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

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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.

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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 10⁵ 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 HindIII and SpeI 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 ([32P]-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 $2^{-ΔΔCT}$
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 $5 \times 10^7$ 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 ($1.0 \times 10^8$ 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
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

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**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.**

| Betaine derivatives | 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 theazole 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].

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**MYCOBIOLOGY**

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| 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 II catalytic subunit                                    |
| CNAG_00609 | –         | Hypothetical protein                                                                    |
| CNAG_00673 | –         | Cytoplasmic protein                                                                    |
| CNAG_00760 | –         | Methyltetrahydrofolate reductase                                                       |
| CNAG_00977 | –         | VHS domain-containing protein                                                          |
| CNAG_01309 | –         | arf/Sar family protein                                                                  |
| CNAG_01407 | –         | 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 |–         | Hypothetical protein                                                                   |
| CNAG_02652 | 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_03604 | SRE1      | Sterol regulatory element-binding protein                                              |
| CNAG_03683 | VPS25     | ESCRT-II complex subunit VPS25                                                         |
| CNAG_04090 | –         | Clathrin heavy chain                                                                   |
| CNAG_05071 | –         | Sulfitase reductase (NADPH) hemoprotein beta-component                                   |
| CNAG_05074 | –         | Hypothetical protein                                                                   |
| CNAG_05122 | –         | Homoserine O-acetyltransferase                                                         |
| CNAG_05282 | APT4      | Phospholipid-translocating ATPase                                                      |
| CNAG_05515 | –         | Hypothetical protein                                                                   |
| CNAG_05550 | –         | Hypothetical protein                                                                   |
| CNAG_05591 | CHS3      | Putative chitin synthase                                                                |
| CNAG_05643 | –         | DNA polymerase delta subunit 4                                                         |
| CNAG_05704 | VPS25     | ESCRT-II complex subunit VPS25                                                         |
| 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 | F2C7      | Hypothetical protein, hypothetical protein, variant                                    |
| CNAG_06224 | MUN2      | 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_07750 | –         | Hypothetical protein                                                                   |
| CNAG_07773 | –         | 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](image1.png)

**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 10⁵ 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 10⁵ cells) were spotted onto the plates and incubated at 30°C for 2 days.

![Figure 2](image2.png)

**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.

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