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SCREENING AND CHARACTERIZATION OF PACLITAXEL-PRODUCING FUNGUS *Talaromyces wortmannii* WQF18 ISOLATED FROM *Cephalotaxus mannii* Hook. f.

Quach Ngoc Tung[1,2], Vu Thi Hanh Nguyen[1,2], Pham Quynh Anh[1], Tran Hong Quang[3], Chu Hoang Ha[1,2], Phi Quyet Tien[1,2*]

[1]Institute of Biotechnology, VAST, Vietnam
[2]Graduate University of Science and Technology, VAST, Vietnam
[3]Institute of Marine Biochemistry, VAST, Vietnam

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ABSTRACT

Cephalotaxus mannii Hook. f. is a rare evergreen conifer native listed in the International Union for Conservation of Nature (IUCN) Red List, which is utilized for leukemia treatment. Although endophytic fungi from *C. mannii* was reported before, their cytotoxic property has not been revealed yet. In the present study, a total of 7 endophytic fungi were isolated from *C. mannii* collected in Ha Giang province, Vietnam, among which the isolate WQF18 was active against 5 tested pathogens with inhibition zones ranging from 18.0 ± 0.7 to 25.0 ± 0.4 mm. In addition, only ethyl acetate extract of isolate WQF18 showed cytotoxicity on A549 and MCF7 cell lines with IC_{50} values of 69.6 ± 2.3 µg/mL and 78.6 ± 1.6 µg/mL, respectively. PCR-based molecular screening revealed that the positive hits for both 10-deacetylbaccatin III-10-O-acetyl transferase (*dbat*) and taxadiene synthase (*ts*) genes involved in paclitaxel biosynthesis were only observed in the WQF18 isolate. Based on morphological and molecular identification, the WQF18 isolate was identified as *Talaromyces wortmannii* WQF18. The presence of paclitaxel in *T. wortmannii* WQF18 was further confirmed by *dbat* sequence alignment, phenotypic, and HPLC-DAD analysis. To the best of our knowledge, this is the first report demonstrating the paclitaxel-producing capability of endophytic fungi from *C. mannii*. These findings provide a new platform for deciphering paclitaxel biosynthesis of endophytic fungi from non-*Taxus* plants and further paclitaxel production.

Keywords: *Cephalotaxus mannii*, cytotoxicity, *dbat*, endophytic fungi, paclitaxel, *Talaromyces wortmannii*.

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[*]*Corresponding author email*: tienpq@ibt.ac.vn

INTRODUCTION

Endophytes are defined as microorganisms colonizing inside the living plant tissues without manifesting diseases in the hosts. *Neotyphodium coenophialum* was the first endophytic fungi isolated from plants, marking a breakthrough in endophyte research history (Bacon et al., 1977). Since then, endophytic fungi have drawn interest from researchers worldwide because of their immense potential to produce bioactive compounds with therapeutic and medicinal applications. One of the lead compounds from fungal endophytes was the anticancer compound paclitaxel which had only been obtained from yews before (*Taxus* species) (Flores-Bustamante et al., 2010). To date, more than 20 genera of paclitaxel-producing fungi have been identified from *Taxus* species such as *Taxus brevifolia* and *Taxus baccata* (El-Sayed et al., 2020). Interestingly, paclitaxel can also be synthesized by fungi localizing inside plants that do not produce paclitaxel. Endophytic fungus *Grammothele lineata* recovered from the jute plant *Corchorus olitorius* was demonstrated to yield taxol (Das et al., 2017). A recent study reported four endophytic fungal strains from *Tsuga chinensis* that belonged to the genus *Penicillium* and *Aspergillus* held the potential of producing paclitaxel (Vu et al., 2022).

Isolation and detection of paclitaxel-producing fungi using traditional screening methods are laborious and time-consuming. To solve this problem, a molecular marker screening assay was discovered as an efficient alternative approach to identify paclitaxel-producing fungi. Paclitaxel molecular markers including genes coding for DBAT, C-13 phenylpropanoid side chain-CoA acyltransferase (BAPT), and TS have been used to screen for paclitaxel-producing endophytic fungi (Zhang et al., 2008). The TS responsible for the formation of the taxane core was considered a useful indicator of paclitaxel biosynthesis in endophytic fungi (Zhou et al., 2007). Moreover, DBAT and BAPT participate in the modification of taxane core and the assembly of paclitaxel, which are efficient PCR markers in detecting paclitaxel-producing fungi (Zhang et al., 2008). However, there are only a few studies screening paclitaxel-producing fungi from non-*Taxus* plants using PCR-based molecular makers.

