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Characterization of Prostaglandin E_2 Production by *Candida albicans*▽

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Candida albicans **produces lipid metabolites that are functionally similar to host prostaglandins. These studies, using mass spectrometry, demonstrate that *C. albicans* produces authentic prostaglandin E_2 (PGE_2) from arachidonic acid. Maximal PGE_2 production was achieved at 37°C in stationary-phase culture supernatants and in cell-free lysates generated from stationary-phase cells. Interestingly, PGE_2 production is inhibited by both nonspecific cyclooxygenase and lipoxygenase inhibitors but not by inhibitors specific for the cyclooxygenase 2 isoenzyme. The *C. albicans* genome does not possess a cyclooxygenase homolog; however, several genes that may play a role in prostaglandin production from *C. albicans* were investigated. It was found that a *C. albicans* fatty acid desaturase homolog (Ole2) and a multicopper oxidase homolog (Fet3) play roles in prostaglandin production, with *ole2*/*ole2* and *fet3*/*fet3* mutant strains exhibiting reduced PGE_2 levels compared with parent strains. This work demonstrates that the synthesis of PGE_2 in *C. albicans* proceeds via novel pathways.**

The eicosanoids are a family of 20-carbon fatty acid metabolites that include prostaglandins and leukotrienes. Prostaglandin E_2 (PGE_2) is an oxygenated metabolite of arachidonic acid (AA) and is produced via a two-step process beginning with the initial action of a cyclooxygenase (COX-1 or COX-2) to form PGH_2 followed by specific PGE synthases in the mammalian host (4, 16, 42, 43; reviewed in reference 26). PGE_2 is a potent regulator of host immune responses, with the ability to elicit both pro- and anti-inflammatory responses, depending on the target cell. PGE_2 acts via one of four different G-protein-coupled receptors (EP1 to EP4). PGE_2 can inhibit Th1-type immune responses, phagocytosis, and lymphocyte proliferation (3, 24, 34, 41). PGE_2 can also promote Th2-type responses, immunoglobulin E production, and tissue eosinophilia (6, 10, 15, 17, 35, 36). During a *Candida* infection, the development of a Th1 response results in protection and clearance while a Th2 response is nonprotective, leading to chronic or disseminating disease (23). Therefore, PGE_2 may be an important factor in shifting host immune responses towards those that promote fungal colonization and chronic infection.

During the interaction of *C. albicans* with the host, lipid mediators coming from both the host and *Candida* have the potential to influence immune responses. *Candida* produces both endogenous oxylipins (the generic term for an oxygenated polyunsaturated fatty acid) and novel eicosanoid products from exogenous AA (8, 30). One of these fungal oxylipins isolated from *Candida* supernatants exhibits cross-reactivity with host PGE_2 (30). This compound, termed PGEx for "PGE cross-reactive compound," is bioactive on mammalian cells in vitro similar to PGE_2, indicating that the fungal oxylipins can modulate host immune responses. Our laboratory and others have previously reported that host PGE_2 and fungal PGEx enhance the morphogenesis of *C. albicans* (18, 30). Candidal oxylipin production is also upregulated during biofilm formation (1). Similarly, mammalian eicosanoid inhibitors also inhibit candidal oxylipin production, morphogenesis, and biofilm formation (2, 30). These last two observations suggest the presence of an eicosanoid/oxylipin pathway in *C. albicans* that plays a role in the control of morphogenesis and biofilm formation. These physiological processes are important in the colonization of host tissues and indwelling medical devices (reviewed in references 31 and 45).

C. albicans does not contain AA as part of its fatty acid repertoire; however, *C. albicans* is known to cause the release of AA from host tissues (5). Additionally, supplementation of *C. albicans* cultures with exogenous AA significantly increased PGEx production (28). Taken together, the evidence suggests an important role for eicosanoid-derived oxylipins in *C. albicans*; thus, the objective of this study was to determine whether *Candida* produces authentic PGE_2 from AA and to identify which growth variables influence PGE_2 production.

MATERIALS AND METHODS

Strains. The *C. albicans* strains used in this study include the following: CHN1 (a clinical isolate), SC5314 (a prototrophic parental strain) (13), CAF2-1 (an auxotrophic parental strain, *URA3*/*ura3*Δ::*imm434*), CAI4 (an auxotrophic parental strain, *ura3*Δ::*imm434*/*ura3*Δ::*imm434*) (13), JA2 (same as CAI4, but *ole2*Δ::*hisG*/*ole2*Δ::*hisG URA3 hisG* (20), JA2r (same as JA2, but with a plasmid containing an *OLE2* overexpression vector, pAP5) (20), and a *fet3*Δ mutant (same as CAI4, but *fet3*::*hisG*/*fet3*::*hisG-URA3*::*hisG*) (9).

