



Isolation of taxol producing endophytic fungus *Alternaria brassicicola* from non-*Taxus* medicinal plant *Terminalia arjuna*

Harman Gill¹ · M. Vasundhara¹

Received: 10 January 2019 / Accepted: 26 April 2019 / Published online: 3 May 2019
© Springer Nature B.V. 2019

Abstract

In the present study, an endophytic fungal strain was isolated from its non-*Taxus* host plant *Terminalia arjuna* and identified as *Alternaria brassicicola* based on its morphological characteristics and internal transcribed spacer sequence analysis. This fungus was grown in potato dextrose broth and analyzed for the presence of taxol by using chromatographic and spectrometric techniques. The ethyl acetate extract of *A. brassicicola* was subjected to column chromatography. Among the different fractions, the fraction 7 showed positive to taxol, which was further confirmed by UV absorption, HPLC, FTIR spectra and LC-ESI-MS by comparing with the authentic taxol (Paclitaxel). The peaks of fraction 7 obtained by UV spectroscopy, FTIR and HPLC analysis were quite similar to that of standard taxol confirming the presence of taxol. A parent ion peak of m/z 854.95 was observed in the LC-ESI-MS spectrum which was similar to paclitaxel with reported m/z of 854 [M+H]⁺ ion. *A. brassicicola* produced about 140.8 µg/l taxol as quantified through HPLC. Present study results suggest that the endophytic fungus *A. brassicicola* serves as a potential source for the production of taxol isolated from non-*Taxus* plant.

Keywords *Terminalia arjuna* · Endophytic fungi · *Alternaria brassicicola* · Paclitaxel · Internal transcribed spacer

Introduction

The fungal endophytes producing secondary metabolites that are effective against the human diseases have attracted many researchers across the world (Abdel-Azeem et al. 2016). Among these, the search for novel cancer therapeutic agents is of great importance due to increase in the number of cancer deaths, high price of the drugs and the side effects associated with cancer therapies (Kharwar et al. 2011). Taxol (paclitaxel), an anticancer drug used for the treatment of different kind of cancer was initially isolated from the bark of *Taxus brevifolia* (Wani et al. 1971). Due to increase in number of cancer cases every year and the low amount of taxol present in *Taxus* species, it is becoming very difficult to meet the required demand of taxol (Wang et al. 2000). Alternative sources such as chemical synthesis, semi-synthesis, and plant tissue and cell suspension culture are being searched to meet the requirement of taxol. However, these methods

have their own limitations, which compel to search for other sustainable alternative sources for the production of taxol.

The ability to produce taxol by an endophytic fungus *Taxomyces andreanae*, isolated from *Taxus brevifolia* (Stierle et al. 1993) has changed the avenue of research in this field. Sometimes, endophytic fungi produce similar novel secondary metabolites that are produced by their host plants. It has been hypothesized that during the evolution, endophytic fungi might have undergone genetic recombination by inheriting the ability to synthesize the metabolites similar to the host plants (Tan and Zou 2001). Further, Li et al. (1996) reported for the first time the production of taxol by an endophytic fungus *Pestalotiopsis microspora* isolated from non-*Taxus* plant. Since then, reports have been published on the production of taxol by endophytic fungi isolated from both *Taxus* and non-*Taxus* plants and their chemical structure was analyzed spectroscopically (Gond et al. 2014; Sah et al. 2017).

The present investigation was aimed to isolate endophytic fungi capable of producing taxol from non-*Taxus* medicinal plants. Very few reports are available on taxol-producing endophytic fungi from tropical medicinal plants. Different endophytic fungi were isolated from the medicinal plant *Terminalia arjuna* and screened for taxol production. One of

✉ M. Vasundhara
mvasundhara@thapar.edu

¹ Department of Biotechnology, Thapar Institute of Engineering & Technology, Patiala, Punjab 147004, India

the endophytic fungus, *Alternaria brassicicola* capable of producing taxol is being reported in this study.

