stained with varying concentrations of BODIPY. At a low concentration of BODIPY (80 μg/L), it was hard to distinguish different TAPS levels based on the MFI. The 200 μg/L of BODIPY generated the MFI proportional to the TAPS levels, but there was no statistical significance between the samples with varying TAPS titers (Fig. 2(a)). In contrast, when the BODIPY concentration was increased to 400 μg/L, an apparent difference in MFI between subpopulations was observed with a strong linear correlation between TAPS levels and MFI (R^2=0.9811, Fig. 2). Taken together, 400 μg/L was selected as the optimal BODIPY concentration for monitoring the TAPS production in *W. ciferrii*.

2. Mutant Library Construction and Screening of High TAPS-producing Strains

To generate a *W. ciferrii* library, random mutagenesis was performed using EMS, which is a mutagenic chemical [25]. The mutated cells were incubated with BODIPY for TAPS labeling prior to multiple rounds of FACS-based screening. At each round, approximately 2% of the cell population with the highest BODIPY fluorescence was obtained and cultivated for the next round (Fig. 3(a)). Note that through the screening, the MFI of the cell populations gradually increased. After five rounds of sorting, a significant enrichment of fluorescence signal was observed (Fig. 3(b)). Since additional cycles of FACS did not result in further increase in fluorescence intensity, fifty individual clones were randomly selected from the population obtained through five rounds of sorting and analyzed by flow cytometry. Among them, seven mutants with the highest MFI were chosen and subjected to TAPS quantification (Fig. 3(c)). Four of the seven mutants (M6, M36, M38 and M40) showed pronounced improvement in their TAPS production, whereas the remaining three mutants (M28, M35, M39) exhibited only a slight increase in TAPS content in the harvested biomass (Fig. 3(d)). While the BODIPY has been commonly used as a probe for cellular lipids, fatty acid methyl ester (FAME) analysis was also performed to determine the lipid content in all mutants. Notably, the mutant strains M28 and M35, whose TAPS titers were similar to that of the wild-type, accumulated relatively large amounts of lipid (Fig. 3(d), Table 1). In these cases, the high fluorescence of BODIPY likely reflected the concentration of other lipid components (i.e., non-TAPS) in the cells. However, in the other mutants, the actual TAPS content seemed to be well correlated with the fluorescent signals (Fig. 3(d)). Although these results suggest that a fluorescence signal should be carefully considered when targeting to screening for TAPS-enriched mutants, TAPS content was increased in all of the mutants obtained in this study. Given that there is no lipophilic stain or dye that specifically binds to TAPS [26], the results reiterate the suitability of BODIPY for the initial screening of a large number of samples to identify potential high-TAPS mutants. Finally, the M40 mutant strain, which showed a 5.23-fold increase in TAPS titer (103.15 mg/L) over the wild-type strain (19.79 mg/L), was selected as a novel cell factory for high-level TAPS production; this mutant was subjected to several rounds of subculture prior to high cell density cultivation.

3. Fed-batch Cultivation of Wild-type and M40 Strain

Fed-batch cultivation was conducted in a 5.6-L bioreactor, and the yeast strains were cultivated in TAPS medium that was previously reported as the optimal medium for yeast-based TAPS production and glucose was supplied as a carbon source [15]. Wild-type and M40 strains shared a similar growth phenotype during cultivation (Fig. 4). When the medium pH level dropped under 3.5, both wild-type and M40 cells showed delayed growth. At this point, ammonia solution was added to the culture to maintain the pH at 5.4, after which cell growth and TAPS titer began to increase. M40 cells displayed a TAPS production yield as high as 1.495 g/L after 62 h of cultivation, which was 1.54-fold higher than that of wild-type cells (0.973 g/L). Moreover, the mutant demonstrated an improvement of 1.6-fold in volumetric TAPS productivity (29.9 mg/L/h) over the wild-type strain (18.7 mg/L/h) (Fig. 4). As a higher production yield and productivity could be expected based on the initial screening results, this indicated that fed-batch cultivation conditions should be further optimized.

