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Isolation of endophytic fungi from *Hiyung* chili peppers of local South Kalimantan (Indonesia) varieties and in vitro tolerance to acidic environment

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Abstract. *Imaningsih W, Ekowati N, Salamiah, Ratnaningtyas NI, Soesanto L. 2023. Isolation of endophytic fungi from* Hiyung *chili peppers of local South Kalimantan (Indonesia) varieties and in vitro tolerance to acidic environment. Biodiversitas 24: 3844-3852. Hiyung* chili (*Capsicum frutescens* L) is a local chili from Hyung Village, Tapin District, South Kalimantan Province, Indonesia which has the advantage of being cultivated with an agricultural system in swampland, with high production and is known to have high capsaicin also. This advantage is thought to be related to its symbiosis with endophytic microbes, especially fungi. The purpose of this study was to isolate and identify non-pathogenic endophytic fungi from *Hiyung* chili plants and determine their ability to tolerate acidic conditions in vitro. Results showed that a total of 41 endophytic isolates were isolated from different parts of chili plant. Of the 41 isolates four non-pathogenic endophytic fungi were isolated from leaf tissue. Based on morphological and molecular identification these four fungi were *Pseudozyma hubeiensis* DM3.1, *Phoma* sp. DT2.2P1, *Letendraea* sp. DM1.2 and *Phoma* sp. DT2.2P4. Four endophytic fungi showed different abilities in an in vitro preliminary study of acidic environmental stress tolerance. Only *P. hubeiensis* DM3.1 isolate had the daily diameter of colony not significantly different based on the Anova test (α :0.05, F: 1.092, P:0.372) both at pH 5, 4 and 3 and without pH treatment.

Keywords: Acid stress, endophytic fungi, *Hiyung* chili local variety

INTRODUCTION

Hiyung cayenne pepper is one of the peppers (*Capsicum frutescens* L.) grown by farmers in South Kalimantan (Wahdah et al. 2016; Hamdani et al. 2017). These chilies are cultivated in the swampland of Hiyung Village, Tapin District and have been registered as a protected variety and also designated as a national variety by the Ministry of Agriculture of the Republic of Indonesia (Pramudyani et al. 2019) and have received a certificate of geographical indication by Ministry of Law and Human Right Republic of Indonesia at 2019 (Rochman and Tarmizi 2020). The superiority of cayenne pepper is listed in the attachment to the Decree of the Minister of Agriculture no. 031/Kpts/SR.120/D.2.7/4/2016 namely high production (6 to 7 tons per hectare) with a high number of fruits per plant (1820-2180), with a very spicy taste (capsaicin: 2333.05 ppm) and long shelf life (10-16 days at room temperature) (Hamdani et al. 2017).

The tolerance of these chilies to grow in swamplands is high, its production is also better when compared to those grown in the dry land. The swampland where *Hiyung* chili is cultivated is acidic, with a pH of 5 (Pramudyani et al. 2019). The ability of plants to adapt to the environment may be related to their relationship with endophytic microbes (Giauque et al. 2019).

Some microbes, both bacterial and fungal, are known to live in plant tissues as endophytes. Endophytes live internally, are associated in healthy plant tissues and their presence does not interfere with plant growth (Rajamanikyam et al. 2017) and does not cause disease symptoms (Mishra et al. 2014). Most endophytic fungi also live as saprophytes in the environment around plants (Wenndt et al. 2021). Fungi are more commonly found in environments with acidic conditions, thus endophytic fungi are easy to find on plants living in acidic swamps because they can adapt well as help plants to survive with these conditions (Vylkova 2017).

Several endophytic fungi have been isolated from *Hiyung* chili. *Trichoderma* sp. ACH1.1, *Trichoderma* sp. ACH1.6, *Trichoderma* sp. ACH2.2, *Botrytis* sp. ACH2.3, *Gliocladium* sp. ACH2.4, *Harmoniella* sp. ACH2.5, *Humicola* sp. ACH2.6, *Cunninghamella* sp. ACH2.7 is entirely derived from the root (Imaningsih et al. 2021) and *Trichoderma* sp. DN3, *Trichoderma* sp. AK2), *Trichoderma* spp. BT1 derived from the root, leaves, and stems of *Hiyung* chili (Budi and Mariana 2016). These endophytic fungi are known to be able to inhibit the growth

of pathogenic fungi. However, they have not been reported to have acid tolerance.

All endophytic fungi isolated from *Hiyung* chili plants have been identified through morphological characters only, however, molecular identification has never been reported. Information on endophytic fungi from *Hiyung* chili to the species level is still challenging to obtain. Molecular identification of endophytic fungi more accurately explains up to the species level. The latest use of DNA barcodes for fungi is to use the Internal Transcribed Spacer (ITS) region of nuclear DNA (rDNA), this region is most often used for identification of fungi at the species to subspecies level (Fajarningsih 2016). Molecular identification of *Hiyung* chili endophytic fungi needs to be done to analyze the ability of endophytic fungi species isolated from these plants.

Information on the ability of endophytic fungi from chili plants to tolerate acidic environments is still not much done. Even acid-tolerant endophytic fungi derived from *Hiyung* chili have never been reported. The purpose of this study was to isolate and identify non-pathogenic endophytic fungi from *Hiyung* chili plants and determine their ability to tolerate acidic conditions in vitro.

