Chapter 1

Introduction

1.1 Background

The increasing food production following the growing global populations has led to the increase of organic waste, reaching 1.4 billion tons. The accumulation of these wastes causes environmental issues, such as emissions of greenhouse gasses and pollution (Dhiman et al., 2025; Adhikari et al., 2024). One of the abundantly generated agri-food industrial byproducts in Taiwan is soybean curd residue (okara), reaching an annual generation of 0.5 million tons; hence, the need for a method to effectively degrade and valorize it (Kuligowski et al., 2024; Quintana et al., 2023; Xiang et al., 2024). Black soldier fly larvae (BSFL) can convert these wastes into biomass with high protein (32-58%) and lipids (15-39%), which can be processed into animal feed, lubricants, biofuel, and pharmaceuticals (Gold et al., 2018; Franco et al., 2021). Although insect bioconversion is promising, the field is still in its infancy, with much research still needed (Eke et al., 2023).

Shifts in BSFL gut microbiome are correlated with BSFL growth performance, but currently BSFL rearing mostly leaves microbial colonization to chance, which may result in inconsistent growth and conversion efficiency (Koch et al., 2025; Ijdema et al., 2025). Thus, recent research is focusing on microbiome engineering strategies, such as the supplementation of probiotics. Probiotics are live microbes that benefit the host when supplemented in adequate concentrations. While the clinical and regulatory definitions of probiotics in insects may differ from those in humans, this study focuses strictly on the functional role of probiotics in the context of BSFL. Probiotics could be acquired from the BSFL gut isolates, BSFL-rearing-associated isolates (i.e., substrate or frass), or commercial probiotics for other species (Gorrens et al., 2023; Ziyadi et al., 2016). Since microbial communities are typically specific with the host, host-derived probiotics are likely more effective (Ward et al., 2019). Hence, it is preferable to isolate potential probiotics from the host's gut microbiome.

Microbes isolated from BSFL guts, however, differ depending on the substrate and developmental stage, as the substrate is one of the main sources of microbes, and nutritional composition can serve as selective pressure favoring certain microbes, while bacterial composition will experience restructuring between the BSFL life stages (Xu et al., 2025; Klammsteiner et al., 2026; Eke et al., 2023). Currently, common BSFL gut microbes, such as *Morganella*, *Providencia*, *Dysgonomonas*, *Enterococcus*, and *Klebsiella*, have not been tested as probiotics, though they are hypothesized to better colonize the gut (Lin & Shelomi, 2024). Most studies evaluating isolated BSFL gut microbes as probiotics focus predominantly on *Bacillus subtilis*, although the reported outcomes vary and appear to be strain-specific (Gorrens et al., 2023). This study will isolate potential probiotics from different developmental stages (neonates, five-day-old larvae, fifth-instar larvae, and prepupae) of BSFL reared on different substrates, okara and dog food, with common BSFL gut microbes as the main target (Auger et al., 2023b; Vong et al., 2016).

Despite their potential, not all BSFL-derived microbes positively affect growth performance (Silvaraju et al., 2024). Hence, there is a need to analyze their effect on BSFL growth performance (i.e., larval weight and length) and conversion efficiency (i.e., bioconversion efficiency and substrate reduction efficiency) (Bosch et al., 2020; Rasdi et al., 2022). Furthermore, most existing studies do not verify gut colonization, even though it is essential to determine whether the effects of probiotics are due to microbial activity in the gut or external fermentation in the feed (Lin & Shelomi, 2024). One such method is through the use of GFP-labeling, which will be done in this study, to make microbes emit green fluorescence that is easily detected (Zhai et al., 2019). As such, this study will assess the effect of the isolated potential probiotics on BSFL growth performance (larval weight and length) and conversion efficiency (bioconversion efficiency and substrate reduction efficiency), as well as verify the ability of these potential probiotics to colonize the BSFL gut.

1.2 Objective

1. To isolate microbes from different developmental stages of BSFL reared on different substrates.
2. To improve larval growth performance (larval weight and length) and conversion efficiency (bioconversion efficiency and substrate reduction efficiency) when BSFL is supplemented with the isolated microbes.
3. To determine if the supplemented microbes successfully colonized the BSFL gut.

1.3 Hypotheses

The null (H_0) and alternative (H_1) hypotheses for the first objective are as follows:

H_0: No microbes are isolated from the different developmental stages of BSFL reared on different substrates.

H_1: At least one microbe is isolated from the different developmental stages of BSFL reared on different substrates.

The null (H_0) and alternative (H_1) hypotheses for the second objective are as follows:

H_0: There is no significant increase in larval growth performance and conversion efficiency when BSFL is supplemented with any of the isolated microbes.

H_1: There is a significant increase in larval growth performance and conversion efficiency when BSFL is supplemented with at least one of the isolated microbes.

The null (H_0) and alternative (H_1) hypotheses for the third objective are as follows:

H_0: None of the supplemented microbes successfully colonizes the BSFL gut.

H_1: At least one of the supplemented microbes successfully colonizes the BSFL gut.