4. Optimization of TAPS Production in Fed-batch Cultivation of M40 Strain

To achieve the maximum TAPS production, the impact of various carbon sources in the fed-batch culture was evaluated. The most widely used substrate for industrial production of yeast is molas-

ses, an abundantly available byproduct of the sugar refining process [27]. In addition to its low price, molasses contains high concentration of fermentable sugars, including sucrose (30-40%), fructose (5-12%), and glucose (4-9%) [28]. First, flask cultivation of wild-type and M40 strains were performed in TAPS medium supplemented with glucose or molasses, and TAPS production yields were determined. Molasses was found to greatly increase the TAPS titer of the M40 strain compared with that achieved with glucose-fed culture (176.43±5.49 vs. 97.31±4.24 mg/L, respectively) (Supplementary Fig. 1). Next, the experiment was repeated for M40 strain on a larger-scale using fed-batch cultivation. Unlike flask cultivation, the yeast displayed growth retardation during the early phase when molasses was used as the sole carbon source. Therefore, fed-batch fermentation of TAPS was performed with two feeding regimes: glucose or glucose+molasses. While adjusting the pH of TAPS medium in the previous section supported a continuous growth of both wild-type and M40 cells, an automated pH control system was deployed to maintain a stable pH value of 5.4 with an aim of avoiding delayed culture growth. As expected, such pH control greatly improved the growth rate and cell density of the cultures (Fig. 5). The highest TAPS titer achieved was 2.895 g/L in glucose+molasses feeding (after 28 h; Fig. 5(b)) and 2.512 g/L in glucose feeding condition (after 36 h; Fig. 5(a)). Moreover, the maximum volumetric TAPS productivity of 169.5 mg/L/h was achieved with glucose+molasses supplementation, which was 5.5-fold higher than that of the initial fed-batch results (Fig. 4(b)).

Next, high-performance liquid chromatography (HPLC) analysis of fermentation products showed some minor peaks that could be meaningful in addition to the major peak of TAPS (Supplementary Fig. 2(a)). The yeast *W. ciferrii* is known for secreting acetylated sphingoid bases, among which TAPS is the major form [15]. Identification of those minor peaks with electrospray ionization mass spectrometry analysis revealed the presence of triacetyl phytosphingosine (TriAPS), a byproduct generated during the fermentations that can also be used as a ceramide precursor (Supplementary Fig. 2(b)). Taken together, the final production yield of ceramide precursors, including TAPS and TriAPS, was 5.114 g/L, which is 2.5-fold higher than that reported in earlier studies (Fig. 5) [15].

DISCUSSION

W. ciferrii is a natural producer of sphingolipids, among which several ceramide precursors have been identified for industrial applications [13]. In this study, high TAPS-producing *W. ciferrii* strains were obtained by EMS mutagenesis followed by FACS screening, and successfully achieving high TAPS titer and productivity in fed-batch cultivation. In summary, a fluorescence-based screening methodology for the detection and sorting of cells with high amounts of TAPS was established using FACS and BODIPY staining. Considering the strong correlation between TAPS titers and MFI values of BODIPY, it is reasonable to conclude that BODIPY can be used as a stain for TAPS in yeast cells. Herein, five out of seven mutants with high fluorescence displayed a substantially enhanced TAPS content, whereas the remaining two strains showed TAPS levels similar to or greater than that of the wild-type cells. Although our screening approach does not allow quantifying the actual TAPS content, this high-throughput screening system can accelerate the development and analysis of mutant strains, as large-scale libraries can be rapidly screened in a cost effective manner.

After the desired mutant strains were generated, their ability to produce high levels of TAPS in large-scale fed-batch cultivation was evaluated. It is important to maintain the pH of the culture near a suitable value (pH 5.4) for optimal cell growth and TAPS production. Moreover, further improvement can be achieved by molasses supplementation. Molasses has been widely used as a feed substrate for the production of ethanol, industrial chemicals, cosmetics, and pharmaceutical compounds [29,30]. Herein, a combination of molasses and glucose was found to accelerate and maximize the biosynthesis and titer of TAPS in a shorter time.

In addition to TAPS, the presence of TriAPS in the medium may provide another source for ceramide production. Both TAPS and TriAPS can be easily converted into phytosphingosine and ceramides through chemical reactions. The method is composed of three steps: (1) TAPS and TriAPS are produced by yeast cells (M40 strain in the present study); (2) phytosphingosine is obtained by chemical deacetylation of TAPS and TriAPS; and (3) ceramides are formed through chemical acylation of the phytosphingosine base and a fatty acid. Hence, large quantities of ready to use phytosphingosine and ceramides can be obtained using this method.