MATERIALS AND METHODS

Sampling, isolation and purification of endophytic fungi

The research was conducted in the laboratory of the Faculty of Mathematics and Natural Sciences, University of Lambung Mangkurat. Healthy samples of *Hiyung* chili pepper were taken from Hiyung Village, Tapin Tengah Sub-district, Tapin District, South Kalimantan, Indonesia from the agricultural land of Karya Baru Farmers Group. The selected chili plants were healthy, without symptoms of disease, about 40 days after planting. Isolation and purification of endophytic fungi were carried out using the direct planting method (Imaningsih et al. 2021) with modification of the seed soaking time. Roots, stems, leaves, fruits, and seeds were washed with running water and cut into 10 mm pieces. These pieces were soaked in sterile distilled water and then soaked in 0.05% NaClO solution and again in sterile distilled water, each for 1 minutes. The pieces of tissues were placed on Potato Dextrose Agar (PDA) medium with 1% chloramphenicol (Erlamycetin) (with a volume of 1 mL in 100 mL medium) (Black 2020), and incubated at room temperature for 3-5 days. fungal colony growth was observed daily for one week. The success of surface sterilization was proven by the absence of growth of microorganisms in the last rinse of distilled water which was dropped on PDA medium. The grown colonies were then purified.

Germination test of *Hiyung* chili seeds infected by endophytic fungi

The pathogenicity test was carried out based on the method (Soesanto et al. 2020). Endophytic fungal isolates were inoculated in PDA medium, incubated for 3 days at a temperature range (28-30°C), while at the same time chili seeds that had been surface sterilized were soaked in sterile distilled water for 1x24 hours, and incubated for 2 days, after the incubation period, 10 seeds were placed in a Petri dish containing endophytic fungi. The control treatment was by placing sterile chili seeds on PDA medium without endophytic fungi, each isolate was subjected to the same treatment with 3 repetitions. Parameters observed were seed germination rates (normal: Grows well, according to seed germination without endophytic fungi as control; abnormal: Grows, but not as well as control; and not growing). The pathogenicity of isolates was observed by their effect on the germination of chili seeds. The formula used to calculate the germination percentage by dividing the number of germinated seeds by the total seeds multiplied by 100 percent. The data were then statistically analyzed using one-way Anova, α: 0.05, with the program SPSS Ver. 24.

Microscopic confirmation of endophytic fungal hyphae infection

Endophytic fungal hyphae infection was carried out by direct observation under a microscope. *Hiyung* chili sprouts from the previous test were transferred on large petri dishes (ø: 15-20 cm) and grown until the first leaves appeared. The sample of radicle and leaves candidates of *Hiyung* chili sprouts, were taken and then observed under a microscope. Staining was done if the tissue was difficult to observe. Tissues were washed in running water, then immersed in 5.25% NaClO solution (5 minutes) and rinsed with distilled water, then immersed in 1% KOH solution (30 minutes) and rinsed again using distilled water. Root staining was carried out by soaking in a solution of 0.5% CH_3COOH: ink (Parker Quink with SOLV-X ®) with a ratio of 1:50 v/v for ± 30 minutes and then rinsed with distilled water. The stained tissue was then placed on a slide and observed under a binocular microscope (Olympus) with an OptiLab Advance camera (Rosas-Moreno et al. 2023). Hyphal infections in root and leaf tissues were observed, among others, whether they were intracellular or intercellular (Terna et al. 2022).

Macroscopic and microscopic identification of endophytic fungi

Endophytic fungal isolates were identified on the basis of macroscopic (color, shape and diameter of the colonies) and microscopic observation (shape and size of conidia, conidiophore, mycelium, pycnidium). Microscopic observation was carried out using the slide culture (Rosana et al. 2014) by placing a small piece of ± 1 cm PDA medium on a glass object placed on top of the U rod in a sterile Petri dish containing moistened filter paper with sterile distilled water, and put 1 ose of fungal mycelium on the top of PDA medium and then covered with a cover glass. Observations under the microscope (binocular (Olympus) with the OptiLab Advance camera) were carried out after incubation for 24-72 hours. Identification of isolates was based on the identification books Illustrated Genera of Imperfect Fungi (K et al. 1972) and A higher-level phylogenetic classification of the Fungi (Hibbett et al. 2007) and other relevant books such as Pictorial atlas of soil and seed fungi (Watanabe 2002).

Molecular identification of endophytic fungi

Molecular identification of fungi was carried out on selected isolates. DNA extraction was performed using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005). DNA concentration was measured using NanoDrop technique. Extracted endophytic fungal DNA was amplified using PCR machine with forward ITS1 (5'–TCCGTAGGTGAACCTGCGG–3') and reverse ITS4 (5'–TCCTCCGCTTAT TGATATGC–3') (Fajarningsih 2016). PCR amplification using (2x) MyTaq HS Red Mix (Bioline, BIO-25048). The PCR master mix consisted of ddH_2O 9.5x 25 µL), MyTaq HS Red Mix, 2x 12.5x25 µL, 10 µM ITS1 Primer 25 µL, 10 µM ITS4 Primer 25 µL, DNA template 25 µL. PCR conditions were carried out with the initial denaturation step at 95°C for 3 minutes for 1 cycle, denaturation (95°C, 15 seconds, 35 cycles), annealing (52°C, 30 seconds, 35 cycles), extension (72°C, 45 seconds, 35 cycles). ITS rDNA PCR results were sequenced by bi-directional sequencing. The sequencing sequences were then analyzed for homology with reference sequences available at GenBank using the Basic Local Alignment Search Tool (BLAST) at http://blast.ncbi.nlm.nih.gov. The phylogenetic tree was constructed using the MEGA-XI program.

