

Chemical constituents of fungus F03 belonging to Basidiomycota

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Abstract: Filamentous fungus F03 belonging to Basidiomycota was obtained and identified as *Phlebiopsis crassa* based on ITS sequence when *Morchella*. sp was isolated from the wild fruiting body by spores releasing method. Chemical constituents were separated by gel chromatography, HPLC and recrystallization. Structures of compounds were confirmed by NMR data. Four products orsellinic acid (1), α -nigerose (2), uridine (3), N-(4-hydroxyphenyl)acetamide (4) were identified and all compounds were isolated from the genus *Phlebiopsis* for the first time.

Keywords: *Phlebiopsis crassa* Basidiomycota chemical constituents ITS sequence.

INTRODUCTION

Microbial secondary metabolites play important role in discovering new drugs or new compounds. Fungi have been proven to be good sources of bioactive compounds. At present, more and more active substances and new compounds with activities such as anti-inflammatory (Zhang *et al.*, 2017), antiplasmodial activity (Huang *et al.*, 2018), inhibition of enzymes (Chen *et al.*, 2017) antibacterial (Aharwal, 2014), anticancer (He *et al.*, 2017), antioxidant (Sadrati *et al.*, 2013) were found from microorganism, especial from fungi. Basidiomycota and Ascomycota are the two large subphyla. Basidiomycota are filamentous fungi composed of hyphae and reproduce sexually via basidia and basidiospore. Compared to Ascomycota, study on chemical constituents of filamentous belong to Basidiomycota (except microfungi) was less and later.

The reports on metabolites from Basidiomycota mainly focus on fruiting body (Habibi *et al.*, 2015; Schuffler and Anke, 2018). A filamentous fungus F03 was obtained when *Morchella*.sp ZY-1 was isolated from wild fruiting body by spores releasing method. The strain was then identified as *Phlebiopsis crassa* (Lev.) Floudas & Hibbett 2015 on the basis of ITS rDNA sequence.

The chemical constituents of F03 was investigated for the first time. Four products were isolated and identified from the genus firstly.

MATERIALS AND METHODS

Instruments and reagents

NMR spectra were recorded on an Agilent DD2400-MR instrument at 400MHz for ¹H-NMR and 100 MHz for

¹³C-NMR. Chemical shifts were showed with reference to TMS. Semi-preparative HPLC was performed with LC3000 system (Beijing, China) using an Ultraviolet detector and ODS column (5 μ m, 10 \times 250mm, YMC, Kyoto, Japan). The detection wavelength was 210 nm. Visualization of the TLC experiments was performed on plates precoated with silica gel GF254 (Qingdao Oceanic Chemicals, China). Column chromatography was performed using silica gel (300-400 mesh, Qingdao Oceanic Chemicals, China). Analytical grade solvents and HPLC grade methanol was purchased from Fuyu Chemical Industry Company, Tianjin, China and Tedia Company, Inc, USA, respectively. The fermentation broth was carried out on BS-2F thermostatic shaker (Changzhou Huaguan Instrument Co., Ltd., Jiangsu, China).

Fungal material

The microbial strain F03 was isolated from *Morchella* (collected from the Tuanze town of Zunyi, Guizhou), when *Morchella* was separated by spores releasing method. When *Morchella* was isolated from the wild fruiting body, three fungal colonies different from *Morchella* were found. Genomic rDNA was extracted according to the instructions of extraction kit for fungal genomic rDNA. Then, primers of ITS1 (TCCGTAGGT GGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGA TATGC) were applied for polymerase chain reaction. The procedure was run as follows: predenatured at 94°C for 5 min, denatured at 94°C for 30 s, annealed at 58°C for 30 s, and extended at 72°C for 1 min, for a total of 30 cycles, 72°C total extension for 7min. The sequence of PCR products was sent to Boyoushun Sequencing Biotechnology Co., Ltd., Beijing, China. Blast software was used to compare the sequence in Genbank database on the NCBI. The phylogenetic tree constructed by a

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neighbor-joining method that revealed the relationship of F03 with a group of *Phlebiopsis* species (fig. 2).

