Journal of Industrial Microbiology, 1 (1986) 187–193
Elsevier
SIM 026

Biosynthesis of superoxide dismutase and catalase in *Saccharomyces cerevisiae:* effects of oxygen and cytochrome *c* deficiency

Fang-Jen Lee and Hosni M. Hassan

Departments of Food Science and Microbiology, North Carolina State University, Raleigh, NC 27695, U.S.A.

Received 15 July 1985
Revised 15 January 1986
Accepted 19 March 1986

Key words: Saccharomyces cerevisiae; Cytochrome *c*; Superoxide dismutase; Catalase; Oxyradicals

SUMMARY

Two strains of *Saccharomyces cerevisiae* were used to study the synthesis of superoxide dismutase. One strain (cytochrome *c*-deficient) contained 5–10% of the normal amounts of total cytochrome *c*, while the other strain was a wild type. The cytochrome *c*-deficient mutant had lower specific growth rate, growth yield, and oxygen uptake than the wild type. The superoxide dismutase and catalase activities, in both strains, were significantly lower under anaerobic than under aerobic conditions. Furthermore, under aerobic conditions the mutant contained higher levels of superoxide dismutase than the wild type which may be attributed to the higher intracellular flux of superoxide radicals caused by the cytochrome *c* deficiency. The mutant also showed a lower level of catalase which was due to glucose repression.

INTRODUCTION

Superoxide dismutases (SOD) catalyze the dismutation of the superoxide radical (O_2^-), to generate hydrogen peroxide (H_2O_2) and oxygen (O_2). The presence of catalase or peroxidase then disposes of the H_2O_2 generated. It is important to keep the concentrations of O_2^- and H_2O_2 very low in order to minimize the risk of forming even more toxic products such as the hydroxyl radical (OH·) [11].

There are three classes of superoxide dismutase: those containing manganese (MnSOD), iron (FeSOD) or both copper and zinc (CuZnSOD). Generally, the MnSOD and FeSOD are characteristic of procaryotes while the CuZnSOD is characteristic of eucaroytes. MnSOD is also found in the mitochondria of eucaryotes and shows a high degree of homology with procaryotic MnSOD [11]. Recent studies have shown that CuZnSOD is also found in some procaryotes [14,24]. At the present time, it is not clear how these procaryotes acquired the CuZnSOD.

The antioxidant properties of superoxide dis-

Paper Number 10007 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695, U.S.A. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned.

mutase have been recognized; it has been patented for use to increase oxidative stability of biological materials, tissue transplants and food-stuffs [19,20]. In the pharmaceutical field, superoxide dismutase has been developed as a potential anti-inflammatory agent [17] and radiotherapy drug [21,23]. Currently, there is increasing interest in the commercial production and the utilization of superoxide dismutases.

Superoxide dismutase found in *Saccharomyces cerevisiae* is similar to that found in bovine blood (i.e., CuZnSOD), which is presently used for its commercial production. In this study, two strains of *S. cerevisiae* were used to study the biosynthesis of the antioxidant enzymes, SOD and catalase. One strain lacks the ability to synthesize cytochrome *c* and has a lower rate of oxidative metabolism [16]. Data are presented to show the effect of this mutation (cytochrome *c* deficiency), compared to the wild type strain, on the specific growth rate, growth yield, oxygen uptake, cyanide-resistant respiration and on the biosynthesis of superoxide dismutase and catalase under anaerobic and aerobic conditions.

MATERIALS AND METHODS

Microorganisms. *Saccharomyces cerevisiae* var. *ellipsoideus* ATCC 18790 (wild type) and ATCC 18789 (cytochrome *c*-deficient) [16] were obtained from the American Type Culture Collection.

Media. The basal growth medium contained (per liter): glucose 50.0 g, $MgSO_4$ $7H_2O$ 0.5 g, KH_2PO_4 1.0 g, $(NH_4)_2SO_4$ 5.0 g, $CaCl_2$ 0.1 g, NaCl 0.1 g and yeast extract 7.0 g (BBL). Solid medium contained 2.0% agar (Difco). When the yeasts were to be grown anaerobically, the medium was supplemented with 1 g of Tween 80 and 30 mg of ergosterol per liter in order to compensate for the inability of these cells to synthesize sterols in the absence of oxygen [22]. For measurements of the molar growth yield, the medium was changed to (per liter): yeast nitrogen base 7 g (Difco), and different concentrations of glucose were added as indicated in the text. The pH of the medium was adjusted to 6.0 before sterilization.

