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Engineering of *Saccharomyces cerevisiae* as a platform strain for microbial production of sphingosine-1-phosphate

In-Seung Jang^{1,2}, Sung Jin Lee¹, Yong-Sun Bahn^{2*}, Seung-Ho Baek^{3*} and Byung Jo Yu^{1*}

Abstract

Background Sphingosine-1-phosphate (S1P) is a multifunctional sphingolipid that has been implicated in regulating cellular activities in mammalian cells. Due to its therapeutic potential, there is a growing interest in developing efficient methods for S1P production. To date, the production of S1P has been achieved through chemical synthesis or blood extraction, but these processes have limitations such as complexity and cost. In this study, we generated an S1P-producing *Saccharomyces cerevisiae* strain by using metabolic engineering and introducing a heterologous sphingolipid biosynthetic pathway to demonstrate the possibility of microbial S1P production.

Results To construct the sphingosine-producing *S. cerevisiae* strain, both the sphingolipid delta 4 desaturase gene (*DES1*) and the alkaline ceramidase gene (*ACER1*) derived from *Homo sapiens* were introduced into the genome of *S. cerevisiae* by deleting the dihydrosphingosine phosphate lyase gene (*DPL1*) and the sphingoid long-chain base kinase gene (*LCB5*) to prevent S1P degradation and byproduct formation, respectively. The sphingosine-producing strain, DDLA, produced sphingolipids containing sphingosine. In flask fed-batch fermentation, the DDLA strain showed a higher production level of sphingosine under aerobic conditions with high initial cell density. The S1P-producing strain was generated by expressing the human sphingosine kinase gene (*SPHK1*) under the control of the inducible promoter, while deleting the *ORM1* gene involved in the regulation of sphingolipid biosynthesis. The S1P-producing strain, DDLAOGS, exhibited the highest sphingosine production level under fed-batch fermentation in a bioreactor, achieving a 2.6-fold increase compared to flask fermentation. S1P biosynthesis in the DDLAOGS strain was verified by qualitative analysis using electrospray ionization mass spectrometry (ESI-MS).

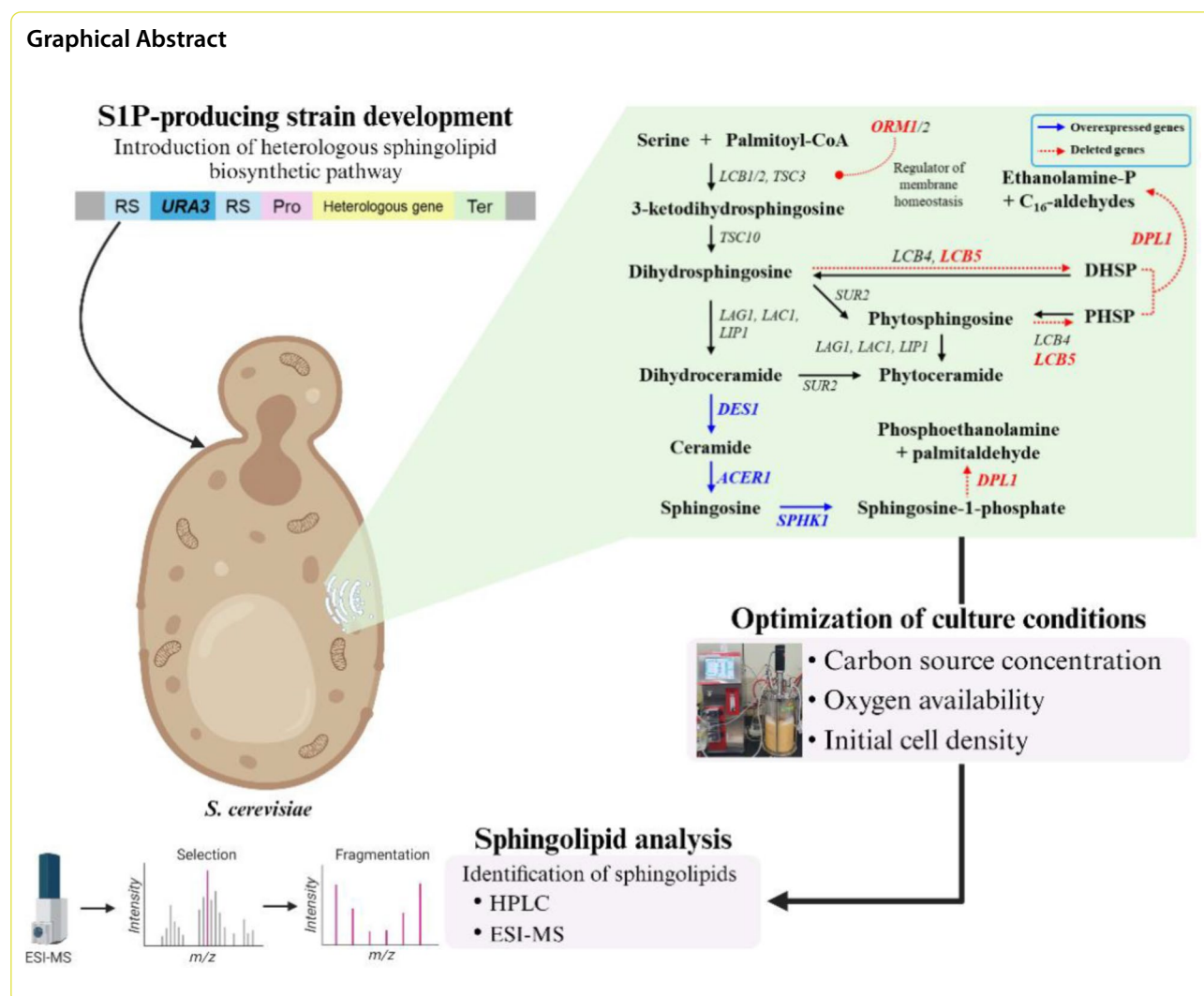
Conclusions We successfully developed a metabolically engineered *S. cerevisiae* as a platform strain for microbial production of S1P by introducing an exogenous pathway of sphingolipids metabolism. The engineered yeast strains showed significant capabilities for sphingolipid production, including S1P. To our knowledge, this is the first report demonstrating that engineered *S. cerevisiae* can be a major platform strain for producing microbial S1P.

