CHAPTER 5

DISCUSSION

1. ANAEROBIC VERSUS AEROBIC

1.1. Temperature

Temperature is one of the most important environmental factors influencing the growth and survival of microorganisms, including yeasts (Watson, 1987). Increasing temperature can cause the rapid growth of cells by increasing chemical and enzymatic reactions within cells and thus stimulating growth. On the other hand, high temperature can damage cells due to irreversible denaturation of proteins, nucleic acids and other cellular components.

Understanding the mechanism(s) by which yeast cells adapt to temperature has fundamental and economic applications. The latter include the commercial application of food preservation, including the effect of heating and cooling on microbial spoilage of foodstuffs. In this respect the heat shock response, characterised by rapid changes in cellular physiology and increased tolerance to various stresses, especially heat, has attracted considerable attention in the past 15 years or so.

In the present studies, the response to heat and peroxide stress of aerobically or anaerobically grown yeast cells, exhibiting different membrane lipid compositions, was examined. As shown in Fig 3.3, heat shock-induced thermotolerance to a heat stress at 48°C was highest in aerobically grown cells followed, in order, by oleic (C18:1) and then linolenic (C18:3) acid supplemented anaerobically grown cells.

The differences in heat stress tolerance could, to a limited extent, be correlated with the degree of membrane lipid unsaturation or unsaturation index (Table 4.2). The indices vary from 0.75 for aerobically grown cells to 0.80 and 1.45 for the anaerobic cells grown on oleic or linolenic acid respectively. The

higher the unsaturation index, the more fluid the membrane. Thus, cells grown anaerobically with C18:3 as lipid supplement have the highest unsaturation index and are the most sensitive to heat stress at 48°C. On the other hand, the aerobic cells have the lowest unsaturation index and are the most heat resistant. These observations are consistent with the widely accepted model, the fluid mosaic model (Singer and Nicolson, 1972), for biological membranes. This model predicts that biological membranes, which consist primarily of lipids and proteins, have to be in a semi-fluid state to be fully functional. According to this model, cell physiology is disrupted at high temperatures when membranes become too mobile or fluid and , conversely, at low temperatures, membranes are too rigid. The greater heat tolerance of aerobic cells, as compared to the anaerobically grown cells, may therefore be correlated with the lower unsaturation index and hence lower membrane fluidity.

In earlier studies on *Escherichia coli*, Overath *et al.* (1970) reported that C18:1 supplemented cells could grow at temperatures above 45°C whereas C18:3 supplemented cells were unable to grow above 40°C. Yatvin (1977), also working with *E. coli,* latter reported that C18:1 supplemented cells were more thermotolerant than C18:3 supplemented cells. These observations agree well with the present studies, at least for the anaerobically grown cells. It is clear, however, that membrane fatty acyl composition is not directly a measure of membrane fluidity. The latter would also be influenced by the type, conformation and arrangement of the membrane proteins as well as the interaction of membrane proteins with the membrane lipids. Recent studies in this laboratory (Swan & Watson, personal communication) have examined the relationship among membrane lipid composition, membrane fluidity and stress tolerance in yeast. These studies have revealed no simple relationship amongst these three parameters. Furthermore, membrane fluidity, as measured by the fluorescence probe 1,6-diphenyl-1,3,5-hexatriene (DPH), was not directly related to the membrane lipid composition. On the basis of these studies, it was

suggested that membrane proteins may play an important role in stress induced cell damage.

In considering other parameters influencing heat stress tolerance in yeast, the role of heat shock proteins and trehalose in particular should be considered. These two parameters were examined in the present studies and the discussion of the results presented in section 3 and 4. Other factors which have recently been proposed to be of importance in yeast stress tolerance include the plasma membrane ATPase (Piper, 1993, Coote *et al.* 1994;) and antioxidant enzymes (Hou, 1992 Steels, 1994) but these are beyond the scope of the present studies.

1.2. Oxidative stress

In order to establish a baseline for the response to oxidative stress of aerobically or anaerobically grown cells, the effects of a H_2O_2 challenge on the two cell-types were investigated. As illustrated in Fig. 3.2 (aerobic cells) and Fig. 3.5 (anaerobic cells), aerobically grown cells were significantly more resistant to H_2O_2 stress than anaerobically grown cells. In very recent studies from this laboratory (Steels *et al.*, 1994; Steels, 1994) it has been confirmed that tolerance to H_2O_2 in yeast is greater in aerobic than in anaerobic cells. Although this conclusion from the present studies and those of Steels *et al.* (1994) are not surprising, no reports on the relative tolerances of aerobic and anaerobic yeast cells appear in the published literature.

These differences may be related to the distinct metabolic activities of the two cell types. In the present studies, anaerobic cells were grown in high glucose media in a constant atmosphere of high purity nitrogen, conditions in which cells were obtaining energy essentially through glycolysis. On the other hand, the aerobic cells, although subjected to glucose catabolic repression, were obtaining energy through both glycolysis and the tricarboxylic acid cycle-respiratory pathways. Cells operating under the latter pathway may be

anticipated to be more able to cope with oxidative stress through production of antioxidant enzymes. In this respect there is evidence in the literature for higher levels of superoxide dismutase (SOD) in aerobic yeast cells as compared to anaerobic cells (Gregory *et al.*, 1974; Lee and Hassan, 1986).

Although there are conflicting data with respect to the precise levels of SOD in yeast cells, the more recent studies from this laboratory (Steels, 1994) have shown distinct differences in SOD levels in aerobic and anaerobic cells. These studies have show a 2-fold higher level of CuZn-SOD in aerobic cells over anaerobic cells. Moreover, the 4-fold higher level of Mn-SOD, associated with the mitochondria, in the former cell type emphasises the differences in levels of antioxidant enzymes in the two cell types. In addition to the SOD enzymes, levels of the antioxidant enzyme cytochrome c peroxidase (Verduyn *et al.*, 1988) are markedly higher, up to 10-fold, in aerobic cells (Steels, 1994).

The levels of antioxidant enzymes in relation to the effects of oxygenation and thermotolerance are discussed further in a later section.

2. ANTIOXIDANTS AND THERMOTOLERANCE

In this series of experiments, the thermosensitivity of cells as influenced by the synthetic antioxidants, butylated hydroxytoluene (BHT) and propyl gallate (PG), and the naturally-occurring antioxidants, glutathione, ascorbate, β-carotene, α–tocopherol and ergosterol was examined.

