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Diversity of fungal community associated with cacao (*Theobroma cacao* L.) top clones from Sulawesi, Indonesia

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Abstract. Cocoa (*Theobroma cacao* L.), one of the most significant smallholder commodities in Indonesia, is mainly planted in the Sulawesi island of Indonesia. The new paradigm approach for improving plant health is the use of indigenous microbes which associates with cocoa tree. One of microbial communities is endophytic fungi. The research aimed to identify of fungal endophyte of cocoa top clones Sulawesi. A total of 27 isolates of fungi was isolated from two clones and three areas; 11 isolates from East Luwu area (MCC-02 Clone was six; Sulawesi-2 (S-2) was five), seven isolates from North Luwu area (MCC-02 clone was four; Sulawesi-2 (S-2) was three), Nine isolates from Bantaeng area (MCC-02 was three; Sulawesi-2 (S-2) was six). The isolates belonged to genera *Penicillium* (two isolates), *Aspergillus* (five isolates), *Geotrichum* (two isolates), *Sordaria*, *Lasiodiplodia*, *Myrothecium*, *Monilia*, *Colletotrichum*, dan and five isolates that have not been identified as not showing conidia on media of PDA. The result revealed that fungi associated with cacao was diverse and every clone and cocoa producing area shows different diversity of fungi. The endophytic fungi were potential to be biological control for controlling cacao diseases.

1. Introduction

Cocoa is a cash crop among perennial crops that planted by mostly smallholder farmers in Sulawesi Island, Indonesia. The plant has proved contributes improve the quality of life of farmers through increased income. Furthermore, market demand for this commodity always high, but in the last five years productivity¹³ cocoa tends to decrease, notably cocoa farm belongs to small farmers because of limiting various factors such as nutritional deficiencies, insect pests, and diseases [1, 2]. The environmental way for improving cocoa tree health to counter plant diseases is the utilization of indigenous microbes that associated with the cocoa plant.

Part of trees such as leaves, stems, branches, and roots are associated with various microbes including bacteria, and fungi, which can colonize the plant tissue endophytically. Huge diversity of endophytic microbes can be found in healthy plant tissue [3-6]. Endophyte fungi are one of¹² e antagonist agents which can be used for controlling plant pathogen, both fungi and bacteria. It was able to colonize the plant tissue and able to produce⁵ ycotoxin, enzymes, and antibiotics [5-7]. Moreover, the endophytes fungi can colonize the plant host without causing any disease symptoms except the host in stress condition [5].

Endophytic fungi that associated with cocoa able to reduc⁶ damage caused by plant pathogen through various mode of action to plant. Specifically, they can inhibit pathogen infection and

proliferation with the host directly through antibiosis, competition, and mycoparasite [8, 9] or indirectly through resistance induction response to the host [7,10].

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2. Materials and methods

2.1. Plant materials

Two selected clones were collected from three areas; Wotu District of East Luwu Regency, Masamba District of North Luwu Regency, and Gantarangkeke District of Bantaeng Regency, Indonesia. The clones were classified as interpopulation hybrids [12]: Masamba cocoa clone (MCC-02) is origin from North Luwu of South Sulawesi and selected by local farm selections H. Andi Mulyadi & M. Nasir for high-yielding types, pod colour is red [12]. In addition, Sulawesi 2 (S-2) is origin from Malaysia and selected in the 1980s by screening the progeny of crosses for resistant, high-yielding types, and the old name is BR25. Pod colour is red.

Currently, the clones were considering as recommended clones from the Indonesian government. Also, the clones have been certified for planting widely in Indonesia and the clones produce high yield, MCC-02 resistance to Cocoa Pod Borer (CPB), and *Phytophthora* pod rot (PPR) Disease and S-2 resistance to PPR, moderate resistance to VSD disease, and susceptible to CPB [12].

2.2. Fungal isolation

Isolations were made from healthy branch material. Initially, samples were rinsed with tap water, and then surface sterilised with NaOCl solution (2.5 %) for three minutes and EtOH (70%) for one minute, then sterilized water for three times. Approximately 3–5 mm diameter pieces of plant material were placed on Potato-Dextrose Agar (PDA) and Water Agar (WA) supplemented with Chloramphenicol and incubated at 25 °C in the dark. Pure cultures were obtained by hyphal tip excision from the colony margins on PDA and WA, and subsequent incubation at 25 °C in the dark.

2.3. Morphological, cultural and molecular characterization

Morphology characterization, which is for the examination of conidial characteristics, was done on every emerged fungus according to Barnet and Hunter [13]. Morphological characterization was conducted by taking into consideration several measurements such as texture, edge, and mycelium colour while reproductive structures (spores), type of hyphae and presence of septa were observed for microscopic feature.