Keywords Sphingosine-1-phosphate, *Saccharomyces cerevisiae*, Metabolic engineering, Sphingolipid biosynthesis, Electrospray ionization mass spectrometry

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Graphical Abstract



Background

Sphingolipids and their metabolites play crucial roles in all eukaryotic cells, serving as both structural components of membranes and signaling molecules [1, 2]. Notably, sphingosine-1-phosphate (S1P) acts as a key signaling molecule required in various biological processes such as cell proliferation, migration, survival, and inflammation [3, 4]. This bioactive mediator is integral to numerous functions in mammalian cells, including immune cell recruitment, blood vessel regeneration, and maintaining endothelial barrier integrity [5].

Given its prominent biological functions, S1P has emerged as a therapeutic molecule for treating cancer, autoimmune diseases, and cardiovascular disorders [3, 4, 6, 7]. Beyond its therapeutic potential, S1P also holds promise for cosmetic applications, particularly in skin regeneration and brightening [8, 9]. Despite the considerable interest in S1P applications across various fields,

an efficient production process for S1P has yet to be reported. Currently, S1P production is predominantly achieved through sophisticated chemical synthesis and biological extraction from blood, methods that are both complex and expensive, thus limiting large-scale production [10, 11].

Consequently, fermentation process involving engineered microorganisms present a promising alternative for S1P production. *Saccharomyces cerevisiae*, extensively used as a model organism for studying lipid metabolism due to its relative simplicity and the conservation of lipid metabolic pathways across eukaryotes, including humans, serves as an ideal candidate [1, 12]. Indeed, numerous enzymes and regulators involved in sphingolipid metabolism were first identified in yeast. Recent advances in synthetic biology and metabolic engineering have further enabled the production of complex lipids in engineered yeast, including ceramide and tetra-acetyl

phytosphingosine [13, 14]. In brief, sphingolipid biosynthesis in *S. cerevisiae* begins with the condensation of serine and palmitoyl-CoA in endoplasmic reticulum (ER) to generate 3-ketodihydrosphingosine. This intermediate is subsequently reduced to dihydrosphingosine, which can be converted to phytosphingosine by hydroxylase (Sur2) or dihydrosphingosine-1-phosphate by kinases encoded by *LCB4* and *LCB5*. Alternatively, dihydrosphingosine can be N-acylated to form dihydroceramide by ceramidase synthases (Lac1 and Lag1). Phytosphingosine can also be converted to phytosphingosine-1-phosphate by phosphorylation or to phytoceramide by N-acylation. Both dihydroceramide and phytoceramide can be further hydroxylated by *Scs7* to form ceramide derivatives [1]. Since *S. cerevisiae* does not naturally synthesize S1P or its precursors, including ceramide and sphingosine, due to the absence of sphingolipid 4-desaturase and ceramidase, S1P biosynthesis can be achieved by introducing a heterologous sphingolipids pathway from mammals [13].

This study aims to construct an engineered *S. cerevisiae*-based platform strain for the production of human S1P by introducing a heterologous sphingolipid biosynthetic pathway. The production level of sphingolipids was further enhanced by optimizing fermentation conditions. S1P production in the resulting strain was confirmed through qualitative analysis using electrospray ionization mass spectrometry (ESI-MS). These findings could provide new insights into microbial production of S1P and facilitate the mass production of S1P for various valuable applications.

Results and discussion

Development of the sphingosine-producing *S. cerevisiae* strains

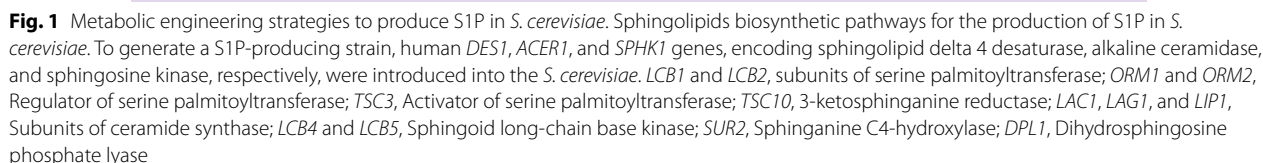
Sphingosine biosynthesis is a highly conserved pathway in all eukaryotes [1, 12]. While plants primarily produce glycosyl inositol phosphor ceramide (GIPC) and glucosylceramide from phytoceramide and phytosphingosine, animal cells generate ceramide and sphingosine as the main precursors for sphingomyelin and glucosylceramide biosynthesis [15, 16]. Although *S. cerevisiae* possesses many enzymes involved in sphingolipid biosynthesis, it does not naturally produce ceramide (N-acyl sphingosine) and sphingosine due to the absence of sphingolipid delta 4-desaturase [13]. Instead, this yeast synthesizes phytosphingosine or dihydrosphingosine-containing ceramides [12]. Since S1P is generated from sphingosine via phosphorylation, an exogenous metabolic pathway for sphingosine biosynthesis needs to be introduced to enable S1P production in *S. cerevisiae*.

