ULTRASTRUCTURAL LOCALIZATION OF PHOSPHOLIPASE ENZYME IN VAGINA CANDIDA ALBICANS

PRINCIPLE INVESTIGATOR : *Pramote Vanittanakom*

CO-INVESTIGATORS : *Nongnuch Vanittanakom*

Sungwal Rugpao

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FINAL REPORT

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BY

*PRAMOTE VANITTANAKOM**
*NONGNUCH VANITTANAKOM ***
*SUNGWAL RUGPAO****

*Department of Pathology,
** Department of Microbiology,
***Department of Obstetrics and Gynaecology,
Chiang Mai University, Chiang Mai, Thailand

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Ultrastructural Localization of Phospholipase Enzyme in Vaginal Candida Albicans

ABSTRACT

Candida albicans is the most common cause of vaginal candidosis. Without the clinical symptom it is difficult to distinguish between the normal flora and pathogenic C. albicans. C. albicans which is a saprophyte under normal condition may cause vaginitis when present in large number or when the condition of host is changed. Much attention has been focused toward defining the pathogenic mechanisms of the organism. This study concerned with phospholipase enzymes which was considered to be one of pathogenic factors. The localization of phospholipase was demonstrated in ten clinical isolates of C. albicans by electron microscopy. Similar results were obtained in all specimens. The phospholipase activity was localized at the cell wall, the wall attached between budding yeast cells, the start of budding, the cell membrane, and in the cytoplasm. However, the enzyme activity was not detected in some yeast cells.

INTRODUCTION

Candida albicans is the most common cause of vaginal candidosis[1-3]. The organism is endogenous as normal flora on the mucocutaneous region in the vagina of about 3.8% of healthy women[4]. Without the clinical symptom it is difficult to distinguish between the normal flora and pathogenic C. albicans. C. albicans may cause vaginitis when present in large number or when the condition of host is changed. The most importance is hormonal change which can be commonly found in pregnant women. Other factors are prolonged antibiotic therapy and presence of some underlying diseases. In some cases the causative agent is exogenously sexual transmitted[3].

Much attention has been focused toward defining the pathogenic mechanisms of the organism. The ability of C. albicans to produce phospholipases is considered to be an important pathogenic feature of this pathogen[5-8]. Phospholipases can damage cell membrane and are active components of bacterial toxins, arthropod poisons and snake venoms which injure or kill their victims[9,10]. Mammalian phospholipases are observed and they are enhanced in patients with bacterial and viral infection[11]. In C. albicans several phospholipases were secreted by growing yeast cells[12]. They were phospholipase A. lysophospholipase, lysophospholipase-transacylase and phospholipase B.

The role of phospholipase activity in the yeast cells of C. albicans either in relation to themselves or in relation to infective processes is unknown. Pugh and Cawson studied the localization of phospholipase in cells of C. albicans one isolate which infected the chick chorio-allantoic membrane[8]. Other experiments had been done in the C. albicans isolates from oral cavity[13,14] from blood, wound and urine[15]. It was suggested that candidal phospholipase seemed to play a complex role in the etiopathology of human candidosis.

This study was emphasized on the ultrastructural localization of phospholipase enzyme in C. albicans isolated from the patients with vaginal candidosis who showed clinical apperance.

MATERIALS AND METHODS

The yeast cells was fixed in cold formalin calcium chloride solution for 2 h at 0°C. After fixation the fixative was removed by centrifugation. The cells were washed once or twice (5 min each) and resuspended in a small volume of cold Ringer solution. Drops of the suspension were added to the freshly prepared incubation mixture consisting at 9 parts of 0.2% lead nitrate in 0.05 M acetate buffer pH 5.0 and 1 part of substrate solution. The substrate solution was 1.0 mg/ml lecithin in 0.1% triton X-100. The cells were incubated for 20-30 min at 37°C. Control incubation were carried out using heat inactivated cells or omitting substrate from the incubation mixture. After incubation the cells were removed by centrifugation, wash once or twice (2 min each) in distilled water, then post fixed in 1% osmium tetroxide buffered to pH 7.2 with cadodylate buffer at 4°C overnight. After osmium imprgenation the cells were rinsed, dehydrated, embedded in Araldite and the sections were prepared for electron microscopy.

