Chapter 1

Introduction

1.1 Background

Pediococcus acidilactici, a lactic acid bacterium (LAB), is widely recognized for its potential applications as a probiotic. Its ability to withstand harsh environmental conditions, including extreme pH levels, temperatures, and osmotic pressures, allows it to colonize the digestive tract effectively (Sharma et al., 2021). However, *P. acidilactici* is a facultative anaerobe that prefers microaerophilic environments with low oxygen concentration. It can still survive aerobically in oxygen-rich conditions but faces challenges from reactive oxygen species (ROS), which can compromise its survival and metabolic functions (Bryukhanov et al., 2022).

To counteract oxidative stress, *P. acidilactici* relies on antioxidant defense mechanisms, which not only protect the bacterium itself but may also enhance the host's antioxidant capacity (Hao et al., 2021). Besides oxidative stress, *P. acidilactici* also encounters growth limitations due to metabolic end-product inhibition, affecting its productivity and viability (Bron & Kleerebezem, 2011). Despite these challenges, studies have shown that *P. acidilactici* maintains a high survival rate (>90%) under various conditions such as low pH (3.0), moderate amounts of bile salts (0.3%), and simulated gastrointestinal (GI) tract environments (Amaretti et al., 2012). This resilience, along with its strong adaptability and probiotic potential, positions *P. acidilactici* as a valuable candidate for gut health applications and food preservation technologies (Feng & Wang, 2020). In addition, *P. acidilactici* TAT-1 was previously isolated from fermented fish and has demonstrated promising antimicrobial and preservative potential, particularly through its cell-free supernatant (CFS) (Nursyam et al., 2007; Kho et al., 2024). Its CFS contains organic acids and bacteriocin-like inhibitory substances (BLIS), indicating its active metabolic profile. In addition to these bioactivities, preliminary studies also suggested this strain can tolerate oxidative conditions, making it a promising candidate for further evaluation of its antioxidant capacity.

Reactive Oxygen Species (ROS) are highly reactive molecules derived from oxygen (O_2) that contain unpaired electrons, making them unstable and capable of disrupting biological systems (Gulcin & Alwasel, 2023). ROS occurs naturally in every species that is exposed to oxygen. While excessive ROS levels can cause significant cellular damage and contribute to various health disorders, low to moderate levels are crucial in regulating essential physiological functions (Jomova et al., 2023). Several key antioxidant enzymes and their corresponding genes play a crucial role in mitigating oxidative stress (Bryukhanov et al., 2022). These enzymes function by ensuring the survival of *P. acidilactici* in oxygen-rich environments by various mechanisms. One way to assess the antioxidant potential of *P. acidilactici*, particularly its ability to inhibit ROS, is through Whole Genome Sequencing (WGS) and antioxidant bioassays.

This project aimed to conduct whole genome sequencing and antioxidant bioassays using *P. acidilactici* TAT-1 CFS to evaluate antioxidant and ROS inhibition activity of *P. acidilactici* TAT-1 The antioxidant bioassays performed in this project aimed to measure the overall activity of antioxidants in the sample with DPPH radical scavenging assay, and also focused on two main stages of ROS scavenging: the scavenging of superoxide and the breakdown of hydrogen peroxide. The results of this study can produce a comprehensive map of the antioxidant mechanisms in *P. acidilactici* TAT-1, allowing a better understanding of the antioxidant activity that can be beneficial for the growth of this species and its potential applications in the food industry.

1.2 Objective

The objectives of this study were:

1. to use Whole Genome Sequencing (WGS) data in identifying genes related to the inhibition activity of Reactive Oxygen Species (ROS) in *Pediococcus acidilactici* strain TAT-1;

2. to identify potential antioxidant activity in the cell-free supernatant of *Pediococcus acidilactici* strain TAT-1 through different antioxidant bioassays.

1.3 Hypothesis

This study hypothesized that *Pediococcus acidilactici* TAT-1 possesses antioxidant defense mechanisms to mitigate oxidative stress. Specifically, the following hypotheses were proposed:

1. Whole Genome Sequencing and Antioxidant Genes
   - H_0 (Null Hypothesis): *Pediococcus acidilactici* TAT-1 did not possess genes encoding antioxidant enzymes.
   - H_1 (Alternative Hypothesis): *Pediococcus acidilactici* TAT-1 possessed genes encoding antioxidant enzymes.
2. Antioxidant Activity in the cell-free supernatant
   - H_0: The cell-free supernatant of *Pediococcus acidilactici* TAT-1 did not exhibit significant antioxidant activity in different antioxidant assays.
   - H_1: The cell-free supernatant of *Pediococcus acidilactici* TAT-1 exhibited significant antioxidant activity in different antioxidant assays.