Growth conditions. Aerobic conditions were achieved by oscillation of 100 ml culture in 500 ml Bellco culture flasks at 30°C, 160 rpm, in a controlled environment incubator shaker (New Brunswick). Anaerobic conditions were performed inside a Coy anaerobic chamber where O_2 was kept at less than 3 ppm and temperature at 30°C.

Chemicals. Bovine serum albumin and xanthine were purchased from Sigma Chemical Co. Hydrogen peroxide (30%) and potassium cyanide were obtained from Fisher Scientific Co. Xanthine oxidase was prepared from fresh cream according to Wand et al. [25].

Growth and pH measurement. Growth was measured in terms of absorbancy at 600 nm (A_{600}) using a Gilford spectrophotometer model 2000. The pH was measured by a glass electrode using a Fisher pH meter model 620.

Dry weight and growth yield measurements. Cell mass was determined from A_{600} measurements using standard curves relating A_{600} to dry weight, or gravimetrically after drying washed cells to a constant weight at 105°C. Gravimetrically determined dry weights were found to be essentially the same as those estimated from the standard curves relating A_{600} to dry weight.

Late log phase cells were used for determining the standard curves relating A_{600} to dry weight. The organisms were grown in yeast-nitrogen base medium using glucose, and cells were separated by centrifugation at 4°C for 20 min in a refrigerated centrifuge at 16 000 × *g*. The cells were resuspended and washed twice in volumes of 0.1 M potassium phosphate buffer (pH 7.0) equal to that of the original growth medium. Different amounts of the washed cells were suspended in the same buffer, A_{600} was measured, and a known volume ($\approx$25–30 ml) of each dilution was placed in a preweighed dish. These samples were dried to constant weights at 105°C. A_{600} values were plotted against dry weights (mg/ml), and regression analysis was used to determined the best fit lines. The relationships between A_{600} and dry weight were the same for the two strains. One A_{600} unit is equal to 213 $\pm$ 2 μg dry weight cells per ml.

Growth yields on glucose were determined by growing the cells to stationary phase in 100 ml of

0.7% yeast-nitrogen base medium containing different concentrations of glucose. Cells, from 25 ml of cultures aliquots, were harvested by centrifugation and washed twice in 0.1 M phosphate buffer as outlined above. The washed cells were resuspended in 25 ml of the same buffer, A_{600} were measured, and the dry weights were determined from the standard curves relating A_{600} to dry weights. Specific glucose molar growth yield values (Y_G) were calculated as grams of dry weight cells produced per mol of glucose used.

Cell-free extract preparation. Cells were collected at 4°C by centrifugation at 12 000 × *g* for 20 min and washed in 50 mM potassium phosphate buffer containing 0.1 mM EDTA, pH 7.8. The cells were disrupted for a total of 6 min using a Heat System W-370 Sonicator, operated at an output of 55 W. The temperature was kept at 4°C by immersion in an ice/salt bath with sonication applied at 45-s intervals intermittently. Cell debris was removed by centrifugation and cell-free extracts were dialyzed against the same buffer (2 × 4 liter) for 36–48 h to prevent interference of low-molecular-weight compound in the enzyme assays. The dialyzed cell-free extracts were clarified by centrifugation at 27 000 × *g* for 30 min.

Assays. Enzyme assays were performed on dialyzed cell-free extracts. Superoxide dismutase was assayed according to McCord and Fridovich [18] with the modification that KCN (10^{-5} M) was added to the reaction mixture in order to inhibit cytochrome oxidase, which otherwise would have interfered with the assay. Catalase was assayed as described by Beers and Sizer [3]. Cell-free extracts were subjected to electrophoresis in 10% polyacrylamide disc gels by the method of Davis [5]. Superoxide dismutase isoenzymes were visualized on the gel using an activity stain [1] and were distinguished by adding 1 mM KCN to inhibit the CuZnSOD [2]. The different SOD isozymes were quantitated on the gels by linear scanning densitometry at 560 nm using a Gilford Model 2600 spectrophotometer [8]. Protein was estimated by the method of Lowry et al. [13] using bovine serum albumin as a standard.

Oxygen uptake was measured at 30°C, in the absence and in the presence of 1.0 mM cyanide, using a Clark Polarographic Electrode. Reaction mixtures contained 5% glucose and cell suspension sufficient to give an A_{600} = 1.0–1.5. The final reaction volume was 5 ml which was buffered at pH 7.0 with 50 mM potassium phosphate.

