Chapter 1

Introduction

1.1 Background

Vibrio natriegens has attracted increasing attention as a microbial platform for molecular biology, synthetic biology, and metabolic engineering (Lima et al., 2024; Weinstock et al., 2016). Its exceptionally rapid growth, broad metabolic capacity, high substrate uptake rate, salt tolerance, and expanding genetic toolbox make it a promising alternative chassis to conventional bacterial systems (Lima et al., 2024). These features are particularly attractive for high-productivity bioprocesses, where rapid biomass accumulation and efficient substrate conversion are desirable. Previous metabolic engineering studies have demonstrated the biosynthetic potential of *V. natriegens*, including production of industrial chemicals such as 1,3-propanediol (Lima et al., 2024), while genome-scale metabolic modeling has further supported its value as an engineered production host (Coppens et al., 2023). However, despite these advantages, high-density cultivation of *V. natriegens* remains technically challenging, suggesting that rapid proliferation may generate physiological stresses that limit culture stability and productivity.

One potential source of this limitation is the coupling between rapid growth, nutrient consumption, and metabolic overflow. Fast-growing bacteria must coordinate carbon uptake, energy production, biosynthesis, and stress adaptation within a narrow temporal window. When nutrient availability changes rapidly, bacteria activate global stress-response programs, including stringent-response-associated transcriptional remodeling (Brauer et al., 2023). At the same time, high glycolytic flux can promote pyruvate overflow and acetate production through pathways including Pta-AckA (Lima et al., 2024). In many bacteria, acetate accumulation is associated with growth inhibition, intracellular acidification, metabolic imbalance, and reduced culture performance (Lima et al., 2024). Therefore, under nutrient-fluctuating or dense culture conditions, *V. natriegens* may experience a dual burden:

reduced nutrient availability together with acetate-associated metabolic stress. However, how these physiological pressures affect single-cell morphology and growth behavior remains poorly defined.

Cell morphology provides a sensitive readout of bacterial physiological state. Changes in cell length, width, division timing, and shape often reflect alterations in growth rate, nutrient status, DNA replication, cell-wall synthesis, and stress-response pathways (Rojas & Huang, 2018). In *V. natriegens*, chromosome copy number and cell volume change dynamically across the growth curve (Bruck et al., 2023), indicating that rapid growth is accompanied by major shifts in cellular organization. However, the regulatory basis and physiological consequences of nutrient-associated morphological transitions remain unclear. In particular, it remains unknown whether these transitions are passive outcomes of slowed growth or active cellular responses controlled by transcriptional programs. This distinction is important because an actively regulated morphological state may represent an adaptive response, a stress-induced failure mode, or a manipulable target for improving culture performance.

In this study, a time-lapse bright-field imaging and Cellpose-based segmentation workflow was established for the quantification of single-cell morphology in *V. natriegens* following acute nutrient downshift. The requirement for active gene expression in the morphological response was investigated using pharmacological inhibitors of transcription and translation, and time-resolved experiments were performed to determine when this requirement emerges during the stress response. Finally, the impact of nutrient deprivation on tolerance to acetate stress was evaluated, providing insight into the relationship between morphological remodeling and growth performance under conditions relevant to high-density cultivation.

1.2 Objective

General Objective:

To investigate how nutrient depletion induces morphological stress and acetate sensitivity in *V. natriegens* at a population and single-cell level and also identify the transcriptional and translational requirements underlying this response.

Specific Objective:

1. To characterize the morphological response of *V. natriegens* to acute nutrient deprivation
2. To determine the transcriptional and translational requirements for nutrient-induced morphological changes
3. To assess the effect of nutrient depletion on the growth of *V. natriegens* under subsequent acetate stress conditions.

1.3 Hypothesis

1. *V. Natriegens* will change morphologically in response to acute nutrient deprivation
2. Nutrient-deprivation-induced morphological changes will be dependent on active transcription and translation
3. Cells pre-exposed to nutrient-depleting conditions will show reduced capacity to resume growth when challenged with acetate, compared to cells maintained in full medium.