

# Identification and Numerical Classification of Keji Beling Leaf Endophytic Fungus (*Strobilanthes crispus* Bl.)

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**Abstract.** This study aims to determine the types of endophytic fungi found on the leaves of vile shard (*Strobilanthes crispus* Bl.). This study used a qualitative descriptive method in the form of exploration by isolating and identifying endophytic fungi from the leaves of the keji beling. The identification process was carried out manually by observing and characterizing the macroscopic and microscopic morphological characteristics of each endophytic fungus isolate. Then the data were analyzed using the Simple Matching Coefficient ( $S_{sm}$ ) in the Multi Variate Statistical Package (MVSP) program, to see the similarity index where each isolate that has a similarity index of  $\geq 70\%$  are grouped into one cluster. Isolation of endophytic fungi on the leaves of the vile shard plant resulted in 24 isolates divided into 4 genera, namely the genera *Alternaria*, *Penicillium*, *Absidia*, and *Aspergillus*.

**Keywords:** Endophytic fungi, identification, keji beling, numerical taxonomy

## 1 Introduction

Endophytic fungi are fungi that live in colonies in plant tissues. In one plant can be found more than one type of endophytic fungi [1]. Endophytic fungi from different plant organs from one host plant may also contain different types of isolates [2]. The presence of endophytic fungi is related to the conditions of the host plant microhabitat and the genotypic match between the host plant and the endophytes. Therefore, it will affect the differences in the composition of the endophytic colonies and the infection rate of the host plants occupied by endophytic fungi at the same location [3].

Endophytic fungi colonize every part of the plant organs, especially the leaves. Several studies have shown that older leaves support more endophytic fungi than relatively young leaves [4],

[5]. Endophytic fungi on old jamblang leaves (*Shyzigium cumini* L.) are more varied than semi-old and young leaves [6]. The number of genera and species of endophytic fungi found in the leaves of old teak (*Tectona grandis* L.) and trembesi (*Samanea saman* Merr.) It is larger with a higher colonization frequency when compared to young leaves [7].

Endophytic fungi can produce various bioactive compounds that act as antibiotics, antivirals, antimicrobials, anticancer, antioxidants, insecticides, antidiabetics, and immunosuppressants. [8]. Utilization of endophytic fungi can also be used as a biological control agent for pests and insect pathogens that cause plant diseases [9]. Based on the description above, the researchers were interested in examining the presence of endophytic fungi isolated from old leaves, half old leaves, and young vile shard leaves. This study aims to isolate the endophytic fungi found on the leaves of keji beling shard and to identify these endophytic fungi based on their phenetic characters.

## **2 Material and methods**

### **2.1 sample Surface Sterilization and Endophytic Fungi Isolation**

The leaf samples of the vile shard that had been collected were washed under running water for  $\pm 5$  minutes and dried, then cut into 1 x 1 cm sizes using a sterile knife. Then the sample was surface sterilized by immersion in 70% alcohol for 1 minute, then immersed in hypochlorite solution (NaOCl) for 5 minutes, then immersed again in 70% alcohol for 30 seconds and finally rinsed with sterile distilled water flowing for 3-5 seconds [10]. The sterilized sample is dried on filter paper. Then the samples were planted in PDA media and incubated at room temperature for 5-10 days. Furthermore, the endophytic fungal isolates were observed for the colony morphology.

### **2.2 Characterization and Identification of Endophytic Fungi**

Identification of endophytic fungi is carried out by macroscopic and microscopic observation referring to the book Introduction to General Tropical Molds [11]. Macroscopic observations of colonies include: color, texture, edge shape, diameter, reverse color of the colony, topography, surface, height (mm), radial lines from the center of the colony on the edge of the colony and concentric circles. Microscopic observation is carried out by taking a pure culture aseptically using a loop needle and placing it on the surface of a glass object and then staining it with lactophenol cotton blue to help observe the microscopic characteristics. Then the preparation was covered with a cover glass and observed under a microscope.

### **2.3 Data analysis**

The characteristics of fungal isolate obtained from the leaves of the keji beling a plant was analyzed using the Simple Matching Coefficient in the Multi Variate Statistical Package (MVSP) 3.22 program to see the similarity index.