Materials and methods

Isolation and screening of taxol producing endophytic fungi

The bark, stem, leaves and fruits of *Terminalia arjuna* were collected from Thapar Institute of Engineering & Technology campus, Patiala, India. For the isolation of endophytic fungi, samples were surface sterilized by giving initial washing in running tap water, followed by rinsing with double distilled water to remove the dust and debris from the surface. Tissues were serially surface sterilized by immersing them in solution of 70% ethanol for 1 min, and then dipped in aqueous solution of 1% sodium hypochlorite for 3 min, followed by rewashing in 70% ethanol for 10 s. Final washing was done with autoclaved double distilled water and then blot dried (Strobel and Daisy 2003). The samples were cut from the edges and outer layer was removed by sterile blade and were transferred onto the potato dextrose agar (PDA) (HiMedia Laboratories Ltd., Mumbai, India) plates containing chloramphenicol (250 µg/ml). The plates were incubated for a period of 7–14 days at $27 \pm 2^\circ\text{C}$ and observed after every 2–3 days for the growth of endophytic fungi. A mixed population of fungal endophytes was subcultured on fresh PDA plates by the hyphal tip method (Strobel et al. 1996a, b). A total of 14 endophytic fungal strains with varying morphotypes were isolated and screened for taxol production (as described below). Among these fungal strains, one of the isolates MVR1 showed positive to taxol production, which was selected for further studies.

Identification of endophytic fungal isolate MVR1

The fungal isolate MVR1 was identified based on its morphological characters and by internal transcribed spacer (ITS) sequence analysis. The fungal strain was grown on potato dextrose agar and the morphology of the culture was observed microscopically after staining with cotton blue in lactophenol. The preliminary identification was done based on the morphology of the fungal colony and the characteristics of the spores produced by the fungal isolate. For molecular characterization, mycelia of freshly grown fungal strain MVR1 was harvested and crushed into a fine powder with liquid nitrogen. Genomic DNA was extracted from the obtained powder by CTAB method (Zhang et al. 2008). DNA was quantified with Nanodrop 1000 spectrophotometer (Thermo Scientific, USA). The ITS region of the DNA was amplified using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3')

primers (White et al. 1990) as described in Vasundhara et al. (2017). The PCR product was sequenced by using ITS1 and ITS4 primers. The obtained sequence was searched for its homologous sequences from NCBI database by performing BLASTN analysis. The homologous sequences obtained from NCBI GenBank were aligned using multiple sequence alignment and the phylogenetic tree was reconstructed by MEGA 7 software (Kumar et al. 2016). Neighbor joining (NJ) method was used to construct the phylogenetic tree. The bootstrap of 1000 replications was used to analyze the definitive level to the nodes of the tree.

Fermentation and solvent extraction

Four to five mycelial discs (5.0 mm diameter) cut from the edges of actively growing mycelium were inoculated aseptically into 1000 ml Erlenmeyer flasks containing 500 ml of the potato dextrose broth (PDB). The flasks were incubated at $27 \pm 2^\circ\text{C}$ under stationary condition for 21 days. After 21 days of incubation, the culture was filtered using sterile cheesecloth to separate the mycelium from the broth (Prabavathy and Nachiyar 2011). To obtain the fungal metabolites, the culture filtrate was extracted with equal volume of ethyl acetate. The organic phase so obtained was collected in a separate flask and the solvent was removed by evaporation under reduced pressure at 35°C using rotary evaporator (Ika® RV 10 digital, Germany). The dried crude extract was dissolved in methanol and the presence of taxol was tested by using necessary chromatographic analysis.

Thin layer chromatography

The ethyl acetate extracts of the fungi were separated along with authentic paclitaxel by using TLC plates coated with silica Gel 60 F₂₅₄ (Merck India, Mumbai, India). The TLC plate was developed with chloroform and methanol (7:1) v/v as described in Strobel et al. (1996a). The R_f values of the bands corresponding to the standard paclitaxel were observed to find the presence of taxol in the extracts. The extract of the fungal isolate MVR1 was further selected for chromatography and LC–MS analysis.

Column chromatography

The glass column (60 cm × 25 mm) was charged with stationary phase (silica gel G, 60–120 mesh) by wet packing method. Crude extract of MVR1 was adsorbed on to the stationary phase and then loaded in the column. The column was eluted with a 100% hexane, gradient of hexane and ethyl acetate (5% ethyl acetate to 100% ethyl acetate), followed by methanol in ethyl acetate (5% ethyl acetate). Final washing of the column was done with pure methanol. The fractions eluted from the column were collected in test

tubes. Each fraction was concentrated and spotted on a thin layer chromatography (TLC) plate. The TLC was developed with mobile phase and visualized in iodine chamber. Based on the TLC profile similar fractions were pooled together and evaporated under reduced pressure as mentioned above.

