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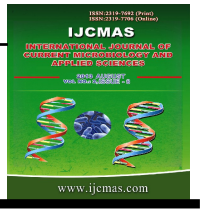

Original Research Article

Diversity of endophytic fungi from root of Maize var. Pulut (waxy corn local variety of South Sulawesi, Indonesia)

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Keywords

Diversity; endophytic fungi; maize var. pulut; waxy corn.

A B S T R A C T

Endophytes are microorganisms that live within plant tissues without causing symptoms of disease. The objective of this investigation was to isolation and identification of fungal endophytes from roots of maize plant var. Pulut (a local variety of south Sulawesi). Sixty three isolates of fungal endophytes were isolated from the root of maize var. Pulut. The isolates belonged to six genera, namely :*Trichoderma* sp., *Fusarium* sp., *Acremonium* sp., *Aspergillus* sp., *Penicillium* sp., and *Botryodiplodia* sp.

Introduction

Waxy corn is a local corn of south sulawesi, and different with several regions in Indonesia. Its consumed as corn on the cob, pie, and roasted corn because it tastes good and savory, taste is due to the content of amylopectin in corn sticky rice of nearly 100%. Although this Waxy corn tastes good and tasty, but very low yield potential of less than 2 tons/ha for small cob size with a diameter a little short 10-12 mm, very sensitive to downy mildew (*Perenosclerospora maydis* L.), but tolerant to drought stress.

Waxy corn is processed in wet milling to produce waxy cornstarch which slowly retrogrades back to the crystalline form of starch. It is grown to make special starches for thickening foods in particularly those that undergo large temperature changes in processing and preparation.

Plants are associated with many different organisms such as bacteria, insects, nematodes, protozoa or fungi (Sieber and Grünig, 2006; Müller and Döring, 2009), which can live endophytically within plant tissues. Endophytic microbes are a very diverse and common group of organisms that can be found in apparently healthy (including functioning but dying off or dead) plant tissue (Saikkonen *et al.*, 1998; Faeth and Fagan, 2002; Sieber, 2002; Vandenkoornhuyse *et al.*, 2002; Piercey *et al.*, 2004; Addy *et al.*, 2005; Porras-Alfaro and Bayman, 2011) and can be located in different plant organs such as leaves, needles, stems or roots (Sokolski *et al.*, 2007; Verma *et al.*, 2007; Grünig *et al.*, 2008b). There are several definitions of the term 'endophyte'. De Bary (1866) was the first to define organisms invading and

residing within healthy host tissue as endophytes. More than a century later, Carroll (1988) defined organisms causing asymptomatic infections within plant tissues as endophytes and excluded pathogenic fungi and special groups of mutualists such as mycorrhizal fungi. Petrini (1991) expanded Carroll`s definition to include all organisms which at certain times in their life inhabit plant organs without causing any harm. Endophytes have co-evolved for a very long period of time with their hosts and therefore usually show low virulence (Sieber, 2007).

The behavior of fungal endophytes can range from mutualistic (Usuki and Narisawa, 2007; White and Torres, 2010) to pathogenic (Tellenbach, 2011; Tellenbach *et al.*, 2011), and endophytes can switch their behavior depending on environmental factors, described as the endophytic continuum (Schulz and Boyle, 2005).

Arnold *et al.,* (2003) could show that fungal leaf endophytes protect *Theobroma cacao* against *Phythophthora* diseases, and similarly Lee e*t al.,* (2009) were able to show that the endophytic *Fusarium verticillioides* reduces disease severity of *Ustilago maydis* on maize.

Endophytes can also be used to make plants more tolerant against abiotic stress like drought or salt, becoming more important nowadays in regards to global warming and to the increasing world population (Saxe *et al.*, 2001; Sherameti *et al.,* 2008; Compant *et al.,* 2010; Redman *et al.,* 2011). The objective of this investigation was to isolation and identification of fungal endophytes from roots of maize plant var. Pulut (a local variety of south Sulawesi).

Materials and Methods

Sample Collection

Healthy plants with roots of maize plant var. Pulut were collected from various places of Takalar Region, Province of South Sulawesi, Indonesia. The samples were collected by aseptic procedures and brought to the laboratory of Plant Protection Department, Faculty of Agriculture, Hasanuddin University, Indonesia and processed within 24 hours of collection.