To construct the sphingosine-producing strain, the heterologous *DES1* gene from *Homo sapiens*, which encodes sphingolipid delta 4-desaturase, was

introduced into the CEN.PK2-1D strain. The *DES1* gene was integrated into the *DPL1* locus under the control of the *TDH3* promoter, resulting in the generation of the DD strain. Previous study has reported that the expression of the human *DES1* gene led to the biosynthesis of sphingolipids containing sphingosine, without phytosphingosine, in engineered *S. cerevisiae* strain [13]. The sphingoid base phosphate lyase, encoded by the *DPL1* gene, is involved in S1P degradation by cleaving it into fatty aldehyde and ethanolamine phosphate [12]. In the previous research, *S. cerevisiae* mutant strain disrupting the *DPL1* gene showed accumulation of the phosphorylated sphingoid bases [17]. Therefore, the introduction of the *DES1* gene alongside the deletion of the *DPL1* gene promotes the accumulation of sphingosine as a precursor for S1P production. As shown in Fig. 1, sphingolipid delta 4-desaturase functions as a bi-functional enzyme that converts dihydrosphingosine and dihydroceramide into sphingosine and ceramide, respectively [18]. Thus, sphingosine can be directly produced from dihydroceramide, bypassing ceramide.

Next, the DDLA strain was constructed by chromosomal integration of the *TDH3* promoter-controlled *ACER1* gene into the *LCB5* locus of the DD strain. Alkaline ceramidase, encoded by the *ACER1* gene, is a key enzyme that increases sphingosine synthesis by catalyzing the conversion of ceramide to sphingosine (Fig. 1). The *LCB5* gene, encoding long-chain base kinase, is involved in the formation of phosphorylated long-chain bases, including dihydrosphingosine-1-phosphate and phytosphingosine-1-phosphate [12]. To further enhance sphingosine production in the DD strain, the *ACER1* gene was introduced by disrupting the *LCB5* gene.

To evaluate the effect of introducing the *DES1* and *ACER1* genes on sphingolipid production, both the DD and DDLA strains were cultivated in flask batch fermentation using YPD medium containing 20 g/L glucose. As expected, the DDLA strain produced sphingolipids containing sphingosine along with dihydrosphingosine and phytosphingosine, whereas sphingosine was not detected in the DD strain (Figure S2). Although ceramide levels could not determine in this study, this result suggests that Des1 expressed in the DD strain might exhibit a higher substrate preference for dihydroceramide over dihydrosphingosine. Additionally, the DDLA strain demonstrated improved sphingosine production in culture medium containing 60 g/L glucose compared to medium containing 20 g/L glucose (Figure S2). Consequently, the DDLA strain was selected as the sphingosine-producing strain for further experiments in this study.



In an effort to optimize cultivation conditions in fed-batch fermentation in flask for improved sphingolipids production, we investigated sphingosine production levels by varying the initial cell inoculum under both aerobic and anaerobic conditions using the DDLA strain. Previous studies have reported significant changes in phospholipid levels in *S. cerevisiae* with oxygen availability, noting increases in phosphatidylinositol, phosphatidylcholine, and phosphatidylethanolamine under anaerobic conditions [19].

that a portion of dihydrosphingosine might be hydroxylated by Sur2 to form phytosphingosine. Under anaerobic conditions, only 42.7 mg/L of sphingosine was produced in high cell density culture (Fig. 2D), whereas no sphingosine was observed in low cell density culture (Fig. 2C). These results demonstrate that oxygen availability and initial cell density are crucial factors in optimizing sphingolipid production in the engineered *S. cerevisiae* strains.

The condensation of palmitoyl-CoA and serine, the first step in the sphingolipid synthesis pathway of *S. cerevisiae*, is mediated by serine palmitoyltransferase. This metabolism is negatively regulated by an enzyme complex consisting Orm1 and Orm2 [1]. To generate an S1P-producing strain, we attempted to integrate the *SPHK1* gene under the control of the *TDH3* promoter into the genome while eliminating the *ORM1* gene. Although we successfully constructed an S1P-producing strain based on the wild-type strain, we were unable to construct S1P-producing strains derived from the DD and DDLA strains. Previous reports indicated that the accumulation