Prostaglandin purification and reverse-phase HPLC. *C. albicans* strain CHN1 was grown in Sabouraud dextrose broth (SDB) for 72 h. Cells were washed twice with 50 ml of phosphate-buffered saline (PBS), pH 6.5, and transferred to a 125-ml culture flask at a concentration of 2×10^7 cells/ml. Cultures were treated with or without 500 μM AA and shaken at 37°C overnight. Supernatants were then passed over PGE_2 affinity columns, and the purified material was collected and dried under N_2 gas to prepare for separation using reverse-phase high-performance liquid chromatography (HPLC). Reverse-phase HPLC analysis was carried out using a Waters 600 HPLC system with a 5-μl sample loop and a Waters Symmetry 2.1- by 150-mm analytical column (Waters Corp., Milford,

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MA). Prior to sample analysis, prostaglandin standards (Cayman Chemicals, Ann Arbor, MI) were separated using an elution gradient starting at 75:25:0.1 (water/acetonitrile/acetic acid) for 10 min, followed by a linear shift to 0:100:0.1 (water/acetonitrile/acetic acid) over 80 min. The elution time of PGE_2 was determined, and the system was set to collect a 1-min fraction containing the PGE_2 peak. Samples were resuspended in 25 μl of 50% methanol in water and separated in the same manner as that described above. The organic compounds were extracted from this fraction by the addition of 500 μl HPLC-grade ethyl acetate (Sigma-Aldrich, St. Louis, MO), followed by vortexing to mix the two layers. The phases were allowed to separate, and the organic phase was removed and transferred to a V vial coated with silane. This step was repeated, and the contents of the vial were dried in a 40°C water bath under a stream of grade 5 nitrogen.

Characterization of *Candida albicans* PGE_2 by MS. To analyze the molecular structure of the *C. albicans* putative PGE_2 compound, *C. albicans* cultures were treated with or without AA, the cells were spun out, and the prostaglandins were purified as described above. The dry sample was then stored at −80°C. All samples for mass spectrometry (MS) were tested the following day.

Liquid chromatography-tandem MS/MS (LC-MS/MS) was carried out using ThermoFinnigan Surveyor HPLC (San Jose, CA) interfaced directly with the electrospray ionization source of a ThermoFinnigan LTQ linear ion trap MS (Thermo-Electron Corp., San Jose, CA). The samples were resuspended in a methanol-water solution (1:1, vol/vol), and 20-μl aliquots were injected onto a Phenomenex Luna phenyl-hexyl column (Phenomenex Corp., Torrence, CA) (2.00 by 150 mm; 3-μm inside diameter). The mobile-phase solvents were 10 mM ammonium acetate, pH 8.5, and methanol. The compounds were separated and eluted from the analytical column with a linear gradient first of 50% to 60% methanol over 12 min and then of 60% to 90% methanol over 2 min at a flow rate of 0.3 ml/min, as described previously (48). The column was heated to 50°C, and the sample tray was cooled to 4°C throughout the analysis.

Determination of prostaglandin concentration by ELISA. To determine the optimal PGE_2 production conditions, we monitored the production in different strains of *C. albicans* over time at different temperatures in both whole cells and lysates. *C. albicans* strains CHN1 (a clinical isolate) and SC5314 were grown overnight at 30°C in SDB (1% enzymatic digest of casein, 2% dextrose; Difco, Detroit, MI). For whole-cell assays, the overnight cultures were used to inoculate fresh SDB to an optical density (OD) at 600 nm of 0.2. Cultures were grown at 25°C or 37°C to an OD_{600} of 0.6 to 0.8. Peroxide-free AA (Cayman Chemicals, Ann Arbor, MI) was added to a final concentration of 500 μM, and growth was continued at room temperature or 37°C. Samples were removed at various time points, centrifuged, and stored at −20°C until an analysis of the PGE_2 concentration was conducted. Culture supernatants were analyzed for prostaglandin production using a monoclonal PGE_2 enzyme-linked immunosorbent assay (ELISA; Cayman Chemicals, Ann Arbor, MI). This ELISA kit is highly sensitive and detects as little as 15 pg/ml of PGE_2. Controls included heat-killed (HK) cultures (boiled for 6 h), cultures grown without AA, and AA alone. Background levels of PGE_2 detected in buffer plus AA alone at time zero were subtracted from experimental samples (~50 pg/ml). In addition, cell viability was measured by dilution plating samples onto Sabouraud dextrose agar plates.

For PGE_2 determination in rat serum, *C. albicans* strain SC5314 was grown overnight in SDB at 30°C. *Candida* cells were counted and added to the serum at a concentration of 10^8 cells/ml and incubated at 37°C for 4 h. No exogenous AA was added. Normal whole rat serum was purchased from Zymed (San Francisco, CA), while analbuminemic rat serum was isolated from Nagase analbuminemic rats (27). A PGE metabolite kit was used to measure PGE_2 levels in serum supernatants (Cayman Chemicals, Ann Arbor, MI). PGE_2 is rapidly degraded in vivo and in ex vivo animal fluids, such as serum, into an unstable intermediate (15-keto-13,14-dihydro-PGE_2). The PGE metabolite kit converts this unstable intermediate into a stable measurable derivative, which serves as a marker for PGE_2 production. This enzyme immunoassay (EIA) kit is highly sensitive and detects as little as 2 pg/ml of PGE metabolites.