Fermentation and extraction

The mycelia of F03 strain were aseptically picked from slants to 250mL Erlenmeyer flasks containing 100mL PDA liquid media and were poured into another 1000mL Erlenmeyer flask containing 400mL PDA liquid media

after 3-4 days at 28°C and 150r/min on a shaker. A total of 22L broth was incubated for 15 days. Then cultures were extracted by EtOAc three times, which was further evaporated under reduced pressure to yield a crude extract (3.1g).

Isolation and identification

The EtOAc extract (3.1g) was subjected to normal phase

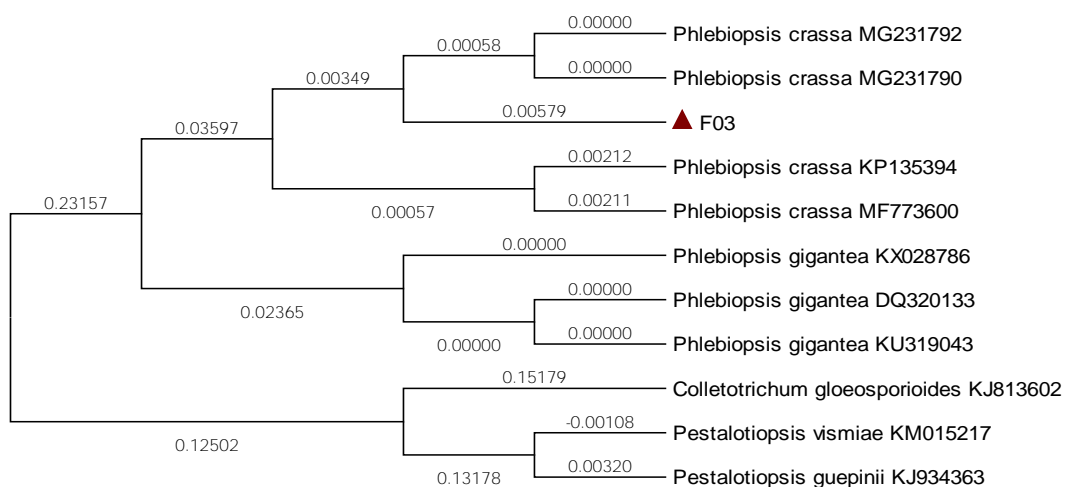


Fig. 1: Phylogenetic tree by Neibor-Joining base on ITS1+5.8S+ITS2



Fig. 2: The colony of F03

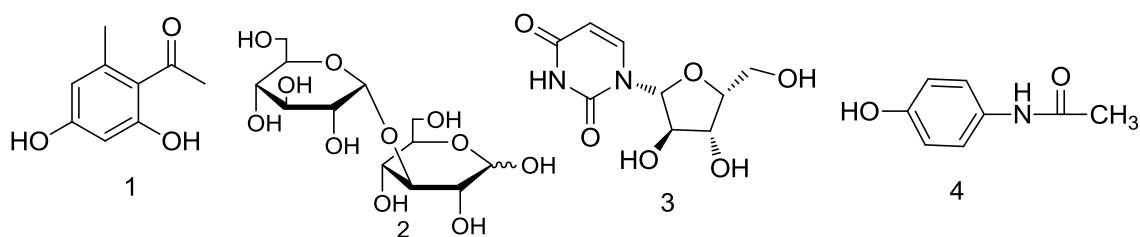


Fig. 3: The structures of compounds 1-4

silica gel column chromatography (120g, 300-400 mesh), gradually eluted with petroleum ether-EtOAc (6: 1→1: 8, v/v) to obtain 5 fractions (Fr.1 ~ Fr.5). Fr.2 was further crystallized by acetone to afford compound 1 (4.7mg). Fr.5 was purified by repeated silica gel column using a gradient elution with CHCl₃-MeOH (15: 1→8: 1, v/v) to afford compound 2 (12.5mg) and further crystallized by acetone to give compound 3 (6.8mg). Fr.3 was purified by preparation of thin-layer chromatography with CHCl₃-MeOH (30: 1, v/v) to afford compound 4 (5.2 mg). The structures of metabolites were confirmed by NMR data and references.

