Disruption of the sphingolipid $\Delta^8$-desaturase gene causes a delay in morphological changes in Candida albicans

Takahiro Oura and Susumu Kajiwara

Ceramides and glycosylceramides, including desaturated long-chain bases, are present in most fungi as well as animals and plants. However, as the budding yeast Saccharomyces cerevisiae is not capable of desaturating long-chain bases, little is known about the physiological roles of these compounds in fungi. To investigate the necessity of desaturation of long-chain backbones in ceramides and glucosylceramides in fungal cells, we have identified and characterized a sphingolipid $\Delta^8$-desaturase (SLD) gene from the pathogenic yeast Candida albicans. Gene disruption of the C. albicans SLD homologue led to the accumulation of (E)-sphing-4-enine, a main substrate for the SLD enzyme. Introducing the Candida SLD gene homologue into these mutant cells resulted in the synthesis of (4E, 8E)-sphinga-4,8-diene and this gene homologue was therefore identified as a Ca-SLD gene. Additionally, the sld disruptant of C. albicans had a decreased hyphal growth rate compared with the wild-type strain. These results suggest that $\Delta^8$-desaturation of long-chain bases in ceramides plays a role in the morphogenesis of C. albicans.

INTRODUCTION

Sphingolipids are abundant compounds in the cells of eukaryotic organisms, some of which are essential for growth. These lipids contain a ceramide which consists of a sphingobase bound to a long-chain fatty acid. The outer leaflet of the plasma membrane is highly enriched with sphingolipids and, recently, in many organisms, they have been proposed to serve as intra- and intercellular second messengers which play an important role in various cellular events, such as proliferation and differentiation (reviewed by Dickson & Lester, 2002). Glucosylceramide (GluCer), which contains a directly linked glucose, is a typical sphingolipid found in most eukaryotes. On the other hand, glycosylinositolphosphorylceramides, which are linked with inositol via a phosphodiester bond, are present only in fungi and plants. Galactosylceramides, which are linked with galactose, are found in animals and fungi (reviewed by Warnecke & Heinz, 2003).

In animals, glycosylceramides (GlcCers) not only are essential for growth but also are believed to play a crucial role in cell differentiation and cell–cell interactions (reviewed by Merrill et al., 1997; Ternes et al., 2002). Plant GlcCer and its derivatives such as ceramide and long-chain bases (LCBs) have also been suggested to function in signal transduction and host–pathogen interactions (reviewed by Sperling & Heinz, 2003; Thevissen et al., 2004; Ramamoorthy et al., 2007). In fungi, two yeast species, Saccharomyces cerevisiae and Schizosaccharomyces pombe, have been used as models for studying higher eukaryotic cells with regard to the metabolism and function of sphingolipids as well as other intracellular components. However, as these yeasts are not capable of synthesizing GlcCer and are not able to desaturate and methylate LCBs, knowledge of the molecular mechanisms responsible for GlcCer function and the role of modified LCBs in fungi remains limited.

Recently, a number of other yeast species and fungi have been reported to contain GluCers which are thought to play important roles in growth and differentiation. For example, the addition of antibodies against a purified Cryptococcus GluCer led to the inhibition of cell budding and growth in Cryptococcus neoformans, and antibodies raised against a purified Pseudallescheria GluCer inhibited the formation of mycelium in Pseudallescheria boydii and germ-tube formation in C. albicans (Rodrigues et al., 2000; Pinto et al., 2002). Additionally, inhibition of UDP-glucose:ceramide glucosyltransferase (GCS), which catalyzes the final step of GluCer synthesis, influences the growth and differentiation of Aspergillus nidulans and A. fumigatus (Levery et al., 2002). However, little is known about which structure of GluCer causes these biological phenomena in fungal cells.

