**CAPABILITY OF SULPHUR OXIDIZING BACTERIA TO INHIBIT BASAL ROT AND INCREASE SHALLOT GROWTH**

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***ABSTRACT***

In few recent years, basal rot desease caused by soil borne pathogen *Fusarium oxysporum f. sp. cepae* (FOCe) be one of the factor that affect shallot production in Indonesia (Fatawi et al., 2009). This research aims to study the potential of S-oxidizing bacteria (SoB) as biological control agents to inhibit FOCe growth and provide available-S to increase shallot growth. The SoB were isolated from shallot rhizosphere of both healthy and suffered from basal rot. The experiment arranged in completely randomized design (CRD) with two factors that are ability of bacteria isolate test to oxidizing S with four times of incubation and to inhibit the growth of FOCe under in vitro and one factor of sulphur oxidizing bacteria inoculation in green house. The result showed that the best combination of bacteria isolates are NBH 12, TBK 3, and PBH 17 able to increase the availability of S and toinhibit the growth of FOCe 100% both in vitro and in vivo 100%, so it hasa tremendous potential when it use as a inoculum of biofertilizer and biological agent of basal rot.

**Keywords:** basal rot, biological agents, S deficiency, sulphur oxidizing bacteria, shallot

**INTRODUCTION**

Diseases and soil nutrients deficiency are two of some factors influence shallot production in Indonesia. Basal or tuber root rot caused by *Fusarium oxysporum* f. sp. *cepae* (FOCe) maybe one of the diseases which often decreasing shallot yield. FOCe, is one of soil borne pathogen fungi which difficult to be control (Visser et al., 2006). Pathogens usually infect the base of the tuber, thus resulting the growth of leaves, roots, and tubers disrupted and then rot eventually.

Biological control of soil born pathogen around plant rhizosphere maybe the safest method and also reduce the use of costly pesticides (Fakhrunissa et al., 2006; Bosah et al,. 2010). The use of antagonistic microbes show an effective method in reduce disease infection which one of the causes is fusarium and increase plant growth parameters significantly (Nawar, 2013; Dawar et al., 2008).

Another problem which also found in shallot cultivation is the tendency of farmers to use high dosages of N, P and K fertilizer, and exclude sulfur (S) element which can stimulate the deficiency of S in soil. While the structure of protein in plant is largely determined by S cluster. Especially, typical aroma of shallot was closely related to sulfur content. It has been developed, in the former research, Biosulfo fertilizer formula which can provide P and S nutrient especially for red onion (Sudadi et al., 2011).,

The use functional microorganism of S-oxidizing which is antagonistic to FOCe can increase the availability of S and its absorption by shallot and also prevent the development of basal rot pathogen microbial. Management of soil microbial communities are very promising to reduce the activity of soil borne pathogens and increase plant growth. Specific functional microbial as nutrient provider can contribute to the suppressiveness of soil as well as microbial activity in rhizosphere (Bahera et al., 2014; Mazzola, 2004).

**MATERIALS AND METHOD**

 The experiment was conducted in April 2013 to May 2014 at Laboratory of Soil Biology and Biotechnology and in green house of Faculty of Agriculture, University of Sebelas Maret, Surakarta. Sulfur oxidizing bacteria (SoB) were isolated from shallot rhizosphere of three soil ordos that were Andisols (from Tawangmangu and Ngargoyoso), Entisols (Bantul), and Vertisols (Palur). Test for potential capability as shallot basal rot inhibitor was conducted in mixed Nutrient Agar -potato dextrose agar (PDA) medium and in NA + polysulphide medium (Wieringa, 1966 ; modified) for their potential as available-S provider. Four SoB isolates with highest potential were selected i.e. NBH 12, TBK 3, PBH 7 and PBH 17.

Two experiments were conducted in order to study their capability to provide available-S (in laboratory) and to inhibit basal rot disease (in green house).

 The laboratory experiments were arranged in completely randomized design with two treatment factors that were kind of sulfur-oxidizing SoB (NBH 12, TBK 3, PBH 7 and PBH 17) isolates and incubation times (0, 3, 6, and 9 day), in liquid sulfur medium. Each treatment combinations replicated four times. Variables observed were water soluble-S consentration, cell number and medium pH.