UV spectroscopic analysis

The purified fractions obtained (7 fractions) after column chromatography were dissolved in methanol and UV absorption was measured between 200 and 500 nm by using UV-Visible spectrophotometer (Shimadzu UV-2600, Japan) and compared with the standard drug Paclitaxel.

Fourier-transform infrared spectroscopy (FTIR)

FTIR of fraction 7 (5 mg) was recorded using KBr pellets on Perkin Elmer RX (Spectrum RX FTIR Spectrometer, USA). The IR spectrum was recorded in the range of 250 to 4000 cm^{-1} . The peaks obtained of fraction 7 and the peaks of standard Paclitaxel were compared.

High pressure liquid chromatography (HPLC)

One of the fractions (fraction 7) showing similar UV spectra to that of authentic Taxol was subjected for HPLC analysis (Dionex Ultimate 3000, Thermo Fisher Scientific Inc., USA) using C18 reverse phase column (4.6×250 mm). The mobile phase used for separation consisted of methanol/acetonitrile/water (25:35:40, v/v/v) run over a period of 30 min at flow rate of 1.0 ml/min and detected with online diode array detector (DAD) at 232 nm. Twenty microlitre of the sample was passed through 0.2 μm microgene syringe filter before injecting into the column. Presence of taxol in the fraction 7 was confirmed by comparing the retention time with that of standard paclitaxel. The amount of taxol present in the crude extract was quantified based on the standard curve prepared by taking known concentrations of authentic paclitaxel.

LC-ESI-MS

HPLC was performed on Waters 2795 HPLC having quaternary pumping configured for flow rates from 0.05 to 5.0 ml/min and detected with diode array detector (DAD) at 232 nm. The auto sampler is configured with a 100 μl syringe. Fraction 7 was dissolved in methanol and used for analysis. Sample was injected into the C18 column and separation was carried out using methanol/acetonitrile/water (25:35:40, v/v/v) over a period of 30 min with flow rate of 1.0 ml/min. This equipment was coupled with Waters micro-mass quadrupole time of flight mass spectrometer equipped with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources having mass Range of

4000 amu in quadruple and 20,000 amu in ToF. The ESI capillary was set at 3.5 kV and cone voltage was 40 V and the spectra were recorded.

Results

Isolation and screening of taxol producing endophytic fungi

A total of 14 fungal endophytes were isolated from different tissues of *T. arjuna* and were screened for the production of taxol. The extracts of these fungi were examined for the presence of taxol by TLC chromatographic analysis. TLC analysis revealed that MVR1 isolated from the leaves showed positive for taxol production which was selected for further studies.

Identification of fungal isolate MVR1

The morphological characteristics of the endophytic fungal isolate MVR1 on PDA plate showed whitish brown colored center surrounded by greyish margin and velvety appearance (Fig. 1a). Microscopic observation of MVR1 showed presence of long chain, branched conidiophores with septate hyphae (Fig. 1b). The morphological and microscopic features were identical with that of *Alternaria* species. The ITS sequence (569 bp) obtained was analyzed by using BLASTN, which showed 100% similarity (100% query coverage) with *A. brassicicola*. The sequence obtained in this study was deposited at NCBI database under the accession number MK158222. Phylogenetic analysis revealed clustering of MVR1 with *A. brassicicola* species (Fig. 2). Based on the morphological and molecular characteristics, the fungal isolate MVR1 was identified as *A. brassicicola*.

Taxol production by *A. brassicicola* MVR1

By using potato dextrose broth as a culture medium, the amount of taxol produced by *A. brassicicola* was about 140.8 $\mu\text{g/l}$ culture filtrate as determined by HPLC. The fungal extract of MVR1 was subjected to column chromatography and the elutions were pooled into 7 fractions based on the TLC profile. These fractions were evaporated under reduced pressure and subjected to chromatography analysis. The presence of taxol in fraction 7 was confirmed by UV spectroscopy, FTIR and HPLC analysis. The UV absorption spectrum of fraction 7 of *A. brassicicola* showed maximum absorbance at 230 nm which was similar to the authentic paclitaxel where the maximum absorption was recorded at 230.20 nm (Fig. 3). FTIR spectrum of fraction 7 was analyzed by comparing wavelength of spectrum with that of functional groups. The IR spectra were recorded in the

Fig. 1 Morphological and microscopic features of MVR1. **a** Front view of MVR1 on PDA plate and **b** hyphae and conidiophores of MVR1 under $\times 40$ microscope

