Chitohexose Induce the Yeast Proliferation of Candida albicans

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Candida albicans generally grows in the hyphae form in RPMI1640 medium. Chitohexose (COS-6) induced the yeast form of C. albicans dose-dependently under this condition. When COS-6 was exposed to C. albicans, yeast proliferation was observed 6 h after starting the incubation. When we observed the growth form of C. albicans cultured with COS-6, yeast proliferation was observed at log-phase. These results showed that COS-6 was useful for the yeast form inducer of C. albicans.

Key words Candida albicans; chitohexose; chitohexose; morphological change; yeast; hyphae

Chitin is a polymer of N-acetylglucosamine and a component of crustacean and insect exoskeletons.3) Generally, chitooligosaccharides are prepared by deacetylation and hydrolysis of chitin. Chitooligosaccharides possess several biological activities such as antimicrobial activity2–4) and elicitation of plant defense reactions.5) Candida albicans is a fungus found in normal microbial flora that generally exists in the oral mucous, skin, intestinal organs and vagina. C. albicans is an opportunistic fungus that has pathogenic activity in immunosuppressed humans such as patients treated with immunosuppressive drugs, acquired immunodeficiency syndrome (AIDS) patients and other immune-compromised hosts.6,7) C. albicans is morphologically changed from the yeast form to the pseudohyphae or hyphae form depending on the growth conditions. C. albicans grows in the hyphae form in the infected region, and the virulence is higher in the hyphae form than in the yeast form.8) Yeast to hyphae transition is triggered by a variety of external factors such as pH,9) temperature10) and oxygen.11) Control of the morphological changing of C. albicans is important for treating Candida infection, thus, we examined the effects of chitohexose as a regulator of hyphae formation in C. albicans.

MATERIALS AND METHODS

Strains The C. albicans NIH A-207, ATCC 1542, ATCC 1621, ATCC 1768, ATCC 2076 and ATCC 2640 strains were routinely grown in Sabouraud’s medium (peptone 10 g, glucose 20 g and yeast extract 5 g per liter) at 27℃. To induce hyphae formation, the cells were grown in RPMI1640 medium (Nissui Pharmaceutical) at 37℃ in 5% CO2.

Reagents Chitohexose (COS-6) was provided by Yaizu Suisankagaku Industry. This solution was acidic in RPMI1640 medium, therefore, each solution was neutralized with 1N NaOH before the assay.

Effect of COS-6 on the Growth Form of C. albicans C. albicans yeast cells were suspended in RPMI1640 medium supplemented with COS-6 (1×10⁷ cells/ml) and incubated at 37℃ in 5% CO2. After incubation, the number of yeast cells was measured using a hemocytometer. The amount of total cells (yeast and hyphae cells) was calculated by measuring optical density at 620 nm. Absorbance was convert to numbers of cells using calibration curves prepared with yeast cells. The accuracy of this measurement was verified using NucleoCounter™ YC-100 (MS Techno Systems). NucleoCounter™ YC-100 can count the amount of nuclei and convert this value into the number of cells. When the number of hyphae cells of C. albicans was counted using this system, the resultant number was similar to the number calculated from absorbance (data not shown).

Statistical Analysis Values are shown as means±S.E., and statistical analysis of these data was performed using Student’s t-test. p<0.05 was considered significant.

RESULTS AND DISCUSSION

To examine the effect of COS-6 on the morphological changing of C. albicans, the cells were cultured with COS-6 and the ratio of yeast cells was measured. Before the examination, the COS-6 solution was neutralized with 1N NaOH because COS-6 is acidic in RPMI1640 medium. A corresponding concentration of neutralized salt solution was used as the negative control. The ratio of yeast cells was dose-dependently increased by COS-6 compared with the negative control (Fig. 1), indicating that COS-6 could inhibit the hyphae growth of C. albicans. The yeast proliferation by COS-6 was also induced in C. albicans ATCC 1542, ATCC 1621, ATCC 1768, ATCC 2076 and ATCC 2640 strains (data not shown). These results showed that COS-6 generally induced the yeast proliferation of C. albicans. To examine when the yeast proliferation was initiated by COS-6, COS-6 was exposed to C. albicans for 1 to 8 h then incubated until 24 h. After incubation, the ratio of the yeast cells was measured. As shown in Table 1, exposure to COS-6 for more than 6 h was needed to induce yeast proliferation. These findings indicated that exposure of COS-6 of C. albicans for more than 6 h induced yeast proliferation. Yeast proliferation of C. albicans treated with COS-6 was not observed until 6 h after the incubation (data not shown). As C. albicans NIH A-207 is at lag-phase until 6 h of incubation, we suggested that COS-6 affected at lag-phase of C. albicans. To observe the style of yeast proliferation at log-phase, the growth form of C. albicans treated with COS-6 was examined photographically. C. albicans generally grows in the hyphae form in RPMI1640 medium, but the addition of COS-6 induced yeast proliferation at log-phase (Fig. 2). As shown in Table 1, the sensitivity

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of C. albicans to COS-6 was enhanced at log-phase. Although, the mechanism of which yeast proliferation was induced by COS-6 remains unclear, we would like to analyze the expression of COS-6 receptor and sugar metabolism in C. albicans. Amphotericin B and neticonazole are also induced the yeast proliferation of C. albicans.12) However, chitoooligosaccharides did not inhibit the growth of C. albicans.

Therefore, we thought that COS-6 was useful as the yeast inducer of C. albicans.

REFERENCES