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Antifungal Mechanism of Action of Lauryl Betaine Against Skin-Associated Fungus *Malassezia restricta*

Eunsoo Do[a]*, Hyun Gee Lee[b]* (iD), Minji Park[a], Yong-Joon Cho[c] (iD), Dong Hyeun Kim[a] (iD), Se-Ho Park[a], Daekyung Eun[b], Taehun Park[b], Susun An[b] and Won Hee Jung[a] (iD)

[a]Department of Systems Biotechnology, Chung-Ang University, Anseong, Korea; [b]Safety Research Institute, Amorepacific R&D Center, Yongin, Korea; [c]Korea Polar Research Institute, Incheon, Korea

ABSTRACT

Betaine derivatives are considered major ingredients of shampoos and are commonly used as antistatic and viscosity-increasing agents. Several studies have also suggested that betaine derivatives can be used as antimicrobial agents. However, the antifungal activity and mechanism of action of betaine derivatives have not yet been fully understood. In this study, we investigated the antifungal activity of six betaine derivatives against *Malassezia restricta*, which is the most frequently isolated fungus from the human skin and is implicated in the development of dandruff. We found that, among the six betaine derivatives, lauryl betaine showed the most potent antifungal activity. The mechanism of action of lauryl betaine was studied mainly using another phylogenetically close model fungal organism, *Cryptococcus neoformans*, because of a lack of available genetic manipulation and functional genomics tools for *M. restricta*. Our genome-wide reverse genetic screening method using the *C. neoformans* gene deletion mutant library showed that the mutants with mutations in genes for cell membrane synthesis and integrity, particularly ergosterol synthesis, are highly sensitive to lauryl betaine. Furthermore, transcriptome changes in both *C. neoformans* and *M. restricta* cells grown in the presence of lauryl betaine were analyzed and the results indicated that the compound mainly affected cell membrane synthesis, particularly ergosterol synthesis. Overall, our data demonstrated that lauryl betaine influences ergosterol synthesis in *C. neoformans* and that the compound exerts a similar mechanism of action on *M. restricta*.

1. Introduction

Betaine (trimethylglycine) is a natural product derived from sugar beet (*Beta vulgaris*), which is common in human diet. It is required for several physiological processes, such as cellular detoxification of homocysteine and adaptation to osmotic and ionic stresses by serving as an osmoprotectant [1–3]. In cosmetic products such as shampoo, eleven alkyl betaines are commonly used as antistatic and viscosity-increasing agents [4]. Furthermore, the antibacterial activity of betaine against *Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus* have been reported [5–8]. Although numerous biological activities of betaine and its derivatives have been reported, its antifungal activity has not yet been studied in detail, especially against the fungi involved in various skin conditions.

Malassezia is a dominant fungal genus on the human skin surface and is considered as a major causative agent for skin diseases such as dandruff,

seborrheic dermatitis, atopic dermatitis, and pityriasis versicolor [9–11]. Among the 17 known species of *Malassezia*, *M. restricta* is well-recognized as the predominant species on human skin [10–13], particularly being associated with dandruff in a number of studies. A recent large-scale microbiome analysis demonstrated that the increased abundance of *M. restricta* in the scalp was significantly correlated with dandruff [11,13,14].

Here, we evaluated the possible anti-dandruff function of betaine derivatives by assessing their antifungal activity against *M. restricta*. The antifungal mechanism of action of the betaine derivatives was also studied. We used *Cryptococcus neoformans*, which is a basidiomycetous yeast like *Malassezia*, as a model organism because genetic manipulation and functional genomics tools have still not yet been developed for *M. restricta*. The genome of *C. neoformans* has been well-annotated and genetic manipulation and functional genomics tools for this species, such as whole gene deletion libraries are readily

available. Taking full advantage of *C. neoformans* as the model fungal organism, we performed a phenotypic assay using gene deletion mutant libraries to understand the mechanism of action of the betaine derivatives. Transcriptome analyses were also performed to support the data obtained from phenotypic screening of the *C. neoformans* gene deletion libraries. Our data demonstrated that lauryl betaine, one of the betaine derivatives tested in the current study, effectively inhibited the growth of both *C. neoformans* and *M. restricta*, mainly by influencing membrane synthesis in the fungi.