Phospholipase activity was localized by coupling the fatty acid released by substrate hydrolysis with a heavy metal (lead) in a Gomori type procedure. Lecithin was used as a substrate to demonstrate the activities of phospholipase A and lysolecithinase.

RESULTS

The localization of phospholipase activity was observed in ten clinical isolates of C. albicans by electron microscopy. Similar results were obtained in all specimens. The substrate used in this experiment was lecithin. Lecithin was hydrolysed by phospholipase A into fatty acid and lysolecithin. The lysolecithin was further split by lysolecithinase into fatty acid and glycerylphosphoryl choline. The enzyme activity was localized by coupling the released fatty acid with lead which could be demonstrated as electron dense material.

The negative control experiments confirmed that there were no enzyme acitvity in the yeast cells (Fig. 1)

The phospholipase activity was slightly localized in the plasma membrane and in the cytoplasm whereas the enzyme activity granules were found at the cell wall (Fig. 2). Some yeast cells showed a numerous fine granules around the cell wall and unevenly heavy deposit at one side of the plasma membrane (Fig. 3). The distribution of enzyme activity seemed to be vary during the cell cycle. At the start of budding, the enzyme activity was strongly localized at the start of budding side and at another side of the yeast cell (Fig. 4). During elongated budding, the yeast cells showed strong enzyme activity at some part of the plasma membrane (Fig. 5). In the budding, the enzyme activity was strongly located at the cell wall attached between budding yeast cells (Fig. 6). However, the enzyme activity was not detected in some yeast cells.

Figure 1. Transverse section of control negative phospholipase yeast cell. Candida albicans showing nucleus (N) with double membrane (NM), small mitochondria (M), cell membrane (CM) and cell wall (CW). (X 60,000)

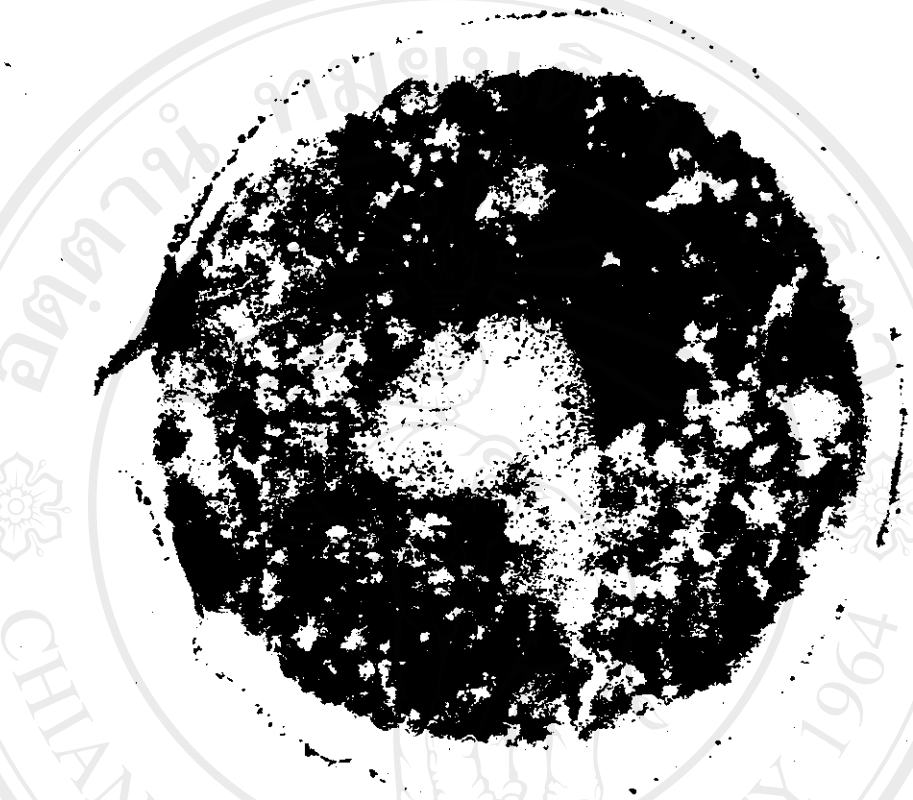

Figure 2. Positive phospholipase Candida yeast cell. Strong enzyme activity granules are found in the cytoplasm and at the cell wall. (X 65,000)