In vitro test of ability of acid-tolerant endophytic fungi

Acid tolerant endophytic fungi were obtained by inoculating fungi from previous tests into PDA medium with the addition of citric acid monohydrate (C6H10O8), (BM: 210, 14 g/mol) with a molarity of 1 M until the pH reached 3, 4 and 5 (Gao et al. 2021). The isolates were incubated at room temperature 28-29°C for 3 to 7 days. The diameter of endophytic fungi was measured daily.

RESULTS AND DISCUSSION

Endophytic fungi from *Hiyung* chili plant origin

A total of 41 isolates of endophytic fungi have been successfully isolated from various parts of the *Hiyung* chili plant. The highest number of endophytic fungi were isolated from old leaf tissue, followed by old stem tissue and medium leaf tissue (Figure 1). This is different from the report by Paul et al. (2012) that endophytic fungi are mostly derived from roots in chili plants from South Korea. This is likely due to differences in host varieties and environment. In accordance with the statement Christian et al. (2016) that endophytic fungi communities are strongly influenced by the characteristics of host plant and the environment in which host grows. The large number of endophytic fungi on leaves in this study may be due to greater dispersal of fungi through the air compared to soil-borne fungi. According to Imaningsih et al. (2023), *Hiyung* chili is grown in swampland farming, where there are various abiotic limiting factors, such as water content and soil acidity. The distribution of *Hiyung* chili endophytic fungi in the soil may be more difficult because the presence of acidic environment.

Germination of chili seeds infected with endophytic fungi

A total of 41 isolates were isolated using the surface sterilization method. The pathogenicity of all isolates was confirmed through Koch's postulates. The percentage of germination from the pathogenicity test results is shown in Table 1.

Based on the pathogenicity test results, it can be seen that the average percentage of normal germination was above 50% in 4 isolates, above 10% in 14 isolates and below 10% in the remaining (22 isolates), among them 14 isolates were pathogenic as they inhibited the germination of *Hiyung* chili seeds. The 4 best endophytic fungal isolates that showed the highest germination percentage were DM3.1, DT2.2P1H, DM1.2, DT2.2P4. Four isolates were of leaf origin with normal germination percentages of 90%, 66.67%, 56.56% and 50% (Table 1). These result are not better than the results of previous research on endophytic fungi from *Hiyung* chili, namely *Harmoniella* sp ACH2.5, *Humicola* sp. ACH2.6, *Cunninghamella* sp. ACH2.7 whose germination percentages ranged from 73 to 90% (Imaningsih et al. 2021). In the present study all the four isolates obtained from leaves. These results Budi and Mariana (2016) and Imaningsih et al. (2021) reported that endophytic fungal isolates can be isolated from the roots of *Hiyung* chili.

The results of microscopic sections of leaves, stems, and roots of *Hiyung* chili infected with selected isolates are shown in Table 2.

Infective hyphae of the DM3.1 isolate were not visible as it belonged to the yeast group (Table 2, Figures 1 and 6.A). This is because most yeasts do not form hyphae (Joubert and Doty 2018). Other isolates showed invading hyphae on both roots and stems. Endophytic fungi have been reported to infect various plant organs such as roots and stems (Terna et al. 2022). The absence of hyphae on the leaves may also due to the young age of the plants. Endophytic fungal invasion on corn leaves was observed at 14 days after pre germination (Terna et al. 2022).

Microscopic and macroscopic characterization and molecular indentification of selected endophytic fungi isolates

The microscopic and macroscopic character of all the four selected endophytic isolates were different (Figures 1-4). On PDA medium, the upper surface of DM3.1 isolate was white with wavy edges and the lower surface was brownish orange. The mycelium was aerial hyaline hyphae, and small spores with a diameter of 1.47 µm (Figure 2).

The colonies of DT2.2P1 isolates on PDA medium were black in color. The reverse side was also black with a brown ring. Hyaline hyphae were dark in color conidia measured 5.1 x 6.79 µm, colorless and unicellular. Chlamydospores are dark brown, subglobose, muriform with an indistinct conidiophore shape (Figure 3).

Table 1. The average germination percentage of *Hiyung* chili seeds treated with endophytic fungi