Data from the present experiments showed that levels as low as 100 ppm PG increased cell thermosensitivity during induction of thermotolerance. On the other hand, BHT at 100 and 200 ppm had marginal effects on cell thermotolerance and was only inhibitory at much higher levels (750 ppm). The present results indicate that BHT and particularly PG, which are used as antioxidants for the prevention of lipid peroxidation in foods (Davidson *et al.*, 1983), also have antimicrobial activity. In one of the very few reports in the published literature on the effects of artificial antioxidants on yeast, Eubank and

Beuchat (1983) reported that PG (at 1000 ppm) enhanced the rate of heat inactivation, measured at 50°C, of *S. cerevisiae*. Other food antioxidants such as butylated hydroxyanisole (at 100-200 ppm) were also found to enhance heat induced death in *S. cerevisiae*.

It has recently been shown that heat shock of yeast cells at 37°C induces lipid peroxidation, as measured by thiobarbituric acid formation (Steels *et al.* 1994). The lipid peroxidation presumably arising from free radical induced damage to membrane phospholipids. Furthermore, a mild heat shock has been reported to induce the antioxidant enzyme superoxide dismutase in *E. coli* (Privalle & Fridovich, 1987), *Halobacterium halobium* (Begonia & Salin, 1991) and, very recently, also in *S. cerevisiae* (Steels *et al.*, 1994; Hou, 1992; Steels, 1994). The induction of antioxidant enzymes and lipid peroxidation in yeast by a mild heat shock would indicate that heat shock and oxidative stress, induced by oxygen-derived free radicals, may share a common mechanism. It might be anticipated, therefore, that antioxidants, acting as free radical scavengers, may protect cells from heat damage.

In the present studies, preincubation of cells, prior to heat treatment, with the naturally-occurring antioxidants, glutathione and ascorbate, had only a marginal effect on cell survival (Fig. 3.3). If anything, at 20 mM glutathione or ascorbate, there was a measurable detrimental effect on cell survival.

A synergistic effect of heat and ascorbate in inducing cell killing in various microorganisms was reported by Mackey and Seymour (1989). In one experiment, these authors reported that addition of isoascorbate (1mM) accelerated thermal death in *Candida zeylanoides* on exposure to a 52°C heat stress. There are few, if any, other reports on heat and antioxidant effects of ascorbate or glutathione on yeast cells. In a relatively recent publication, Cheng and Wilkie (1991) reported a protective effect by vitamin E (α–tocopherol), ascorbate and glutathione on survival of *S. cerevisiae* cells exposed to vitamin A. The authors speculated that the toxicity of vitamin A may be related to

generation of free radical intermediates and that protection could be induced by antioxidant detoxification by ascorbate, glutathione and vitamin E.

It would appear, however, from the present studies that heat stressed yeast cells, at least under the present experimental conditions, are not associated with the generation of free radicals. On the other hand, oxidative stress imposed by H_2O_2 would be expected to be associated with free radical damage to cells. In the present studies, preincubation of cells with ascorbate or glutathione prior to heat stress improved cell survival, at least in the short term (15 to 60 min), by 5-10% (Fig. 3.9 and 3.10).

It is debatable if this degree of apparent protection against heat can be taken as any indication, certainly not a strong one, that ascorbate and glutathione were functioning as antioxidants and thereby mopping up free radicals. It is more likely that these antioxidants were acting non-specifically, perhaps as growth promoters. Support for this concept comes from experiments in which various antioxidants were incorporated into agar media. In particular, addition of ascorbate (Fig. 3.11) and to a much lesser extent α-tocopherol (Fig. 3.12) to agar media resulted in higher percentage survival to heat stress than in control media.

Overall, the present experiments demonstrated that, for the antioxidants tested, there was marginal protection against heat or oxidative stress. There may be several explanations for this observation. One is that heat stress is not associated with production of free radicals or at least of the kind that can be scavenged by the antioxidants tested in the present studies. On this particular point, it should be emphasised that there is little indication, at least in the published literature, of what naturally-occurring antioxidants actually function in *Saccharomyces*. On the other hand, highly pigmented yeasts, especially of the genera *Rhodotorula*, *Sporobolomyces* and *Phaffia* contain high concentrations of β-carotene, a known antioxidant (Moore *et al.*, 1989; Schroeder and Johnson, 1993). The fact that β-carotene had little effect on cell survival of

stressed *Saccharomyces* cells, may simply reflect the absence of any suitable mechanism whereby β-carotene could be assimilated and/or utilised as an antioxidant in this particular yeast species. Similarly, ascorbate (vitamin C) and α-tocopherol (vitamin E) are not found in significant amounts in yeasts generally (Reed and Nagodawithana, 1991) and thus may not be suitable as experimental antioxidants. Historically, β-carotene (vitamin A), ascorbate (vitamin C) and α-tocopherol (vitamin E) have been extensively examined as potential antioxidants in higher eucaryotes, acting as free radical scavengers (Sies, 1986; Halliwell and Gutteridge, 1989). There is accumulating evidence that these antioxidants act as oxygen-derived free radical scavengers in a variety of situations, including human diseases (Halliwell and Gutteridge, 1989).

By comparison, there is evidence that glutathione, a known antioxidants in lower and higher eucaryotes (Meister and Anderson, 1983; Penninckx and Elskens, 1993), may play a role in protecting yeast cells against some stresses. The latter include the detoxification of some chemicals and protection against metal-chelating and thio-reactive agents (Murata and Kimura, 1987a). Glutathione, although not essential for yeast viability, has been shown to play a key role in cell proliferation (Ohtake *et al.*, 1990). At this point in time, there is little evidence in *Saccharomyces* for any naturally-occurring antioxidant which may play a role in protecting cells against a particular stress, be it heat, ethanol, oxidative or some other stress. There is scant evidence in the literature for precise identification of free radicals or their intermediates in yeast cells, apart from the indirect observation of lipid peroxidation (Steels *et al.*, 1994) and antioxidant enzymes (Hou, 1992; Steels, 1994) induced by heat and H_2O_2. Nevertheless, the fact that oxygen-derived free radicals and antioxidants, both enzymatic and non-enzymatic, are ubiquitous in nature would warrant further investigation of the yeast system.

3. OXYGENATION

A major finding of the present studies was that oxygenation of cells during stress treatment was highly beneficial to cell survival. Furthermore, oxygenation after heat stress also resulted in improved cell survival or perhaps more correctly, cell recovery.

These observations were somewhat unexpected in that the prediction was that a combination of stress, e.g. heat, and oxygen should have resulted in increased, rather than decreased, sensitivity to the stress. Hyperoxic conditions induce formation of oxygen-derived free radicals, including superoxide radical and the highly reactive hydroxyl radical. These radicals are thought to cause extensive damage to all cell types (Halliwell and Gutteridge, 1989).

