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### **Influence of Cultural Conditions on Glutathione Peroxidase Synthesis in *Candida albicans***

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**Abstract:** The influence of cultural conditions that affect GPX production in *Candida albicans* grown in Lee's medium was investigated. Optimum temperature and pH for GPX activity were 25°C and 7.2, respectively. Substrate specificity for *C. albicans*. Glutathione peroxidase was in the order of cumene hydroperoxide>t-butyl hydroperoxide>hydrogen peroxide>benzoyl peroxide. Aeration as well as large head space volume enhanced the growth of *C. albicans* and GPX production. Arabinose and ammonium sulphate significantly increased the GPX synthesis. Among nitrogen sources, polypeptone enhanced both the growth and GPX synthesis. Various cellular activities are regulated by the level of GSH. Therefore, the level of GPX might be used as one of the criteria in developing new drugs against *Candida albicans*.

**Key words:** *Candida albicans*, glutathione peroxidase, opportunistic yeast pathogen, cultural conditions, antioxidant glutathione

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### **INTRODUCTION**

Glutathione peroxidase (GPX; EC 1.11.1.9) is one of the important antioxidant enzymes that protects the cell against oxidative damage. Glutathione peroxidase converts the reduced glutathione (GSH) to its oxidized form (GSSG) in the following reaction:



In animals, this enzyme was studied extensively and found in two forms namely selenium (Se) dependent GPX and selenium independent GPX (Wendel, 1980; Arthur, 2000; Li *et al.*, 2004). The Se-dependent enzyme contains selenocysteine at the active site and catalyzes the reduction of peroxides as well as organic hydroperoxides with glutathione as its hydrogen donor (Brown *et al.*, 2007; FU *et al.*, 2007). The Se-independent GPX is active with organic hydroperoxides only and is identical with some GSH transferase (Prohaska, 1980). Missall *et al.* (2005) reported two glutathione peroxidases in the fungal pathogen *Cryptococcus neoformans* are expressed in the presence of specific substrates. In mammalian studies selenium is known to protect the cells against the toxic effects of heavy metals (Nordberg *et al.*, 1978). The activity of GPX in general, is known to be influenced by age, species and several environmental and nutritional factors (Manavathu *et al.*, 1996).

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*Candida albicans* an opportunistic yeast belongs to the class fungi Imperfecti and is present as a part of normal flora. This opportunistic yeast is capable of invading and colonizing the body tissues when the host immunity is impaired (Prasad, 1991). This organism releases different metabolites into the bloodstream, causing varying symptoms such as lethargy, chronic diarrhea, yeast vaginitis, bladder infections, muscle and systemic candidiasis (Prasad, 1991).

*Candida albicans* possesses two distinct morphological forms as yeast and mycelium and this phase transition plays a major role in its pathogenicity (Prasad, 1991). These morphologic changes are due to various environmental and nutritional conditions such as pH and temperature, carbon and nitrogen sources (Gunasekaran *et al.*, 1995). This study reports on the influence of cultural conditions on growth and GPX production in *C. albicans* cells grown in a synthetic medium amended with various testing compounds (such as nutrients, inducers and inhibitors) under different environmental (temperature and oxygen stress) conditions.

## MATERIALS AND METHODS

### Organism and Culture Medium

The strain of *Candida albicans* (3153A) was maintained in Sabouraud dextrose agar (Difco) at 37°C and utilized in the present investigation (Gunasekaran *et al.*, 1995). Each 250 mL Erlenmeyer flask containing Lee synthetic medium (Lee *et al.*, 1975) was seeded with 1.0 mL of yeast suspension and incubated in a rotary shaker at 100 rpm and 25°C for 72 h or the time specified in each set of experiments.

