

The Potential of Proteolytic Bacteria Isolated from Peat Soil against *Salmonella Typhi* and *Escherichia Coli*

Rizni Syahputri¹, Rachmiati¹, Sartini¹ and Kiki Nurtjahja²

¹Department of Biology, Universitas Medan Area, Medan, Indonesia

²Department of Biology, Universitas Sumatera Utara, Medan, Indonesia

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Abstract: Peat soil consisted of organic organic soil that developed of plant residue in wet condition. The aim of this study was to investigate the ability of proteolytic bacteria originated from peat soil in inhibiting pathogenic bacteria (*Salmonella typhi* and *Escherichia coli*). The soil samples were obtained from an area ±200 m² at Labuhan Batu, North Sumatera, Indonesia. Each soil samples (200 g) were homogenized and serial dilution was conducted to isolate soil bacteria. All of the bacteria then were cultured in skim milk agar at 37°C for 48 h. Bacterial colony with clear zone were isolated and identified. Nutrient agar plate medium was used to examine antagonist characteristics between pathogenic and proteolytic bacteria. Results showed that total 12 isolates bacteria were successfully isolated. Among 12 isolates, six isolates (PSP7, PSP8, PSP9, PSP10, PSP11 dan PSP12) were proteolytic characteristics. Among the proteolytic isolates, PSP7b and PSP11 were the highest clear zone. However, antagonism of isolate PSP10 (14.88 mm) against *E. coli* was higher than that of PSP9 (10.25 mm) against *S. typhi*.

1 INTRODUCTION

As a tropical country and high rainfall, Indonesia has high proportion of peat soil (Warren et al. (2017). The distribution of peat soil in Indonesia were in Sumatera (35%), Kalimantan (30%), Papua (30%) and Sulawesi (3%) (Wahyumto et al. (2006). Mahdiyah (2015) reported that peat soil contain microorganisms and potential for health and agriculture development.

The presence of cellulolytic and proteolytic microorganisms in peat soil is crucial in degrading high organic compound. Peat soil bacteria is potential source of antibiotic (Yuliar et al. (2011), Enzyme production (Ravi et al. 2015). Mahdiyah (2015) was reported the presence of proteolytic bacteria isolated from peat swamp soil in Banjarmasin, South Kalimantan. Afriyani et al. (2009) reported that *Lactobacillus brevis* was proteolytic that potential to inhibit *Staphylococcus aureus*. Whereas, Erlindawati et al. (2015) was successful to isolate three isolates peatsoil bacteria that inhibit *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter gergoviae*, and *Proteus rettgeri*.

The importance peat soil that support ecosystem can prevent damage by human activities (Warren et al. (2017). High biodiversity of microorganism

particularly bacteria that potential for human life required to be explored. The aim of recent study was to isolate proteolytic bacteria from peat soil that potential in inhibiting pathogenic bacteria *Salmonella typhi* and *Escherichia coli*.

2 MATERIALS AND METHOD

2.1 Sampling of Peat Soil

The research was conducted from August to Nopember 2020 at Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan. As much as 200 g from five sites of peat soil as samples were obtained from sampling area 200 m² at Labuhan batu, North Sumatera. Three replicates for each site. All soil samples then were packed in steril polyethylene bag.

2.2 Screening and Isolation of Proteolytic Bacteria

Each soil sample was homogenized and 25 g of the homogenized sample in 500 ml flask was mixed in

250 ml sterilized distilled water. The suspension was shaken and 1 ml of the aliquot was poured with skim milk agar on to petri dish (9 cm in diameter). Four dilution was made (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4}) for each sample with three replicates. All plates were incubated for 24 h at 37°C. Separate bacterial colonies with clear zone were enumerated and isolated in nutrient agar slant.

2.3 Examination of Proteolytic Bacteria

Each isolate of proteolytic bacteria was examined in skim milk plate agar. Each culture was incubated for 24 h at 37°C. The presence of clear zone surrounding the colonies was determined.

