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## RESEARCH NOTE

# Screening of Indole-3-Acetic Acid (IAA) Productions by Endophytic *Fusarium oxysporum* Isolated from *Phyllanthus niruri*

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**ABSTRACT**

Ten newly isolated endophytic fungi from *Phyllathus niruri* Linn. were identified as *Fusarium oxysporum*. These isolates were screened out for their productivity of indole acetic acid (IAA) by the salkowski's method. Out of these isolates, two isolates showed high amount of indole acetic acid production, which were FO9 and FO10 with concentration of 23.52 μg/ml and 5.95 μg/ml, respectively.

Keywords: *Phyllathus niruri,* endophytic *Fusarium oxysporum,* IAA, Salkowski's methods

*Phyllanthus niruri* Linn. has been reported to have several medicinal properties. This plant is popular in folk medicine for over 2000 years to treat jaundice, gonorrhea, frequent menstruation, and diabetes and topically as a poultice for skin ulcers, sores, swelling, and itchiness (Naik & Juvekar, 2003). Fresh leaves, fruits and also whole plant can be used to treat various diseases, particularly hepatitis and other viral infection (Paithankar *et al*., 2011).

Endophytes are microbes that colonized plant tissue while showing no external sign of infection or negative effect toward the host plant (Prasad & Dagar, 2014). Schulz and Boyle (2006) also stated that endophytes were considered mutualistic and non-pathogenic. Based from previous reports, endophytes that were isolated from medicinal plant for instance *Tinospora cordifolia, Calotropis procera, Coscinum fenestum,* and *Gynura procumbens* showed great potential (Bhore *et al*., 2010; Goveas *et al*., 2011; Kedar *et al*., 2014). An example of endophyte that were considered potential is endophytic *Fusarium oxsysporum*.

Based from various reports, endophytic *F. oxsyporum* was known as beneficial endophytes towards the some plant (Martinuz *et al*., 2013). This species was known able to enhance plant growth by inducing systemic resistance towards *Radopholus similis* on banana (zum Felde *et al*., 2009). Also, this species reported to produce phytohormone IAA (Cohen *et al*., 2002). Pytohormone IAA is a type of plant hormone known to stimulate cell elongation by modifying certain conditions such as increasing osmotic contents of the cell, increasing permeability of water into cell, decreasing wall pressure, an increasing cell wall synthesis and inducing protein synthesis (Mohite, 2013).

Several methods were developed to identify the presence of IAA such as High-Performance Liquid Chromatography (HPLC) or Gas Chromatography (GC). Nevertheless, these methods were time consuming and tedious (Glickmann & Dessaux, 1995). Alternative simple, rapid and cheap method to detect the presence of IAA is by using Salkowski's protocol (Glickmann & Dessaux, 1995). The solution to conduct the analysis known as Salkowski's reagent as the interaction with IAA will yields a pink colour on the solution (Werner & Gainess, 1967). According to Fierro-Coronado *et al.* (2014), Salkowski's reaction has a greater specificity for detecting IAA than other tested auxins.

In this experiment, ten endophytic *F. oxysporum* that were isolated from *P. niruri*

were screened with Salkowski's reagent to determine the production of IAA by these isolates.

*Phyllanthus niruri* samples were collected within the same area but from three different locations i.e. Dahlia College, External Laboratory of Faculty of Resource Science and Technology, and Tunku Abdul Rahman Putra Hall (DeTAR), Universiti Malaysia Sarawak (UNIMAS), Kota Samarahan.

The isolation was based on the procedure derived from Khan *et al.* (2012) with a few modifications. The plant samples were washed with tap water to remove the debris and other sand particles on the leaves and stem surfaces. As for the root surfaces, they were washed with running tap water for 12 hours to remove tiny sand particles before isolation. Then, the samples were immersed in 30% Clorox for 2 minutes and followed by 70% ethanol for 1 minute. Finally, the samples were rinsed with sterile distilled water and dried on sterile filter paper to remove excess water. Sterilized samples were printed on the media to prove the surface sterilize was achieved. After surface sterilization, the leaf, stem, and root segments were cut into approximately 0.5 $cm^2$ pieces using flame-sterilized scalpel. 10 segments were placed on each Petri dish containing water agar (WA) media and potato dextrose agar (PDA) media. The Petri dishes were sealed with Parafilm and incubated at 25$^oC$ for three to seven days. Obtained *F. oxysporum* isolates were then inoculated into new PDA media and incubated for further study.