Preparation of *C. albicans* cell-free lysates. For lysates, *C. albicans* was grown for 24 h at 37°C in SDB. Cells were counted using a hemacytometer and concentrated to 2×10^9 cells/ml. Cells were washed twice in 25 ml 1× PBS, and 1 ml of the concentrated culture was resuspended in 1.5 ml lysis buffer (1 mM EDTA in distilled water). Approximately 500 μl acid-washed glass beads (Sigma-Aldrich, St. Louis, MO) was added, and cells were vortexed at high speed for 1 min and then incubated on ice for 1 min for five cycles. Lysates were collected, and the beads were washed with an additional 500 μl of lysis buffer. The two samples were pooled and then centrifuged at 14,000 rpm for 30 min at 4°C to remove cell debris. For prostaglandin production, 100 μl of lysate was incubated with 500 μM AA for 90 min at 37°C. To determine the efficiency of the lysis procedure, lysate cell debris pellets were resuspended, and the remaining viable cells were counted by dilution plating onto Sabouraud dextrose agar plates. The efficiency of lysis ranged from 97 to 98% for all samples. The protein concentrations of the lysates were normalized to a standard using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL) prior to the addition of AA for prostaglandin production. Lysates were analyzed for prostaglandin production using a monoclonal PGE_2 ELISA (Cayman Chemicals, Ann Arbor, MI). Controls included boiled lysates (boiled for 6 h), lysates without AA, and AA alone. Background levels of PGE_2 detected in buffer plus AA alone at time zero were subtracted from experimental samples (~50 pg/ml).

COX and LO inhibitors. Cyclooxygenase (COX) inhibitors (aspirin, indomethacin, resveratrol, and CAY10404) and a lipoxygenase (LO) inhibitor (nordihydroguaiaretic acid [NDGA]) were purchased from Cayman Chemicals (Ann Arbor, MI). All inhibitors were dissolved in dimethyl sulfoxide for stock solutions and diluted in 1× PBS or 1 mM EDTA prior to addition to *C. albicans* whole cells or lysates, respectively.

Statistical analysis. The Student's *t* test (two-tailed with unequal variance) was used to analyze the significance of differences between the two experimental groups. Data with a *P* value of 0.05 or less were considered significant.

RESULTS AND DISCUSSION

The analysis of *Candida* PGEx using LC-MS/MS determined that PGEx (Fig. 1A) possessed a mass and an elution time that were identical to those of the commercial PGE_2 standard (Fig. 1B). In the absence of AA, there was no detectable production of PGEx from *C. albicans* supernatants (data not shown); however, in the presence of AA, the PGEx product was readily detectable. Further analysis using MS/MS (Fig. 1B and inset) revealed that the fragmentation patterns for the PGEx and the PGE_2 standard were identical. Although a small amount of auto-oxidation takes place, the *Candida* PGE_2 is distinguishable from a product of the auto-oxidation of AA, since 8-iso-PGE_2 elutes at a different time than PGE_2 (Fig. 1C). Thus, these data demonstrate conclusively that *C. albicans* is capable of synthesizing authentic PGE_2 from exogenous AA.

C. albicans PGE_2 production and accumulation in culture supernatants from whole cells increased over time, reaching maximum levels at 48 h and 37°C for both strains CHN1 and SC5314 (Fig. 2a). Growth curves for these samples with or without the addition of AA to SDB were similar; therefore, the increased PGE_2 production observed in the presence of AA is not due to increased culture growth (data not shown). To test whether the AA levels found in serum would be sufficient for *Candida* prostaglandin production in vivo, we measured PGE_2 production by *Candida* in the presence of serum ex vivo without the addition of exogenous AA (Fig. 2b). It is known that PGE_2 is rapidly metabolized by serum proteins (12, 47), so to measure the levels of PGE_2 produced by *Candida* incubated in serum and to quantify these unstable by-products, a PGE metabolite assay was employed. Serum from analbuminemic rats was also tested, since serum albumin is involved not only in the breakdown of PGE_2 but also in the limitation of the bioavailability of fatty compounds by binding free fatty acids (including AA and PGE_2) (14). Indeed, we observed PGE_2 production by live *Candida* in the presence of analbuminemic rat serum without AA but not normal rat serum (Fig. 2b). This indicates that sufficient quantities of AA can be found in serum for *Candida* to potentially produce PGE_2 in vivo using host AA. In addition, PGE_2 could be detected when lysates were incubated with exogenous AA, indicating that enzymatic activity may occur intracellularly (Fig. 2c). Furthermore, significantly less PGE_2 was detected in the supernatants of HK *C. albicans* and boiled lysates, indicating the presence of a denaturable enzymatic

