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Hap4 System-Regulated Plasma Membrane Proton Fluxes are Crucial for *Saccharomyces cerevisiae* Adaptation to Varying pH, Oxygen, and Glucose Concentrations

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ABSTRACT

The heme-activated Hap4 complex is a transcriptional factor of respiration in *Saccharomyces cerevisiae*, which acts as a global regulator of TCA cycle, complexes III and IV of the electron transport chain, and mitochondrial biogenesis. This study aims to investigate the role of the Hap4 in yeast growth and proton flux regulation across the cell membrane depending on external pH (3.0 or 5.0), glucose concentrations (0.5% or 2%), and oxygen availability (aerobic or oxygen-limited conditions). Growth performance was evaluated using the specific growth rate (SGR), and proton flux across the whole-cell membrane was assessed using a potentiometric method. Results demonstrated that aerobic conditions and pH 5.0 supported the highest SGR (0.4 h^{-1} in *S. cerevisiae* W303-1B and 0.2 h^{-1} in *Δhap4*) during aerobic growth with 2% glucose. The *Δhap4* strain exhibited significantly prolonged lag phase (~7 h compared to 2 h in W303-1B) and an 8-fold decrease in SGR compared to wild-type strain. The highest proton flux rate (4.5 mM H^+ min^{-1} CFU^{-1}) was observed in W303-1B strain during aerobic growth and had remained constant at pH 3.0 - 5.0, whereas *Δhap4* displayed a 6-fold reduction of SGR in microaerophilic growth. Both strains exhibited a similar pattern of proton flux during aerobic growth at pH 3.0 -independent of glucose concentration—with a 3-fold inhibition of proton flux upon treatment with DCCD. This suggests that under aerobic conditions, DCCD-sensitive proton fluxes are not dependent on the Hap4 system, indicating a Hap4-independent mechanism of pH adaptation. In contrast, under microaerophilic conditions, the *Δhap4* showed a complete absence of proton flux compared to the wild-type strain, indicating a critical role of Hap4 in pH adaptation under these conditions. The results obtained can be used in metabolic engineering strategies, to increase yeast robustness and industrial fermentation efficiency under varying environmental conditions.

Keywords: Hap4 system, *S. cerevisiae*, proton flux, DCCD-sensitive transport, metabolic adaptation, mitochondrial regulation

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