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3. Results and discussion

3.1. Results

3.1.1. Associated fungi with cocoa clone of MCC 02 and S2. 27 isolates of endophytic fungi were obtained from MCC02 and S2 Clone originally from three central cocoa producer regencies namely East Luwu, North Luwu, and Bantaeng. In East Luwu, six (6) isolates were obtained from MCC-02 and five (5) isolates from (S-2); in North Luwu, four (4) isolates were obtained from MCC-02 and three (3) isolates S-2; and in Bantaeng, three (3) isolates were obtained from MCC-02 and six (6) isolates from S-2 (table 1-7). The isolates belonged to genera *Penicillium*, *Aspergillus*, *Geotrichum*, *Sordaria*, *Lasiodiplodia*, *Myrothecium*, *Monilia*, and *Colletotrichum* respectively.

Table 1. Endophytic fungal association with clones

Origin	Clone	Isolate	Taxa
EAST LUWU	MCC-02	6	<i>Penicillium</i> sp., <i>Aspergillus</i> spp. (two isolates), <i>Geotrichum</i> sp., <i>Sordaria</i> sp., and Isolate T7.45.LT
	S-2	5	<i>Gliocephalotrichum</i> sp., <i>Aspergillus</i> spp., <i>Penicillium</i> sp., Isolate T4.S2.LT, and Isolate T5.S2.LT
NORTH LUWU	MCC-02	4	<i>Lasiodiplodia</i> sp. (two isolates), <i>Geotrichum</i> sp., and Isolate T2.45.LU
	S-2	3	<i>Lasiodiplodia</i> sp. (two isolates), <i>Penicillium</i> sp.
BANTAENG	MCC-02	3	<i>Myrothecium</i> spp., <i>Geotrichum</i> spp. (two isolates)
	S-2	6	<i>Aspergillus</i> spp. (two isolates) <i>Monilia</i> spp., <i>Colletotrichum</i> sp., <i>Lasiodiplodia</i> sp., and Isolate T6.S2.B

Table 2. Endophytic fungal association with MCC-02 clone from East Luwu


Fungal Isolates	Macroscopic characterization	Microscopic feature
1. <i>Aspergillus</i> spp. 	dark green upward and army green backwards of colony, thin and uneven colony growth but even colony edge	Hypha with septa, hyaline conidiophore, many branches and rounded conidia
2. <i>Aspergillus</i> spp. 	dark green upward and green backward of colony, soft and powdery colony and uneven colony	Hypha with septa, long conidiophore, many branches and ovoid conidia
3. <i>Geotrichum</i> sp. 	white up and backwards of colony, even colony growth, smooth surface and even of colony edge	Hypha with septa, segmented hypha forming conidiophore
4. <i>Sordaria</i> sp. 	dark up and backwards of colony, even colony growth, smooth surface and even of edge of colony	Hypha with septa, dark and hyaline conidiophore, forming ascus
5. <i>Penicillium</i> sp. 	dark green upward and, dark yellow backward of colony, uneven colony growth and uneven colony edge	Septation hypha, long conidiophores and ovoid conidia
6. T7/45/LT 	white, smooth and even mycelium, uneven colony growth and uneven colony edge	Septation hypha

Table 3. Endophytic fungal association with S-2 clone from East Luwu






Fungal Isolates	Macroscopic characterization	Microscopic feature
1. <i>Aspergillus</i> spp. 	Dark upward and greenish white backward of colony, even mycelium spread, rough colony surface and even colony edge	Hyphal septation and branching, upright and prolong conidiophore but branchless, covering whole vesicle mycelium surface and phialide conidium formation. Spherical and hyaline hypha with 1 cell sterigmata.
2. <i>Gliocephalotrichum</i> sp. 	White upward and creamy white backward of colony, even and smooth mycelium, even colony growth and even colony edge	Hyphal septation with tall conidiophore, hyaline and blong-elliptical conidia
3. <i>Penicillium</i> sp. 	dark with green spot of upward and dark backward of colony, irregular formation of mycelium, rough and even colony growth and uneven colony edge	Hyphal septation, conidiophore branching and hyaline and globose conidia
4. T4/S2/LT 	Dark upward and backwards of colony, flat colony and even colony edge	Hyphal septation and fusiform conidia
5. T5/S2/LT 	Dark and white upward and backward colony, flat colony and uneven colony edge	Hyphal septation

Table 4. Endophytic fungal association with MCC-02 clone from North Luwu

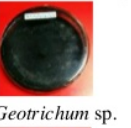



Fungal Isolates	Macroscopic characterization	Microscopic feature
1. <i>Lasiodiplodia</i> sp. 	Dark colony, smooth and even colony growth with even edge	Hyphal septation and obovoid
2. <i>Geotrichum</i> sp. 	White and transparent colony, even and smooth growth of colony and smooth colony surface with even edge	Hyphal septation and hyphal segmentation forming conidia
3. <i>Lasiodiplodia</i> sp. 	Dark colony and uneven colony growth and its edge	Hyphal septation and obovoid
4. 2/45/LU 	The colony is blackish, The colony growth is uneven, and the edge of colony is uneven	Hyphae is septa, conidia ovoid

