A Possibility of Proteolytic Bacteria Utilization to Control 
*Ralstonia solanacearum* 59 In Vitro

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Abstract. *Ralstonia solanacearum* is a bacterium that causes wilting in chili plants. This bacterium can damage the tissue and cause a decrease in production. One of the controls using antagonistic bacteria with the mechanism of antibiosis is the production of secondary metabolites. Secondary metabolites are protease enzymes. The purpose of this study was to examine the ability of proteolytic bacteria from tofu wastewater to inhibit the growth of *Ralstonia solanacearum* 59. The study was conducted at the Biology Laboratory, Institut Teknologi Sumatera from April-October 2019. The research method used was experimental testing of bacterial antagonists in vitro on media Mueller Hinton Agar. The results showed that of the 28 proteolytic isolates tested, only three bacterial isolates could inhibit the growth of *R. solanacearum* 59, namely BLT-15, BLT-17, and BLT-27. Of the three isolates, BLT-17 showed the highest inhibition zone against *R. solanacearum* of 23 mm. This inhibition zone activity showed that BLT-17 isolate was able to be used as a biocontrol agent of *R. solanacearum* 59, the cause of bacterial wilt disease.

1. Introduction

Bacterial wilt caused soil-borne, namely, *Ralstonia solanacearum* is a plant disease that is very damaging and reduces production. This bacterium can attack more than 200 species of plants, including important crops such as chili, tomatoes, potatoes, and beans [1, 2]. Bacterial wilt attack on chili plants caused by *Ralstonia solanacearum* in Indonesia tends to increase since 2004-2011, from 216.90 ha to 504.05 ha [3]. Control of *R. solanacearum* is quite difficult because the pathogen has a wide range of hosts, can survive on the rest of the host tissue, in the soil in a dormant state even though there is no host for years and is easily spread by water flow and can move actively while at surface water layers [4]. The recommended control technique and considered environmentally friendly for this disease is biological control. Biological control is based on the use of antagonistic microbes that can be direct (competition, predation, and antibiosis) or indirectly through the induction of host plant resistance.

Soil-borne diseases can be controlled using beneficial bacteria. The bacteria that are often used are Plant Growth Promoting Bacteria (PGPB) such as *Bacillus amyloquefaciens*, *B. licheniformis*, and *B. subtilis*. PGPB can increase plant growth through by two mechanisms i.e direct and indirect mechanisms. Phytohormone production, acquisition of nutrients such as phosphate and nitrogen, control of pathogens in various ways including the production of hydrolytic enzymes (proteases, chitinases, and glucanases), antifungal compounds, lipopeptides or antibiotics are the most common direct mechanisms of plant growth promoting bacteria [5]. In general, the control of pathogenic fungi only uses the ability of antibiotics, while the use of hydrolytic enzymes such as proteases is still very...
rare, so it needs to be studied further. Previous studies [6] have succeeded in isolating 28 protease-producing bacterial isolates from tofu wastewater, which also can dissolve phosphate in pikovskaya medium. Protease-producing bacteria are thought to be able to suppress the growth of \textit{R. solanacearum} by degrading its cell walls to reduce its incidence rate. This study aims to determine the ability of proteolytic bacterial isolates from tofu liquid waste to control the growth of \textit{R. solanacearum} 59 in vitro.

2. Methods
The research was conducted at the ITERA Biology Laboratory in April to October 2019. The materials used in this study were 28 isolates of proteolytic bacteria resulting from the isolation of tofu liquid waste [10] and \textit{Ralstonia solanacearum} 59 obtained from the PT. Arara Abadi, Sinarmas Forestry, Riau.

\textit{Bacterial Rejuvenation}
A total of 28 proteolytic bacterial isolates were rejuvenated on Nutrient Agar + Skim Milk 1% and incubated at 30°C. As confirmation, the bacteria were stained for gram method. In contrast, \textit{Ralstonia solanacearum} 59 was grown on Nutrient Agar media and incubated at room temperature.

