Antifungal Spectra of Activity of Actinomycetes Strains Against *Rhizoctonia solani*

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ABSTRACT

Biological control offers an environmentally friendly alternative to the use of antifungal for controlling damping-off diseases. A collection of about 24 actinomycete strains from peat soil Siak-Riau was screened for the ability to produce metabolites that inhibit *R. solani* growth in vitro. Seven isolates showed strong in vitro antagonistic against *R.solani* in agar disc and well-diffusion methods by producing extra cellular antifungal metabolites.

Keywords

antifungal, actinomycetes, *R.solani*

1. INTRODUCTION

The actinomycetes are known saprophytic bacteria that decompose organic matter, especially polymers such as lignocelluloses, starch and chitin, in soil. While there role in other microbial mediated soil processes has not been so extensively studied, evidence indicates that actinomycetes are quantitatively and qualitatively important in the rhizosphere, where they may influence plant growth and protect plant roots against invasion by root pathogenic fungi (Lynch and Crook, 1992). *Streptomyces* species and other actinomycetes have been shown to protect several different plants to various degrees from soil-borne fungal pathogens or damping-off. The application of biocontrol agents to suppress disease caused by *R.solani* has received considerable attention in current years. Actinomycetes have been recognize as sources for several secondary metabolites, antibiotics and lytic enzymes (Shahrokhi et al., 2005). Here, we report screened actinomycetes from peat soil Siak-Riau antagonism towards a fungal root pathogen, *R.solani*, which causes damping-off.

2. METHOD

2.1 Preparation of Actinomycetes: A synthetic medium, Casein Glycerol Agar (glycerol 10 g, casein 0,3 g, KNO_3 2 g, NaCl 2 g, KH_2PO_4 2 g, $MgSO_4$ $7H_2O$ 0,05 g, $CaCo_3$ 0,02 g, $FeSO_4$ $7H_2O$ 0,01 g and agar 20 g in 1 L distilled water) was used for isolating and screening of actinomycetes.

2.2 Preparation of *R.solani* Tested fungi was kind gift as follows: *R.solani* from Plant pathology Lab, Faculty Agriculture, University of Riau. The fungus was grown room temperature and maintained on potato dextrose agar (PDA).

2.3 Screening Antifungal Bioassays Actinomycetes: Each actinomycetes isolate was smeared on Casein Glycerol Agar medium as a single streak and after incubation at room temperature for 7 days, from well-grown streaks 6 mm agar disk of actinomycetes colony. Disk were then aseptically transferred to PDA plates having fresh lawn cultures *R.solani*. Plates were incubated room temperature for 6-7 days and bioactivity was evaluated by measuring the diameter on inhibition zones (Aghighi et al., 2004).

2.4 Preparation of crude extract from submerged cultures : Active strains of actinomycetes were grown in Casein Glycerol Broth on shakers under 150 rpm at 28 °C. To monitor the activity, aseptically small aliquots of culture media were taken every 24 h for 15 days and the activity was evaluated by well diffusion method.

Isolates actinomycetes that showed activity against test fungal *R.solani* were grown in submerged culture in 100 ml flask containing 50 ml of liquid medium CG. The flask were inoculated with 1 ml actinomycetes culture (10^6 CFU/ml) on shakers under 150 rpm at 28 °C. To prepare crude extracts, depend to actinomycetes strains, between 7 to 10^{th} days of post inoculation which the activity reached maximum. Antifungal activity of the strains was measured by diffusion agar method or ethyl acetate extracts and culture medium filtrates.

3. RESULTS AND DISCUSION

Table 1. Antifungal inhibitory effect of active actinomycetes strains against *R.solani*

Actinomycetes Strains	Zone of inhibition	Actinomycetes Strains	Zone of inhibition
L11	-	L321	-
L12	2,1	L421	-
L13	-	L513	-
L15	2,1	SM11	2,1
L17	-	SM12	1,9
L18	3,2	SM13	-
L121	-	SM14	-
L221	-	SM15	-
L223	-	SM16	-
L225	-	SM17	-
L311	-	MH11	-
L313	1,0	MH23	2,2

In screening for actinomycetes having antifungal activity, 24 isolates were screened from which 7 isolates showed activity against *R. solani*. Table 1 shows antifungal activity of active actinomycetes strains. Cell of morphology actinomycetes strains is shown Figure 1.

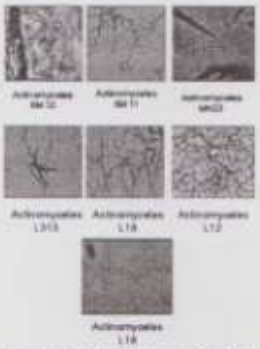

Figure 1. Spore chain structure of actinomycetes with ligh microscop (400x)

Each mechanism has actinomycetes attack different targets of the fungus. According, Soares et al. (2006) actinomycetes can produce antibiotics and bioactive compound which can control various phytopathogens. For examples, *Streptomyces* sp. has been reported to suppress the growth mycelium *Fusarium moniliforme* and *Streptomyces galbus* can suppress germination spore of *Alternaria solani*, *Aspergillus niger*, *Curcularia pallesceuse* and *Helminthosporium oryzae*.

Actinomycetes having antifungal activity, further testing is done using casein glycerol broth. Curves compound bioactive production actinomycetes strains can be seen in Figure 2.

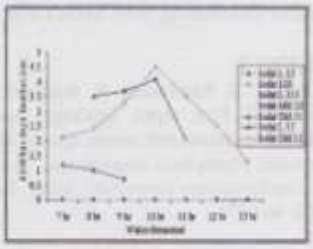

Figure 2. Activity versus post seeding time in submerged media culture of actinomycetes against *R.solani*.

Actinomycetes strains L12, L18 and L313 to produce compound bioactive highest in the fermentation medium in 10 days, actinomycetes MH23 in 9 days and SM 11 in 7 days. Strains L15 and SM 12 do not have the activity. This may be caused by a compound bioactive produced strains L15 and SM 12 in the amount not so much concentration not able to suppress the target fungi. Factors that influence the work have not been suspected of optimum growth conditions such as media, temperature and incubation time.

Extraction results of the fermentation media using ethyl acetate to 6 shows have actinomycetes strains activity (medium and mycelium). Antifungal testing method with paper disc can be seen in Table 2. Actinomycets strain SM11 (1.6 cm) had a bigger clear zone compared to that of the other isolates. Actinomycetes stain L15 (media and mycelia) does not show clear zones. Although the diameter of the clear zone in solid media is not always correlated with the high activity of bioactive production in liquid media or extraction, the method is often used to precisely identify. Each of the actinomycetes strains of the same genus produces a different quality and bioactive compound different characteristic.

Table 2. Zone of inhibition of the isolates actinomycetes used ethyl acetat extracts

Actinomycetes Strains	Zone of inhibition (cm)	
	Media	Mycelia
Control	0	0
L12	1,3	0,8
L15	0	0
L18	1,1	0,9
L313	1,2	0,8
MH23	0,9	0,9
SM11	1,6	0,9
SM 12	1,3	0,7

3. ACKNOWLEDGMENTS

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