CONCLUSION

This study describes a successful screening system for high TAPS-producing mutants of *W. ciferrii*. The isolated mutant M40 displayed TAPS volumetric productivity increased by 60% compared with wild-type cells. Enhanced production of ceramide precursors was achieved by fed-batch cultivation of the M40 strain, with a 2.5-fold higher titer than previously reported for nonconventional yeast *W. ciferrii* [15]. Hence, this study provides a platform for the development and screening of mutants with improved productivity of ceramide precursors in yeast.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be

construed as a potential conflict of interest.

AUTHORS' CONTRIBUTION

Y. J. Lee conceived of this study. Y. J. Lee and S. Park conducted overall experiments. Q. Tran and A. J. Ryu wrote the manuscript. K. K. Kwon performed cytometric analysis and FACS. Y. J. Lee, J. Yun and H. Kim finalize the manuscript. All authors read and approved the final manuscript.

SUPPORTING INFORMATION

Additional information as noted in the text. This information is available via the Internet at http://www.springer.com/chemistry/journal/11814.

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Supporting Information

Fluorescence-activated cell sorting-mediated directed evolution of *Wickerhamomyces ciferrii* for enhanced production of tetraacetyl phytosphingosine

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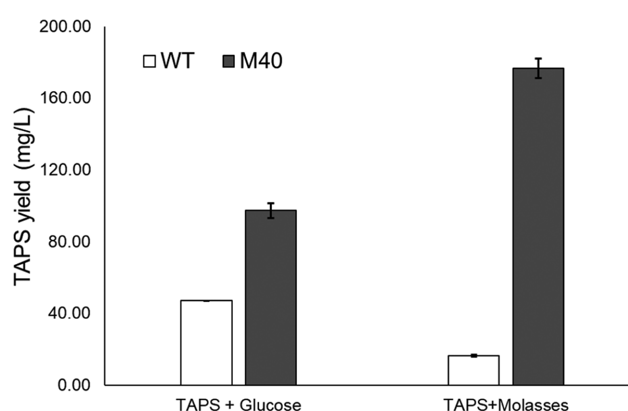

Fig. S1. Influence of carbon sources on TAPS production in wild-type and M40 strain.

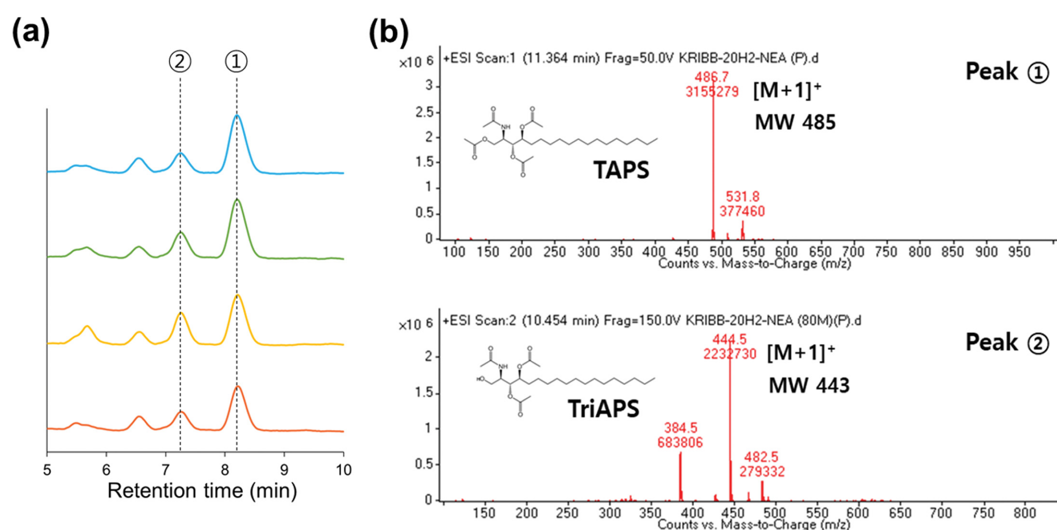

Fig. S2. Evidence of TAPS and TriAPS co-production from the M40 strain. (a) HPLC profiles of fermentation samples of M40 strain. (b) Electrospray ionization mass spectrometry analysis of the different peaks from the HPLC analysis.