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ANTAGONISM COMPETENCE OF *Trichoderma* spp. ISOLATES AGAINST *Rhizoctonia solani* KUHN

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Abstract

Antagonism test in-*vitro* of *Trichoderma* spp. against *Rhizoctonia solani* Kuhn had been carried out. This study was aimed to clarify the discrepancy antagonistic function inside species biodiversity. Enzyme activities of *Trichoderma* spp. such as cellulase, chitinase, ligninase, lipase, and protease were observed, while the IAA (indole acetic acid) hormone and phosphate (Ca-phosphate) dissolving capability examined, too. All isolates inhibited *R. solani* growth with restraint rate between 20.0 to 82.1%. The isolates produced cellulase activity but they did not have any ligninase at all. Forty six isolates produced lipase, 38 isolates of protease, and one isolate of chitinase. Twenty nine isolates released IAA between 0.0958 to 9.2575 ppm and one isolate dissolved phosphate minerals. Every single potential of uniqueness antagonism behavior at each isolate had a chance to future application in agriculture work, particularly to impede population of *Rhizoctonia solani* as soil born pathogen in the horticulture activity.

Key words: Antagonim, enzyme activities, plant growth hormone, phosphate dissolving, *Rhizoctonia solani*, *Trichoderma* spp.

1. Introduction

Rhizoctonia solani is a soil borne pathogen that very common and widely distributes as due to owned by many host plants (Ogoshi and Ui, 1983). Controlling the pathogenic fungus by using chemical fungicides in soil can not effective because of that fungal propagules distributed in soil are often beyond the reach of fungicides (Campbell, 1989). In the other hand, fungicides application lead to kill non-target microorganisms, make strains resistant to fungicides, and harm the environment and health (Djatnika *et al*., 2003).

Genus of *Trichoderma* is free-living fungus and highly accommodated in plant parts such as leaf, root, decaying wood, and rhizosphere soil. The genus become an opportunistic fungus, plant symbiont, and may have a parasitic function to other fungi. Saba *et al*. (2012) informed that some strains of *Trichoderma* colonized with strong and durable on the root surface, and penetrated into epidermis through few cells below epidermis. The fungi released variety of compounds inducing localized or systemic resistance responses. Few species of *Trichoderma* produced extracellular enzymes such as β-1.3-glucanase, chitinase, lipase and protease playing role in mycoparasitism action for the competition (Haran *et al*., 1996). *Trichoderma* is applicable as biological control agent against plant pathogens. *Trichoderma harzianum* reduced other fungal plant pathogens such as root rot of sugar beets (Ciccarese *et al*., 1992), stem rot of ground nuts (Cilliers *et a*l., 2000), damping-off and stem rot of cowpea plants (Adandonon *et al*., 2004), and neck rot of chickpeas caused by *Sclerotium rolfsii* (Maurya *et al*., 2008).

In the other function, *Trichoderma* has been widely used as biofertilizer purposes for undertaking action in the plant nutrient mineralization and also to produce plant growth hormone. Kapri and Tewari (2010) observed that some of *Trichoderma* isolates dissolved tricalsium phosphate at different levels. Application of *Trichoderma* inoculant increased chickpeas (*Cicer arietinum*) growth includes the roots and shoots length, dry and wet weight of roots and shoots, although the plant was cultivated in P-deficient soils containing only tricalsium phosphate. Saba *et al.* (2012) found out that *Trichoderma* colonized on the roots and increased root improvement, crop productivity, resistance to abiotic stresses, and also in the nutrients absorption and utilization by plants.

Considering the above mentioned, so here the antagonism investigation throughout 82 *Trichoderma* isolates had been completed. The purpose of this study was to consider antagonistic potential of *Trichoderma* spp., at their species biodiversity. The result could be useful to evaluate the potential of *Trichoderma* spp. for antagonist agents against *R. solani*, as well as to take the benefit over its capacity in ecological function because of releasing enzymes and plant growth hormone; and dissolving phosphate for plant growth improvement. The selected *Trichoderma* isolates could be useful for agriculture benefit, particularly in the tropical soil environment.

2. Materials and Methods

2.1 Microbial culture

Microorganisms as living culture used in the study (*Trichoderma* spp. and *Rhizoctonia solani* Kuhn) were deprived from InaCC (Indonesian Culture Collection, Research Center for Biology, Indonesian Institute of Sciences) as a depository representative of microbial agent. The pure isolates of 82 *Trichoderma* were isolated from various sources such as soil substrate, dead larva and termite, plant root, decaying materials of wood, bamboo, and fabric, and as aerial spore contamination. Isolates identification based on morphological character on branching of conidiophores, shape of the phialides, emergence of phialospores, and shape of phialospores (Rifai, 1969).