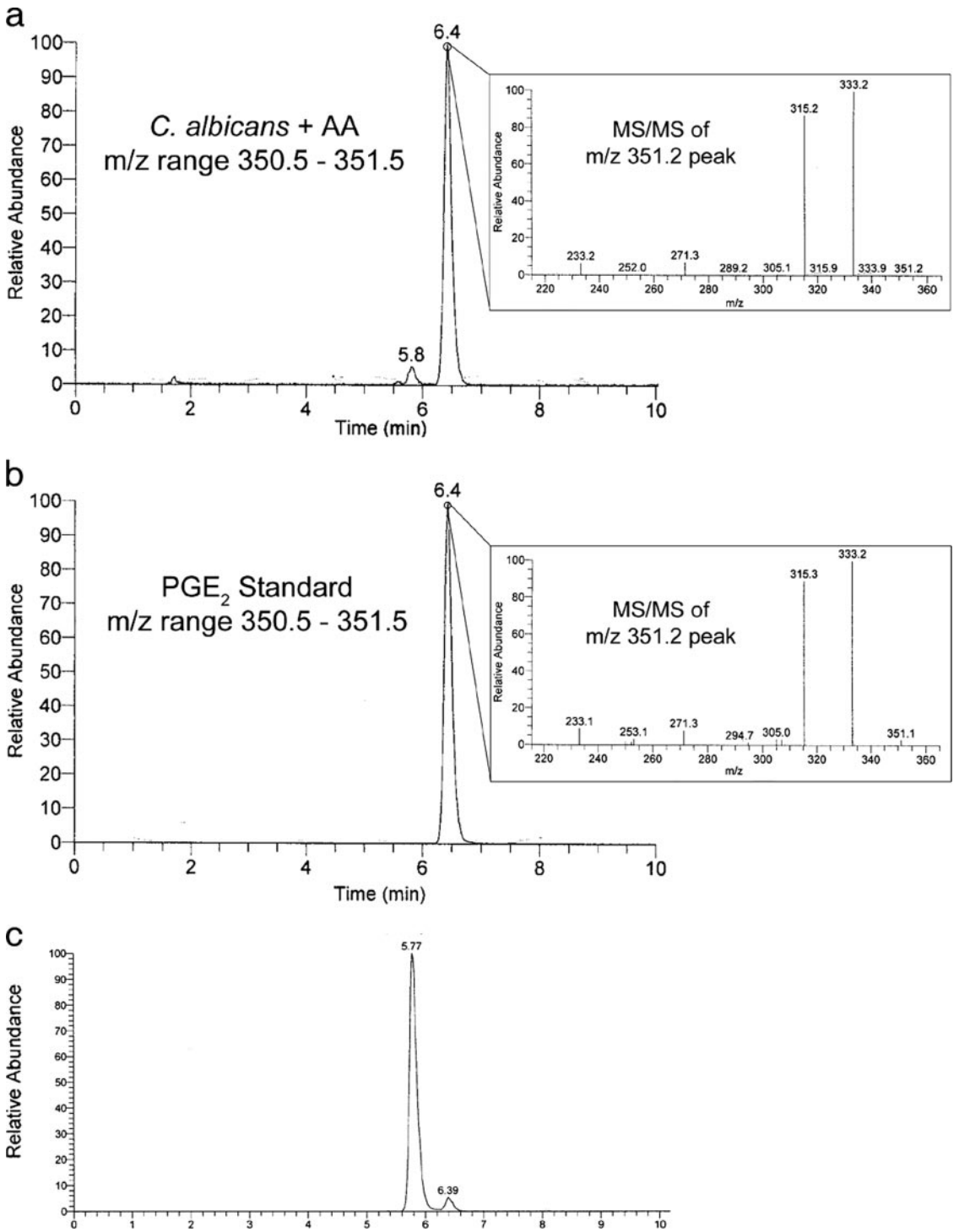

FIG. 1. MS of PGE_2 from *C. albicans*. *C. albicans* strain CHN1 was incubated with AA, and putative PGE_2 was purified from the supernatants and subjected to LC-MS/MS. (A) Elution profile of PGEx focusing on the *m/z* range of 350.5 to 351.5. (B) Elution profile of a purified PGE_2 standard. Insets in panels A and B demonstrate the MS/MS fragmentation pattern seen at the major peak at 6.4 min. (C) Elution profile of an 8-iso-PGE_2 standard (a stereoisomer of PGE_2 formed by auto-oxidation of AA).

pathway for prostaglandin production (Fig. 2a and c). There was an unexpectedly high signal from controls containing the fatty acid alone (perhaps the products of the auto-oxidation of AA or perhaps simply nonspecific binding); however, significantly more PGE_2 was detected in the presence of *C. albicans.* The difference in the background signals observed with AA alone and in the presence of boiled lysates may be a result of the binding of denatured lysate proteins to AA or its by-products.

