J. Microbiol. Biotechnol. (1998), **8**(3), 291 - 294

JOURNAL OF
MICROBIOLOGY AND
BIOTECHNOLOGY

NOTE

Deer Antler Extract Selectively Suppresses Hyphal Growth in Dimorphic Fungus, *Candida albicans*

PARK, HYUNSOOK[1], GIL-JA JHON[2,3], AND WONJA CHOI[1,3]*

[1]*Department of Biological Sciences,* [2]*Department of Chemistry, and*
[3]*Research Institute for Life Sciences, Ewha Womans University, Seoul 120-750, Korea*

Received: March 25, 1998

Abstract Transfer of *Candida albicans* grown in Sabouraud medium to the RPMI medium induces the transition from a nonpathogenic yeast form to a pathogenic hyphal form. This transition was severely inhibited in a dose-dependent manner when deer antler extract was added to the RPMI medium in a nontoxic range (up to 500 μg). In that range, deer antler extract inhibited the hyphal transition and cell growth, whereas no effect was observed on the yeast growth. When hydrophobic or hydrophilic fractions were prepared by detergent-solubilization of deer antler extract, the hydrophobic fraction showed a large degree of inhibition of the hyphal growth in *Candida albicans*. Neither fraction affected the growth in the yeast form. The pattern of chitin localization in the culture of the yeast form grown in RPMI in the presence of deer antler extract was confirmed by calcofluor staining and this exhibited strongly the suppression of hyphal transition.

Key words: Deer antler extract, *Candida albicans*, antifungal activity

Deer antler has been traditionally used as an invigorant rather than a medicine in Korea and China. A lot of efforts to investigate the pharmacological effects of antler has been made, but little information is available about its molecular and cellular pharmacological action. There are many reports on the physiological functions of antler: regulation of the level of glucose [4] and insulin in the blood [13]; increase of the iron concentration in red blood cells for curing anemia [9]; enhancement of the production of antibodies [5] and natural killer cells [4, 10]; reduction of cholesterol concentration in blood cells to reduce artherosclerosis [15]; release of stress [18]; relief of symptoms of senility [16].

*Corresponding author
Phone: 82-2-360-2892; Fax: 82-2-360-2385;
E-mail: wjchoi@mm.ewha.ac.kr

As mentioned above, one of the effects of antler is the augmentation of immunological functions [4, 5, 10]. The effect of antler on the immunological function may be caused by either the stimulation of the cellular immune system or the suppression of pathogenic activity of the microbes. Despite many reports on the immunological effects of deer antler on cultured cells or animals, no evidence has been reported about the effect on microbes at the biochemical level. In this study, the antifungal activity of antler was tested in relation to the suppression of pathogenicity of *Candida albicans*.

As a major etiologic agent of candidiasis, *C. albicans*, which is one of the normal human flora, shows opportunistic infections which cause life-threatening systemic and disseminated diseases in immunocompromised patients such as AIDS patients or those who have undergone prolonged administration of antibiotics [7, 12, 14]. Azole compounds are commonly used for the treatment of candidiasis [3, 6, 17] but side effects as well as relapses are serious [1, 11]. The major disadvantage of currently available antifungal agents is toxicity which causes severe side effects in the immunocompromised patients [1, 11]. Therefore, the development of new drugs specific to this yeast is needed.

Here, we report the effect of deer antler on the hyphal growth of *C. albicans*.

C. albicans is found in both the yeast form and the hyphal form in infected tissue, but it is widely believed that the hyphal form is responsible for the virulence [12]. One of the virulent factors is a self-morphology transition from yeast to hyphal form [12, 17]. This factor provides the rationale that *C. albicans* could serve as a model to test the effect of deer antler extract. Some agents in the extract which disturb the hyphal transition may suppress the spread or aggravation of the disease. This study was focused on the antifungal activity of deer antler extract.

Whole extract of deer antler (*Cervus nippon*) was prepared by reflux with *n*-hexane and chloroform followed

by extraction with ethanol. The extract was resuspended in water. The whole extract was further fractionated with $CHCl_3/CH_3OH$/2.5 M NH_4OH (20/10/2). The soluble and insoluble parts were designated subfraction A and subfraction B, respectively.