\textit{Proteolytic bacteria antagonism test against \textit{R. solanacearum} 59 in vitro}
\textit{R. solanacearum} was grown on Nutrient Broth medium and then incubated by shaker incubator at 120 rpm for 24 hours. Proteolytic bacterial isolates were grown on the Mueller Hinton Agar medium, which had been mixed with \textit{R. solanacearum} 59 suspension (200 microliters of bacterial suspension) through a bottle using a toothpick — subsequently incubated for 24-48 hours at room temperature. The inhibitory ability is seen from the clear zone formed around the colony.

3. Results and Discussion

\textit{Proteolytic bacteria antagonism test against \textit{R. solanacearum} 59 in vitro}

Of the 28 proteolytic bacterial isolates tested, only 3 (three) isolates could inhibit the growth of \textit{R. solanacearum} 59. The inhibitory ability was demonstrated by the formation of clear zones around a colony of antagonistic bacteria (proteolytic) (Figure 1). The three bacteria are BLT-15, BLT-17, BLT-26, and BLT-27. The ability of these four bacteria to inhibit the growth of \textit{R. solanacearum} 59 is quite varied. These can show from the diameter of the inhibition zone formed (Table 1). The higher the diameter of the inhibitory zone, the more potential the bacterium is in inhibiting the growth of pathogenic bacteria. Of the three bacteria, BLT-17 isolates showed the best inhibition against \textit{R. solanacearum} 59 with inhibition zones, and the highest inhibition zone parameters were shown by BLT 17 isolates with inhibition zones of 23 mm. Differences in the ability of bacterial isolates to inhibit the growth of \textit{R. solanacearum} 59 due to differences in the strains of each isolate.

| Isolate | Diameter of Inhibition (mm) | Diameter of Colony (mm) | Inhibition Zone (mm) | Antimicrobial Index |
|---------|-----------------------------|-------------------------|---------------------|-------------------|
| BLT 15  | 17.00                       | 12.50                   | 4.50                | 0.36              |
| BLT 17  | 30.00                       | 7.00                    | 23.00               | 3.29              |
| BLT 27  | 8.00                        | 5.00                    | 3.00                | 0.60              |

Inhibition zones begin to form 24 hours after inoculation, but clear zones that are formed are not yet clearly visible. Inhibition zones begin to appear clearly after 48 hours of incubation. The formed barrier zone shows that the mechanism of BLT-17 as a biological agent is antibiosis. This shows that potential bacterial isolates inhibit \textit{R. solanacearum} 59 by a direct mechanism [7]. The amount of
inhibition zone formed in BLT-17 isolates showed very high ability. When compared with \textit{B. subtilis} B315 which only has an inhibition zone of 14 mm [8] and \textit{B. subtilis} MTCC-8114 with a 14-16 mm inhibition zone [9] with the same inoculation method namely inoculation spot, this BLT-17 isolate is a proteolytic bacterium that has a proteolytic index of 2.100 and is non-pathogenic in plants [10]. In this study, there was no correlation related to the ability of protease as an antibiotic to \textit{R. solanacearum} 59. However, these results indicate that proteolytic bacteria from tofu wastewater have potential as antimicrobial properties against pathogenic bacteria. \textit{Streptomyces} sp. which is known to have the potential for antibiosis is also reported to be able to produce protease enzymes, one of which is \textit{Streptomyces} sp S-4. The ability of \textit{Streptomyces} sp. S-4 in inhibiting \textit{R. solanacearum} is lower than BLT-17 bacterial isolate which is only 4.53 mm [11]. The presence of proteolytic enzymes in bacterial isolates does not indicate that these isolates have the ability of antibiosis against pathogenic bacteria, but the three types of bacterial isolates tested have antibiotic abilities and produce proteases, especially BLT-17 isolates.

![Image](image.png)

\textbf{Figure 1.} Inhibition zone of proteolytic bacteria against \textit{R. solanacearum} 59

\section*{4. Conclusion}
Based on the description, it can be concluded that BLT-17 isolate can inhibit \textit{R. solanacearum} 59 in vitro with a 23 mm inhibition zone so that it can be used as a potential isolate in suppressing bacterial wilt disease in chili plants.

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