Abbreviations: GCS, UDP-glucose, ceramide glucosyltransferase; GlcCer, glycosylceramide; GluCer, glucosylceramide; LCB, long-chain base; SLD, sphingolipid $\Delta^8$-desaturase.
Biosynthesis of GluCer starts with condensation of palmitoyl-CoA and serine to synthesize 3-ketosphinganine by serine palmitoyltransferase (reviewed by Warnecke & Heinz, 2003). 3-Ketosphinganine reductase catalyzes the conversion of 3-ketosphinganine to sphinganine. Ceramide synthase catalyses the condensation of sphinganine to saturated acyl-CoA for synthesis of ceramides. Next, sphingolipid Δ⁶-desaturase (DES) catalyses the Δ⁶-desaturation of LCBs in ceramides (Terne et al., 2002). Moreover, in plants and many fungi, sphingolipid Δ⁶-desaturase (SLD) catalyses the Δ⁶-desaturation of LCBs in ceramides, while sphingolipid 9-methyl-transferase (SLM) introduces a methyl group in carbon-9 of LCBs in ceramides (Sperling et al., 1998; Takakuwa et al., 2002; Ternes et al., 2006). The desaturation of fatty acyl groups in ceramides has also been detected in some organisms. Finally, GCS catalyses the reaction of these ceramides with UDP-glucose, resulting in the synthesis of GluCer (Leipelt, 2001). Since it has been suggested that most of the genes that encode enzymes for GluCer synthesis are absent in Sac. cerevisiae and Sch. pombe, little is known at present about fungal genes involved in GluCer synthesis. It was recently reported that, in Cryptococcus neoformans, a pathogenic fungus, a strain lacking the GCS gene is not able to synthesize GluCer is incapable of growing in neutral and alkaline solutions in vitro, which reduces its pathogenicity in its host (Rittershaus et al., 2006). In contrast, disrupting the GCS gene in C. albicans does not affect the growth of the yeast in its unicellular or filamentous forms (Leipelt et al., 2001).

In this study, we have focused on the gene modifying an LCB moiety of GlCcER, namely the gene encoding the SLD enzyme. We identified a C. albicans SLD gene from the Candida genome database and constructed an sld null mutant. We then investigated the effect of the SLD disruption on the morphogenesis of fungal cells. Interestingly, the C. albicans sld mutant appeared to have an obvious delay in filamentous growth compared with isogenic wild-type strains.

**METHODS**

**Media, growth conditions and basic techniques.** The C. albicans strains used and constructed in this study are shown in Table 1. Cells were typically grown at 30 °C in YPD medium [1 % Bacto yeast extract, 2 % Bacto peptone, 2 % glucose (pH 5.6)] or complete minimal (CM) medium (Ausubel et al., 1992) without uracil and/or arginine. The speed of growth was measured as OD₆₀₀ by using an Ultrospec 3000 spectrophotometer (Pharmacia Biotech). The yeast-to-hypha transition was induced at 37 °C in both solid and liquid media. For the solid transition medium, we used either Spider medium (Liu et al., 1994) or 10 % fetal bovine serum medium (Hanaoka et al., 2005). For the liquid transition medium, we used 10 % fetal bovine serum in YPD, adjusted to pH 7.2. Filamentation in the liquid medium was induced by inoculating 2 × 10⁴ to 5 × 10⁴ cells ml⁻¹ and incubating at 37 °C. For spot tests, cells were grown overnight at 30 °C in YPD medium and tenfold serial dilutions were then spotted onto the indicated YPD agar plate and grown for 31 h at 30 °C. Antifungals used in this study were amphotericin B (Wako Pure Chemical Industries), fluconazole (LKT Laboratories), miconafungin (Astellas Pharma) and terbinafine (LKT Laboratories).

Escherichia coli DH5α and cloning vector pBluescript II SK + (Stratagene) were used for DNA manipulation. General recombinant DNA procedures were performed as described by Sambrook et al. (1989). C. albicans was transformed by the method described by Umeyama et al. (2005).

**Plasmid construction.** The primers used in this study are listed in Table 2. The Ca-SLD DNA fragment was amplified by PCR using primers sld-clb5 and sld-clb3, with TUA4 chromosomal DNA as a template, and cloned into the EcoRV site of pbBlueScript II SK + to generate pBS-SLD. Nucleotide sequencing confirmed that there were no PCR errors. The 1.8 kb BglII–Xhol DNA fragment from pBS-SLD was inserted into the BamHI/Xhol sites of pFLAG-Act1 (Umeyama et al., 2002) to generate pFLAG-Act1-SLD.

A plasmid, pBS-hph200-URA3, was constructed for gene replacement. The 2.0 kb PsvII DNA fragment from pUC19-hph200-URA3 (Umeyama et al., 2005) was inserted into the EcoRV site of pbBlueScript II SK + to generate pBS-hph200-URA3.