### Preparation of Cell Free Extract

Yeast cells grown in Sabouraud dextrose broth were harvested at 72 h of growth by centrifugation at 7,000x g washed with 0.5 M of potassium phosphate buffer, pH 7.5 containing phenylmethylsulfonyl fluoride (1 mM), EDTA (4 mM) and 2-mercaptoethanol (5 mM) and resuspended in the same buffer. Cells were homogenized in the same buffer and the homogenate was centrifuged at 20,000x g for 20 min at 4°C. The supernatant was used as the cell free extract for enzyme assay.

### Enzyme Assay

#### Glutathione Peroxidase Activity

Glutathione-peroxidase activity was measured in a coupled enzyme method by measuring the decrease of NADPH at 340 nm (Aisaka *et al.*, 1983). The reaction mixture, which initially contained 570 µL of 0.1 M potassium phosphate buffer with 1 mM of EDTA (pH 7.0), 100 µL of crude enzyme, one hundred microliters of 10 mM GSH and 30 µL of glutathione reductase from Baker's yeast (50 units of lots [129H7465 and 78H7430]) was incubated at 37°C for 10 min. One hundred microliters of pre-warmed 12 mM t-butyl hydroperoxide was incubated accordingly following incubation. One hundred microliter of 1.5 mM NADPH were added to the pre-warmed assay mixture and the peroxide independent consumption of NADPH was monitored every 30 sec for 3 min with a Hitachi U-3000 spectrophotometer. The reaction was initiated by adding pre-warmed 12 mM t-butyl hydroperoxide to the assay mixture. The decrease in absorption was monitored every 30 sec for 5 min. The GPX activity in the enzyme sample was determined using the appropriate extinction coefficient and the liner slope of the absorbance values obtained from the t-butyl hydroperoxide consumption of NADPH (Flohe and Gunzler, 1984). Protein was measured by the method of Bradford (1976). All the experiments were repeated 3 times and the values represent the mean of triplicates and these values are given in the Fig. 1-6.

#### **Effect of Carbon and Nitrogen Sources on GPX Production**

The organism was grown in Lee medium for 72 h at 25°C to study the influence of four types of carbon sources arabinose, sucrose, glucose and gluconic acid at two concentrations (1.25 and 10%, 86 and 690 mM, respectively) on GPX production was tested. To determine the effect of different nitrogen source, *C. albicans* was grown at 25°C for 72 h in Lee medium substituted for ammonium sulphate, with potassium nitrate, urea, glutamic acid and the media were prepared as previously described, with the addition of the respective nitrogen at 38 mM concentration.

#### **Effect of Temperature on GPX Production**

To determine the optimum temperature for GPX production, the organism was grown at four different temperatures (15, 25, 37 and 45°C) for 72 h in Lee medium, harvested and the crude enzyme extracts were assayed as described earlier.

#### **Effect of Oxygen Tension on GPX Production**

The effect of oxygen tension on GPX production, four sets of 250 mL flasks containing 15, 25, 50 and 100 mL of Lee medium were prepared and inoculated with yeast cells and grown as a shake culture in yeast form at 25°C for 72 h.

#### **Effect of Peroxides on GPX Production**

To determine the substrate specificity of GPX, five types of peroxides (cumene peroxide, t-butyl peroxide, hydrogen peroxide, potassium peroxide and benzoyl peroxide) at 12 mM concentration were measured.

#### **Kinetics of Growth on GPX Activity**

For determination of the changes in GPX activity at different growth phases, flasks with Lee medium were inoculated with *C. albicans* and incubated at 25°C for 120 h. At 24 h interval cell growth was measured.

#### **Effect of pH on GPX Production**

To determine the influence of pH on GPX production, the yeast was grown in Lee media with pH values ranging from 3.0 to 8.5 at 25°C for 72 h. At the end of growth period, cells were harvested and the dry weight was determined.

#### **Effect of Media**

To study the influence of media on the growth and GPX activity in *C. albicans*, the organism was grown in three types of media, Lee synthetic medium, Sabouraud Dextrose Broth (SDB) and Tryptic Soy Broth (TSB) were prepared as per manufacturer's instructions (Difco) and grew the organism at 25°C for 72 h. The growth and GPX activity were measured.