2.2 Chemical material

Some chemicals used in this study consisted of carboxy methyl cellulose (CMC), $CaCl_2$, $CaCl_2.2H_2O$, $CoCl_2.6H_2O$, congo red, ferric sitrate, glycerol, glucose, HCl (37%), KH_2PO_4, K_2HPO_4, $MgSO_4$, $MgSO_4.7H_2O$, $MnSO_4.4H_2O$, NaCl, NaOH, Na_2CO_3, Na_2HPO_4, NH_4Cl, $(NH_4)_2$-tartrate, peptone, Salkowski reagent (0.5 M $FeCl_3.6H_2O$ in concentrated H_2SO_4 solution), poly-R, potato dextrose agar (PDA), potato dextrose broth (PDB), skimmed milk, thiamine-HCl, tri-calcium phosphate (TCP), triglycerides, tween 80, yeast extract, and $ZnSO_4.7H_2O$.

2.3 Antagonistic assessment

In-*vitro* test was targeted to select antagonism potential of *Trichoderma* against *R. solani* growth inside a media culture. Antagonism performance tested in dual culture technique follows in Upadhyay and Rai (1987). Seed culture of *Rhizoctonia solani* and *Trichoderma* were grown on PDA medium then printed onto the tip of culture growth with sterilized plastic straw (6 mm diameter disk-shape) and inoculated into another PDA medium inside new petridish in opposite direction, respectively. The culture was incubated for 5 to 7 days in the room laboratory temperature around 27-28^{o}C (Figure 1). The growth interaction was calculated as percentage inhibition of colony radial refer to Fokkema (1976) formula:

$$\text{Percentage inhibition} = \frac{(R1 - R2) \times 100\%}{R1}$$

R1 = radius of *R. solani* growth towards the edge of the petridish
R2 = radius of *R. solani* growth toward *Trichoderma*

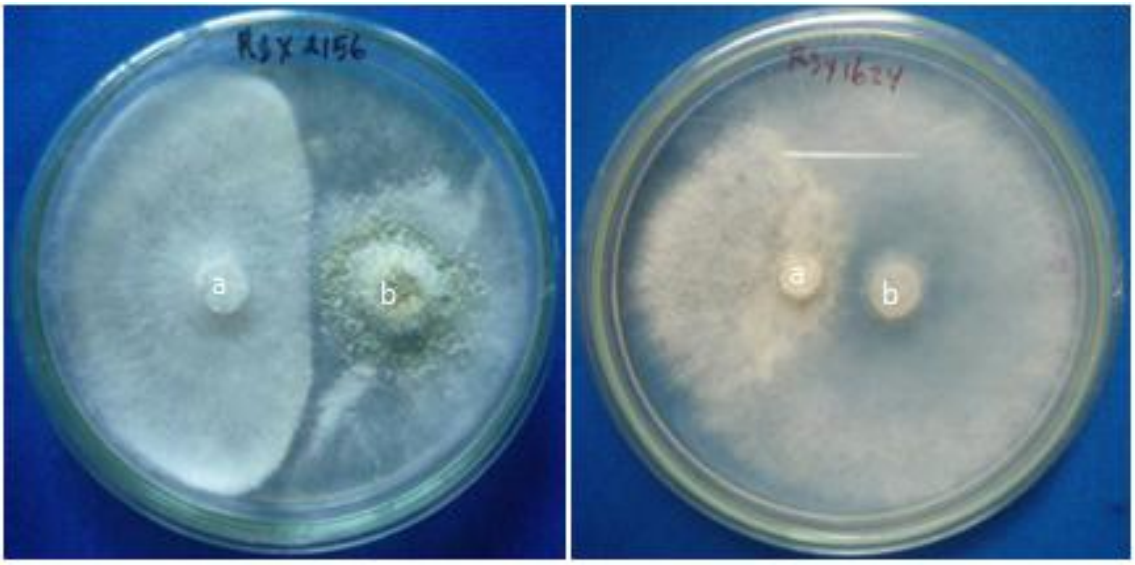

Figure 1. Antagonism character was shown by growth competition of *Trichoderma* sp. 2156 (b) against *Rhizoctonia solani* (a) as in the left photo, and compared with growth performance of *R. solani* (a) versus *Trichoderma* sp. 1624 (b) in the right photo

2.4 Enzymes activities

Determination of enzyme activities accomplished with several methods based on specific media culture preparation for several enzymatic tests (Figure 2). Chitinase activity was tested following Lingappa and Lockwood (1962) technique. Medium containing colloidal chitin was used as carbon and nitrogen sources for *Trichoderma* culture. The medium was poured into petridish (5 cm diameter) and inoculated with each isolate of *Trichoderma*, then incubated in the room for 3 days. Chitinase activity was indicated by the present of a clear zone around or even under the growing colony.

