Screening of Endophytic Fungi from *Hypselandra variabilis* (Coll & Hems) Pax & Hoffm and their Starch Hydrolysis Activities

Hla Myo Thein[*], Khin Thida Swe[**] , Swe Zin Win[***]

Abstract

The endophytic fungi were isolated from various parts of *Hypselandra variabilis* (Coll & Hems) Pax & Hoffm (Thamon) plant grown at Tha-Yet- Kan village, Madayar Township, Mandalay Region, during July to September 2021. The endophytic fungi were isolated by surface sterilization method (NITE, 2004). A total fifteen fungi were isolated according to their morphological characters. The amylase enzyme activities of fungal isolates were detected by method of NITE, 2004. The pure endophytic fungi were tested by iodine for the study of starch hydrolyzing activities. Among them, eleven endophytic fungi showed the starch hydrolyzing activities. And then, pure isolated fungi were inoculated into test tubes containing isolation medium for the further research such as antimicrobial activities.

Keywords: endophytic fungi, starch hydrolyzing activities

Introduction

Endophytes are microbes existing within the plants without causing any disturb to their growth and development. Unlike disease-causing microorganisms, endophytes are non-pathogenic and many of them are known to promote their plant host's fitness (Mendes *et al*., 2013). In recent years, it is gradually conceded that endophytic fungi or endophytes have played a very important role in affecting the quality and quantity of the crude drug through a particular fungus-host interaction, indicating that more appreciating on the particular relationships between endophytic fungi and medicinal plants is required for promoting crude drug production (Faeth and Fagan, 2002).

Hypselandra variabilis (Coll & Hems) Pax & Hoffm is a tropical species belongs to family Capparaceae. It is indigenous to Myanmar and its local name is Thamon. The leaves and roots are used in aching, sedema, cold extremities and also used as stomachic, expectorant and counter irritant. Fruits and vegetables are rich in minerals, vitamins and fibre and low in saturated fat (Batta, 2016). Since this plant is consumed as seasonal food by local people in Myanmar, it is required to examine the nutritional and medicinal value of this plant (San and Han, 1998). The crown gall of the Thamon is used to relieve eye sore. It is believed that eating Tha-Mon once a

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year can keep the good health along the lifespan. Wild edible plants are still consumed traditionally by different coummunities mainly in rural and suburban areas (Pinela *et al*., 2017).

Thamon flowers contain a number of beneficial antioxidants including polyphenols. Antioxidants against and free radicals, which are by products of cell oxidation. Free radicals are associated with causing a number of health problems, including breast, prostate and lung cancers (Bhowmik and Lombardi, 2014).

In the present study, an attempt has been made to offer an endophytic fungus as source of enzymes for industrial essential. The aim of the present study was to screen the different endophytic fungi for amylase production isolated from *Hypselandra variabilis* (Coll & Hems) Pax & Hoffm (Thamon) plant. The isolated endophytic fungi will have antimicrobial activities in a later article and will synthesize compound the best of them.

Materials and Methods

Collection of Plant Sample

The *Hypselandra variabilis* (Coll & Hems) Pax & Hoffm (Thamon) plants were collected from Tha-Yet-Kan Village, Madayar Township, Mandalay Region(N 22º 4´21'' and E 96º 6´31''). Sample collection was carried out in July to September 2021. Disinfected plant samples were collected and sealed in the polyethylene bags. The sample preparations were performed at Microbiology laboratory of Botany Department, Yadanabon University.