Origin of Isolates	Codes	Germination average percentage (%)		
		Normal	Abnormal	Not growing
Control	K	90.00±8.16	3.33±4.71	6. 67±4.71
Young Leaf	DM3.1	90.00±8. 16	6.67±4.71	3. 33±4.71
Old Leaf	DT2.2P1	66.67±12.47	10.00±8.16	23.33±9.43
Young Leaf	DM1.2	56.67±26.25	16.67±12.47	26.67±17.00
Old Leaf	DT2.2P4	50.00±8.16	30.00±8.16	20.00±0.00
Medium Leaf	DS1.1P1	46.67±12.47	30.00±8.16	23.33±12.47
Old Leaf	DT2.2P2.2	40.00±8.16	16.67±12.47	43.33±12.47
Seeds	Bj2.2	30.00±21.60	20.00±14.14	50.00±8.16
Ripe fruit	BM3.1P1	30.00±24.49	10.00±8.16	60.00±32.66
Medium Leaf	DS1.1	26.67±37.71	0.00±0.00	73.33±37.71
Medium Leaf	DS2.2	26.67±37.71	20.00±16.33	53.33±24.94
Old Leaf	DT2.2P1P	26.67±18.86	16.67±12.47	60.00±29.44
Old Stem	BT2.2	23.33±17.00	16.67±12.47	60.00±16.33
Old Leaf	DT2.2P1H	20.00±14.14	20.00±8.16	60.00±16.33
Old Stem	BT1.1P	20.00±28.28	3.33±4.71	76.67±26.25
Root	A2.1	20.00±14.14	33.33±17.00	46.67±30.91
Old Leaf	DT1.1P	13.33±18.86	16.67±17.00	70.00±35.59
Young Stem	BM1.1	13.33±9.43	20.00±21.60	66.67±4.71
Old Leaf	DT2.2P1H	10.00±14.14	6.67±4.71	83.33±17.00
Old Leaf	DT2.1	6.67±4.71	56.67±17.00	36.67±12.47
Root	A1.1P1	6.67±9.43	50.00±21.60	43.33±17.00
Old Leaf	DT3.1P2	6.67±9.43	16.67±17.00	76.67±26.25
Old Stem	BT2.1P1	6.67±4.71	13.33±4.71	80.00±0.00
Old Leaf	DT2.2P3	3.33±4.71	3.33±4.71	93.33±4.71
Old Stem	BT3.2	3.33±4.71	3.33±4.71	93.33±9.43
Old Leaf	DT2.2P4	3.33±4.71	6.67±9.43	90.00±14.14
Medium Leaf	DS1.2	3.33±4.71	60.00±14.14	16.67±12.47
Old Leaf	DT3.1	0.00±0.00	0.00±0.00	100.00±0.00
Medium Leaf	DS1.2P2	0.00±0.00	13.33±18.86	86.67±18.86
Medium Leaf	DS3.1	0.00±0.00	3.33±4.71	96.67±4.71
Old Leaf	DT1.1H	0.00±0.00	6.67±4.71	93.33±4.71
Old Leaf	DT1.1P12	0.00±0.00	3.33±4.71	96.67±4.71
Ripe fruit	BM 1.1	0.00±0.00	13.33±12.47	86.67±12.47
Ripe fruit	BM1.2P1	0.00±0.00	0.00±0.00	100.00±0.00
Old Stem	BT1.1C	0.00±0.00	1.00±8.16	90.00±8.16
Old Stem	BT1.2P1	0.00±0.00	0.00±0.00	100.00±0.00
Old Stem	BT2.2	0.00±0.00	0.00±0.00	100.00±0.00
Old Leaf	DT3.1	0.00±0.00	0.00±0.00	100.00±0.00
Old Stem	BT2.1P1	0.00±0.00	0.00±0.00	100.00±0.00
Old Stem	BT3.1	0.00±0.00	0.00±0.00	100.00±0.00
Old Stem	BT3.2P1	0.00±0.00	10.00±8.16	90.00±8.16

Note: Normal: Grows well, according to seed germination without endophytic fungi as control; Abnormal: Grows, but not as control

Table 2. Infection of selected endophytic isolates into the tissues of *Hiyung* chili sprout

Isolates	Infected tissues		
	Root	Stem	Leave
DM3.1	-	-	-
DT2.2P1	+	+	-
DM1.2	+	+	-
DT2.2P4	+	+	-

Note: +: Presence of fungal hyphae in tissues, -: Absence of fungal hyphae in tissues

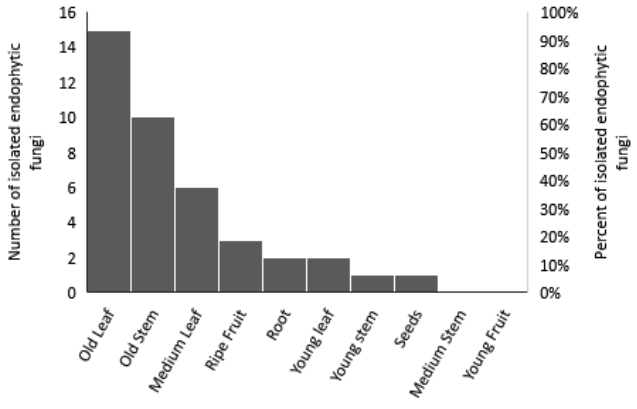

Figure 1. The number of endophytic fungi isolated from each part of the plant (roots, stems, leaves, fruits and seeds)