Figure 3. Candida yeast cell.
The phospholipase activity is localized as a fine granules around the cell wall and unevenly concentrated at one side of the plasma membrane. (X 65,000)

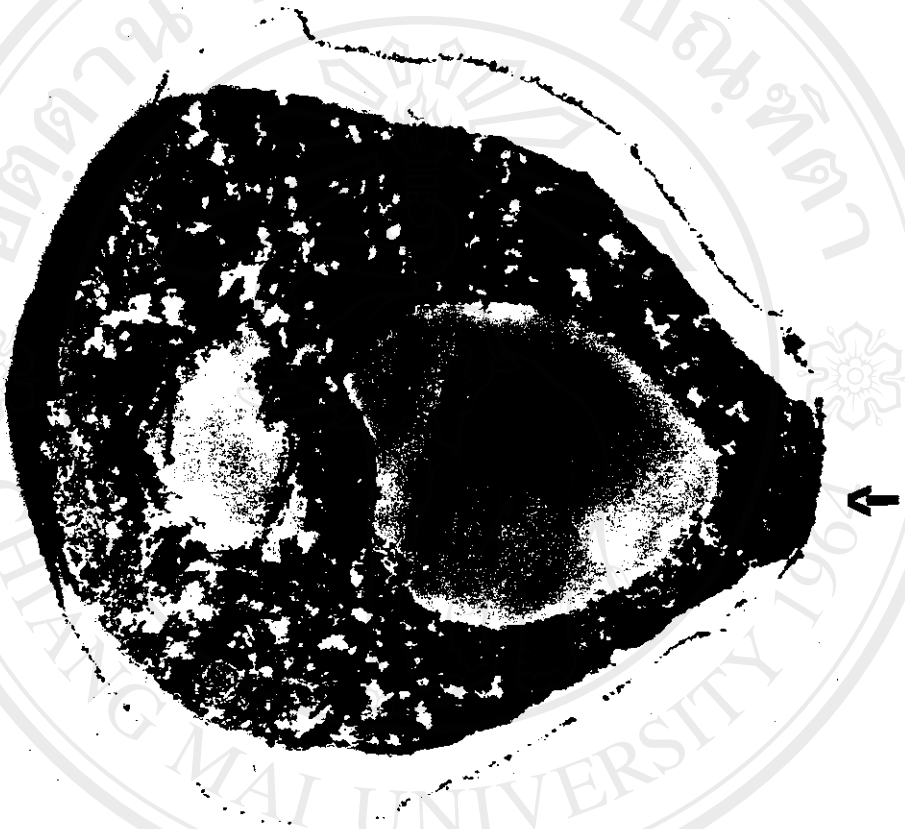

Figure 4. Candida yeast cell showing strongly enzyme activity at the start of budding (arrow) and at another side of the cell. The distribution of enzyme activity is also found in plasma membrane and cytoplasm. (X 65,000)

