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Potency Microbial Unicellular Filoplan Chili and Its Inhibitory to *Collectotrichum capsici* Causal Antraknose Disease on Chili (*Capsicum annum* L.)

Potensi Mikroba Uniseluler Filoplan Cabai serta Penghambatannya Terhadap *Collectotrichum capsici* Penyebab Penyakit Antraknosa Cabai (*Capsicum annuum* L.)

Ahmad Ilham Tanzil[1)*], Syamsuddin Djauhari[2)], Liliek Sulistyowati[2)]

[1)] Agrotechnology Undergraduate Study Program, Faculty of Agriculture, Jember University, Jember 68121 Indonesia
[2)] Agroecotechnology Undergraduate Study Program, Department of Plant and Diseases, Faculty of Agriculture, Brawijaya University, Malang 65145 Indonesia

**Corresponding Author*: aitanzil@unej.ac.id

ABSTRACT

Exploration of unicellular microbes can be used as biological control agents. Biological control is an alternative control so that it is safe for human health and the environment. This study was conducted to isolate filoplan unicellular microbes which have antibiosis ability and characterise the secondary metabolite produced to inhibit Collectotrichum capsici in-vitro. This research was conducted at the Departement Plant Pest and Diseases, University of Brawijaya. Microbe isolated from surface of chilies in different age. Isolates microbes were tested for antagonistic potential against C. capsici. Persistence of the potential antagonist under different conditions of pH, temperature and light were also examined. Antagonist test was conducted to confirm antibiosis mechanism. Furthermore, extracts were characterized using pH, light exposure and temperature. The most potential antagonist was identified as isolate C3C with 36.20% inhibition. pH 5, 35^{0}C temperature and light exposure of 0% is the best media optimization with the percentage of inhibition 37.5%, 38.2% and 42.3%.

Keyword : *Unicellular microbe, Collectotrichum capsici, chili, secondary metabolite, antifungal*

ABSTRAK

Explorasi dari mikroba uniseluler dapat digunakan menjadi agens pengendali hayati. Hal tersebut menjadi alternatif pengendalian sehingga aman bagi kesehatan manusia dan lingkungan. Penelitian ini bertujuan untuk mendapatkan mikroba uniseluler filoplan yang memiliki kemampuan antibiosis dan karakterisasi senyawa metabolit sekunder yang dihasilkan dalam menghambat *Collectotrichum capsici*. Penelitian ini dilaksanakan di Laboratorium Penyakit Tanaman, Universitas Brawijaya, Malang. Mikroba uniseluler filoplan diisolasi dari permukaan buah cabai besar hijau, hijau kemerahan, merah, merah tua. Semua mikroba uniseluler yang diisolasi diuji antagonis melawan *C. capsici*. Mikroba terpilih diuji karakter pengaruh senyawa antijamur terhadap *C. capsici* dengan perlakuan optimasi pH media, suhu dan cahaya. Kemudian dikarakterisasi senyawa antijamur menggunakan pengaruh pH, intensitas cahaya dan suhu. Ditemukan isolat mikroba uniseluler terbaik yang teridentifikasi sebagai isolate C3C dengan daya penghambatan 36,20%. pH 5, suhu 35^{0}C dan paparan cahaya 0% merupakan optimasi media terbaik dengan persentase penghambatan 37,5%, 38,2% dan 42,3%.

Kata kunci: Mikroba uniseluler, *Collectotrichum capsici,* cabai, senyawa metabolisme sekunder, antijamur

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INTRODUCTION

Chili peppers (Capsicum annuum L.) is a type of fruit of vegetables which has the potential to be developed. So as to meet the needs of consumers needs to be done intensification and extension [1]. One of the major problems in the cultivation of pepper plants that affect productivity that is the fungal pathogen has invaded anthracnose pathogen causing.When chilies tend to dry out and shrivel up [2].

Losses caused by fungal attacks can be avoided if crops are exempt from these pathogens. The use of fungicides in controlling pathogenic fungi have an impact that is not good for the environment, human health, and even cause resistance and death of non-target microorganisms. Therefore, the new technology to control pathogenic fungi that cause anthracnose more environmentally friendly need to be developed. Biological agents or also called biological control of plant diseases getting more attention as an alternative to the control of chemical pesticides.

Biological agents of plant diseases involving microorganisms lucrative benefits such as fungi (molds and yeasts) or bacteria to control the diseases caused by plant pathogens [3]. The positive impact of the use of biological control that is safer for the environment and does not accumulate in the food chain. It has a positive impact in improving plant growth and even protection against pathogens, that enhances the plant. The growth of pathogens can decrease and increase resistance to the host plant as a result of activities undertaken by microbes antagonist [4].

