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Resveratrol Impaired the Morphological Transition of *Candida albicans* Under Various Hyphae-Inducing Conditions

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The ability of the human fungal pathogen *Candida albicans* to undergo the morphological transition from a single yeast form to pseudohyphal and hyphal forms in response to various conditions is known to be important for its virulence. Many studies have shown the pharmacological effects of resveratrol, a phytoalexin polyphenolic compound. In this study, we investigated the antifungal activity of resveratrol against *C. albicans*. Both yeast-form and mycelial growth of *C. albicans* were inhibited by resveratrol. In addition, normal filamentation of *C. albicans* was affected and yeast-to-hypha transition under serum-, pH-, and nutrient-induced hyphal growth conditions was impaired by resveratrol.

Keywords: *Candida albicans*, resveratrol (*trans*-3,4',-5-trihydroxystilbene), dimorphism

Candida albicans, a major fungal pathogen causing mucosal and systemic infections in immunocompromised hosts [5, 16], is dimorphic and grows as yeast as well as filamentous modes in host organisms and *in vitro* [19]. A key property of this fungus is its ability to switch from the yeast to the hyphal form in the host, and this has been implicated in the process of pathogenesis since mutants defective in hyphal growth are known to be less virulent in systemic infections [15]. This transition is known to occur in response to a variety of environmental conditions, such as the presence of serum, body temperature (37°C), neutral pH, and growth with a poor carbon source [19].

Resveratrol (*trans*-3,4',-5-trihydroxystilbene) is a phytoalexin polyphenolic compound produced by the innate host defense systems of plants [3, 9]. Although many studies have shown various pharmacological effects of resveratrol, for instance, antiviral properties, protective effects against inflammation, enhancement of stress resistance, and lifespan extension [3, 4, 7], little is known about its effects on fungi. The antifungal activity of resveratrol was first demonstrated by Jung *et al*. [10, 11]. However, Weber *et al*. [21] recently mentioned that the potential candidacidal activity of resveratrol was not confirmed. The effective concentration of resveratrol against *C. albicans* observed by other groups varies considerably [6, 13]. This suggests the difficulties still remain in the treatment with such biological agent. Therefore, there is a great interest in the effects of resveratrol on *C. albicans.* The present study was designed to gain a better understanding of the antifungal activity of resveratrol. We focused our attention on the examination of the effects of resveratrol on *C. albicans* growth, particularly the effects on morphological transition from single yeast cells to hyphal filaments under various hyphae-inducing conditions.

Fig. 1 and 2 represent the inhibitory effects of resveratrol (Sigma-Aldrich, St. Louis, MO, U.S.A.) on the yeast forms as well as on mycelial growth of *C. albicans* strain SC5314. *C. albicans* yeast (1×10^4 cells/ml in YPD) was incubated with various concentrations of resveratrol at 30°C for 16 h. The yeast growth was then assessed using a XTT {sodium3'-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate} reduction assay by measuring the colorimetric change at 490 nm, which is based on the cleavage of the yellow tetrazolium salt XTT to form an orange formazan dye by metabolically active cells, and is suitable for determination of *Candida* cell proliferation [8, 20]. The results were expressed as percentages of the untreated control. Resveratrol inhibited the growth of *C. albicans* yeast-form cells in a dose-dependent manner (Fig. 1). Significant inhibition of yeast-form growth was observed when *C. albicans* was treated at a concentration of 100 or 200 μg/ml. Specifically, yeast-form growth was reduced in the presence of 200 μg/ml resveratrol by >50% compared with culturing without resveratrol. To determine the effects of resveratrol on mycelial growth of *C. albicans*, a crystal violet (CV; Wako

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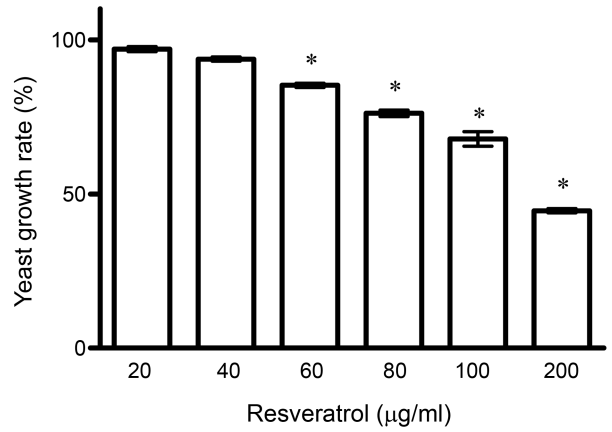

Fig. 1. Effects of resveratrol on the yeast-form growth of *C. albicans*.
Data represent means ± SD of three independent experiments performed in duplicate. Student's *t*-test *$p<0.01$ *vs.* resveratrol-untreated control.