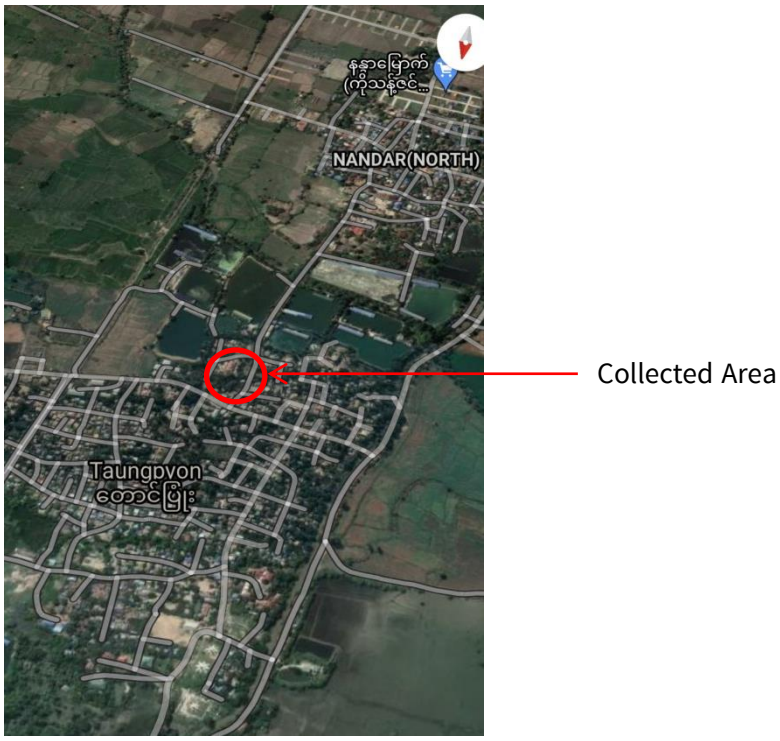


Figure.1 Map of Madayar Township

(Source: Google Map)

Identification of Plant Sample

The collected plant samples were identified according to the reference books (Backer and Bakhuizen Van Deen Brink, 1963) and (Dassanayake, 1980).

Isolation of Endophytic Fungi (Ando, et al., 2004)

The surface sterilization method (NITE, 2004) was used for the isolation of endophytic fungi. The endophytic fungi were isolated from leaves, stems and flowers of the *Hypselandra variabilis* (Coll & Hems) Pax & Hoffm (Thamon) plant. Plant sample was rinsed carefully in tap water for 15 mins and air dried before it was processed. The plant materials were then surface sterilized by soaking them consecutively in 70% ethanol for 60 seconds and then, also soaked 10% sodium hypochloride for 60 seconds and rinsed carefully with sterile distilled water. Then, with a sterile scissors, outer tissue was removed and the inner tissues of 0.5 cm size were delicately cut and placed on Czapek–Doz Agar (CZA) medium (Sucrose 3.0 g, $NaNo_3$ 0.2 g, K_2HPO_4 0.1 g, $MgSO_47H_2O$ 0.05 g, KCL 0.05 g, $FeSO_47H_2O$ 0.001 g, Agar 1.8 g, Distilled water 100 mL)and Glucose Yeast Extract Peptone Agar(GYA)medium(Glucose 1.0 g, Yeast Extract 0.3 g, Peptone 0.3g, K_2HPO_4 0.001 g, $MgSO_47H_2O$ 0.001 g, Agar 1.8 g, Distilled water 100 mL). After autoclaving, the medium was added with chloramphenicol to suppress bacterial growth. The Petri-disc was incubated at room temperature for 3 days to 7 days until fungal growth appeared.

Starch hydrolyzing activities of Isolated Fungi (NITE, 2004)

The liquid medium (Soluble Starch 1.0 g, KH_2PO_4 0.1 g, $MgSO_47H_2O$ 0.1 g, NaCl 0.1 g, $(NH_4)_2SO_4$ 0.2 g, $CaCO_3$ 0.1 g and distilled water 100 mL) was prepared for the amylase activity test according to the given composition and sterilized. Then about 20 mL of this medium was poured into each test tube and autoclaved at 121ºC. After sterilization, these test tubes were cooled at room temperature. The isolated fungi were inoculated into each test tube respectively and incubated for 7 days. Then, Iodine solution (drop by drop 0.1 mL to 0.3mL) was poured gently into each test tube containing liquid culture medium.

After adding the iodine solution, if the liquid culture medium changed to blue black colour, it indicates that the isolated fungi cannot hydrolyse the starch. If the blue black colour changed into pale colour or colourless, the isolated fungi can hydrolyse the starch.

Results

Identification of Plant Sample

Hypselandra variabilis (Collect & Hems) Pax & Hoffm

Local name : Thamon

English name : Unknown

Flowering period : January to March

Perennial unarmed shrubs; stems and branches terete, pubescent when young. Leaves palmately compound, alternate, stipulate; leaflet 3-5, obovate-oblong, petiolate. Inflorescences terminal or axillary, corymbose cymes, flowers bisexual, actinomorphic, tetramerous, hypogynous, creamy-white, about 1.5 cm in diameter at anthesis, apetalous, bisexual, bracts leafy, subtending the flowers. Calyx campanulate, deeply 4 partite. Disc fleshy, green, surrounding the androgynophore. Stamens 8-10, free, exserted; filaments filiform, white; anthers ovoid. Ovary superior, ellipsoid, unilocular, with many ovules on the parietal placentae; stigma discoid. Baccate ovoid, glabrous; seeds fleshy.