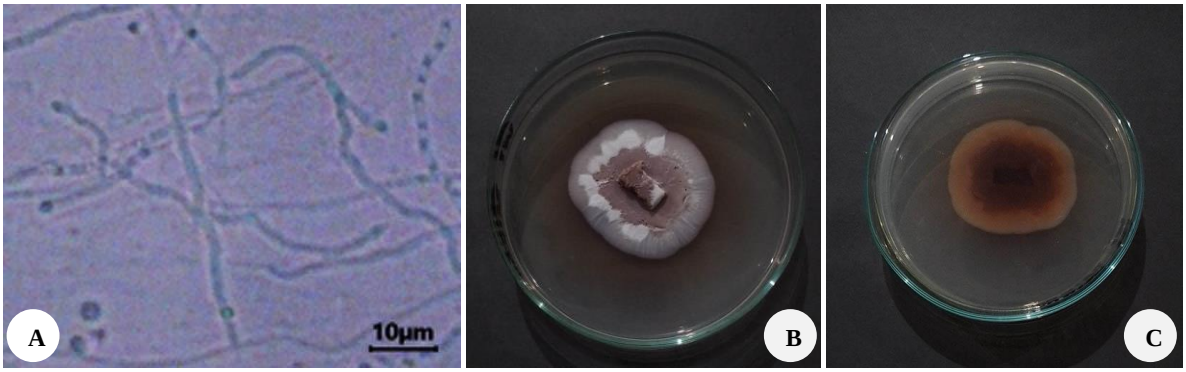

Figure 2. A. Hyphae and conidia, B. Upper surface and C. Reverse surface of *Pseudozyma hubeiensis* DM3.1 colony on PDA medium

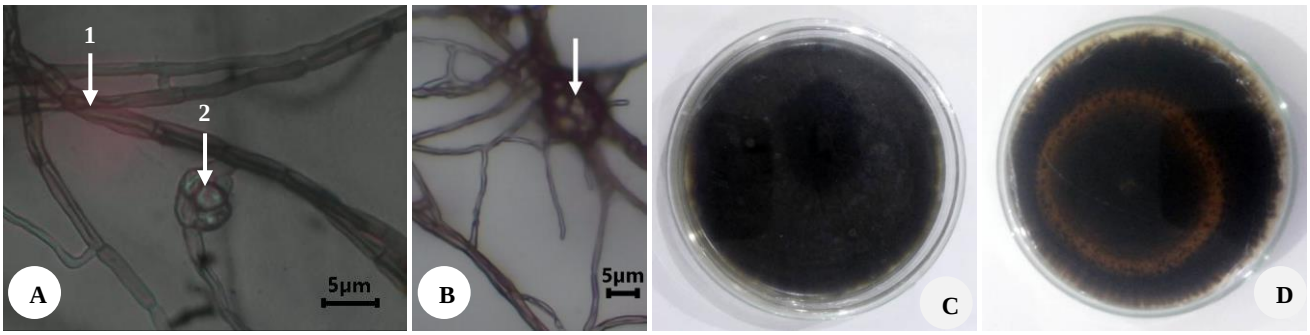

Figure 3. A. 1. Hyphae and 2. Conidia, B. Chlamydospores (arrow) C. Upper surface, D. Reverse surface of *Phoma* sp. DT2.2P1 colony on PDA medium

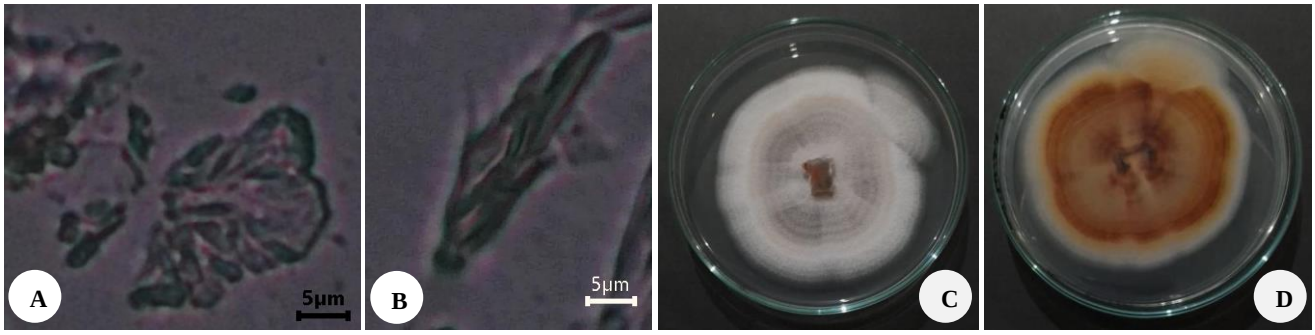

Figure 4. A. Ascospores, B. Hamathecium (hyphae that develop into ascus candidates), C. Upper surface, D. Reverse surface of *Letendraea* sp. DM 1.2 colony on PDA medium

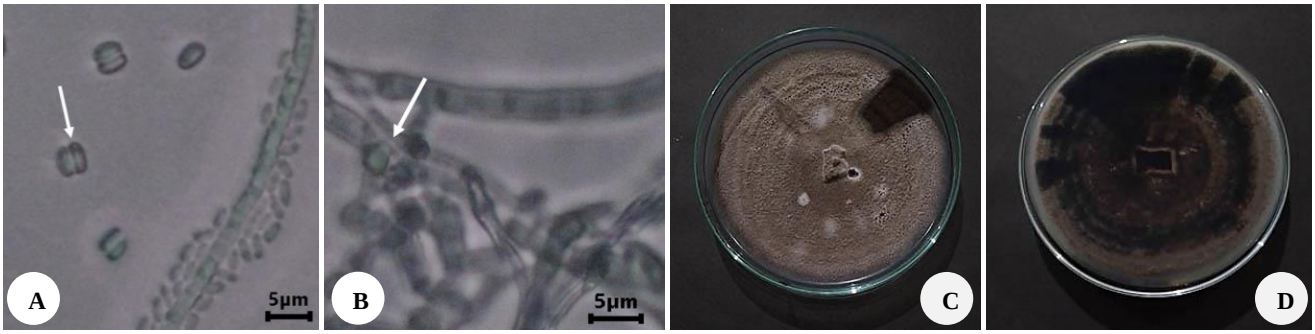

Figure 5. A. Conidia and B. Chlamydospores, C. Upper surface, D. Reverse surface of of *Phoma* sp. DT2.2P4 colony on PDA medium