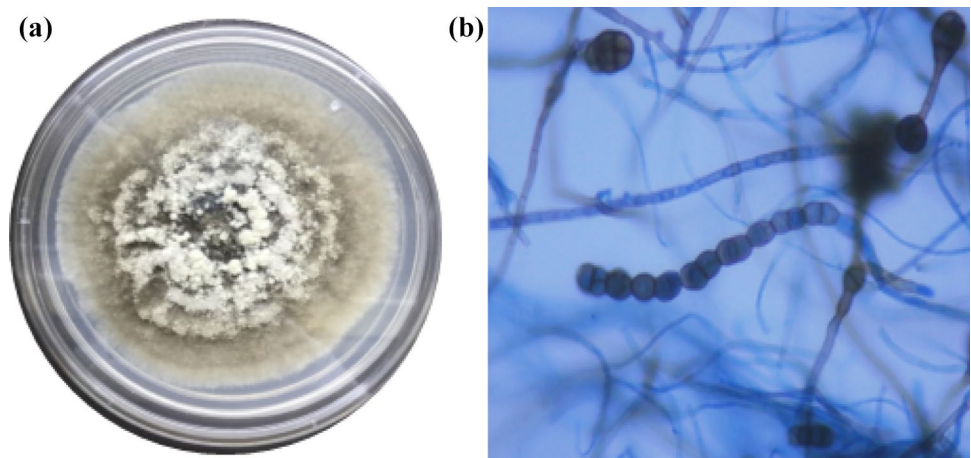
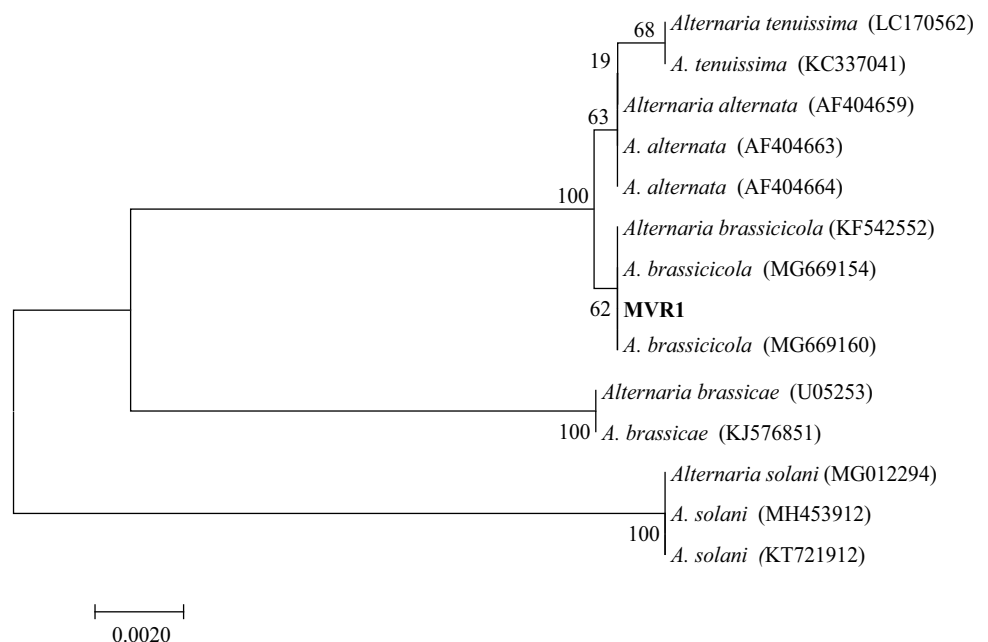


Fig. 2 Neighbor Joining tree showing the relationships between the internal transcribed spacer (ITS) sequence of the present study isolate (MVR1) (shown in bold) and those of related *Alternaria* species retrieved from GenBank



region $4000\text{--}500\text{ cm}^{-1}$. The IR spectral data of fungal taxol from *A. brassicicola* showed a broad peak in the region of 3418.3 , which is due to presence of $(-\text{NH})$ amine group, the peak at 1574.8 region because of $(-\text{C}=\text{C})$ indicating the presence of cyclic alkene. The alcohol $(-\text{OH})$ and ether groups $(\text{C}-\text{O})$ were observed in the range of 1416.5 and 1022.2 regions, respectively. The obtained spectrum of fraction 7 was similar to the authentic taxol confirming the presence of taxol (Fig. 4).

