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Original article

Diversity and biotransformative potential of endophytic fungi associated with the medicinal plant *Kadsura angustifolia*

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Abstract

This study investigated the diversity and host component-transforming activity of endophytic fungi in medicinal plant *Kadsura angustifolia*. A total of 426 isolates obtained were grouped into 42 taxa belonging to Fungi Imperfecti (65.96%), Ascomycota (27.00%), Zygomycota (1.64%), Basidiomycota (0.47%) and Mycelia Sterilia (4.93%). The abundance, richness, and species composition of endophytic assemblages were significantly dependent on the tissue and the sampling site. Many phytopathogenic species associated with healthy *K. angustifolia* were found prevalent. Among them, *Verticillium dahliae* was dominant with 16.43% abundance. From 134 morphospecies selected, 39 showed remarkable biocatalytic activity and were further identified as species belonging to the genera *Colletotrichum*, *Eupenicillium*, *Fusarium*, *Hypoxylon*, *Penicillium*, *Phomopsis*, *Trametes*, *Trichoderma*, *Umbelopsis*, *Verticillium* and *Xylaria* on the basis of the sequence analysis of the internal transcribed spacer (ITS1-5.8S-ITS2). The results obtained in this work show that *K. angustifolia* is an interesting reservoir of pathogenic fungal species, and could be a community model for further ecological and evolutionary studies. Additionally, the converting potency screening of some endophytic fungi from this specific medicinal plant may provide an interesting niche on the search for novel biocatalysts.

Keywords: Biotransformation; Diversity; Endophytic fungi; *Kadsura angustifolia*; *Verticillium dahliae*

1. Introduction

Accumulating evidence has demonstrated that fungal endophytes are hyperdiverse and abundant groups. Fungal endophytes can be found colonizing the tissues of all plants, irrespective of their taxonomical affiliation or environmental preferences. Structure and composition of fungal communities is known to be influenced by many factors such as geographic locations, climatic patterns, seasonality, host plant identity, structure and diversity of surrounding vegetation, physiology and specificity of the colonized tissues, etc. [1,2]. Consequently, different fungi forming distinctive endophytic communities are specific to each environmental condition and tissue type as a result of adaptation to these different environmental conditions. These fungi have profound impacts on plant hosts in many ways: some impact plant fitness, some affect plant disease resistance and susceptibility, and some decompose plant litter [3]. However, the vast majorities of fungal endophytes and their ecological significance have yet to be adequately characterized but are assumed to vary according to host and environment.

It is known many of these endophytic fungi have been sought and characterized for their ability to produce biologically active secondary metabolites with potential uses in

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medicine, agriculture, and other areas. It has been proposed that endophytes have acquired a diversity of biosynthetic capabilities through years of coevolution and genetic recombination with their host plant [4]. Furthermore, the symbiotic relation with the plant forces them to deal with several toxic compounds produced by their hosts as defense against other organisms. The existence of biodegradation and biotransformation processes of the toxic substances by the help of certain specific enzymes has enabled the endophytes to survive. In 1997, Werner and coauthors [5] reported the first discovery on the biotransformation of endophytic fungi. They found aphelandrine, a macrocylic polyamine alkaloid obtained in the roots of different species of the genus *Aphelandra*, could be metabolized by several endophytes from the roots of host plant. Later, several authors also reported some endophytic fungi were capable to transform some natural products, and metabolize drugs [4,6]. Compared with chemical synthesis, the biotransformation process provides a number of advantages such as producing novel compounds, enhancing the yield of a desired compound, overcoming the problems associated with chemical analysis, and leading to basic information to identify their biosynthetic pathway [7]. Therefore, more recently, endophytes as an underexplored source of biocatalytic diversity have received attention as potential sources of novel biocatalysts in the chemical transformation of natural products and drugs [8].

