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Abstract. Endophyte bacteria have several beneficial including growth hormone, biofertilizer and biocontrol. The sample of rubber plant leaf was successfully isolated nine endophyte bacteria with direct method. This study aims to screening endophyte bacteria that can chitinase activity. The result of the research showed that strain D5 and D35 can produce chitinase which was accomplished by forming clear zone on medium chitin 2% (w/v) after 5 days incubation with Congo red stain. Bacteria strains D5 and D35 produce chitinolytic Index (IC) of 1.66 and 2.08. Identification based on the 16s rRNA gene showed the D35 strain bacteria showing that it has maximum homology 99% with the *Klebsiella variicola* strain F2R9.

1. Introduction

Endophyte bacteria is organism that lives in association with plant for whole or partial of their life cycle. Endophytic bacteria is living by colonization in inner tissue of plant without causing interference to plant and most of endophytic bacteria are beneficial because they are able to act as produce hormone promoting growth [1], antimicrobial [2] natural product resource for medicine [3].

Rubber plants are often exposed to white root diseases caused by *Rigidoporus microporus* [4], leaf deciduous disease caused by *Phytophthora meadii* [5], and tree stem rot caused by *Fusarium oxysporum* [6]. Endophytic bacteria that have the ability to biocontrol against pathogenic fungi are indicated to have chitinolytic activity known as chitinase. chitinase can degrade chitin compounds in the polymer form of β-1,4 N-cetyl-D-glucosamine [7]. Therefore, chitinase enzymes are receiving increased attention in biotechnology applications such as in medicine especially in agriculture [8].

The study [9] reported *Streptomyces hygroscopicus* can hydrolyze chitin with 0.9 mm clear zone that can inhibit the growth of *Rhizoctonia solani*, *Fusarium oxysporum*, *Alternaria alternate, Aspergillus niger, Aspergillus flavus, Sclerotinia scleotiorum, Phytophthora parasitica* and *Botrytis cinerea*. In addition, endophytic bacteria of the rubber plant (*Hevea brasiliensis*) is known *Alcaligenes* sp. identification based on the 16s rRNA gene which is resistant to *Phytophthora meadii* [10]. This study aims

to isolate and select endophytic bacterial isolates from rubber plant leaves that have the ability to produce chitinase.

2. Materials and Methods

2.1 Isolation of endophytic bacteria from leaf of rubber plant
Endophytic bacteria isolated refer [11]. The samples leaf of rubber plant were done by surface sterilization [1]. Each sample was cut with a size of 2 cm x 2 cm and then inoculated on a Petridish containing Nutrient Agar (NA) medium and incubated for 48 hours at room temperature. As a positive control, the sample was rinsed using sterile distilled water, then 1 ml suspense was inoculated in Petridish containing NA media. Petridish are not overgrown by microorganisms are sterile.

2.2 Characterisation morphology of endophytic bacteria
The morphology observation of nine endophytic bacteria included bacteria colony color, colony shape, elevation and margin. Morphological observation on bacteria cell included cell shape and Gram staining [12].

2.3 Screening of chitinase-producing bacteria
Chitinase-producing bacteria endophytic from leaf of rubber plant was selected in media colloidal chitin agar (2%) . Each endophytic bacteria was grown on colloidal chitin agar media and incubated for 5 days at room temperature. The plates were stained with Congo red (0.1%) and the chitinase-producing bacterial made a clear zone. Citinolitic Index (CI) = diameter clear zone/ diameter colony.

2.4 Identification of endophytic chitinolytic bacteria based on 16S rRNA partial sequencing
Identification of the chitinolytic bacteria was conducted with 16S ribosomal DNA. Extraction of the DNA isolates bacteria D35 were conducted using protokol standar kit dari *Presto™ Mini gDNA Bacteria Kit*. DNA of endophytic bacteria was amplified by using primer 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') [1]. DNA sequencing of amplified was carried out at 1st BASE laboratory. Sequencing result data was matched with NCBI Gen Bank data using BLAST where it can be acesed on http://www.ncbi.nlm.nih.gov/.

3. Result and Discussion

Isolation of chitinase-producing bacteria endophyte from rubber plant leaf (*Hevea brasiliensis)* at Siabu village N $00^0$14.347' E 101^0 01.762', Kampar District, Riau Province. A total of nine morphologically different bacteria were isolated from two people's rubber plantation. The bacteria strains were isolated with direct method (**Figure.1**). The result of characterization Gram's stain of four bacterial strains is Gram positive and five bacterial strains Gram negative. The study of [13] have been reported that there are five of chitin bacteria isolated from the waste water, three strains bacteria are Gram negative. The result of characteristic are seven bacteria strains of coccus-shaped (77.78%) and two bacteria strains of rod-shaped. Morphology colony; strains bacteria with color yellow and white, form of circular, elevation of flat and margin entire and undulate (**Table 1**).

