

EFFECTS OF OXIDATIVE STRESS ON  
THE BIOSYNTHESIS OF  
ANTIOXIDANT ENZYMES AND ON  
PLASMID STABILITY IN  
SACCHAROMYCES CEREVISIAE

LEE, FANG-JEN SCOTT

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North Carolina State University at Raleigh

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Effects of Oxidative Stress on the Biosynthesis of  
Antioxidant Enzymes and on Plasmid Stability in  
Saccharomyces cerevisiae

by

Fang-Jen Scott Lee

A thesis submitted to the Graduate Faculty of  
North Carolina State University  
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Approved By:



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Horri M. Hassan  
Chairman of Advisory Committee

### Abstract

LEE, FANG-JEN SCOTT. Effects of Oxidative Stress on the Biosynthesis of Antioxidant Enzymes and on Plasmid Stability in Saccharomyces cerevisiae. (Under the direction of Dr. H. M. Hassan).

The effects of oxygen concentration, paraquat (0.5 mM), and copper (0.1 mM) on the growth and the biosynthesis of the antioxidant enzymes, superoxide dismutase (SOD) and catalase, were studied in S. cerevisiae grown in glucose-limited chemostat cultures. The effect of dilution rates ( $D$ ,  $\text{hr}^{-1}$ ) on cell mass, glucose consumption, ethanol production, oxygen uptake, and specific activities of SOD and catalase were also investigated at each steady state. SOD was optimally produced at  $D$ -values between 0.22 and 0.26  $\text{hr}^{-1}$  in the presence of oxygen or paraquat, and at  $D$ -values greater than 0.17  $\text{hr}^{-1}$  when copper was present. On the other hand, catalase activity decreased with increasing  $D$ -values. However, the presence of copper or 100% oxygen repressed catalase activity at low  $D$ -values ( $D < 0.1 \text{ hr}^{-1}$ ), and decreased the rate of oxygen uptake at all  $D$ -values tested. The presence of paraquat affected the rate of oxygen uptake only at high  $D$ -values ( $D > 0.22 \text{ hr}^{-1}$ ). We also studied the effect of oxygen concentration on the biosynthesis of SOD and catalase at  $D = 0.1 \text{ hr}^{-1}$ . The data clearly show that synthesis of SOD and catalase, though correlated with changes in oxygen tension, are independent

of one another.

We also investigated the effects of extended growth on plasmid stability in yeast cells. Therefore, a chimeric plasmid (pYT760-ADH1) containing the yeast killer toxin/immunity cDNA was transformed into a leu/his mutant (AH22) and into four industrial toxin-sensitive yeasts. Plasmid stability was dependent on the presence of the 2 u plasmid which is naturally present in some yeasts. The chimeric plasmid was very stable and expressed toxin production ( $89.5 \pm 4.8\%$  killer cells) in two of the transformed yeasts that contained the 2 u plasmid, but was lost within 10 generations from two other transformed pickle yeasts that did not contain the 2 u plasmid. However, the plasmid was extremely stable (100% killer cells) and expressed more toxin in the mutant strain AH22. The effects of dilution rate ( $D$ ,  $\text{hr}^{-1}$ ) on plasmid stability and toxin expression were studied in transformed AH22 (AH22/T3) and Montrachet 522 (522/T1) wine yeast grown in glucose-limited chemostat cultures. The results show that killer toxin production by AH22/T3 cells increased as a function of  $D$ ,  $\text{hr}^{-1}$  and that plasmid stability reached 100% at  $D \geq 0.09 \pm 0.01 \text{ hr}^{-1}$ . However, with Montrachet 522/T1 transformed cells, 100% plasmid stability was seen at  $D \geq 0.18 \pm 0.02 \text{ hr}^{-1}$ . We also challenged the AH22/T3 in chemostat culture ( $D = 0.25 \text{ hr}^{-1}$ ) with an equal number of untransformed cells (AH22). Transformed cells dominated the



population (100%) within 8 to 10 hours of growth.

The effects of oxygen tension and dilution rate on expression and stability of killer toxin chimeric plasmid in transformants 522/T1 were investigated at each steady state. Expression of the killer toxin increased as a function of dilution rate when the transformants were grown in  $N_2$  or air, but not in 100%  $O_2$ . Killer transformants grown in the presence of  $N_2$  have 2-4 fold and 10-15 fold higher toxin expression than that in the presence of air and 100%  $O_2$ , respectively. Plasmid stability increased as a function of dilution rate, regardless of the oxygen tension used. However, the transformants grown in air gave the highest plasmid stability. These studies showed that the effect of oxygen tension on cell metabolism can be extended to affect the expression and stability of recombinant DNA cultures.

### Biography

Fang-Jen Scott Lee was born in Taipei, Taiwan, Republic of China on April 20, 1957. He received his elementary and secondary education in Taipei, graduating from Cheng-Kuo high school in 1976.

He received his B.S. degree with a major in Agriculture Chemistry from National Taiwan University in 1980. The following two years, the author served with the ROTC as a supplementary officer.

He was accepted into the graduate school of North Carolina State University in August 1982 to pursue a Master of Science degree in Food Science, emphasizing in Microbial Physiology, working under the insightful direction of Dr. H. M. Hassan. He completed his degree in May 1984. He chose to continue his graduate education in Food Science at NCSU by pursuing a Ph.D. degree.

The author was inducted into the honorary societies of Phi Tau Sigma and Gamma Sigma Delta in 1984 and was elected to full membership in Sigma Xi in 1986.

The author was married to Lee-Wen Lin on January 21, 1984. She is pursuing her Ph.D. degree in Biochemistry at NCSU.

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## Introduction

The oxygen supply to a microorganism can determine, in several ways, the routes whereby an organic substrate is catabolized to generate ATP. Oxygen is also important in the synthesis of structural components (e.g., cholesterol, heme, tyrosine) and trace constitutive components (e.g., steroid hormones) of living organisms, as well as in the degradation of foreign compounds (e.g., drug detoxifying systems) (Babior, 1980). However, it is now generally accepted that the causes of oxygen toxicity to microbes are several, and that inactivation of key metabolic components (including enzymes and cofactors) as a consequence of direct or indirect interaction with oxygen are important (Hassan and Fridovich, 1980).

Yeast metabolism was investigated in the era of classical biochemistry. As a result, important contributions to our general knowledge of central metabolic pathways emerged from these studies and, consequently, yeast technology has developed to an appreciable degree (Cook, 1958; Rose and Harrison, 1969, 1970, 1971). For the last few years, yeasts have been used to express foreign polypeptides of industrial importance. Indeed, S. cerevisiae possesses several benefits as a host. Among other advantages it is nonpyrogenic, it can glycosylate foreign proteins and it can, in some cases, secrete foreign