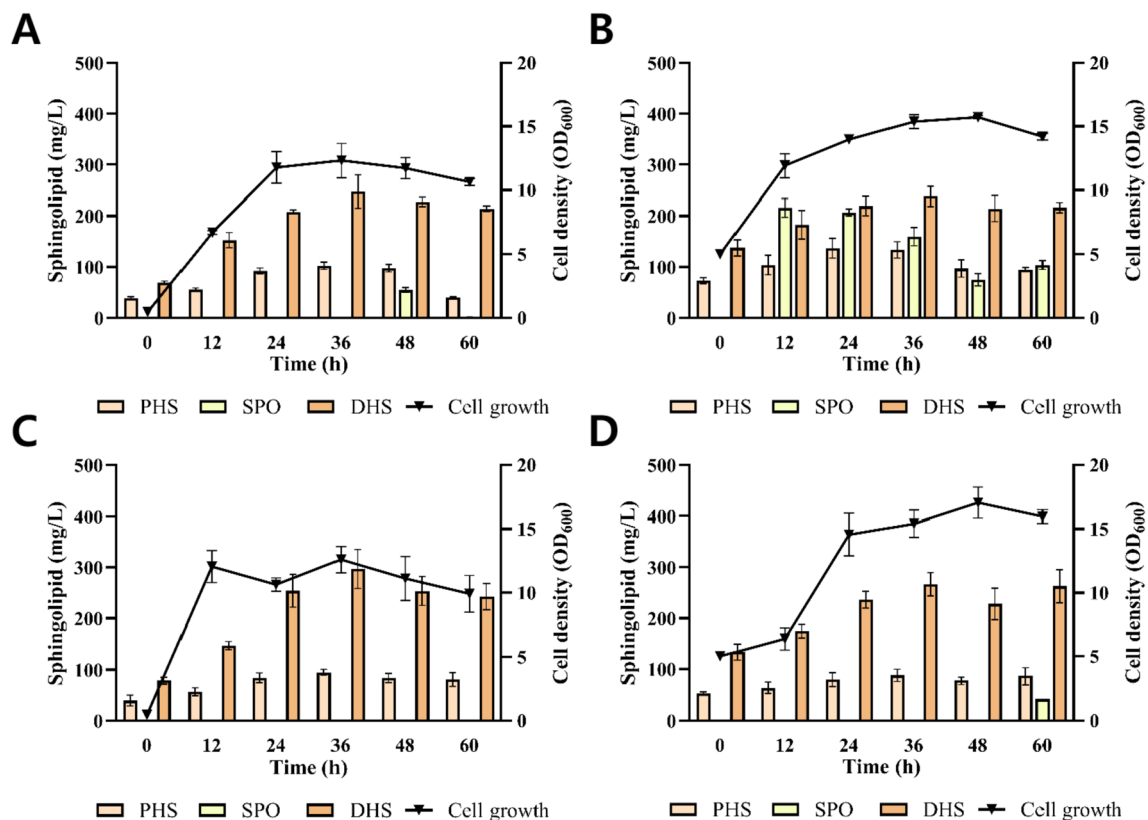


Fig. 2 Comparison of sphingolipids production levels by flask fed-batch fermentation under various culture conditions. Sphingosine-producing strain DDLA was cultivated in aerobic conditions with low initial cell density (A), aerobic conditions with high initial cell density (B), anaerobic conditions with low initial cell density (C), and anaerobic conditions with high initial cell density (D), respectively. Cell growth and sphingolipids level were determined for 60 h of cultivation. Error bars indicate standard deviations of three independent experiments

of phosphorylated sphingoid long-chain bases leads to growth inhibition in *S. cerevisiae* [17]. Therefore, the constitutive expression of sphingosine kinase might be associated with unsuccessful strain development. Specifically, S1P and ceramide are implicated as signaling molecules mediating cell proliferation regulation. Accordingly, the *SPHK1* gene was expressed under the control of the *GAL1* and *SUC2* promoter in the DDLA strain, resulting in the DDLAOGS and strain DDLAOS strains, respectively.

To profile sphingolipid biosynthesis in the S1P-producing strains, we examined the sphingolipid production levels of the DDLAOGS and DDLAOS strains. Flask fermentations were conducted under optimized conditions, including aerobic cultivation with high-density cells. Galactose or sucrose was fed every 12 h for inducible expression of sphingosine kinase after depleting the initial glucose over 60 h (Fig. 3). The DDLAOGS strain produced up to 402.7 mg/L of dihydrosphingosine with a decreased phytosphingosine level, exhibiting a 1.7-fold higher concentration

compared to the DDLA strain. After the addition of galactose, the dihydrosphingosine level gradually decreased in the DDLAOGS strain. Sphingosine production was undetectable after the initial glucose was exhausted at 12 h. However, sphingosine accumulated up to 167.1 mg/L after 36 h of cultivation.

Conversely, in the DDLAOS strain, the introduction of the *SPHK1* gene led to a decrease in overall sphingolipid production compared to the DDLA and DDLAOGS strains, although the sphingosine level gradually increased regardless of *SPHK1* gene expression. This reduction in sphingolipid production in DDLAOS strain suggests that cell growth and the activity of sphingosine kinase might affect the sphingolipid biosynthesis. Accordingly, these results indicate that the *GAL1* promoter is more suitable for *SPHK1* gene expression in the context of S1P production in *S. cerevisiae*. Thus, based on sphingosine production levels, the DDLAOGS strain was selected as the S1P-producing strain for further experiments.

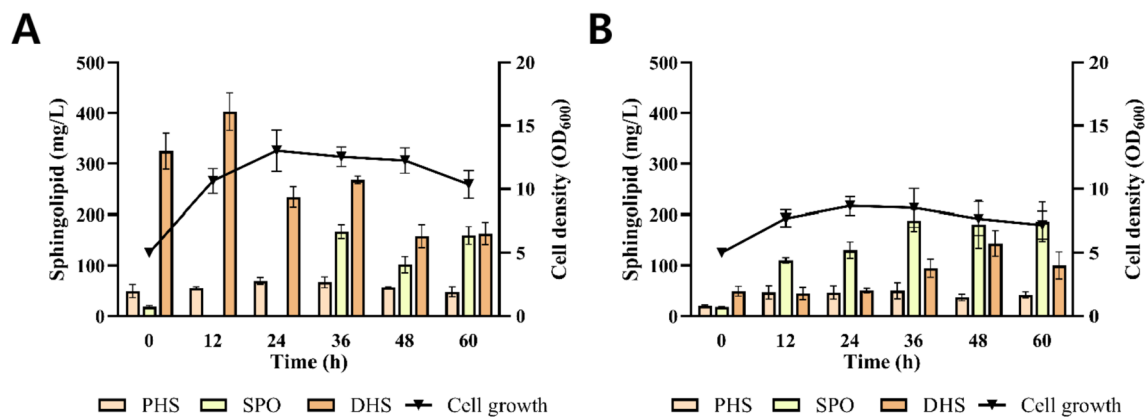


Fig. 3 Identification of sphingolipids production in S1P-producing strains by flask fed-batch fermentation. Both the DDLAOGS (A) and DDLAOGS (B) strains were cultivated in YPD medium containing 60 g/L glucose under aerobic conditions with high initial cell density. After depletion of initial glucose, galactose or sucrose was feed for inducing sphingosine kinase, respectively. Cell growth and sphingolipids levels were measured during 60 h of cultivation. Error bars indicates standard deviations of three independent experiments

Identification of sphingolipids production capability in bioreactor

To evaluate the robustness and scalability of sphingolipid production in engineered strains, we further investigated sphingolipid production levels using fed-batch fermentation in a 10 L-scale fermenter. The DDLA strain, which produces sphingosine, reached a production level of up to 647.5 mg/L sphingosine at the early stage of fermentation. Additionally, dihydrosphingosine and phytosphingosine levels in this strain gradually accumulated to 714.3 mg/L and 401.4 mg/L, respectively, at 36 h (Fig. 4A). This trend in sphingolipid biosynthesis showed a correlation with the results from flask fed-batch fermentation under aerobic conditions with high cell density. Regardless of the fermentation scale, the sphingosine level in the DDLA strain gradually decreased after 12 h of cultivation.