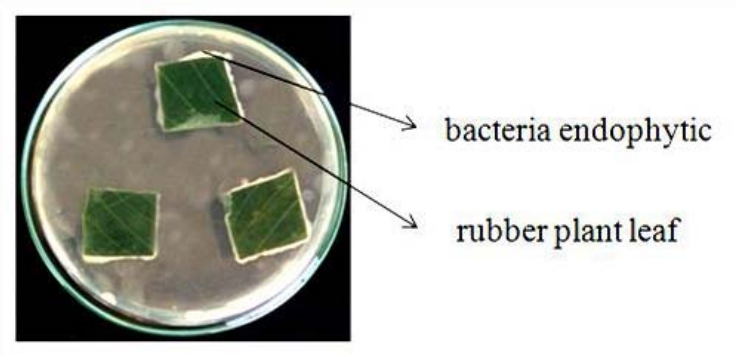

Figure 1. Isolation of bacteria from rubber plant leaf (*Hevea brasiliensis)* with direct method

Table 1. Characterization of isolate endophytic bacteria isolated from rubber plant leaf (*Hevea brasiliensis)*

Isolate	Gram's stain	Morphology cell	Morphology colony			
			color	form	elevation	margin
D5	positive	coccus	yellow	circular	flat	entire
D6	negative	coccus	white	circular	flat	entire
D7	negative	coccus	white	circular	flat	undulate
D8	negative	coccus	white	circular	flat	undulate
D9	positive	rod	yellow	circular	flat	entire
D10	positive	rod	white	circular	flat	entire
D34	positive	coccus	white	circular	flat	entire
D35	negative	rod	white	circular	flat	entire
D36	negative	coccus	white	circular	flat	undulate

The results of screening of endophytic bacterial strains from rubber leaf are known to produce qualitative chitinase enzyme there are two isolates, namely isolate D5 and D 35. Strains bacteria were found to produce clear zone when incubated in chitin media and further stained with Congo red (**Figure 2**). The clear zone surrounding the colony bacteria indicates chitinase activity to break down chitin compound in agar medium. Chitinolytic bacteria were selected based on Chitinolytic Index (CI) at various range of 1.66 to 2.08. The results of CI in this study were higher than those reported by [14] *et al*. They reported strains chitinolytic bacteria successfully isolated from *Cricula trifenestrata* silkworm cocoon with chitinolytic index 0.21 to 1.81. Interestingly in this study, both chitinolitic isolates (D3 and D35) have no inhibitory properties against *Rigidoporus microporus* (data unpublished). Bacteria strain D5 and D35 may be able to inhibit the growth of other pathogenic fungi.

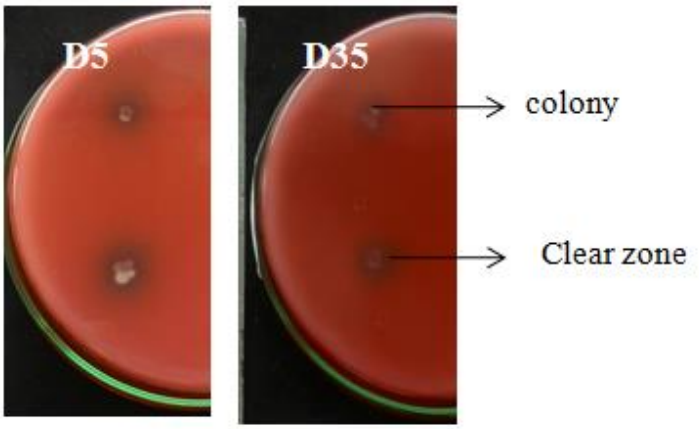

Figure 2. Chitinolytic test on colloidal chitin media (2% w/w) with Congo red stain

Table 2. Chitinolytic bacteria isolates based on Chitinolytic Index (CI).

Isolate	Inhibition activity of *Rigidoporus microporus*	∅ colony	∅ clear zone	chitinolytic index (CI)
D5	-	4.69	7.84	1,66
D35	-	4.23	8.8	**2,08**

This study bacteria strains of chitinolytic that gave CI < 2.0 further identification the ribosomal RNA (16S rRNA). At present, the sequence analysis technique of 16S rRNA, genes encoding small-subunit ribosomal RNA were commonly used for the phylogenetic classification and reconstructing of prokaryotic phylogenies [15]. The analysis of 16S rRNA gene sequences provides ways to investigate the dissimilarity between strains bacteria natural communities and strains collections. Isolates D35 was subjected to 16S rRNA sequence method for bacteria strain identification. The 16S RNA nucleotide using BLAST showed D35 isolates belong to family Enterobcteriaceae and with genus *Klebsiella.* PCR amplification of 16S rRNA gene D35 produced a DNA fragment measuring 1462 bp (**Figure 3**) and accession description for bacteria strain D35 **Table 3**.