Modified method according to Peterson and Johnson (1949) was used to quantify lipase activity. Medium containing trygliceride was inoculated with each isolate of *Trichoderma* and incubated in the room for 3 days. The clear zone formation under growing colony inside the petridish means that the isolate showed lipase activity.

Skimmed milk was used as a substrate to refer protease activity by using the method of Uria *et al*. (2006). Agar medium containing protein sources was poured into petridish and then inoculated with each isolate of *Trichoderma*, and incubated in the room for 3 days. Protease activity was produced by *Trichoderma* by creating a clear zone surround the culture growth.

Ligninase activity was tested by using Peterson and Bridge (1994) method. Poly-R 4.78 medium was poured into petridishes then inoculated with each of *Trichoderma* isolate, and incubated in the room for 3 days. Ligninase activity was indicated by the present clear zone surround or under the growing colony.

Fungal ability in dissolving phosphate was tested by using Pikovskaya's medium (Thakuaria *et al*., 2004). Fungal culture was inoculated into media containing tricalcium phosphate and incubated in room condition for 3 days. The existence of a clear zone around the colony growing indicated that *Trichoderma* isolates dissolved phosphate source for mineralization.

Carboxy Methyl Cellulose (CMC) was used as a substrate for cellulase test following the Andro *et al*. (1984) method. Medium was poured into petridishes and then inoculated with each of *Trichoderma* isolate. After incubation in the room for 3 days, poured 10 ml of congo red solution on the top media surface and it was left for 10 minutes. The solution was discarded and replaced with 10 ml of 1 M NaCl solution for 15 minutes. Cellulase activity was noticed by a clear zone surround the colony growing. Cellulase index was calculated by dividing the clear zone diameter with fungal growth diameter.

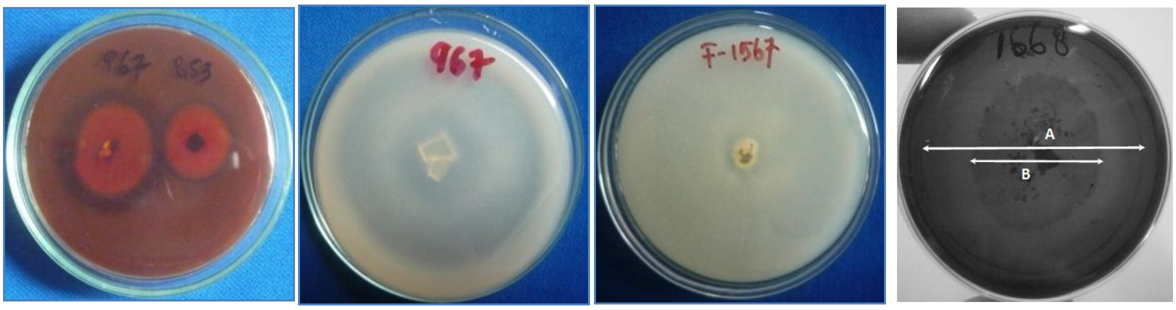

Figure 2. Test method was complied with activities of lipase (967 & 853), protease (967), cellulase (1668), and dissolving phosphate (F-1567) by *Trichoderma* spp. Cellulase index was calculated by dividing the clear zone diameter (A) with fungal growth diameter (B)

2.5 Indole Acetic Acid (IAA) acquisition

IAA was released by *Trichoderma* and measured by using the Gordon and Webber (1950) technique. *Trichoderma* isolates which were grown on PDA medium then transferred into a bottle containing Potato Dextrose Broth (PDB) media, aseptically. Three milliliter of culture were taken from the bottles (after 5 days incubation) and then inserted into Eppendorf tube for each of isolates. The culture was precipitated by centrifuge the Eppendorf tubes for 10 minutes at 11000 *g*. One milliliter supernatant from each tube was put into a glass test-tube, add with 2 ml Salkowski reagent, and lastly mixed on vortex. The solution was left for 30 minutes to develope color, and it was measured by using UV-VIS spectrophotometer 530 nm. Value of IAA in the solution was referred to linear regression standard.