Oxygen also has the opposite effect of inducing antioxidant enzymes. In yeast, exposure of cells to 100% oxygen is known to increase SOD activity (Lee and Hassan, 1986). More recently, Steels (1994) has reported increased SOD activity, especially the mitochondrial associated Mn-SOD, of 40% and 20% in anaerobic and aerobic yeast cells respectively exposed to 100% oxygen. Conversely, it has been reported that yeast SOD activity, especially the cytoplasmic CuZn-SOD, decreases on transition from aerobiosis to anaerobiosis (Clarkson *et al.*, 1991). Interestingly, mutants lacking CuZn-SOD (Bilinski *et al.*, 1985) or Mn-SOD (Van Loon *et al.*, 1986) are adversely affected in the presence of hyperoxia and fail to grow normally. In the case of Mn-SOD-deficient mutants, the cells were particularly sensitive to oxidative stress (Van Loon *et al.*, 1986).

Other antioxidant enzymes of importance in oxidatively stressed yeasts are glutathione peroxidase (Galiazzo *et al.*, 1987) and cytochrome c peroxidase (Verduyn *et al.*, 1988). The latter enzyme in particular increases markedly on exposure of cells to hyperoxia (Steels, 1994). In the present studies, levels of antioxidant enzymes were not measured as time and the scope of the thesis

did not permit this major undertaking. Nevertheless, it could be implied, from previous work in this laboratory (Steels, 1994), that increases in antioxidant enzymes, particularly Mn-SOD and cytochrome c peroxidase, would have occurred during oxygenation. However, an obvious and a most important parameter, in the present studies with respect to oxygen and heat stress was the temperatures at which the cells were exposed to oxygen. It should be noted that the solubility of oxygen in aqueous solutions decreases markedly with increasing temperature. The solubility of oxygen at 25°C is 211 mM, at 37°C it is 167 mM and at 48°C it is 120 mM (Hitchman, 1978). At higher temperatures, therefore, oxygen may become rate limiting. This effect would be further accentuated by a higher rate of metabolic activity.

Oxygenation at the different temperatures would presumably lead to increased levels of oxygen available to cells. The increased oxygen levels may allow cells to better cope with heat stress, especially at the higher temperatures (37°C and 48°C) at which increased metabolic activity may require increased oxygen supply. This is almost certainly an over-simplified explanation of the oxygen effects observed in the present studies.

In an early study, Graumlich and Stevenson (1979) reported that endogenous respiration of heat damaged yeast cells was substantially higher than non-heated controls. However, in these studies it was questionable whether the cells were actually severely damaged or not (heat stressed at 56°C for 1 to 2 min), as survival was essentially 100% as determined by viable plate counts. By contrast, experiments in this laboratory have shown that even a mild heat shock at 37°C for 30 to 45 min leads to a 50% decrease in whole cell respiration (Swan, personal communication) and also decrease in mitochondrial respiration and function (Ferrari, 1994). The latter studies have demonstrated that mitochondria are a key target for heat stressed induced damage. Furthermore, a mild heat shock (37°C for 30 min) provided substantial

protection of mitochondrial oxidative phosphorylation against heat stressed induced damage (Ferrari, 1994).

The oxygen effects as observed in the present studies may therefore be related to protection of key mitochondrial functions by oxygen during heat stress. In this respect it should be noted that Van Uden (1984) has previously proposed the mitochondrial inner membrane as target sites for thermal and alcohol induced damage. Support for this concept comes from the studies of Jimenez and Benitez (1988) which suggest that the stability of yeast mitochondrial DNA may be a key factor in determining cell viability under conditions of high temperature and ethanol concentrations.

The concept that oxygen may be protecting key mitochondrial functions, especially at high temperatures, would also hold in the case of yeast grown anaerobically. Under conditions of strict anaerobiosis, yeast still retain mitochondrial profiles, the so-called promitochondria, although these are structurally distinct as observed in the electron microscope from those observed in the aerobic cell (Plattner and Schatz, 1969; Watson *et al.*, 1970; 1971). Furthermore, it has been demonstrated that these promitochondria, which contain mitochondrial DNA (Criddle and Schatz, 1969; Watson *et al.*, 1971), do perform important, if not essential, cellular functions and these include transport processes and energy-requiring (ATP) reactions (Subik *et al.*, 1972, Visser *et al.*, 1994). It is thus envisaged that even in the case of anaerobic cells, oxygen may function to protect against heat-induced damage to key mitochondrial functions. Oxygenation or aeration of anaerobic yeast is known to stimulate the synthesis of unsaturated fatty acids and ergosterol, both of which are essential cellular components and require oxygen for synthesis (Andreasen and Stier, 1953, 1954). Moreover, it has been proposed that the fermentation performance of brewing yeasts is positively correlated with lipid composition, especially ergosterol content, which in turn is dependent on oxygen levels (Molzahn, 1989). The higher the oxygen level, the greater the ergosterol

content and the higher the fermentation efficiency (Molzahn, 1989). The viability and fermentative capacity of such cells are reportedly much improved (Boulton and Quain, 1987). Interestingly, the oxygen induced synthesis of ergosterol shows good correlation with accumulation of trehalose (Callaerts *et al.*, 1993).

Oxygen limitation has been shown to result in loss of cryotolerance in yeast (Gelinas *et al.*, 1989) and this factor was more important than trehalose content. Gelinas and Goulet (1991) extended these observations to examine the morphology of such cells subjected to oxygen saturation. These authors reported that cells grown under excess oxygenation exhibited extensive invaginations in their cytoplasmic membranes and, more importantly, possessed giant mitochondria. It was speculated that the lower trehalose content and relatively poor mitochondrial development in oxygen-deprived growth media could be involved in the greater cryosensitivity of such yeasts (Gelinas *et al.*, 1989). Survival in frozen and thawed *E. coli* has also been reported by Swartz (1970) to be enhanced to some extent by the presence of oxygen.

In the present studies, oxygenation was extremely beneficial to cell survival not only during heat shock and heat stress but also after the heat treatments. This observation would therefore lead to the conclusion that oxygen was contributing in some way to cellular repair mechanisms. The nature of the target sites for heat damage and mechanisms of repair remain unknown. An understanding of the oxygen effects, would mark a substantially advance in our knowledge into the mechanism of the heat shock response.

The use of cycloheximide to block normal and heat shock protein synthesis gave a clear indication that some aspect of protein synthesis was involved in stress tolerance (Fig. 4.1). This observation was in general agreement with the view that heat shock proteins are somehow involved in induced thermotolerance in a wide range of organisms (Lindquist and Craig, 1988; Watson, 1990).

The function of heat shock proteins (hsp) and trehalose is highly controversial and to date there is no general consensus as to the relative contributions which these two components make towards either intrinsic or induced stress tolerance (Piper, 1993). Nevertheless, it is clear that under some conditions, such as in the case of specific mutant studies, it can be demonstrated that hsp play a role in protecting yeast cells against heat and other stresses. The work of Sanchez *et al.* (1992) on a yeast mutant deficient in hsp 104 synthesis has shown that hsp 104 is important for tolerance against heat and other stresses. However, it should be noted that even in the case of hsp 104, there have been conflicting data. One group (Winkler *et al.*, 1991) have emphasised the importance of hsp 104 in thermotolerance and another group, using exactly the same wild-type and hsp 104-deficient strains, have reported that trehalose was the major factor governing tolerance to heat stress (DeVirgilio *et al.*, 1990).