Sphingosine, produced by ceramide hydrolysis, is an important bioactive signaling molecule involved in cell growth and viability [20]. This molecule can be recycled through re-acylation to ceramide and its derivatives, a process known as the salvage pathway. This pathway is a pivotal metabolism involving various enzymes, including sphingomyelinase, ceramidase, and ceramide synthase, which modulate ceramide synthesis and cellular signals. In a previous study, S. Murakami et al. demonstrated the ability to convert sphingosine to ceramide using in vivo labeled sphingolipids in engineered *S. cerevisiae* strains [13]. Thus, a portion of the sphingosine accumulated in the DDLA strain might be utilized for ceramide regeneration via the native salvage pathway.

When the DDLAOGS strain was cultivated in a bioreactor, the sphingosine production level reached up to 431.3 mg/L, while dihydrosphingosine and

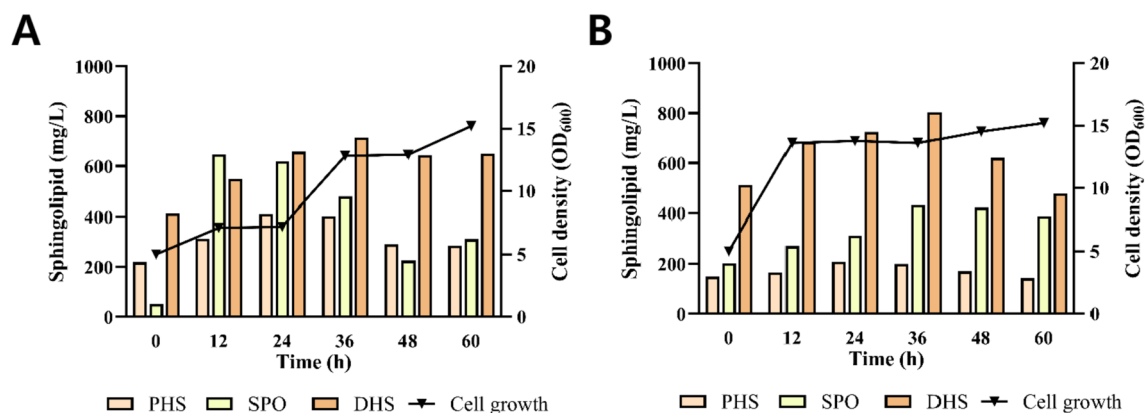


Fig. 4 Improvement of sphingolipids production levels in bioreactor. Sphingosine-producing strain DDLA (A) and S1P-producing strain DDLAOGS (B) were cultivated in a 10 L bioreactor by fed-batch fermentation. Cell growth and sphingolipids levels were determined during 60 h of fermentation

phytosphingosine accumulated to 803.4 mg/L and 207.7 mg/L, respectively. The DDLAOGS strain showed a 12% increase in dihydrosphingosine production and a 48% decrease in phytosphingosine production compared to the DDLA strain. As expected, the sphingosine production level in the DDLAOGS strain was lower than that of the DDLA strain, but a substantial amount of sphingosine still accumulated in the cells after 36 h of cultivation (Fig. 4B). This result suggests that the expression level of *SPHK1* might not be sufficient to prevent sphingosine accumulation. Therefore, further studies aimed at optimizing the expression of the *SPHK1* gene, including fine-tuning the promoter, might be an efficient strategy to reduce sphingosine levels in the DDLAOGS strain.

Qualitative analysis of S1P production using ESI–MS

In general, sphingolipid analysis via high performance liquid chromatography (HPLC) utilizes sphingoid bases-1-fluoro-2,4-dinitrobenzene (DNP) derivatives; however, S1P cannot be converted to a DNP derivative due to its negatively charged phosphate group [13, 21]. Consequently, we employed ESI–MS analysis for the identification of S1P biosynthesis in the engineered strain [22]. For qualitative analysis, ESI–MS was performed using S1P and sphingosine as standard preparations. As shown in Fig. 5A, the ESI–MS spectrum of S1P exhibited a characteristic peak at 202.17 m/z, distinguishing it from sphingosine.

To investigate the S1P production capability of the DDLAOGS strain, samples from fed-batch fermentation in a bioreactor were analyzed using ESI–MS. The spectra obtained from these samples confirmed the phosphorylation of sphingosine to form S1P in the DDLAOGS strain, as indicated by the distinct peak at 202.17 m/z (Fig. 5B). Additionally, the peak height increased over the fermentation period, suggesting that the engineered *S. cerevisiae* strain with the heterologous *SPHK1* gene can produce sphingosine to S1P as fermentation progresses. The successful detection of S1P in the fermentation samples validates the effectiveness of our metabolic engineering strategy, which involved integrating heterologous genes (*DES1*, *ACER1*, and *SPHK1*) to construct a biosynthetic pathway for S1P production.

S. cerevisiae is a well-characterized eukaryotic model organism with advanced genetic manipulation tools. Additionally, it offers significant advantages as a prominent chassis for the biosynthesis of lipid-derived molecules, including microbial lipids. Several studies have suggested that *S. cerevisiae* is capable of accumulating considerable amounts of sphingolipids, such as microbial ceramides [13, 23]. The primary objective of this study was to develop a platform strain for producing human

S1P. For this purpose, we selected *S. cerevisiae* as the host strain.

