Study on Antimicrobial Metabolite Producing Fungi Isolated from Mangrove Plants

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Abstract

In the isolation of fungi, six different mangrove plants *Aegialitis rotundifolia* Roxb., *Avicennia alba* L., *Avicennia marina* (Frosk.) Vierh., *Aegiceras corniculatum* (L.) BL., *Avicennia officinalis* L. and *Ceriops decandra* (Griff) Ding Hou. were collected in the Pathein area. Eighteen endophytic fungi were isolated from these mangrove plants. In the screening program, endophytic fungi ET-11 from the leaf *Aegiceras corniculatum* (L.) BL. exhibited the highest activity on *Bacillus subtilis* KY-327.Therefore, the strain ET-11 was selected for identification, fermentation and purification of antibacterial compound. This stain was isolated from the leaf of *Aegiceras corniculatum* (L.) BL. collected at Pathein-Chaung Tha Road (N16° 52' 36" E 94° 23' 42").

Keywords: Isolation, endophytic fungi, mangrove plants, Pathein area, biological properties

Introduction

Plants can be considered as a new isolation source of microorganisms. This means that there is much possibility of findings of new microorganisms (Scott and Lori, 1996). An endophyte is literally defined as one organism living inside the healthy plant parts. Most procedures for isolating endophytes are comparatively simple and routine for one skilled in basic plant pathological or microbiological technique. However, the process of designing an analytical study of an endophytic community, handling and maintaining often hundreds of isolates, characterizing them taxonomically, and quantitatively interpreting the results can be burdensome and overwhelming. The techniques and materials used for isolation, maintenance, identification and preservation of endophytes of grasses were reviewed recently (Bacon, 1990).

Endophytes are an extremely host-specific and specialized subset of endophytes with their own peculiar life cycles. The isolation and identification methods for endophytes was last reviewed in 1986 in which presented a useful table listing surface sterilization protocols for various kinds of plants and plant organs (Petrini, 1986).

The mangrove plants are the rich sources of microorganisms (Jones, 1997). Endophytic fungi inhabiting leaf and stem tissue in Angiosperms can dominate early stages of litter decomposition (Aoki *et al.*, 1990; Kendrick, 1962). The typical materials for microbial sources are soil, living and fallen leaves, leaf litters, dung, fresh water, marine water. In order to find new antibacterial bioactive compound, it is very important to set up an effective screening system which has a unique target and deals with unique microorganisms.

Materials and Methods

In the isolation of fungi, six different mangrove plants collected in the Pathein area were employed (Fig. 1). Endophytic fungi were isolated by the method of Ando and Inaba, 2004, Low Carbon Agar medium (LCA) medium as shown in (Fig. 2).

The isolated fungi were grown at 25° C for 7 days on Potato Dextrose Agar (PDA) medium. The isolated fungi were inoculated on seed medium (GPY medium) and incubated at 25° C for 3 days. Five ml of seed culture was transferred into the fermentation medium and

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incubated at 25° C for 7 days on rotary shaker (100 rpm). After the end of fermentation, the fermented broth (20□l) was used to check the antimicrobial activity against test organisms by paper disc diffusion assay. Paper disc having eight millimeter diameter (Advantec, Toyo Roshi Kaisha Co., Ltd., Japan) were utilized for antimicrobial assays.

The assay medium (Glucose 1%, Polypepton 0.3%, KNO_3 0.1%, Agar 1.8%, Distilled water 100 ml, pH 6.5-7.0) was used for the antimicrobial activity test. Clear zones (inhibitory zones) surrounding the test discs indicate the presence of bioactive metabolites which inhibit the growth of test organisms.

The test organisms used in paper disc diffusion assay were *Agrobacterium tumefaciens* IFO5431, *Aspergillus flavus* IFO3290, *Aspergillus paraciticus* IFO5123, *Bacillus pumalis* NITE47239, *Bacillus subtilis* KY-327, *Candida albicans* NITE09542, *Clostridia sp.* NITE86491, *Curvularia oryzae* NITE52906, *E. coli* AHU5436, *Magnaporthe grisea* NITE86512, *Micrococcus luteus* NITE8329, *Micrococcus roseus* IFO51384, *Microsporum gypseum* IFO43906, *Pseudomonas fluores* IFO94307, *Saccharomyces cerevisiae* NITE52847, *Salmonella typhi* AHU7943, Methicillin Resistant *Staphylococcus aureus* (MRSA) NITE1308 (IFO25196), *Tricophyton mentagrophyte* NITE10275, *Tricophyton rubrum* NITE 10683 and *Xanthomonas oryzae* IFO93517. These test organisms were obtained from NITE (National Institute of Technology and Evaluation, Kisarazu, Japan).