In the present studies, a mild heat shock at 37°C for 45 min, induced the synthesis of classical hsp of yeast belonging to the hsp 100, hsp 90, hsp 70 and hsp 60 groups (Fig 4.16). In addition, protein bands were also observed at between 30 and 44 kDa and many of these bands have been previously reported in yeast (Watson, 1990; Mager and Ferreira, 1993). However, it was beyond the scope of the present studies to further characterise the nature of these proteins. For the purposes of the present studies, the major objective in examining the protein pattern of cells subjected to stress was to determine if these were any additional and/or enhanced synthesis of protein(s) associated with oxygenation and stress tolerance. Although there were indications of enhanced protein synthesis in the region of 40 and 36 kDa (Fig. 4.16) on oxygenation of cells at 25°C, it should be noted that these conditions did not lead to an increase in thermotolerance. On the contrary, cells oxygenated at 25°C prior to heat stress were in fact more sensitive to subsequent heat treatment, induced (Fig 4.5) or intrinsic (Fig 4.6). Interestingly, Collinson and

Dawes (1992) have reported H_2O_2-induced synthesis in yeast of several polypeptides in the 38-45 kDa region. The nature of these polypeptides remain to be elucidated.

Kim *et al.* (1989) reported the induction of a 27 kDa protein in *S. cerevisiae* cells grown anaerobically and subjected to a hyperaerobic atmosphere (95% O_2 : 5% CO_2). Antibodies to this protein were also recognised in rat tissues and this observation lead the authors to speculate that the 27 kDa protein may represent an adaptive response that evolved to protect cells from various oxidative stresses. Despite numerous attempts, the present studies did not reveal any newly synthesised or enhanced synthesis of proteins inducible by a combination of heat shock plus oxygen as compared to a heat shock alone (Fig 4.16 and 4.17). It is unlikely that the laboratory techniques used to examine new (or enhanced) protein synthesis in the present work were inappropriate. The incorporation of radiolabelled ^{35}S-amino acid into protein followed by autoradiography was highly sensitive in detecting heat shock inducible proteins. There is no reason to assume that oxygen-inducible proteins would not be detected using the same techniques. On the other hand, it could be argued that western blotting using antibodies to specific proteins would be a more sensitive method to detect potential oxygen-inducible proteins. However, the question then arises as to which antibodies for which proteins. Nevertheless, using antibodies to yeast CuZn-SOD and Mn-SOD, which are known to be heat shock and oxidative shock inducible (Steels *et al.*, 1991; 1994; Hou, 1992, Costa *et al.*,1993), would be a good starting point.

Another key factor in yeast stress tolerance appears to be levels of trehalose (Wiemken, 1990), which have been shown to be increased upon a mild heat shock (Attfield, 1987; Hottiger *et al.*, 1987). Trehalose levels were examined in the present studies to ascertain possible correlations with oxygen-induced stress tolerance. One interesting aspect of these studies was that although a heat shock did induce trehalose synthesis, the increased synthesis

was not significant if cells were heat shocked in the absence of exogenous glucose, as in the case for washed cells (Fig. 4.15). A similar observation was noted by Winkler *et al.* (1991) and earlier studies by Hottiger *et al.* (1987) and DeVirgilio *et al.* (1990) indicated that glucose was required for accumulation of trehalose during a heat shock. Oxygenation during heat shock of cells did result in a measurable increase in the concentration of trehalose although it is doubtful if this increase was significant as to contribute substantially to the marked increase in stress tolerance of oxygenated cells. However, the most intriguing observation for trehalose in the current experiments was the extremely high concentration (8-10% w/w) found in anaerobically grown cells. It is difficult to relate these results with literature values as no comparable data (anaerobic cells grown with C 18:1 and ergosterol) could be found although high trehalose concentrations in yeast are not unusual under certain similar conditions such as partial aerobiosis (Gelinas *et al.*, 1989). In this latter study, it was shown that cryotolerance of bakers' yeast, although partially related to trehalose levels, was greatly influenced by aeration. Although the reason (s) for the positive effect of oxygenation on cryotolerance was not identified it was proposed that mitochondria may somehow be involved. However, what is clear from the present studies is that levels of trehalose, at least in comparing aerobic and anaerobic yeast, are not directly correlated with stress tolerance. Aerobic cells have relatively low levels of trehalose compared with anaerobic cells (Fig. 4.15) and yet are more stress tolerant.

REFERENCES

Alexandre, H., I. Rousseaux a ıd C.Charpentier (1994) Relationship between ethanol tolerance, lipid composition and plasma membrane fluidity in *Saccharomyces cerevisiae* and *Kloeckera apiculata*. *FEMS Microbiology Letters*, 124: 17-22.

Andreasen, A.A. and T.J B. Stier (1953) Anaerobic nutrition of *Saccharomyces cerevisiae*, I. Ergosterol requirement for growth in a defined medium. *Journal of Cellular and Comparative Physiology*, 41: 23-36.

Andreasen, A.A. and T.J B. Stier (1954) Anaerobic nutrition of *Saccharomyces cerevisiae*, II Unsaturated fatty acid requirement for growth in a defined medium. *Journal of Cellular and Comparative Physiology*, 43: 271-281.

Andreoli, S.P., C.P. Mallet and J.M. Bergstein (1986) Role of glutathione in protecting endothelial cells against hydrogen peroxide injury. *Journal of Laboratory Clinical Medicine*, 108: 190-198.

Aruoma, O.I. and B. Halliwell (1991) *Free radicals and food additives.* Taylor and Francis, London.

Attfield, P.V. (1987) Trehalose accumulates in *Saccharomyces cerevisiae* during exposure to agents that induce heat shock response. *FEBS Letters*, 225: 259-263.

Attfield, P.V., A. Raman a ıd C.J. Northcott (1992) Construction of *Saccharomyces cerevisiae* strains that accumulate relatively low concentrations of trehalose, and their application in testing the contribution of the disaccharide to stress tolerance. *FEMS Microbiology Letters*, 94: 271-276.

Beggs, W.H., F.A. Andrews and G.A. Sarosi (1978) Synergistic action of amphotericin and antioxidants against certain opportunistic yeast pathogens. *Antimicrobial Agents and Chemotherapy*, 13: 266-270.

Begonia, G.B. and M.L. Salin (1991) Elevation of superoxide dismutase in *Halobacterium halobium* by heat shock. *Journal of Bacteriology*, 173: 5582-84.