C. albicans grows in the yeast form in Sabouraud medium [12]. When the whole extract, subfraction A, and subfraction B of deer antler were added in three different concentrations to the culture, the yeast growth was not affected at all (Fig. 1). Regardless of initial cell density of the cultures, the growth pattern of each culture was the same as control. This confirms the result that the cytotoxicity of added extracts was not observed when tested by colony forming unit (CFU) assay (data not shown).

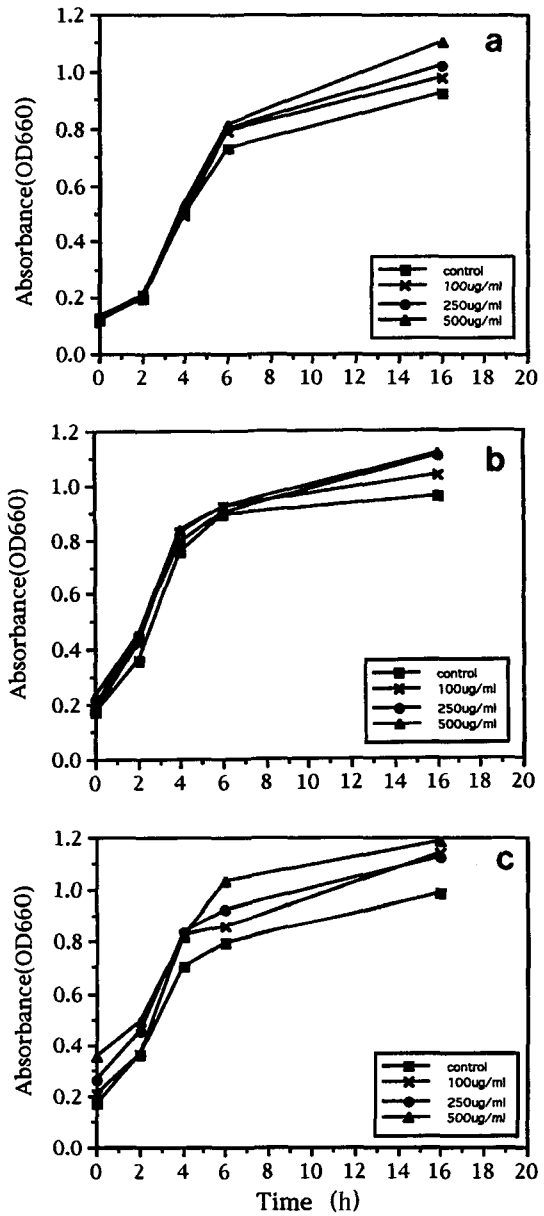

Fig. 1. Growth rate of SC5314 in Sabouraud-Dextrose media supplemented with deer antler extract.

Cultures with 100 μg, 250 μg, and 500 μg of each fraction were measured by the absorbancy at 660 nm; a, whole extract; b, subfraction A; c, subfraction B.

Transfer of a *C. albicans* culture grown in Sabouraud medium to RPMI medium induces hyphal growth for unknown reasons [12]. When RPMI medium was supplemented with whole extract of deer antler, the hyphal growth was suppressed in a dose-dependent manner (Fig. 2 and Table 1). At a concentration of 250 μg/ml of the whole extract, the hyphae were shorter, and the frequency of hyphae formation was reduced (Fig. 2C). However at a higher concentration of whole extract, 500 μg/ml, hyphae formation was severely suppressed with the increment of single blastoconidia cells (data not shown).

The supplement of subfractions of whole extract, the same results were obtained (Figs. 2D, 2E). Subfraction A suppressed hyphal growth to the same degree as whole extract, whereas addition of subfraction B resulted in a more severe inhibition of hyphal growth. Some morphological differences between these subfractions were observed. Firstly, formation of germ tubes occurred in the cultures with subfractions, however, the frequency was decreased in subfraction B. Secondly, the branch formation observed in subfraction A was not induced in subfraction B. Thirdly, only subfraction B grew in clusters, but not in subfraction A in our culture (Fig. 2F).