Distribution : This species was recorded in Myanmar (Kress *et al*. 2003).

Habit Leaf L.S of flower T.S of ovary

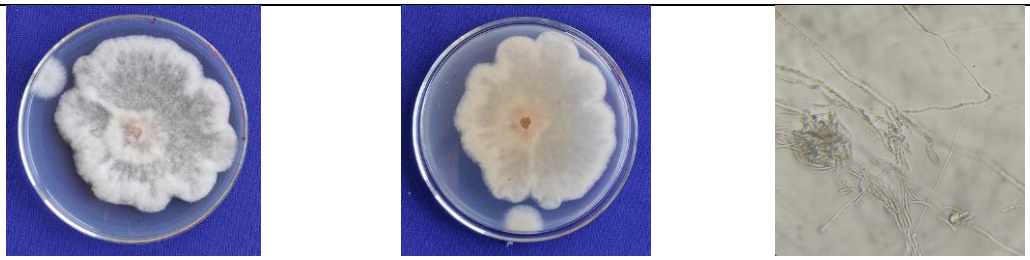
Figure.2 Morphological Characters of *Hypselandra variabilis* (Collect & Hems) Pax & Hoffm

Isolation of Endophytic Fungi

Fifteen endophytic fungi were isolated from leaves, stems and flowers of Thamon plant. Pure culture of isolated endophytic fungi were named as HMO-01 to HMO-15 (Figure - 3 to 14).

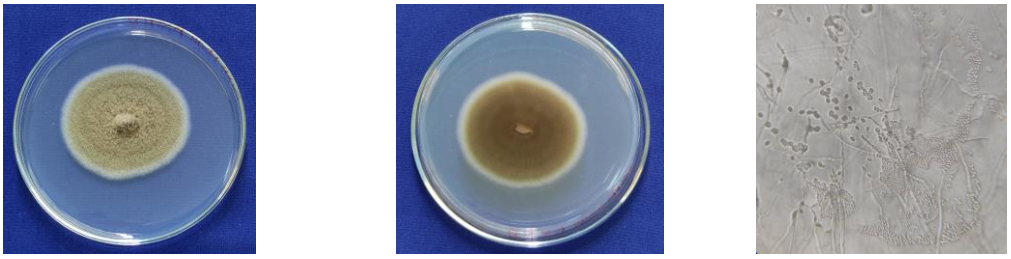
Table.1 List of Endophytic Fungi Isolated from parts of *Hypselandra variabilis* (Coll & Hems) Pax & Hoffm (Thamon)

Media	Plant Parts	Fungi Number	Isolated Fungi
GYA	Leaves	HMO – 01, 02, 03	3
GYA	Stems	HMO- 04, 05	2
GYA	Flowers	HMO- 06, 07	2
CZA	Leaves	HMO-08, 09,10	3
CZA	Stems	HMO-11,12,13	3
CZA	Flowers	HMO- 14, 15	2
Total isolated fungi			15