Cephalotaxus mannii Hook. f. is slow-growing evergreen conifer listed in the IUCN Red List, which distributes in China, India, and Vietnam (Saithong et al., 2010). *Cephalotaxus* spp. are eminent producers of various alkaloids with anticancer potentials such as cephalotaxine, harringtonine, homoharringtonine, and their derivatives (Pérard-Viret et al., 2017). Endophytic fungi were previously isolated in the bark of *Cephalotaxus mannii* collected in Thailand and China (Saithong et al., 2010). Endophytic fungus *Asperigillus* sp. CM9a recovered from the stems of *C. mannii* in China was found to produce cyclopentenedione, diketopiperazines, lactone, benzophenone, terpene, anthraquinone, diphenyl ethers, alkaloid, and isotryptoquivaline F, among which only isotrptoqivaline F had anti-TNF-α activity (Xue et al., 2014). However, the antimicrobial and cytotoxic potential of endophytic fungi residing in *C. mannii* has not been explored yet. This study aims to isolate and characterize paclitaxel-producing fungi isolated from *C. manni* in northern Vietnam. The effort led to the identification of a new paclitaxel-producing fungus, identified as *T. wortmannii* WQF18 through genetic, chemical, and phenotypic analysis. To the best of our knowledge, *T. wortmannii* is the first member of *Talaromyces* genera capable of producing paclitaxel.

MATERIALS AND METHODS

Sampling and isolation of endophytic fungi

The bark of *C. mannii* Hook. f. was collected in the Bat Dai Son Nature Reserve (23°8’16”N - 105°0’44”E; 1,230 meters in height) Ha Giang province, Vietnam with the help of expert plant gatherers and local ethnic minority peoples. The samples were placed in sterile plastic bags and transported to the

laboratory of the Institute of Biotechnology, Vietnam Academy of Science and Technology. The Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology supported for identification of *C. mannii* Hook. f. The collected bark was sterilized following the procedure previously described (Vu et al., 2022). In brief, the bark was treated with 70% ethanol for 30 s, 3.5% sodium hypochlorite solution for 2 min, 70% ethanol for 2–5 s, and rinsed with sterile distilled water. The sterilized samples were cut into small segments and placed in 9-cm diameter Petri dishes containing Potato Dextrose Agar (PDA) supplemented with 100 mg/L streptomycin for 4–6 days at 28 ^{o}C. The single hyphal tip method was employed to obtain pure fungi that are preserved in 15% (v/v) glycerol at -80 ^{o}C for long-term preservation.

Fungal extraction and antimicrobial activity

Fungal endophytes were fermented in 200 mL of Potato Dextrose Broth (PDB) and incubated in dark at 28 ^{o}C. After 14 days, the cultures were filtered through a clean piece of filter paper to obtain the culture filtrates that were later extracted by twice the volume of ethyl acetate. The resulting solutions were dried at 40 ^{o}C with the evaporator R300 (Buchi, Flawil, Switzerland) to collect the fungal crude extract. The dried extract was dissolved in 1% (v/v) dimethyl sulfoxide (DMSO) for antimicrobial and cytotoxic assays.

The antimicrobial activity of the crude extracts obtained from the fungal isolates was evaluated using an agar-well diffusion assay (Gonelimali et al., 2018). Seven microbial pathogens used in this study included *Escherichia coli* ATCC 11105, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus cereus* ATCC 11778, methicillin-resistant *Staphylococcus epidermidis* (MRSE) ATCC 35984, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591, *Enterococcus faecalis* ATCC 29212, and *Candida albicans* ATCC 10231. Erythromycin and nystatin were used as positive controls and antimicrobial activity was expressed as a zone of inhibition (mm).

Cytotoxic activity

The cytotoxic potential of crude extracts was evaluated by using the sulforhodamine B (SRB) test with the human lung cancer A549 and human breast adenocarcinoma MCF7 cell lines (Skehan et al., 1990). The tested cell lines were grown on 96-well plates with starting density of around 10^4 cells for 24 h. Fungal crude extracts were added with different concentrations and left for 24 h before the cells were fixed with cold 10% (w/v) trichloroacetic acid for 1 h. Plates were gently shaken for 10 min and then read at the optical density of 540 nm in a microplate reader (BioTek EXL800). The IC_{50} value which means inhibition of 50% of cancer cell survival by comparison with control is calculated by Table Curve 2Dv4 computer software.