**Strain construction.** To disrupt Ca-SLD, two different markers were used for two different alleles (see Fig. 2). The 0.6 kb DNA fragment corresponding to the 3’ end of Ca-SLD was amplified using primers sld-mut-Not5 and sld-mut-Sac3, digested with NotI and SacI, and then cloned into the NotI–SacI site of pBS-hph200-URA3 and pUC19-ARG4 (Hanaoka et al., 2005) to generate pBS-hph200-URA3-NS and pUC-ARG4-NS, respectively. The 0.6 kb DNA fragment corresponding to the 5’ end of Ca-SLD was amplified using primers sld-mut-Kpn5 and sld-mut-Xho3, digested with KpnI and Xhol and then cloned into the KpnI/Xhol site of pBS-hph200-URA3-NS to generate pBS-hph200-URA3-disSLD. The 0.6 kb DNA fragment corresponding to the 5’ end of Ca-SLD was amplified using primers sld-mut-Sph5 and sld-mut-Kpn3, digested with SphI and KpnI and then

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**Table 1. Yeast strains used and constructed in this study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
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<tbody>
<tr>
<td>TUA4</td>
<td>ura3Δ::imm434/ura3Δ::imm434 arg4Δ::hisG200/arg4Δ::hisG200</td>
</tr>
<tr>
<td>TUA6</td>
<td>ura3Δ::imm434/ura3Δ::imm434 arg4Δ::hisG200/arg4Δ::hisG200 RP10::p3HA–ACT1</td>
</tr>
<tr>
<td>SLD101</td>
<td>ura3Δ::imm434/ura3Δ::imm434 arg4Δ::hisG200/arg4Δ::hisG200 sldΔ::hph200–URA3–hph200/SLD</td>
</tr>
<tr>
<td>SLD102</td>
<td>ura3Δ::imm434/ura3Δ::imm434 arg4Δ::hisG200/arg4Δ::hisG200 sldΔ::hph200–URA3–hph200/SLD::ARG4</td>
</tr>
<tr>
<td>SLD103</td>
<td>ura3Δ::imm434/ura3Δ::imm434 arg4Δ::hisG200/arg4Δ::hisG200 sldΔ::hph200/SLD::ARG4</td>
</tr>
<tr>
<td>SLD104</td>
<td>ura3Δ::imm434/ura3Δ::imm434 arg4Δ::hisG200/arg4Δ::hisG200 sldΔ::ARG4 RP10::pFLAG–ACT1</td>
</tr>
<tr>
<td>SLD105</td>
<td>ura3Δ::imm434/ura3Δ::imm434 arg4Δ::hisG200/arg4Δ::hisG200 sldΔ::hph200/SLD::ARG4 RP10::pFLAG–ACT1–SLD</td>
</tr>
</tbody>
</table>
Table 2. Oligonucleotide primers used in this study

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence (5'–3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>sld-clo3</td>
<td>AACCTCGAGCTATATTAGTGCTTTGTTG</td>
</tr>
<tr>
<td>sld-mut-Kpn3</td>
<td>AAGGTACCGGTATCTCAGAGGATTGTTGAAAG</td>
</tr>
<tr>
<td>sld-mut-Sac3</td>
<td>ATGGGCGCGAGATCTCAGGTTTCTAAGGTTTACGAG</td>
</tr>
<tr>
<td>sld-mut-Kpn5</td>
<td>AAAGGTACCTATACCTTTGGAAGGTTTGTTAACGAAG</td>
</tr>
</tbody>
</table>

Cloned into the Sph/KpnI site of pUC-ARG4-NS to generate pUC-ARG4-disSLD.

The 3.3 kb KpnI–SacI fragment of pBS-hph200-URA3-disSLD was used to transform the C. albicans Arg+ Ura- strain TUA4 to generate a Ura- transformant (SLD101). The 3.2 kb SphI–SacI fragment of pUC-ARG4-disSLD was then used to transform Arg+ strain SLD101 to generate SLD102. The resulting Ura- Arg+ transformants of SLD102 were plated on medium containing 5-fluoroorotic acid to isolate the Ura- segregant (SLD103). To confirm the gene disruption, genomic DNA was isolated from each strain, digested with HpaII, run in a 1% agarose gel and then transferred onto a Hybond-N+ nylon membrane (Amersham Biosciences). Southern hybridization was performed using a 32P-labelled probe in a solution containing 5 × SSC, 6 × Denhardt’s solution and 0.1 mg herring sperm DNA ml⁻¹ at 65°C according to the methods of Sambrook et al. (1989). The SLD gene was reintroduced into the Ca-RP10 locus of the null mutant using AatI-digested pFLAG-Act1-SLD to transform strain SLD103 to generate SLD105. The AatI-digested empty vector pFLAG-Act1 was integrated into strain SLD103 to generate SLD104 as a control.