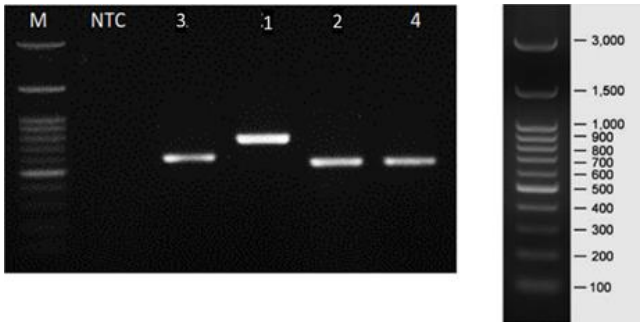

Figure 6. PCR products. 1 µL PCR product using electrophoresis with 0.8% TBE agarose, M: 100 bp DNA ladder (2.5 µL), sample number is at the top, NTC: Non Template Control, 1: DM3.1 (741bp), 2: DT2.2P1 (527bp), 3: DM1.2 (652bp), 4: DT2.2P4 (530bp)

On PDA medium, colonies were round, white on the edges and brown in the center with flat edges with concentric rings. The reverse color of colony was brownish-orange with lines in the center towards the edge of colony. Hyphae hyaline, septate, ascospores were oval and pointed at both ends, measuring 4-6 x1-4 µm (Figure 4).

Colonies of DT2.2P4 on PDA medium were black on both the surfaces, with slightly wavy edges (Figure 5). The microscopic feature of isolate DT2.2P4 was hyaline, septate, fusiform hyphae with spores measuring 3-5µm. Both macroscopic and microscopic observations show that DM3.1 isolates are yeast, having hyphae and blastoconidia chains. According to Barnett et al. (2000), these traits are characters of *Pseudozyma* genus. While isolates DT2.2P1 and DT2.2P4 were belonged to genus *Phoma* with characteristics according to the identification book by Watanabe (2002). The microscopic features of DM1.2 isolate were different from the previous isolate. According to de Silva et al. (2021), the characters of the genus *Letendraea* is light brown velvety colony on PDA media, Hyphae are branched cephal, hyalins, with brown ascospores, ellipsoidal to fusiform, straight or slightly curved, one septate and pointing to both ends.

Molecular identification of endophytic isolates was carried out using forward primer ITS1 (5'–TCCGTAGGTGAACCTGCGG–3') and reverse primer ITS4 (5'–TCCTCCGCTTAT TGATATGC–3'). PCR products were carried out using electrophoresis to produce DNA sample bands of varying sizes (Figure 6). Based on the Basic Local Alignment Search Tool (BLAST) analysis, 10 isolates showed the best suitability for each sample. The DM3.1 isolate had a sequence length conformity (Query coverage) of 100% with *Pseudozyma hubeiensis* strain ABS2, with a match between sequences (Percentage Identity) of 99.73%. Isolate DT2.2P1 had 100% Query coverage with *Phoma* sp. strain DTO 421-F6, with 100% percentage identity. DM1.2 isolate had 99% Query coverage with *Letendraea* sp. XXMF000004 Isolate with 98.15% percentage identity. The DT2.2P4 isolate had 100% *Phoma* sp. strain DTO 421-F6 with 100% percentage identity. *Pseudozyma hubeiensis* DM3.1 was yeast (OQ780778), while *Phoma* sp. DT2.2P1 (OQ780779), *Letendraea* sp. DM1.2 (OQ780780) and *Phoma* sp. DT2.2P4 (OQ780781) all three were molds.

The phylogenetic relationship of each isolate with the 10 species from BLAST results is shown in Figure 7. DM3.1 isolate was a yeast isolated from young leaf tissue with 90% germination, and similar to the genus *Pseudozyma*. The genus *Pseudozyma* i.e. *Pseudozyma churashimaensis* is reported to be associated with chili leaves (Lee et al. 2017). Yeast getting into the tissues of plants is allegedly carried by air, water and insects. The non-motile nature of yeast causes yeast to be very passive, so the mechanism of infection is also thought to begin with its colonization of plant parts and multiplying to allow it to reach xylem and circulate to all parts of the plant. Yeast also has the property of being able to produce substances capable of destroying plant cell walls. This makes it easier for yeast to colonize plant roots (Joubert and Doty 2018).

Phoma sp. DT2.2P1 and *Phoma* sp. DT2.2P4 was isolated from old leaf tissue. These isolates had an average of 66.67% and 50% of normal germination percentages, respectively. This genus has been widely reported as an endophyte that provides many advantages to plants (Baron and Rigobelo 2022; Soltani Nejad et al. 2022). *Phoma* infection in plant tissues usually occurs as a wound, through stomata or directly into the epidermis. Sometimes this genus also forms external hyphae, when the growth phase of its host plant is exhausted, this genus mostly becomes saproba (Deb et al. 2020).