Surface view Reverse view Photomicroscopy X 40

Figure.3 Colony morphology and photomicroscopy of endophytic fungus HMO-01

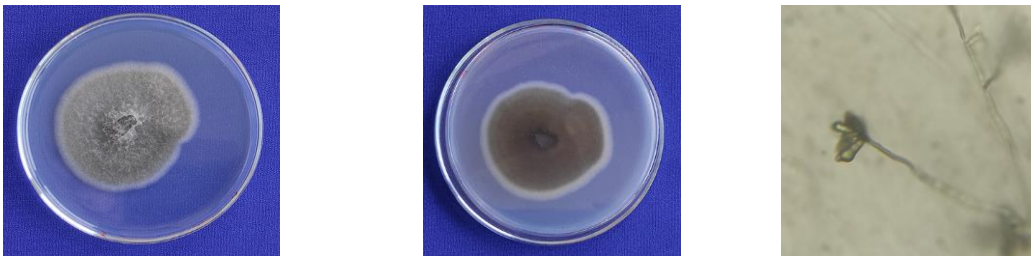


Surface view Reverse view Photomicroscopy X 40

Figure.4 Colony morphology and photomicroscopy of endophytic fungus HMO-02

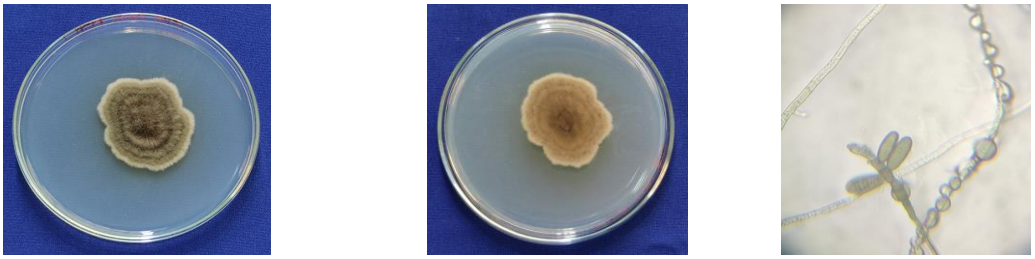
Surface view Reverse view Photomicroscopy X 40

Figure.5 Colony morphology and photomicroscopy of endophytic fungus HMO-03



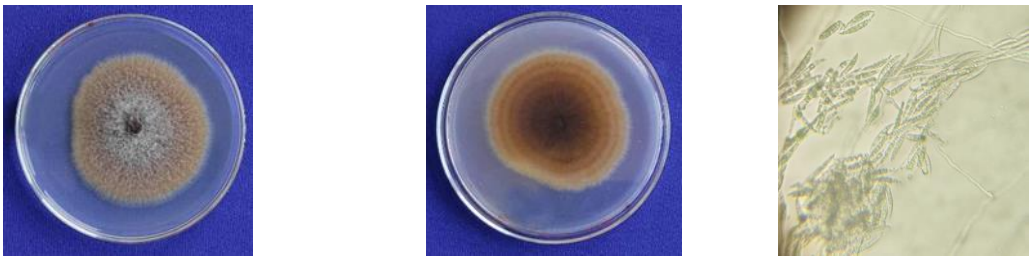
Surface view Reverse view Photomicroscopy X 40

Figure.6 Colony morphology and photomicroscopy of endophytic fungus HMO-04



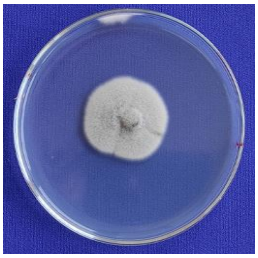
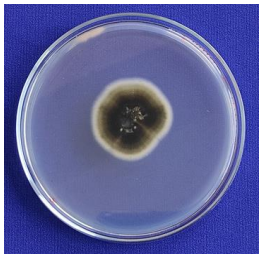
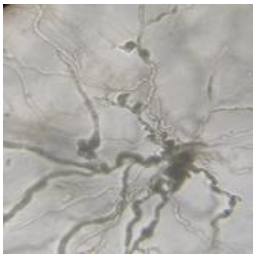
Surface view Reverse view Photomicroscopy X 40

Figure.7 Colony morphology and photomicroscopy of endophytic fungus HMO-05



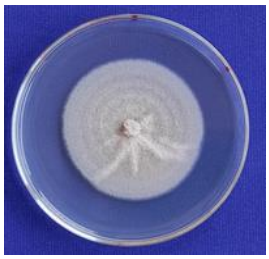
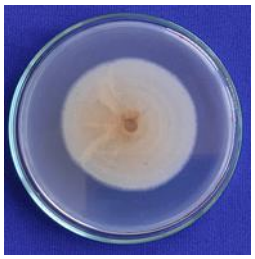
Surface view Reverse view Photomicroscopy X 40

Figure.8 Colony morphology and photomicroscopy of endophytic fungus HMO- 06

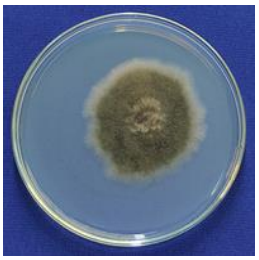
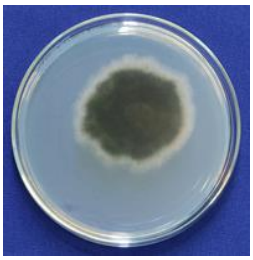
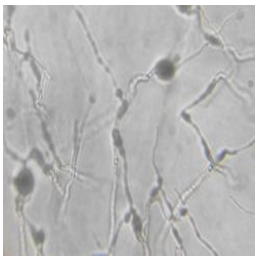
Surface view Reverse view Photomicroscopy X 40