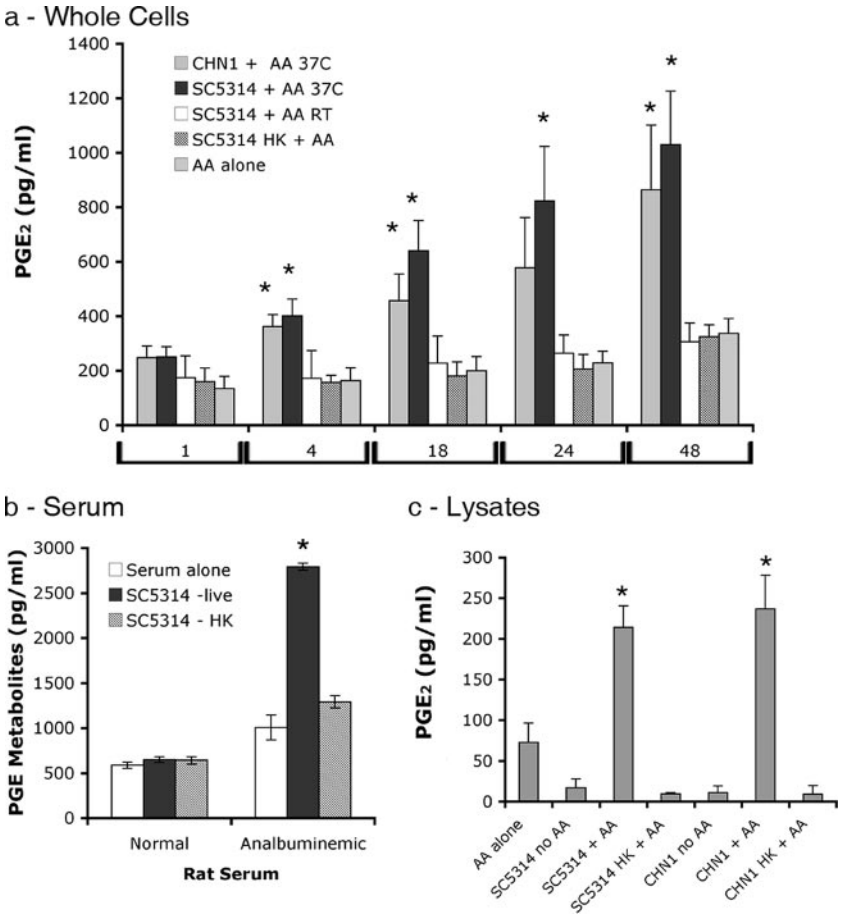

FIG. 2. PGE_2 production in *C. albicans* whole cells and cell-free lysates. (a) *C. albicans* strains SC5314 and CHN1 were grown in SDB at room temperature or 37°C with or without 500 μM AA and sampled at various times. Controls included no AA, AA alone, and HK *Candida* (boiled, 6 h). (b) For whole-cell prostaglandin production in serum, *C. albicans* strain SC5314 was incubated in normal or analbuminemic rat serum for 4 h at 37°C. (c) For *Candida* cell-free lysates, 24-h cultures of *C. albicans* strains SC5314 and CHN1 were lysed by vortexing with acid-washed glass beads in 1 mM EDTA buffer. Lysates were centrifuged, protein concentrations were normalized to a standard, and the lysates were incubated at 37°C with or without 500 μM AA for 90 min. Controls included buffer alone, buffer with AA alone, and lysates without AA. Both whole-cell supernatants and cell lysates were assayed for PGE_2 production using a monoclonal PGE_2 EIA kit (Cayman Chemicals, Ann Arbor, MI), while PGE_2 production in serum was measured using a PGE metabolite EIA kit (Cayman Chemicals, Ann Arbor, MI). Experiments were performed and measured in duplicate, and values are the averages of results of two to three independent experiments. *, $P < 0.05$, live versus HK *C. albicans*.