The green house experiment was conducted in polybag pot Ø 25 cm filled with sterile fine soil (Ø 2 mm) of Andisols, arranged in completely randomized design with single factor there were SoB isolate combinations, each with three replications. Growth of shallot as well as basal rot disease incident were observed periodically. Data were analyzed used *F* test at 95% level confidance followed with *Duncan Multiple Range Test* (*DMRT*) if any significant differences.

**RESULTS AND DISCUSSION**

**Potential capability of SoB to inhibit growth of FOCe**

 From the antagonism test against FOCe, 20 bacterial isolates was identified had capability to inhibit growth of FOCe in NA-PDA medium. According to Rajkumar et al. (2005), in vitro test accurately proven to test a biological agent to prevent the development of plant pathogen which further tested in vivo. The inhibition capability of bacterial isolate against plant pathogen maybe as result of various mechanisms, such as antibiosis, for example by removing antibiotic substance of 2,4-diasetilfloroglusinol (phl), pectinase enzyme (Santoso et al., 2007; Mukarlina et al., 2010; Gohel et al., 2006). Sulfur classified as derivative organosulfur compound of aliin and alisin amino acid’s constituent in shallot. Organosulfur compound are anti-microbial which can inhibit the growth of certain microbial, for example FOCe (Hernawan and Setiawan, 2003). Inhibition capability characterized by the formation of inhibition zone between the colony of isolates and FOCe.

Table 1. Inhibition of *Fusarium oxysporum* f. sp. *cepae* colony by bacteria isolates from shallot rhizosphere

|  |  |  |  |
| --- | --- | --- | --- |
| Soil sample location | Shallot condition | Code of bacteria isolates\*) | Inhibition (%) |
| Bantul | Healthy | BBH 11 | 46.67 | b |
| BBH 15 | 37.78 | b |
| BBH 17 | 44.44 | b |
| Infected by FOCe | BBK 5 | 22.22 | a |
| BBK 11 | 17.78 | a |
| BBK 14 | 36.67 | b |
| Ngargoyoso | Healthy | NBH 6 | 53.33 | c |
| NBH 12 | 37.78 | b |
| NBH 14 | 45.56 | b |
| Infected by FOCe | NBK 10 | 38.89 | b |
| Palur | Healthy | PBH 7 | 50.00 | c |
| PBH 11 | 55.56 | c |
| PBH 17 | 70.00 | d |
| Infected by FOCe | PBK 8 | 18.89 | a |
| PBK 9 | 38.89 | b |
| Tawangmangu | Healthy | TBH 5 | 38.89 | b |
| TBH 18 | 22.22 | a |
| TBH 15 | 33.33 | b |
| Infected by FOCe | TBK 3 | 33.33 | b |
| TBK 8 | 37.78 | b |

\*) Explanation : B = soil samples from Bantul, N = Ngargoyoso, P = Palur, T = Tawangmangu. H = healthy plant, K = infected plant. Number followed by same letter is not significantly different by DMRT at 95 % of level confidence.

 Inhibition capability of isolates from healthy plant rhizosphere were significantly higher than one isolated from infected plant rhizosphere (*P*<0.005). That’s way the plant was healthy. The isolate of PBH 17 has the highest capability to inhibit the growth of FOCe. The ability of bacteria to inhibit the growth of FOCe was different allegedly cause of amount and type of antibiotic substances they produced, and also the mechanisms to suppress the growth of pathogen with different way. The wider of inhibition zone formed, the more sensitive are the bacteria (Andini, 2011), and of course, higher their potential capability.

**Potential capability to oxidize S**

Thecapability of bacteria isolates to oxidize sulfur were examined by culturing them in NA-polysulphide medium. The result showed that there were four bacterial isolates able to oxidize S as indicated by the clear zone they made around their colony on NA + polysulphide medium. Clear zone is an early sign to indicate the capability of bacteria to oxidize sulfur (S0). This element is usually stable and oxidize chemically very slow.