Sphingoid base analysis. Analysis of the sphingoid base composition was performed as described by Tani et al. (2004) with optimizations. Lysophosphilized cells were treated in chloroform–methanol (2:1, v/v) for 10 min with a subsonic homogenizer (HOM-100; Asahi Glass). Total lipids were then extracted with 4 vols chloroform–methanol (2:1, v/v) and chloroform–methanol (1:2, v/v) and hydrolysed in 0.4 M KOH–methanol at 37°C for 2 h. After washing, the organic phase was dried on a rotary evaporator to yield the alkali-hydrolysed sphingoid bases which were extracted with diethyl ether and reacted with 0.2 M sodium periodate for 2 h to obtain fatty aldehydes. The alkali-hydrolysed fatty aldehydes were extracted in dichloromethane and analysed by GLC (GC-18A; Shimadzu) on a 0.25 mm x 50 m CP-Sil 88 capillary column (Varian). The initial column temperature of 150°C was maintained for 2 min, increased by 2°C min⁻¹ to 190°C and then maintained for 6 min.

RESULTS

Identification of a C. albicans SLD gene homologue and its sequence analysis

From the Candida genome database website (http://www.candidagenome.org/), we detected one sequence having high similarity with fungal SLD proteins from Saccharomyces klyveri (Sk-SLDp), Kluyveromyces lactis (Kl-SLDp) and Pichia pastoris (Pp-SLDp). The resulting amino acid sequence, Ca-SLDp (orf19.260), was predicted to be a 584 amino acid polypeptide. The deduced amino acid sequence of Ca-SLDp had high identities with Sk-SLDp (58.5%), Kl-SLDp (56.5%) and Pp-SLDp (53.7%). The haem-binding motif (HPGG) in cytochrome b6 and three histidine motifs (HXXXH, HXXHH, QXXHH), considered essential for desaturase activity and as potential ligands for iron atoms, are found in Ca-SLDp (Fig. 1).

Construction of Ca-sld mutants

To investigate the functions of Ca-SLD in C. albicans, we constructed Ca-sld mutants as described above. For the first allele, Ca-SLD was replaced with an hph200–URA3–hph200 cassette (Fig. 1). To confirm that loss of SLD function was responsible for the phenotypes observed, the SLD expression plasmid pFLAG-Act1-SLD and the pFLAG-Act1 vector alone were reintroduced into the Ca-RP10 locus (Murad et al., 2000) of sld mutant strain SLD103, yielding strains SLD105 and SLD104, respectively.

Sphingoid base analysis of Ca-sld mutants

To investigate the profile of sphingoid base residues in C. albicans transformants, GluCers were isolated from SLD104 (sld mutant), SLD105 (SLD transformant) and the corresponding wild-type strain, TUA6. Their sphingoid base compositions were compared (Fig. 3). The GluCers of the wild-type strain contained the three sphingoid bases (E)-sphing-4-enine, (E,E)-sphinga-4,8-diene and (E,E)-9-methylsphinga-4,8-diene. In contrast, the sld mutant contained only one sphingoid base, (E)-sphing-4-enine. The sphingoid base profile of the SLD transformant strain was similar to that of the wild-type. These results show that Ca-SLDp is the only enzyme that introduces a double bond at the Δ⁸ position of the LCB in ceramides.
**Fig. 1.** Amino acid sequence alignment of Ca-SLDp and closely related sphingolipid Δ^8-desaturases. Sk-SLD, Sac. kluveyi SLD (Takakuwa et al., 2002); Kl-SLD, K. lactis SLD (Takakuwa et al., 2002); Pp-SLD, P. pastoris SLD (Ternes et al., 2006). Sequences were aligned using the FASTA algorithm. Conserved amino acids are in white on black. Positions of amino acid residues relative to the start codon are given to the right. The haem-binding motif (HPGG) in cytochrome b₅₆ and three histidine motifs (HXXXH, HXXHH, QXXHH), considered essential for desaturase activity and as potential ligands for iron atoms, are boxed.