2.4 Antimicrobial Activity of Proteolytic Bacteria

Pathogenic bacteria, *Escherichia coli* and *Salmonella typhi* used were culture collection of Laboratory Microbiology, Pharmacy Department, Universitas Sumatera Utara. Subculture each of the bacteria was tested against isolates of proteolytic bacteria using Kirby-Bauer method in petri dish (9 cm in diameter). Each suspension of the bacteria was spread out at nutrient agar plate medium and paper disc containing 0.1 ml proteolytic bacteria was put on the plate. All plates were incubated at 24 h for 37°C. Three replicates for each culture. The presence of clear zone surrounding paper disc was observed.

3 RESULTS AND DISCUSSION

3.1 Proteolytic Bacteria in Peat Soil

A total of 12 bacterial isolates (Psp1, PSp2, PSp3, PSp4, PSp5, PSp6, PSp7, PSp8, PSp9, PSp10, PSp11, PSp12) of proteolytic bacteria were successfully isolated from peat soil. However, only 6 isolates (PSp7, PSp8, PSp9, PSp10, PSp11, PSp12) were proteolytic characteristics that indicated by the presence of clear zone surrounding their colonies in skim milk agar medium. The characteristic each of the isolates was indicated by clear zone (Table 1).

Table 1: Isolates of proteolytic bacteria isolated from peatland in skim milk agar medium after incubation 24 hour at 37°C

Isolates of proteolytic bacteria	Diameter of clear zone (mm) in skim milk agar
PSp7	9.0
PSp8	8.5
PSp9	8.0
PSp10	8.5
PSp11	10.0
PSp12	8.5

As shown in Table 1 each isolate has different proteolytic activity as indicated by different diameter clear zone. However, isolates such as PSp11 and PSp7 were the highest diameter 9.0 mm and 10.0 mm respectively. Based morphological characteristic all isolates has gram positive, streptococci except PSp11 has bacil form. Our results were in line with Mahdiyah (2015) that most bacteria isolated from peat soil with streptococci and gram negative. Hidayat (2004) reported most bacteria isolates from peat soil at Tanjung Puting, Kalimantan was genus of *Bacillus* that rod shape and proteolytic activity. Whereas, isolation of proteolytic bacteria from soil by Ravi et al. (2015) stated most of the species was *Bacillus subtilis*.

3.2 Antibacterial Activity of Proteolytic Bacteria against Pathogenic Bacteria

The potential of antibacterial isolated from peatland against *Salmonella typhi* and *Escherichia coli* was indicated by the presence of clear zone Table 2.

Table 2: Antagonistic activity of proteolytic bacteria

Bacterial isolates	Pathogenic bacteria/ Diameter of inhibition (mm)	
	<i>Salmonella typhi</i>	<i>Escherichia coli</i>
PSp7	0,00	12.45
PSp8	8.50	10.37
PSp9	10.25	10.10
PSp10	9.35	14.88
PSp11	0.00	10.50
PSp12	8.67	10.50

Table 2 showed that all proteolytic bacteria inhibit both *Salmonella typhi* and *Escherichia coli* as indicated by inhibition zone. The potential inhibition of all isolates on *E. coli* is higher than that of *S. typhi*. Even, two strain PSp7 and PSp11 both are proteolytic and have no inhibition on *S. typhi*. However each strain showed different inhibition, strain PSp9

showed the inhibition (10.25 mm) on *S. typhi*, whereas, strain PSp10 on *E. coli* (14.88 mm). The inhibition might occur the proteolytic have hydrolytic compounds. Erlindawati (2015) and Agustono et al. (2021) reported that peat soil bacteria produce protease and anti-microbe. In our results indicate that all isolate proteolytic bacteria were gram positive and showed antibacterial activity as previously reported by Ong et al. (2015).

4 CONCLUSION

Different strains of proteolytic bacteria isolated from peat soil have antagonis activity againts *Salmonella typhi* and *Escherichia coli*.

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