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Capability of Sulphur Oxidizing Bacteria to Inhibit Basal Plate Rot and Increase Shallot Growth on Andisols

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ABSTRACT

In the recent years, basal plate rot (BPR) caused by Fusarium oxysporum f. sp.cepae (FOCe) is one of the important constraints of shallot production in Indonesia. This research aimed to study the potential of S-oxidizing bacteria (SOB) as biological control agents to inhibit FOCe and provide available-S to shallot on Andisols. An experiment in laboratory was conducted to evaluate the capabilty of SOB in oxidizing S in liquid medium and a pot experiment was aimed to evaluate the capability of SOB in providing available-S and inhibiting infection of shallot by FOCe in Andisols. All treatments were arranged by a Completely Randomized Design with three replications. The data were analyzed by F test, followed by Duncan's Test.The results showed that the capability of oxidizing S in a liquid medium increased after the incubation for six days, with the highest concentration of soluble-S was taken from SOB2 isolate. The isolate provided the highest available-S, while the highest capability of decreasing the disease incidence was taken by SOB3 and SOB1+2+3. SOB3 isolates promoted the highest growth of shallot. So it has a tremendous potential if they are used as an inoculum of bio-fertilizer and biological control agent of BPR of shallot.

INTRODUCTION

Diseases and soil nutrients deficiency are two factors which influence the shallot production in Indonesia. Basal plate rot (BPR) caused by *Fusarium oxysporum* f. sp. *cepae* (FOCe) is one of the diseases which often decreases shallot yield. FOCe is one of soil borne pathogen that is difficult to control (de Visser, van den Broek, & van den Brink, 2006). Prakoso, Wiyatiningsih, & Nirwanto (2016) reported that the disease intensity of BPR on some cultivars of shallot could reach by 83.3 %. The pathogenic fungi usually infects shallot at the root and plate causing leaves grow abnormally and resulting a specific symptom of twisting leaf. Biological control of soil born pathogen around plant rhizosphere maybe the safest method and also can reduce the usage of costly pesticides (Bosah, Igeleke, & Omorusi, 2010; Fakhrunnisa, Hashmi, & Ghaffar, 2006). The usage of antagonistic microbes showed an effective method in reducing such disease infection caused by Fusarium and increasing plant growth significantly (Dawar, Hayat, Anis, & Zaki, 2008; Nawar, 2013).

Another problem which also found in shallot cultivation is the tendency of farmers to use high dosage of N, P and K fertilizer, but exclude sulphur (S) element which can stimulate the deficiency of S in soil. Most tropical soils are defisit in sulphur (Rego, Sahrawat, Wani, & Pardhasaradhi, 2007; Sahrawat, Wani, Rego, Pardhasaradhi, & Murthy, 2007; Tisdale, Nelson, & Beaton, 1985). The sulphur problem tend to increase significantly so that it is crucial to overcome. Sulphur is a secondary macro nutrient needed by plants as part of the essential amino acids (cystine, cysteine and methionine), and important for protein synthesis, chlorophyll production and carbohydrate metabolism (Kasno, Anggria, & Rostaman, 2017; Tan, 1994). It is also a functional group of sulfhydryl (-SH) which plays an important role in many biochemical processes of plant metabolisms (Smith & Siregar, 1983) especially, typical aroma of shallot is closely related to sulphur content.

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RESULTS AND DISCUSSION

Potential Capability of SOB Isolates in Inhibiting Growth of FOCe

From the antagonism test against FOCe, 20 isolates of sulphur oxidizing bacteria identified, had a capability of inhibiting the growth of FOCe in NA-PDA medium (Table 1). According to Rajkumar, Wang, & Kui (2005), in vitro accurately proven to test a biological agent to prevent the development of plant pathogen which is further tested in vivo. The inhibition capability of the isolates against plant pathogen maybe as the result of various mechanisms, such as antibiosis, for example by producing antibiotic substance such of 2,4-diasetilphloroglucinol (phl) and pectinase enzyme (Mukarlina, Khotimah, & Rianti, 2010; Santoso, Soesanto, & Haryanto, 2007). Sulphur classified as a derivative organosulphur compound of aliin and alisin amino acid constituent in shallot. Organosulphur compound is an anti-microbial which can inhibit the growth of certain microbial pathogenic fungi, such as FOCe (Hernawan & Setyawan, 2003). The inhibition capability was characterized by the formation of inhibition zone between the colony of bacteria isolates and FOCe. The inhibition zone is an indication that the antagonists produce one or some chemical compounds released into media around the bacterial colony that are able to inhibit the growth of pathogen by forming a clear zone area.