We next examined the effects of various COX and LO inhibitors on the PGE_2 production by *C. albicans*. Inhibitors were added to the cultures at the same time as the AA. Interestingly, both the nonselective COX inhibitors (aspirin, indomethacin, and resveratrol) and the lipoxygenase inhibitor (NDGA) inhibited PGE_2 production in whole cells at 48 h in a dose-dependent manner without significantly affecting fungal viability (Fig. 3a and data not shown). NDGA does not inhibit prostaglandin production in mammalian systems; however, in *Candida*, this molecule proved to be an effective inhibitor. Both NDGA and resveratrol share similar structural features in that both are plant polyphenols that are antioxidant defense compounds, which may explain their inhibitory effects on fungal PGE_2 production. A highly selective COX-2 inhibitor (CAY10404; Cayman Chemicals, Ann Arbor, MI) had no effect on PGE_2 production by whole cells, supporting the idea that drug-mediated inhibition of PGE_2 production is at the enzymatic level and is not due to nonspecific toxicity effects (Fig. 3a). Both COX and LO inhibitors also diminished PGE_2 production in lysates (Fig. 3b). In addition, CAY10404 exhibited a slight but insignificant inhibitory effect on lysates but not on whole cells, suggesting either that this drug is unable to gain access to the *C. albicans* prostaglandin biosynthetic machinery or that it loses effectiveness once inside the cell (Fig. 3b). Overall, these data suggest that an enzyme exists in *C. albicans* that is structurally distinct from both mammalian COX and LO enzymes but that may contain some features of both types of enzymes. BLAST searches of the *Candida* genome have not yielded any genes or proteins with any significant homology to mammalian eicosanoid biosynthetic enzymes (44). Therefore, the search for the enzymes involved in prostaglandin biosynthesis has been difficult. Recently, two homologs of the *Saccharomyces cerevisiae* Δ9 fatty acid stearyl-coenzyme A desaturase gene (*OLE1*) were characterized for *C. albicans* and termed *OLE1* and *OLE2* (20). As expected, OLE1p exhibits fatty acid desaturase activity (required for oleic acid synthesis), and the gene encoding it is essential in *Candida*. However, a mutant lacking both alleles of *OLE2* had no apparent pheno-

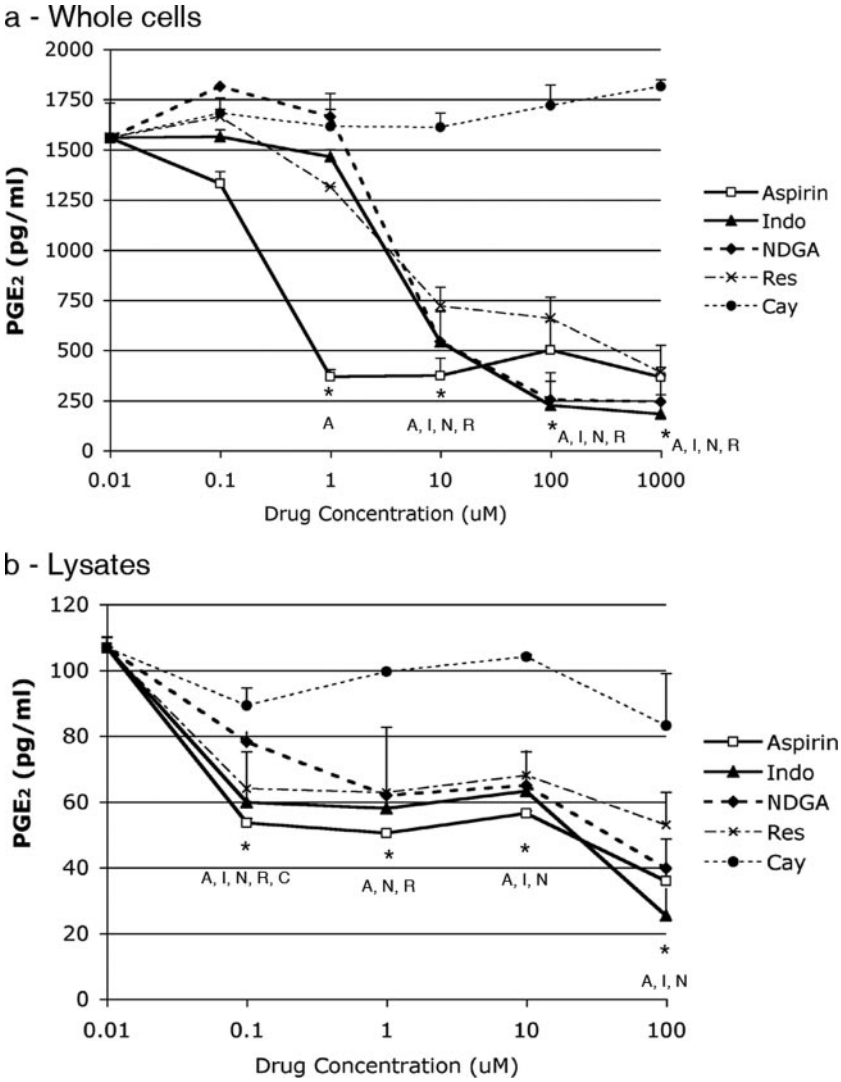

FIG. 3. Effect of COX and LO inhibitors on *C. albicans* PGE_2 production in whole cells and lysates. *C. albicans* whole cells (a) and cell-free lysates (b) were treated as described in the legend to Fig. 2. Inhibitors dissolved in dimethyl sulfoxide were added at the time of AA addition. Both whole-cell supernatants and cell-free lysates (with protein concentrations normalized) were assayed for PGE_2 production using a monoclonal PGE_2 EIA kit (Cayman Chemicals, Ann Arbor, MI). Experiments were performed in duplicate, and results are the averages for two independent experiments which were repeated three times with similar results. Initials indicate which drugs exhibit statistically significant inhibition at each concentration. A, aspirin; I or Indo, indomethacin; N, NDGA; R or Res, resveratrol; C or Cay, CAY10404. *, $P < 0.05$, no inhibitor versus inhibitor.