PCR-based molecular screening for paclitaxel-producing fungi

The primary search for paclitaxel-producing fungi was conducted by PCR amplification, using specific primers for three essential genes involved in paclitaxel production. They included *ts* (ts-F: 5'–ATCAGTCCGTCTGCATACGACA–3' and ts-R: 5'–TAAGCCTGGCTTCCCGTGTTGT–3'), *dbat* (dbat-F: 5'–ATGGCTGAC ACTGACCTCTCAGT–3' and dbat-R: 5'–GGCCTGCTCCTAGTCCATCACAT–3'), and *bapt* (bapt-F: 5'–CCTCTCTCCGCCATTGACAACAT–3' and bapt-R: 5'–GTCGCTGTCAGCCATGGCTT–3') (Kumar et al., 2019). The PCR reaction was carried out in a 25 µL final volume consisting of 0.1 µg of genomic DNA, 0.4 µM of forward and reverse primers, 0.2 mM dNTPs, 1×Taq DNA polymerase buffer, and 1 U of Taq DNA polymerase. The amplification condition was conducted as described previously (Das et al., 2017). The resulting PCR product was visualized on 2% (w/v) agarose gel by electrophoresis followed by purification and Sanger sequencing. The obtained sequence was analyzed using

BLAST (www.ncbi.nlm.nih.gov/BLAST) and the *dbat* sequence of endophytic fungal strain WQF18 was aligned with the *dbat* gene of paclitaxel-producing fungi including *Lasiodiplodia theobromae* SKJM 1101 (KP136287.1), *Aspergillus candidus* MD3 (EU883596.3), and *Cladosporium cladosporioides* MD-2 (EU375527.1) using Jalview 2.11.2.5.

Characterization of fungal paclitaxel

To observe paclitaxel-producing fungus, 21-day fungal culture was stained with Sudan IV and observed microscopically followed by microscope observation (Soliman & Raizada, 2018). The presence of paclitaxel in the crude extract WQF5 was detected using HPLC-DAD analysis with the support of an Agilent 1200 HPLC system (El-Bialy & El-Bastawisy, 2020). The presence of paclitaxel in fungal extract WQF5 was determined by comparison with retention times (Rt) and UV spectra of the standard paclitaxel (Sigma).

RESULTS AND DISCUSSION

Evaluation of the antimicrobial activity of fungal isolates

Table 1. Antimicrobial activity and cytotoxicity from ethyl acetate crude extracts of fungal isolates recovered from the bark of *Cephalotaxus mannii*

Strain	Antimicrobial activity (D-d, mm)							Cytotoxicity IC_{50} (µg/mL)	
	1	2	3	4	5	6	7	A549	MCF7
WQF13	7.6 ± 0.6	4.9 ± 0.5	-	-	7.3 ± 1.5	-	-	-	-
WQF14	-	-	-	-	-	-	-	-	-
WQF15			11.8 ± 1.0	-	-	-	6.5±0.4	-	-
WQF16	-	12.5 ± 1.3	-	13.5 ± 0.8	-	-	-	-	-
WQF17	-	-	-	-	-	-	-	-	-
WQF18	-	18.0 ± 0.7	18.0 ± 0.9	25.0 ± 0.4	-	20.0 ± 0.4	19.0 ± 0.7	69.6 ± 2.3	78.6 ± 1.6
WQF19	8.5 ± 0.8	-	-	-	-	15.5 ± 0.8	-	> 100	> 100
Erythromycin	22.1 ± 1.0	17.6 ± 1.5	29.7 ± 1.5	7.4 ± 0.6	4.3 ± 0.5	27.3 ± 1.5	-		
Nystatin	-	-	-	-	-	-	21.3 ± 1.5		
Ellipticine								0.5 ± 0.04	0.5 ± 0.05

Notes: *Microorganisms: (1) *Escherichia coli* ATCC 11105; (2) *Pseudomonas aeruginosa* ATCC 9027; (3) MRSE ATCC 35984; (4) *Bacillus cereus* ATCC 11778; (5) MRSA ATCC 33591; (6) *Enterococcus faecalis* ATCC 29212; (7) *Candida albicans* ATCC 10321. * Result: (-), no activity/inhibition.

