MICROBIOLOGY
INDONESIA
ISSN 1978-3477, eISSN 2087-8575
Vol.15, No.4, December 2021, p 135-138

Available online at
http://jurnal.permi.or.id/index.php/mionline
DOI: 10.5454/mi.15.4.3

SHORT COMMUNICATION

Anti Fungal Activity of Chitinolytic Bacteria *Lysinibacillus fusiformis* and *Brevibacillus reuszeri* against the Fungal Pathogens *Rhizoctonia solani* and *Fusarium oxysporum*

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Chitinolytic bacteria can produce chitinase, reported as a biocontrol agent against plants. This research aims to observe chitinolytic activity in inhibiting the growth of *Rhizoctonia solani* and *Fusarium oxysporum*. Anti fungal testing in dual culture test by growing each of the chitinolytic bacteria, *Lysinibacillus fusiformis* and *Brevibacillus reuszeri,* with the pathogenic fungi, *F. oxysporum* and *R. solani,* in petri dishes containing chitin agar media facing a distance of 3 cm. The results showed that chitinolytic bacterial isolates were capable inhibit the fungus by having the activity of each index inhibition of *L. fusiformis* isolates (30%), *B. reuszeri* (77%) against *F. oxysporum*, and *R. solani* fungi isolates (100%) for each chitinolytic bacterial isolate.

Key words: anti fungal, chitinolytic bacteria, pathogenic fungi

Bakteri kitinolitik dapat menghasilkan enzim kitinase dan dilaporkan sebagai agen biokontrol terhadap tanaman. Penelitian ini bertujuan untuk melihat aktivitas kitinolitik dalam menghambat pertumbuhan *Rhizoctonia solani* dan *Fusarium oxysporum*. Pengujian anti-cendawan menggunakan teknik kultur ganda dengan menumbuhkan masing-masing bakteri kitinolitik, *Lysinibacillus fusiformis* dan *Brevibacillus reuszeri*, dengan jamur patogen, *F. oxysporum* dan *R. solani*, dalam cawan petri yang berisi media agar mengandung kitin secara berhadapan dengan jarak 3 cm. Hasil penelitian menunjukkan bahwa isolat bakteri kitinolitik mampu menghambat cendawan dengan indeks penghambatan *L. fusiformis* (30%), *B. reuszeri* (77%) terhadap cendawan *F. oxysporum* dan *R. solani* (100%) untuk setiap isolat bakteri kitinolitik.

Kata kunci: anti cendawan, bakteri kitinolitik, cendawan patogen

Horticultural crops have important potential in fulfilling nutrition and increasing farmers' income so as to support community welfare (Malhotra 2016; 'Kerutagi *et al.* 2019). However, in cultivation, there are obstacles in the form of plant diseases caused by fungal pathogens that can pose a risk of plant damage and decreased production (van Bruggen *et al.* 2016; Panth *et al.* 2020). This can cause economic losses in agriculture and the horticultural industry. Therefore, it is necessary to take preventive measures to control these pathogens such as chemical and biological control.

Chemical control is a control that can be done quickly but has a bad long-term effect on the environment and can cause pathogen resistance. Therefore, biological control is the main solution in preventing fungal pathogens that destroy horticultural crops. *Fusarium oxysporum* and *Rhizoctonia solani* are two types of pathogens that often attack horticultural crops (Liu *et al.* 2017; Zhao *et al.* 2014). The main composition of fungal cell walls is chitin so that biological control strategies can be carried out by utilizing chitinolytic enzyme-producing bacteria.

Chitinolytic bacteria are a group of bacteria that are capable of producing the chitinase enzyme (Asif *et al.* 2020; Moon *et al.* 2017; Wang D *et al.* 2018). The enzyme functions to catalyze the chitin degradation reaction by cutting the glycosidic bonds between N-residues. acetylglucosamine. Chitinolytic bacteria have strong antagonistic activity against fungal pathogens with hyperparasitism and antibiotic mechanisms (Pliego *et al.* 2011).The use of chitinolic bacteria as biological control agents is expected to degrade the cell walls of pathogenic fungi so that it can inhibit the growth of pathogenic fungi in horticultural crops. Two *Chitinolic* bacteria, namely *Lysinibacillus fusiformis* and *Brevibacillus reuszeri,* were tested for their antifungal abilities against *F. oxysporum* and *R. solani*.