RESULTS

Identification of F03

On PDA medium, hyphae of F03 were dense and aerea hyphae grew very well, white, loosely. The edges of the colonies were neat and the old mycelia were pale (fig. 1).

Blast on Genbank database, F03 was identified as *Phlebiopsis crassa* belonging to Basidiomycotina with 99% similarity. Phylogenetic tree by Neibor-Joining sequence by Mega 5.1 (fig. 2).

Metabolites isolated from strain F03

Four compounds were obtained from the EtOAc extract (fig 3). Compound 1: white crystals. There were two aromatic proton signals δ 6.12 and δ 6.07 in ¹H-NMR. Correspondingly, six aromatic carbon signals were observed in ¹³C-NMR. It was four substituted benzene rings. ¹H-NMR (CD₃OD, 400 MHz) δ : 6.18 (1H, s, H-5), 6.13 (1H, s, H-3), 2.48 (3H, s, H-8); ¹³C NMR (CD₃OD, 100 MHz) δ : 175.2, 167.0, 163.7, 145.3, 112.2, 105.3, 24.4. The NMR data were consist with reference (Miao et al., 2012), thus, compound 1 was orsellinic acid.

Compound 2: Yellow gum. ¹H NMR(400 MHz, CD₃OD) δ : 4.84 (d, *J* = 20.8 Hz, 3H), 3.99 ~ 3.55 (m, 1H), 3.31 (s, 1H), 1.32 (d, *J* = 19.5 Hz, 1H). ¹³C NMR (100 MHz, CD₃OD) δ : 98.19 (C-1), 93.94 (C-2), 78.05 (C-3), 76.28 (C-4), 75.68 ~ 74.98 (C-5), 71.79(C-6), 62.80 (C-7), 49.00 (C-8). The NMR data were consist with reference (Huang et al., 2016), compound 2 was α -nigerose.

Compound 3: white powder. ¹H NMR (400 MHz, DMSO-*d*₆) δ : 5.78 (d, *J* = 4.6 Hz, 1H), 5.64 (d, *J* = 8.9 Hz, 1H), 5.37 (d, *J* = 5.0 Hz, 1H), 5.09 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ : 163.09 (C-4), 150.73 (C-2), 140.70 (C-6), 101.73 (C-5), 87.66 (C-1'), 84.82 (C-4'), 73.52 (C-3'), 69.87 (C-2'), 60.84 (C-5'). The NMR data were consistent with references (Ai et al., 2010), compound 3 was identified as uridine.

Compound 4: ¹H NMR (400 MHz, CD₃OD) δ : 7.29 (d, *J* = 8.8 Hz, 1H), 6.72 (d, *J* = 8.8 Hz, 1H), 1.28 (s, 3H). ¹³C NMR (100 MHz, CD₃OD) δ : 171.37 (C-1), 155.37 (C-4'),

131.65 (C-1'), 123.34 (C-2', C-6'), 116.19 (C-3', C-5'), 23.49 (C-2). The NMR data were consist with reference(Deng et al., 2015), compound 4 was confirmed as N-(4-hydroxyphenyl)acetamide.

DISCUSSIONS

Phlebiopsis crassa that used to be named as *Porostereum crassum* grew to 2-3 cm with white, loose and spider like colony on PDA media for 2 days at 28 °C. At the later stage of growth, the mycelia changed from white to black gray. Microorganisms usually accumulate secondary metabolites in the late growth stage. In this study, cultures of 13 days maybe not enough. Compound 1 orsellinic acid, a kind of phenol compound is widely distributed in the secondary metabolites of fungi. Compound 2, 3 and 4 was usually isolated from plants and microbes while all compounds were identified from *P. crassa*.

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