Table 1. Growth inhibition of *Fusarium oxysporum* f. sp. *cepae* by sulphur oxidizing bacteria on potato dextrose agar

Soil orders/ location	Shallot condition	Isolate code of bacteria *)	Growth Inhibition (%)	
Entisols/Bantul	Healthy	BH 11	46.67	c
		BH 15	37.78	c
		BH 17	**44.44**	**c**
	Diseased	BI 5	22.22	d
		BI 11	17.78	d
		BI 14	36.67	c
Andisols/Ngargoyoso	Healthy	NH 6	53.33	b
		NH 12	**37.78**	**c**
		NH 14	45.56	c
	Diseased	NI 10	38.89	c
Vertisols/Palur	Healthy	PH 7	50.00	b
		PH 11	55.56	b
		PH 17	**70.00**	**a**
	Diseased	PI 8	18.89	d
		PI 9	38.89	c
Andisols/Tawangmangu	Healthy	TH 5	38.89	c
		TH 18	22.22	d
		TH 15	33.33	c
	Diseased	**TI 3**	**33.33**	**c**
		TI 8	37.78	c

Remarks: *) B = shallot rhizosphere soil samples from Bantul District, N = Ngargoyoso, P = Palur, T = Tawangmangu. H = healthy shallot, I = diseased shallot. Values followed by same letter are not significantly different based on DMRT at 95 % of level confidence

The type of isolate has a significant influence ($P < 0.005$) on the capability of bacteria isolates to oxidize S indicated by the clear zone diameter. Table 2 shows that the isolate of TI3 has the highest clear zone diameter (0.83 cm). The criteria for choosing the isolate that used in this experiment were based on the formation of a clear zone around the colony. This was due to there is not all bacteria which was able to inhibit the growth of FOCe were able to oxidize sulphur in NA + polysulfide medium. It can be presumed that the broader and clearer of a clear zone, qualitatively the ability of bacteria to oxidize S is higher (Saraswati, Husen, & Simanungkalit, 2007).

Capability of Oxidizing S in Liquid Sulphur Medium

The potential capability of bacterial isolates to oxidize S quantitatively were examined in liquid Sulphur medium. Soil microbes which were able to oxidize S will produce SO_4^{2-} ions in oxidative condition (Sudadi, Ernawati, Sumarno, Dewi, & Widijanto, 2013). The result is presented in Fig. 1.

Isolate combinations, incubation times and their interaction were highly significant ($P = 0.000$) on dissolved-S. Inoculation treatment of SOB_2 was able to produce the highest level of dissolved S (0.1855 ppm) on the sixth days of incubation time (Fig. 1). The increasing of dissolved-S level on the third - to sixth days of incubation time proved that the bacteria isolates inoculated were still able to dissolve S, maybe because the medium condition was still favorable for the isolates growth. Otherwise, the decreasing of dissolved-S concentration showed that the decrease of bacteria activity in dissolve S was possibly because the liquid medium condition had less favorable to growth. For example, a high population density will stimulate a higher competition to acquire nutrient and space to grow. Elemental sulphur existed in the medium will be oxidized by Sulphur oxidizing bacteria to form sulfate which can increase the acidity of a medium characterized by the decrease in pH medium (Hong & Valix, 2014).