### **RESULTS AND DISCUSSION**

Among the four tested carbon source, glucose supported the maximum growth followed by sucrose. However, maximum GPX production was observed from the cells grown in arabinose followed by gluconic acid (Fig. 1). At higher concentration GPX production was reduced regardless of the type of carbon source.

The influence of various nitrogen sources on GPX production revealed that polypeptone supported the maximum growth and enzyme production (Fig. 2). Among the other nitrogen sources, the least amount of growth and enzyme production was found in cells grown in potassium nitrate.

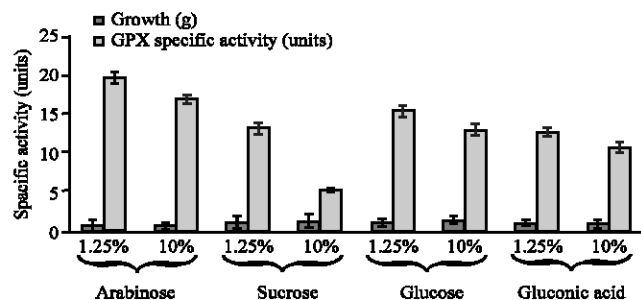


Fig. 1: Effect of carbon source on the GPX synthesis in *C. albicans*

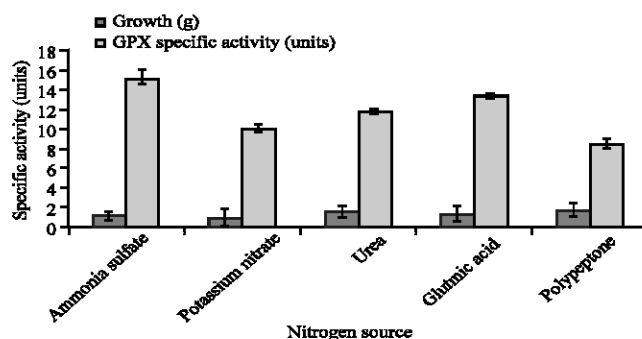


Fig. 2: Effect of nitrogen source on the GPX synthesis in *C. albicans*

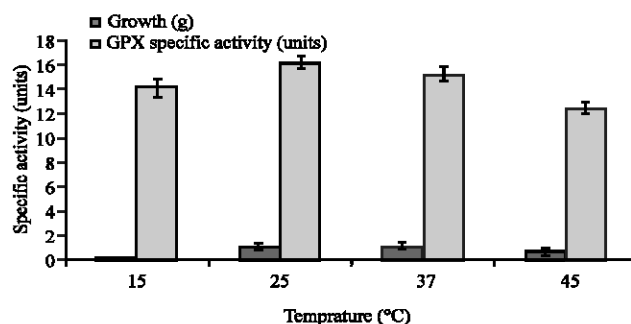


Fig. 3: Effect of temperature on the GPX synthesis in *C. albicans*

Under assay conditions, optimum temperature for growth and GPX production was found to be 25°C (Fig. 3). Very little difference was observed in enzyme activity between the cells grown at other temperatures.

The influence of growth of *C. albicans* on GPX activity revealed that the enzyme activity reached its maximum at 48 h followed by a steady decrease in later growth periods. The highest observed GPX activity during the early part of *C. albicans* growth could be due to its active metabolism.

The study on substrate specificity of *Candida* GPX, on different hydroperoxides showed that cumene peroxide was the preferred substrate for the enzyme as compare to other substrates tested (Fig. 4). The least effective substrate for GPX was found benzoyl peroxide.