2. Materials and methods

2.1. Strains and growth media

C. neoformans var. *grubii* H99 and *M. restricta* KCTC 27527 strains were used in this study [15,16]. *C. neoformans* mutant strains used are listed in the Supplementary Table S1. *C. neoformans* strains were cultured in yeast extract-peptone- dextrose (YPD) medium (1% yeast extract, 2% bacto-peptone, and 2% glucose) at 30 °C and *M. restricta* strain was cultured in Leeming and Notman agar (LNA) at 34 °C as previously described [17]. To evaluate the antifungal sensitivity against betaine derivatives, *C. neoformans* strains were grown in YPD medium overnight, 10-fold serial dilutions of cell suspensions (starting at 10^5 cells) were spotted onto the YPD medium containing the compound and incubation was performed at 30 °C for 2 days.

2.2. Construction of the mutant strain

To construct the *sre1* mutant, the gene-specific knock-out (KO) cassette was prepared by overlapping polymerase chain reaction (PCR) using primers listed in Supplementary Table S2 with the wild-type genomic DNA and the plasmid pCH233 as templates. The constructed KO cassette was introduced into the wild-type strain by biolistic transformation as previously described [18]. Replacement of the wild-type *SRE1* coding region with the KO cassette containing the nourseothricin acetyltransferase (*NAT*) gene in the *sre1* mutant was confirmed by PCR. The deletion of *SRE1* in the mutant was also confirmed by Southern blot analysis using the genomic DNA samples digested with *Hind*III and *Spe*I restriction enzymes (Supplementary Figure S1). The digested DNA fragments were separated in an agarose gel and were transferred to an UltraBind transfer membrane (Pall-Gelman Laboratory, Washington, NY). The gene-specific probe was amplified by PCR from the wild-type genomic DNA using the primers Sre1probe_F and Sre1probe_R listed and labeled with phosphorus32-deoxycytidine

triphosphate ([^{32}P]-dCTP). The membrane was hybridized with the probe, exposed to a phosphor screen (PerkinElmer, Waltham, MA) overnight and scanned using a Packard cyclone phosphor imager (PerkinElmer).

2.3. Determination of minimum inhibitory concentration against betaine derivatives

Minimum inhibitory concentration (MIC) was determined to evaluate the sensitivity of *C. neoformans* and *M. restricta* KCTC 27527 to betaine derivatives. A standard broth serial dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines was used [19]. For *C. neoformans*, the final concentrations of the betaine derivatives and fluconazole ranged from 0.28 to 288 μg/mL and from 0.125 to 128 μg/mL, respectively. Fluconazole was used as a reference antifungal drug. For *M. restricta* KCTC 27527, MIC was determined using the method suggested by Sugita et al. [20] with slight modification. Briefly, compounds were diluted in 980 μL of melted LNA medium, resulting in final concentrations ranging from 0.375 to 6 mg/mL. *Malassezia* cells were incubated for 3 days at 34 °C and then the MIC was determined.

2.4. Screening of the C. neoformans gene deletion library

To identify the mutants that show increased sensitivity to lauryl betaine, we performed a phenotypic screen for two sets of *C. neoformans* gene deletion libraries, designated UCSF-2015 and UCSF-2016, which were generated by Dr. Hiten Madhani's group at the University of California, San Francisco [21]. The mutants were grown in YPD medium using 96-well microtiter plates at 30 °C for 3 days and 10-fold serial dilutions were spotted onto YPD medium containing 15 μg/mL of lauryl betaine. The plates were incubated at 30 °C for 2 days and then photographed. This experiment was performed in triplicate.