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Table 1 Activity data of chitinolytic bacteria against pathogenic fungi

Chitinolytic Bacteria	Pathogenic Fungi	Inhibition Radius		Inhibition Index
		R1	R2	
Lysinibacillus fusiformis	*Rhisoctonia solani*	0	0	100%
Lysinibacillus fusiformis	*Fusarium oxyforum*	3.6	2.2	30%
Brevibacullus reuszeri	*Rhisoctonia solani*	0	0	100%
Brevibacullus reuszeri	*Fusarium oxyforum*	3.6	0.8	77%

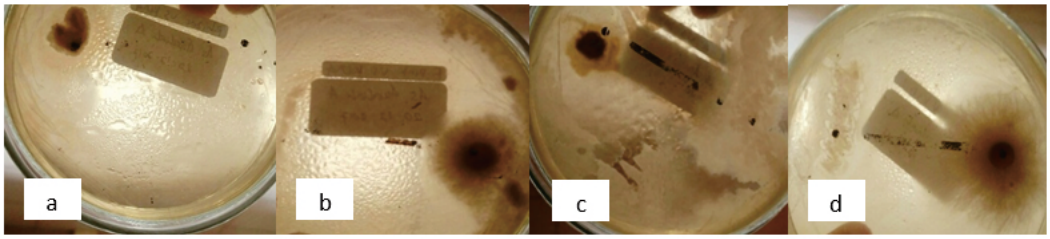

Fig 1 Anti-fungal activity of chitinolytic bacteria. a. *Lysinibacillus fusiformis* against *Rhisoctonia solani*, b. *Lysinibacillus fusiformis* against *Fusarium oxysporum,* c. *Brevibacullus reuszeri* against *Rhisoctonia solani*, d. *Brevibacullus reuszeri* against *Fusarium oxysporum*.

The isolates of the fungus *R. solani* and *F. oxysporum* (Patimah 2018) were cultured on Potato Dextrose Agar (PDA) media and incubated for 7 days at room temperature. Chitinolytic bacteria isolates, namely *L. fusiformis* and *B. reuszeri* (Aditia 2016) were cultured on Nutrient agar (NA) media and incubated for 1x24 hours at 37 °C.

The antifungal test of chitinolytic bacterial isolates was carried out using a multiple culture test. Previously isolated chitinolytic bacteria, *R. solani* and *F. oxysporum*, were scratched at a distance of 2 cm from the edge of the media. After the bacteria were 24 hours old, the bacteria were tested in the Dual Culture test against fungi using Chitin Agar Media. Chitin Agar is used to break down chitin compounds in bacteria. Pathogenic fungal mycelium with a diameter of 0.5 cm was placed in a petri dish with a distance of 2 cm from the edge of the media using a needle loop. Then the chitinolytic bacteria were scratched lengthwise at a distance of 3 cm from the mycelium of the pathogenic fungi, the cultures were incubated at room temperature for 7 days (Anesini and Perez 1993; Yurnaliza *et al.* 2012). This test was repeated 1 time. The inhibition index used in this study was carried out by observing the resulting inhibition zone on the 7 days calculated using the formula:

$$\left(\frac{R1 - R2}{R1}\right) \times 100\%$$

R1: Growth radius towards the edge of the Petri
R2: Growth radius towards antagonistic bacteria

Based on the results of the anti-fungal activity test, it was found that *L. fusiformis* produced an inhibitory index of 100% against *R. solani* and 30% against *F. oxysporum.* Meanwhile, *B. reuszeri* produced an inhibitory index of 100% against *R. solani* and 77% against *F. oxysporum* (Table 1). The inhibition index of 100% meant that there was no growth of pathogenic fungi when the antagonist was tested with chitinolytic bacteria *L. fusiformis* (Fig 1a) and *B. reuszeri* (Fig 1c). Inhibition index <100% means that there is still growth of pathogenic fungi. This growth was indicated by the presence of hyphae formed on the surface of the growing medium when antagonist testing with chitinolytic bacteria was carried out (Fig 1b and 1d).