Hyphal suppression by deer antler extract was confirmed when stained by calcofluor [3]. Both subfractions A and B exhibited yeast growth, which was characterized by staining of the chitin ring and bud scar by calcofluor (Figs. 3A, 3D).

The degree of inhibition by whole extract and subfraction A was the same when determined by the percentage of hyphae (Table 1). At a concentration of 100 μg/ml, 250 μg/ml, and 500 μg/ml of whole extract and subfraction A, the hyphae formation was reduced by 75%, 60%, and 40%, respectively. In contrast, subfraction B reduced hyphae formation to 66%, 42%, and 28%, respectively, at the same concentrations.

In general, the yeast form is not virulent while the hyphae form is responsible for the virulence. None of the three fractions from deer antler had any effect on the yeast growth. In other words, deer antler did not affect the growth of the nonpathogenic form. This result was in contrast to the fact that deer antler extract exerts many effects by stimulating host cells or by its cytotoxicity to the hyphal growth of pathogenic fungi. It might be possible to develop deer antler as a new medicine to treat the immunocompromised patients whose immune system is hampered too much to be

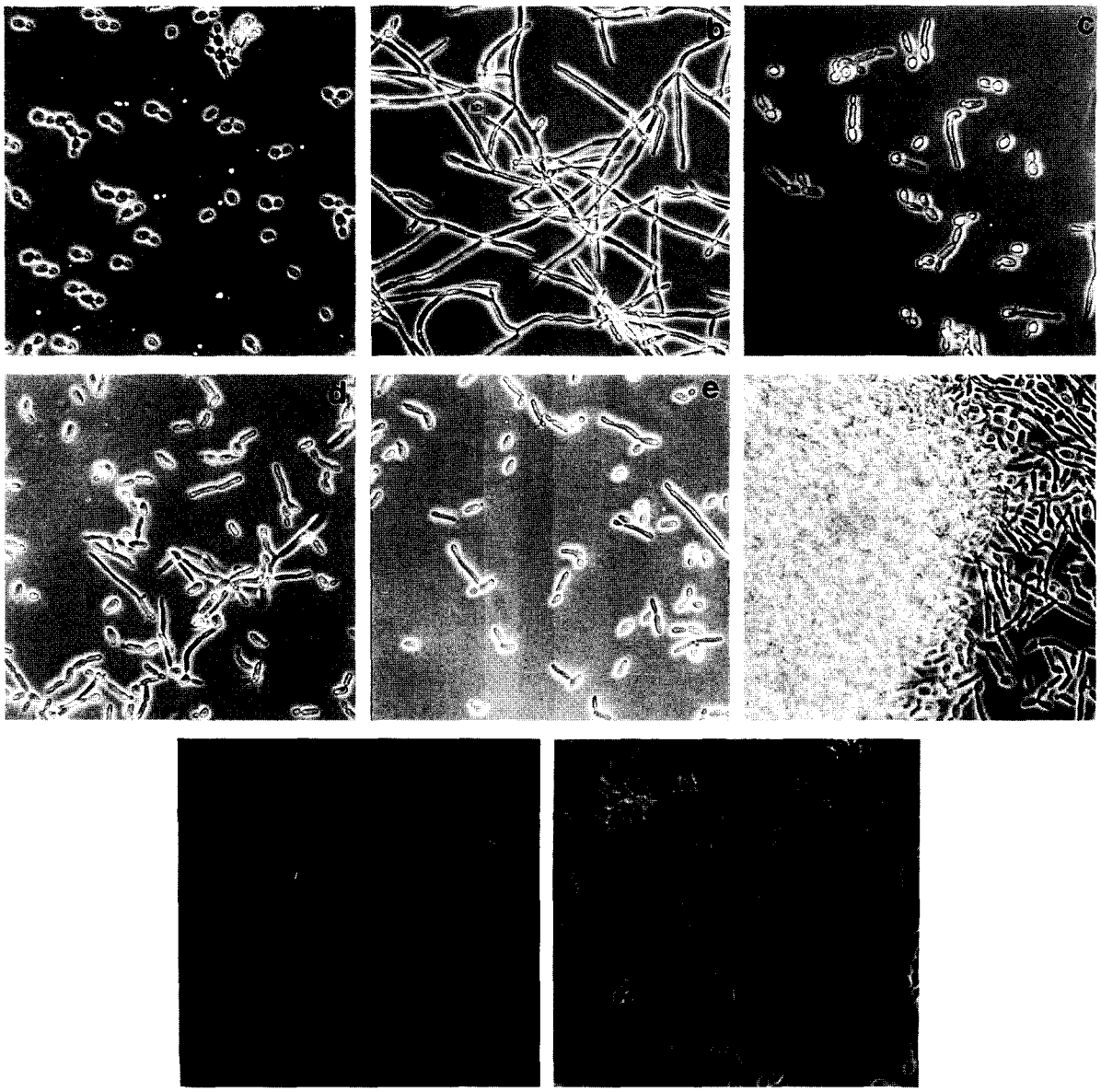

Fig. 2. Morphology of SC5314 by phase contrast microscopy.
a. cultures grown in Sabouraud-Dextrose media at 30°C without deer antler extract. b-f. the cultures grown in RPMI at 37°C; b, no deer antler extract as a control; c, 250 μg of whole extract; d, 250 μg of subfraction A; e, 250 μg of subfraction B; f, cluster part under same condition as e; g, 500 μg of subfraction A; h, 500 μg of subfraction B.