In addition, the combination of isolate, incubation time, and their interaction were highly significant ($P = 0.000$) to pH value of liquid sulphur medium. The pH values showed a sharp decline at the beginning to third days of incubation time (Fig. 2) caused by the increase of water soluble-S (H_2SO_4) concentration in the medium as a result of elemental sulphur (S^0) oxidation. The elemental sulphur was oxidized enzymatically by microbes based on the following reaction (Tisdale, Nelson, & Beaton, 1985).

$$2\ S^0 + 3\ O_2 + 2\ H_2O \xrightarrow[\text{S-oxidizer microbes}]{} 2\ H_2SO_4$$

Sulphuric acid (H_2SO_4) will produce $2H^+$ in liquid medium which increases H^+ concentration and decreases the pH medium. Furthermore, the pH value becomes relatively constant at around sixth to ninth days of incubation. The pH value decreased as the length of incubation from 6.2 at initial to 5-5.2 at the end of incubation time. The indication showed

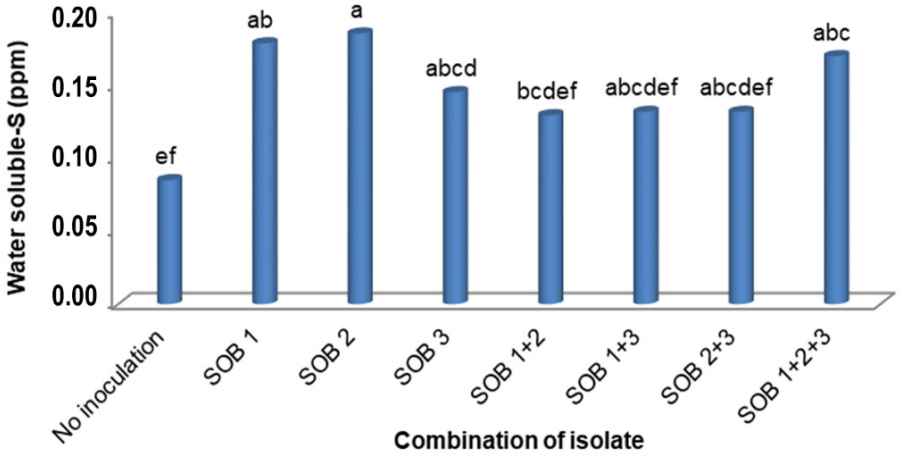


Fig. 1. Influence of sulphur oxidizing bacteria isolates on six days incubation times on water soluble-S concentration of liquid sulphur medium. The same letter above the barchart indicated that the values were not significantly different among treatment based on DMRT at 95 % of confidence level