Table 2. The capbility of some bacterial isolates from shallot rhizosphere to oxidize S in NA + polysulphide medium (diameter of clear zone).

|  |  |
| --- | --- |
| Isolate | Diameter of clear zone average (cm) |
| NBH 12 | 0.37 a |
| TBK 3 | 0.83 c |
| PBH 7 | 0.23 a |
| PBH 17 | 0.50 b |

Description: Isolate NBH 12 = as sulfur oxidizing bacteria (SOB) 1, TBK 3 = SOB 2, PBH 17 = SOB 3. Number followed by same letter showed no different significant in 95% of level confidence.

 The result of *F* test showed that type of isolate has significant influence (*P*<0.005) to average of clear zone diameter. Table 2 showed that isolate of TBK 3 has the highest average of clear zone diameter which is 0.83 cm. The selection of these three types of bacteria based on the formation of clear zone around the colony, because not all bacteria which able to inhibit the growth of FOCe capable to oxidize sulfur in NA + polysulphide medium. The wider and clearer of xlear zone, qualitatively it can presumed that the ability of bacteria to oxidize S is bigger (Saraswati et al., 2007).

**Capability to oxidize S in Liquid Sulfur Medium**

The potential capability of bacteria to oxidize S quantitatively were examined in liquid sulfur culture medium. Soil microbes which able to oxidize S will produce SO42- ions in oxidative condition (Sudadi, 2013). The result is presented in Figure 1.

Day

Day

Day

Day

Figure 1. Influence of sulfur oxidizing bacteria isolate combinations and period of incubation on water soluble-S concentration of liquid sulfur medium. Number followed by same letter were not different significantly by DMRT at 95% of level confidence. Description: Isolate NBH 12 = as sulfur oxidizing bacteria (SOB) 1, TBK 3 = SOB 2, PBH 17 = SOB 3.

Day 0

Day 3

Day 6

Day 9

Figure 2. Effect of sulfur oxidizing bacteria’s isolate combination and period of incubation to pH in liquid sulfur medium. Number followed by same letter were not different significantly by DMRT at 95% of level confidence. Isolate NBH 12 = as sulfur oxidizing bacteria (SOB) 1, TBK 3 = SOB 2, PBH 17 = SOB 3.

The result of *F* test showed that combination of isolate, period of incubation and interaction between combination of isolate and period of incubation was highly significant (*P*=0.000) to dissolved S. Inoculation treatment of SOB 2 able to produced the highest level of dissolved S which is 0.1855 ppm on the sixth day of incubation period (Fig. 1). The increasing of dissolved S level on the third day to sixth day of incubation period proved that bacteria which inoculated still able to dissolve S in liquid medium with medium condition which still fulfill the need of bacteria in it such as nutrients.

The decreasing of dissolved S level showed the deterioration of bacteria activity in dissolve S possible because the liquid medium condition with the nutrient content was less and bacterial cell divided so population in medium was higher so the competition was occurred to acquire nutrient and space to grow. Sulfur on the medium will be oxidized by sulfur oxidizing bacteria to form sulfate. Sulfate formed can reduce the acidity level of medium characterized by decreasing of pH.

The result of *F* test showed that combination of isolate, period of incubation, and their interaction was highly significant (*P*=0.000) to pH value of liquid medium. pH value of liquid medium shown by Fig. 2 experienced a sharp decline at the beginning of incubation of third day. Furthermore, pH value becomes relatively constant at around sixth day to ninth say of incubation period. pH value showed decreasing as the length of incubation. Range of pH value from the initial incubation period was 6.2-6.4 and at the end of incubation period was 5-5.2. That indication showed that sulfur oxidizing bacteria used was acidophilic, which is bacteria that can grow well at pH under 5.5 (Mc Kane and Kandel, 1996). In accordance with research of Heydarnezhad et al. (2012) which showed the use of elemental sulfur can lower soil pH and increase sulfur content in soil and pH value decrease faster.

**Potential capability to provide available-S and inhibit basal rot of Shallot**

 To determine the potential of sulfur oxidizing bacteria as S provider for plant conducted analysis of soil’s available S during vegetative maximum at 40 days after planting. S level which dissolved by S-oxidizing microbes very depend by the amount of energy and carbon sources which converted into organic acids. The ability of sulfur oxidizing bacteria to provide S in soil presented in Fig. 3.

