

CHAPTER 1

INTRODUCTION

1.1 Introduction

Green revolution has prompted significant increases in agricultural output, obtained via the use of genetically improved crop types, chemical herbicides, and fertilisers. Yet that success is tainted by a number of unsustainable practices, including the deterioration of soil and human health (Sharma and Singhvi, 2017; Münzel et al., 2023), soil erosion and degradation (Hossain et al., 2022), groundwater contamination (Srivastav, 2020), and loss of soil biodiversity (Tong et al., 2019). Consequently, utilising specialised strategies, plant microbiome exploitation is expected to potentially revolutionise the agriculture and food industries. These include (i) incorporating crop health and upgraded management practices in ensuring specific climatic conditions for increased productivity and quality; (ii) implementing eco-friendly pest and pathogen control methods, thereby reducing chemical pesticide usage and its negative effects on the environment and human health; (iii) giving thought to astute and effective approaches for implementing natural resources (i.e. soil and water); and (iv) producing higher-quality goods (Singh and Trivedi, 2017). Research on environmentally acceptable alternatives of plant growth-promoting chemicals has been sparked by rising concerns about the detrimental impacts of excessive

chemical usage in agriculture (Padmini et al., 2017; Wang et al., 2017; Behera et al., 2022; Ye et al., 2022). Under such unique environmental conditions, the mangrove habitat contains abundant and characteristic of microbial resources which make mangrove known to host diverse microbial communities. Microbial resources are capable to produce enzyme, bioactive compounds, antibiotic and other value substances (Selim et al., 2021; Danquah et al., 2022; Janatiningrum and Lestari, 2022). The complex and unique habitat of mangrove forests supports the growth of a wide range of bacteria with diverse metabolic capabilities. This biodiversity increases the likelihood of discovering novel and potentially useful bacteria (Liu et al., 2019).

Furthermore, mangrove soil bacteria have developed unique adaptations to thrive in the challenging conditions of the mangrove environment. These conditions include high salinity, fluctuating tidal conditions, low oxygen levels, and nutrient limitations. Bacteria from such environments are often resilient and possess unique traits that may be beneficial in various applications. Next, the bacteria present in mangrove soil are essential for breaking down organic matter and recycling nutrients, contributing to the overall health and stability of the ecosystem. The ability of these bacteria to efficiently decompose complex organic compounds suggests that they may have valuable enzymatic capabilities that can be harnessed in biotechnological applications (Nimnoi and Pongsilp, 2022). Microbes serve as a source of biotechnologically valuable and important products and contribute to biogeochemical cycles and serve as a primary nutrition source to plants. Some of these studies have isolated and identified microbial strains with the potential for biotechnological resources such as microbial cellulase, endophytes and thermostable and

salinity-tolerant glucanase enzyme (Behera et al., 2016; Castro et al., 2018; Paixão et al., 2021). Selecting mangrove soil as a source of bacteria offers the opportunity to tap into the diverse microbial communities that have adapted to the challenging conditions of this unique ecosystem. The exploration of these bacteria holds promise for discovering novel traits and potential applications in various fields, while also promoting the conservation and understanding of mangrove habitats.

Duckweeds are among the world's tiniest free-floating aquatic plants. Duckweeds are members of the family *Lemnaceae*, which includes five genera and 36 species divided into two subfamilies: *Lemnoidea* (15 species) and *Wolffioideae* (21 species). Duckweed species reproduce genetically identical clones vegetatively, with daughter fronds sprouting from mother fronds (Baek et al., 2021). Duckweeds are abundant in protein, essential amino acids, and micronutrients (Appenroth et al., 2017). Duckweed can be a useful biological model for studying plant-microbe interactions due to its ease of inoculation. Because of their tiny size and rapid development, duckweed can alter the genetic of the rhizosphere (Demmig-Adams et al., 2022). Therefore, studying the complex relationships between bacteria derived from mangroves and duckweed not only provides valuable knowledge for sustainable agriculture, but also opens up possibilities for advanced biotechnological uses, improving our comprehension of the symbiotic relationship between plants and microorganisms in unique ecosystems.

1.2 Problem Statement

It's undeniable that widespread use of chemical fertilisers and pesticides has increased agricultural output and moved the world closer to its food security and poverty reduction goals. In any case, their uncontrolled and excessive use has led to contaminated food, negative environmental effects, and disease resistance, all of which pose serious risks to human health and food security (Singh and Trivedi, 2017). The long-term viability of today's agricultural sector is also under question due to the widespread and often immoral usage of chemical fertilisers and pesticides. As a result, food security and safety become more of an uphill battle as farming costs soar and the average farmer's income drops (Kapari et al., 2023).

Constant reliance on chemical fertilisers to develop agriculture is associated to severe soil quality decline, water pollution and contamination hazards, and overall economic stagnation (Bisht and Chauhan, 2021). The three most widely grown cereal grains (corn, rice, and wheat) account for around 57% of global agricultural calorific consumption, although their yields have been flat or declining for quite some time now, according to the available studies (Erenstein et al., 2022). Increasing environmental factors such as drought, heavy metal pollution, soil salinity, and heat stress exacerbate the problem (Islam and Sandhi, 2022). It is estimated that biotic stressors, such as those caused by pests and pathogens, reduce agricultural output by a further 30%, costing the world economy \$40 billion annually (Syed Ab Rahman et al., 2018).

On the other hand, research has shown that only 0.1% of chemical pesticides successfully kill the target pests, while the rest leach into the soil and water nearby, polluting the ecosystem (Sathish Kumar et al., 2024). Moreover, genetically modified (GM) plants provide a promising option for low-input and sustainable agriculture, but the expense and time required for creating new varieties is daunting (Syed Ab Rahman et al., 2018). High yields can be reached through the use of sustainable agricultural approaches that prevent depletion of soil nutrients by prioritising their absorption in plant cells (Sankar et al., 2017). Therefore, natural resources, such as phytomicrobiomes and their utilisation are generally considered as an efficient replacement for guaranteeing long-term agricultural production and food quality, hence supporting good environmental and social goals (Singh and Trivedi, 2017). In general, biologically oriented agriculture refers to a broad manufacturing and management system that emphasises and increases agro-ecosystem features such as robustness, biodiversity, biological cycles, and biological soil activity.

1.3 Aim and Objectives

The aim of this study was to develop a biofertilizer by combining specific mangrove-associated bacteria to enhance the growth rate and protein contents of duckweed plants.

Hence, the objectives of this study were:

1. To identify a wide range of microbial species and function of genes from three soil samples collected at the freshwater riverine mangrove at Sungai Lukut, Negeri Sembilan, Malaysia using metagenomic Whole Genome Sequencing (mWGS).
2. To isolate and identify selected bacterial species which grown on selective media which are nitrogen-fixing bacteria, potassium solubilizing bacteria and phosphorus solubilizing bacteria by using Polymerase Chain Reaction (PCR) and sequencing of the 16s rRNA gene.
3. To determine the selected bacterial species that can affect duckweed plants through growth rate and protein contents analyses.