Figure.9 Colony morphology and photomicroscopy of endophytic fungus HMO-07

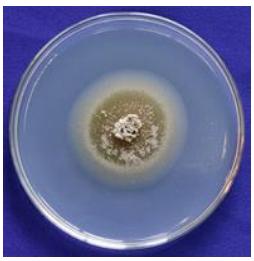
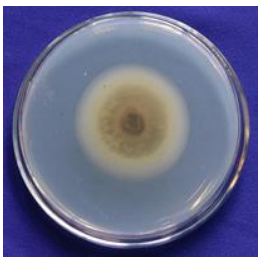

Surface view Reverse view Photomicroscopy X 40

Figure.10 Colony morphology and photomicroscopy of endophytic fungus HMO-08

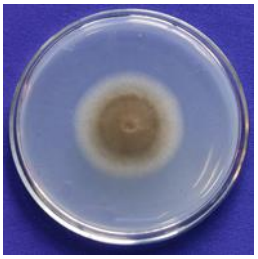
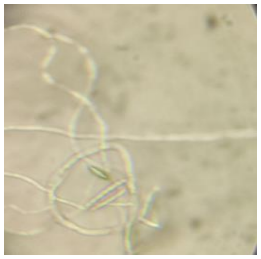
Surface view Reverse view Photomicroscopy X 40

Figure.11 Colony morphology and photomicroscopy of endophytic fungus HMO-09


Surface view Reverse view Photomicroscopy X 40

Figure.12 Colony morphology and photomicroscopy of endophytic fungus HMO-10

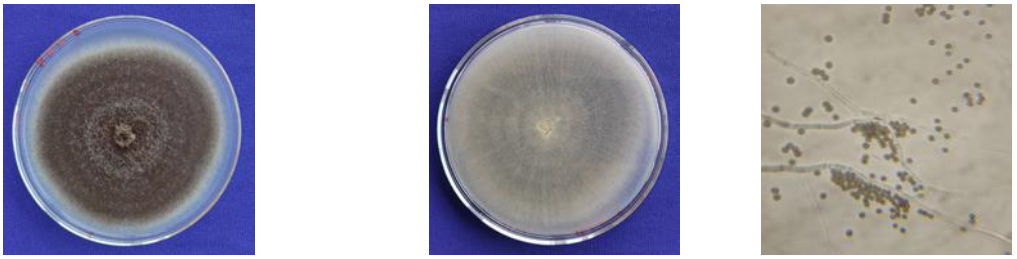




Surface view Reverse view Photomicroscopy X 40

Figure.13 Colony morphology and photomicroscopy of endophytic fungus HMO-11

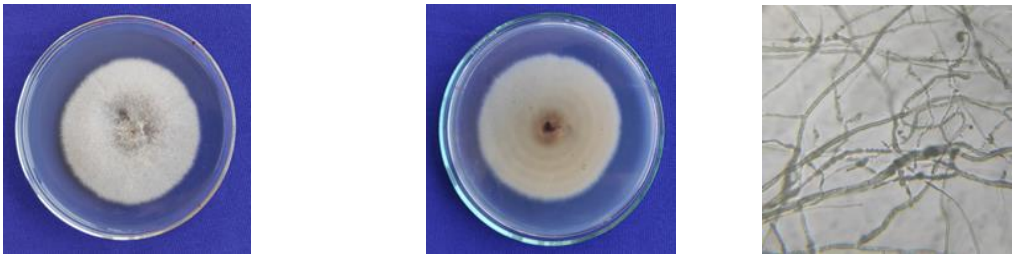
Surface view Reverse view Photomicroscopy X 40

Figure.14 Colony morphology and photomicroscopy of endophytic fungus HMO-12



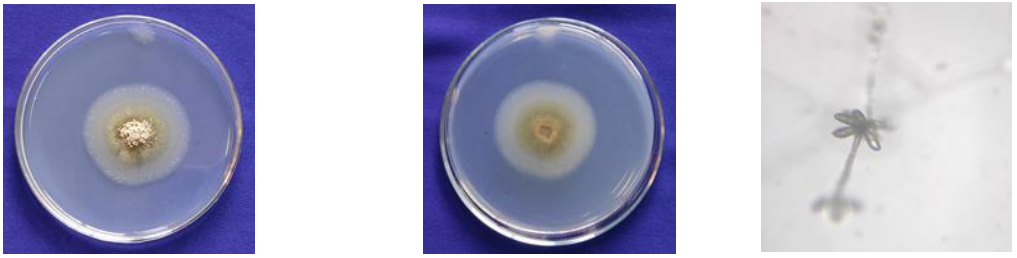
Surface view Reverse view Photomicroscopy X 40