### **3 Results and discussion**

#### **3.1 Endophytic Fungal Isolate**

Isolation of endophytic fungi on keji beling leaves, which were incubated for 5-10 days at room temperature showed that there was fungal growth from the leaf surface. Endophytic fungi grow on the fifth day and have dark green, white, brown, gray and blackish brown colonies. In young leaves, 6 isolates were found. 7 isolates in semi-old leaves, and 12 isolates in older leaves.

#### **3.2 Identification of Strains**

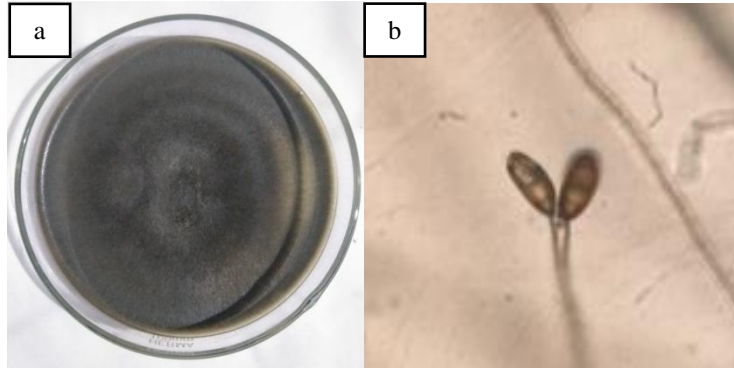
Based on the results of observations of the keji beling a leaf endophytic fungus by looking at the macroscopic and microscopic characteristics by referring to the classification manual according to [12], [11].

##### **3.2.1 Genus *Alternaria***

Macroscopically, EDT 11 and EDS 6 isolates to have blackish green colonies. The color of the reverse of the colonies is dark green to brownish. The diameter of the colonies is 8.3 – 9.1 mm in 5 days, the edges of the colonies are like threads. The shape of the colonies is circular, the texture of the colonies like velvet. The topography of the colony is wrinkled and wrinkled, and has concentric circles.

Microscopically, isolates EDT 11 and EDS 6 have septate hyphae, hyaline in color, spiral shaped and have smooth walls. Asexual spore production, conidia, elliptical in shape and produced singly. The conidiophores are branched, smooth-walled, brown, and septate. Conidia are elliptical, brown, single-celled, branched and septate, and have stolon.

Based on the macroscopic and microscopic characteristics of isolates EDT 11 and EDS 6, the two isolates to belong to the genus *Alternaria*. The *Alternaria* genus found in this study is presented in **Figure 1**. below.



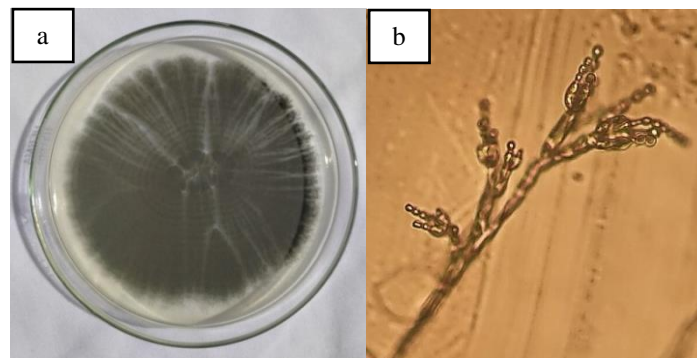
**Fig. 1.** (a) *Alternaria* colonies and (b) their microscopic morphology

### 3.2.2 Genus *Penicillium*

Macroscopically, isolates EDT 2, EDT 6, EDS 4, EDT 3, EDT 9, EDM 4, EDS 7, EDT 4, and EDM 3 had been green with brownish colonies, whereas the colonies were dark green to brownish, colony diameter 3, 6 – 9.1 in 5 days, the edges of the colony are like threads. The shape of the colony is circular to circular. The texture of the colony is like flour. The topography of the colony is tangled and wrinkled, and does not have concentric circles.

Microscopically, isolates EDT 2, EDT 6, EDS 4, EDT 3, EDT 9, EDM 4, EDS 7, EDT 4, and EDM 3 had septate hyphae, hyaline in color, spiral shaped and smooth walls. It produces special asexual spores, conidia type, round in shape and produced in clusters. Conidiophores are branched, have several metulae, are smooth-walled and hyaline. Conidia are semispherical to round, hyaline, and have smooth walls. Has stolon and rhizoid.