Table 5. Endophytic fungal association with S-2 clone from North Luwu


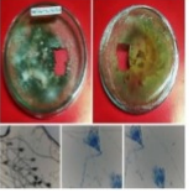

Fungal Isolates	Macroscopic characterization	Microscopic feature
1. <i>Lasiodiplodia</i> sp. 	dark grey colony, smooth and even colony growth with even of its edge	Hyphal septation and obovoid conidia
2. <i>Penicilium</i> sp 	The colony is deep green and white and even of edge	Hyphal septation and hyaline and ramify conidiophore
3. <i>Lasiodiplodia</i> sp. 	Dark and smooth colony with even of its edge	Hyphal septation obovoid colony and two cell and deep brown colony

Table 6. Endophytic fungal association with MCC-02 clone from Bantaeng










Fungal Isolates	Macroscopic characterization	Microscopic feature
1. <i>Myrothecium</i> spp. 	Greenish grey upward of colony, and smooth, thick and even colony growth with even of its edge	Hyphal septation and subhyaline and ovoid conidia
2. <i>Geotrichum</i> sp. 	White upward and backward colony and smooth and even colony growth with even of its edge	Hyphal septation and hyphal segmentation forming conidium
3. <i>Geotrichum</i> sp. 	whitish grey upward and backward colony and smooth and uneven colony growth with uneven of its edge	Hyphal septation and hyphal segmentation forming conidia

Table 7. Endophytic fungal association with S-2 clone from Bantaeng

Fungal Isolates	Macroscopic characterization	Microscopic feature
1. <i>Aspergillus</i> spp. 	Dark green upward and backward colony surface with light green colony edge. Uneven colony growth with uneven its edge	Hyphal septation, mycelium branching, upright and prolong conidiophore but branchless. Covering whole vesicle mycelium surface and forming phialide and conidia formed subsequently in the phialide. Spherical and hyaline colony with 1 cell sterigmata.
2. <i>Aspergillus</i> spp. 	Dark green upward and light green backward colony surface, evenly spread mycelium, rough colony surface with even its edge	Hyphal septation, mycelium branching, upright and prolong conidiophore but branchless. Covering whole vesicle mycelium surface, forming phialide and conidia formed subsequently in the phialide. Spherical and hyaline conidia with 1 cell sterigmata
3. <i>Colletotrichum</i> sp. 	Grey upward and backward colony. Smooth, thick and even colony growth with even its edge of colony	Hyphal septation, oblong conidium with 1-cell and hyaline
4. <i>Siodiplodia</i> spp. 	Deep dark upward and back ward colony, smooth and even growth of colony with even its edge	Hyphal septation
5. <i>Monilia</i> spp. 	Grey colony upward and backward colony surface, smooth and even colony growth with even its edge	Hyphal septation with tan and mass septa
6. T6/S2/B 	White and dark upward colony. Smooth and even colony growth with even its edge	Hyphal septation and globose conidia

12. Discussion

These findings revealed that number of fungi associated with healthy cocoa tissue, and the taxa and number of fungi can be different among the clones even though the clones are planted in the same area or farm. Similarly, number and taxa diversity can be distinguished among the area despite that the cocoa clones are same.

Numerous factors affect the composition of the microbial communities in plant tissue both structure and taxa in the plant and habitat interaction. Previous research revealed that microbes associated with

plant vary spatially [14], microbes associated with plant communities might depend on the interaction among others associated microbial endophyte [11] pathogen [15, 16].

High diversity of fungi associated with cocoa can be related to the level of resistance to cocoa diseases. The fungi may be protected plant from pathogen invasion through direct and indirect resistance, particularly the pathogen which has the endophytic mechanism. However, among the fungi isolates, some of them are reported to cause diseases on cocoa and other plants such as *Lasiodiplodia* and *Colletotrichum*.

The endophytic microorganisms are omnipresent and may enhance the plant health through improving tolerance to physical factors such as heavy metals and drought, and reducing biotic factors like pests and plant pathogen [17], and also assist the plant to grow well [18].

The genera *Aspergillus* and *Penicillium* were isolating in high frequency in East Luwu area both MCC-01 and S-2 Clones. Meanwhile, in North Luwu the genus *Lasiodiplodia* was a dominant group in both clones whereas in Bantaeng area no predominant genus. However, at S-2 clone in Bantaeng area isolated more diverse of fungi than other areas such as *Aspergillus* spp. (two isolates) *Monilia* spp., *Colletotrichum* sp., *Lasiodiplodia* sp.

4. Conclusion

Irrespective of cocoa planting area, several fungi associated with cocoa in different clone and the number and taxa of fungi may different and similar among the clones.

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