Residual glucose was measured in the growth medium using a glucose kit based on glucose oxidase/peroxidase coupled assay (Sigma Chemical Co.).

RESULTS

Growth and molar growth yields

The effects of cytochrome *c* deficiency on the specific growth rate ($k \cdot h^{-1}$) and the molar growth yield on glucose (Y_G) are presented in Figs. 1 and 2. Data in Fig. 1 show that the wild type grew at a slightly faster rate than the mutant. The specific growth rate for the wild type and the mutant were 0.58 h^{-1} and 0.43 h^{-1}, respectively. This corresponded to generation times equal to 72 min for the wild type and 97 min for the mutant. The wild type was also more efficient in utilizing glucose (Fig. 1)

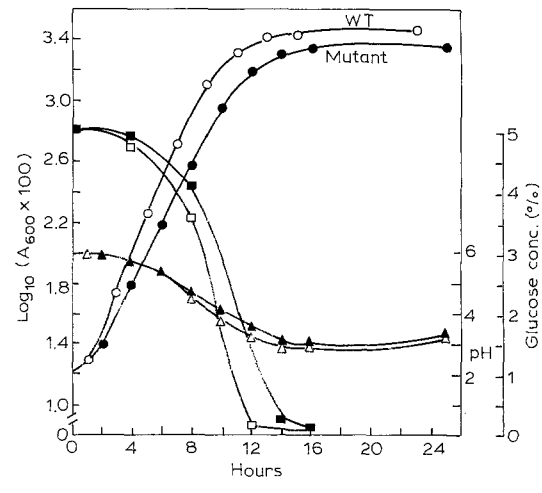

Fig. 1. Kinetics of growth and changes in pH and residual glucose for wild type (WT) and mutant strains. Cells were grown in the basal growth medium containing 5% glucose A_{600}, pH, and glucose were determined at the specified time intervals. ○ ●, A_{600}; △ ▲, pH; □ ■, glucose concentrations; open symbols, wild type; close symbols, mutant strain.

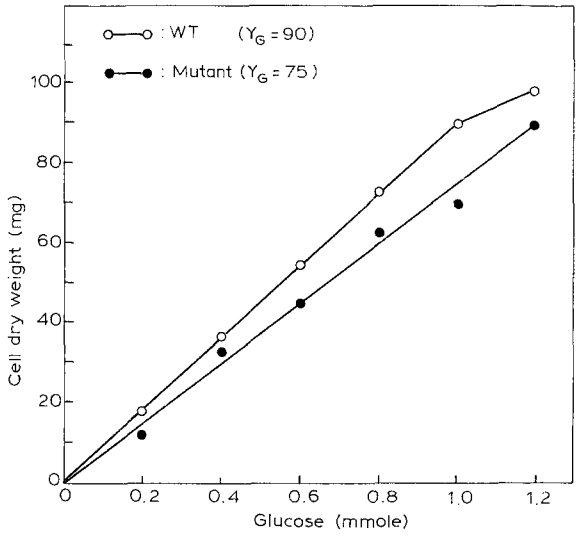

Fig. 2. Specific glucose molar growth yields for wild type (WT) and mutant strains. Cells were grown to stationary phase in 0.7% yeast-nitrogen base medium containing the specified concentrations of glucose. Cell dry weight and Y_G were determined as described in Materials and Methods.

and in making cell materials (Fig. 2). Thus, the molar growth yields on glucose (Y_G) were 90 and 75 g dry wt. cells per mol glucose utilized by the wild type and the mutant, respectively.

Oxygen uptake

The mutant used in this study is about 90% deficient in its cytochrome *c* content [16], and therefore is able to respire and use oxygen as a terminal electron acceptor. Cyanide is known to inhibit oxygen uptake by inhibiting cytochrome oxidase. A positive correlation between the rate of cyanide-resistant respiration and the fluxes of O_2^- and H_2O_2 in living cells has been documented and been taken as an indirect index for superoxide and hydrogen peroxide generated in the cells [9,11]. It was reasoned that, since the mutant is largely deficient in its cytochrome *c* content, it might have a higher percent cyanide-resistant respiration than its counterpart (the wild type). Data in Table 1 show the rates of oxygen uptake for the wild type and the mutant cells taken from late log phase and stationary phase cultures. It is interesting to note that cells taken from late log phase cultures respired at a lower rate than cells taken from stationary phase cultures. This is due to the presence of glucose which inhibits the reconstitution of the mitochondria [15]. Data in Table 1 also indicate that the mutant respired at a lower rate than the wild type. At late log phase, the mutant was 21.5% lower than the wild type; however, the difference was much greater