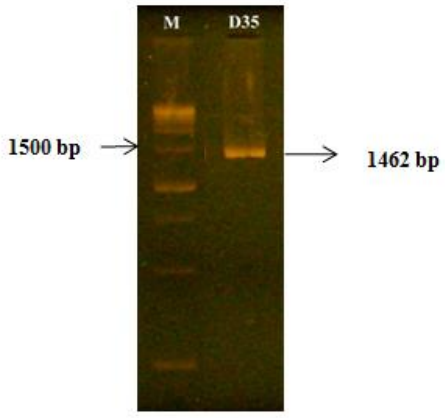

Figure 3. Electrogram product PCR *16S rRNA*. (M) *Gen ruller* 1 kb DNA *ladder*, D35= Fragment gen 16S *rRNA* isolate D35

The homology of the partial 16S rRNA gene sequence of the isolates was analyzed using the BLAST algorithm in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (**Table 3**). Phylogenetic analyses were conducted using a multiple sequence alignment tool (Clustral W). Only the highest-scored BLAST result was considered for phylotype identification. BLAST showed that the isolate D35 linear DNA has maximum homology (99%) with *Klebsiella variicola* strain F2R9 with accession NR-025635.1 (**Figure 4**). Previous research *K. variicola* is known to be found in plants such as banana trees [16], sugarcane [17]. Moreover, the Strain *K. variicola* has been known to be opportunistic pathogen of humans [18]. The result study [19] reported *K. variicola* have also been isolated from cows

Table 3. Accession description for bacteria strain D35

Description	Max score	Total score	Identity (%)	Accession
Klebsiella variicola strain F2R9	2495	2495	99%	NR-025635.1
Klebsiella pneumoniae strain DSM 30104	2477	2511	99%	NR-117686.1
Klebsiella pneumoniae strain JCM1662	2477	2511	99%	NR-112009.1
Klebsiella pneumoniae strain ATCC 13883	2475	2475	99%	NR-119278.1
Klebsiella pneumoniae strain NBRC 14940	2466	2466	99%	NR-113702.1

The study [1] reported was endophytic bacteria from rubber plants (from leaf, shaved bark and feeder root) identified based of partial sequencing 16S rRNA on had found *Bacillus cereus, Pseudomonas aureginosa, Brachybacterium paraconglomeratum, Bacterium* and *Providencia vermicola*.

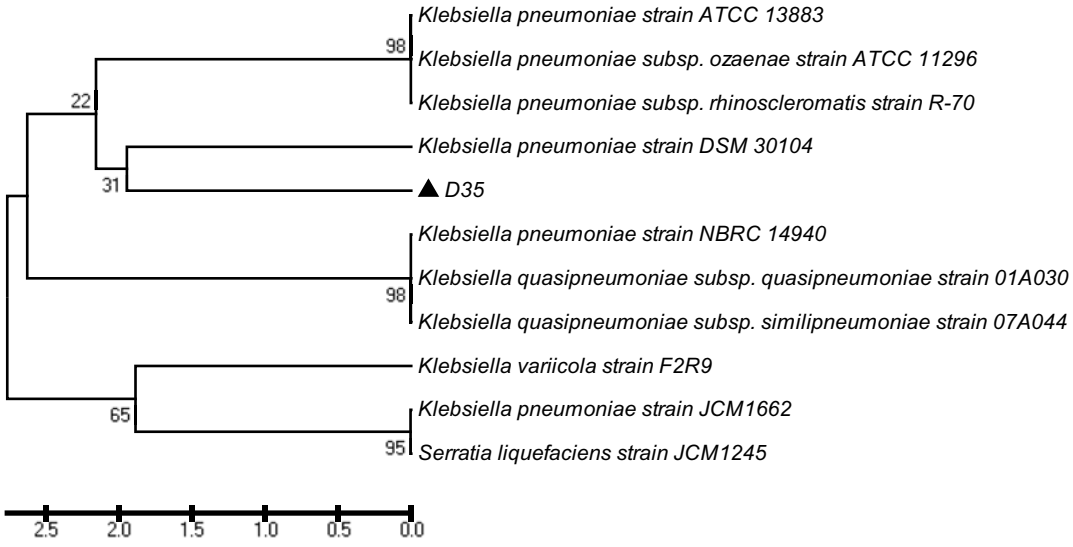

Figure 4. Phylogenetic tree of strain D35 showing the similarity with *Klebsiella variicola FR29*

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