

Original Research Article

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Selection and Characterization of Rhizosphere Fungi Producing Siderophore

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ABSTRACT

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Iron has low solubility in nature so that the role of siderophore as chelating iron and inhibiting a pathogenic enzyme is needed for plant growth. In this study, we selected and characterized fungi producing siderophore with the aim can be used as biofertilizer and biocontrol. There were 10 fungal isolates in total from rhizosphere which were obtained produced various siderophores. Catechol siderophore emitted was between $5,07 \mu\text{g l}^{-1}$ and $14,87 \mu\text{g l}^{-1}$ while the salicylate siderophore ranged from $3,89 \mu\text{g l}^{-1}$ to $7,21 \mu\text{g l}^{-1}$. ETR17 which produced the highest rate of catechol siderophore which was $14,87 \mu\text{g l}^{-1}$ and salicylate siderophore $7,21 \mu\text{g l}^{-1}$. The isolate was identified and characterized as *Aspergillus* sp

Introduction

Iron is a micronutrient which is highly necessary for almost all organisms because it has the role in enzymatic and metabolism process like respiration and DNA synthesis (Kraemer, 2003; Miethke, 2017). Similarly, plants require iron nutrient for their growth and development. In nature, iron in forms of oxides and hydroxide are abundant but difficult to be absorbed by plants because Fe^{3+} is less soluble. As a result, they are difficult to be used by organisms. They are different from some microorganisms like bacteria (Arora,

2017; Patil 2014, Chakraborty, 2014), yeast (Ghosh, 2015) and fungi (Ghosh, 2017; Usha, 2013) that can secrete secondary metabolite siderophore with less iron compound (Hussein, 2019; Prema, 2013). Some fungi producing siderophore are *Trichoderma harzianum*, *T. viride*, *T. asperellum* (Ghosh, 2017), *Aspergillus niger* and *A. flavus* (Usha, 2013).

Microorganisms release siderophore to scavenge mineral iron by forming dissolved Fe^{3+} complex through active transport mechanism (Kraemer, 2003). Siderophore

secreted by microorganisms is the strongest binder of Fe^{3+} (Raymond, 2003) and can act as an iron chelator (Prema, 2013; Kraemer, 2003), absorber, and solvent (Miethke, 2007).

Besides that, siderophore also has potential to control phytopathogenic fungi and bacteria (Prema, 2013) through antagonist mechanism by inhibiting pathogenic enzyme activities (Ghosh, 2017) and mechanism of nutrient competition (Ferramola, 2013).

Symbiotic microorganisms with plant roots can boost the availability and the absorbance of iron because of its ability to emit siderophore that can bind iron and change it to be dissolved Fe^{3+} so that it becomes easy to be absorbed by plants (Kraemer, 2003; Ferramola, 2013). Therefore, this study aims to isolate and characterize fungi that can produce siderophore that is potential to be used as biofertilizer and biocontrol for plants.

Materials and Methods

Fungal Isolates from Rhizosphere

Fungal isolates were obtained by isolating soil from the rhizosphere area with a dilution of 10^{-4} – 10^{-6} . Then, the isolate was grown in the Potato Dextrose Agar (PDA) medium to get pure isolates.

Siderophore production

The siderophores produced by fungal isolates were tested using the method by (Reeves, 1983; Sivasakthivelan and Stella, 2012). Media used in this study was the Potato Dextrose Broth (PDB). Three cork borers of fungal isolates were added into each flask and incubated at 37°C for 7 days. After 7 day incubation, fungal isolate culture was centrifuged at 10.000 rpm for 2 minutes. Supernatant was used to estimate the salicylate-type siderophore. 20 ml of

supernatant culture was taken and the pH was adjusted to be 2.0 using HCl. After that, 20 mL of ethyl acetate was added into the supernatant and extracted two times. 5 mL of tested solution added with 5mL of heatway reagent, 1 ml of 0,1 M iron chloride and 1 ml of 0,1 N HCl were put into 100 ml of distilled water mixed with 1 ml of 0,1 M Potassium Ferricyanide. Then, the absorbance was measured at 560 nm using sodium salicylate as the standard to estimate the salicylate-type siderophore.

To measure the concentration of catechol-type siderophore, 5 ml of test solution was mixed with 5 ml Hathway's reagent and the absorbance was set at 700 nm with 2.3 DHBA as the standard. The concentration of culture filtered was determined and stated in $\mu\text{g l}^{-1}$

Identification

Fungal isolates from rhizosphere were identified macroscopically by observing their colony morphologies including texture, color, reverse color, and radial and concentric lines. The microscopic identification was performed by viewing the hypha, sporangium, conidiophore, and conidia (Watanabe, 2002) at 40x magnification.

Results and Discussion

Isolation of soil in the rhizosphere area resulted in 10 isolates with various morphological characteristics in the PDA media (Table 1). The color of isolates is predominantly white while reverse colony colors varied (white, yellow, green, and brown) with some different textures e.g. cottony, granular, velvety, and powdery.

The measurement of siderophore concentration showed that those 10 isolates produced various catechol and salicylate siderophore (Graph 1).