Table 1

Oxygen uptake and cyanide-resistant respiration in two strains of *Saccharomyces cerevisiae*

Two strains of *S. cerevisiae* were grown under the conditions described in Materials and Methods. Cells were taken at late log phase and stationary phase for oxygen uptake measurements. Cyanide was added to 1 mM final concentration. Cyanide-resistant respiration (%) values were calculated as:

$$\frac{O_2 \text{ uptake in presence of } CN^-}{O_2 \text{ uptake in absence of } CN^-} \times 100$$

Strains	O_2 uptake (nmol/min per mg dry wt.)			
	$-CN^-$		$+CN^-$	
	late log	stationary	late log	stationary
Wild type	38.87	96.59	6.65 (17.1%)	6.78 (7.0%)
Mutant	30.52	47.43	4.9 (16.1%)	4.51 (9.5%)

Table 2

Effect of oxygen on superoxide dismutase and catalase biosynthesis in two strains of *Saccharomyces cerevisiae*

Two strains of *S. cerevisiae* were grown in the glucose basal medium in the presence and the absence of oxygen. Cell-free extracts were prepared and assayed as described in Materials and Methods.

Strains	Growth condition	SOD (U/mg protein)	Catalase (U/mg protein)
Wild type	Anaerobic	0.88	0.18
	Aerobic	11.8 ± 2.0	17.4 ± 1.0
Mutant	Anaerobic	0.93	0.20
	Aerobic	22.4 ± 5.2	6.7 ± 2.0

(51%) for cells taken from stationary phase. This again indicates that the wild type is able to reconstitute its mitochondrial respiratory system during stationary phase (i.e., after removal of the glucose effect) better than the mutant. The percent cyanide-resistant respiration was about the same in the wild type and in the mutant cells taken from the late log phase of growth, but was 36% higher in the mutant cells taken from the stationary phase of growth.