3. Result and Discussion

All *Trichoderma* isolates had variation value against *R. solani*, and as well as cellulase index value, but had no ligninase activity. Only one isolate of *Trichoderma* sp. (2178) produced chitinase, and isolate number 1567 dissolved Ca-phosphate in agar media. Evaluation on antagonism rate with their percent inhibition of growth ranged between 20.0 to 82.1%. The highest inhibition was shown by isolate of *T. harzianum* (521) whereas the lowest one was made by *Trichoderma* sp. (2236), and both of isolates were isolated from soil sample. Cellulase enzyme accession ranged at 0.42 for the lowest and 2.44 for the highest index. Number collection of both isolates was *Trichoderma* sp. (1646) and *T. harzianum* (626), respectively (Table 1). Both of fungal species were isolated from soil sample as well. According to all the data observed in the study, they had no a correlation between antagonist potency compared to cellulase index value.

Twenty nine isolates released IAA hormone (Table 2). Antagonist potency among 29 isolates was not have any significant data connection with IAA value. Isolate producing the higest IAA and positively releasing protease and lipase was *T. virens* (2160) which is isolated from plant root. Isolate of *Trichoderma* sp. (2178) had certain physiological character to release chitinase, cellulae, and moderately producing IAA hormone (1.9162 ppm). Refer to some of potential characters that were owned by the species, so it could have potential for biocontrol and at once useful to biofertilizer application.

Some isolates of *Trichoderma* actively produced lipase and protease. In other study, the species only positively had protease activitiy but without lipase, or in the vice versa (Table 3). Observation work of 62 species based on enzymes and hormones released by the species, each isolate was grouped into six groups of the isolate characters (Figure 3). The isolates producing lipase and protease become strong competitor and may have parasitic potential against *R. Solani* or even some other fungi.

3.1 Antagonism character

Dual culture method has been used extensively to study the antagonism character of *Trichoderma* isolates against pathogenic fungi by several researchers (Benhamou and Chet, 1993; de Melo and Faull, 2000; Mishra, 2010; Gaigole *et al*., 2011; Bhale *et al*., 2013; Suciatmih and Rahmansyah 2013). According to El-Katatny (2001), the formation of inhibition zone at the contact point between *Trichoderma* and *R. solani* in dual culture was caused by the production of volatile and unvolatile metabolites. Extracellular hydrolytic enzyme production by *Trichoderma* was assumed to degrade cell membrane and cell wall of *R. solani*. Some isolates of *Trichoderma* in this study secreted lipase and protease enzymes and were able to inhibit *R. solani* growth in-*vitro.*

Antagonism of *Trichoderma* against *R. solani* has been reported intensively by Choudary *et al*. (2007), Gaigole *et al*. (2011), Bhale *et al*. (2013) and Khang *et al*. (2013). Szekeres *et al*. (2004) inform mechanism of *Trichoderma* that was involved in biocontrol as due to 1) stimulate plant defense mechanism; 2) competition of the substrate; 3) antibiosis by producing anti-fungal metabolites; and 4) mycoparasitism through the production of cell wall degrading enzymes.

Phenomenon of several isolates of *Trichoderma* against *R. solani* in the observation indicated that their rapid growth to compete for substrate performance, while in 15 isolates number (521, 532, 837, 850, 953, 1626, 1631, 1672, 1681, 2127, 2174, 2178, 2195, and 2257) had combination both of rapid growth and mycoparasitism potential as due to enzymatic system in isolates. Twenty two isolates of *Trichoderma* in the investigation had potency in mycoparasitism through the cell wall and cell membrane degrading enzyme such as protease and lipase, respectively. The level of inhibition to *R. solani* growth by *Trichoderma* spp. was varied (20.0-82.1%). Barbosa *et al.* (2001) and Misra (2010) found out that every single isolate of *Trichoderma* produced different inhibitory effectiveness against *Cladosporium herbarum* and *Pythium aphanidermatum,* correspondingly.

Table 1. Antagonism (Ant.) character of *Trichoderma* spp. against *Rhizoctonia solani*, and their cellulase index (CI) value of the isolates (see the collection number/CN) deprived from various resources