type and was viable. It was suggested that this gene might play a role in fungal prostaglandin production (20). We therefore examined PGE_2 production in JA2 (*ole2*/*ole2*) and a reconstituted mutant strain, JA2r (*ole2*/*ole2* pAM2-*OLE2*). Compared with CAF2-1 (*OLE2*/*OLE2*), mutant strain JA2 (*ole2*/*ole2*) produced less PGE_2 in whole cells at 48 h (Fig. 4a). There were no differences in the levels of growth between the strains (data not shown). This defect was rescued in the reconstituted strain JA2r (*ole2*/*ole2* pAM2-*OLE2*). In addition, PGE_2 production in the mutant strain was decreased by approximately 25% in *C. albicans* cell-free lysates compared with the parent strain or reconstituted mutant (Fig. 4b). This suggests that *OLE2* plays a role in candidal prostaglandin production. Ole2 is a putative Δ9 desaturase; this type of enzyme is involved in unsaturated fatty acid production in yeast. However, Ole2 must exhibit some other enzymatic activity during prostaglandin production because the unsaturated fatty acid precursor was provided in these experiments. BLAST searches using the Ole2 protein sequence revealed the presence of two conserved domains, a Δ9 desaturase domain and a cytochrome B domain. Cytochrome B enzymes exhibit a wide range of properties and function in a large number of different redox processes (22). Therefore, Ole2 may be involved in PGE_2 production at the level of oxidation of AA. However, the lack of complete inhibition of prostaglandin production in the *OLE2* mutant indicates the involvement of other enzymes in fungal PGE_2 synthesis.

Previous studies of prostaglandin production by *Cryptococcus neoformans* have implied that the enzyme laccase plays a primary role in the early stages of prostaglandin synthesis (J.

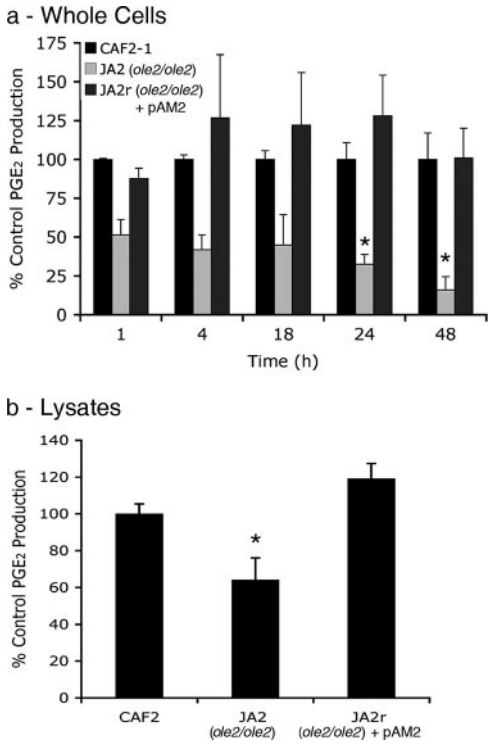

FIG. 4. Role of *C. albicans OLE2* in PGE_2 production. (a) *C. albicans* strain CAF2-1 (parent), JA2 (*ole2*/*ole2*), and JA2r (*ole2*/*ole2* pAP5-*OLE2* [overexpression plasmid]) whole cells (a) or cell-free lysates (b) were treated as described in the legend to Fig. 2 (20). Both whole-cell supernatants and lysates (with protein concentrations normalized) were assayed for PGE_2 production using a monoclonal PGE_2 EIA kit (Cayman Chemicals, Ann Arbor, MI). The amount of detectable oxidized AA observed in control samples containing AA alone for each time point was subtracted from the experimental samples. The percentage of control PGE_2 production was determined by the formula [(PGE_2 produced by mutant strain)/(PGE_2 produced by parental strain)] $\times$ 100. Experiments were performed in duplicate, and results are the averages for three independent experiments. *, $P < 0.05$, CAF2-1 versus JA2.