Beuchat, L.R. and W.K. Jor es (1978) Effect of food preservatives and antioxidants on colony formation by heated conidia of *Aspergillus flavus*. *Acta Alimentaria*, 7: 373-384.

Bilinski, T., Z. Krawiec, A. Liczmanski and J. Litwinska (1985) Is hydroxyl radical generated by the Fenton reaction in vivo?. *Biochemical and Biophysical Research Communications*, 130: 533-539.

Bomhard, E.M., J.N. Bremmer and B.A. Herbold (1992) Review of the mutagenicity/ genotoxicity of butylated hydroxytoluene. *Mutation Research*, 277: 187-200.

Boulton, C.A. and D.E. Quain (1987) Yeast, oxygen and the control of brewery fermentations. *Yeast and Fermentation, EBC congress* 40: 401-408.

Bradford, M.M. (1976) A rapic and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248-254.

Callaerts, G. , D. Iserentant and H. Verachtert (1993) Relationship between trehalose and sterol accumulation during oxygenation of cropped yeast. *Journal of the Institute of Brewing*, 51: 75-77.

Casey, G.P. and W.M. Ingledew (1986) Ethanol tolerance in yeasts, *CRC. Critical Review Microbiology*, 13: 219-226.

Cavicchioli, R. and K. Watson (1986) Loss of heat shock acquisition of thermotolerance in yeast is not correlated with loss of heat shock proteins. *FEBS Letters,* 107: 149-152.

Chang, H.C. and A.L. Branen (1975) Antimicrobial effect of butylated hydroxyanisole (BHA). *Journal of Food Science*, 40: 349-351.

Cheng, L.L. and D. Wilkie (1991) Protective effect of antioxidant. *Biochemical Pharmacology,* 2: 1237-1240.

Chung, K.T., S.E. Stevens, W-F. Lin and C.I. Wei (1993) Growth inhibition of selected food-borne bacteria by tannic acid, propyl gallate and related compounds. *Letters in Applied Microbiology*, 17: 29-32.

Clarkson, S.P., P.J. Large, C.A. Boulton and C.W. Bamforth (1991) Synthesis of superoxide dismutase, catalase and other enzymes and oxygen and superoxide toxicity during changes in oxygen concentration in cultures of brewing yeast. *Yeast*, 7: 91-103.

Collinson, L.P. and I.A. Dawes (1992) inducibility of the response of yeast cells to peroxide stress. *Journal of General Microbiology*, 138: 329-335.

Coote, P.J, M.V. Jones, I.J. Seymour, D.L. Rowe, D.P. Ferdinando, A.J. McArthur and M.B. Cole. (1994) Activity of the plasma membrane H^+-ATPase is a key physiological determinant of thermotolerance in *Saccharomyces cerevisiae*. *Microbiology*, 140: 1881-1890.

Costa, V., E. Reis, A. Quintanilha and P. Moradas-Ferreira (1993) Acquisition of ethanol tolerance in *Saccharomyces cerevisiae*: the key role of mitochondrial superoxide dismutase. *Archives of Biochemistry and Biophysics*, 300: 608-614

Coutinho, C. and E. Bernardes, D. Felix and A.D. Panek (1988) Trehalose as cryoprotectant for preservation of yeast strains. *Journal of Biotechnology*, 7: 23-32.

Craig, E.A., B.D. Gambil and R.J. Nelson (1993) Heat shock proteins: Molecular chaperones of protein biogenesis. *Microbiological Reviews*, 57: 402-414.

Criddle, R.S. and G. Schatz (1969) Promitochondria of anaerobically grown yeast. *Biochemistry*, 8:332-338.

Crowe, J.H., F.A. Hoekstra and L.M. Crowe (1992) Anhydrobiosis. *Annual Review of Physiology,* 54: 579-599.

D'Amore, T., R. Crumplen and G.G. Stewart (1991) The involvement of trehalose in yeast stress tolerance. *Journal of Industrial Microbiology*, 2: 191-196.

Davidson, P.M. and A.L. Branen (1980a) Inhibition of two psychrotrophic *Pseudomonas* species by butylated hydroxyanisole. *Journal of Food Science*, 45: 1603-1606.

Davidson, P.M. and A.L. Branen (1980b) Antimicrobial mechanisms of butylated hydroxyanisole against two *Pseudomonas* species. *Journal of Food Science*, 45: 1607-1613.

Davidson, P.M., L.S. Post, A.L. Branen and A.R. Mc Curdy (1983) Naturally occurring and miscellaneous food antimicrobials. In *Antimicrobials in Foods*. (Eds. A.L. Branen and P.M. Davidson), pp. 371-419, Marcel Dekker, New York .

De Virgilio, C., P. Piper, T. Boller and A. Wiemken (1991) Acquisition of thermotolerance in *S. cerevisiae* without heat shock protein hsp 104 and in the absence of protein synthesis. *FEBS Letters*, 288: 86-90.

De Virgilio, C., U. Simmen, T Hottiger, T. Boller and A. Wiemken (1990) Heat shock induces enzymes of trehalose metabolism, trehalose accumulation, and thermotolerance in *Schizosaccharomyces pombe*, even in the presence of cycloheximide. *FEBS Letters*, 273: 107-110.

Demain, A.L. and N.A. Solomon (1981) Industrial microbiology. In *Industrial Microbiology and the Advent of Genetic Engineering*. Scientific American Books, W.H. Freeman and Co, San Fransisco.

Emerit, I and B. Chance (1992) *Free Radicals and Aging*. Birkhauser Verlag, Berlin.

Eubank, V.L. and L.R. Beuchat (1982) Increase sensitivity of heat-stressed *Saccharomyces cerevisiae* cells to food-grade antioxidants. *Applied and Environmental Microbiology*, 44: 604-610.

Eubank, V.L. and L.R. Beuchat (1983) Combined effects of antioxidants and temperature on survival of *Saccharomyces cerevisiae*. *Journal of Food Protection,* 46: 29-33.

Farr, S.B. and T. Kogoma (1991) Oxidative stress response in *Escherichia coli* and *Salmonella typhimurium*. *Microbiological Reviews*, 55: 561-585.

Ferrari, B (1994) *The Role of Mitochondria in the Stress Response*. Honours thesis, Department. of Biochemistry, Microbiology and Nutrition, University of New England, Armidale, Australia.

Finley, D., E. Ozkaynak and A. Varshavsky (1987) The yeast ubiquitin gene is essential for resistance to high temperature, starvation and other stresses. *Cell*, 48: 1035-1046.

Fridovich, I. (1989) Superoxide dismutases. An adaptation to a paramagnetic gas. *Journal of Biological Chemistry*, 266: 7761-7764.

Fung, D.Y.C., S. Taylor and J. Kahan (1977) Effects of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on growth and aflatoxin production of *Aspergillus flavus*. *Journal of Food Safety*, 1: 39-51.

