

Chemical Engineering Thesis Defense

Long-term Evolution of E. Coli Chimeras ATP Synthesis

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Abstract

Adenosine triphosphate (ATP) is the currency that defines biological energy in living organisms, driving the metabolic cycle and sustaining the cell, which enables it to continue functioning. Most of the energy within the cell is produced by F1F0 ATP synthase. The proton motive force (pmf), an electrochemical gradient across the membrane, serves as the energy source for synthesizing ATP in the F1 domain by driving the enzyme's rotation. The c-ring in the F0 membrane plays an active role in translocating protons down the pmf gradient, where the rotational force required to synthesize ATP is directly proportional to the number of c-subunits in the c-ring. The number of c-subunits varies among species, ranging from c8 to c17. The 360-degree rotation of the c-ring consistently produces 3 ATPs, with the number of protons entering the c-ring equaling the number of c-subunits.

This thesis investigates the evolutionary changes of the genetically modified c-ring in E. coli F1F0 ATP synthase that occurred over a 60-day period. These strains were grown in succinate medium, which forced them to rely on aerobic respiration for energy, focusing on the efficiency of ATP synthase. A primary objective is to compare ATP production rates in these evolved bacterial populations at Day 0 and Day 60, evaluated under three different pmf conditions. This comparison will explain how various energy environments favor specific c-ring sizes or adaptive strategies that improve ATP production.

Additionally, this research aims to identify the specific genetic changes that occurred in these E. coli chimera populations over a 60-day evolutionary period. By comparing the genomic analysis between day 0 and day 60 cultures, this study seeks to identify the mutations, especially those affecting the ATP synthase subunits or their regulatory elements, that have been selected in response to the succinate-limited growth conditions. Ultimately, this study aims to establish a direct connection between genetic evolution and changes in ATP synthesis efficiency. This will offer valuable insights into the adaptive landscape and resilience of this essential energy-transducing molecular machine.

July 7, 2025; 9:00am; WLSN 237