PROCEEDING

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“Advancing Biodiversity for Sustainable Food Security”

July 26 - 27, 2016

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held by
Faculty of Mathematics and Natural Sciences Udayana University, Bali
Postgraduate Study Program of Biology Udayana University, Bali
North Dakota State University, USA
School of Biology, Udayana University, Bali
PROCEEDING

THE INTERNATIONAL CONFERENCE ON BIOSCIENCES
“Advancing Biodiversity for Sustainable Food Security”

Udayana University, Bali, 27th - 28th July 2016

Held by:
Postgraduate Study on Biology, Faculty of Mathematics and Natural Sciences, Udayana University, Bali, Indonesia
and
The North Dakota State University, United States of America

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PREFACE - CHAIRMAN OF THE ORGANIZING COMMITTEE

This proceeding compiles all papers presented in the International Conference on Biosciences 2016 held at the Udayana University, Bali on 27th - 28nd July 2016, which was aimed to gather scientists, government officers, and industries in Biosciences-related disciplines, so that they could discuss and share their expertise, experience and expand networking.

This International conference was an implementation of MoU between the Postgraduate Study on Biology and The North Dakota State University and held in accordance to the 54th Anniversary of Udayana University. The conference consisted of 5 plenary sessions in which all honorable invited speakers delivered their works covering general aspects of Biosciences related topics. They came from Australia, India, Indonesia, Japan, Malaysia, and USA. Besides these plenary sessions, we also had four satellite symposia, covering areas of: (1) Ecology and environmental biology, (2) Physiology and developmental biology, (3) Biotechnology, genetics, molecular biology, (4) Health and microbiology, and (5) Food and agriculture. Totally more than 100 contribution papers (oral and poster presentation) were presented in this conference. The efforts of the presenters to prepare their contribution papers for this conference are highly appreciated.

This Conference was financially supported by the Rector of Udayana University, Faculty of Mathematics and Science, Udayana University, Postgraduate Study on Biology, Udayana University, and NDSU through GIFSA Institute founded by Prof. Kalidas Shetty. Therefore, in this occasion, on behalf of the committee, I would like to thank their generous supports on this conference.

My special thanks should also go to all people who have been involved in the committee of the conference. Without their hard working and efforts, I am afraid we would not be able to make this event to happen.

We hope that all papers presented in this proceeding will prove useful for further studies in Biosciences-related areas. Once again, thank you very much for your participation in this conference, and see you again in 2018.

Chairman of the Organizing Committee

Drs. Yan Ramona, M. App.Sc., Ph.D.
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MECHANISMS OF ANTIBIOTIC FILTRATE

*Streptomyces thermocarboxydus* AGAINST *Fusarium oxysporum*  
F020 ULTRASRUCTURE THROUGH SCANNING ELECTRON MICROSCOPE AND TRANSMISSION ELECTRON MICROSCOPE

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Abstract

*Fusarium oxysporum* F020 is the leaf rot fungus on Aloe vera (*Aloe barbadensis* Mill) in Bali (Kawuri et al 2012). To control the pathogen Kawuri (2013) found the bacterium *Streptomyces thermocarboxydus* able to inhibit the growth of *F.oxysporum* both in vitro and in vivo. This research was to examine *S. thermocarboxydus* ability to produce antibiotics and mechanisms to inhibit the growth *F.oxysporum* Fo2010 using Scanning Electrone microscop (SEM) and Transmission Electrone microscop (TEM). The study was conducted at the College of Agriculture Ibaraki University Japan. The concentration of the filtrate *S.thermocarboxydus* antibiotic that is used to test the inhibition of 100%, 75%, 50% and 25% in the untreated control filtrate. *S.thermocarboxydus* and *F.oxysporum* Fo2010 colonies grown on YMA media and PDA for 5 days at 25 °C. Colony was cut with a size of 1-3 mm and then fixed with a solution of 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 4 °C for 4 hours and then allowed to stand at room temperature (25°C) for 1 hour. Samples that have been fixed washed with sodium cacodylate buffer (pH 7.2) and continued fixation with 1% osmium tetroxide in 0.1 M cacodylate buffer and allowed to stand at room temperature for 5 hours. Furthermore, the dehydration process by using ethyl alcohol serially (40%, 60%, 80%, 99.5% and 100%). Samples were cut using a cutting device freeze (TF-2, Eiko, Japan) and treated with a solution of t-butylalcohol, and placed in the vacuum freeze-drying apparatus (ID-2, Eiko, Japan). Samples were dried and placed in a special place coated with osmium tetroxide (OPC 60A, Filgen, Japan) and platinum (JUC-5000, JEOL, Japan). The coated samples were observed for SEM by using JSM-6701F, JEOL, Japan with 5 kV acceleration voltage and TEM (JEM-2100, JEOL, Japan) (Hall, 1978; Hayat, 1981). In this study by using antibiotics filtrate *S.thermocarboxydus* with different levels of the percentage of 100%, 75% and 50% can lead to cell death macrokonidia, microconidia, chlamydospores and changes in the morphology of hyphae of *F.oxysporum*. Using TEM is known that filtrate *S.thermocarboxydus* mechanism is capable of damaging the cell membrane both the outer membrane and the inner membrane of the cell, so the cell becomes lysis, release of organelles and resulted in the death of cells *F.oxysporum*