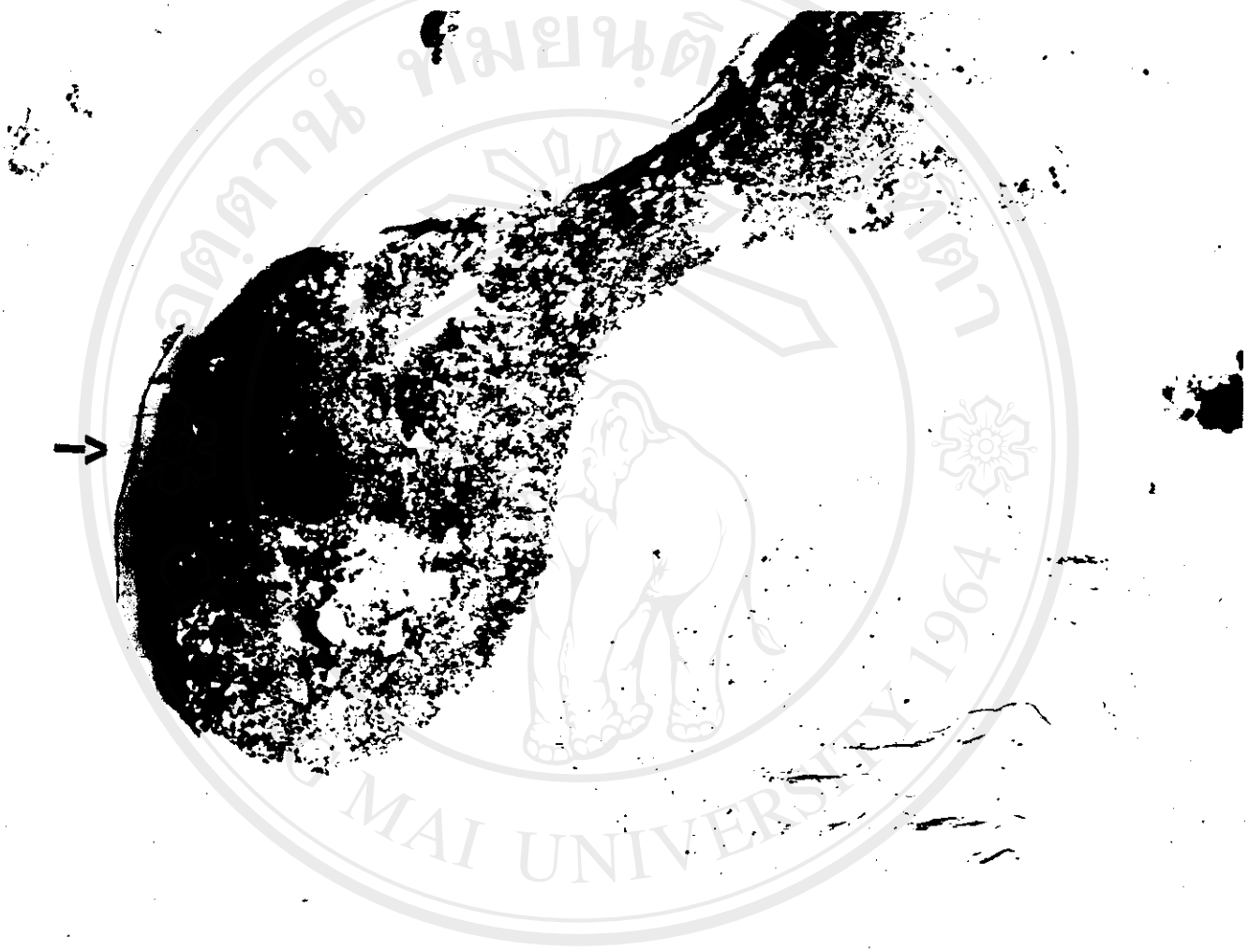

Figure 5. Elongated budding yeast cells. The enzyme activity is strongly localized at some part of the plasma membrane (arrows). (X 32,000)

Figure 6. Budding yeast cell of Candida albicans, showing strongly phospholipase activity at the cell wall attached between budding cells. The enzyme is also found in the plasma membrane and cytoplasm.
(X 82,000)

DISCUSSION

These experiments show that the phospholipase activity was localized at the cell wall, the wall attached between budding yeast cells, the start of budding, the cell membrane and in the cytoplasm. These results correspond to the report of Pugh and Cawson[7]. The distribution of enzyme activity seems to be vary during the yeast cell cycle. The enzyme activity is localized in the periphery of the yeast cell at the region where a bud will develop. The phospholipase activity is associated with bud formation. It seem to be that the phospholipase activity in the yeast cells of C. albicans plays a role in relation to infective process. Phospholipase damages the cell membrane of host cells and causes lesions of candidiasis[9,10].

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