Figure.15 Colony morphology and photomicroscopy of endophytic fungus HMO-13



Surface view Reverse view Photomicroscopy X 40

Figure.16 Colony morphology and photomicroscopy of endophytic fungus HMO-14



Surface view Reverse view Photomicroscopy X 40

Figure.17 Colony morphology and photomicroscopy of endophytic fungus HMO-15

Table.2 Morphological and Microscopical Characters of Endophytic Fungi

Strain No.	Morphological characters		Microscopical characters			Media
	Surface view	Reverse view	Size (5 Days old culture)	Shape	Conidia	
HMO – 01	White colour	Yellowish pink colour in the center and edge white colour	7.3cm	Spindle	Many	GYA
HMO – 02	Brownish gray in the center and edge white colour	Dark brownish gray in the center and edge white colour	6.3 cm	Spherical	Many	GYA
HMO – 03	White colour	Yellowish pink colour in the center and edge white colour	8.6 cm	Spindle	2	GYA
HMO – 04	Light Gray in the center and edge white colour	Dark Gray in the center and edge white colour	5.6 cm	Ovoid to short cylindrical	4	GYA
HMO – 05	Pale Gray in the center and edge white colour	Antique bronze in the center and edge white colour	6.3 cm	Ovoid to short cylindrical	3	GYA
HMO – 06	Pale brown colour	Pale black in the center and edge yellowish pink colour	6.3 cm	Spindle	Many	GYA
HMO – 07	Cream colour	Black in the center and edge white colour	3.7 cm	Globose phialides	Singly or in groups	GYA
HMO – 08	Cream colour	Pale pink	5.9 cm	Ovate -oblong	2	CZA
HMO – 09	Brownish gray in the center and edge pale gray colour	Pale black in the center and edge white colour	6.0 cm	Globose	1	CZA
HMO – 10	Olive colour in the center and edge creamy colour	Olive colour in the center and edge yellowish pink colour	5.0 cm	Ovoid -oblong	1	CZA
HMO – 11	Antique bronze colour	Antique bronze in the center and edge pale Antique bronze colour	6.0 cm	Globose	1	CZA
HMO – 12	White colour	White colour	7.3 cm	Crescent	1- 3	CZA
HMO – 13	Neutral gray colour	Pale Neutral gray colour	7.3 cm	Spherical	many	CZA
HMO – 14	Titanium white	Titanium white	9.0 cm	Globose phialides	Singly or in groups	CZA
HMO – 15	Creamy colour in the center and titanium white colour	Creamy colour in the center and titanium white colour	6.5 cm	Ovoid to short cylindrical	5	CZA

Starch hydrolyzing activities of Isolated Fungi (NITE, 2004)

In the screening of amylase enzymes from isolated endophytic fungi were carried out, eleven endophytic fungi (HMO-02, HMO-03, HMO-05, HMO-06, HMO-09, HMO-10, HMO-11, HMO-12,

HMO-13, HMO-14 and HMO-15) showed starch hydrolyzing activities. The rest four fungi (HMO-01, HMO-04, HMO-07, HMO-08) do not show the starch hydrolyzing activities(Figure-18).

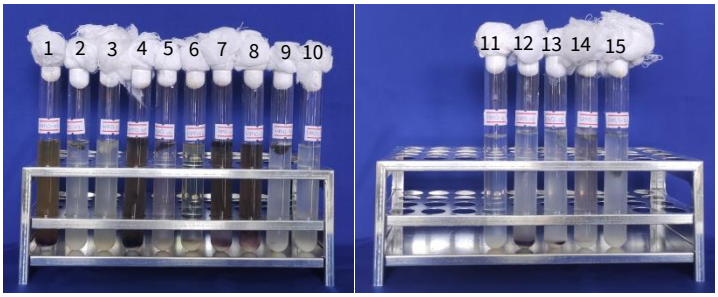


Figure.18 Starch hydrolyzing activity of endophytic fungi HMO-01 to HMO-15

Discussion and Conclusion

Studies have shown that some medicinal properties of plants may be related to their endophytic fungi (Zou *et al*., 2000). Endophytic fungi have been explored from various plant sources for their industrial potential. Therefore researchers focused on terrestrial plants for endophytic (Bindu *et al.,* 2021).