No	CN	Names	Ant. (%)	CI	Samples of origin	No	CN	Names	Ant. (%)	CI	Samples of origin
1	521	*T. harzianum*	82.1	1.70	soil	42	1677	*Trichoderma* sp.	69.7	1.14	soil
2	531	*T. harzianum*	56.3	0.66	soil	43	1678	*Trichoderma* sp.	67.9	0.54	soil
3	532	*T. harzianum*	72.7	1.25	soil	44	1679	*Trichoderma* sp.	53.1	1.13	soil
4	534	*T. aureoviride*	60.7	1.86	soil	45	1680	*Trichoderma* sp.	54.5	0.54	soil
5	548	*T. atroviride*	60.0	1.26	soil	46	1681	*Trichoderma* sp.	72.7	1.72	soil
6	607	*T. harzianum*	76.9	1.72	soil	47	1682	*Trichoderma* sp.	61.3	1.14	soil
7	608	*T. harzianum*	40.0	1.35	soil	48	1683	*Trichoderma* sp.	69.7	0.73	fabric
8	620	*T. virens*	66.7	0.66	soil	49	1715	*Trichoderma* sp.	50.0	1.86	pencil
9	621	*T. harzianum*	40.0	1.31	soil	50	1891	*T. harzianum*	44.4	1.28	bamboo
10	626	*T. harzianum*	56.3	2.44	soil	51	1963	*T. virens*	59.4	1.46	door
11	679	*Trichoderma* sp.	66.7	1.15	termite	52	2075	*Trichoderma* sp.	33.3	1.86	leaf
12	695	*T. virens*	60.6	1.25	soil	53	2076	*Trichoderma* sp.	54.3	1.19	leaf
13	708	*Trichoderma* sp.	43.3	1.43	termite	54	2127	*Trichoderma* sp.	75.8	1.39	conta.*
14	714	*T. virens*	66.7	0.63	termite	55	2135	*Trichoderma* sp.	75.9	1.33	root
15	718	*T. virens*	69.7	1.72	soil	56	2156	*Trichoderma* sp.	43.3	1.28	conta.
16	720	*Trichoderma* sp.	60.6	0.73	soil	57	2160	*T. virens*	48.3	1.20	root
17	837	*Trichoderma* sp.	72.7	1.15	larvae	58	2174	*Trichoderma* sp.	76.7	1.31	conta.
18	849	*T. virens*	50.0	1.10	larvae	59	2175	*Trichoderma* sp.	33.3	1.35	conta.
19	850	*Trichoderma* sp.	75.8	1.22	termite	60	2178	*Trichoderma* sp.	75.8	1.46	conta.
20	858	*Trichoderma* sp.	68.0	1.33	termite	61	2195	*Trichoderma* sp.	76.7	1.26	conta.
21	862	*Trichoderma* sp.	68.8	1.26	termite	62	2196	*Trichoderma* sp.	36.0	1.59	conta.
22	947	*T. virens*	60.6	1.43	termite	63	2229	*Trichoderma* sp.	35.7	1.31	soil
23	953	*Trichoderma* sp.	72.4	1.10	termite	64	2230	*Trichoderma* sp.	65.5	1.33	soil
24	966	*Trichoderma* sp.	33.3	1.13	larvae	65	2232	*Trichoderma* sp.	61.5	1.40	soil
25	967	*Trichoderma* sp.	48.5	0.54	termite	66	2235	*Trichoderma* sp.	61.3	1.28	soil
26	1567	*Trichoderma* sp.	53.3	1.10	soil	67	2236	*Trichoderma* sp.	20.0	1.22	soil
27	1622	*Trichoderma* sp.	46.9	1.22	soil	68	2238	*Trichoderma* sp.	35.7	1.19	soil
28	1624	*Trichoderma* sp.	69.0	0.73	soil	69	2252	*T. pseudokoningii*	33.3	1.46	soil
29	1625	*Trichoderma* sp.	52.0	1.74	fabric	70	2253	*T. harzianum*	68.0	1.22	soil
30	1626	*Trichoderma* sp.	75.8	1.14	fabric	71	2257	*Trichoderma* sp.	78.1	1.32	conta.
31	1631	*Trichoderma* sp.	71.4	0.54	fabric	72	2264	*Trichoderma* sp.	69.7	0.54	root
32	1646	*Trichoderma* sp.	43.3	0.42	soil	73	2286	*Trichoderma* sp.	69.0	1.10	soil
33	1667	*Trichoderma* sp.	69.7	1.13	soil	74	2292	*Trichoderma* sp.	66.7	1.20	soil
34	1668	*Trichoderma* sp.	60.0	1.43	soil	75	KG-4	*Trichoderma* sp.	40.7	1.43	soil
35	1669	*Trichoderma* sp.	54.5	1.10	soil	76	TDG-2	*T. virens*	46.7	1.50	soil
36	1670	*Trichoderma* sp.	68.8	1.20	soil	77	TDG-10	*Trichoderma* sp.	50.0	1.39	soil
37	1671	*Trichoderma* sp.	40.0	1.31	soil	78	TTDG-10	*Trichoderma* sp.	33.3	1.35	soil
38	1672	*Trichoderma* sp.	75.0	1.74	soil	79	TTDG-12	*T. virens*	40.7	1.26	soil
39	1674	*Trichoderma* sp.	54.5	1.72	soil	80	TTDG-19	*Trichoderma* sp.	39.4	1.33	soil
40	1675	*Trichoderma* sp.	69.7	1.33	soil	81	TTDG-21	*Trichoderma* sp.	56.3	1.26	soil
41	1676	*Trichoderma* sp.	72.7	1.22	soil	82	SPT-1	*Trichoderma* sp.	48.3	1.50	fabric

*contaminant in PDA media as due to laboratory aerial spore

3.2 Physiological character

Some isolates of *Trichoderma* tested in this study showed their lipase and protease enzyme activities, but did not show chitinase activity. Inability of *Trichoderma* in producing chitinase could be due to weakness or inactivity of isolates. Environmental factors such as acidity, temperature, and incubation time might affect the physiological factor. Biological factors such as genetics might also affect the biochemistry and metabolism of *Trichoderma*, so disturbed the enzyme production system (Bhale and Rajkonda, 2012).

