SHORT COMMUNICATION

The Effects of Xanthorrhizol on the Morphology of Candida Cells Examined by Scanning Electron Microscopy

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The incidence of invasive fungal infections, particularly those caused by Candida sp., has increased over the past few decades (Hsu et al. 2005). Recently, strains of Candida sp., such as C. albicans, C. glabrata, C. guilliermondii, and C. parapsilosis are showing increased resistance to traditional antifungal agents (Hawser and Dauglas 1995; Nguyen et al. 1996; Barchiesi et al. 1999; Dauglas 2003). This demonstrates the great importance of identifying novel antifungal agents (Ficker et al. 2003). Recent years have seen a growing interest in the use of natural antifungal agents isolated from the medicinal plants.

Xanthorrhizol, a novel bioactive compound isolated from the rhizome of temulawak or java turmeric (Curcuma xanthorrhiza Roxb.) on the morphology of four human pathogenic Candida species, i.e., C. albicans, C. glabrata, C. guilliermondii, and C. parapsilosis was examined by scanning electron microscopy (SEM). The SEM analysis showed that, unlike control cells representing normal oval to spherical with smooth surface, treatment of Candida strains with xanthorrhizol at 1 x MICs (minimum inhibitory concentration) significantly affected the external morphology, exhibiting deformation, and protrusions on the cell surface. The potent antifungal activity of xanthorrhizol may support the use of medicinal plants for the treatment of candidal infections.

Key words: antifungal, Candida sp., scanning electron microscopy, xanthorrhizol

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Fig 1 Structure of xanthorrhizol (Hwang et al. 2000a).
min. The final pellets were then resuspended in sterile water. A drop of each suspension was transferred onto glass cover slips and fixed onto aluminium SEM stubs (Agar Science Ltd, Standstead, UK). The drop was spread thinly on the slip and dried in the air for 2 hour at room temperature. Graded concentrations of ethanol (70, 80, 90, and 95%, vol/vol), were applied, 2-5 min each, to ensure complete dehydration of specimens. The specimens were coated with gold in a low-pressure argon atmosphere employing a model ES500 Polaron Sputter Coating Unit (Polaron Equipment Ltd, New Haven, West Sussex, UK). A JEOL JSM-840 scanning electron microscope (Jeol Technics Ltd, Tokyo, Japan) was used to evaluate samples, operating at accelerating voltages of 20-25 kV (Helal et al. 2006).

SEM analysis showed that, unlike control cells (antifungal-agent free) showing normal oval to spherical shapes with smooth surfaces, treatment of Candida species with MIC of xanthorrhizol affected the external morphology of these yeasts (Fig 2). Control cells displayed well-formed cells with smooth unadulterated surface (Fig 2a, c, e, g). In contrast, cells incubated in the presence of xanthorrhizol demonstrated a greater tendency to clump compared with the control cultures (e.g., C. albicans - Fig 2b). Xanthorrhizol-treated C. glabrata cells showed minor abnormalities without a smooth or a slightly awkward surface (Fig 2d). Xanthorrhizol-treated Candida cells exhibited deformation and protrusions on the cell surface, which was more clearly demonstrated with C. guilliermondii and C. parapsilosis (Fig 2f, h).

Electron micrographs revealed the existence of a recognizable affected external morphology of Candida cells caused by xanthorrhizol. Visible deformation, protrusion, or clumping was noted for each species at concentration MICs for 1 h treatment. In general, Candida exposed to xanthorrhizol at concentrations 1 x MICs exhibited substantial ultrastructural abnormalities such as shape deformation, protrusion, rugged cells surface, and clumping. Although, we were not able to identify the underlying molecular changes caused by the compounds by scanning electron microscope after 1 h treatment, we were able to show that the observable cell wall changes were generally obtained following exposure of the isolates to concentrations of xanthorrhizol equal to 1 x MICs. Analysis of electron micrograph at the appropriate exposure time and higher concentrations (2 x MICs or 4 x MICs) may result in more detailed analyses of the activities and effects of antifungal agents (Klepser et al. 1998). Further studies have been conducted examining the effect of xanthorrhizol on the morphology Candida cells at 2 x MICs and 4 x MICs for 2 and 4 h of incubation.

In summary, the potent anticandidal action of xanthorrhizol against strains of four human pathogenic Candida species was demonstrated by scanning electron microscopy analysis. The results showed the usefulness of xanthorrhizol, a promising new antifungal agent for the topical treatment of candidiasis.

Fig 2  SEM of Candida albicans (a and b), C. glabrata (c and d), C. guilliermondii (e and f), and C. parapsilosis (g and h) after treating by xanthorrhizol at 1 x MIC for 1 h of incubation.
REFERENCES


