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Short Communication

Identification of a taxol-producing endophytic fungus EFY-36

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Morphological and molecular methods were used to identify the statues of an isolate, EFY-36, a taxol-producing endophytic fungus. Based on the morphology of the fungal colony, the mechanism of spore production and the characteristics of the spores, the isolate is *Mucor* spp. Furthermore, the analysis of 18S ribosome RNA sequence of the isolate was achieved using PCR technology. The results showed that the 18S ribosome RNA sequence of the isolate has a higher homology relationship with other taxol-producing endophytic fungi; *Pestalotiopsis* sp., *Seimatoantlerium* sp. and *Pestalosphaeria* sp.

Key words: Endophytic fungus, fungi classification, *Mucor* spp., molecular identification.

INTRODUCTION

Produced by and purified from *Taxus brevifolia*, taxol (paclitaxel) has become a widely used cancer drug in clinical treatments of breast and ovarian cancers. Due to the rapid growing market, current industrial production of taxol by semi-synthesis that consumes large amount of taxus trees cannot meet the requirement of the market (Ji et al., 2006). Therefore, scientists around the world had found a new approach to the industrial production of taxol. Since 1993 when the first taxol-producing endophytic fungus was reported, scientists have attempted to find a high taxol-producing endophytic fungus so as to apply it to the industrial production of taxol. Up to now, no fungus strain is applied to the industrial production process because the taxol contents from isolates are too low for the industrial process. In order to solve the problem, there are two approaches: one is to improve taxol-producing fungi which have been isolated and the other is to continue isolating new endophytic fungus from natural sources. However basic researches for endophytic fungi are necessary. In our study laboratory, two projects are been carried out at the same time. While a series study results have been reported (Guo et al, 2006; Zhou et al., 2006; Wang et al., 2007; Zhou et al., 2008). In this paper, we report the experimental result of the identification of a taxol-producing endopfytic fungus EFY-36 with two different methods. Although the isolate was reported in previous publications (Zhou at al., 2007), the biological characteristic of the fungus was not particularly described. This study provides information roundly on morphological identification and laid the foundation to further exploring molecular techniques in fungi identification.

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EXPERIMENTAL PROCEDURES

The primary organism used in this study was isolate EFY-36, anamorph, originally obtained as an endophyte of *Taxus chinensis var. mairei* near Chongqing, Southwest, People's Republic of China. Isolation and identification of endophytic fungus was adopted with conventional method. The morphometrics of endophytic fungus was classified according to the morphology of the fungal colony, the mechanism of spore production and the characteristics of the spores. The analysis of endophytic fungus 18S ribosome RNA sequence used PCR cloning technology. DNA was extracted by the CTAB method. 18S ribosome RNA sequence was isolated by PCR using primers 18SPF (5'-GGAAGGGRTGTATTTATT AG-3') and 18SPR (5'-CCTCTAAATGACC AAGTTTG-3'). The amplification was performed in a GeneAmp PCR System 2400 for 30 cycles with 35 sec at 94℃, 35 sec at 50℃ and 90 sec at 72℃. After the final cycle, the amplification was extended for 10