Seven endophytic fungi were successfully recovered from the surface sterilized barks of *C. mannii*. No colonies grown from the last wash of the sterilization procedure indicated the effectiveness of the method. All strains were grown onto PDB for 14 days and extracted with ethyl acetate to assess antimicrobial potential. Out of 7 crude extracts, 5 crude extracts (71.4%) were active against at least one tested pathogen (Table 1). MRSA ATCC 33591 was quite resistant to all fungal extracts, except for the WQF13 extract, while *P. aeruginosa* ATCC 9027 showed sensitivity to 3 fungal extracts such as WQF13, WQF16, and WQF18. Among all extracts with antimicrobial activity, only WQF18 displayed inhibitory effects toward 5 pathogens. Of note, the zones of inhibition

ranged from 18.0 ± 0.7 to 25.0 ± 0.4 mm making WQF18 the most potent antimicrobial strain from the bark of *C. mannii.* The antifungal effect of WQF18 was also demonstrated with an inhibition zone of 19.0 ± 0.7 mm, comparable to that of nystatin (21.3 ± 1.5 mm). In agreement with this result, 80.1% of endophytic fungi isolated from *C. hainanensis* Li in China were highly active against pathogenic bacteria (Yang et al., 2015). In contrast, 33.3% of fungal isolates from *Tsuga chinensis* (Franch.) Pritz. in Ha Giang province, Vietnam exhibited antimicrobial activities with microbial pathogens (Vu et al., 2022). Hence, these supported that *C. mannii* is rich in endophytic fungi with antimicrobial properties.

Anticancer activity test of fungal ethyl acetate extracts

Among 7 isolates, only WQF18 extract (14.2%) showed anticancer activity against both tested cancer cell lines A549 and MCF7 with the respective IC_{50} values of 69.6 ± 2.3 µg/mL and 78.6 ± 1.6 µg/mL (Table 1). Similar to a previous study, 18.5% of fungal extracts associated with *C. hainanensis* had cytotoxic activity against at least one tumor cell line (Liu et al., 2016).

Since endophytic fungi from non-*Taxus* species also produced paclitaxel (Vu et al., 2022), the capability of synthesizing paclitaxel from endophytic fungi involved in *C. mannii* was evaluated using PCR-based molecular makers. It revealed that 5 fungal strains such as WQF13, WQF15, WQF16, WQF18, and WQF19 contained at least one of three key genes (Table 2). Surprisingly, the positive hits for both *dbat* and *ts* genes were only observed in the isolate WQF18. It is believed that the gene *ts* is responsible for the formation of a paclitaxel core as the first step in paclitaxel biosynthesis, which is not diagnostic (Kumar et al., 2019). On the contrary, the presence of *dbat* more likely results in the production of baccatin III or paclitaxel. These findings strongly supported the cytotoxicity of WQF18 on both A549 and MCF7 cell lines. Even though paclitaxel-producing fungi were previously found in *Taxus* plants, fungal endophytes from non-*Taxus* plants such as *Ginkgo biloba* and *Hibiscus rosa-sinensis* were proven to produce paclitaxel (Kumaran et al., 2009; Abdel-Fatah et al., 2021). They suggest that endophytic fungi may evolve to have distinct metabolic strategies to synthesize paclitaxel from non-*Taxus* plants, which remain poorly characterized to date.

Table 2. PCR-based molecular screening for paclitaxel-producing fungi

Strain	Paclitaxel biosynthetic genes		
	dbat	*bapt*	*ts*
WQF13	-	-	+
WQF14	-	-	-
WQF15	+	-	-
WQF16	-	-	+
WQF17	-	-	-
WQF18	+	-	+
WQF19	+	-	-

Notes: Result: (-), negative hit; (+), positive hit.

Identification of bioactive isolate WQF18

Morphological identification revealed that WQF18 grew well with a diameter of 23–26 mm on PDA within 7 days at 28 ^{o}C. Ascoma color was yellow to orange and ascospores were ellipsoid with thick walls. Conidiophores were verticillate, or sometimes had subterminal branches (Fig. 1A). These observations indicated that fungal isolate WQF18 showed high similarity with the *Talaromyces* genus.