Some unicellular microbes such as yeasts and bacteria can produce metabolites as a biological control against plant pathogenic fungi. Class Actinomycetes produce antifungal compounds against pathogenic fungi through the destruction of the cell membrane [5]. Besides yeasts used as cosmetic ingredients, chemical industry and agriculture [6]. Exploitation of unicellular microbes can now be used as a biological agent and became one of the basic biomedical sciences. Therefore it is necessary to study the isolate of unicellular microbes and the potential control of anthracnose chili.

MATERIAL AND METHOD

This study was conducted in Departement of Plant Pest and Diseases, Faculty of Agriculture, University of Brawijaya, Malang. The healthy chili fruits were collected from chili plantation at Dau district, Malang.

Chili 50 g was placed into erlenmeyer containing 100 ml of sterile distilled water. The erlenmeyer was shaken using rotary shaker at 120 rpm for 24 hours. The soaking water was then diluted into 10^0, 10^3, 10^4 and 10^5. 100 µl of suspension was planted on YMA media (without antibiotic). The growing uniceluller microbes was then purified and identification base on their morphological characters.

Method of antagonistic test referred to [7]. Each isolated yeast was scratched on PDA media exactly in the middle of petri dish vertically 1 inoculation loop. Taken out colony of *C. capsici* was then placed on ± 3 cm of right and left side from colony of unicelluler microbe on PDA media. The percentage of inhibition was measured and calculated using formula [8]:

$$PH = \frac{rp - rk}{rp} \times 100\ \%$$

Note: PH : the percentage of unicelluler microbe inhibition against *C. capsici,* rp: the spoke amount of pathogen without unicelluler microbe treatment (r1+r2) and rk: the spoke amount of pathogen colony with unicelluler microbe treatment (r1+r2).

Data of antagonistic test result was analyzed using F test on the level 5%. If the data of test result had significantly difference, Duncan Multiple Range Test (DMRT) on the level 5% would be conducted to see the differences between treatments.

RESULT AND DISCUSSION

Identifikasi of C. capsici

C. capsici on PDA media had gray - blackish color and it had unconcentric circle. The colony texture was rough and it had uneven edges. Hyphae of *C. capsici* was aseptate and was hyaline. Morphology *C. capsici* had setae and conidia. The size of conidia had oval slightly built were 10.01-11,34 µm. According to [7] *C. capsici*

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colony in PDA media had slightly rough texture and had gray-blackish color.

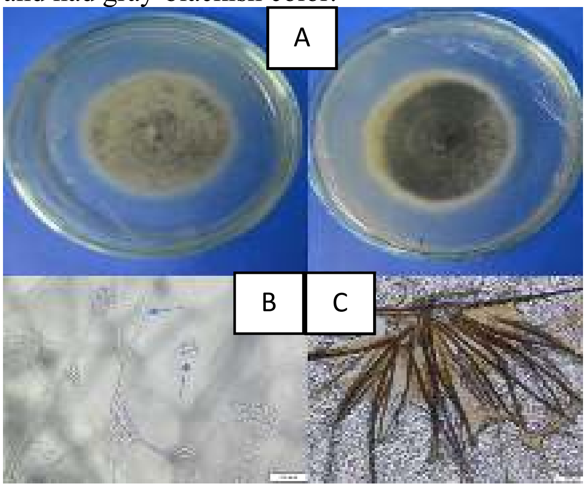

Figure 1. Colony of *C. capsici* A. macroscopically (1) on PDA media age of 7 days after incubation (dai). B. morphology (2) hyphae and conidium (3) setae.

Antagonistic Test

All of the isolated yeast were capable to inhibit growth of *C. capsici* on PDA medium, with percentage of inhibition about 19,17-36,20 %. The most potential unicelluler microbes to inhibit growth of *C. capsici* on PDA media i.e., C3C isolate, with percentage of inhibition about 36,20%. According to [8], filoplan microbe generally had higher antagonistic ability because it adapts easily and it grows faster. This is what causes filoplan unicelluler microbe more superior in controlling the nutrition and living space than pathogen fungi.

Base on the pattern resulted from inhibit growth of *C. capsici,* it was suggested that microbe was capable to poduce antibiosis compound. To ensure the existence of antibiosis compound, the study was then continued to investigate whether the compound was produced by unicelluler microbe actively by effect of pH, temperature and light.

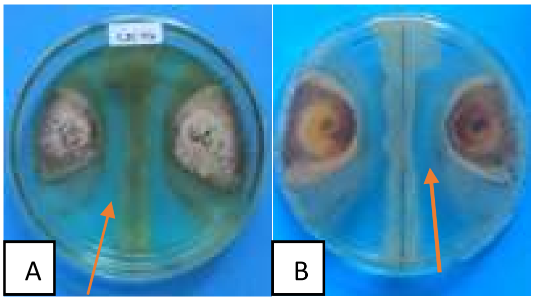

Figure 2. Antagonist Test Isolate C3C with *C. capsici* on PDA media age of 12 days after incubation (dai). A. Front, B. Behind.