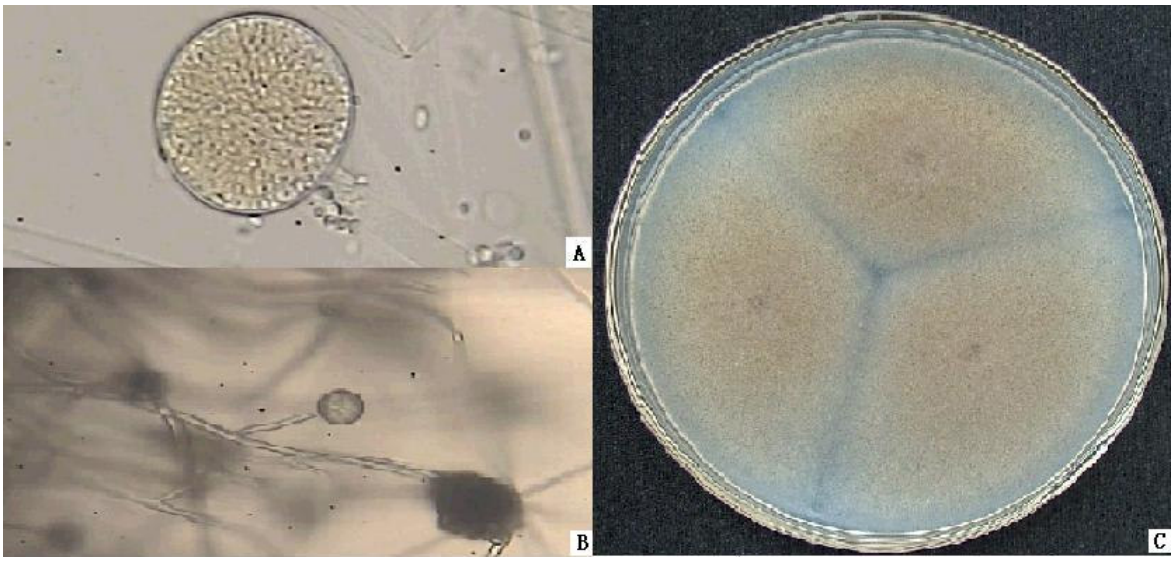

Figure 1. A. Culture characteristic of the fungus EFY-36: The sporangium and spores with two shape: rotundity and ellipsoid. **B.** Culture characteristic of the fungus EFY-36: The sporangiophore and its branch; magnified micrograph of the fungal mycelium (magnification: 400 ×). **C.** Culture characteristic of the fungus EFY-36: The morphology of the fungal colony.

min at 72°C. The PCR product was cloned into the pMD18-T vector (TaKaRa) and sequenced with DYEnamic Direct dGTP Sequencing Kit (Amersham) and a 373A DNA sequencer. The analysis and comparison of the sequence were performed with nucleotide blast of GenBank (http://www.ncbi.nlm.nih.gov). The phylogenetic tree was produced using BLAST pairwise alignments.

RESULTS

Fungal taxonomy

After culture, the brown grey colony had a loose texture and a height of 0.2 - 0.5 cm (Figure 1C). Some of the sporangiophore had a small branch and was 7 - 12.5 µm in diameter. Sporangia were spherical, yellow and about 27.5 – 45 µm in diameter. After maturity, the sporangia wall was digested. The columella was nearly spherical or nearly oval. The former was 20 - 32.5 µm in diameter, and the later 22.5 – 30 × 27.5 - 35 µm. There was no receptacle at the junction of columella and sporangiophore. Sporan-giospore was nearly spherical or oval to nearly oval. Nearly spherical spore was 3 – 5 µm in diameter and oval to nearly oval spore was 3 – 5 × 4 - 7.5 µm in size (Figures 1A and B).

18S ribosome RNA sequence analysis

A 1555-bp fragment was amplified from the DNA of EFY-36 strain, the sequence of which showed similarity with other endophytic fungus as revealed by nucleotide blast search. The percentages of identity between *Pestalotiopsis* sp. NG12-30, *Pestalotia rhododendri*, *Seimatoantlerium* sp. and *Pestalosphaeria* sp. NE-32 were found to be 99%. Phylogenetic tree analysis indicated that 18S ribosome RNA sequence of EFY-36 strain is closely related to *Pestalotiopsis* (Figure 2).

DISCUSSION

According to earlier literatures, it had a diversity of the taxol-producing endophytic fungus. Since 1993, observations of taxol-producing fungi, *Taxomyces andreanae* and *Pestalotiopsis microspora* had been reported. From then on, although more than a decade, fungi isolated from various plants was filamentous fungus the classification status and characteristic of which was different and included *P. microspora*, *Nodulisporium sylviforme*, *Fusarium lateritium, Mucor, Penicillium, Ozonium* and *Tubercularia* (Metz et al., 2000; Strobel et al., 2003; Wang et al., 2006; Zhou et al., 2007; Chi et al., 2008; Chakravarthi et al., 2008). Among them, *Mycelia sterilia* is predominant (Chen et al., 2002), but their improvement by genetic engineering is relatively difficult.

