Antagonistic Endophytic Fungi of *Globba pendula* Roxb. from Taman Hutan Raya, North Sumatra against *Staphylococcus aureus* ATCC® 29213™

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Abstract. *Globba pendula* Roxb. is a species in Zingiberaceae with prospective source of novel bioactive compounds. Traditionally, the roots or rhizomes have been used to treat several ailments in South Sumatra, Indonesia. Bioprospecting of its bioactive compounds is still limited along with its associated microorganisms, especially the natives one in North Sumatera. In this study, we conducted a preliminary screening of 16 (sixteen) fungal endophytic isolates from rhizome of *G. pendula* in Taman Hutan Raya, North Sumatera. The isolates were subjected against *Staphylococcus aureus* ATCC® 29213™ using dual culture plate assay. The sixteen isolates were differentiated morphologically by considering characters such as: Form, Elevation, Margin, Color, Texture, Concentric ring and Radial lines. All isolates were antagonists of *S. aureus* with different performances. Two isolates exhibited very strong antagonistic activity (+++), 13 isolates with strong activity (++), and only one with mild activity (+). The results suggest that fungal endophytes from *G. pendula* may be used as source of antimicrobial compounds. The molecular identification of endophytes is currently under investigation.

1. Introduction

*Globba* spp. are members of Zingiberaceae with potential use as either ornamental or medicinal plant in Southeast Asia, comprising about 100 species distributed around region [1,2]. In India, some of the species were utilized as traditional medicine. The rhizome of *G. bulbifera* is known to possess anti-asthma, cough reliever and anti-venom activities by the local tribes [3]. In Thailand, *G. williamsiana* was propagated to produce a large industrial scale of products as ornamental plants with high economical value [4]. In Indonesia, some tribes from South Sumatra have been using *G. pendula* as ethnobotanical medicine to treat post-partum flatulence and mouth ulcer [5]. Despite being reported in separate study among region, there is still limited information regarding its bioprospective study regarding the aspect of plant-microbial interaction in the form of endophytic associations.

Being relatives with other members of Zingiberaceae, members of *Globba* spp. may exhibit the same potential as source of bioactive natural compounds. There has been an increasing interest to uncover the inner microorganisms living within medicinal plants. The rationale of studying the microorganisms so called endophytes is supported by evidence that endophytes also synthesize an intermediate or even complete metabolites as host [6]. Yet, a novel compound is suspected to exist among strains producing the natural compound especially in fungal endophytes [7].
A comprehensive report on Sumatran *Globba* has shown that Sumatra is a potential biodiversity spot. Sixteen species of *Globba* with five varieties may be found across Sumatra island [1]. In North Sumatera, four species of *Globba* are reported in Hutan Sibayak, namely *Globba aurantiaca*, *G.paniculata*, *G.patens* and *G.pendula* [8]. Based on our exploration, *Globba pendula* Roxb. is the most frequently found species of *Globba* in Taman Hutan Raya. In this study, we presented a preliminary result of antagonistic fungal endophytes from rhizome of *Globba pendula*, as one aspect of our comprehensive study to unravel the fungal endophytes residing within each species of Zingiberaceae found in North Sumatera.

2. Materials and Methods

2.1 Plant Material

Fresh *Globba pendula* rhizome were collected randomly from Taman Hutan Raya, Deli Serdang regency, North Sumatera province, Indonesia. Plant rhizomes were removed from soil debris and stored in plastic bags. Specimens were identified to species level at Herbarium Medanese, Universitas Sumatera Utara.