In an effort to further enhance S1P production, additional metabolic engineering strategies to regulate sphingolipid metabolism are necessary. The sphingolipid C4-hydroxylase enzyme, encoded by the *SUR2* gene, is critical for controlling the intracellular levels of both phytosphingosine/dihydrosphingosine and phytoceramide/dihydroceramide in *S. cerevisiae*. Therefore, deleting the *SUR2* gene may improve sphingosine levels by reducing phytosphingosine formation from dihydrosphingosine. Additionally, engineering ceramidase activity can be an effective strategy for developing S1P-producing strains. In *S. cerevisiae*, dihydroceramide is degraded into dihydrosphingosine via Ydc1, which is localized to the ER. Thus, deletion of *YDC1* gene may contribute to enhancing S1P production levels. Investigating S1P toxicity and enhancing S1P tolerance can also be powerful strategies for improving S1P production capabilities. Regarding the use of *Yarrowia lipolytica* as a host chassis for S1P production may be also an attractive strategy for improving S1P production level. In our future research, we intend to develop an S1P-producing strain based on *Y. lipolytica* to further improve S1P productivity.

Further optimization of fermentation process is another crucial factor for improving production levels. Our results indicated that sphingolipid titers were higher in a bioreactor compared to flask-level fermentation, suggesting that sphingolipid production capability can be further improved by regulating medium pH, maximizing oxygen transfer rate, optimizing agitation speed and substrate feeding strategies.

Based on qualitative analysis, we plan to develop quantitative methods for determining the concentration of S1P produced by the engineered strains. Additionally, optimizing extraction and purification methods will be essential for enhancing detection sensitivity and enabling more precise quantification of this valuable sphingolipid metabolite.

Conclusions

In this study, we successfully developed an engineered *S. cerevisiae* platform strain for microbial production of S1P by introducing a heterologous sphingolipid biosynthetic pathway and optimizing fermentation conditions. To generate sphingosine-producing strains, we integrated the sphingolipid delta 4-desaturase gene (*DES1*) and alkaline ceramidase gene (*ACER1*) from *Homo sapiens* into the genome, replacing the *DPL1* and *LCB5* ORFs, respectively. The resulting strain, DDLA, produced sphingosine as a major sphingolipid, along with dihydrosphingosine and phytosphingosine. Subsequently,