cured with available antifungal agents. The colony-forming unit (CFU) test suggested that the effect of deer antler on *C. albicans* was a suppressive activity (data not shown). Therefore, deer antlers could be used as a supplement to the patients to whom strong antifungal agents are not recommended for treatment.

According to the differences in the degree of inhibition and morphology of culture, it is likely that subfraction B is more effective than subfraction A in terms of hyphal suppression. It seems that the B fraction contains some components involved in the inhibition of transition to the hyphae form. Further fractionation will enable the identification of the compounds responsible for the anti-fungal activity.

In Korea, deer antler has been used as an invigorant widely. However, this study suggested the possibility to develop deer antler as a new antifungal medicine by showing the suppressive effect on the pathogenic growth of the microbe which is a major etiologic agent of candidiasis. Further study is necessary to identify the compound to develop a new medicine and reveal the molecular mechanism of deer antler.

Table 1. The inhibition of hyphal growth by deer antler extract.

Deer antler (μg/ml)	Hyphae formation (%*)		
	Whole extract	subfraction A	subfraction B
100	78	77	66
250	64	60	42
500	40	37	28

*Hyphae formation was obtained by the ratio of the number of hyphae to the number of total cells, both of which were counted with a hemacytometer.

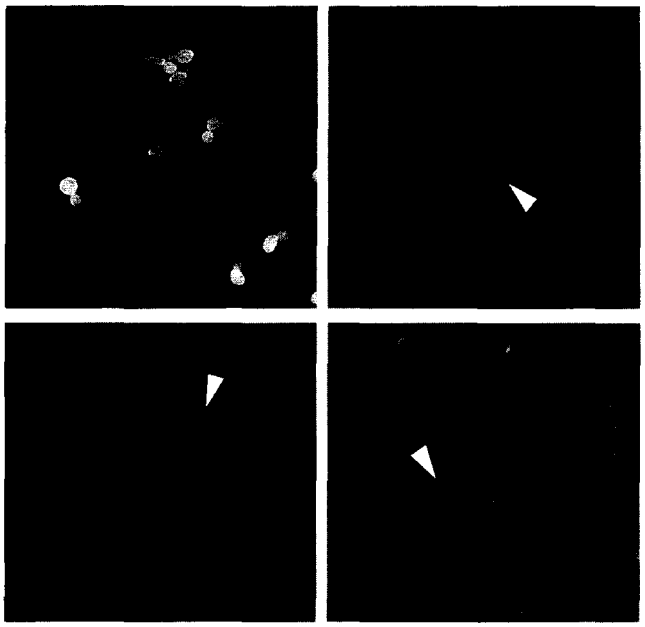

Fig. 3. Calcofluor staining of SC5314.
Washed cells were suspended in 0.01% calcofluor and the fluorescence was measured with an excitation filter at 365 nm. a, culture grown in Sabouraud-Dextrose without deer antler extract; b, the culture grown in RPMI without deer antler extract; c, subfraction A; d, subfraction B. Arrows indicate chitin in hyphal septum (b), and chitin ring and bud scar (c and d).