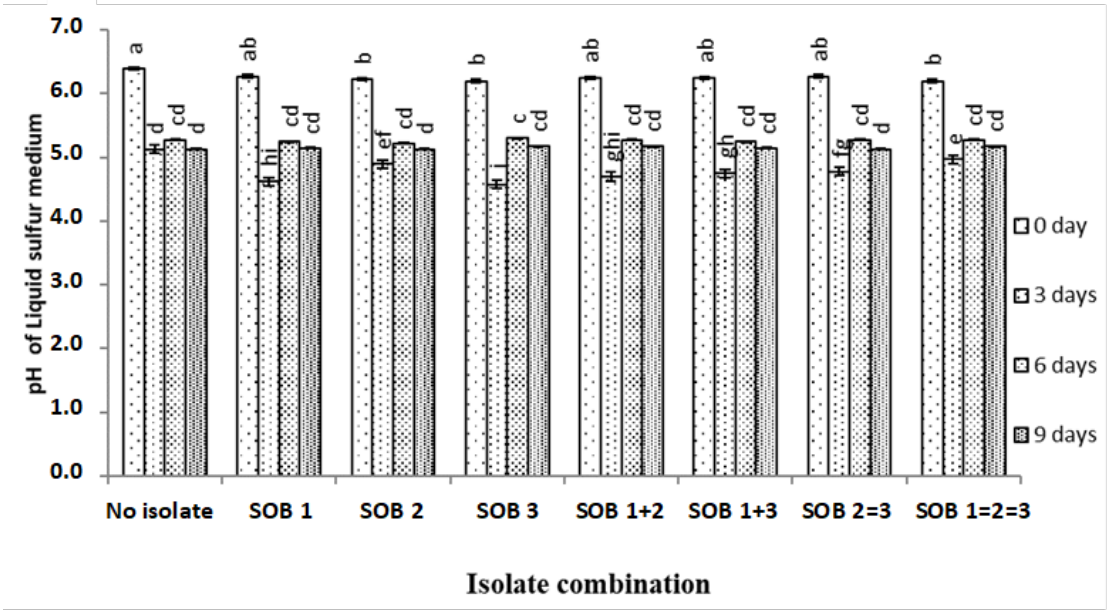
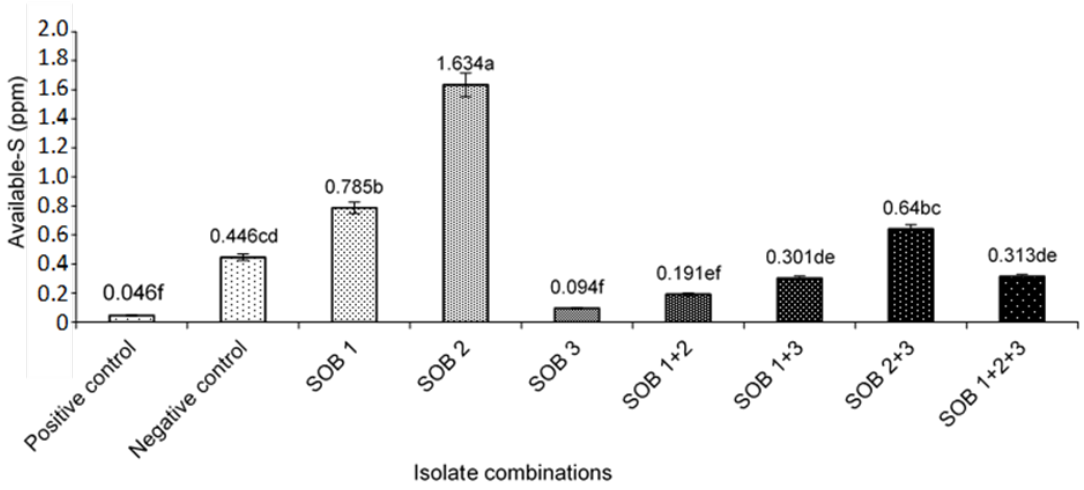


Fig. 2. Influence of Sulphur oxidizing bacteria isolates and incubation times on liquid Sulphur medium pH. The same letter above the barchart indicated that the values were not significantly different among treatment based on DMRT at 95 % of confidence level



Remarks: The same letter above the barchart indicated that the values were not significantly different among treatment based on DMRT at 95 % of confidence level

Fig. 3. Influence of isolate combinations to available-S in soil

that sulphur oxidizing bacteria was acidophilic, that could grow well at pH under 5.5 (Hong & Valix, 2014; Mangold, Valdés, Holmes, & Dopson, 2011). In accordance with the research of Heydarnezhad, Shahinrokhsar, Vahed, & Besharati (2012) which showed that the usage of elemental Sulphur can lower the soil pH and increase the sulphur content in soil and pH value will decrease faster.

Potential Capability of Providing Available-S and Inhibiting Basal Rot of Shallot

Available-S level which dissolved by S-oxidizing microbes depends on the amount of available energy and carbon sources. The ability of sulphur oxidizing bacteria to provide available-S in Andisol soil is presented in Fig. 3.

Table 3. Influence of Sulphur-oxidizing bacteria to growth and disease incidence on shallot inoculated by FOCe on Andisols

SOB	Biomass weight (g/plant)				Disease incidence (%)
	Fresh		Dry		
No Inoculation FOCe	8.80	C	1.04	B	0.00c
No SOB	8.70	C	1.12	Ab	33.33 b
SOB_1	8.10	C	1.73	Ab	66.67 a
SOB_2	9.51	C	1.64	Ab	66.67 a
SOB_3	20.35	A	2.02	A	0.00c
SOB_{1+2}	7.00	C	1.68	Ab	66.67 a
SOB_{1+3}	13.85	C	0.94	B	33.33 b
SOB_{2+3}	11.24	Cb	1.27	Ab	33.33 b
SOB_{1+2+3}	18.89	B	1.83	Ab	0.00 c