To determine the amount of IAA produced by each isolate, a spectrophotometric technique was performed using the modified Salkowski's method (Bhagobaty & Joshi, 2009). The isolates were sub-cultured on PDA and incubated for seven days. Seven discs of fungal colony from the subculture were grown in potato dextrose broth (PDB) and incubated on a shaker at 28$^oC$ with 150 rpm/min for seven days. The broth was centrifuged at 5000 rpm for 25 min. 1 ml of supernatant was mixed with 4 ml of Salkowski's reagent (1 ml 0.5 $FeCl_3$; 50 ml distilled water; 30 ml concentrated $H_2SO_4$) and then incubated at room temperature and dark condition for 30 minutes. The colour change from colourless to pink or red indicates that the fungal endophytes have secreted IAA in the extract. The potential endophytes that had secreted IAA were quantified by using a spectrophotometer at the wavelength of 540 nm. IAA concentration of each potential fungal endophytes was compared to a standard curve.

The isolation of fungi from surface sterilized *P. niruri* normally allows the recovery of endophytic fungi. All isolates, obtained from *P. niruri*, were identified through molecular and morphological characteristics. In the present study, a total of 10 endophytic *F. oxysporum* isolates were isolated from *P. niruri.*

Ten *F. oxysporum* isolates were screened out for their productivity of IAA on spectrophometer. Out of these 10 isolates, two isolates (FO9 and FO10) showed ability to produce IAA. The isolate F09 produced the highest amount of IAA (23.52 μg/ml). Table 1 shows the results of ten endophytic *F. oxysporum* isolates after tested with Salkowski's reagent with their respective collection site and IAA concentrations.

In this study, most endophytic *F. oxysporum* isolates were isolated from the root. This is because *F. oxysporum* commonly found in the soils (Aimé *et al*., 2013). Thus, giving them advantage to colonize the plant root (Bacon & Yates, 2006). Moreover, plant roots are the most favourable habitat for microbes including endophytes (Dasri *et al*., 2014).

Fungal extracts from FO9 and FO10 were the only two isolates that changed their colour from colourless to pink which were the isolates from DeTAR and External Laboratory (Faculty Resource Science and Technology), respectively, while others remain colourless. Several earlier reports showed that endophytic *F. oxysporum* has the ability to produce IAA (Cohen *et al*., 2002; Hasan, 2002). But, in this experiment, only 20% of the endophytic *F. oxysporum* isolates had the ability. Mohite (2013) suggested that endophytes producing IAA were varied among strains. Furthermore, Khalid *et al.* (2003) also suggested that secretion ability of

**Table 1.** Colour assays and IAA concentration of *F. oxysporum* isolates that were isolated from samples collected from three different places (Dahlia College, Faculty Resource Science and Technology External Laboratory [FRST-EL] and Tunku Abdul Rahman Putra Hall [DeTAR]) and occurrence (leaf, stem, and root).

| Isolates | Plant Samples collected | Occurrence in plant parts | Changes in colour after treated with Salkowski's reagent | IAA concentration (μg/ml) |
|---|---|---|---|---|
| FO1 | FRST-EL | Root | - | 0 |
| FO2 | FRST-EL | Root | - | 0 |
| FO3 | Dahlia College | Root | - | 0 |
| FO4 | FRST-EL | Root | - | 0 |
| FO5 | FRST-EL | Stem | - | 0 |
| FO6 | FRST-EL | Root | - | 0 |
| FO7 | FRST-EL | Root | - | 0 |
| FO8 | DeTAR | Root | - | 0 |
| FO9 | DeTAR | Root | + | 23.52 |
| FO10 | FRST-EL | Root | + | 5.95 |

metabolites by endophytes could be affected by different genetic makeup, growth kinetics and enzymatic activities involved in auxin synthesis under given cultural conditions. FO9 showed the highest IAA concentration with 23.52 μg/ml followed by FO10 with only 5.95 μg/ml. This difference may be influenced by several factors such as culture condition, growth stage and substrate availability (Mohite, 2013).

As the conclusion, fungal endophytes from the same species but different strains have different ability on producing metabolites. This may also affect other fungal characteristic, abilities and modes. This shown that endophytic *F. oxysporum* has bright future for biotreatment in agriculture development.

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