The presence of an inhibitory index showed that chitinolytic bacteria *L. fusiformis* and *B. reuszeri were* able to inhibit the growth of pathogenic fungi. This is because chitinolytic bacteria produce chitinase enzymes that are able to degrade fungal cell walls. The main component of fungal cell walls is chitin. Chitin is a linear polymer composed of monomers, namely β-1,4- N-acetyl-glucosamine (Cabib *et al.* 2001; Roncero 2002; Moon *et al.* 2017). In the presence of chitinase enzymes produced by chitinolytic bacteria, hydrolysis of chitin compounds occurs at -1,4-glycosidic bonds and produces oligosaccharides or N-acetyl-glucosamine monomers (Tronsmo and Harman 1993; Herdyastuti *et al.* 2010).

The magnitude of the inhibitory index produced depends on how much N-acetyl-glucosamine

monomer is produced from the chitin hydrolysis process using the chitinase enzyme. The greater the number of N-acetyl-glucosamine monomers produced, the greater the inhibitory index that will be formed.

Testing of the anti-fungal ability of chitinolytic bacteria was carried out simultaneously, but there were differences in the inhibitory index of each isolate of chitinolytic bacteria. This is due to the different species of chitinolytic bacteria and fungal pathogenic species used. Each chitinolytic bacteria certainly produces different chitinase enzymes so that it affects the anti-fungal activity which is characterized by the presence of an inhibition zone. In addition, each pathogenic fungus certainly has a specific defense mechanism so that it affects self-defense against foreign compounds that interfere with its existence.

Based on Table 1, it can be seen that the larger the inhibition zone produced, the chitinase produced by chitinolytic bacteria is able to hydrolyze large amounts of chitin so that the growth of pathogenic fungi is inhibited. The fungus *F. oxysporum is* more resistant to chitinase because the cell wall composition of the fungus *F. oxysporum* in the outer layer contains glycoprotein compounds that protect the surface of the mycelium. The glycoprotein content in the cell wall is 50-60% of the total cell wall mass (Schoffelmeer *et al.* 1999; Yurnaliza *et al.* 2012).

Chitinase is widely used as a biocontrol agent, especially for plants that are often infected with fungi, it is because chitin, which is the main component of fungal cell walls, can be degraded by the chitinase enzyme to produce an environmentally friendly product compared to chemicals (Asif *et al.* 2020). The chitinase enzyme's role is widely used as an effective antifungal against *R. solani* on transgenic cotton (*Nicotiana tabacum L*) (Broglie *et al.* 1991), Transgenic elite indica rice (Datta *et al.* 2001), or *F. oxysporum* on strawberries (Wang Y *et al.* 2003), *F. oxysporum* f. sp. *Udum* on pigeon pea (Bapat and Shah 2000), *Bacillus thuringiensis* can inhibit the growth of several fungal pathogens and fungal cell wall degradation(Hollensteiner *et al.* 2017).

Many species of *Bacilli* are well known as plant growth-promoting bacteria (PGPB), biocontrol of pests and diseases. It was reported that species of *B. brevis* are effective antagonists for pathogenic fungi such as *F. oxysporum* f. sp. *Udum* (Bapat and Shah 2000), *Bacillus licheniformis* for *Aspergillus niger*, *Magnaporthe oryzae* and *R. solani* (Cui *et al.* 2012), *Virgibacillus marismortui*, Bacillus *subtilis, Bacillus pumilus, Bacillus licheniformis, Terribacillus halophilus, Halomonas elongata, Planococcus rifietoensis, Staphylococcus equorum and Staphylococcus* sp. for *Botrytis cinerea* (Essghaier *et al.* 2009), *Bacillus licheniformis* for F. *graminearum, Bipolaris sorokinianum, Sclerotinia sclerotiorum, Botrytis cinerea, Phytophthora capsici, R. cerealis, Bipolaris maydis, Gaeumannomyces graminis*, and *Pseudoperonospora cubensis* (Wang Z *et al.* 2014).

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