Pure Chemical Industries, Osaka, Japan)-staining assay developed by Abe *et al*. [1, 2] was performed. *C. albicans* (1×10^4 cells/ml in RPMI1640 containing FCS) was cultured for 3 h and then incubated with various concentrations of resveratrol for 16 h under 5% CO_2 at 37°C. The photometrical absorbance at 590 nm of the *Candida*-bound CV extract, which reflected the number of viable hyphal *Candida*, was measured. The results were expressed as the percentage of the untreated control. Mycelial growth of *C. albicans* was affected by resveratrol. Resveratrol ranging from 40 to 200 μg/ml was capable of inhibiting mycelial growth of *C. albicans* but not in a dose-dependent manner (Fig. 2).

For experiments involving hyphal growth, *C. albicans* yeast cell suspensions were spread on hyphae-inducing solid media without or with resveratrol and the colonies

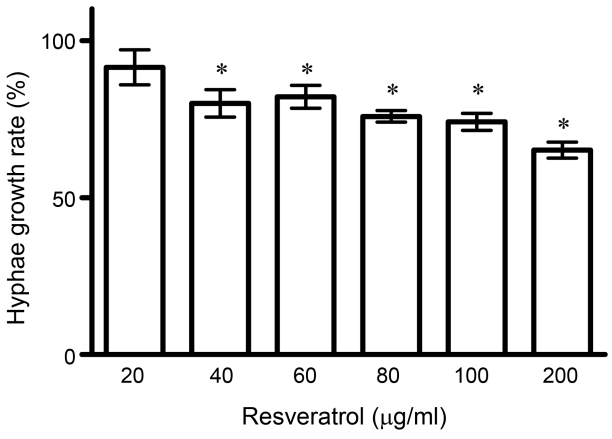

Fig. 2. Effects of resveratrol on mycelial growth of *C. albicans*.
Data represent means ± SD of three independent experiments performed in duplicate. Student's *t*-test *$p<0.01$ *vs.* resveratrol-untreated control.

were photographed after a 6-day incubation at 37°C. Resveratrol impaired the yeast-to-hyphae transition and induced colony morphological changes of *C. albicans* (Fig. 3). Normally, *C. albicans* cells on hyphae-inducing medium at 37°C are able to form extensive hyphae [14, 18]. However, under all conditions examined, resveratrol blocked hyphal outgrowth of mature colonies and the extent of hyphal growth was significantly reduced (Fig. 3). On YPD (1% yeast extract, 2% peptone, and 2% glucose per liter)+10% fetal calf serum (FCS, Sigma-Aldrich) agar plates that induce serum-mediated filamentation [19], the *C. albicans* colonies exhibited indistinct and diminished hyphal growth (Fig. 3A). The filamentation induced by the pH environment on Lee's medium (1% nutrient broth, 0.2% K_2HPO_4, and 1% glucose per liter) at pH 7 was markedly reduced when resveratrol was added. The hyphae around the colony cultured with resveratrol were shorter and less abundant than those of untreated colonies (Fig. 3B). Moreover, the colonies grown with resveratrol on the nutrient-limited media, such as synthetic low-ammonium-dextrose (SLAD) containing 50 μM ammonium sulfate as the sole nitrogen source or Spider medium that was modified from the liquid formulation by the substitution of glucose for mannitol, failed to form complete and wrinkle hyphae and the colonies were flat and small (Fig. 3C and 3D). To observe *C. albicans* hyphal development in liquid media, late-exponential-phase cultures grown in YPD at 30°C were inoculated into fresh hyphae–inducing media without or with resveratrol and incubated at 37°C for 1, 3, and 5 h. Preliminary experiments revealed that <40 μg/ml resveratrol was sufficient to inhibit hyphae formation by *C. albicans* (data not shown). Therefore, 40 μg/ml of resveratrol was added to the media. *C. albicans* (1×10^4 cells/ml in hyphae-inducing liquid media) was incubated without or with 40 μg/ml resveratrol at 37°C for 1, 3, and 5 h. After incubation, the numbers of hyphal cells were counted microscopically as previously described and the hyphae ratio was expressed as the percentage of each control [17]. Resveratrol affected the normal filamentation of *C. albicans* under various conditions (Table 1). The ratio of filamentation was significantly inhibited by resveratrol in all media examined. The ratio of hyphal cells compared with untreated control cells after incubation for 5 h was decreased by approximately 50% under serum-inducing condition, by 70% with pH-inducing condition, and by 80% under nutrient-limited condition, when resveratrol was added to the media. In addition, *C. albicans* generated short hyphae in the presence of resveratrol rather than the long and straight hyphae observed in control cells (data not shown). Taken together, resveratrol treatment resulted in incomplete hyphal morphology and significantly decreased hyphal formation under various filament-inducing conditions.