Isolation of Fungal Endophytes

Endophytic fungi were isolated according the protocols described by Petrini 1986, which were slightly modified based on preliminary tests. The root of maize plant var. Pulut taken from the field were washed twice in distilled water then surface sterilized by immersion for 1 minute in 70% (v/v) ethanol, 5 minutes in sodium hypochlorite (2.5 % (v/v) available chlorine) and 30 seconds in 70% (v/v) ethanol and then washed three times in sterilized distilled water for 1 minute each time. After surface sterilization, the samples were cut into 5 mm pieces and aseptically transferred to plates containing potato dextrose agar (PDA, pH 6.8, containing (g/l): potato 200; dextrose 20; agar 15.), which had been autoclaved for 15 minutes at 121ºC and then aseptically supplemented with 100 mg/ml of chloramphenicol (Pfizer) to suppress bacterial growth. Aliquots from the third wash were plated onto PDA to check that surface sterilization had been effective and they were then incubated at 28ºC. Any fungi present was isolated, purified and then maintained at 4ºC on PDA slopes for further identification. For tentative identification, microscopic slides of each

fungal endophyte were prepared, examined under light microscope (Olympus, USA) and identified with reference to Barnet and Hunter (1998) and Dugan (2006).

Results and Discussion

Sixty-three isolates of endophytic fungi were collected from root of maize plant var.Pulut. All endophytic fungi could be cultivated on artificial media and maintained as a pure culture. They exhibited characteristic colony and microscopic morphology that could be used to differentiate them. All isolates were identified as belonging to 6 genera, namely *Trichoderma* sp. (15 isolates, 23.8%), *Fusarium* sp. (12 isolates, 19.1%), *Aspergillus* sp. (18 isolates, 28.6%), *Penicillium* sp. (6 isolates, 9.5%), *Acremonium* sp. (6 isolates, 9.5%), and *Botryodiplodia* sp. (6 isolates, 9.5%) (Figure 1).

Some results of characterisation of colony and microscopic morphological study are shown in Figures 2 and 3 respectively. *Trichoderma* sp. characterized by green colonies, many branching of conidiophores and konidia formed on conidiophores clustered on the cell surface (Figure 1a,1b). *Trichoderma* sp. have oval conidia, produced from a single or clustered phialaid with approximately 2.9 to 3.2 x 2.4 to 2.8 µm. Hyphae of *Aspergillus* sp. are characterized by rapid growth, green and black, colonies on PDA media diameter up to 9 cm in 5 days, heavy sporulation which formed in the early growth of the solid layer. The beginning of sporulation conidiophores are yellowish-brown, which quickly turned into a greenish-brown. Conidiophores clear stalk, and generally thick-walled and flashy (Figure 2a, 2b). *Fusarium* sp. characterized by very rapid growth of hyphae and orange, dark red clamidospora, which stick to the walls of petridishes if the age of 3 days. Microscopic identification results showed that the crescent-shaped makrokonidia with 3-5 septa and clamidospora is round and slightly oval (Figure 3a, 3b). *Peniccilium* sp. characterized by conidiophores up and emerge from the substrate or from hyphae, septae clear and colorless, branching at the ends, has a head shape and produce spores as peniculate fialid and sometimes bottle-shaped (Figure 4a, 4b). The growth rate of *Acremonium* colonies is moderately rapid, maturing within 5 days. The diameter of the colony is 1-3 cm following incubation at 25°C for 7 days on potato glucose agar. The texture of the colony is compact, flat or folded, and occasionally raised in the center. It is glabrous, velvety, and membrane-like at the beginning. Powdery texture may also be observed. By aging, the surface of the colony may become cottony due to the overgrowth of loose hyphae. The color of the colony is white, pale grey or pale pink on the surface. The reverse side is either uncolored or a pink to rose colored pigment production is observed. *Acremonium* spp. possess hyaline, septate hyphae which are typically very fine and narrow. Vegetative hyphae often form hyphal ropes.

Unbranched, solitary, erect phialides are formed directly on the hyphal tips, the hyphal ropes, or both. The phialides are separated from hyphae by a septum and taper towards their apices. At the apices of the phialides are the hyaline conidia 2-3x4-8µm in size. They usually appear in clusters, in balls or rarely as fragile chains. The conidia are bound by a gelatinous material. They may be single or multicellular, fusiform with a slight curve or resemble a shallow crescent.

Figure.1 Number of Isolates of Fungal endophytes

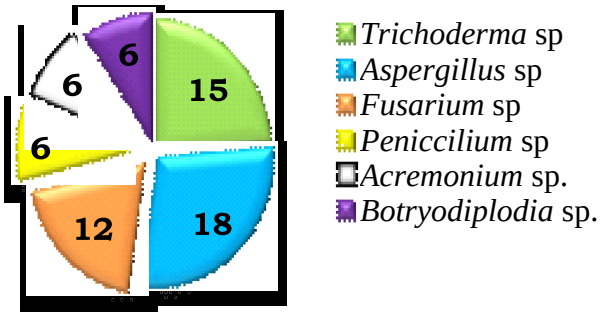


Figure.2 Colour Variation Among Colonies on PDA Medium Plates at 5 days: (1a) *Trichoderma* sp (2a) *Aspergillus* sp.; (3a) *Fusarium* sp.; (4a) *Penicillium* sp.; (5a) *Acremonium* sp.; (6a) *Botryodiplodia* sp.

