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Morphological and Molecular Identification of *Beauveria bassiana* as Entomopathogen Agent from Central Kalimantan Peatland, Indonesia

Ici Piter Kulu[1,2]*, Abdul Latief Abadi[3], Aminudin Afandhi[3] and Nooraidawati[4]

[1]Doctoral Student of Agriculture Faculty, Brawijaya University, Malang, Indonesia
[2]Faculty of Agriculture, Palangka Raya University, Indonesia
[3]Faculty of Agriculture, Brawijaya University, Indonesia
[4]Faculty of Agriculture, Lambung Mangkurat Univeristy, Indonesia

Abstract: This aim of this research was to indentify *B. bassiana* by morphological and molecular identification that isolated from monoculture and polyculture field in central Kalimantan, Indonesia. Isolate of *B. bassiana* was explored from monoculture and polyculture field in Palangkaraya peatland, Central Kalimantan. Soil samples was taken from 60 sampling point nearby mustard plant root, 30 samples from monoculture field and 30 samples from polyculture field. Isolate of *B. bassiana* was identified by morphological and molecular characterization. The result showed that eight isolates of *B. bassiana* were found in Palangka Raya, Central Kalimantan from both monoculture and polyculture. There are no significant differences between *B. bassiana* isolates which isolated from monoculture and polyculture based on morphological and molecular characterization according PCR techniques. All of *B. bassiana* isolates has three size of band 800bp, 550bp, 400bp. *B. bassiana* could be potential entomopathogen agent from peatland in Palangka Raya, Central Kalimantan, Indonesia.
Keywords: *B. bassiana*, entomopathogen, PCR, Peatland Central Kalimantan.

Introduction

Plutella xylostella Linn (Lepidoptera; Plutellidae) is the main pest that infected mustard farm in Kalimantan Indonesia, especially in Palangka Raya City as the biggest vegetable producer. This pest cald causing 70-80% yield losses, moreover it can causing 100% of damage if it was not controlled with synthetic pesticide.

Mustard farmer in Peatland use synthetic pesticide for controlling *P. xylostella* population. Survey of Faculty of Agriculture Palangka Raya[1] in Kalampangan mustard farm, Palangka Raya, Central Kalimantan showed that there was increasing level of synthetic insecticides application. High population of *P. xylostella* cause synthetic insecticide application up to three times every week in high concentration/doses.

The increasing of synthetic insecticides level not only contributed on food resources yield, but also gave bad effects into environment and non-target organisms. So food resources production with eco-friendly pest controlling was needed. Some of pathogen microbes, such as bacteria, fungi, virus, protozoa, and entomopathogenic nematodes were not clearly studied yet. Studied and evaluation about beneficial pathogen lead to the exploration of microbes that has potential to control *P. xylostella* population [2].

B. bassiana is one of the best-known genera of entomopathogenic fungi which used for control of insect pests[3]. Exploration of *B. bassiana* is one of strategy to know the potential as entomopatogen agent in palangkaraya city. This research focus on the morphological characterization and molecular analysis of *B.bassiana* isolate from monoculture and polyculture field in Central Kalimantan, Indonesia.

Materials and Methods

B. bassiana Isolate Exploration

Isolate of *B. bassiana* was explored from monoculture and polyculture field in Palangkaraya peatland, Central Kalimantan. Soil samples was taken from 60 sampling point nearby mustard plant root. The sampling area was categorized as dry peatland (B1) and wet peatland (B2). The total of 30 samples from monoculture field and 30 samples from polyculture field were collected and identified.

Morphological Identification of *B. bassiana* Isolate

All soil samples were examined at Department of pests and plant diseases, Faculty of Agriculture, University of Brawijaya. *B. bassiana* were isolated using Dilution plate method in Potato Dextrose Agar yeast and Peptone Dextrose Agar Yeast. Isolate of *B. bassiana* was identified by macroscopy and microscopy methods. Barnett and Hunter [4] determination was referred in this study.