Letendraea sp. DM1.2 was isolated from young leaf tissue. The average normal germination was only 56.67%. Its infectious hyphae were present in the stems and roots. The genus *Letendraea* has also been reported as endophytic in *Magnolia candolli* and *M. garrettii* (de Silva et al. 2021). *Letendraea* was reported also as endophytic and isolated from the leaves of *Hedychium spicatum* (Sarma et al. 2020). These isolates are also known to be associated with insects (Xu et al. 2021). The entry of fungi associated with insects into plants can be through direct invection of roots, stems and leaves (Francis et al. 2022).

In vitro tolerance of *Hiyung* chili endophytic fungi to acidity

The diameter of endophytic fungi increased at each pH treatment from days 1 to 7 after incubation (Figure 8a-d). The average daily colony diameter of *P. hubeiensis* DM3.1 was not significantly affected by the differences in pH treatment based on the Anova assay (α :0.05; F: 1.092, P:0.372). However the average daily diameter of *Phoma* sp. DT2.2P1 in various pH treatments, significantly influenced by pH differences based on the Kruskal Wallis test (α: 0.05; P: 0.01), the fungi had stunted growth at pH3. Likewise the average daily diameter of *Letendraea* sp. DM1.2 in various pH treatments was significantly influenced by pH differences based on the Anova test (α: 0.05; F: 17.75; P: 0.00). Even on pH3 this endophytic fungi do not grow. While the average daily diameter of *Phoma* sp. DT2.2P4 was the same as the previous two isolates, significant in various pH treatments based on the Kruskal Wallis Test (α:0.05; P:0,02).

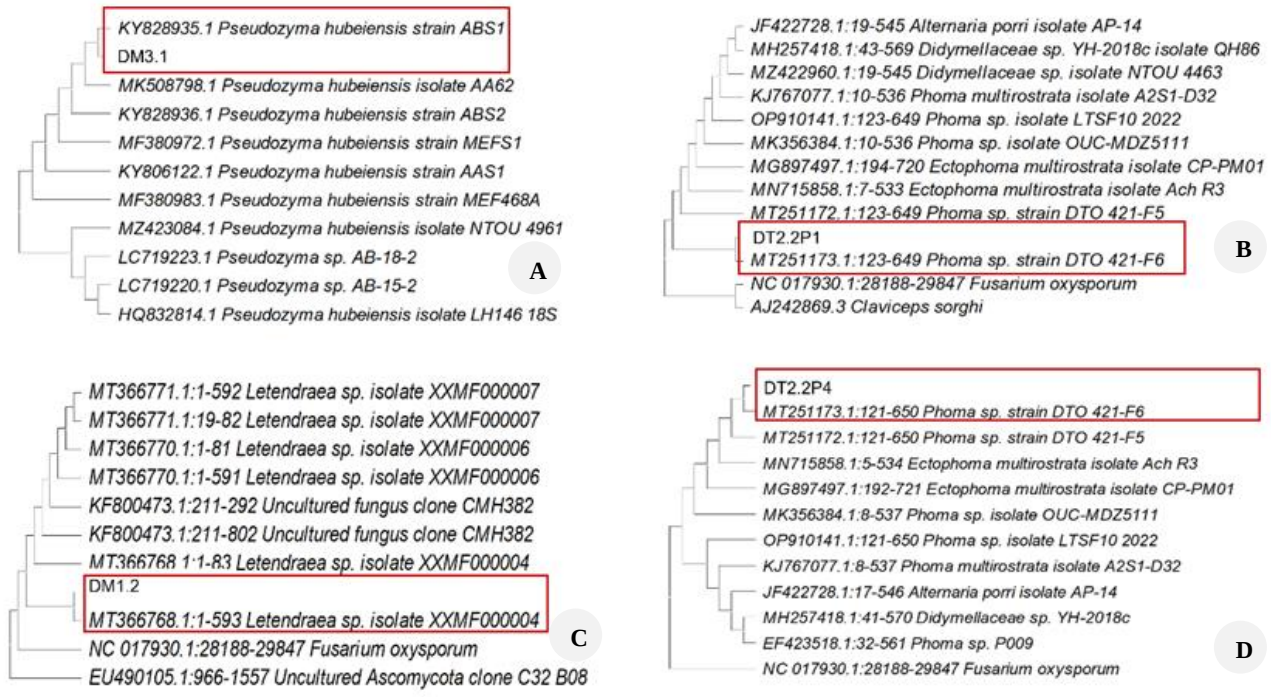

Figure 7. Phylogenetic relationships of endophytic isolates using the Neighbor-Joining