Remarks: Values followed by the same letter, in the same column, are not significantly different by DMRT at 95 % of confidence level

Inoculation of SOB isolates were highly significant (P = 0.000) to increase the level of soil available-S with SOB_2 inoculation treatment was the highest (Fig. 3) although this available-S is still very low (Blakemore, Searle, & Daly, 1987). This result is corresponded with the result of laboratory experiment (Table 2 and Fig. 1). Vidyalakshmi & Sridar (2006) said that Sulphur is one of the fourth major essential nutrient elements for plant growth, that the transformation is driven by soil microbial biomass. The activity of SOB to oxidize elemental Sulphur and reduced S compound will increase phosphorus (P) availability (Ullah, Jilani, Khan, Akhtar, & Rasheed, 2016). One indicator of plant growth is the fresh and dry biomass of plant which is affected by the magnitude of photosynthetic supplied to the plant body (Wahyuni, Mulawarman, & Damiri, 2015).

The SOB isolate combination influenced the fresh and dry biomass significantly (P = 0.005). SOB_3 isolate was the highest in promoting growth of shallot showed by the weight of fresh and dry biomass (Table 3) although the isolate was the lowest in providing available-S in the soil. It is not supported a theory that Sulphur is an important nutrient required for plant to synthesize chlorophyll (Nikiforova et al., 2005). It is possible that promoting the growth of shallot is not just determined by the available-S in the soil but also the ability to provide the available other nutrients, some growth promoting regulators, and antagonism to the disease. The latest is the most logic because of SOB_3 shows no disease on the inoculated shallots. It is also supported by the capability of the isolate in inhibiting growth of the pathogen in vitro (Table 1). The plants infected by pathogen such Fusarium, will be wilting, decreasing photosynthesis or even stop before the plant dies completely (Wu et al., 2008).

The visual infected shallot, FOCe causes primer symptom of basal plate rot with some secondary symptoms such as yellowish of leaves, and thin plant (Baswarsiati, 2009). The result of F test showed that the isolate combination significantly influenced the disease incidence of BPR (P = 0.000) with the results that isolate combination was less effective than the single one such as SOB_3 which was excepted for the combination with three isolates. Possibly, between the isolates are antagonistic each other but it needs a further study to prove it. The treatments which were no disease or the disease incidence were 0 (zero) showed that the biological control agents worked well. This may be caused by their capability of inhibiting growth of FOCe in soil. The two treatments were applied with SOB3 so it is possible that the isolate took part in the zero disease incidences. It is also supported by the evident that SOB_3 was the most effective in inhibiting growth of FOCe. The effectiveness of SOB_3 in controlling the disease was due to the soil used in the testing was Andisols from where the bacterium was isolated. Commonly, biological control agents will be well established if the agents are isolated from the same ecological niche (Pal & Gardener, 2006). SOB_3 was isolated from shallot rhizosphere planted on Andisols soil. SOB_3 is a promising isolate to further study as a biological control agent of BPR of shallot especially planted on Andisols soil. According to Ramadhina, Lisnawita, & Lubis (2013)use of multimedia methods was found to be most effective. There exist a statistically significant association was found only in gender with regular oral examinations (X2 = 4.5, df = 1, p = 0.03, an organism can be considered as biological agent if it has a capability to inhibit the development or growth of other organisms. Sintayehu, Ahmed, Fininsa, & Sakhuja (2014) stated that biological control could be used in BPR management by reducing the disease incidence at 21 and 30 %.

CONCLUSION

Sulphur Oxidizing Bacteria isolate of SOB_1, SOB_2 and SOB_3 have capability of oxidizing Sulphur

in liquid medium, inhibiting growth of FOCe on solid medium and increasing the available-S as well as reducing BPR of shallot in Andisol soil. SOB_2 isolate has the highest capability to oxidize S in liquid medium, while isolate of SOB_3 is the most promising as a biological control agent of BPR and promoted the highest growth of shallot on Andisol soil.

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