Molecular Identification of *B. bassiana* Isolate

A. Polymerase Chain Reaction (PCR) Gene Amplification

Isolate genotype was amplified with PCR method using thermocycler machine. PCR cocktail was 20µl in volume which consisted 6 µl of ddH_2O, 10 µl of PCR kit GoTaq® Green Master Mix (10 x buffer taq polymerase, dNTP, $MgCl_2$, primer, Taq DNA polymerase, ddH_2O), 1 µl of forward primer, 1 µl of reverse primer, and 2 µl of DNA sample. Genomic samples were amplified using primer forward 5'-GACTAAATGGTAGTGGGTTCTGC-'3 and reverse primer 5'- CCATCCTTTCAGAGTGGGAGT-'3. Amplification temperature was referred to Correa [5] which thermocycler machine was set in 95^0C for minutes 5 pre-denaturation and followed by 30 cycles w for hich consisted of 95^0C for 30 seconds denaturation, 57^0C for 30 seconds annealing, and 72^0C for 30 seconds extension. Cycle was ended up by post-extenssion in 72^0C for 10 minutes and followed by 4^0C for 5 minutes.

B. Amplified Gene Confirmation

All amplified DNA sample was confirmed using agarose gel electrophoresis. Agarose gel was prepared with 1.5% concentration which made of 0.6 gram of agarose powder and 40 ml of TBE buffer. Electroforesis running was set on 65 Volt for 1.5 hours. DNA samples which has been separated on gel then visualized using UV transilluminator and documented using polaroid camera.

Results and Discussion

Morphological identification of *B. bassiana*

B.bassiana which has been isolated are 8 isolates from 60 isolate from monoculture and polyculture in Palangkaraya, Kalaimantan, Indonesia. It shows that *B. bassiana* not only find in monoculture only but also in polyculture. Macroscopic characters of the colonies that observed were growth pattern, color, shape, surface texture, colony elevation, and time that colony needed to cover up the pettri dish. Macroscopic characters of *B. bassiana* isolates is on Table 1.

Table 1. Macroscopic characters of *B. bassiana* isolates on peatland

Isolate Code	Colony Observation					
	Growth pattern	Color	Shape	Texture	Elevation	Age (day)
B1M3T3S1	Disperse	White	Round	Smooth	Raised	10
B1M3T3S2	Disperse	White	Dotted	Smooth	Flat	7
B1M3T3S3	Disperse and dense	White	Round	Smooth	Raised	7
B1M3T3S5	Disperse	White	Round kecil and wide	Smooth	Flat	8
B2M2T2S4	Disperse	White	Wide round	Smooth	Flat	9
B2M3T3S3	Disperse	White	Wide round	Smooth	Flat	10
B1P1T1S1	Disperse	White	Round	Smooth	Flat	9
B2P3T3S1	Disperse and dense	White	Medium Round	Smooth	Raised	8

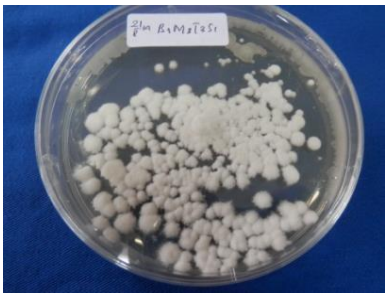
Macroscopic morphology character shown that there were no significantly differences among eight isolates (Figure 1). All of *B. bassiana* colonies color were white, there was no significantly differences on color, texture was smooth like powder which more found by colony growth. Colony growth pattern was disperse, without pattern and not concentrical. These results were related to study of Ahmad[6] and Utami et al[7] which shown that *B. bassiana* will grow on PDA medium as white mycelium and form white powder layer.

Characteristic differences of *B. bassiana* were on colony shape and elevation. Mold colony tend to round with size variation, colony elevation were thick raised and thin flat. Characterictic differences also shown on time that needed by colony to cover up medium surface. B1M3T3S3 and B1M3T3S2 isolates were covered up the medium on day 7, while other isolates were on day 7 up to day 10.

Microscopic characters observation of *B. bassiana* were shape, size, color and thickness of hyphae, conidiophore, and conidium. Microscopic characters of *B. bassiana* was shown on figure 2.

Microscopic observation result show that hyphae size about 1-2 µm which grouped on conidiogene cells with 3-6 µm in size. Hyphae then branched and formed conidiogene cells with bottle like form, small neck, and branch long were up to more than 20 µm and 1 µm wide. Fertile hyphae was found on branch, circular and normally thicken or swollen. While mycelium which is hyphae aggregate of *B. bassiana* was white and insulated.

