CHAPTER I. INTRODUCTION

1.1 Background

Indonesia has a vast karst area, covering 15.4 million hectares from Sumatra to Papua (Sulistiyowati & Haryono, 2021). The formation of karst, including the development of caves and various formations within them, is strongly influenced by the dissolution of limestone or calcium carbonate ($CaCO_3$). The solubility of calcium carbonate can decrease or increase depending on various factors, both abiotic factors such as evaporation, changes in temperature and pressure, and biotic factors, especially microbial precipitation activity. The influence of biotic factors, especially those from microorganisms, has a greater impact than abiotic factors in this environment (Castanier et al., 2000).

Various bacterial species contribute to the precipitation of carbonate minerals in natural environments such as soils, geological formations, freshwater biofilms, oceans and high-salinity lakes. Calcium carbonate precipitation involves a combination of mineralization processes on microbial cell surfaces, chemical precipitation from saturated solutions, and erosion of existing carbonate layers (Riding, 2000). During the Microbial Induced Calcium Carbonate Precipitation (MICP) process, organisms secrete metabolic products such as carbonate ions (CO_3^{2-}) that react with calcium ions (Ca^{2+}) in the environment to form calcium carbonate minerals. Both autotroph and heterotroph bacteria are capable of inducing calcium carbonate precipitation in their surroundings (Anbu et al., 2016).

Biomineralization is defined as biologically induced precipitation, where organisms create a local microenvironment with conditions that allow optimal extracellular chemical precipitation of minerals (Hamilton, 2003). MICP is a biogeochemical process that facilitates calcium carbonate precipitation in the soil matrix, resulting in minerals that bind to sand particles through surface contact, thereby increasing soil strength and stiffness (Mortensen et al., 2011).

The urease enzyme is found in ureolytic bacteria and has been widely applied in various fields. It also plays a role in catalysis of biomineralization processes, especially in calcite precipitation, which can be used in construction (Krishnapriya & Venkatesh, 2015). Previous studies have demonstrated that ureolytic bacteria, through the production of urease enzyme, are able to convert urea to ammonia (Phang et al., 2018). For example, bacterial isolate TSB12, isolated from a limestone cave, showed a urease activity of 8.7×10^6 μmol ammonia/min (Omoregie et al., 2016). The ability of urease (urea amidohydrolase; EC 3.5.1.5) to induce carbonate precipitation in microorganisms has been discussed by several researchers.

Urease activity is found in a variety of microorganisms, but some strains produce very high levels of urease. For example, *Sporosarcina pasteurii* (formerly *Bacillus pasteurii*) is a non-pathogenic, with a growth optimum pH of 9.0 that can survive in extreme conditions. Therefore, many researchers have extensively studied the use of this strain for MICP. The MICP process is an effective and environmentally friendly technology that can be applied to solve various environmental problems, including heavy metal and radionuclide remediation, bioconsolidation, biocementation, CO_2 sequestration, and other applications (Anbu et al., 2016).

Several previous studies have highlighted that bacteria from caves have the potential to precipitate $CaCO_3$, playing a role in the formation of cave formations through their urease enzyme activity, despite the very low availability of nutrients in the cave environment. These $CaCO_3^-$ producing bacteria are referred to as ureolytic bacteria, which have the ability to induce large amounts of $CaCO_3$ precipitation in a short period of time. The capacity of these bacteria in $CaCO_3$ precipitation offers various benefits, including applications in bioconstruction technology. Therefore, this study aims to isolate ureolytic bacteria from Ngalau Tarang Cave, Agam, West Sumatra.

However, research on the isolation and characterization of ureolytic bacteria from speleothem in Ngalau Tarang Cave, Kamang Magek, West Sumatra, is remains scarce. This research is important to understand the role of ureolytic bacteria in the formation of speleothem in the region and its potential application in the field of biotechnology, such as biogrouting for concrete structure repair. By examining ureolytic bacteria in speleothem that produce urease, which plays a role in the formation of calcite ($CaCO_3$), it is expected to contribute to the development of biotechnology in the field of construction.

1.2 Problem Formulation

1. Do bacterial isolates occur in the speleothem of Ngalau Tarang Cave, Agam, West Sumatra?
2. Do the bacterial isolates exhibit urease activity and possess the potential to precipitate calcium carbonate ($CaCO_3$)?

3. What are the characteristics of bacterial isolates found from Ngalau Tarang cave speleothem, Agam, West Sumatra?

1.3 Research Objectives

1. Isolation of bacterial isolates of bacteria in Ngalau Tarang , Agam, West Sumatra that can produce urease enzyme.
2. Testing the ability of bacterial isolates to produce urease enzyme qualitatively and calcium carbonate ($CaCO_3$) precipitation potential through quantitative measurement of the bacterial isolates.
3. Macroscopic and microscopic observation of bacterial isolates obtained from Speleothem of cave.

1.4 Benefits of Research

The benefit of this research is to provide insight into bacteria in cave spleothem that produce the enzyme urease, which plays an important role in calcium carbonate precipitation. These bacteria not only contribute to speleothem formation in nutrient-limited cave environments, but also have significant potential for application in construction biotechnology.