**Fig. 2.** Gene disruption of Ca-SLD. (a) Disruption strategy by homologous recombination. (b) Restriction maps for each modified allele. WT, Wild-type. (c) Confirmation of disruption constructs by Southern hybridization using the probe indicated in (b). Lanes: 1, SLD/SLD, ura3, arg4; 2, sld/SLD, URA3, arg4; 3, sld/sld, URA3, ARG4; 4, sld/sld, ura3, ARG4.
Phenotype analysis of the Ca-sld disruptant

At first, to investigate the necessity of $\Delta^8$-desaturation of the LCB in ceramides and GluCers, we examined the growth rate of the sld disruptant and the control strains at 30 and 10 °C in liquid YPD medium (Fig. 4). There was no significant difference in the growth rate among these strains at 30 °C. However, the growth rate of the sld disruptant was lower than that of the wild-type at 10 °C. These results indicate that the fungal ceramide and GluCer containing a double bond at the $\Delta^8$-position in the LCB play some role in growth of C. albicans at 10 °C.

In order to observe hyphal formation of the disruptant, overnight cultures were inoculated into liquid YPD medium (pH 7.2) plus 10 % serum medium. Time-course observation of growth at 37 °C with shaking demonstrated that hyphal growth of the sld mutant was similar to that of the wild-type, and there was no significant difference in germ-tube formation (Fig. 5). In contrast, on agar media such as 10 % serum agar at 37 °C or spider medium at 25 °C, filamentation of the sld disruptant was slower than that of the wild-type strain (Fig. 6). This phenotype was restored by the reintroduction of Ca-SLD to the sld mutant.

To investigate other effects of the loss of $\Delta^8$-desaturation of GluCer in C. albicans, growth of the sld mutant in various agents or environments was examined by drop assays (Fig. 7). The sld disruptant was sensitive to 0.02 % SDS in comparison with the wild-type. Other stresses such as heat shock (42 °C), salt (1 M NaCl or 1 M KCl), 6 % ethanol or 50 µg Calcofluor white (CFW) ml$^{-1}$ had no effect. Moreover, the sld disruptant was more susceptible than the wild-type to the lanosterol 14x-demethylase inhibitor flucnonazole, while other antifungals such as amphoterin B, micafungin and terbinafine had no effect. These results indicate that $\Delta^8$-desaturated GluCers may have a role in membrane integrity in C. albicans.

DISCUSSION

In this study, we characterized the sphingolipid $\Delta^8$-desaturase gene from C. albicans (Ca-SLD) and also constructed a disruptant of this gene. This is the first report on the physiological characterization of an SLD gene disruptant in any organism. The deduced amino acid sequence of the gene product encoded by orf19.260 in the C. albicans genome had high identities with those of other yeasts, confirming that the ORF encodes a C. albicans sphingolipid $\Delta^8$-desaturase (Ca-SLDp).

We constructed the sld disruptant by replacing two Ca-SLD ORFs with two different marker genes. From the result of the analysis of LCB in GluCers, the sld mutant contained only the sphingoid base (E)-sphing-4-ene. In contrast, the SLD transformant strain contained the three sphingoid bases (E)-sphing-4-ene, (E,E)-sphinga-4,8-diene and (E,E)-9-methylsphinga-4,8-diene in GluCers, a result that was similar to the wild-type strain. The amounts of GlCer from these cells were unlikely to make much of a difference. These results show that Ca-SLDp is required for desaturation at the $\Delta^8$ position of C. albicans glucosylcer-
**Fig. 5.** Germ-tube formation in liquid YPD (pH 7.2) plus 10% serum at 37 °C. Cells from strains TUA6 (SLD/SLD), SLD104 (sld/sld) and SLD105 (sld/sld SLD) were grown overnight at 30 °C. For induction of filamentation, 2×10^6 to 5×10^6 cells ml^−1 were inoculated into YPD (pH 7.2) with 10% serum and incubated at 37 °C with shaking.

**Fig. 6.** Morphology of *C. albicans* strains grown on solid agar medium. Cells from strains TUA6 (SLD/SLD), SLD104 (sld/sld) and SLD105 (sld/sld SLD) were grown overnight at 30 °C and 10^6 cells were then spotted onto the indicated agar plate and grown for 7 days at 37 °C on agar medium containing 10% serum or at 25 °C on Spider medium. Results of a representative experiment are shown; four further independent experiments showed the same results.
amides. Additionally, Δ⁸-desaturation by Ca-SLDp is necessary for 9-methylation by sphingolipid 9-methyltransferase that occurs in LCBs within ceramides.