Effect of oxygen on superoxide dismutase and catalase biosynthesis

Previous studies have shown that the levels of

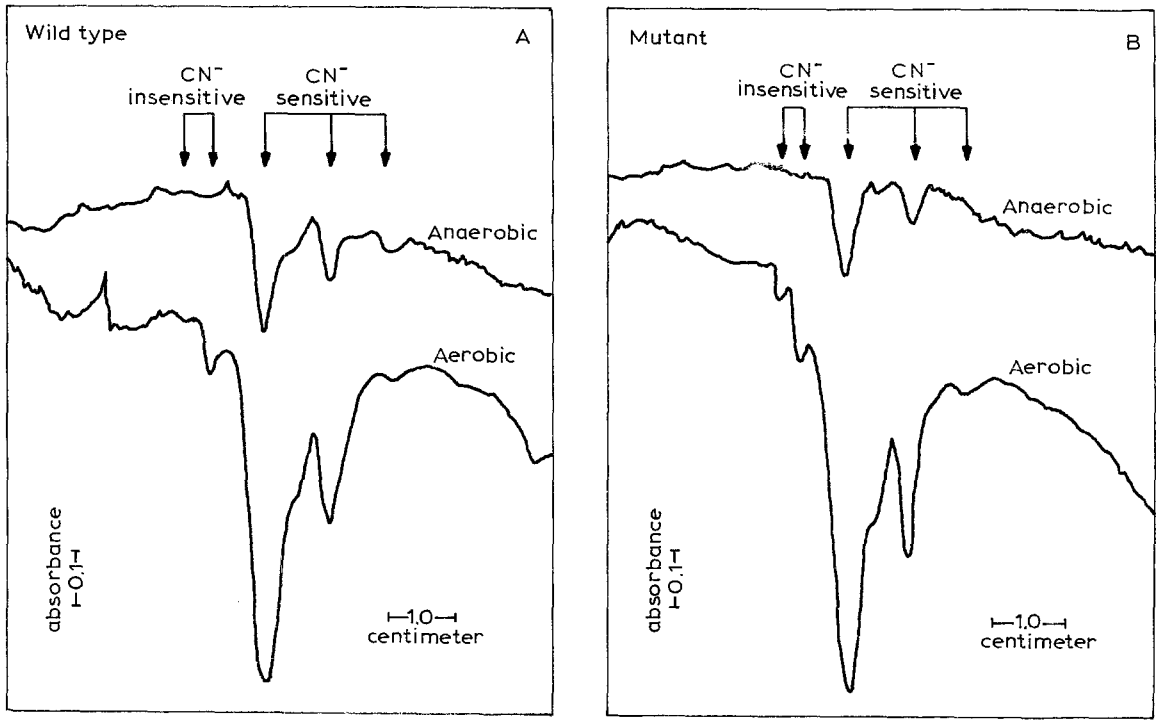

Fig. 3. Densitometric scans of polyacrylamide gel electropherograms stained for SOD activity. The cultures were grown in the absence or in the presence of air, and cell-free extracts were prepared as in the Materials and Methods. Samples (100 μg) were applied to 10% gels and after electrophoresis and staining for SOD activity the gels were scanned at 560 nm. (A) wild type, (B) cytochrome *c*-deficient mutant.

SOD in procaryotes are regulated by the rate of O_2^- (the substrate for SOD) generated intracellularly and that the synthesis of SOD is not under a catabolite repression type of regulation [8,9]. However, catalase biosynthesis has been shown to be regulated by catabolite repression (glucose effect), both in procaryotes and eucaryotes [4,10,12]. Data in Table 2 clearly show that both strains responded to the presence of molecular oxygen by increasing their intracellular levels of both SOD and catalase. These findings correlate with previous studies that have shown that these enzymes are important in protecting the cells against oxygen toxicity [6,7,9,11]. The level of SOD in the mutant cells exposed to oxygen was about twice that seen in the wild type. This might indicate that the intracellular flux of O_2^- in the mutant cells is significantly higher than that experienced in the wild type cells. On the other hand, catalase levels were much higher in the wild type than in the mutant cells which may be explained by the persistent presence of glucose in the culture of the mutant.

Disc-gel electrophoresis of extracts prepared from both strains grown in the absence and presence of oxygen demonstrated the presence of five SOD activity bands (Fig. 3). Two bands were cyanide insensitive and were identified as MnSOD, while three bands were cyanide sensitive and were identified as CuZnSOD. Both types of SOD (i.e. MnSOD and CuZnSOD) were induced by the presence of oxygen. However, when grown in the presence of oxygen, the CuZnSOD accounted for 90% of the total SOD activity present in the cells.

DISCUSSION

During the oxidation of a metabolite, electrons are released and must be accepted by an oxidizing agent. In aerobic metabolism, oxygen serves as the preferred electron acceptor where a stepwise electron carrier system (the respiratory chain) mediates the transfer of electrons to oxygen to generate water. In *S. cerevisiae*, cytochrome *c* is an important component of the respiratory chain and a deficiency in cytochrome *c* biosynthesis is known to affect oxidative metabolism [15,16]. In the absence of a fully functional electron transport chain, reduced electron carriers have the potential to react spontaneously with molecular oxygen and generate the partially reduced oxygen intermediates (O_2^-, H_2O_2 and $OH\cdot$) [11].

In this paper, we demonstrated that a deficiency in cytochrome *c* biosynthesis in *S. cerevisiae* caused decreases in the specific growth rate, specific glucose molar growth yield, and total oxygen uptake. On the other hand, this deficiency correlates with increases in superoxide dismutase. This is likely due to an increase in the intracellular flux of O_2^- caused by the cytochrome *c* deficiency.

S. cerevisiae, grown in the presence of glucose, is subjected to the glucose effect also known as the Crabtree effect [15]. The presence of excess glucose, therefore, inhibits the synthesis of the mitochondria and of the respiratory enzymes [15]. The cytochrome *c*-deficient mutant is expected to be under the influence of the Crabtree effect longer than the wild type strain because of its slow metabolism of glucose (Fig. 1). The synthesis of catalase has been shown to be under catabolite repression [4,10,12], and data presented in Table 2 clearly show that, under aerobic conditions, the mutant synthesized less catalase than the wild type. In conclusion, the cytochrome *c*-deficient mutant increased the level of CuZnSOD in response to increased intracellular flux of superoxide anion.

ACKNOWLEDGEMENT

This work was supported in part by grant PCM-8213853 from the National Science Foundation.