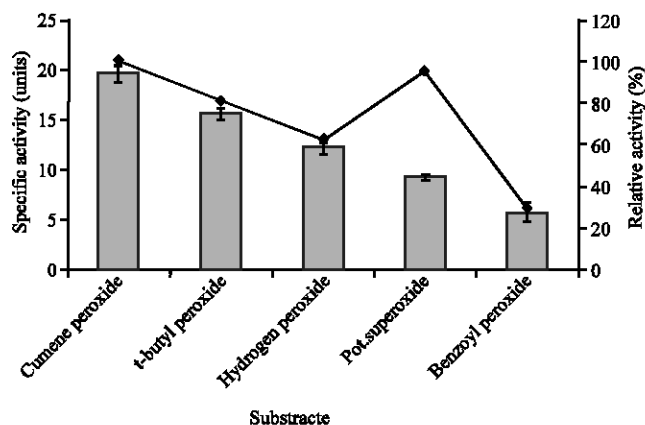


Fig. 4: Substrate specificity of GPX in *C. albicans*

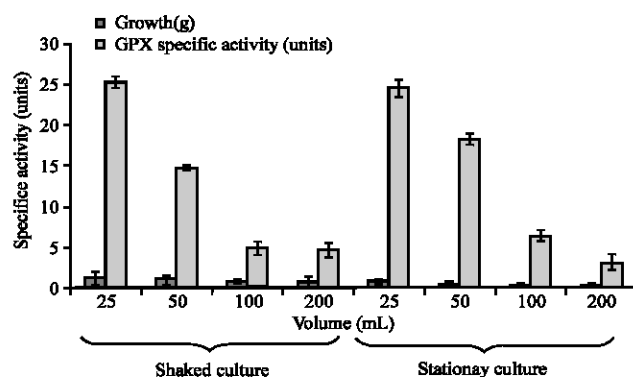


Fig. 5: Effect of oxygen tension on the GPX synthesis in *C. albicans*

The results on the influence of oxygen tension on GPX are shown in Fig. 5. Maximum GPX activity was observed from cells grown in flasks containing 25 mL of medium in either shaking or stationary conditions. However, more enzyme activity was observed from the shake culture. The enzyme production was reduced as the volume of medium increased regardless of the type of culture. Similar findings were observed with GPX from *Mucor hiemalis* (Aisaka *et al.*, 1983).

In order to determine the inductive effect of the substrates of GPX, cumene peroxide, t-butyl hydroperoxide and hydrogen peroxide were added to the warm medium under sterile conditions. For comparison, cells were grown in Lee medium without any of the tested with compounds. Maximum induction was observed by cumene peroxide as compared to other hydroperoxides (Fig. 6). Although, an increase in enzyme activity was found at higher concentration (1 mM), except in the case of cumene peroxide, there is no correlation between the concentration and the activity.

Maximum growth occurred between pH 6.0-6.5 and the GPX activity was found to be maximum at pH 6.0. Among the three tested media, maximum growth found maximum in SDB followed by Lee medium. On the other hand GPX activity was maximum in Lee medium followed Sabouraud dextrose broth and GPX activity were maximum in Lee medium followed by TSB. The factors that influence the synthesis and the properties of *C. albicans* GPX are very similar to other organisms reported earlier (Sundquist and Fahley, 1988; Stohs, 1990).

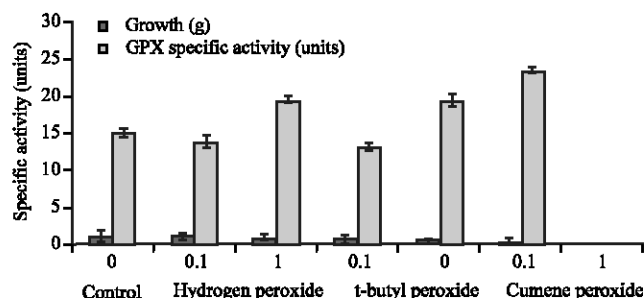


Fig. 6: Effect of peroxide on the GPX synthesis in *C. albicans*

## CONCLUSION

In the present study, we found that GPX production was influenced by various cultural and nutritional factors. Future experiments are planned to study on the mechanism(s) of antioxidants on GPX as well as the purification and characterization of GPX from *Candida albicans*.

## ACKNOWLEDGMENTS

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