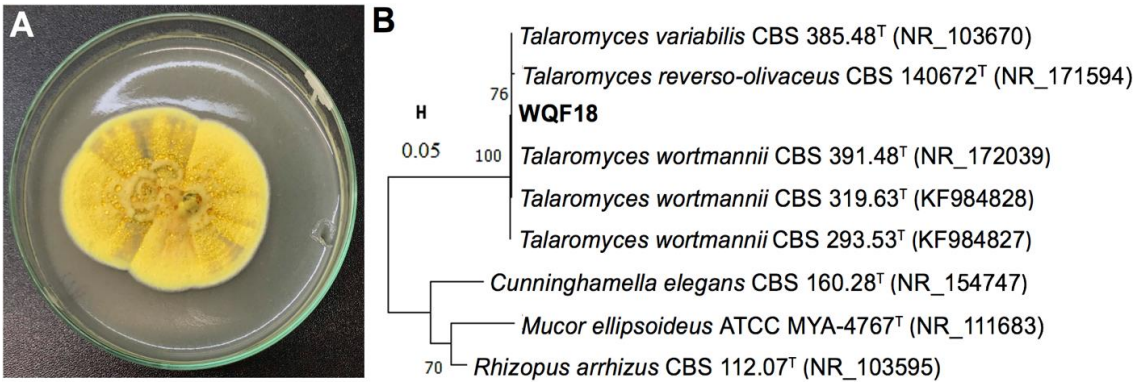

Figure 1. Morphology (A) and phylogenetic tree based on the ITS gene sequences (B) of fungal strain WQF18 with nearest type strains

ITS sequence analysis was used to confirm isolate identification at the species level. The ITS sequence data was trimmed and aligned using BLAST. ITS sequence of WQF18 showed 99.4–99.8% similarity with *T. wortmannii* CBS 391.48^T, *T. wortmannii* CBS 319.63^T, and *T. wortmannii* CBS 293.5^T, respectively. Lower similarities were observed in *Talaromyces reverso-olivaceus* CBS 140672^T (96.9%) and *Talaromyces variabilis* CBS 385.48^T (98.5%). ITS sequence of WQF18 was deposited onto GenBank (NCBI) under accession number OP482182. Phylogenetic analysis also clustered WQF18 with a member of *T. wortmannii* species (Fig. 1B). Thus, WQF18 belonged to *T. wortmannii.*

T. wortmannii is frequently isolated as an endophytic fungus from different plants such as *Aloe vera* and *Tripterygium wilfordi.* Of note, *T. wortmannii* from *Aloe vera* produced 6 compounds including flavomannin AB, 2 new unsymmetrical dimer, and 2 new mixed dihydroanthracenone/anthraquinone dimers actively against *S. aureus*, *Streptococcus*, *Enterococcus*, and *Bacillus* (Bara et al., 2013c). Another research showed that atropisomer, skyrin, and rugulosin A from *T. wortmannii* displayed remarkable activity against MRSA, *S. epidermidis*, *S. pneumonia*, and *Enterococcus faecalis* (Bara et al., 2013a). Moreover, two new cyclic peptides, talaromins A and B, with no biological activity were also obtained from *T. wortmannii* associated with *Aloe vera* (Bara et al., 2013b). In support of these studies, the fungal genome comprises up to 90 biosynthetic gene clusters, many of which are silent and paclitaxel has not been reported in *T. wortmannii* yet (Clevenger et al., 2017). Thus, it is interesting to continue exploiting the metabolic potential of *T. wortmannii* from *C. mannii*.

Determination of paclitaxel in *T. wortmannii* WQF18

To confirm paclitaxel biosynthesis in the molecular aspect, a 300-bp fragment of *dbat* was sequenced and analyzed using BLAST (NCBI). BLAST analysis of the *dbat* sequence exhibited moderate similarity to truncated sequences of *L. theobromae* (49.4%), *C. cladosporioides* (51.1%), and *A. candidus* (50.0%) (Fig. 2A). Morphological observation using Sudan IV under light microscope indicated paclitaxel production of *T. wortmannii* WQF18 (Fig. 2B). In addition, using HPLC-DAD the chromatogram of WQF18 showed a peak with Rt at 35.781 min, which overlapped with the standard paclitaxel (35.783 min) (Fig. 2C). Similar to the Rt result, the UV spectra of WQF18 extract also matched that of the standard paclitaxel. Taken together, these results suggest that *T. wortmannii* WQF18 most likely produces paclitaxel.