Table 2. *Trichoderma* potential for biological control and biofertilizer utilization based on antagonism character and the release of hormone IAA of the strain

No	Collection number	Species names	Antago -nism (%)	IAA (ppm)	No	Collection number	Species names	Antago-nism (%)	IAA (ppm)
1	548	*T. atroviride*	60.0	0.0958	16	2127	*Trichoderma* sp.	75.8	0.3353
2	607	*T. harzianum*	76.9	2.7305	17	2135	*Trichoderma* sp.	75.9	0.6766
3	608	*T. harzianum*	40.0	1.4012	18	2156	*Trichoderma* sp.	43.3	0.6647
4	620	*T. virens*	66.7	0.8748	19	2160	*T. virens*	48.3	9.2575
5	621	*T. harzianum*	40.0	0.5868	20	2174	*Trichoderma* sp.	76.7	1.4012
6	679	*Trichoderma* sp.	66.7	0.6766	21	2178	*Trichoderma* sp.	75.8	1.9162
7	695	*T. virens*	60.6	0.7725	22	2196	*Trichoderma* sp.	36.0	1.2216
8	714	*T. virens*	66.7	1.0599	23	2229	*Trichoderma* sp.	35.7	0.2635
9	953	*Trichoderma* sp.	72.4	0.2575	24	2235	*Trichoderma* sp.	61.3	1.8024
10	1622	*Trichoderma* sp.	46.9	0.3353	25	2236	*Trichoderma* sp.	20.0	0.7964
11	1625	*Trichoderma* sp.	52.0	0.9401	26	2238	*Trichoderma* sp.	35.7	0.1677
12	1626	*Trichoderma* sp.	75.8	0.5449	27	2252	*T. pseudokoningii*	33.3	0.9820
13	1670	*Trichoderma* sp	68.8	1.6946	28	2257	*Trichoderma* sp.	78.1	0.8204
14	1677	*Trichoderma* sp.	69.7	3.2874	29	2286	*Trichoderma* sp.	69.0	0.5269
15	1678	*Trichoderma* sp.	67.9	0.4731					

Table 3. Sixty two potential species that have lipase (Lip.) and protease (Prot.) activities, in relation to the present or absent of hormone IAA in the strain

Collection number	Names	IAA (ppm)	Presence of:		Collection number	Names	IAA (ppm)	Presence of:	
			Lip.	Prot.				Lip.	Prot.
620	*T. virens*	0.8748	+	+	2229	*Trichoderma* sp.	0.2635	+	–
2160	*T. virens*	9.2575	+	+	2238	*Trichoderma* sp.	0.1677	+	–
2196	*Trichoderma* sp.	1.2216	+	+	2252	*T. pseudokoningii*	0.9820	+	–
2257	*Trichoderma* sp.	0.8204	+	+	521	*T. harzianum*	–	+	–
626	*T. harzianum*	–	+	+	532	*T. harzianum*	–	+	–
849	*T. virens*	–	+	+	534	*T. aureoviride*	–	+	–
967	*Trichoderma* sp.	–	+	+	718	*T. virens*	–	+	–
1646	*Trichoderma* sp.	–	+	+	862	*Trichoderma* sp.	–	+	–
1667	*Trichoderma* sp.	–	+	+	947	*T. virens*	–	+	–
1668	*Trichoderma* sp.	–	+	+	1680	*Trichoderma* sp.	–	+	–
1672	*Trichoderma* sp.	–	+	+	1682	*Trichoderma* sp.	–	+	
1675	*Trichoderma* sp.	–	+	+	2075	*Trichoderma* sp.	–	+	–
1681	*Trichoderma* sp.	–	+	+	2076	*Trichoderma* sp.	–	+	–
1715	*Trichoderma* sp.	–	+	+	2175	*Trichoderma* sp.	–	+	–
1963	*T. virens*	–	+	+	2195	*Trichoderma* sp.	–	+	–
2253	*T. harzianum*	–	+	+	679	*Trichoderma* sp.	0.6766	–	+
2264	*Trichoderma* sp.	–	+	+	695	*T. virens*	0.7725	–	+
KG-4	*Trichoderma* sp.	–	+	+	1670	*Trichoderma* sp	1.6946	–	+
TDG-2	*T. virens*	–	+	+	2235	*Trichoderma* sp.	1.8024	–	+
TTDG-12	*T. virens*	–	+	+	2286	*Trichoderma* sp.	0.5269	–	+
TTDG-19	*Trichoderma* sp.	–	+	+	708	*Trichoderma* sp.	–	–	+
SPT-1	*Trichoderma* sp.	–	+	+	837	*Trichoderma* sp.	–	–	+
621	*T. harzianum*	0.5868	+	–	850	*Trichoderma* sp.	–	–	+
953	*Trichoderma* sp.	1.1000	+	–	858	*Trichoderma* sp.	–	–	+
1622	*Trichoderma* sp.	0.3353	+	–	1624	*Trichoderma* sp.	–	–	+
1625	*Trichoderma* sp.	0.9401	+	–	1631	*Trichoderma* sp.	–	–	+
1626	*Trichoderma* sp.	0.5449	+	–	1671	*Trichoderma* sp.	–	–	+
1677	*Trichoderma* sp.	3.2874	+	–	1891	*T. harzianum*	–	–	+
2127	*Trichoderma* sp.	0.3353	+	–	TDG-10	*Trichoderma* sp.	–	–	+
2156	*Trichoderma* sp.	0.6647	+	–	TTDG-10	*Trichoderma* sp.	–	–	+
2174	*Trichoderma* sp.	1.4012	+	–	TTDG-21	*Trichoderma* sp.	–	–	+