In the present study, an attempt has been made to offer an endophytic fungus as source of enzymes for industrial requirements**.** A total of fifteen endophytic fungal strains were screened from leaves, stems and flowers of *Hypselandra variabilis* (Coll & Hems) Pax & Hoffm (Thamon) inoculated on Glucose Yeast extract Agar (GYA) and Czapek- Dox Agar **(**CZA) media. In this study, HMO-01, HMO-03, HMO-12, HMO-13, and HMO-14 showed excellent growth on PGA (Potato Glucose Agar) medium. However, HMO-02, HMO-04, HMO-05, HMO-06, HMO-08, HMO-09, HMO-10, HMO-11 and HMO-15 showed moderate growth and only HMO-07 showed to be the least (poor) growth on PGA medium (Table-2).

According to the results of starch hydrolyzing activities, these eleven endophytic fungal strains (HMO-02, HMO-03, HMO-05, HMO-06, HMO-09, HMO-10, HMO-11, HMO-12, HMO-13, HMO-14 and HMO-15) changed into pale colour or colourless, therefore, they showed the starch hydrolyzing activities (Figure-18). However, HMO-01, HMO-04, HMO-07 and HMO-08 showed blue black colour, it indicates that the isolated endophytic fungi cannot hydrolyse the starch (Figure-18).

It was concluded that the medicinal plant *Hypselandra variabilis* (Coll & Hems) Pax & Hoffm (Thamon) has numerous types of endophytic fungi. The isolated endophytic fungi will be tested antimicrobial activities in a later article. Among them, a strain that exhibit the highest antimicrobial activity will be selected for further study.

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References

Ando, K., M. Suto and S.Inaba, 2004. Isolation and identification of fungi, Workshop, BDBRC-Pathein University.

Backer C.A and R. C. Bakhuizen Van Deen Brink, 1963. **Flora of Java**, Vol.1-3.Rijksherbarium, Lelyden, N. V.P. Noordhoff.

Batta A. 2016. **A review on phytochemicals and their activities**. Int. J. Curr. Res. Med. Sci. 2 (1): 20-28.

Bindu, N., S.K. Goyol, A. D. Tripathi and V. Kumar. 2021. **Exploring the diversity of endophytic fungi and screening for their pullulanase-producing capabilities.** Journal of Genetic Engineering and Biotechnology. https:// doi.org/10.1186/s 43141-021-00208-0.

Bhowmik, M and P. Lombardi.2014. **Recent asvances in Antitumor Berberine**. J. Cancer Sci Ther. Vol.7 910), pp. 11- 15.

Dassanayake M.D.,1980. **A revised handbook of the flora of Ceylon**. Vol.1-14, University of Peradeniya.

Faeth, S.H., and Fagan, W.F. 2002. **Fungal endophytes: common host plant symbionts but uncommon mutualists**. Integr. Comp. Biol. 42, 360-368.dio:10.1093/icb/42.2.360.

Kress, J., Defilipps. Robert A., Farr E. and Yin Yin Kyi, 2003. **A checklist of the trees, shrubs and climbers of Myanmar. Department of systematic Biology – Botany**. National Museum of Natural Historal History, Washington DC.USA.

Mendes, R., Garbeva, P., and Raaijmakers, J.M. 2013. **The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms**. FEMS Microbiol.Rev.37,634-663.doi:10.1111/1574-6976.12028.

Pinela J., Carvalho A. M. and Ferreira Isabel C. F.R. 2017. **Wild edible plants: Nutritional and toxicological characteristics, retrieval strategies and importance for today's society**. Food Chem. Toxicol.110: 165-188.

San P. P and Han Y. Y. 1998. A study on morphological and anatomical characteristics of some species of the family Capparidaceae.

Zou. W.X., J.C. Meng, H.Lu, G.X. Chen, G.X. Shi, T.Y. Zhang, R.X. Tan.2000. Metabolites of *Colletotrichum gloeosporioides*, an endophytic fungus in Artemisia mongolica. Journal of Natural Products, 63,pp.1529-1530.

NITE (National Institute of Technology and Evaluation).2004. **Surface sterilization and baiting method.**