Keywords: Filtrate antibiotic, *S.thermocarboxydus*, *F.oxysporum* Fo2010, ultrastrucure cell
BACKGROUND

Aloe vera (Aloe barbadensis Mill.) is a herb type of plant and belongs to the Liliaceae family. Among approximately 400 Aloe species existed, A. barbadensis is cultivated the most and has high quality. Leaf of Aloe vera plant contains gel which is the most important component of the plants, as it contains amino acid, antraquinon, enzyme, lignin, mineral, mono and polysaccharide, salisilic acid, saponin, sterol and various vitamins beneficial for health (Barcroft and Myskja, 2009).

Disease that have destroyed Aloe vera plant is leaf rot, with the symptom of rotten leaf. The infected leaf becomes rotten and dry with brown color crescent type. The disease was found for the first time in Aloe plantation in a few regions in Bali in 2010. Infection, with diameter reaches 1- 4 cm, starts from leaf edge causing leaf to dry, rot with brown color, shrink and finally damage, broken with dry and rotten leaf tip. The cause has been identified, using molecular identification assessment, as Fusarium oxysporum F020 (Kawuri et al 2012).

Synthetic fungicides are currently utilized as disease control agent for diseases caused by pathogenic fungi, that can cause a number of problems such as decreases in soil pH, environmental pollution and health problems. Moreover, synthetic fungicide can cause pathogen resistance and decrease in non target organism population (Brimer and Boland, 2003). To overcome problems of synthetic fungicide used, an alternative safe way to control disease on plants must be found. Microorganism can be used as biocontrol for plant pathogen due to more environmental friendly and also affable to non target microorganism.

Prapagdee et al. (2008) reported that many genus Streptomyces have been used as antifungi agents to control several pathogenic fungi on plants. The cap capability of Streptomyces in inhibiting growth of pathogenic fungi is due to its capability to produce both antifungal agent and extracellular hydrolytic enzyme those are able to degrade fungi’s cell wall.

To control the pathogen Kawuri (2012) found the bacterium Streptomyces thermocarboxydus able to inhibit the growth of F.oxysporum both in vitro and in vivo .This research was to examine S. thermocarboxydus ability to produce antibiotics and mechanisms to inhibit the growth F.oxysporum Fo2010 using Scanning Electrone microscop (SEM ) and Transmission Electrone microscop (TEM).

MATERIALS AND METHODS

The study was conducted at the College of Agriculture Ibaraki University Japan. The concentration of the filtrate S.thermocarboxydus antibiotic that is used to test the inhibition of 100, 75, 50 and 25% in the untreated control filtrate. S.thermocarboxydus and F.oxysporum Fo2010 colonies grown on YMA media and PDA for 5 days at 25°C. Colony was cut with a size of 1-3 mm and then fixed with a solution of 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 4 OC for 4 hours and then allowed to stand at room temperature (25°C) for 1 hour.

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process by using ethyl alcohol serially (40, 60, 80, 99.5 and 100%). Samples were cut using a cutting device freeze (TF-2, Eiko, Japan) and treated with a solution of t-butylalcohol, and placed in the vacuum freeze-drying apparatus (ID-2, Eiko, Japan). Samples were dried and placed in a special place coated with osmium tetroxide (OPC 60A, Filgen, Japan) and platinum (JUC-5000, JEOL, Japan). The coated samples were observed for SEM by using JSM-6701F, JEOL, Japan with 5 kV acceleration voltage and TEM (JEM-2100, JEOL, Japan) (Hall, 1978; Hayat, 1981).