Modern phytochemical and pharmacological studies have shown that Schisandraceae plant is a rich sources of lignans and lanostane- and cycloartane-type triterpenoids, which possess various beneficial pharmacological effects such as anti-inflammatory, anti-tumor, anti-HIV activities, etc. [9,10]. Over the past ten years or so, the discovery of a series of highly oxygenated triterpenoids with different skeletons has further increased the interest in this family. Of these Schisandraceae plants, we assumed that *Kadsura angustifolia* could be a community model for further ecological and evolutionary studies, as well as studies of the interactions among endophytic microorganisms and their relationship with the host plant due to its relative single chemical composition, in which the content of an A-ring-secocycloartene triterpenoid, nigranoic acid in the root and stem of *K. angustifolia* collected at Xichou of Yunnan province can reach up to 3.8% (unpublished). Previously, our study has indicated that nigranoic acid may be a precursor of biosynthesizing those highly oxygenated triterpenoids [11]. Here, we wonder if any endophytic fungi may biotransform nigranoic acid to highly oxygenated triterpenoids. A crucial first step for understanding the relationship between plant and endophytic fungi, and the influence by their environment is to clarify the fungal community of the *K. angustifolia* plant. Thus, our present research was to isolate and characterize the maximum number of fungal isolates in selected plants to determine the diversity, distribution of endophytes and their host component-transforming activities. To the best of our knowledge, this is the first report of the endophyte community associated with *K. angustifolia*.

2. Materials and methods

2.1. Plant material

Healthy and asymptomatic stems and roots of *K. angustifolia* were collected in November 2012 at Xichou and Maguan of Yunnan province of China, lying approximately 55.8 km apart. Samples were taken from five *K. angustifolia* plants per site (a total of ten plants) separated by a variable distance ranging from 100 to 500 m. The plants were chosen randomly, without regard to their age or their size. All plant materials were immediately brought to the laboratory, and stored at 4 °C in refrigerator. Each sample tissues were used within 24 h from collection. Finally, 100 segments from each tissue part were plotted for the isolation of endophytic fungi.

2.2. Fungal isolation and identification of morphology

To eliminate epiphytic microorganisms, all the *K. angustifolia* samples were thoroughly washed in running tap water to remove debris, and then air-dried naturally. The dry samples were individually surface sterilized with 75% ethanol for 5 min and rinsed with sterile distilled water 3 times, followed by immersion in 0.1% mercuric chloride (HgCl) (v/v) for 3–5 min. Afterwards, the samples were rinsed 3 times in sterile distilled water and then the stems and roots of the samples were cut into 0.5-cm lengths, and transferred to potato dextrose agar (PDA) medium supplemented with 60 μg/ml of streptomycin and 100 μg/ml of ampicillin using an aseptic technique. The inoculated plates were incubated at 28 °C in darkness for 2–15 days to allow the growth of endophytic fungal hyphae, and checked regularly. Pure isolates were checked for purity and transferred to another PDA plate by the hyphal tip method [12]. To ensure that the surface sterilization had removed all hyphae and chlamydospores externally adhering to the segments, they were placed in PDA agar plates and incubated at 28 °C in darkness. Only segments that were negative in this test were used for isolation of endophytes. All pure isolates were numbered and stored on the surface of PDA slants at 4 °C.

All fungal endophytes were identified based on morphological characterizations. Fungal characterizations were made according to their macroscopic characteristics of the colonies (color, aspect and presence of pigmentation) and microscopic characteristics (morphology of vegetative spores' structures). Standard taxonomic manuals were used to identify the fungal genera and species [13–16]. Some isolates sporulated readily on PDA media after 7 days of incubation in darkness at 28 °C. Isolates that did not sporulate were placed in darkness at room temperature for up to 20 days to stimulate sporulation. Isolates that failed to produce spores were treated as sterile mycelia.

2.3. Statistical analysis of data

Colonization frequency (CF) of fungal genera was calculated using the following formula: $CF = N_{COL}/N_t \times 100$,