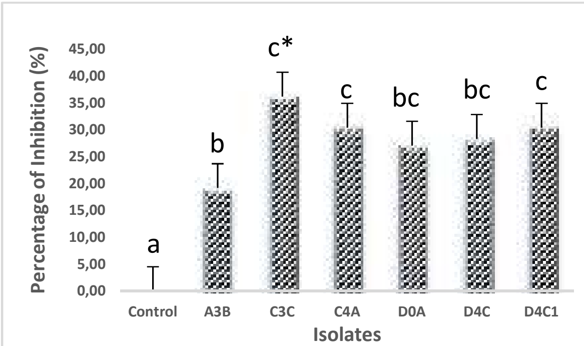

Figure 3. Percentage of unicelluler microbe inhibiotion against *C. capsici* after 7 dai. (*) antibiosis activity

Effect of pH, Temperature and Light against Antagonistic Ability of Unicelluler Microbe

C3C isolate could inhibit growth of *C. capsici* at temperature of pH 5, 6, 7, 8, temperature 20ºC, 25ºC, 30ºC, 35ºC and light 0%, 25%, 50%, 75%, 100%. The highest inhibition of *C. capsici* occurred on pH 5, temperature 35ºC and light 0% incubation by C3C, with percentage of inhibition about 37,5%, 38,2% and 42,3%. According to [9] and [10] antagonistic bacteria in tropical can grow well and can express its ability at pH 5, 5-43ºC and light 0%. On pH of acidic or alkaline is too high can lead to the inactivation of the antibiosis compound was produced by yeast, especially if the compound is produced in the form of enzymes. If the temperature is too high and too low, the growth of microbe will be inhibited so the bacteria can't produce antibiosis compound aptimally. The 100% light could made bacteria broken cause UV effect. Furthermore, antibiosis compound in form of enzyme may be destroyed or inactivated.

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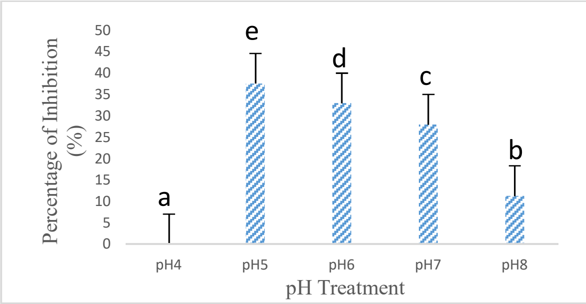

Figure 4. Effect of pH on the percentage of *P. aeruginosa* inhibition against *C. capsici* after 7 dai

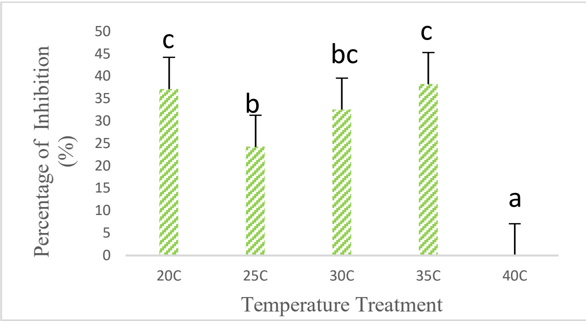

Figure 5. Effect of temperature on the percentage of *P. aeruginosa* inhibition against *C. capsici* after 7 dai

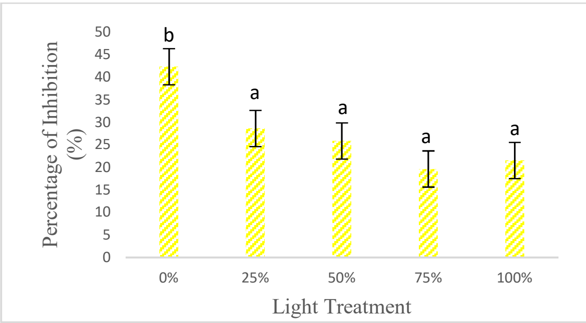

Figure 6. Effect of light on the percentage of *P. aeruginosa* inhibition against *C. capsici* after 7 dai

CONCLUSION

The best inhibition was C3C isolate within antibiotic activity. The highest inhibition of *C. capsici* occurred on pH 5, 35°C and 0% light incubation. Based on the pattern resulted from antagonistic test, it was suggested that the active compound(s) was actively produced by C3C isolate.

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