Galiazzo, F., A. Schiesser and G. Rotolio (1987) Glutathione peroxidase in yeast. Presence of the enzyme and induction by oxidative conditions. *Biochemical and Biophysical Research Communications*, 147: 1200-1205.

Galiazzo, F., A. Schiesser and G. Rotolio (1988) Oxygen-independent induction of enzyme activities related to oxygen metabolism in yeast by copper. *Biochimica et Biophysica Acta*, 965: 46-51.

Gelinas, P and J. Goulet (1991) Morphology of bakers' yeast and dissolved oxygen saturation during fed-batch growth. *Letters in Applied Microbiology*, 12: 164-170.

Gelinas, P., G. Fiset, A. Le Duy and J. Goulet (1989) Effect of growth conditions and trehalose content on cryotolerance of bakers' yeast in frozen doughs. *Applied and Environmental Microbiology*, 55: 2453-2459.

Gething, M.J. and J. Sambrook (1992) Protein folding in the cell. *Nature*, 355: 33-45.

Graumlich, T.R. and Stevenson, K.E. (1979) Respiration and viability of thermally injured *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, 38: 461-465.

Greenberg, J.T. and B. Demple (1986) Glutathione in *Escherichia coli* is dispensable for resistance to H_2O_2 and gamma radiation. *Journal of Bacteriology*, 168: 1026-1029.

Gregory, E.M., S.A. Goscin and I. Fridovich (1974) Superoxide dismutase and oxygen toxicity in a eukaryote. *Journal of Bacteriology*, 117: 456-460.

Hall, B.G. (1983) Yeast thermotolerance does not require protein synthesis. *Journal of Bacteriology*, 156: 1363-1365.

Halliwell, B. and J.M.C. Gutteridge (1989) *Free Radicals in Biology and Medicine*. Clarendon Press, Oxford.

Harris, C., M.R. Juchau and P.E. Mirkas (1991) Role of glutathione and Hsp 70 in the acquisition of thermotolerance in post implantation rat embryos. *Teratology*, 43: 229-239.

Hartl, F.U., J. Martin and W. Neupert (1992) : Protein folding in the cell: the role of molecular chaperones Hsp 70 and Hsp 60. *Annual Review of Biophysics and Biomolecular Structure*, 21: 293-322.

Hitchman, M.L. (1978) Measurement of dissolved O_2. *Chemical Analysis*, 49: 19-27.

Hottiger, T., C. De Virgillo, W. Bell, T. Boller and A. Wiemken (1992) The 70-kilodalton heat-shock proteins of the SSA subfamily negatively modulate heat-shock-induced accumulation of trehalose and promote recovery from heat stress in the yeast, *Saccharomyces cerevisiae*. *European Journal of Biochemistry*, 210: 125-132.

Hottiger, T., P. Schmutz and A. Wiemken (1987) Heat-induced accumulation and futile cycling of trehalose in *Saccharomyces cerevisiae*. *Journal of Bacteriology*, 169: 5518-22.

Hou, Y. (1992) *Heat and Oxidative Stress as a Function of Respiratory and Fermentative Metabolism in Yeast*. MSc thesis. Department. of Biochemistry, Microbiology and Nutrition, University of New England, Armidale, Australia.

Inai, K.T., T. Kobuke, S. Nambu, T. Takemoto, E. Kou, H. Nishima, M. Fujihara, S. Yonehara, S. Suehiro, T. Tsuya, K. Horiuchi and S. Tokuoka (1988) Hepatocellular tumorigenicity of butylated hydroxytoluene administered orally to $B6C3F_1$ mice, Japanese. *Journal of Cancer Research*, &9: 49-58.

Ingram, L.O. and T.M. Buttke (1984) Effects of alcohols on microorganisms. *Advances in Microbial Physiology*, 25: 253-300.

Inoue, Y., L.T. Tran and A. Kimura (1993) Role of glutathione peroxidase against oxidative stress in yeast: phenotypic character of lipid hydroperoxide-sensitive mutants derived from *Hansenula mrakii*. *Journal of Fermentation and Bioengineering*, 75: 229-231.

Jimenez, J. and T. Benitez (1988) Yeast cell viability under conditions of high temperature and ethanol concentrations depends on the mitochondrial genome. *Current Genetics*, 13: 461-469.

Kates, M. and R.M. Baxter (1962) Lipid composition of mesophilic and psychrophilic yeast (Candida species) as influenced by environmental temperature. *Canadian Journal of Biochemical Physiology*, 40: 1213.

Kim, I.H., K. Kim, and S.G. Rhee (1989) Induction of an antioxidant protein of *Saccharomyces cerevisiae* by O_2, Fe^{3+}, or 2-mercaptoethanol. *Proceedings of the National Academy of Sciences, USA* 86: 6018-6022.

Konings, A.W.T. and A.C.C. Ruifrok (1985) Role of membrane lipids and membrane fluidity in thermosensitivity and thermotolerance of mammalian cells. *Radiation Research*, 102: 86-98.

Konings, A.W.T. (1988) Membranes as targets for hyperthermic cell killing. *Cancer Research*, 109: 9-21.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.

Laemmli, U.K. and M. Favre (1973) Maturation of the head of bacteriophage T4, I. DNA packaging events. *Journal of Molecular Biology*, 80: 575-599.

Lee, F.J.S. and H.M. Hassan (1986) Biosynthesis of superoxide dismutase and catalase in *Saccharomyces cerevisiae*: Effect of oxygen and cytochrome c deficiency. *Journal of Industrial Microbiology*, 1: 187-193.

Lee, F.J.S. and H.M. Hassan (1987) Biosynthesis of superoxide dismutase and catalase in a chemostat culture of *S. cerevisiae*. *Applied Microbiology and Biotechnology*, 26: 531-536.

Letters, R. (1968) Phospholipids of yeasts. In *Aspects of Yeast Metabolism*. (Eds. A.K. Mills).pp: 303-319. Blackwell Scientific Publications, Oxford.

Lewis, J.G., R.P. Learmonth and K. Watson (1993) Role of growth phase and ethanol in freeze-thaw stress resistance of *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, 59: 1065-1071.

Lewis, J.G., R.P. Learmonth and K. Watson (1995) Induction of heat, freezing and salt tolerance by heat and salt shock in *Saccharomyces cerevisiae*. *Microbiology*, 141: 1-8.

Lewis J.G. (1994) *Physiological aspects of stress tolerance in baking strains of Saccharomyces cerevisiae*. PhD thesis, Department of Biochemistry, Microbiology and Nutrition, University of New England, Armidale, Australia.

Lillie, S.H. and J.R. Pringle (1980) Reserve carbohydrate metabolism in *Saccharomyces cerevisiae*: response to nutrient limitation. *Journal of Bacteriology*, 143: 1384-1394

Lindquist, S and E.A. Craig (1988) The heat-shock proteins. *Annual Review of Genetics*, 22:631-677.