A survey of literature on paclitaxel-producing fungi highlights that this is the first

study describing a member of the *Talaromyces* which is able to produce paclitaxel. To date, many members of the genera *Alternaria, Pestalotiopsi, Beauveria, Epicoccum, Fusarium, Geotrichum, Phoma, Nodulasporium, Phomopsis,* and *Cladosporium* derived from *Taxus* species have been considered as paclitaxel producers (Flores-Bustamante et al., 2010). In contrast, only *Penicillium polonicum, Phyllosticta dioscoreae, Grammothele lineata,* and *Alternaria brassicicola* isolated from non-*Taxus* species were shown to synthesize paclitaxel (Kumaran et al., 2009; Das et al., 2017; Gill & Vasundhara, 2019; Abdel-Fatah et al., 2021), leading to the elimination of horizontal gene transfer reported before. The underlying mechanism of how endophytic fungi from non-*Taxus* species synthesize paclitaxel is an interesting subject for future studies.

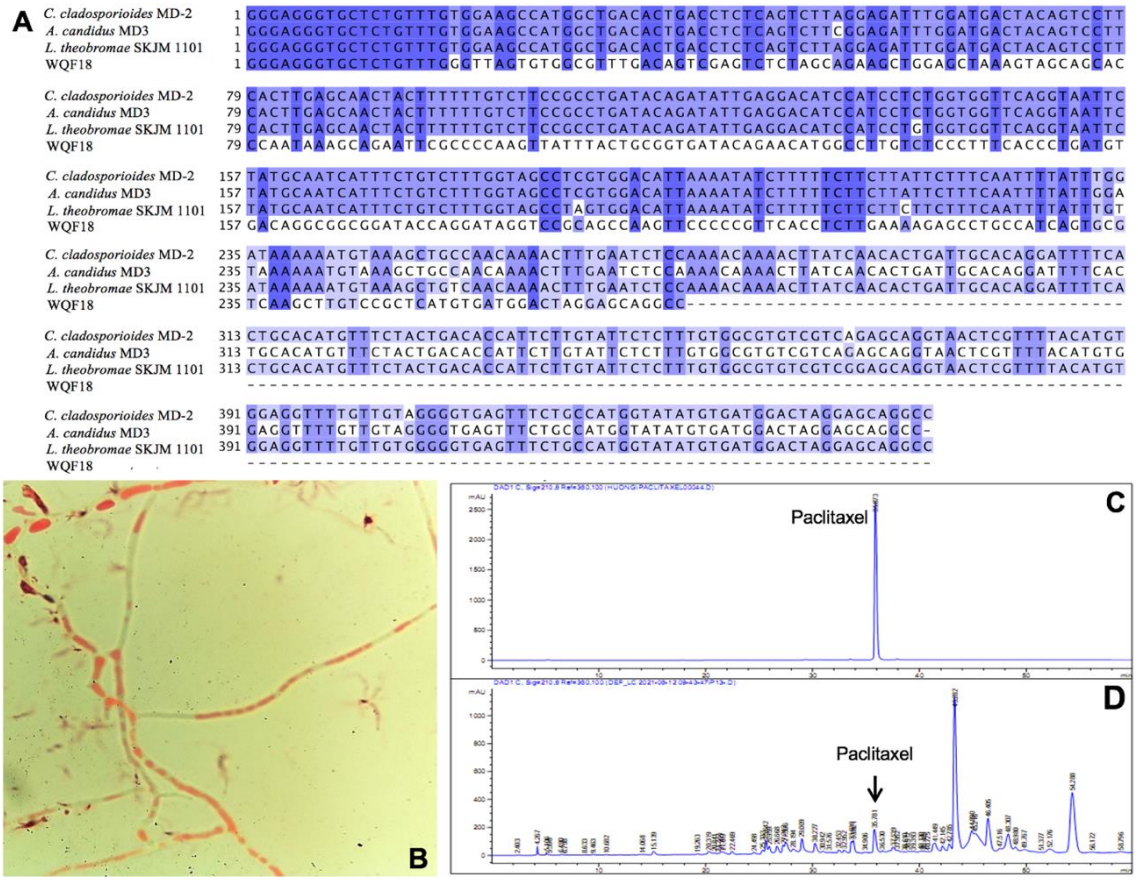

Figure 2. Determination of paclitaxel present in the extract of *Talaromyces wortmannii* WQF18. (A) Multiple sequence alignment of truncated *dbat* of paclitaxel-producing fungi aligned with truncated *dbat* of *Talaromyces wortmannii* WQF18. (B) Staining of WQF18 cells with Sudan IV. Determination of paclitaxel in the *Talaromyces wortmannii* WQF18 (D) based on comparison to standard paclitaxel (Sigma) (C) using HPLC-DAD