RESULTS AND DISCUSSIONS

Observation using Scanning Electron Microscope (SEM) showed that *Fusarium oxysporum* Fo2010 had curve shape of macroconidia of 4 septa with foot cell, 31 µm length with smooth surface. Microconidia had rough surface, 4.6 µm length (Picture 1). Hypha morphology had not curvy with 13 µm in diameter and chlamydospora intercalar had wavy surface and 37 µm length. Research by Torres *et al.* (2003) shows that hypha of *Fusarium verticillioides* has smooth surface, while Alberthini *et al.* (2003) reported that hypha of *F. culnorum* has rough surface.

![Picture 1. Scanning Electrone Microskop (SEM) *Fusarium oxysporum* Fo2010. A. macroconidia bar 10 µm; B. microconidia bar 1 µm; C. Chlamidospora bar 10 µm D. Hypha bar 10 µm](image)

Colony *F. oxysporum* treated with antibiotic filtrate of *S. thermocarboxydus* (100%, 75%,50%) showed the diameter colony was different with the control (untreated colony), however colony of *F. oxysporum* treated with 25% antibiotic filtrate was same as control. Observation on macroconidia showed the mechanism based on SEM and TEM was the capability of filtrate to damage cell wall and plasma membrane of the macroconidia (Picture 2). It can be seen through SEM, that hypha’s surface of *F. oxysporum* on control, was not curvy (Picture 1D).

Treatment with *S. thermocarboxydus* antibiotic filtrate, under SEM showed broken hypha, hypha surface seemed to be curvy as seen on Picture 3.ABC. SEM data was supported by TEM analyses, through where it showed perforated cell wall and plasma membrane, organelle under pressure and wider vacoule causing cell became lysis and died (Picture 3.AA, BB, CC).

Under Scanning electrone microscope, Clamydospore interalar treated with 100% and 75% filtrate antibiotic can cause morphology damage, however in this reasearch clamydospore treated with 50% filtrate antibiotic was not found.
Figure 2. SEM and TEM macroconidia (A) and microconidia (B) of *F. oxysporum* treated with 100 % antibiotic filtrate (arrow, broken plasma membran)

Figure 3. SEM and TEM hypha of *F. oxysporum* treated with 100 % A, 75% (B), 50%(C) antibiotic filtrate (arrow, broken plasma membran)

Figure 4. SEM Clamidospore intercalar of *F. oxysporum* treated by 75% (A) and 50% (B) antibiotic filtrate.

Study on fungi with the same genus of *Fusarium* was done by Liyong *et al.* (2009) who found that chitosan treatment of 0.5% concentration over *Fusarium sulphureum* causing dry rot on potato roots. It was seen through SEM, that
morphology of hypha was changed and become tangled, swollen and branching. TEM results showed the cytoplasm distribution was not normal, thickened hypha membrane, formation of new septa was also on broken hypha, but microconidia and macroconidia were not damaged.

Significant damage also occurred in intercalary chlamydospores which occurred for all treatments (Figure 4). There are similarities results of research conducted by Domínguez et al. (2011) which is damage to the cell membrane of hyphae and destruction makrokonidia thoroughly, but targets different fungi. Goh et al. (2009) reported the formation of chlamydospores is an indicator that the yeast cells experience stress. Yuan et al. (2011) reported research with the antibiotic ciprofloxacin concentration of ≥40 g / ml was able to induce the formation of chlamydospores in F. graminearum and F. avenaceum.

CONCLUSIONS

In this study by using antibiotics filtrate S.thermocarboxydyus with different levels of the percentage of 100%, 75% and 50% can lead to cell death macroconidia, microconidia, chlamydospores and changes in the morphology of hyphae of F.oxysporum. Using TEM is known that filtrate S.thermocarboxydyus mechanism is capable of damaging the cell membrane both the outer membrane and the inner membrane of the cell, so the cell becomes lysis, release of organelles and resulted in the death of cells F.oxysporum.

REFERENCES