The growth rate of the sld disruptant at 30 °C in liquid YPD medium was similar to that of the wild-type. However, the sld mutant showed slower growth than the wild-type strain at 10 °C. These results indicate that Δ⁸-desaturation of LCBs in ceramide and GluCer is necessary for growth of *C. albicans* at 10 °C. *(E,E)-9-Methylsphinga-4,8-dienine in GluCers is assumed to be essential in *Kluyveromyces lactis* to maintain sufficient membrane fluidity at low temperatures (Tanji *et al.*, 2004). Therefore, the loss of this molecule in the cell membrane may result in the sensitivity of the sld mutant to low temperatures.

When grown in liquid media to induce the hyphal form, the sld disruptant was able to elongate and its germ tubes evaginated normally. However, when grown on hyphal-inducing solid medium, the *C. albicans* sld disruptant showed slower elongation than the wild-type. These results suggest that Δ⁸-desaturation of LCBs in ceramide and GluCer is closely related to hyphal elongation on solid medium. Sphingolipids, a major component of the lipid raft, are known to be important for normal hyphal growth in *C. albicans* (Martin & Konopka, 2004). In this latter report, myriocin, a specific inhibitor of serine palmitoyltransferase, which catalyses the first step of sphingolipid biosynthesis, affected hyphal elongation in *C. albicans*.

Therefore, GluCer containing Δ⁸-desaturated LCB, among many kinds of sphingolipids, may play an important role in hyphal elongation in *C. albicans*. Moreover, some GlcCers of fungi and plants are thought to be highly active in inducing fruiting body formation in the fungus *Schizophyllum commune* (Kawai & Ikeda, 1985; Kawai *et al.*, 1986). Structural analysis of these GlcCers has revealed that a *cis- or trans-Δ⁸* double bond in the sphingoid base moiety is essential, while the sugar moiety has no effect. However, a *C. albicans* gcs mutant that is unable to condense ceramides with glucose grew in both yeast and filamentous forms (Leipelt *et al.*, 2001). These results and our finding suggest that Δ⁸-desaturated and 9-methylated ceramides may be active in inducing hyphal morphogenesis of *C. albicans*.

The *C. albicans* sld disruptant was sensitive to SDS in comparison with the wild-type but CFW, a cell-wall stress inducer, had no effect on this mutant. Moreover, the *C. albicans* sld disruptant was more susceptible than the wild-type to the lanosterol 14α-demethylase inhibitor fluconazole. We surmise that the defect of Δ⁸-desaturation may have led to a disturbance in the membrane integrity of *C. albicans*. Sphingolipids, including GluCer, are enriched in the detergent-resistant membrane microdomains known as lipid rafts. Lipid rafts have been implicated in numerous cellular processes, including signal transduction, protein and lipid sorting, cellular entry by toxins and viruses and viral budding (reviewed by Chazal & Gerlier, 2003; Bollinger *et al.*, 2005). In addition, antimicrobial peptide
inhibitors of fungal plasma membrane ATPase (Pma1p), which is known to be associated with the lipid raft, block the azole resistance of C. albicans (Monk et al., 2005). Therefore, the sensitivity to SDS and susceptibility to fluconazole of the sld disruptant may result from some defect of lipid rafts in C. albicans. In future experiments, the detergent-resistant microdomain in the membrane of the C. albicans sld mutant will be analysed in detail.

The sld disruptant did not contain (E,E)-9-methylsphinga-
4,8-diene or (E,E)-sphinga-4,8-diene. (E,E)-Methylsphinga-
4,8-diene is the most abundant sphingoid base in ceramide and GluCer of the C. albicans wild-type strain. Therefore, it may be that 9-methylation of the LCB and its relation with filamentous growth of C. albicans may be more important than the Δ4-desaturation. A C. albicans ORF (orf19.4831) encoding a homologue of sphingolipid C9-methyltransferase of Pichia pastoris has been found in the C. albicans genome (Ternes et al., 2006). Analysis of this gene disruptant will further clarify the most important sphingoid base in C. albicans.

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REFERENCES


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