2.5. Quantitative real-time PCR

Total RNA was extracted using TransZolUp (Transgen Biotech, Beijing, China) and the complementary DNA (cDNA) was synthesized using the RevertAid First Strand cDNA synthesis Kit (Thermo Fisher Scientific, Waltham, MA) following the manufacturer's instructions. Primers for qRT-PCR was designed using Primer Express software 3.0 (Applied Biosystems, Foster, CA) and are listed in Supplementary Table S3. Relative quantitation of gene expression was performed using the $2^{-\Delta\Delta CT}$

method with the 7500 system (Applied Biosystems) [22]. The expression levels of *TEF2* and *ACT1* were used as internal controls for *C. neoformans* and *M. restricta*, respectively.

2.6. RNA sequencing and transcriptome data analysis

C. neoformans strains were grown in YPD overnight and 5×10^7 cells were transferred to fresh YPD medium containing lauryl betaine. The cells were incubated at 30 °C for 3 h and harvested for RNA extraction. *M. restricta* KCTC 27527 cells (1.0×10^8 CFU/mL) were cultured in the presence or absence of 3 mg/mL lauryl betaine, incubated at 34 °C for 12 h and were harvested for RNA extraction. Total RNA was extracted using RiboPure Yeast RNA extraction kit (Ambion, Foster, CA) and the RNA integrity was evaluated using BioAnalyzer 2100 (Agilent Technologies, Palo Alto, CA). Libraries for RNA sequencing were constructed using the TruSeq Stranded Total RNA Sample Prep Kit (Cat. RS-122-2201; Illumina, San Diego, CA) following the manufacturer's instructions. The libraries were then sequenced by the Illumina HiSeq 2500 instrument (Illumina) following the manufacturer's instructions and 75-bp paired-end reads were generated. Raw sequences were subjected to adapter sequence removal and quality-based trimming using Trimmomatic v0.36 with default parameters [23]. Cleaned reads were mapped to the reference genome by bowtie2 with the "–very-sensitive" option [24]. FeatureCounts in the Subread package was used to count the reads mapped to each coding sequence [25]. Finally, the counts from each coding sequence (CDS) were normalized to fragments per kilobase million (FPKM) and transcripts per million (TPM) values [26]. The transcriptome data have been deposited to the Gene Expression Omnibus database of NCBI under accession no. GSE124734.

3. Results and discussion

3.1. Antifungal activity of betaine derivatives

Antifungal activity of betaine derivatives against *M. restricta* was determined using the modified broth serial dilution method [20] and the sensitivity of the fungus to each compound was presented as the MIC (see Section 2). *M. restricta* was chosen due to its association with dandruff. Six betaine derivatives, namely capryl/capramidopropyl betaine, coco-betaine, cetyl betaine, oleyl betaine, lauryl betaine, and cocamidopropyl betaine, were tested (Table 1). It should be noted that the lauryl betaine used in the current study was mixed with myristyl betaine, which did not show any antifungal activity (data not

shown), at a 2:1 ratio to increase solubility. Among the betaine derivatives tested, cocamidopropyl betaine showed the strongest antifungal activity (MIC of 0.075–1.5 mg/mL), followed by lauryl betaine (MIC of 1.5–3 mg/mL) (Table 2). Although cocamidopropyl betaine showed the lowest MIC against *M. restricta*, the compound was excluded in our study because of its cytotoxicity and allergenic property according to the American Contact Dermatitis Society [27,28]. Instead, we selected lauryl betaine as the best betaine derivative possessing the strongest antifungal activity against *M. restricta*.