CONCLUSION

In conclusion, the present study sheds light for the first time on the paclitaxel-synthesizing potential of endophytic fungi isolated from *C. mannii*. From the bark of *C. mannii*, 7 endophytic fungi were recovered, among which WQF18 extract showed

remarkable antimicrobial and cytotoxic activities. Using PCR-based molecular screening, *T. wortmannii* WQF18 was found to contain *dbat* and *ts* genes that are involved in paclitaxel biosynthesis. In addition, *dbat* sequence alignment and HPLC-DAD further confirmed the presence of paclitaxel in *T. wortmannii* WQF18 at the genetic and phenotypic levels. Genomic and transcriptomic analyses are interesting subjects for future studies to further explore a paclitaxel biosynthesis of *T. wortmannii* WQF18.

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REFERENCES

Abdel-Fatah S. S., El-Batal A. I., El-Sherbiny G. M., Khalaf M. A., El-Sayed A. S., 2021. Production, bioprocess optimization and γ-irradiation of *Penicillium polonicum*, as a new Taxol producing endophyte from *Ginko biloba*. *Biotechnol Rep*, 30: e00623. https://doi.org/10.1016/j.btre.2021.e00623

Bacon C. W., Porter J. K., Robbins J. D., Luttrell E. S., 1977. Epichloë typhina from toxic tall fescue grasses. *Applied and environmental microbiology*, 34(5): 576−581.https://doi.org/10.1128/aem.34.5.576-581.1977

Bara R., Aly A. H., Pretsch A., Wray V., Wang B., Proksch P., Debbab A., 2013a. Antibiotically active metabolites from *Talaromyces wortmannii*, an endophyte of *Aloe vera*. *J Antibiot*, 66(8): 491–493. https://doi.org/10.1038/ja.2013.28

Bara R., Aly A. H., Wray V., Lin W., Proksch P., Debbab A., 2013b. Talaromins A and B, new cyclic peptides from the endophytic fungus *Talaromyces wortmannii*. *Tetrahedron Lett.*, 54(13): 1686–1689. https://doi.org/10.1016/j.tetlet.2013.01.064

Bara R., Zerfass I., Aly A. H., Goldbach-Gecke H., Raghavan V., Sass P., Mándi A., Wray V., Polavarapu P. L., Pretsch A., Lin W., Kurtán T., Debbab A., Brötz-Oesterhelt H., Proksch P., 2013c. Atropisomeric dihydroanthracenones as inhibitors of multiresistant *Staphylococcus aureus*. *J Med Chem*, 56(8): 3257–3272. https://doi.org/10.1021/jm301816a

Clevenger K. D., Bok J. W., Ye R., Miley G. P., Verdan M. H., Velk T., Chen C., Yang K., Robey M. T., Gao P., Lamprecht M., Thomas P. M., Islam M. N., Palmer J. M., Wu C. C., Keller N. P., Kelleher N. L., 2017. A scalable platform to identify fungal secondary metabolites and their gene clusters. *Nature chemical biology*, 13(8): 895–901. https://doi.org/10.1038/nchembio.2408

Das A., Rahman M. I., Ferdous A. S., Amin A., Rahman M. M., Nahar N., Uddin M. A., Islam M. R., Khan H., 2017. An endophytic Basidiomycete, *Grammothele lineata*, isolated from *Corchorus olitorius*, produces paclitaxel that shows cytotoxicity. *PLOS ONE*, 12(6): e0178612. https://doi.org/10.1371/journal.pone.0178612

El-Bialy H. A., El-Bastawisy H. S., 2020. Elicitors stimulate paclitaxel production by endophytic fungi isolated from ecologically altered *Taxus baccata*. *Radiat Res Appl Sci*, 13(1): 79–87. https://doi.org/10.1080/16878507.2019.1702244

El-Sayed A. S. A., El-Sayed M. T., Rady A. M., Zein N., Enan G., Shindia A., El-Hefnawy S., Sitohy M., Sitohy B., 2020. Exploiting the biosynthetic potency of taxol from fungal endophytes of conifers plants; Genome mining and metabolic manipulation. *Molecules*, 25(13): 3000.