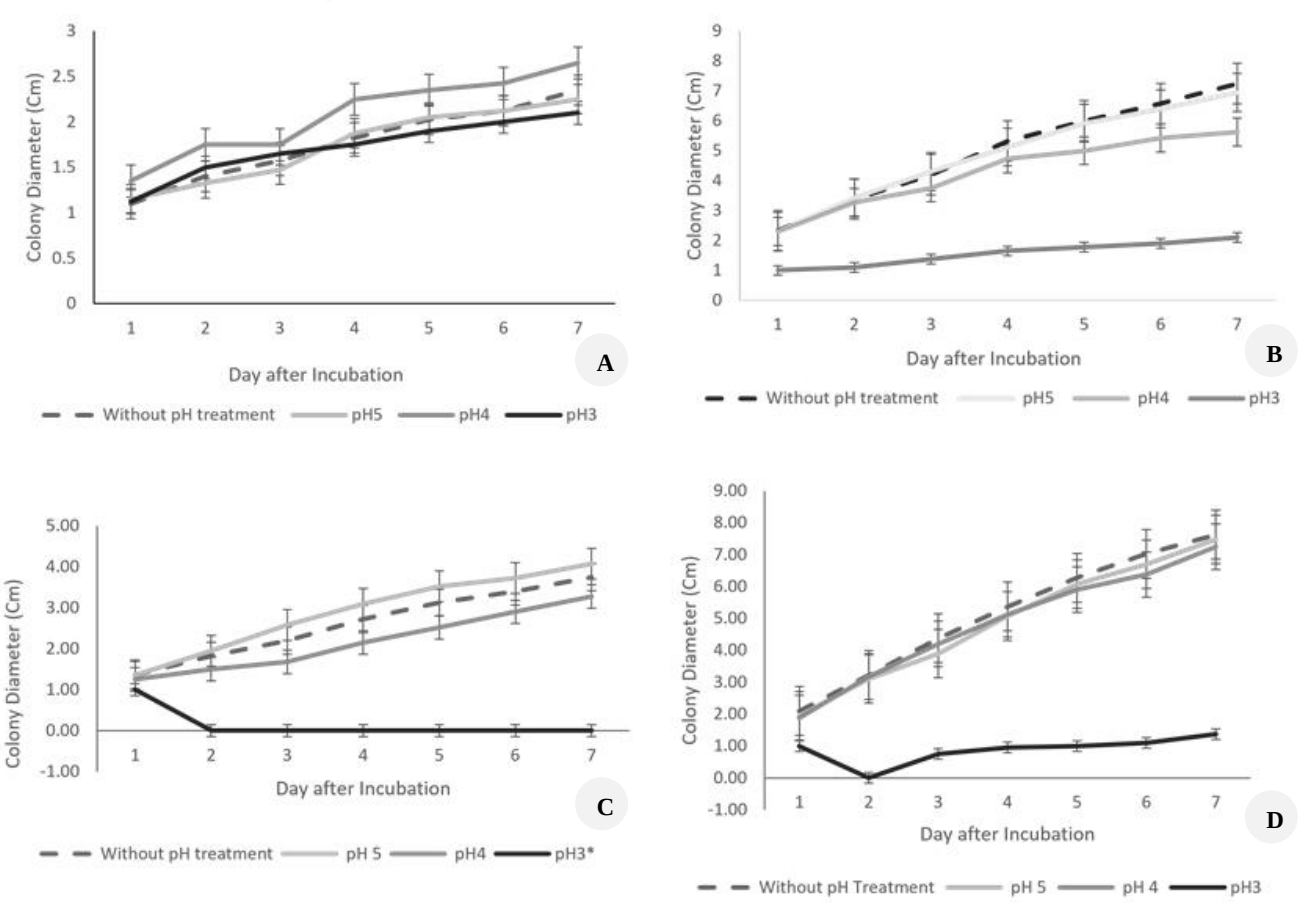

Figure 8. A. The average daily diameter of *P. hubeiensis* DM3.1, B. *Phoma* sp. DT2.2P1, C. *Letendraea* sp. DM1.2, D. *Phoma* sp. DT2.2P4 at various pH treatments. (*) sign indicates significance based on the ANOVA test with α: 0.05

The four isolates showed different diameters in each pH treatment. Each isolate may be different in tolerating pH stress. The average daily diameter of colonies of *P. hubeiensis* DM3.1 was increased in all pH treatments and there was no significant difference between treatments based on ANOVA test (α: 0.05, F: 1.092, P: 0.372). This is because yeast is reported to have acidophile tendencies (Palma et al. 2018). *Phoma* was also reported to have tolerance to acidic environmental conditions (Aguilera and González-Toril 2019), although in this study its growth was stunted at pH3. Only isolate of *Letendraea* sp. DM1.2 did not grow at pH3. Colony diameters of all endophytic isolates on pH 3 to 5 were achieved respectively (*P. hubeiensis* DM 3.1: 1.5-2.5 cm, *Phoma* sp. DT2.2P1: 4-6 cm, *Letendraea* sp. DM1.2: 2-3 cm, and *Phoma* sp. DT2.2P4: 6-7 cm). *Aspergillus niger* had colony diameters ranging from 1.4-3.1 cm at pH 3 and 0.75-3.75 at pH 5 (Rosas-Moreno et al. 2023). This is probably because the endophytic fungus from *Hiyung* chili leaves has adapted to the acidic host growing environment in South Kalimantan peatlands. Fungal tolerance to acidic environments has also been reported in fungi from mangrove sediments (Gao et al. 2021).

This study was successful in isolating endophytic fungi derived from the roots, leaves, stems, fruits, and seeds of the *Hiyung* chili plant. Of the 41 isolates, most isolates were derived from leaves. Only four isolates were chosen because they produce good seed germination and do not cause disease. Out of the four isolates, *P. hubeiensis* DM.31 was a yeast, *Phoma* sp. DT2.2P1, *Letendraea* sp. DM1.2 and *Phoma* sp. DT2.2P4 were molds. The four fungi were able to grow in acidic conditions with pH 4 and 5 in vitro. These results can be used as a basis for future research on the application of endophytic fungi to *Hiyung* chili plants, especially their tolerance to acidic environments through symbiosis with endophytic fungi.

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