Genome-wide reverse genetics using a gene deletion mutant library has been successfully used to study a number of physiological responses of fungi against environmental stimuli, including antifungal drugs. Indeed, identification of mutants through the screening of a gene deletion library that displayed altered fitness against antifungal drugs has provided solid evidence for the connection between a gene and a drug in *Saccharomyces cerevisiae* and *Candida albicans* [29–31]. However, despite the attention that has been paid to *M. restricta* because of its association with several skin diseases, no genetic manipulation tool has been developed for this species. Therefore, the direct molecular genetic approach was not possible to study the mechanism of action of the antifungal drug against *M. restricta*. Alternatively, we utilized *C. neoformans*, a human fungal pathogen classified under the same fungal phylum as *Malassezia* species (Basidiomycota) and took advantage of the well-developed genetic manipulation tools and gene deletion libraries available for this fungus to understand the antifungal mechanism of action of lauryl betaine. We determined the antifungal activity of lauryl betaine against *C. neoformans* and found that the MIC of the compound was 4.5 μg/mL, which was lower than that of fluconazole (16 μg/mL; Table 3). This result suggested that *C. neoformans* is highly susceptible to lauryl betaine and that this species can be used as the model fungal organism to study the mechanism of action of the compound against *M. restricta*.

3.2. Systematic screenings of gene deletion mutant libraries revealed that lauryl betaine inhibits cell membrane synthesis and integrity

To understand the mechanism of action of lauryl betaine, we screened two sets of gene deletion libraries of *C. neoformans*, designated UCSF-2015 and UCSF-2016, which were generated by Dr. Hiten Madhani's group [21]. *C. neoformans* gene deletion mutants were spotted on media containing lauryl betaine and their growth was monitored; a total of 77 mutants displayed significant growth defects in

Table 1. Betaine derivatives used in this study.

Betaine derivatives	Commercial name (manufacturer)	Chain length
Capryl/capramidopropyl betaine	Tego Betain 810 (The Evonik Industries Co.)	8
Coco-betaine	Chembetaine ACB Surfactant (The Lubrizol Co.)	12 − 18
Cetyl betaine	CDB Special (Stepan Co.)	16
Oleyl betaine	Chembetaine OL-30 Surfactant (The Lubrizol Co.)	18
Lauryl betaine	Chembetaine BW Surfactant (The Lubrizol Co.)	12 − 14
Cocamidopropyl betaine	Mitaine CA (Miwon Commercial Co.)	12 − 18

Table 2. Antifungal activity of betaine derivatives against *M. restricta* KCTC 27527.

M. restricta KCTC 27527	MIC (mg/mL)
Capryl/capramidopropyl betaine	>6
Coco-betaine	6
Cetyl betaine	6
Oleyl betaine	6
Lauryl betaine	1.5–3
Cocamidopropyl betaine	0.75–1.5

Table 3. Antifungal activity of lauryl betaine and fluconazole against *C. neoformans*.

C. neoformans H99	MIC (μg/mL)
Lauryl betaine	4.5
Fluconazole	16

the medium containing lauryl betaine (Figure 1(A) and Table 4). Interestingly, we found that the mutants lacking *CFO1* (CNAG_06241), *CFT1* (CNAG_06242), and *FRE4* (CNAG_07334), which encode ferroxidase, iron permease, and ferric reductase, respectively, showed growth defects in the presence of lauryl betaine. In *C. neoformans*, these genes play important roles not only in the high-affinity reductive iron uptake, but also in cell membrane synthesis, particularly ergosterol synthesis, because numerous enzymes involved in membrane synthesis require iron as a cofactor. Indeed, previous studies have shown that the *C. neoformans* mutants lacking *CFO1*, *CFT1*, or *FRE4* were more sensitive to the azole antifungal drug fluconazole compared to the wild type, mainly due to a deficiency in ergosterol synthesis [32–34]. To confirm these results, we challenged the independently constructed *cfo1*, *cft1*, and *fre4* mutants, which were used in our previous studies [32–34], with lauryl betaine and observed the same phenotypes for the mutants (Figure 1(B)).