Based on the macroscopic and microscopic characteristics of isolates EDT 2, EDT 6, EDS 4, EDT 3, EDT 9, EDM 4, EDS 7, EDT 4, and EDM 3, these isolate belong to the *Penicillium* genus. The genus *Penicillium* found in this study is presented in **Figure 2** below.



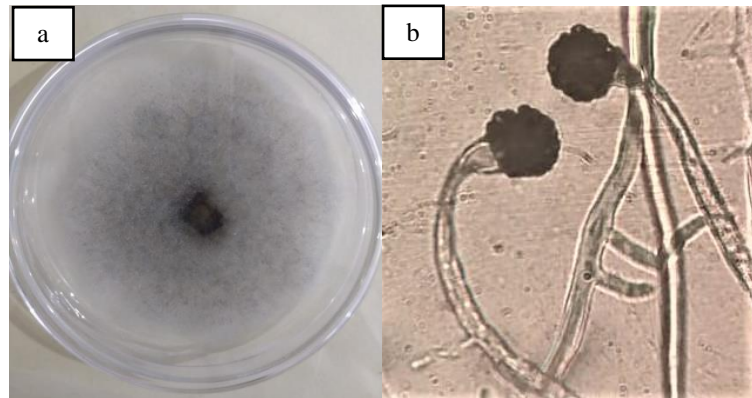
**Fig. 2.** (a) *Penicillium* colonies and (b) their microscopic morphology

### 3.2.3 Genus *Absidia*

Macroscopically, isolate EDT 10 has light gray colonies with thread-like edges. The reverse color of the colonies is gray. The diameter of the colonies is 8.2 cm. The colony shape is circular. The texture of the colonies is like cotton. The topography of the colonies is wrinkled and wrinkled and does not there are concentric circles.

Microscopically, isolate EDT 10 has hyphae that are not septate, hyaline in color, spiral shaped and have smooth walls. It produces special asexual spores. The conidia are round in shape and produced in clusters. Microscopically, isolate EDT 10 has hyphae that are not septate, hyaline in color, spiral shaped and have smooth walls. It produces special asexual spores. The conidia are round in shape and produced in clusters.

Based on the macroscopic and microscopic characteristics of the EDT 10 isolate, this isolates belongs to the genus *Absidia*. The *Absidia* genus found in this study is presented in **Figure 3** below.



**Fig. 3.** (a) *Absidia* colonies and (b) their microscopic morphology

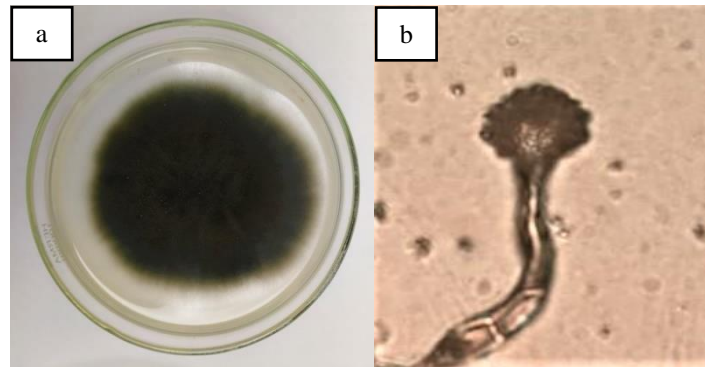
### 3.2.4 Genus *Aspergillus*

Macroscopically, isolates EDT 8, EDT 7, EDT 5, EDS 2, EDS 1, EDS 5, EDT 1, EDS 3, EDM 5, EDM 6, EDM 2, and EDM 1 had been green with brownish colonies, whereas colored colony's dark green to brownish, colony diameter 4.3 – 8.7 in 5 days, circular colony shape, colony texture like velvet, colony topography wrinkled and wrinkled and there are no concentric circles.

Microscopically, the *Aspergillus* genus have septate hyphae, hyaline in color, spiral in shape and has smooth walls. It produces special asexual spores, conidia type, round in shape. The sporangium is around, brown and found at the tip. Conidiophores are simple (unbranched), rough-walled, hyaline and single. The columella at the tip of the conidiophore are large and round. Conidia are around, brownish green, and smooth. Has hyaline colored stolon and does not have rhizoid.

