ROLE OF OXYGEN AND ANTIOXIDANTS IN THE RESPONSE OF YEAST TO HEAT AND OXIDATIVE STRESSES

BY

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A thesis submitted for the degree of Master of Rural Science
In the Department of Biochemistry, Microbiology and Nutrition
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July, 1995

STATEMENT

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree.

I certify that to the best of my knowledge any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

Jeane B. Tjandiagung
July, 1995

ACKNOWLEDGEMENTS

I am deeply grateful to my supervisor, Associate Professor Ken Watson, for his patience, helpful and friendly assistance and guidance. Without his continuous encouragement, it would have been very difficult to complete my study program on time.

Sincere thanks also to all the members of the yeast group for their help and advice on my work, especially to Tracey Swan, Debbie Webster and Claudia Gross. Thanks also go to Dr. D. Evans and Mrs. R. Muldoon for reading some of my earlier drafts of the thesis.

My appreciation goes to the Australian Government, through the Equity and Merit Scholarship Scheme (EMSS), for the opportunity given to me for acquiring higher academic competence.

My special thanks go to my mum, my husband, Tris and my wonderful daughter, Audesia for their encouragement, understanding and patience while I undertook this study.

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ABSTRACT

A study was conducted on the physiological effects of heat and oxidative stress on the yeast *Saccharomyces cerevisiae.* The effects of oxygenation and synthetic and naturally-occurring antioxidants on cell survival were examined. Two cell types were used as experimental systems at 25°C. In one case, cells were grown aerobically in an orbital shaker and, in the other, cells were grown anaerobically in water-jacketted fermenters under a constant stream of high purity nitrogen. Cells were subjected to a mild heat shock from 25°C to 37°C for 45 min before exposure to a heat stress at 48°C over a time course. The ability of cells to survive, this heat protocol, as determined by plate counts, was termed induced thermotolerance. On the other hand, the ability of cells to survive a direct heat stress from 25°C to 48°C over a time course was termed intrinsic thermotolerance. Oxidative stress was measured by incubation of cells at 25°C with H_2O_2 (5-10 mM).

In general, aerobically grown cells were more stress tolerant than anaerobically grown cells. This observation was correlated, to some extent, with the membrane fatty acyl composition of the cells. The addition of synthetic antioxidants (butylated hydroxytoluene, propyl gallate) was detrimental to cell survival against heat. By contrast, naturally-occurring antioxidants (ascorbate, α-tocopherol, β-carotene) in general appeared to act as growth promotants. Oxygenation, either during or after the heat treatments, was highly beneficial (3 to 15-fold) to cell survival. However, oxygenation prior to thermal treatment was detrimental as was oxygenation in the presence of cycloheximide which inhibited protein synthesis.

Heat shock induced (37°C/45 min) protein synthesis was monitored by ^{35}S-methionine labelling and SDS-PAGE analysis. The classical heat shock proteins (hsp) hsp 100, hsp 90, hsp 70, and hsp 60 were identified in aerobic and anaerobic cells. Additional bands at 36 and 40 kDa were observed in cells

oxygenated (45 min) at 25°C but no additional bands were observed in cells subjected simultaneously to heat treatment and oxygenation.

Trehalose concentration in anaerobic cells (8-12% w/w) was relatively high compared to aerobic cells (< 0.5% w/w). Heat shock induced a modest increase in trehalose levels. These results indicated that heat shock proteins but not trehalose may play a role in stress tolerance.

The major conclusions from the present studies were that exogenous antioxidants, synthetic or naturally-occurring, had no significant protective effect on yeast thermotolerance or oxytolerance. The marked beneficial effects on cell survival by oxygenation were proposed to be partly due to protection of key mitochondrial functions.