Starch hydrolysing activity was undertaken by the method of NITE, 2004. The isolated strains were inoculated in the liquid medium containing soluble starch and incubated at 27˚C for 3 days. The control was also done. The iodine solution was poured slowly onto the liquid cultures, if the colour was not changed, the microorganisms can hydrolyse the starch.

Aegialitis rotundifolia Roxb.
(တုပ်ပင်) Plumbaginaceae

Avicennia alba L. .
(သမဲ့/လမဲ့)Avicenniaceae

Avicennia marina (Frosk) Vierh.
(သမဲ့/လမဲ့) Avicenniaceae

Aegiceras corniculatum (L.) BL.
(ဗျာပင်) Myrsinaceae

Avicennia officinalis L.
(သမဲ့/လမဲ့) Avicenniaceae

Ceriops decandra (Griff) Ding Hou.
(ဖျိုင်းတောက်ဖြူ) Rhizophoraceae

Figure (1) Morphologies of Mangrove plants employed for the isolation of endophytic fungi.

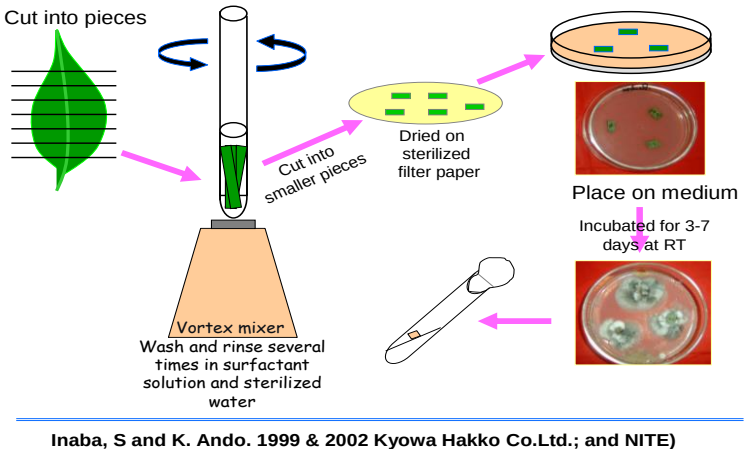


Figure (2) Procedure of isolation of endophytes (Ando, K. and S. Inaba (2004).

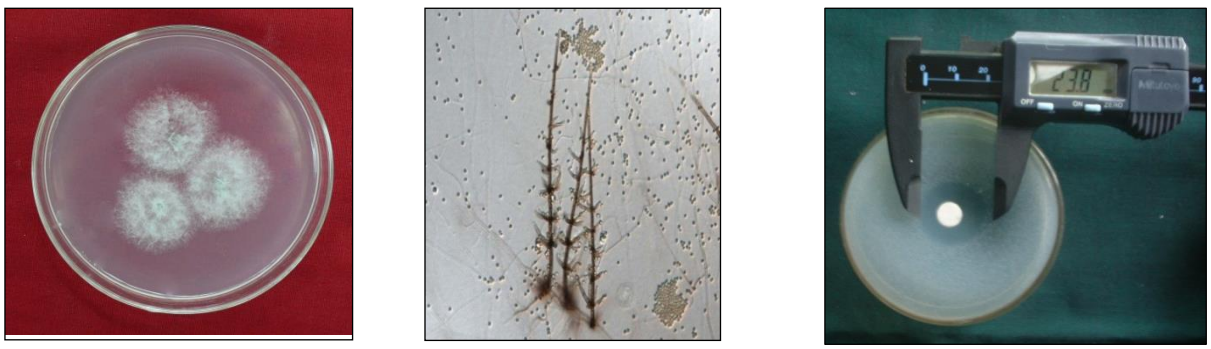
Results

Isolation of Endophytic Fungi

In the isolation of fungi, 18 endophytic fungi were isolated from six different mangrove plants (Table 2). Three fungi were isolated from *Aegialitis rotundifolia* Roxb., two fungi from *Avicennia alba* L., four fungi from *Avicennia marina* (Frosk.) Vierh., two fungi from *Aegiceras corniculatum* (L.) BL., three fungi from *Avicennia officinalis* L., and four fungi from *Ceriops decandra* (Griff) Ding Hou.