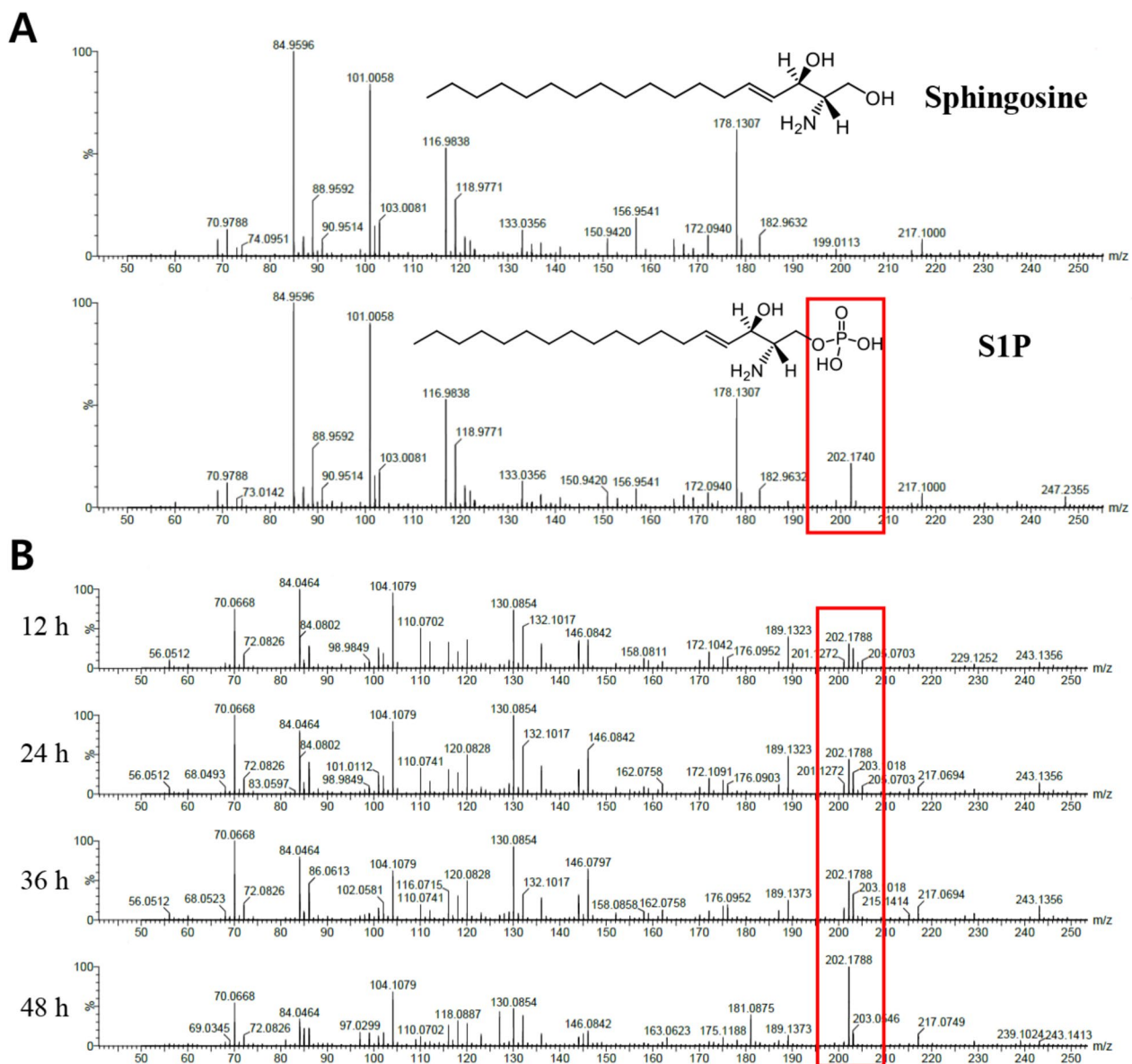


Fig. 5 Qualitative analysis of S1P production in S1P-producing strain by ESI-MS. **A** ESI-MS spectra of sphingosine (up) and S1P (down) standards. **B** ESI-MS analysis of sphingolipids produced by S1P-producing strain. Fed-batch fermentation for S1P production was conducted in bioreactor with DDLAOGS strain. S1P was detected by ESI-MS at 12 h intervals

introducing the *GAL1* promoter-controlled human sphingosine kinase gene (*SPHK1*) into the *ORM1* locus significantly improved sphingolipid production levels, validating the effectiveness of our metabolic engineering strategy employed in this study. Furthermore, we demonstrated that aerobic cultivation with high initial cell density enhances sphingolipid production in the engineered strains. The DDLAOGS strain exhibited the highest levels of sphingolipid production in fed-batch fermentation using bioreactor, achieving maximum concentrations of 431.3 mg/L sphingosine, 803.4 mg/L dihydrosphingosine,

and 207.7 mg/L phytosphingosine, respectively. Additionally, S1P biosynthesis in engineered strain was verified by ESI-MS analysis.

This study highlights the potential of producing microbial S1P in metabolically engineered yeast. Given a pivotal role of S1P in regulating anti-apoptosis in cell, microbial-derived S1P can be a valuable biomolecule for pharmaceutical and cosmetic applications, including immune cell regulation, blood vessel regeneration, skin regeneration and whitening. Moreover, this research provides insights into genetic manipulation strategies for

beneficial sphingolipid derivatives and demonstrates the feasibility of yeast-based human S1P production. To the best of our knowledge, this is the first report describing an engineered *S. cerevisiae* strain capable of producing the S1P.

Methods

Strains and media

Escherichia coli strain DH5α [F[−] Φ80*lacZ*ΔM15 Δ(*lacZYA-argF*) U169 *recA1 endA1 hsdR17* (rK[−], mK⁺) *phoA supE44 λ[−]thi-1 gyrA96 relA*] was used for plasmid construction and replication. *E. coli* was cultivated in LB medium (5 g/L yeast extract, 10 g/L tryptone, 10 g/L sodium chloride) supplemented with 100 μg/mL ampicillin. All yeast strains used in this study were derived from *S. cerevisiae* strain CEN.PK2-1D (*MATα ura3-52 trp1-289 leu2-3,112 his3Δ1 MAL2-8C SUC2*). Yeast cells were cultivated in YP medium (10 g/L yeast extract and 20 g/L peptone) supplemented with 20 or 60 g/L dextrose. To select cells containing the integrated DNA cassette with *URA3* auxotrophic marker, synthetic complete-Ura (SC-Ura) solid medium (6.7 g/L yeast nitrogen base without amino acids, 1.92 g/L yeast synthetic drop-out medium supplement without uracil, 15 g/L bacto agar, and 20 g/L dextrose) was used.

Plasmid construction

Plasmids for expressing heterologous genes were constructed by general molecular cloning. Codon optimized genes from *Homo sapiens*, *DES1* (sphingolipid delta 4 desaturase), *ACER1* (alkaline ceramidase 1), and *SPHK1* (sphingosine kinase 1), were synthesized by Cosmogenetech (Korea). To generate the gene integration cassette, each gene was amplified from synthetic DNA fragment by polymerase chain reaction (PCR), and

then cloned between *Xba*I and *Xho*I restriction sites of pUC-URA3 plasmid as a backbone vector. The resulting plasmids, pUC-URA3-P_{TDH3}-*DES1*, pUC-URA3-P_{TDH3}-*ACER1*, and pUC-URA3-P_{TDH3}-*SPHK1*, were isolated from the transformants and confirmed by DNA sequencing, respectively. Plasmids for expressing *SPHK1* gene via inducible promoter were constructed as follow. Both inducible promoters, *GAL1* (galactokinase 1) and *SUC2* (β-fructofuranosidase 2), were prepared by PCR amplification from the genomic DNA of CEN.PK2-1D strain. Each PCR product was cloned into the *Xba*I and *Sac*I restriction sites of pUC-URA3-P_{TDH3}-*SPHK1* plasmid to replace the *TDH3* promoter, resulting in pUC-URA3-P_{GAL1}-*SPHK1* and pUC-URA3-P_{SUC2}-*SPHK1*, respectively. All plasmids used in this study are described in Table 1.

Construction of engineered *S. cerevisiae* strains

Various genetically manipulated strains were generated based on PCR-mediated homologous recombination method. To construct integration strains, gene-specific integration cassettes were amplified from each plasmid containing the required gene using target-specific primer pairs, and then introduced into *S. cerevisiae* strains via general transformation protocol. After identifying the correct genomic integration of the DNA cassette by colony PCR, the *URA3* auxotrophic marker was excised subsequently by incubating the strain on SC-Ura solid medium containing 1 g/L of 5-fluoroorotic acid for 3 days. The genetically manipulated strains and their genotypes are listed in Table 1.