The production of catechol siderophore was between 5,07 $\mu\text{g l}^{-1}$ and 14.87 $\mu\text{g l}^{-1}$ while the salicylate siderophore ranged from 3.89 $\mu\text{g l}^{-1}$ to 7,21 $\mu\text{g l}^{-1}$. From the graph, it can be seen that ETR 17 isolate released the highest rate of catechol and salicylate siderophore among other isolates which were 14.87 $\mu\text{g l}^{-1}$ and 7.21 $\mu\text{g l}^{-1}$ respectively. The amount of catechol siderophore emitted by ETR17

isolate was higher than the one by the isolate of Sivasakhthivelan (2012) which was 8.26 $\mu\text{g l}^{-1}$. Meanwhile, salicylatesiderophore was lower than by isolate from Sivasakhthivelan (8.96 $\mu\text{g l}^{-1}$).On the other hand, Kesaulya (2015) identified it to be between 2.772 $\mu\text{g l}^{-1}$ and 4.214 $\mu\text{g l}^{-1}$. This amount is smaller compared with the one produced by ETR17.

Table.1 Morphological characteristics of fungal isolates from rhizosphere

Isolate Shape	Texture	Colony Colors	Reverse Colors	Concentris Lines	Radial Lines
ETR5	Powdery	Green	White	Have	No Have
ETR10	Velvety	White	Brown	Have	No Have
ETR11	Powdery	White	Brown	Have	No Have
ETR16	Granular	Black	White	Have	No Have
ETR17	Granular	Brown	Yellow	Have	No Have
ETR18	Granular	Green	White	Have	No Have
ETR20	Cottony	White	White	Have	No Have
ETR25	Velvety	Grey	Yellow	No Have	Have
ETR26	Granular	Green	White	Have	No Have
ETR29	Granular	Green	Green	Have	No Have

Graph.1 Measurement of catechol and salicylate producing isolates

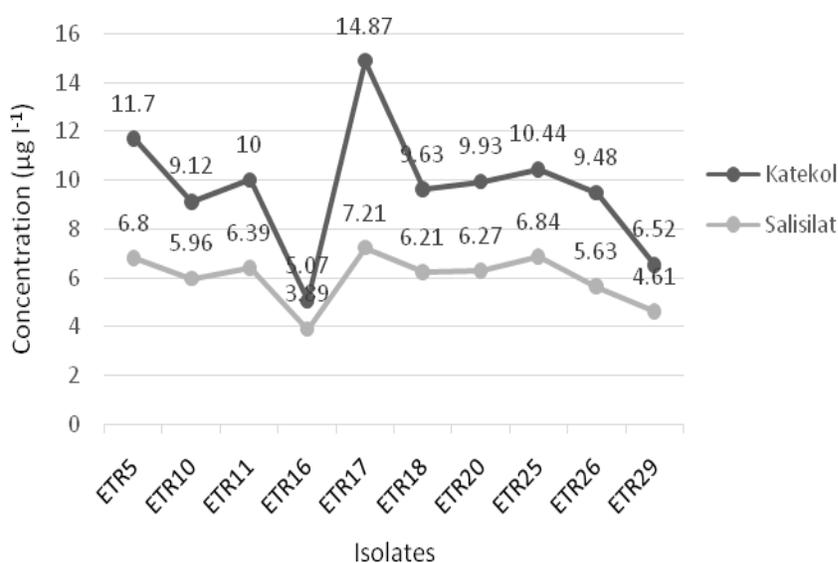
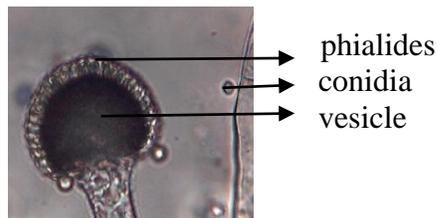
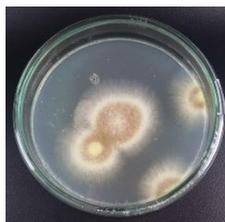


Fig.1 Microscopic of ETR 17 (40x magnification)



Macroscopically, surface of ETR17 have brown center and white margin of colony culture color. Isolate has granular texture, yellow reverse colony, and concentric line but no radial line. The characteristic of the microscopic isolates is that they have vesicle, phialide, and conidia (Fig. 1). Based on those macroscopic and microscopic features, the type of isolate obtained was *Aspergillus sp.* This finding confirms the study by Usha (2013) that *Aspergillus niger* and *Aspergillus flavus* are two isolates which can secrete high siderophore ($12 \mu\text{g l}^{-1}$ dan $10 \mu\text{g l}^{-1}$) in black pepper. Therefore, ETR17 isolate can be utilized as biofertilizer and biocontrol to manage fungal phytopathogens.

ETR17 is the isolate that can produce the highest catechol and salicylate siderophore which are $14.87 \mu\text{g l}^{-1}$ and $7.21 \mu\text{g l}^{-1}$ respectively. Macroscopic and microscopic observations showed that the type of ETR17 isolate was *Aspergillus sp* and has the potential to be developed as biofertilizer and biocontrol.

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