Screening (or) Preliminary study for antibacterial activity by paper disc diffusion assay

In the screening program, it was observed that fungus ET-01 showed the activity on *Micrococcus luteus* (15.3 mm), fungus ET-07 against *E. coli* (16.7 mm), fungus ET-08 against *E. coli* (14.1 mm), fungus ET-09 against *Bacillus pumalis* (13.3 mm), fungus ET-11 against *Bacillus subtilis* KY-327 (23.5mm), fungus ET-13 against *E. coli* (17.1 mm), fungus ET-14 against *Bacillus pumalis* (15.1 mm), fungus ET-16 against *Xanthomonas oryzae* (15.0 mm) and fungus ET-17 had the activity on *Bacillus pumalis* (12.7 mm). Among them, endophytic fungus ET-11 exhibited the highest antibacterial activity on *Bacillus subtilis* KY-327 (23.5mm) (Fig. 3). Therefore, this fungus ET-11 was selected for further investigations such as identification, fermentation and purification of metabolite. Fungus ET-11 was isolated from the leaf of *Aegiceras corniculatum* (L.) B.



A. Morphology of Fungus ET-11

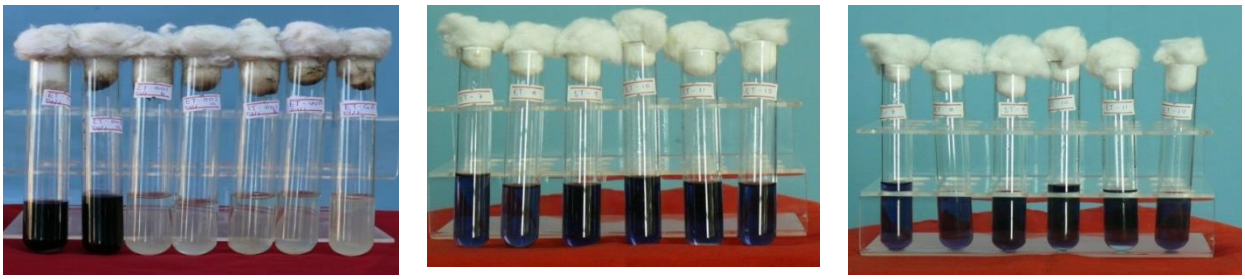
B. Photomicrograph of Fungus ET-11 (X 1000)

C. Antibacterial activity against *Bacillus subtilis* KY-327 (23.8 mm, clear zone)

Figure (3) Morphology, photomicrograph and activity of fungus ET-11.

Starch Hydrolysing Activity Test

It was observed that endophytic fungi ET-02, 03, 04, 05 and 06 showed the starch hydrolysing activities although other fungi did not show the starch hydrolysing activity (Fig. 4). Therefore, it was considered that fungi ET-02, 03, 04, 05 and 06 can be used for hydrolysis of starch to glucose.



Control ET-01 02 03 04 05 06 ET-07 08 09 10 11 12 ET-13 14 15 16 17 18

Figure (4) Starch hydrolysing activities of endophytic fungi.

Discussion and Conclusion

In the isolation of fungi, six different mangrove plants collected in the Pathein area were employed. Endophytic fungi were isolated by the method of Ando and Inaba, (2004).

In this study, 18 endophytic fungi were isolated from six different mangrove plants. Three fungi were isolated from *Aegialitis rotundifolia* Roxb., two fungi from *Avicennia alba* L., four fungi from *Avicennia marina* (Frosk) Vierh., two fungi from *Aegiceras corniculatum* (L.) BL., two fungi from *Avicennia officinalis* L., and four fungi from *Ceriops decandra* (Griff) Ding Hou.

In these tests, it was observed that endophytic fungus ET-11 exhibited the highest antibacterial activity on *Bacillus subtilis* KY-327 (23.5mm). So, fungus ET-11 exhibited more activities than other isolated fungi. Fungus ET-11 was isolated from the leaf of *Aegiceras corniculatum* (L.) BL. collected on Pathein-Chaung Tha Road (N 16° 52' 36" E 94° 23' 42"). Therefore, this fungus ET-11 was selected for further investigations such as identification, fermentation and purification of metabolite. Then, it was also found that fungus ET-02, 03, 04, 05 and 06 had the starch hydrolysing activities.

Test organism *Bacillus subtilis* KY-327 possesses DNA topoisomerase. According to Japan Kyowa Hakko Pharmaceutical Co. Ltd. (1993), DNA topoisomerase is synthesized during the tumor cell replication. On the other hand, much of this enzyme causes to replicate the tumor cell. If the synthesis of DNA topoisomerase can be inhibited, the growth of tumor cells or cancer cells will stop or cannot replicate.

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