Chapter 1

Introduction

1.1 Background

Endophytic bacteria are bacteria that reside inside plants without showing any signs of disease. They keep parasitic or commensal relationships with their host plants and are progressively being acknowledged for their ability to produce a wide variety of bioactive secondary metabolites with big biotechnological, medicinal, and agricultural applications. Secondary metabolites are bioactive substances that are generated by plants or microorganisms and have significant ecological or protective functions despite not being necessary for basic growth or reproduction. Besides that, they have antimicrobial, antioxidant, and even anticancer properties (Zotchev, 2024; Semenzato & Fani, 2024): Alkaloids, phenolics, terpenoids, lipopeptides, and polyketides. Endophytes live inside plant tissues, frequently populating areas where diseases might otherwise invade. By inhabiting these areas early on, they can limit pathogen establishment and compete for resources. *Bacillus subtilis* endophytes, for example, have been demonstrated to inhibit Fusarium moniliforme invasion by competing for space and nutrients (Xia et al., 2022). Endophytic microbes are thought to have antimicrobial properties due to their ability to produce a variety of bioactive substances such as phenazines, alkaloids, lipopeptides, and hydrolytic enzymes which can directly inhibit the growth of bacterial and fungal pathogens. These substances not only inhibit competitors but also help to protect the host plant from infection (Chaudhary et al., 2022). Aside from direct antagonism, many endophytes interfere with microbial quorum-sensing systems, a process known as quorum quenching, which disrupts pathogen communication, reduces virulence, and, in some cases, improves the efficacy of conventional antibiotics acting as potential antibiotic adjuvants (Moussa, 2024). Complementarily, endophytes routinely induce a systemic resistance in their host plants. This effect is achieved through the precise activation of signaling cascades centered around salicylic acid (SA) and jasmonic acid (JA), as well as the upregulation of class-specific defense proteins and enzymes, thereby fortifying the plants' immune apparatus against a spectrum of invading pathogens (White et al., 2019). Generally, the development of antibiotic resistance among pathogenic microbes that has been escalating is the reason that the search for plant-derived endophytic microorganisms as a source of new antibacterial and antibiofilm agents has become a focus of attention (Nimbulkar et al., 2025). Consequently, the investigation of endophytic bacteria is rapidly becoming a method to discover new bioactive compounds in a sustainable and eco-friendly manner. Moreover, the extract's antibacterial and antibiofilm properties make it suitable for treating both acute and chronic infections simultaneously, eliminating free-floating bacteria and inhibiting or degrading biofilms that cause recurring, chronic, and antibiotic-resistant infections.

Piper crocatum Ruiz & Pav., also called red betel plant, is a fragrant medicinal plant in Indonesia and Southeast Asia. It has well-documented ethnomedicinal usages that include wound healing, antioxidant replenishment, and antibacterial therapy, related to its high level of phenolic compounds, flavonoids, and essential oils. Scientific research has shown that *P. crocatum* extracts have antibacterial activity (Astyka et al., 2024; Ni et al., 2023) in comparison to its fungal counterparts; the role of its bacterial endophytes in various bioactivities is yet unknown. Although preliminary findings suggest endophytic bacteria isolated from other Piper spp. have strong antibacterial and antibiofilm activities, indicating that *P. crocatum* may offer a similar opportunity. This study fills this gap by isolating bacterial endophytes from various plant tissues (leaves, stems, and roots) through surface sterilization and selective culture.

The anticipated impacts of this study extend well beyond characterizing microbial diversity within *P. crocatum* to discovering endophytic bacteria producing antibacterial chemicals, with the potential to help an international effort to produce new drugs against multidrug-resistant infection, which is an important priority in contemporary medicine (Semenzato & Fani, 2024). Unlike previous synthetic antibiotics, natural bioactives from endophytes often have complex chemical structures with diverse modes of action that make them less prone to resistance. Furthermore, microbial sources are often consistent with sustainable drug authorization paradigms as they can be grown easily in controlled laboratory conditions, without overharvesting their host plants and destroying ecological balance (Nimbulkar et al., 2025). Beyond pharmaceutical effects, the discoveries have the potential to inform agricultural biotechnology by discovering endophytes that act as both plant growth boosters and disease suppressants. Finally, this research emphasizes the necessity of preserving medicinal plants as reservoirs of unique microbial biodiversity and encourages the use of endophyte-based bioproducts in future healthcare management initiatives

1.2 Objective

- Identifying the characteristics of endophytic bacteria from different tissues of red betel plant
- Identifying the isolate's antibacterial activity against specified pathogenic bacteria using the agar well diffusion technique
- Determining the antibiofilm inhibition activity using the crystal violet microtiter plate test

1.3 Hypothesis

Endophytic bacteria isolated from the leaves, stems, and roots of *Piper crocatum* produce secondary metabolites that exhibit significant antibacterial and antibiofilm activity against selected pathogenic bacteria.