REFERENCES

1 Beauchamp, C.O. and I. Fridovich. 1971. Superoxide dismutase: improved assay and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276–287.

2 Beauchamp, C.O. and I. Fridovich. 1973. Isozymes of superoxide dismutase from wheat germ. Biochim. Biophys. Acta 317: 50–64.

3 Beers, R.F. and I.W. Sizer. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 159: 133–140.

4 Cross, H.S. and H. Ruis. 1978. Regulation of catalase synthesis in *Saccharomyces cerevisiae* by carbon repression. Mol. Gen. Genet. 166: 37–43.

5 Davis, B.J. 1964. Disc electrophoresis. II. Methods and application to human serum proteins. Ann. N.Y. Acad. Sci. 121: 404–436.

6 Gregory, E.M. and I. Fridovich. I. 1973. Induction of superoxide dismutase by molecular oxygen. J. Bacteriol. 114: 543–548.

7 Gregory, E.M., S.A. Goscin and I. Fridovich. 1974. Superoxide dismutase and oxygen toxicity in a Eukaryote. J. Bacteriol. 117: 456–460.

8 Hassan, H.M. and I. Fridovich. 1977. Regulation of superoxide dismutase synthesis in *E. coli*: Glucose effect. J. Bacteriol. 132: 505–510.

9 Hassan, H.M. and I. Fridovich. 1977. Regulation of the synthesis of superoxide dismutase in *E. coli*: Induction by methyl viologen. J. Biol. Chem. 252:7667–7672.

10 Hassan, H.M. and I. Fridovich. 1978. Regulation of the synthesis of catalase and peroxidase in *Escherichia coli*. J. Biol. Chem. 253: 6445–6450.

11 Hassan, H.M. and I. Fridovich. 1980. Superoxide Dismutase: Detoxication of a Free Radical. Enzymatic Basis of Detoxication. Vol. 1. pp. 311–332. Academic Press Inc., New York.

12 Ishida,. A., T. Sasaki and T. Matsusaka. 1981. Biosynthesis of catalase in aerobically grown *Saccharomyces cerevisiae*: Effect of glucose and cyclic 3′,5′-adenosine monophosphate. J. Gen. Appl. Microbiol. 27: 209–217.

13 Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

14 Martin, J.P. and I. Fridovich. 1981. Evidence for a natural gene transfer from the ponyfish to its bioluminescent bacterial symboit *Photobacter leiognathi*. J. Biol. Chem. 256: 6080–6089.

15 Matile, Ph., H. Hoor and C.F. Robinow. 1969. Yeast Cytology: the mitochondria. The Yeasts. Vol. I. pp. 266–271. Academic Press Inc., New York.

16 Mattoon, J.R. and F. Sherman. 1966. Reconstitution of phosphorylating electron transport in mitochondria from a cytochrome *c*-deficient yeast mutant. J. Biol. Chem. 241: 4330–4338.

17 McCord, J.M. and D. English. 1981. Superoxide dismutase: An antiinflammatory drug. Enzyme as Drugs. pp. 353–364. John Wiley & Sons Inc., New York.

18 McCord, J.M. and I. Fridovich. 1969. Superoxide dismutase: an enzymatic function of erythrocuperein. J. Biol. Chem. 244: 6049–6055.

19 Michelson, A.M. 1977. Superoxide dismutase and its application as an oxidation inhibitor. US Patent 4,029,819.

20 Michelson, A.M. and J. Monod. 1975. Superoxide dismutase and its application as an oxidation inhibitor. US Patent 3,920,521.

21 Misra, H.P. and I. Fridovich. 1976. Superoxide dismutase and the oxygen enhancement of radiation lethality. Arch. Biochem. Biophys. 176: 577–581.

22 Park, L.W. and P.R. Starr. 1963. A relationship between ergosterol and respiratory competency in yeast. J. Cell. Comp. Physiol. 61: 61–65.

23 Petkau, A. 1978. Radiation protection by superoxide dismutase. Photochem. Photobiol. 28: 765–774.

24 Steinman, H.M. 1985. Bacteriocuprein superoxide dismutases in *Pseudomonads*. J. Bacteriol. 162: 1255–1260.

25 Wand, W.A., F.O. Brady, R.D. Wiley and K.V. Rajagopalan, 1975. A new purification procedure for bovine milk xanthine oxidase: effect of proleolysis on the subunit structure. Arch. Biochem. Biophys. 169: 695–701.