C. albicans is the predominant species of yeast isolated from patients with oropharyngeal candidiasis, which is

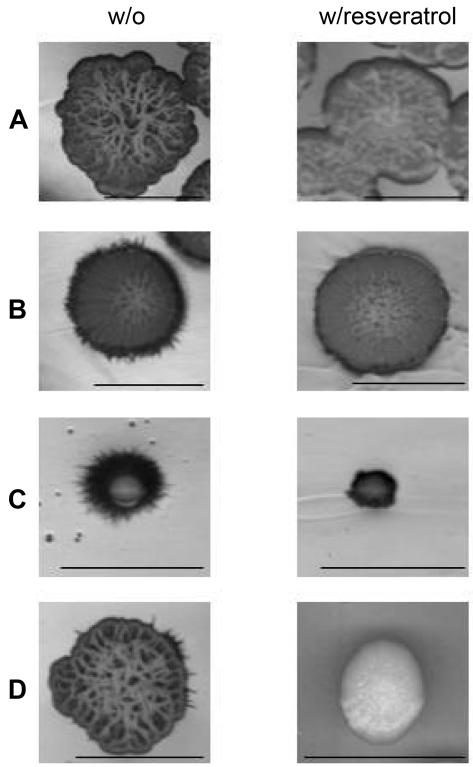

Fig. 3. Inhibition of hyphal outgrowth of *C. albicans* by resveratrol on hyphae-inducing plates: YPD+10% FCS containing 100 μg/ml resveratrol (**A**), Lee's at pH 7 containing 40 μg/ml resveratrol (**B**), Spider containing 100 μg/ml (**C**), and SLAD containing 40 μg/ml resveratrol (**D**).
Scale bars represent 1.0 cm.

a frequent symptom of human immunodeficiency virus infection. Relatively few classes of antifungal drugs are currently available for clinical treatment of oral and systemic candidiasis. Increased use of these antifungal agents to treat candidiasis has resulted in a dramatic increase in the emergence of drug-resistant candidal species [12]. Because of the toxic side-effects of these antifungal drugs, there is a need to evaluate novel antifungal agents as alternative drug therapies. This study showed that resveratrol was effective in the control of both cell types of *C. albicans* because it inhibited not only the growth of yeast-forms but also mycelial growth. In addition, the induction of yeast-to-hyphae morphological switching in *C. albicans* cells under various conditions such as serum induction, nutrient starvation, and neutral pH was impaired by resveratrol. It is not clear if resveratrol has a specific inhibitory effect on hyphal formation or simply attenuates mycelial growth-dependent hyphal formation. Additional approaches will be required to delineate the molecular basis. Nevertheless, our findings suggest that resveratrol has the potential to serve as an anti-candidal agent and as the basis for the development of new antifungal treatments.

Table 1. Hyphal ratio of *C. albicans* treated with resveratrol.

Medium	Incubation time (min)	Hyphae ratio (%)	
		Resveratrol (-)	Resveratrol (+)
YPD+10% FCS	60	77.1±1.3	19.6±0.6
	180	89.3±0.7	24.6±3.0
	300	95.8±1.1	44.8±3.1
Lee's	60	42.8±0.5	18.3±0.9
	180	77.5±1.0	21.5±2.1
	300	97.6±0.7	26.4±2.6
SLAD	60	36.8±1.3	15.4±0.7
	180	51.6±3.8	16.8±0.2
	300	92.6±1.0	17.7±0.7
Spider	60	45.3±2.3	15.2±1.5
	180	74.0±0.7	14.3±1.1
	300	93.9±0.9	16.8±0.9

Data represent means ± SD of three independent experiments performed in duplicate.

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