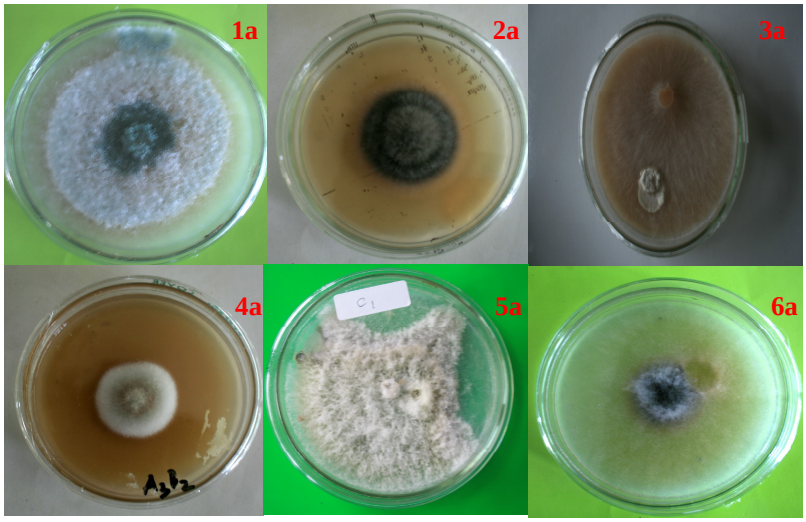
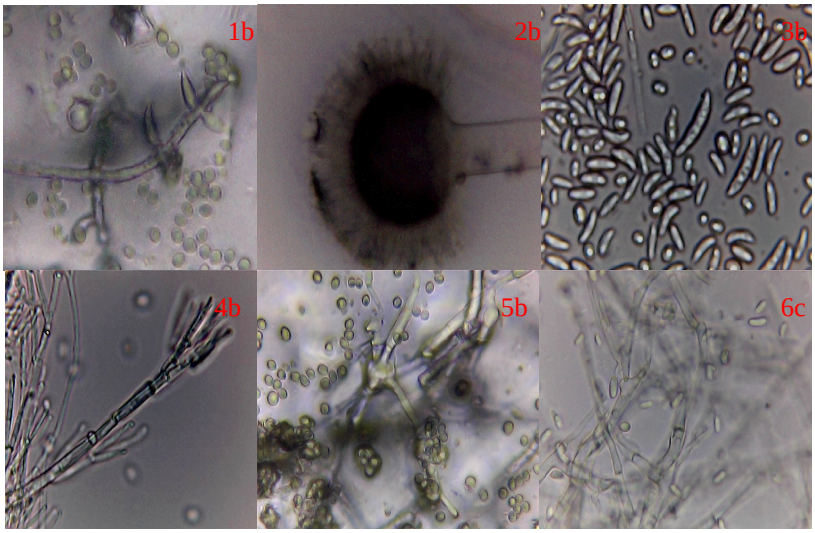


Figure.3 Light Microscopic Observation of Fungi: (1b) *Trichoderma* sp.; (2b) *Aspergillus* sp.; (3b) *Fusarium* sp.; (4b) *Penicillium* sp.; (5b) *Acremonium* sp.; (6b) *Botryodiplodia* sp.



Botryodiplodia sp., also in culture medium of PDA the fungus formed dark brown to black pycindia. They were globosely, ostiolate, singular and scattered, which extruded conidia through the ostiole. Data also show that the endophytic isolate *Botryodiplodia* sp. produced conidia. Young conidia were hyaline and one celled (20-30 μ length and 10-18 μ width). Mature conidia were dark and have rounded ends and central septum with approximately similar length (20 to 26 μ) and width (10 to 14 μ) (Figure 6a, 6b).

In this study six genera of endophytic fungi were isolated from maize roots, namely: *Fusarium*, *Trichoderma*, *Acremonium*, *Aspergillus*, *Penicillium*, and *Botryodiplodia*. In previous studies various endophytic fungi had been isolated from different plant hosts. Ten genera of endophytic fungi were isolated from root system of palm trees (Nur Amin, *et al* 2008). Three of those genera, *Trichoderma* sp.; *Aspergillus* sp and *Botryodiplodia* sp. were also found in the current study. *Fusarium* sp. had also been isolated from root systems of tomato and banana (Hallmann, 1994; Nur Amin, 1994). *Acremonium* sp. was isolated from tomato and rye grass roots (Bargmann and Schonbeck, 1992; Clay, 1986; Schuls and Boyle, 2005). While *Beaveria bassiana*, *Trichoderma koningii*, *Alternaria alternata*, *Phoma* sp., *Acremonium strictum* were isolated from maize roots (Orole and Adejumo, 2009).

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