2.2 Isolation of Endophytic Fungi

Standard surface sterilization is used as isolation method of endophytic fungi of *G.pendula* [9]. Pieces of rhizome, *G.pendula* were dipped consecutively into following sterilization solutions: 75% ethanol (2 min), 5.3% NaOCl (5 min) and 75% ethanol (30 secs). Sterilized pieces were washed with sterile distilled water to remove remaining solutions. Sterilized pieces were blotted to dry using Whatman filter paper and cut into a further smaller pieces (1–2 cm). The pieces were plated on Potato Dextrose Agar (Oxoid™) supplemented with 0.1% chloramphenicol as antibiotic for bacterial growth. Isolation plates were incubated at 25 – 28 °C for 1 wk to maximize fungal growth from each pieces. Observed fungal mycelium were observed daily and small portion was inoculated into new medium to preserve the pure fungal cultures. Morphological characteristics of each fungal isolates were recorded such as: form, elevation, margin, color (surface and bottom), texture, concentric ring and radial line.

2.3 Antagonism Assay of Endophytic Fungi

As a preliminary screening of antagonistic fungal endophytes, we used a sensitive strain of *Staphylococcus aureus* subsp. aureus Rosenbech ATCC® 29213™. Antagonism assay performed based on colony v.s colony in dual culture plate assay with modification of techniques [10]. Colony suspensions of *S.aureus* were made by picking up colonies into sterile physiological saline solution (0.95% NaCl) to obtain OD_{600}=0.5. Aliquot of suspension were mixed with 15 mL molten PDA (45°C), supplemented with 1% (w/v) yeast extracts for the growth of pathogen. Based on our observation, ratio of cell suspension with molten agar must be adjusted in 1 : 15 to make a compact lawns. Three plugs of fungal endophytes mycelium were placed on top of PDA as replicates. Plates were incubated at 25 – 28 °C for 2 days. Mycelial plugs exhibiting clear zones around fungal colonies were designated as antagonistic isolate and diameter of inhibition zone (IZ) were in a millimetre unit (mm).

3. Results and Discussion

Sixteen fungal morphotypes were recovered from rhizomes of *G.pendula* sampled from Taman Hutan Raya as one of representative spot of *Globba* diversity in North Sumatera (Table 1). The results of antagonism assay against *S.aureus* ATCC® 29213™ are presented in Table 2. Majority of antagonistic isolates were categorized as strong antagonists (21 ≥ IZ ≥ 30 mm) (Figure 1).
Table 1. Morphological characteristics of isolated fungal endophytes from *G. pendula*

| Code  | Form       | Elevation | Margin    | Color         | Texture   | Concentric Ring | Radial Line |
|-------|------------|-----------|-----------|---------------|-----------|-----------------|-------------|
| S6G4  | Irregular  | Flat      | Undulate  | Black         | Grey      | Flourish        | -           |
| S6C5  | Irregular  | Raised    | Filiform  | Pinkish White | Reddish White | Flourish        | -           |
| S6A5  | Filamentous| Raised    | Entire    | Black         | Grey      | Flourish        | -           |
| S6B1  | Irregular  | Raised    | Undulate  | Yellowish White | Brown        | Smooth          | -           |
| S6D1  | Irregular  | Raised    | Undulate  | White         | White     | Smooth          | -           |
| S6C4  | Rhizoid    | Umbonate  | Lobate    | White         | White     | Smooth          | -           |
| S6B4  | Filamentous| Raised    | Filiform  | Grey          | Black     | Cottony         | -           |
| S6B2  | Irregular  | Raised    | Undulate  | Black         | Black     | Dull            | -           |
| S6A1  | Irregular  | Flat      | Undulate  | Light orange  | Light orange | Cottony         | -           |
| S6A2  | Irregular  | Flat      | Undulate  | Black         | Cream     | Flourish        | -           |
| S6G1  | Irregular  | Raised    | Undulate  | White         | Light brown | Cottony         | -           |
| S6C3  | Circular   | Umbonate  | Filiform  | Grey          | Black     | Flourish        | -           |
| S6D2  | Circular   | Raised    | Filiform  | White         | White     | Smooth          | -           |
| S6G2  | Irregular  | Flat      | Undulate  | Light pink    | Pink      | Smooth          | -           |
| S6G3  | Circular   | Umbonate  | Entire    | Grey          | Brown     | Flourish        | -           |
| S6C2  | Filamentous| Flat      | Filiform  | Pinkish White | Orange    | Flourish        | -           |