Single cell *B. bassiana* conidium was oval and tend to round or ellipse with hialin color and 2-3 µm diameter[8]. Conidium was formed on sympodial shape from parental cells which will be stopped on its peak. Conidium was growth with longer shape because as growing point. Conidium was attached on tip and conidiophore side or on its branches [9]. Next growth was began from next bottom conidia then will be formed young conidia series with longer conidial head. When all of conidia formed, peak of conidium connector from conidiogenous cells were growth on zig-zag pattern and same as first growth[10, 11].



B1M3T3S1

B1M3T3S2

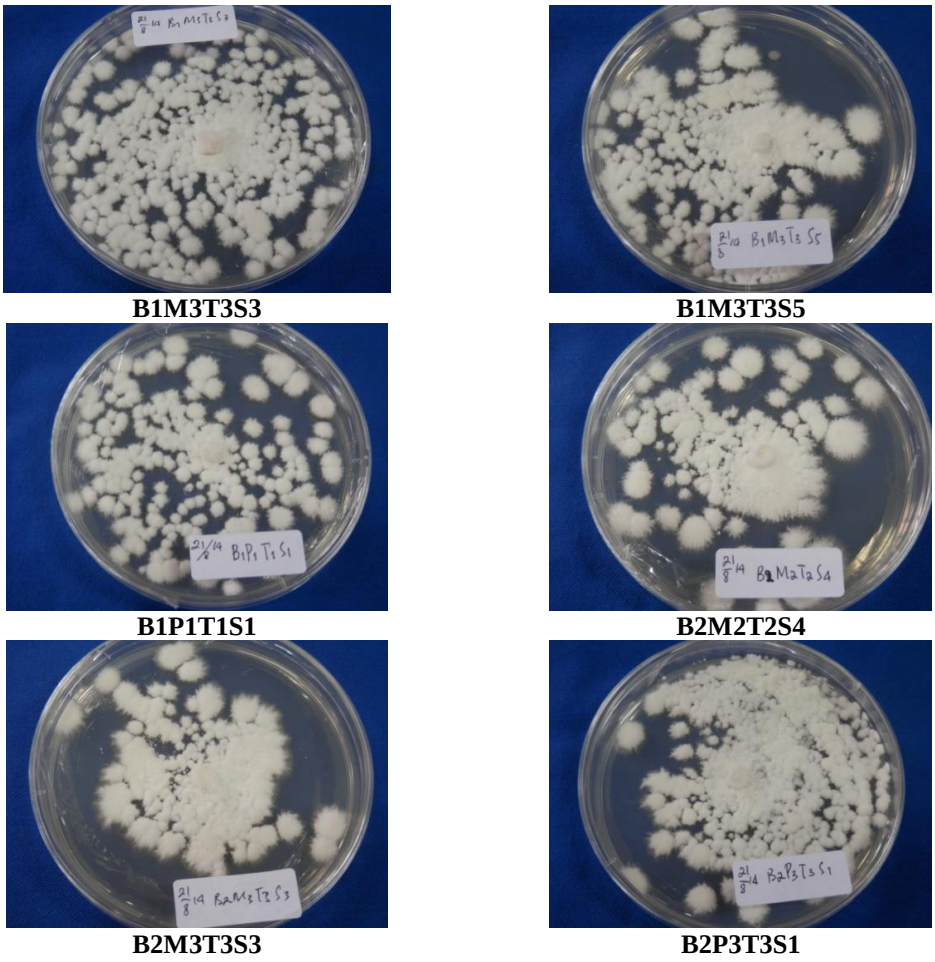


Figure 1. Eight *B. bassiana* isolates growth pattern from peatland on PDAY medium after 7 days of culture

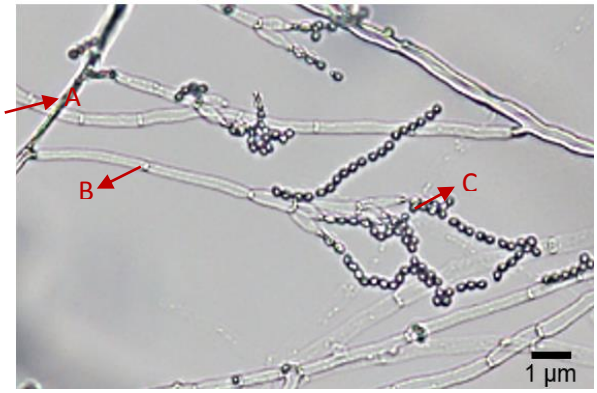


Figure 2. Microscopic *B. bassiana*, A: hyphae, B: conidiophore, C: conidium.