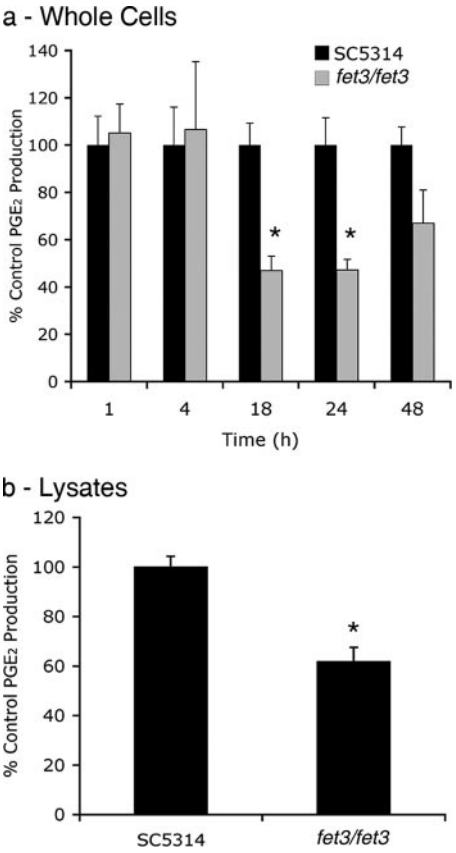

FIG. 5. Role of *C. albicans FET3* in PGE_2 production. (b) *C. albicans* strain SC5314 and *fet3*/*fet3* mutant whole cells (a) or cell-free lysates (b) were treated as described in the legend to Fig. 2 (9). Both whole-cell supernatants and lysates (with protein concentrations normalized) were assayed for PGE_2 production using a monoclonal PGE_2 EIA kit (Cayman Chemicals, Ann Arbor, MI). The amount of detectable oxidized AA observed in control samples containing AA alone for each time point was subtracted from the experimental samples. The percentage of control PGE_2 production was determined by the formula [(PGE_2 produced by mutant strain)/(PGE_2 produced by parental strain)] $\times$ 100). Experiments were performed in duplicate, and results represent the average of three independent experiments. *, $P < 0.05$, SC5314 versus *fet3*/*fet3* mutant.

Erb-Downward, unpublished observation). Cryptococcal laccase is a member of the multicopper oxidase family of polyphenol oxidases and is known to be critical for melanin formation and virulence (33, 46). One remarkable feature of the inhibitors resveratrol and NDGA is that they are both polyphenols with structures very similar to that of caffeic acid, a substrate for the cryptococcal laccase enzyme (40). *Candida* can also melanize in vivo, and laccase activity can be detected in cytoplasmic yeast extracts (25). *Candida* does possess a family of laccase homologs (the Fet family of multicopper oxidases), with several exhibiting >45% similarity to Lac1 at the protein level (data not shown). This family of oxidases also includes the Fet proteins (Fet3 and Fet5) in *S. cerevisiae*, which are ferroxidases involved in iron transport (7, 39). *S. cerevisiae* Fet3 exhibits polyphenol oxidase activity as well, indicating that it possesses enzymatic activity similar to that of *C. neoformans* laccase (37). To test the role of *C. albicans* laccase homologs in prostaglandin production, PGE_2 levels were measured in a *FET3* null mutant (*fet3*/*fet3*) (9). The *fet3*/*fet3* mutant strain produced less PGE_2 in both whole cells and cell-free lysates than did wild-type *C. albicans*, with no differences in cell growth (Fig. 5a and b and data not shown). This indicates that Fet3 also may play a role in PGE_2 production, possibly in a role similar to that which has been observed for *C. neoformans* (Erb-Downward, unpublished observation). PGE_2 production was not completely abrogated in the *fet3*/*fet3* mutant strain, which indicates that other enzymes are involved. Studies are currently under way to construct null mutants with other *C. albicans FET* family genes and to test for defects in PGE_2 production.

C. albicans poses a significant clinical threat to both immunocompetent and immunocompromised individuals. Chronic colonization of mucosal surfaces with *Candida* is common, with carriage rates among healthy adults ranging from 30 to 70% (19, 38). Mechanisms by which *C. albicans* persists at mucosal surfaces in the face of an adaptive immune response are relatively unknown. We have demonstrated that *C. albicans* can produce an immune system signaling compound (PGE_2)

using fatty acids available within the host. PGE_2 is known to downregulate Th1 inflammatory responses and promote Th2-type responses or allergic responses, which are nonprotective during candidiasis (3, 11, 21, 32, 36). This local dampening of Th1 responses may also facilitate chronic mucosal persistence via the maintenance of immunologic "ignorance" in an otherwise immunocompetent host. Once conditions favor the outgrowth of *C. albicans* (as would occur with immunocompromise or antibiotic treatment), PGE_2 production may play a role as a virulence factor by downregulating the innate effector phase or the protective Th1 and response to the infection.

Mammalian inhibitors of eicosanoid production are effective at inhibiting *C. albicans* PGE_2 production. In addition, this class of inhibitors is also effective in inhibiting morphogenesis and biofilm formation as well as exhibiting antifungal activity on biofilms (1, 2, 29). The development of drugs that specifically target the fungal prostaglandin pathways may be one strategy to combat fungal colonization and infection. The identification of enzymes (encoded by *OLE2* and *FET3*) that participate at some level of PGE_2 biosynthesis will provide insights into the biochemistry of fungal prostaglandin production. However, a "prostaglandin null" mutant, which would definitively address the role of fungal eicosanoids in the pathogenesis of fungal diseases, remains to be discovered.

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