Ergosterol is the main component of the fungal cell membrane and the deficiency of its synthesis can disrupt cell membrane integrity. Moreover, deletion of many gene required for ergosterol synthesis is known to be lethal [35]. Interestingly, in addition to the aforementioned genes, the gene deletion mutant lacking *SRE1* (CNAG_04804), which is the gene encoding the sterol response element-binding protein (SREBP), showed increased sensitivity to lauryl betaine. In fungi, SREBP is a major regulatory protein that controls ergosterol synthesis and homeostasis, and it is well-known that the *C. neoformans* mutant lacking *SRE1* is hypersensitive to azole antifungal drugs that inhibit ergosterol synthesis [36,37]. To

confirm our observation, we separately constructed a *sre1* mutant and evaluated its sensitivity to lauryl betaine (Supplementary Figure S1). As expected, the *sre1* mutant generated in the current study also showed increased sensitivity to lauryl betaine compared to the wild type, further suggesting that lauryl betaine inhibits ergosterol synthesis (Figure 1(C)).

3.3. Lauryl betaine caused global changes in the transcriptome of the fungal cells

In addition to utilizing a gene deletion mutant library, analysis of antifungal drug-induced transcriptome changes can provide useful information for understanding the mechanism of action of the drug [38–40]. Therefore, we analyzed the transcriptome of *C. neoformans* grown in medium containing lauryl betaine compared with that of cells grown in the absence of the drug. To extract total RNA, the *C. neoformans* cells were cultured in the medium containing 4.5 μg/mL of lauryl betaine, which was the MIC determined. We also included the total RNA from the fungal cells grown in the presence of 9 μg/mL of lauryl betaine to observe any concentration-dependent expression changes. Transcriptome analysis was also applied to *M. restricta* cells to investigate the mechanism of action of lauryl betaine on the fungus directly. The *M. restricta* cells were grown in the medium containing lauryl betaine (3 mg/mL) and their transcriptome was compared with that of the cells grown in the medium without the drug (see Section 2).

The results of our transcriptome analysis suggested that the expression levels of a significant number of genes in *C. neoformans* were altered by treatment with lauryl betaine. With a fold-change cut-off of 1.5-fold, 1791 genes were up-regulated and 194 genes were down-regulated in the cells grown in the presence of 4.5 μg/mL lauryl betaine, while 3067 genes were up-regulated and 154 genes were down-regulated in the cells grown in the presence of 9 μg/mL lauryl betaine, suggesting that the drug triggers global transcriptomic changes in *C. neoformans* (Supplementary Table S4). In contrast, comparisons of the transcriptomes of the *M. restricta* cells grown in the presence or absence of lauryl betaine showed that 44 genes were up-regulated and 299 genes were down-regulated, and that the total number of differentially expressed genes

Table 4. List of genes identified from the screening of the *C. neoformans* gene deletion library.