Molecular Identification of *B. bassiana*

Genome of pure isolate was identifed using PCR method with primer specific for identification *B. bassiana*. PCR results show that *B. bassiana* has three DNA bands which appeared on gel with size 800 bp, 550 bp, and 400 bp (Figure 3). Both of *B. bassiana* which isolated from monoculture field and polyculture field were have same size DNA band.

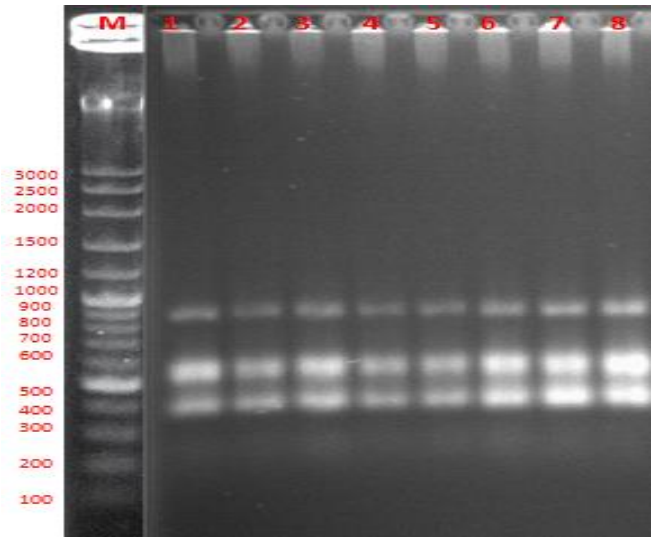


Figure 3. Each isolate has the same bands on 400bp, 550bp, and 800bp (M=marker, 1= B1M3T3S1, 2= B1M3T3S2, 3= B1M3T3S3, 4= B1M3T3S5, 5= B2M2T2S4, 6= B2M3T3S3, 7= B1P1T1S1, 8= B2P3T3S1).

Molecular identification showed positive result that all of eight isolates were *B. bassiana* mold which correspond with earlier study that *B. bassiana* has DNA size about 320-2300 bp[12] and 494-1900[13]. While, other more relevant studied showed that DNA band of *B. bassiana* which separated using agarose gel electrophoresis was about 750bp[14]. Amplification through PCR method of 28S ribosomal DNA also done by Viaud et al[15] show that *B. bassiana* isolates were on 500bp.

All samples have the same base pair showed that all isolates were *B. bassiana*. Molecular analysis confirmed that sample from polyculture and monoculture are *B. bassiana*. mold which can be found in all around the world and has many variations of trader among the other entomopathogen mold. Insect from order Lepidoptera, Coleoptera, Hemiptera, Diptera, and Hymenoptera are the main trader of *B. bassiana*[16].

Strain or isolates was difficult to be distinguished morphologically, they have different genetic information also different physiology activity. Species diversity of *B. bassiana* is determined by their pathogenicity[17]. Varela and Morales[18] showed that the differentiation of virulence level of mold that infected coffee *Hypothenemus hampei* is related to the physiological and biochemistry reaction that happened between *B. bassiana* isolates.

Biological control using *B. bassiana* was not fully succeeded because the availability of mold was low because of its persistent conidia and also the environmental factor which influence the insect mortality. Lacey *et al* [19] stated that the key of success in biological control using entomopathogen mold were depend on the right strain use, formulation, dosage, and time. Application time is depending on the availability of trader, environment condition, and the compatibility of farming technique.

B. bassiana mold can be applied by inundation or being sprayed same as the application of synthetic insecticides. One of problem of low persistence mold application, is physical factor, such as sun radiation. Persistence upgrading efforts of *B. bassiana* is by making the formula as persistence bioinsecticides stocks. According to Suwahyono[20] the formulation using rice and corn can elevate the *B. bassiana* persistence.

Conclusion

Based on morphological and molecular identification of *B. bassiana* were found in Palangkaraya, Central Kalimantan from both in monoculture and polyculture. There are eight isolates of *B. bassiana* that collected from dry and wet peatland. All of *B. bassiana* isolates has three size of band 800bp, 550bp, 400bp. There is no significant differences between *B. bassiana* isolate which isolated from monoculture and

polyculture based on morphological and molecular identification. *B. bassiana* can be potential entomopathogen agent to control *P.xylostella* in Palangkaraya, Central Kalimantan, Indonesia.

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