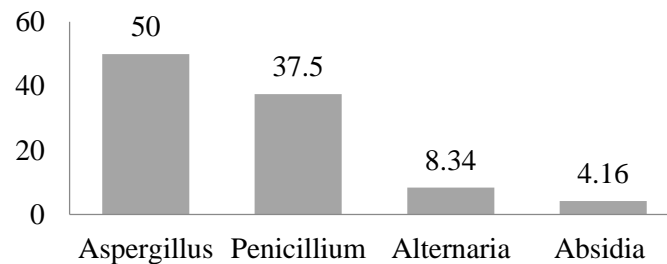
Based on the macroscopic and microscopic characteristics possessed by isolates EDT 8, EDT 7, EDT 5, EDS 2, EDS 1, EDS 5, EDT 1, EDS 3, EDM 5, EDM 6, EDM 2 and EDM 1, these

isolate belong to the genus *Aspergillus*. The genus *Aspergillus* found in this study is presented in **Figure 4** below.



**Fig. 4.** (a) *Aspergillus* colonies and (b) their microscopic morphology

Based on **Figure 5**, it can be seen that the genus *Aspergillus* has the highest frequency of presence, namely 50%, then the genus *Penicillium* with a frequency of presence of 37.5%, the genus *Alternaria* with a frequency of presence of 8.34% and the genus *Absidia* with a frequency of presence of 4.16%. *Aspergillus* is genus of fungus that is a cosmopolitan because it can be easily found, such as in soil, plant remains and in the air [14].



**Fig. 5.** Bar diagram of types of endophytic fungi on the leaves of the keji beling (*Strobilanthes crispus* Bl.)

The types of endophytic fungi on old leaves are higher than on semi-old and old leaves. On young leaves, there are two genera of endophytic fungi, namely the *Aspergillus* and *Penicillium* genera. *Penicillium* can be found widely in various substrates and is also often referred to as a cosmopolitan mold. On semi-old leaves, there are three genera of endophytic fungi, namely the genera *Aspergillus*, *Penicillium*, and *Alternaria*. Meanwhile, in old leaves there are four genera of endophytic fungi, namely the genera *Aspergillus*, *Penicillium*, *Alternaria*, and *Absidia*. Characteristics of the isolate of the keji beling a leaf endophytic fungus (*Strobilanthes crispus* Bl.) **Tabel 1.**

**Table 1.** Characteristics of the isolate of the keji beling a leaf endophytic fungus (*Strobilanthes crispus* Bl.)

Code Isolate	Colony Color	Colony Reverse Color	Colony Form	Edge Colony	Circle Cocentric	Margins Colony
EDM 1	green old	blackish green	circular	velvet	no	flat
EDM 2	green old	blackish brown	circular	velvet	no	flat
EDM 3	green old	blackish dark green	irregular	rough	no	jagged
EDM 4	green old	yellowish green	circular	like flour	no	wavy
EDM 5	green old	blackish brown	circular	velvet	no	like thread
EDM 6	brownish green	blackish green	circular	like woolen cloth	yes	like thread
EDS 1	soft brown	brown	circular	velvet	yes	flat
EDS 2	soft brown	brown	circular	fine	yes	flat
EDS 3	soft brown	brown old	circular	velvet	no	like thread
EDS 4	grayish brown	blackish brown	circular	like woolen cloth	ye	like thread
EDS 5	green old	blackish green	circular	fine	no	like thread
EDS 6	green old	blackish green	circular	velvet	no	Like thread
EDS 7	green old	blackish green	irregular	rough	no	Jagged
EDT 1	brown has white spots	blackish brown	circular	Like woolen cloth	yes	Like thread
EDT 2	brown	brown old	circular	velvet	no	like thread
EDT 3	green old	blackish green	circular	like woolen cloth	no	like thread
EDT 4	green old	blackish green	irregular	rough	no	jagged
EDT 5	green old	blackish dark green	circular	fine	no	like thread
EDT 6	green old with gray spots	blackish dark green	circular	like woolen cloth	yes	like thread
EDT 7	green old	blackish green	circular	fine	no	like thread
EDT 8	green extinct	green extinct	circular	like flour	no	flat
EDT 9	green young	light yellowish green	circular	like flour	no	flat
EDT 10	gray	gray	circular	like cotton	no	like thread
EDT 11	green old	blackish dark green	circular	velvet	yes	like thread
EDM 3	green old	blackish dark green	irregular	rough	no	jagged
EDM 4	green young	yellowish green	circular	like flour	no	wavy