Protease activity in this study detected in 38 isolates belonging to *T. harzianum* (3 isolates), *T. virens* (7 isolates) and *Trichoderma* spp. (28 isolates). The same results were reported that protease enzyme was produced respectively by *T. harzianum* (de Marco and Felix, 2002; Haggag *et al*., 2006; Shakeri and Foster, 2006; Mishra, 2010), *T. reesei* NTG-17 (Zambare, 2010), *T. virens* (Mishra, 2010), and *T. viride* (Šimkovič *et al*., 2008; Mishra, 2010). *Trichoderma* producing protease in addition to deactivation of other fungal pathogens, can also be used to control plant diseases, which degrades protein components inside the cell wall of the host fungal pathogens (Elad and Kapat, 1999; Haggag *et al*., 2006). Controling phatogenic *Botrytis cinerea* by *T. harzianum* was made possible by the protease action. Protease will break down hydrolytic enzymes produced by *B. cinerea* into a peptide chain. Therefore, the constituent of amino acids will decrease its capacity to act in disturbing plant cells (Bhale and Rajkonda, 2012).

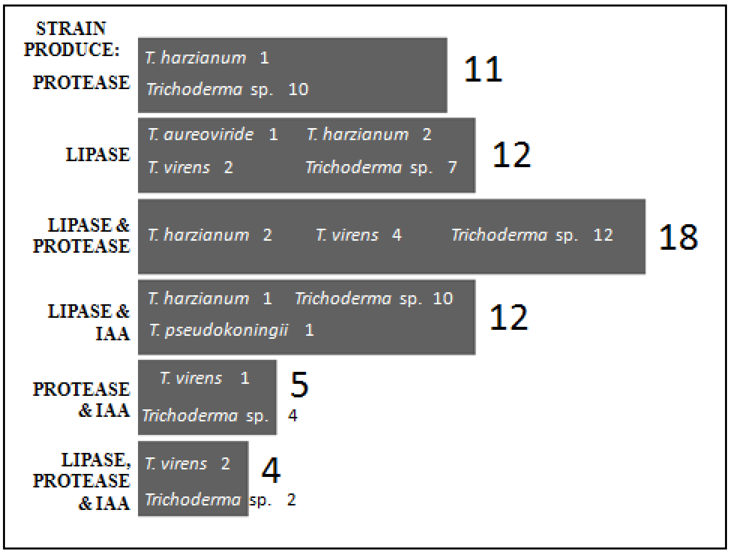

Figure 3. Quantity of characterized species that were appraised with enzymes activities and the production of hormone IAA released by *Trichoderma* strains

Lipase activity in this study detected among 46 isolates belonging to *T. aureoviride* (1 isolate), *T. harzianum* (5 isolates), *T. pseudokoningii* (1 isolate), *T. virens* (8 isolates) and *Trichoderma* spp. (31 isolates). The same results were already published that lipase was produced by *T. atroviride* 676 (Marques *et al*., 2014), *T. harzianum* (Cuervo-Parra *et al*., 2011; Ülker *et al*., 2011; Toscano *et al*., 2013), *T. reesei* (Rajesh *et al*., 2010), *T. viride* (Kakde, 2011), and *Trichoderma* sp. (Nwuche and Ogbonna, 2011). While lipase in some species such as *T. harzianum*, *T. koningii*, *T. pseudokoningii*, *T. virens* and *T. viride* reported by Bhale and Rajkonda (2012) had antagonistic potential to other pathogenic fungi. Lipase enzyme produced by *Trichoderma* can degrade the possibility of lipid components of *R. solani* membranes cell.