Gene ID	Gene name	Product description
CNAG_00248	VPS36	ESCRT-II complex subunit VPS36
CNAG_00368	–	Vacuolar-sorting protein 53 long isoform
CNAG_00561	–	Histone acetyltransferase type B catalytic subunit
CNAG_00609	–	Hypothetical protein
CNAG_00673	–	Cytoplasmic protein
CNAG_00760	–	Methylenetetrahydrofolate reductase
CNAG_00977	–	VHS domain-containing protein
CNAG_01309	–	arf/Sar family protein
CNAG_01399	–	Hypothetical protein
CNAG_01556	–	Cytoplasmic protein
CNAG_01923	–	ATPase GET3
CNAG_02007	ADK1	Adenylate kinase 1
CNAG_02029	WSP1	Wiskott-Aldrich syndrome protein
CNAG_02270	MET2	Homoserine O-acetyltransferase
CNAG_02313	–	Hypothetical protein, hypothetical protein, variant
CNAG_02568	–	UBA/TS-N domain-containing protein
CNAG_02702	CLC1	Putative voltage-gated chloride channel
CNAG_02795	–	Phosphoribosyl glycinamide formyltransferase
CNAG_02826	–	Mitochondrial amino-acid acetyltransferase
CNAG_02905	–	Hypothetical protein
CNAG_03235	–	THO complex subunit 1
CNAG_03269	–	Aldehyde dehydrogenase
CNAG_03325	–	ChAPs family protein
CNAG_03333	–	Cytoplasmic protein
CNAG_03348	–	Hypothetical protein
CNAG_03370	–	Calcium-binding protein NCS-1
CNAG_03380	–	Hypothetical protein
CNAG_03528	–	AP-2 complex subunit alpha
CNAG_04174	PEX6	Peroxin-6
CNAG_04388	SOD2	Mitochondrial manganese superoxide dismutase
CNAG_04450	–	Chromodomain-helicase-DNA-binding protein 1
CNAG_04678	YPK1	Protein kinase
CNAG_04751	–	Hypothetical protein
CNAG_04804	SRE1	Sterol regulatory element-binding protein
CNAG_04863	VPS25	ESCRT-II complex subunit
CNAG_04904	–	Clathrin heavy chain
CNAG_05071	–	Sulfite reductase (NADPH) hemoprotein beta-component
CNAG_05074	–	Hypothetical protein
CNAG_05122	–	Homoserine O-acetyltransferase
CNAG_05282	APT4	Phospholipid-translocating ATPase
CNAG_05512	–	Hypothetical protein
CNAG_05515	–	Hypothetical protein
CNAG_05560	–	–
CNAG_05579	–	Hypothetical protein
CNAG_05581	CHS3	Putative chitin synthase
CNAG_05643	–	DNA polymerase delta subunit 4
CNAG_05704	VPS22	ESCRT-II complex subunit VPS22
CNAG_05721	MFE2	Multifunctional beta-oxidation protein
CNAG_05837	–	Hypothetical protein
CNAG_05839	–	Cytochrome c oxidase subunit 6b, cytochrome c oxidase subunit 6b, variant
CNAG_05899	–	Pyrroline-5-carboxylate reductase
CNAG_06078	–	Hypothetical protein
CNAG_06080	–	Inositol/phosphatidylinositol phosphatase
CNAG_06156	FZC7	Hypothetical protein, hypothetical protein, variant
CNAG_06224	MLN2	Nuclear movement protein nudC
CNAG_06241	CFO1	Ferroxidase/laccase
CNAG_06242	CFT1	Major iron permease
CNAG_06334	–	Hypothetical protein
CNAG_06383	–	Cytoplasmic protein
CNAG_06507	–	Hypothetical protein
CNAG_06511	–	Hypothetical protein
CNAG_06568	SKS1	RAN protein kinase
CNAG_06631	–	Myosin heavy chain
CNAG_06672	–	Formate dehydrogenase
CNAG_06731	–	Hypothetical protein
CNAG_06792	–	Hypothetical protein
CNAG_06910	–	Beta-lactamase
CNAG_07334	FRE4	Ferric-chelate reductase
CNAG_07362	–	Nucleolin
CNAG_07373	–	Carbamoyl-phosphate synthase, large subunit
CNAG_07414	PAN6	Pantoate-beta-alanine ligase
CNAG_07600	–	Beta-glucosidase
CNAG_07636	CSR2	Putative chitin synthase regulator
CNAG_07643	–	Hypothetical protein
CNAG_07647	–	Voltage-gated chloride channel protein
CNAG_07709	–	Hypothetical protein
CNAG_07733	–	Hypothetical protein

in *M. restricta* were less than that in *C. neoformans* (Supplementary Table S5). We speculated that the thick cell wall and membrane of *M. restricta*, as well as its capability for biofilm formation, might have contributed to its less susceptible phenotype and to the observed changes in the transcriptome of the fungus in response to lauryl betaine [41].