Table 2. Antagonism result of endophytic fungi against *S. aureus* ATCC® 29213™

| Locality       | Isolate Code | Diameter of Inhibition Zone (mm) |
|----------------|--------------|----------------------------------|
| Taman Hutan Raya | S6A1         | 27.5                             |
|                | S6A2         | 25.07                            |
|                | S6A5         | 27.3                             |
|                | S6B1         | 30.63                            |
|                | S6B2         | 26.33                            |
|                | S6B4         | 26.9                             |
|                | S6C2         | 32.47                            |
|                | S6C3         | 20.37                            |
|                | S6C4         | 26.6                             |
|                | S6C5         | 19.03                            |
|                | S6D1         | 27.0                             |
|                | S6D2         | 29.87                            |
|                | S6G1         | 26.53                            |
|                | S6G2         | 26.77                            |
|                | S6G3         | 25.2                             |
|                | S6G4         | 23.57                            |

Information regarding the plant-microbial association in *Globba* spp. is still limited. A novel actinomycete namely *Micromonospora globbae* was isolated from the roots of *Globba winitii* C. H. Wright [11]. Contributing to previous report, this study obtained fungal endophytes which need a further molecular identification to uncover a possible novel strains.

Most studies of *Globba* spp. were focused on characterizing the essential oils within certain plant parts. Essential oils recovered from whole plants of *G.schomburgkii* and *G.ophioglossa* were identified as major group of β-caryophyllene and caryophyllene oxide [16]. Rhizomes of *G.reflexa* from India were reported to contain a labdane-type diterpene [17]. The volatile constituents of the rhizomes and aerial parts of *G.sessiliflora* were also reported to contain β-caryophyllene followed with
minor constituents of other diterpenes. Effort to link the presence of fungal endophytes in \textit{G.pendula} with synthesis of major diterpene compounds by the plant in the form of $\beta$-caryophyllene, will be further investigated.

Endophytic fungi isolated from medicinal plants are proved to be an effective source to produce new antibiotics against bacterial infection. Ayurveda practice of theurapetics in India is now followed by an extensive laboratory studies and evidence leading to the rising development of endophytic fungi from medicinal plants [12]. Four medicinal plants i.e. \textit{Rheum emodi}, \textit{Hypericum perforatum}, \textit{Diocoria deltodia} and \textit{Artemisia annua} were reported to be inhabited by 28 fungal endophytes. The fungal endophytes were further screened as antibacterial-producing strain based on MICs value from tested Dichloromethane extracts [13].

Crude extracts of fungal endophytes isolated from \textit{Cymbopogon caesius} is highly active against \textit{S.aureus} using agar well diffusion method. The extracts were obtained from liquid-liquid partition using ethyl acetate as organic solvent [14]. Relative to \textit{Globba} spp., the \textit{Zingiber cassumunar} was reported to harbor forty-four fungal endophytes exhibiting antibacterial activity. The ethyl acetate extracts of mycelial extracts were found to highly active against \textit{S.aureus} and \textit{Escherichia coli} with MIC value of 31.25 and 7.81 $\mu$g/mL, respectively[15]. In general, further step in screening potential strain must be evaluated through extraction of either mycelial or mycelial-free fermentation broth to give a concise result of antibacterial activities.

4. Conclusions

In this study, we successfully isolated 16 species of fungal endophytes from \textit{Globba pendula} Roxb. rhizome with locality from Taman Hutan Raya, North Sumatera. All isolates were antagonists of \textit{S.aureus} with high inhibitory performances. Most fungal endophytes (13 out of 16) showed strong
antagonism against Staphylococcus aureus ATCC® 29213™ while two strains namely S6B1 and S6C2 were the strongest antagonists displaying Inhibition Zone (IZ) > 30 mm.

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