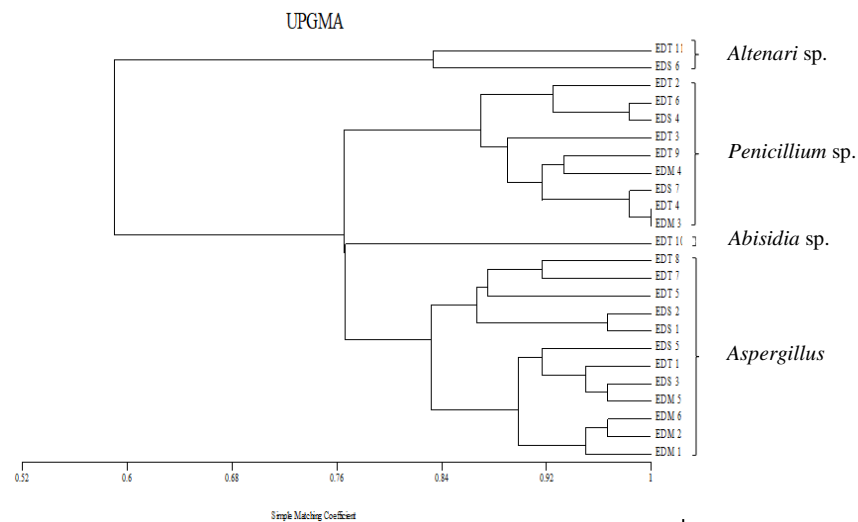
### 3.3 Dendrogram Construction

Based on Figure 6 above, it can be seen that 4 genera of endophytic fungi were obtained from the 24 isolates of endophytic fungi that were successfully isolated from the leaves of the keji beling plant. The four genera includes *Alternaria*, *Penicillium*, *Absidia*, and *Aspergillus*. Endophytic fungi that have the same morphological characters are grouped into one genus using Simple Matching Coefficient (Ssm) analysis in the Multi Variate Statistical Package (MVSP) 3.22 software program. Based on the taxospecies concept, every fungal isolate that has a similarity index of  $\geq 70\%$  can be classified into one genus [13].

It isolates EDT 11, and EDT 6 was grouped in one genus because the two isolates had a similarity index based on Ssm analysis, namely 83.3% and based on their macroscopic and microscopic morphological characteristics, both isolate belonged to the genus *Alternaria* **Figure 6.**

The isolates EDT 2, EDT 6, EDS 4, EDT 3, EDT 9, EDM 4, EDS 7, EDT 4, and EDM 3 were grouped into one genus because the nine isolates had a similarity index based on Ssm analysis of 86.9% and based on characteristics. The macroscopic and microscopic morphology of the nine isolates belongs to the genus *Penicillium*. Isolate EDT 10 has a similarity index based on Ssm analysis, namely 76.7%. It isolates EDT 8, EDT 7, EDT 5, EDS 2, EDS 1, EDS 5, EDT 1, EDS 3, EDM 5, EDM 6, EDM 2, and EDM 1 were grouped in one genus cluster because the twelve isolates had a similarity index based on Ssm analysis was 83.2% and based on macroscopic and microscopic morphological characteristics, the twelve isolates belonged to the *Aspergillus* genus.

Based on the macroscopic and microscopic morphological characteristics, the isolate belongs to the genus *Absidia*.



**Fig. 6.** Dendrogram of endophytic fungal isolates from the leaves of the keji beling plant (*Strobilanthes crispus* Bl.) using simple matching coefficient analysis



## 4 Conclusion

Based on the research that has been carried out, it can be concluded that: Isolation of endophytic fungi on the leaves of the keji beling plant found 24 isolates of endophytic fungi. The types of endophytic fungi found on the leaves of the keji beling plant are the genera *Alternaria*, *Penicilium*, *Absidia*, and *Aspergillus*. Types of endophyl endophytic fungus on old leaves are higher in number than on semi-old and young leaves.

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