Trichoderma harzianum (2 isolates), *T. virens* (4 isolates) and *Trichoderma* spp. (12 isolates) secreted lipase and protease enzymes. All isolates inhibited *R. solani* growth through the secretion of that enzymes. Three isolates number 1672, 2253 and 2257 also grew rapidly and released lipase and protease; meaning that the isolates competed for substrate utilization, too. The isolates that had potential biocontrol agent against *R. solani* need to be further tested in a field scale.

All isolates of *Trichoderma* produced cellulase activity in this study. The same result was reported by other researchers that cellulase enzyme was produced by *T. atroviride* (Kovács *et al*., 2008), *T. aureoviride* 7-121 (Zaldívar *et al*., 2001), and *Trichoderma* sp. (Reanprayoon and Pathomsiriwong, 2012). *Trichoderma longibrachianum* grown on sorghum straw released cellulase enzymes such as CM-cellulase and β-glucosidase (Velazquez-Cedeno *et al*., 2004). Beldman *et al*. (1985) informed that *T. viride* produced some types of cellulase enzyme and acted synergistically to break down the material of cellulose. The enzyme had 6 types of endoglukanase, 3 types of exoglukanase, and one type of β-glucosidase. Salma and Gunarto (1999) reported that cellulase produced by *Trichoderma* could also damage pathogenic fungi Pythiaceae groups, such as *Phytophthora infestans*.

Phosphate minerals dissolving fungus in this study detected only on *Trichoderma* sp. (1567). The same result was reported that capability to dissolve phosphate (Ca-phosphate) was showed by *T. harzianum* (Altomare *et al*., 1999; Tallapragada and Gudimi, 2011), *T. virens* and *T. viride* (Rudresh *et al*., 2005), and also in *Trichoderma* sp. (Kapri and Tewari, 2010; Saravanakumar *et al*., 2013). Capability of *Trichoderma* to dissolve phosphate minerals will facilitate the absorption of phosphate compounds by plant. Phosphate compounds could also suppress fusarium wilt on bananas (Stover, 1962).

One of the phytohormones produced by microorganisms is indole acetic acid (IAA), as essential hormone for plant growth and development (Frankenberger and Arshad, 1995). Metabolite product like IAA in this study was produced by *T. atroviride* (1 isolate), *T. harzianum* (3 isolates), *T. pseudokoningii* (1 isolate), *T. virens* (4 isolates) and *Trichoderma* sp. (20 isolates) (Table 2). The same result was reported by Gravel *et al.* (2007) that *T. atroviridae* produced IAA. Contreras-Cornejo *et al*. (2009) reported that *T. virens* produced materials related to auxin to trigger the growth of plants, such as indole-3-acetic acid, indole-3-acetaldehyde and indole-3-ethanol; while *T. atroviride* or *T. virens* increased biomass and stimulated the formation of lateral roots of *Arabidopsis thaliana* seedling. Sofo *et al.* (2011) informed that 10 days after inoculation of *T. harzianum* (T-22) on cherry plant (*Prunus cerasus* x *P. canescens*) increased IAA and Gibberellic acid (GA3) at the top of plant 49% and 71%, respectively; and in the root 40% and 143%, as well. Mwangi *et al.* (2011) reported that inoculation of *T. harzianum* onto tomato seedlings in sterilized soil increased root dry weight and plant height.

4. Conclusion

- Antagonist potential of *Trichoderma* spp. against *R. solani* in the study, mostly because of its producing lipase and protease, or both of the enzyme activities, and also as due to rapid growth character of isolates. Combination of whole character owned by the isolate could strongly stimulate against pathogenic fungi.

- *Trichoderma* spp. could support the growth of plants since they had the characters of cellulase enzyme activity, hormone IAA, and dissolving phosphate existing in the isolates. Three isolates of *Trichoderma* (1672, 2253 and 2257) were obtained as candidates of potential biocontrol agents against *R. solani*; while 29 isolates of *Trichoderma* as agents for supporting plant growth because of releasing IAA. Those of potential isolates need to be further investigated in the field trial conditions.

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