Lumpkin, C.K., K.J. Henle and G. Sammartino (1988) Expression of thermotolerance following microinjection of glutathione disulfide. *Radiation Research*, 115:201-210.

Mackenzie, K.F., K.K. Singh and A.D. Brown (1988) Water stress plating hypersensitivity of yeasts Protective role of trehalose in *Saccharomyces cerevisiae*. *Journal of General Microbiology*, 134: 1661-1666.

Mackey, B. M. and D. A. Seymour (1989) The bactericidal effect of isoascorbic acid combined with mild heat. *Journal of Applied Bacteriology*, 67: 629-638.

Mager, W.H. and P.M. Ferreira (1993) Stress response of yeast. *Biochemical Journal*, 290: 1-13.

Mansure, J.T.C., A.D. Panek, L.M. Crowe and J.H. Crowe (1994) Trehalose inhibits ethanol effects on intact yeast cells and liposomes. *Biochimica et Biophysica Acta*, 1191: 309-316.

McAlister, L. and D.B. Finkelstein (1980) Heat shock proteins and thermal resistance in yeast. *Biochemical and Biophysical Research Communication*, 93 (3): 819-824.

McAlister, L., S. Strausberg, A. Kulaga and D.B. Finkelstein (1979) Altered patterns of protein synthesis induced by heat shock of yeast. *Current Genetics*, 1: 63-74.

Meister, A. and M.E. Anderson (1983) Glutathione. *Annual Review of Biochemistry*, 52: 711-760.

Meyer, F., R.J. Light and K. Bloch (1963) Fatty acid metabolism in anaerobic yeast. *Journal of Biological Chemistry*, 238: 2654-2659.

Miller, M.J., N.H. Xuong and F.E. Guidushek (1982) Quantitative Analysis of the heat shock response of *Saccharomyces cerevisiae*. *Journal of Bacteriology*, 151: 311-327.

Molzahn, S.W. (1989) Control of quality and costs in fermentation. *Abstracts European Brewery Convention,* Zurich, pp. 135-150.

Moore, M.M., M.W., breedveld and A.P. Autor (1989) The role of carotenoids in preventing oxidative damage in the pigmented yeast, *Rhodotorula mucilaginosa*. *Archives of Biochemistry and Biophysic*, 270: 419-431.

Morimoto, R.J., A. Tissieves and C. Georgopoulos (1990) *Stress Proteins in Biology and Medicine*. Cold Spring Harbor Laboratory Press, New York.

Murata, K and A. Kimura (1987a) Relationship between glutathione contents and generation times in *Saccharomyces cerevisiae*. *Agricultural Biological Chemistry*, 50: 1055-1056.

Murata, K and A. Kimura (1987b) Effect of L-buthionine-sulphoximine on the cellular glutathione level and growth of *Saccharomyces cerevisiae*. *Agricultural Biological Chemistry*, 51: 2827-2829.

Nover, L. (1984) *Heat Shock Response of Eucaryotic Cells*. Springer-Verlag, Berlin.

Ohtake, Y., A. Satou and S. Yabuchi (1990) Isolation and characterisation of glutathione biosynthesis-deficient mutants in *Saccharomyces cerevisiae*. *Agricultural Biological Chemistry*, 54: 3145-3150.

Olsen, P., O. Meyer, N. Bille and G. Wurtzen (1986) Carcinogenicity study on butylated hydroxytoluene (BHT) in Wistar rats exposed in utero. *Food Chemistry Toxicology,* 24 1-12.

Overath, P., H.U Schairer, U. and W. Stoffel (1970) Correlation of *in vivo* and *in vitro* phase transitions of membrane lipids in *Escherichia coli*. *Proceedings of the National Academy of Sciences*, 67: 606-612.

Panaretou, B. and P.W. Piper (1992) The plasma membrane of yeast acquires a novel heat shock protein (Hsp 30). *European Journal of Biochemistry,* 206: 635-640.

Panek, A., J.J. Vania, M.F. Paschoalin and D. Panek (1990) Regulation of trehalose metabolism in *Saccharomyces cerevisiae* mutants during temperature shifts. *Biochimie*, 72: 77-79.

Parsell, D.A. and S. Lindquist (1993) The function of heat shock proteins in stress tolerance. *Annual Review of Genetics*, 27: 437-496.

Penninckx, M.J. and M.T. Elskens (1993) Metabolism and functions of glutathione in microorganisms. *Advances in Microbial Physiology*, 34: 239-301.

Petko, L. and S. Lindquist (1986) Hsp 26 is not required for growth at higher temperature, nor for thermotolerance, spore development or germination. *Cell*, 45: 885-894.

Piper, P.W. (1993) Molecular events associated with acquisition of heat tolerance by the yeast *Saccharomyes cerevisiae*. *FEMS Microbiology Review*, 11: 339-356.

Plattner, H. and G. Schatz,. (1969) Promitochondria of anaerobically grown yeast III Morphology. *Biochemistry*, 8: 339-343.

Plesset, J., C. Palm and C.S. Mc Laughlin (1982) Induction of thermotolerance by ethanol in *Saccharomyces cerevisiae. Biochemical and Biophysical Research Communications* 108: 1340-1345.

Praekelt, U.M. and P.A. Meacock (1990) Hsp 12, a new small heat shock gene of *S. cerevisiae*: Analysis of structure, regulation and function. *Molecular and General Genetics*, 223: 97-106.

Privalle, C.T. and I. Fridovich (1987) Induction of superoxide dismutase in *Escherichia coli* by heat shock. *Proceedings of the National Academy of Sciences U.S.A.*, 84: 2723-2726.

Proudlock, J.W., L.W. Wheeldon, D.J. Jollow and A.W. Linnane (1968) Role of sterols in *Saccharomyces cerevisiae. Biochimica et Biophysica Acta*, 152: 434-437.

Reed, G. and Nagodawithana (1991) *Yeast Technology.* 2nd Ed. Van Nostrand Reinhold, New York.

Regnacq, M. and H. Boucherie (1992) Isolation and sequence of a hsp 30, a yeast heat shock gene coding for a hydrophobic membrane protein. *Current Genetics*, 23: 435-442.

Rice-Evans, C. (1993) *Richelieu Free Radical Series*, volume I-VII, Richelieu Press, London.

Rose, A.H. (1980) Recent research on industrially important strains of *Saccharomyces cerevisiae*. In *Biology and Activities of Yeasts* (Eds. F.A. Skinner, S.M. Passmore and R.R. Davenport) pp. 103-121. Academic Press, London.

Sanchez, Y., J. Taulien, K.A. Borkovich and S. Lindquist (1992) Hsp 104 is required for tolerance to many forms of stress. *The EMBO Journal*, 11: 2357-2364.