Acknowledgments

We thank Dr. R. Calderon for the strain SC5314. We thank Dr. E. Cabib for a gift of calcofluor white. This work was sponsored by the Korean Ministry of Education through research fund (BSRI-97-3442).

REFERENCES

1. Aabo, K. and R. Decoster. 1987. Hypertension during high-dose ketoconazole treatment: A probable mineral corticosteroid effect. *Lancet* **2:** 637–638.
2. Castanheira, G. 1988. Open study with oral ketoconazole in the treatment of acute vaginal candidiasis. *Curr. Ther. Res.* **43:** 369–373.
3. Roncero, C., M. H. Valdivieso, J. C. Ribas, and A. Duran. 1988. Effect of calcofluor white on chitin synthases from *Saccharomyces cerevisiae. J. Bacteriol.* **170:** 1945–1949.
4. Cho, H. J. and G. J. Jhon. 1994. Changes of gangliosides metabolism in streptozotocin-induced diabetic rats and effect of deer antler. *J. Appl. Pharm.* **2:** 223–228.
5. Choe, P. N. and K. H. Kim. 1987. Effect of *Cervi cornu* on depression of immune response induced by methotrexate in mice. *K. H. Univ. O. Med. J.* **10:** 589–604.
6. Dismukes, W. E., S. Shadomy, S. C. White, and H. P. Yu. 1985. Treatment of systemic mycoses with ketoconazole; *in vitro* susceptibilities of clinical isolates of systemic and pathogenic fungi to ketoconazole. *Ann. Intern. Med.* **103:** 861–872.
7. Giger, D., J. E. Domer, and J. T. Jr. McQuitty. 1978. Experimental murine candidiasis: Pathological and immune responses in T-lymphocyte-depleted mice. *Infect. Immun.* **21:** 729–737.
8. Kim, N. J. 1985. Effects of deerhorn extracts on the antibody production against *Escherichia coli* antigen. *J. Kor. Ori. Med. Soc.* **6:** 119–129.
9. Kim, Y. H., M. K. Shin, S. I. Lee, H. I. Lee, and W. H. Kim. 1979. Influence of the administration of *Cervi cornu* on the serum protein contents and the prothrombin time in rats. *K. H. Univ. O. Med. J.* **2:** 61–66.
10. Ko, B. H. and I. B. Song. 1990. The effects of deer antler, ginseng and ogapy on the immune response and NK cell activity. *Korean J. Medicine* **7:** 157–173.
11. Lewis, J. H., H. J. Zimmerman, G. D. Benson, and K. G. Ishak. 1984. Hepatic injury associated with ketoconazole therapy. *Gastroenterology* **86:** 503–513.
12. Odds, F. C. 1988. *Candida and Candidiasis.* 2nd ed. Bailliere Trindall, London.
13. Roe, J. S. 1981. Aqua-acupuncture effects of *Cervuc elaphus*, ginseng radix, in alloxan-induced diabetic mice. Thesis, Kyung Hee University.
14. Shepherd, M. G., R. T. M. Poulter, and P. A. Sullivan. 1985. *Candida albicans*: Biology, genetics, and pathogenecity, *Annu. Rev. Microbiol.* **39:** 579–614.
15. Shin, K. H., E. B. Lee, J. H. Kim, M. S. Chung, and S. Y. Cho. 1989. Research on the pharmaceutical activity of powered deer antler (I). *Korean J. Pharmacology* **20:** 180–187.
16. Son, N. W., M. K. Shin, and H. I. Lee. 1986. The effects of deer antler on the neuro response of starved mice. *Korean J. Oriental Medicine* **7:** 174–183.
17. Vanden Bossche, H. 1985. Biochemical targets for antifungal azole derivatives: hypothesis on the mode of action. *Drug Development Res.* **1:** 313–351.
18. Wang, B. X., H. O. Zhou, and Y. Wang. 1992. Purification and partial characterization of anti-inflammatory peptide from pilose antler of *Cervus nippon* temminck. *Acta Phamaceutica Sinical* **27:** 321–324.