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学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称 博士（理学） 氏名 何 佳倫

学 位 論 文 題 名

Study on Microbial Extracellular Electron Transfer Mechanism Under Maintenance State
(メンテナンス代謝状態における細胞外電子移動に関する研究)

Microorganisms can sustain viability in oxygen-limited environments where energy throughput is severely restricted. This dissertation investigates how bacteria exploit extracellular electron transfer (EET) to sustain long-term viability under anaerobic, energy-limited conditions. Using *Pseudomonas aeruginosa* as a model organism, I examined how redox-active metabolites (RAMs) mediate electron shuttling and how efflux pumps together with membrane permeability govern the efficiency of this process. I further determined how these transport and energetic constraints shape survival over extended periods of metabolic limitation. Across four research chapters, the work follows a logical progression:

Chapter 1 introduces the fundamental concepts of bacterial survival under low-energy conditions and outlines how such physiological states influence cellular responses to antibiotics. This chapter provides the conceptual background necessary to understand how EET supports long-term viability under oxygen-limited environments.

Chapter 2 establishes a quantitative platform for measuring maintenance metabolism. Using a 96-well electrochemical system, phenazine-1-carboxamide (PCN)-driven extracellular electron transfer was quantified across defined concentrations under strictly anaerobic conditions. Charge integration normalized to viable cells yielded a cell-specific metabolic rate of 3000 electrons/ sec/ cell, defining the minimal energetic requirement to sustain viability without growth. This platform enables high-resolution assessment of low-power metabolism.

Chapter 3 demonstrates that efflux pumps are the rate-limiting control point of PCN-mediated redox cycling. By manipulating efflux activity (Δ mexS and inducible mexGHI-opmD strains) and comparing adaptation (0–1 day) and maintenance (1–5 days) phases, I showed that efflux capacity determines metabolic throughput once PCN exceeds 75 μ M. During adaptation, enhanced efflux increases metabolic flux in the high-PCN plateau regime. During maintenance, efflux overexpression dissipates PMF and triggers metabolic compensation—higher ATP generation—to restore membrane potential. Efflux pumps thus act as bioenergetic regulators rather than simple detoxification systems.

Chapter 4 demonstrates that membrane permeability is a key parameter governing electron-shuttle efficiency and the magnitude of RAM-supported metabolism. By comparing PCN, PYO, and a broader panel of RAMs, I found that molecules with high membrane permeability—and therefore high passive diffusion capability—enable cells to sustain elevated metabolic rates even at high extracellular concentrations. Strikingly, this elevated metabolic state increases the susceptibility of cells to antibiotic killing.

Chapter 5 extends these mechanistic insights to environmental microbiology by developing a

rapid tool to identify electroactive bacteria. Having shown in earlier chapters that EET is essential for survival under low-energy conditions, I asked which environmental microbes naturally possess strong EET capability. To answer this, I developed osmium-grafted magnetic nanobeads (M@FA-Os NPs) that selectively capture cells capable of DAB-polymer-mediated redox activity on their outer membranes. This culture-free method rapidly enriches EET-capable bacteria from environmental microbiota, producing communities with significantly faster current rise, 16-fold higher peak current, and enhanced long-term stability. The approach enables accelerated discovery of naturally occurring electroactive taxa and supports studies of redox-based survival strategies in complex ecosystems.

Chapter 6 provides an overall summary of this dissertation and outlines future directions, including the energetic requirements of membrane maintenance during long-term survival and the application of rapid EET-enrichment tools to explore the universality of EET-supported survival strategies across natural microbial environments.

Together, these findings define transport-governed EET as a key determinant of survival strategies under energetic scarcity and provide methodological advances for uncovering EET-linked metabolic strategies across diverse microbial systems.