Flores-Bustamante Z. R., Rivera-Orduña F. N., Martínez-Cárdenas A., Flores-Cotera L. B., 2010. Microbial paclitaxel: advances and perspectives. *J Antibiot*, 63(8): 460–467. https://doi.org/10.1038/ja.2010.83

Gill H., Vasundhara M., 2019. Isolation of taxol producing endophytic fungus

Alternaria brassicicola from non-*Taxus* medicinal plant *Terminalia arjuna*. *World journal of microbiology & biotechnology*, 35(5): 74. https://doi.org/10.1007/s11274-019-2651-8

Gonelimali F. D., Lin J., Miao W., Xuan J., Charles F., Chen M., Hatab S. R., 2018. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Front Microbiol*, 910.3389/fmicb.2018.01639

Kumar P., Singh B., Thakur V., Thakur A., Thakur N., Pandey D., Chand D., 2019. Hyper-production of taxol from *Aspergillus fumigatus*, an endophytic fungus isolated from *Taxus* sp. of the Northern Himalayan region. *Biotechnol Rep*, 24: e00395. https://doi.org/10.1016/j.btre.2019.e00395

Kumaran R. S., Muthumary J., Kim E.-K., Hur B.-K., 2009. Production of taxol from *Phyllosticta dioscoreae*, a leaf spot fungus isolated from *Hibiscus rosa-sinensis*. *Biotechnol Bioproc E*, 14(1): 76–83. https://doi.org/10.1007/s12257-008-0041-4

Liu Y. H., Hu X. P., Li W., Cao X. Y., Yang H. R., Lin S. T., Xu C. B., Liu S. X., Li C. F., 2016. Antimicrobial and antitumor activity and diversity of endophytic fungi from traditional Chinese medicinal plant *Cephalotaxus hainanensis* Li. *Genetics and molecular research: GMR*, 15(2). https://doi.org/10.4238/gmr.15028633

Pérard-Viret J., Quteishat L., Alsalim R., Royer J., Dumas F., 2017. Cephalotaxus alkaloids. *The Alkaloids. Chemistry and biology*, 78: 205–352. https://doi.org/10.1016/bs.alkal.2017.07.001

Saithong P., Panthavee W., Stonsaovapak S., Congfa L., 2010. Isolation and primary identification of endophytic fungi from *Cephalotaxus mannii* trees. *Maejo Int J Sci Technol*, 4: 446–453.

Skehan P., Storeng R., Scudiero D., Monks A., McMahon J., Vistica D., Warren J. T., Bokesch H., Kenney S., Boyd M. R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute*, 82(13): 1107–1112. https://doi.org/10.1093/jnci/82.13.1107

Soliman S. S. M., Raizada M. N., 2018. Darkness: A crucial factor in fungal taxol production. *Front Microbiol*, 9. https://doi.org/10.3389/fmicb.2018.00353

Vu T. H. N., Pham N. S., Le P. C., Pham Q. A., Quach N. T., Nguyen V. T., Do T. T., Chu H. H., Phi Q. T., 2022. Distribution, cytotoxicity, and antioxidant activity of fungal endophytes isolated from *Tsuga chinensis* (Franch.) Pritz. in Ha Giang province, Vietnam. *Ann Microbiol*, 72(1): 36. https://doi.org/10.1186/s13213-022-01693-5

Xue H., Xu Q., Lu C., Shen Y., 2014. Isotryptoquivaline F, a new quinazolinone derivative with anti TNF-α activity from *Aspergillus* sp. CM9a. *Drug discoveries & therapeutics*, 8(5): 208–211. https://doi.org/10.5582/ddt.2014.01039

Yang H. R., Hu X. P., Jiang C. J., Qi J., Wu Y. C., Li W., Zeng Y. J., Li C. F., Liu S. X., 2015. Diversity and antimicrobial activity of endophytic fungi isolated from *Cephalotaxus hainanensis* Li, a well-known medicinal plant in China. *Letters in applied microbiology*, 61(5): 484–490. https://doi.org/10.1111/lam.12483

Zhang P., Zhou P. P., Jiang C., Yu H., Yu L. J., 2008. Screening of Taxol-producing fungi based on PCR amplification from *Taxus*. *Biotechnology letters*, 30(12): 2119–2123. https://doi.org/10.1007/s10529-008-9801-7

Zhou X., Wang Z., Jiang K., Wei Y., Lin J., Sun X., Tang K., 2007. Screening of taxol-producing endophytic fungi from *Taxus chinensis* var. mairei. *Prikladnaia biokhimiia i mikrobiologiia*, 43(4): 490–494