3.4. Fungal cell membrane synthesis is the main target of lauryl betaine

Previous studies have suggested that the genes involved in the possible target pathway of an antifungal drug are normally upregulated in a compensatory response to the drug [38,39]. Therefore, particular attention was paid to the genes that were upregulated upon treatment with lauryl betaine in our transcriptomic data. Moreover, among the upregulated genes in *C. neoformans*, the genes showing dose-dependent changes in the cells grown in the presence of lauryl betaine were included for further analysis. The differential expression of a total of 1430 genes in *C. neoformans* met our selection criteria and the results of the analysis of functional categories suggested that the genes involved in lipid, fatty acid, and isoprenoid metabolism were the most
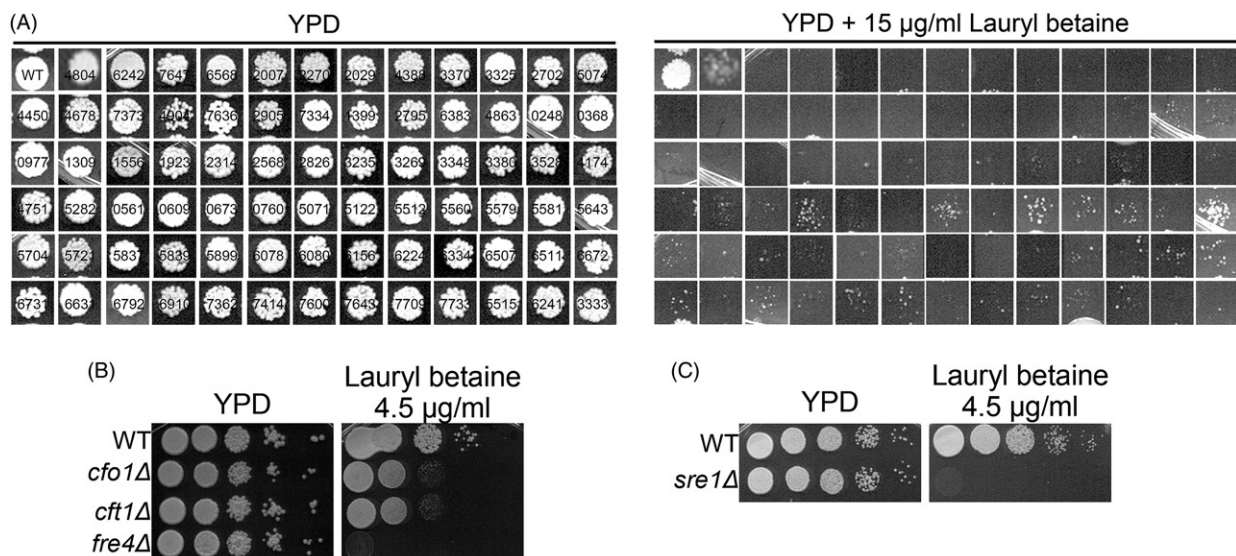