Schindler , D. and J. Davies (1975) Inhibitors of macromolecule synthesis in yeast. In *Methods in Cell Biology*, volume xll (Eds. D.M. Prescott), pp. 17-38. Academic Press, New York.

Schroeder, W.A. and E.A. Johnson (1993) Antioxidant role of carotenoids in *Phaffia rhodozyma. Journal of General Microbiology*, 139: 907-912.

Seah, T.C. and J.G. Kaplan (1973) Purification and properties of the catalase of baker's yeast. *Journal of Biological Chemistry*, 48: 2889-2893.

Sies, H. (1986) Biochemistry of Oxidative Stress. *Angewandte Chemie*, International Edition in English, 25: 1058-1071.

Singer, S.J. and G. Nicolson (1972) The fluid mosaic model of biological membranes. *Science,* 175: 720-731.

Steels, E.L. R.P. Learmonth and K. Watson (1991) Stress-inducible antioxidant enzymes in *Saccharomyces cerevisiae*. *Proceedings of the Australian Society for Biochemistry and Molecular Biology*, 23: SP 21.

Steels, E.L (1994) *Relationship between heat and oxidative stress in Saccharomyces cerevisiae and human tumour cells*. PhD thesis, Department. of Biochemistry, Microbiology and Nutrition, University of New England, Armidale, Australia.

Steels, E.L., R.P. Learmonth and K. Watson (1994) Stress tolerance and membrane lipid unsaturation in *Saccharomyces cerevisiae* grown aerobically or anaerobically. *Microbiology*, 140: 569-576.

Stokes, J.L. (1971) Temperature effects in yeasts. In *The Yeasts*, volume 2, 1st ed. (Eds. A.H. Rose and J.S. Harrison) , pp. 119-114. Academic Press, London.

Subik, J., J. Bolarov and ... kovac (1972) Obligatory requirement of intramitochondrial ATP for normal functioning of the eucaryotic cell. *Biochemical and Biophysical Research Communications*, 49: 192-198.

Swartz, H. (1970) Effect of oxygen on freezing damage. *Cryobiology*, 6: 546-555.

Tran, L., Y. Inoue and A. Kimura (1993) Induction of glutathione peroxidase by reactive oxygen in the yeast *Hansenula mrakii*. *Journal of Fermentation and Bioengineering*, 76: 64-66.

Van Laere, A. (1989) Trehalose, reserve and/or stress metabolite?. *FEMS Microbiological Review*, 63: 201-210.

Van Loon, A.P.G.M., B. Pesold-Hurt and G. Schatz (1986) A yeast mutant lacking mitochondrial manganese-superoxide dismutase is hyper sensitive to oxygen. *Proceedings of the National Academy of Sciences, USA*, 83: 3820-3824.

Van Uden, N. (1984) Temperature profiles of yeasts. *Advances in Microbial Physiology,* 25: 195-251.

Verduyn, C., M.L. Giuseppin, W.A. Schedders and J.P. Van Dijken (1988) Hydrogen peroxide metabolism in yeasts. *Applied and Environmental Microbiology*, 54: 2086-2090.

Visser, W., W. A. Scheffers, W.H.B. Van der Vegte and J.P. van Dijken (1990) Oxygen requirements of yeasts. *Applied and Environmental Microbiology*, 56(12): 3785-3792.

Visser, W., A.A. van der Baan, W. Batenburg-van der Vegte, W.A. Scheffers, R. Kramer and J.P. van Dijken (1994) Involvement of mitochondria in the assimilatory metabolism of anaerobic *Saccharomyces cerevisiae* cultures. *Microbiology*, 140: 3039-3046.

Watson, K., G. Dunlop and R. Cavicchioli (1984) Mitochondrial and cytoplasmic protein synthesis are not required for heat shock acquisition of ethanol and thermotolerance in yeast. *FEBS Letters*, 172: 299-302.

Watson, K. and R. Cavicchioli (1983) Acquisition of ethanol tolerance in yeast cells by a heat shock. *Biotechnology Letters*, 5: 683-688.

Watson, K and A.H. Rose (1980) Fatty-acyl composition of the lipids of *Saccharomyces cerevisiae* grown aerobically or anaerobically in media containing different fatty acids. *Journal of General Microbiology*, 117: 225-233.

Watson, K. (1984) Membrane lipid adaptation in yeast. In *Membrane Fluidity* (Eds. M. Kates and L.A. Manson), pp. 517-542. Plenum Publishing, New York.

Watson, K. (1987) Temperature Relations. In *The Yeasts* (Volume 2) (Eds. Rose, A. H. and J. S. Harrison). pp. 41-65. Academic Press, London.

Watson, K. (1990) Microbial stress proteins. *Advances in Microbial Physiology*, 31:183-223.

Watson, K., J.M. Haslam and A.W. Linnane (1970) Biogenesis of mitochondria xIII. The isolation of mitochondrial structures from anaerobically grown *Saccharomyces cerevisiae*. *Journal of Cell Biology*, 46: 88-96.

Watson, K., J.M. Haslam, B. Veitch and A.W. Linnane (1971) Mitochondria precursors in anaerobically grown yeast. In *Autonomy and Biogenesis of Mitochondria and Chloroplast* (Eds. N.K. Boardman, A.W. Linnane and R.M. Smillie), pp. 162-174, North Holland Press, Amsterdam.

Westerbeek-Marres, C.A.M., M. Moore and A.P. Autor (1988) Regulation of manganese superoxide dismutase in S. cerevisiae. *European Journal of Biochemistry*, 174: 611-620.

Whiffen, A.J. (1948) The production, assay, and antibiotic activity of actidione an antibiotic from *Streptomyces griseus*. *Journal of Bacteriology* 56:283-291

Wiemken, A. (1990) Trehalose in yeast, stress protectant rather than reserve carbohydrate. *Antonie van Leeuwenhoek*, 58: 209-217.

Wieser, R., G. Adam, A. Wagner, C. Schüller, G. Marchler, H. Ruis, Z. Krawiec and T. Bilinski (1991) Heat shock factor-independent heat control of transcription of the CTT1 gene encoding the cytosolic catalase T of *Saccharomyces cerevisiae*. *The Journal of Biological Chemistry*, 266: 12406-12411.

Winkler, K., I. Kienle, M. Burget, J.C. Wagner and H. Holzer (1991) Metabolic regulation of the trehalose content of vegetative yeast. *FEBS Letters*, 291: 269-272.

Yatvin, M.B. (1977) The influence of membrane lipid composition and procaine on hyperthermic death of cells. *International Journal of Radiation Biology*, 32: 513-521.

Zitomer, R.S. and C.V. Lowry (1992) Regulation of gene expression by oxygen in *Saccharomyces cerevisiae*. *Microbiological Review*, 56:1-11.