The systematic classification of fungi has been challenging, because it almost does not have existing fossil. Their morphology is simple and most of their classification was based on the morphology; although the systematic classification of fungi does not consist of a systematic evolution relationship (Miao et al., 2007). Our morphological results show that the isolate, EFY-36 is *Mucor* spp. Although it is not fully important to identify the isolate for industrial production of taxol-producing fungi, the basic researches are necessary. DNA, as a vector of

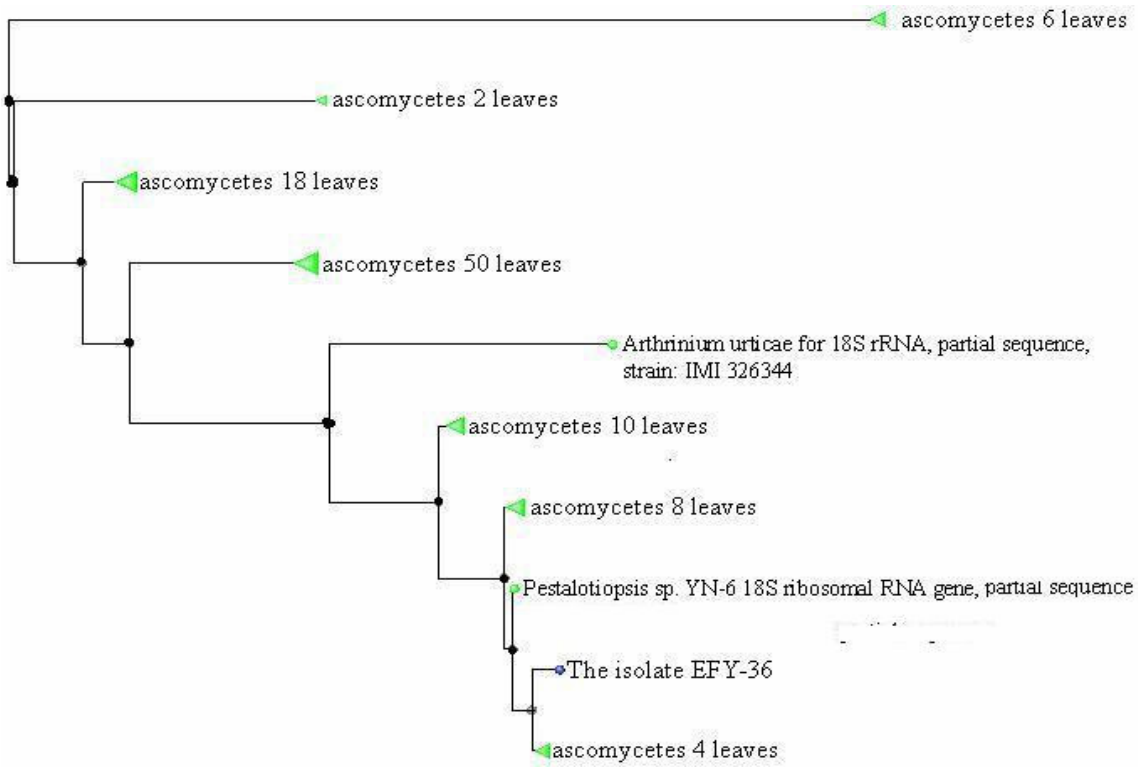

Figure 2. Phylogenetic tree based on 18S ribosome RNA sequence of EFY-36. This phylogenetic tree was produced using BLAST pairwise alignments.

genetic information is the basis of fungal morphology and also the evidence of classification and determination. Many molecular research methods based on genomic DNA have their limitations in application; so molecular classifications replacing the traditional morphological classification have a long way to go. In this work, molecular techniques were further applied to identify the isolate.

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