Figure 1. Phenotypic screening of the *C. neoformans* gene deletion library. (A) *C. neoformans* gene deletion mutants that showed increased sensitivity to lauryl betaine. The numbers indicate the last four digits of the gene ID of *C. neoformans* var. *grubii* H99; (B) Confirmation of increased sensitivity of the mutants lacking genes involved in iron uptake to lauryl betaine. Ten-fold serial dilutions of cells (starting at 10^5 cells) were spotted onto the plates and incubated at 30 °C for 2 days; (C) Sensitivity of the mutant lacking *SRE1* was monitored in the medium containing lauryl betaine. Ten-fold serial dilutions of cells (starting at 10^5 cells) were spotted onto the plates and incubated at 30 °C for 2 days.
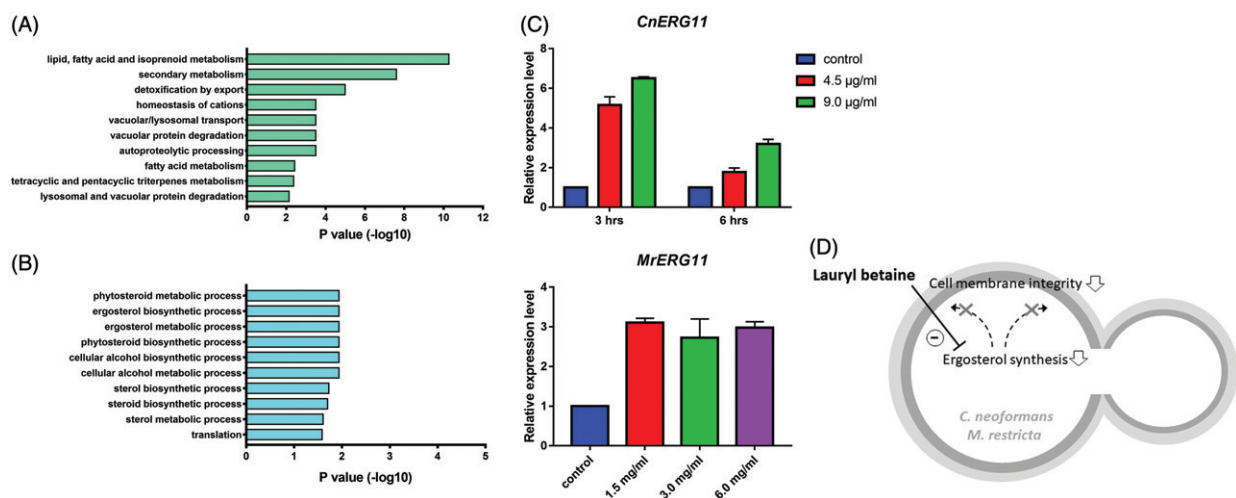

Figure 2. Transcriptome analysis upon treatment of lauryl betaine. (A) Analysis of functional categories of the genes showing differential expression in *C. neoformans* grown in the medium containing lauryl betaine. The analysis was performed using FunCat from the web server FungiFun2; (B) Analysis of functional categories of the genes showing differential expression in *M. restricta* grown in the medium containing lauryl betaine. The analysis was performed using GO-term (biological process); (C) The transcript levels of the *ERG11* homologs were measured using qRT-PCR. Data were normalized against either *TEF2* or *ACT1* for *C. neoformans* and *M. restricta*, respectively; (D) The suggested mechanism of action of lauryl betaine.

significantly influenced by lauryl betaine (Figure 2(A)). These results agreed with the data obtained from screening the gene deletion libraries as the genes required for ergosterol synthesis belong to the functional category of lipid, fatty acid, and isoprenoid metabolism, thus confirming that cell membrane synthesis and integrity is the main target of lauryl betaine. Similarly, we investigated which functional categories are enriched among the differentially expressed genes in the transcriptomic data of *M. restricta* and found that the genes involved in ergosterol biosynthetic and metabolic processes were highly enriched (Figure 2(B)). To further confirm that ergosterol synthesis is the main target of lauryl betaine in both *C. neoformans* and *M. restricta*, we separately analyzed the transcript levels of homologs of *ERG11*, which encodes lanosterol 14-alpha-demethylase and is the major gene in the ergosterol synthesis pathway, in the fungal cells grown in the presence or absence of lauryl betaine using qRT-PCR. The results showed that the transcript levels were highly increased in the cells grown in the presence of lauryl betaine, supporting our findings (Figure 2(C)).

Lauryl betaine is a betaine derivative that is widely used in personal hygiene products such as shampoos because of its low irritation property to the skin and eyes [4]. The present study showed that lauryl betaine possesses a strong inhibitory effect on *M. restricta*, which is the causative fungal agent for dandruff. Furthermore, as shown in Figure 2(D), our study revealed that lauryl betaine mainly inhibits membrane synthesis, particularly ergosterol synthesis, in the fungal cell as established using *C. neoformans* as the model system.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Hyun Gee Lee http://orcid.org/0000-0003-0122-0972
Yong-Joon Cho http://orcid.org/0000-0003-3191-2670
Dong Hyeun Kim http://orcid.org/0000-0003-4641-8448
Won Hee Jung http://orcid.org/0000-0002-6920-0233

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