37. FLOW CYTOMETRIC EVALUATION OF OXIDATIVE STRESS AND CELLULAR VIABILITY IN SACCHAROMYCES CEREVISIAE

Author: Cristina Stahi

Scientific adviser: Cristina Ciobanu, PhD in Pharmaceutical Sciences, Associate Professor, Department of Pharmaceutical Technology, *Nicolae Testemitanu* State University of Medicine and Pharmacy of the Republic of Moldova

Introduction. Oxidative stress is a key adaptive mechanism influencing microbial survival under antimicrobial pressure. Accumulation of reactive oxygen species (ROS) disrupts cellular redox homeostasis, activates apoptosis-related pathways, and modulates stress-response signaling. These adaptive responses contribute to microbial persistence and may promote antimicrobial resistance (AMR). Saccharomyces cerevisiae is a well-established eukaryotic model for investigating oxidative stress-mediated cellular responses relevant to the fungal resistance mechanisms.

Aim of study. To evaluate oxidative stress and cellular viability in Saccharomyces cerevisiae using flow cytometry and to explore their association with mechanisms involved in antimicrobial resistance.

Methods and materials. Yeast cultures (0.8 OD) were prepared as single-cell suspensions. Oxidative stress was induced using hydrogen peroxide (H_2O_2). Experimental groups included: control cells, cells treated with H_2O_2, cells treated with H_2O_2 and ascorbic acid, and cells treated with ascorbic acid alone. Cell viability and ROS production were assessed using propidium iodide (PI) for detection of membrane-compromised cells and 2',7'-dichlorofluorescein (DCF) for intracellular ROS quantification. Samples were analyzed on a BD FACSCalibur flow cytometer with acquisition of 10,000 events per sample. Gating strategies were applied to distinguish viable (DCF-positive), non-viable (PI-positive) and stressed subpopulations. Statistical analysis was performed using RStudio and Excel.

Results. Exposure to H_2O_2 increased intracellular ROS levels, demonstrated by elevated DCF fluorescence intensity, and increased the proportion of PI-positive cells, indicating membrane damage and apoptosis. Antioxidant co-treatment with ascorbic acid reduced ROS accumulation and partially restored cell viability, confirming the oxidative nature of the induced injury. Flow cytometric analysis revealed distinct fluorescence shifts between control, stressed and antioxidant-treated groups. Increased ROS generation correlated with decreased viability, supporting the hypothesis that oxidative imbalance contributes to cellular damage and stress adaptation.

Conclusions. Flow cytometry is a sensitive method for quantifying oxidative stress and viability changes in Saccharomyces cerevisiae. Elevated ROS levels are associated with reduced cell survival, highlighting oxidative stress as a determinant of microbial adaptation. As oxidative stress responses activate antioxidant defenses and survival pathways, these mechanisms may promote antimicrobial resistance. Understanding ROS dynamics provides insight into resistance development and supports the identification of new therapeutic strategies.

Keywords. Saccharomyces cerevisiae, oxidative stress, flow cytometry

https://doi.org/10.66603/medespera2026.074