Figure 3. Influence of isolate combination on soil available-S concentration. Number followed by same letter were not different significantly by DMRT at 95% of level confidence. Isolate NBH 12 = as sulfur oxidizing bacteria (SOB) 1, TBK 3 = SOB 2, PBH 17 = SOB 3.

 Inoculation of SOB isolate was highly significant (*P*=0.000) to the level of soil’s available S. Fig. 3 showed that SOB 2 inoculation treatment was significantly different to all other treatments. Inoculation of SOB 2 was able to produced the highest soil’s available S which is 1.634 ppm from initial soil’s available S level which is 1.033 ppm.

 One of indicator plant growth which can be observed are fresh and dry biomass of plant. Weight biomass of plant is affected by the magnitude of photosynthate result supplied to the plant body (Wahyuni et al., 2004). Fresh and dry weight of biomass presented in Table 3.

Table 3. Effect of sulfur oxidizing bacteria’s isolate combination to biomass and disease incident of shallot

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment | Weight of biomass (g) |  | Disease incident (%) |
| Fresh | Dry |  |
| Positive control | 8.80 | a | 1.04 | a | 0 a |
| Negative control | 8.70 | a | 1.12 | ab | 33.33 b |
| SOB 1 | 8.10 | a | 1.73  | ab | 66.67 c |
| SOB 2 | 9.51 | a | 1.64 | ab | 66.67 c |
| SOB 3 | 20.35 | b | 2.02 | b | 0 a |
| SOB 1+2 | 7.00 | a | 1.68 | ab | 66.67 c |
| SOB 1+3 | 13.85 | a | 0.94 | a | 33.33 b |
| SOB 2+3 | 11.24 | ab | 1.27 | ab | 33.33 b |
| SOB 1+2+3 | 18.89 | b | 1.83 | ab | 0 a |

Description: Number followed by same letter, in the same colum, were not different significantly by DMRT at 95% of level confidence.

Based on *F* test, the giving of isolate combination to fresh and dry biomass was significantly different (*P*=0.005). Table 3 showed the giving of SOB 3 isolate treatment has the highest fresh and dry biomass weight. According to Abadi (2003), on plant infected pathogen cause wilt, the amount of chlorophyll reduce, photosynthesis will stop even before the plant dies completely. The main function of S for plant required to chlorophyll production (Goeswono, 1983).

 Observation on basal rot incident of shallot observed at harvest by pull the plant. The observation of the disease incident by observed the symptoms visually. Fusarium oxysproum causing basal rot of shallot has symptom such as yellowish thin plant and rot in base part and plant easily uprooted because of root growth disturbed and rotten (Baswarsiati, 2009).

Result of *F* test, the giving of isolate combination was highly significant to disease incident (*P*=0.000). Table 3 showed that the giving of SOB 3 and SOB 1+2+3’s isolate treatment did not show the symptom of basal rot. In those treatment proved that bacteria of SOB 3 (PBH 17) and the whole combination of bacteria were able to prevent the development of FOCe in soil. One of the requirements o f an organism can be considered as biological agent according to Ramadhina et al. (2013) is to have the ability of antagonism by inhibit the development or growth of other organisms. Low of disease incident suspected that antagonism bacteria able to master the surface of rhizosphere widely and produce antibiotics, so pathogen disturbed it development. Research result of Sintayehu et al. (2014), biological control significantly can be used to basal rot management by reduce the disease incident at 21% and 30%.

**CONCLUSION**

 Bacterial isolate of NBH 12, TBK 3, and PBH 17 have the highest ability to inhibit the growth of FOCe in PDA medium, oxidize sulfur both in solid and liquid medium, and increase the availability of S in soil. Bacteria isolate of PBH 17 or bacteria isolate combination of NBH 12, TBK 3, and PBH 17 were able to prevent basal rot incident at 100% in in vivo test so very potential when used as biological fertilizer inoculums of S oxidizing and biological agent of basal rot.

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