Culture conditions

To perform fed-batch fermentation in flask, each *S. cerevisiae* strain was inoculated into 20 mL of YPD medium

Table 1 Yeast strains and plasmids used in this study

Strain/Plasmid	Genotype and description	Reference
<i>S. cerevisiae</i> strains		
CEN.PK2-1D	<i>MATα ura-352 trp-1289 leu2-3, 112 his3Δ1 MAL2-8^C SUC2</i>	EUROSCARF
DD	CEN.PK2-1D <i>dpl1Δ::P_{TDH3}-DES1-T_{CYC1}</i>	This study
DDLA	DD <i>lcb5Δ::P_{TDH3}-ACER1-T_{CYC1}</i>	This study
DDLAOGS	DDLA <i>orm1Δ::P_{GAL1}-SPHK1-T_{CYC1}</i>	This study
DDLAOS	DDLA <i>orm1Δ::P_{SUC2}-SPHK1-T_{CYC1}</i>	This study
Plasmids		
pUC-URA3	pUC57 plasmid containing the recyclable hisG-URA3-hisG fragment	[24]
pUC-URA3-P _{TDH3} - <i>DES1</i>	Plasmid containing P _{TDH3} - <i>DES1</i> -T _{CYC1} integration cassette	This study
pUC-URA3-P _{TDH3} - <i>ACER1</i>	Plasmid containing P _{TDH3} - <i>ACER1</i> -T _{CYC1} integration cassette	This study
pUC-URA3-P _{TDH3} - <i>SPHK1</i>	Plasmid containing P _{TDH3} - <i>SPHK1</i> -T _{CYC1} integration cassette	This study
pUC-URA3-P _{GAL1} - <i>SPHK1</i>	Plasmid containing P _{GAL1} - <i>SPHK1</i> -T _{CYC1} integration cassette	This study
pUC-URA3-P _{SUC2} - <i>SPHK1</i>	Plasmid containing P _{SUC2} - <i>SPHK1</i> -T _{CYC1} integration cassette	This study

and cultivated overnight on a 100 mL baffled flask at 30 °C with shaking at 225 rpm. 1% (v/v) of the pre-cultured medium was transferred into 400 mL of YPD medium in a 2 L baffled flask and cultivated at 30 °C on a shaking incubator (225 rpm) for 24 h to achieve high-density cells. An initial cell density of 0.5 or 5 cells were inoculated into 100 mL of YPD medium containing 20 or 60 g/L dextrose in a 500 mL baffled flask and cultivated for 72 h at 30 °C with shaking at 225 rpm. The feeding solution (30 g/L dextrose or 30 g/L sucrose or 30 g/L galactose) was added to the culture medium at 12 h intervals.

Fed-batch fermentation in bioreactor was performed in 3 L YDP medium containing 60 g/L dextrose using a 10 L fermenter (Sartorius, Germany). Inoculated cells were cultivated in 100 mL of YPD medium in a 500 mL baffled flask at 30 °C with shaking at 225 rpm for 24 h. 1% (v/v) of the pre-cultured cells were transferred into 3 of YPD medium in a 5 L fermenter and incubated for 24 h to achieve high-density cells, and then cell density of 5 cells was transferred and cultivated in 3 L of YPD medium supplemented 60 g/L dextrose for 60 h at 30 °C with agitation speed of 225 rpm and air flow rate of 1.0 vvm. When initial glucose was exhausted, the feeding solution containing 600 g/L dextrose or galactose was continuously added.

Analytical methods

Cell growth was monitored by measuring optical density at 600 nm using spectrophotometer (Thermo fisher scientific, USA). For quantitation of dihydrosphingosine, phytosphingosine, and sphingosine, 10 mL of culture medium was harvested and resuspended in 5 mL of methanol with 2 g of glass beads. Cell homogenization was vigorously carried out by bead beating at 6.0 m/sec of speed for 40 s through 18 cycles. After addition of 5 mL of chloroform, the organic solvent layer was collected by centrifugation. The sphingolipid extract was treated with 5 µL of DNP and 4 mL of 2 M potassium borate buffer (pH 10.5) for 30 min at 60 °C, and then sphingolipid-DNP derivatives were prepared by adding 6 mL solvent mixture containing chloroform, methanol, and deionized water (8:4:3) and collecting the organic solvent layer. The samples were analyzed by HPLC using an Waters 2695 system equipped with ODS Hypersil RP-C18 column (Thermo electron, 4.6 × 250 mm, 5 µm) and UV detector (wavelength 350 nm). Two mobile phases, deionized water (A) and methanol/acetonitrile/isopropanol (B, 10:3:1, v/v), were run at a flow rate 1.0 mL/min with a linear gradient of mobile phase B from 80 to 90% for 40 min.

For qualitative analysis of intracellular sphingosine-1-phosphate, the sphingolipid extract from cultured cells were filtered through a 0.22 µm syringe filter, and

qualified by ESI-MS using CHEMCOBOND 5-ODS-H column (ChemcoPak, 5 µm, 50 × 2.1 mm) and UV detector (wavelength 254 nm). The column was eluted with 0.1% formic acid in methanol as a flow rate of 0.5 mL/min with isocratic condition.

Abbreviations

DNP	1-Fluoro-2,4-dinitrobenzene
ER	Endoplasmic reticulum
ESI-MS	Electrospray ionization mass spectrometry
GIPC	Glycosyl inositol phosphor ceramide
HPLC	High performance liquid chromatography
LB	Luria-Bertani
PCR	Polymerase chain reaction
S1P	Sphingosine-1-phosphate
YP	Yeast extract-peptone
YPD	Yeast extract-peptone-dextrose

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12934-024-02579-8>.

Supplementary Material 1.

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Author contributions

BJY, SHB, and YSB participated in the design of this study. ISJ and SJL performed the experiments including the construction of strains and the data analysis. YSB and SHB participated in the interpretation of data. ISJ and SHB wrote the manuscript. BJY and YSB participated in review and editing. All authors have read and approved the final manuscript.

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Availability of data and materials

The datasets used in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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