HPLC analysis confirms the identity of taxol from the fraction 7 of extract of *A. brassicicola* showing a peak with the retention time of 3.91 min that matched with the authentic paclitaxel with a retention time of 4.12 min (Fig. 5a, b). The UHPLC-QTOF-MS analysis of the fraction 7 obtained from extract of *A. brassicicola* showed a parent ion peak of m/z 854.95 (Fig. 6). Characteristically, mass spectra of

standard taxol yielded m/z value 854 $[\text{M}+\text{H}]^+$ ion. Hence, it is confirmed that taxol was present in fraction 7 obtained from extract of *A. brassicicola*.

Discussion

Paclitaxel is a mitotic inhibitor that has been used in chemotherapy for curing many types of cancers since many years (Zhao et al. 2009). The present study aimed to isolate new endophytic fungal strains, capable of producing paclitaxel from non-*Taxus* plants. In the present study, 14 different endophytic fungi were isolated from *T. arjuna* from various tissues. Among these isolates, one of the isolates MVR1, isolated from leaves of *T. arjuna* showed positive for paclitaxel production. Paclitaxel production by a wide range of

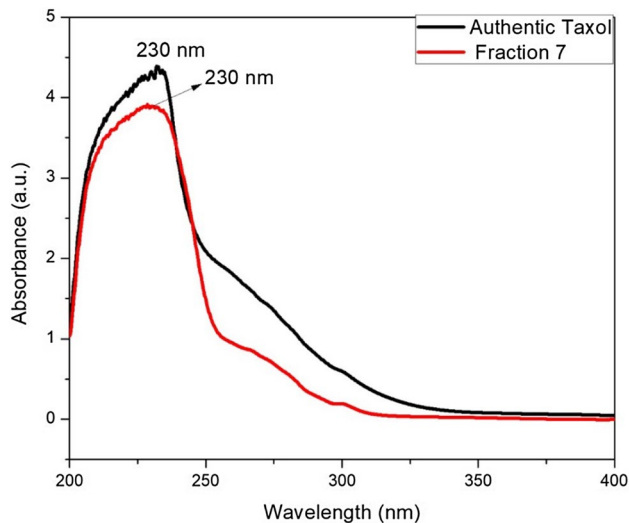


Fig. 3 UV absorption spectra of authentic taxol and fungal fraction 7 from *Alternaria brassicicola*. Spectra were recorded in methanol over the wavelength range 200–300 nm

endophytic fungi were reported by many researchers isolated from both *Taxus* and non-*Taxus* plants (Gond et al. 2014) with maximum being isolated from *Taxus* spp. Some of the non-*Taxus* plants where taxol producing endophytic fungi isolated includes *Aegle marmelos*, *Cardiospermum halicacabum*, *Justicia gendarussa*, *Podocarpus* sp., *Ginkgo biloba*, *Corylus avellana* and *T. arjuna* (Gond et al. 2014). Three endophytic fungi, *Pestalotiopsis terminaliae* CP-4 (Gangadevi and Muthumary 2009a), *Chaetomella raphigera*

TAC-15 (Gangadevi and Muthumary 2009b) and *Alternaria tenuissima* TER995 (Ismaiel et al. 2017) have been reported as paclitaxel producers from *T. arjuna*.

Alternaria species are known to produce various metabolites that exhibit a variety of bioactivities such as phytotoxic, cytotoxic, and antimicrobial properties (Lou et al. 2013 and references therein). Very few reports are available on the production of paclitaxel by *Alternaria* species. *Alternaria alternata* (Tian et al. 2006; de Andrade et al. 2018), *Alternaria* sp. (Strobel et al. 1996b) and *A. tenuissima* (Ismaiel et al. 2017) are some of the species reported for paclitaxel production. Production of some of the steroids (Cerevissterol), terpenoids (Brassicene A-I) and pyranones (Phomapyrone A, Altechromone A) have been reported from *A. brassicicola* (Lou et al. 2013). Matsumoto et al. (1992) reported the production of Depudecin, an inhibitor of histone deacetylase (HDAC) from *A. brassicicola*, which also showed its antitumor potency. Gu (2009) reported antimicrobial and xanthine oxidase inhibitory activities from the endophytic fungus *A. brassicicola* associated with *Malus halliana*. In the present study, we are reporting *A. brassicicola* associated with *T. arjuna* as paclitaxel producer for the first time. The fungus was identified as *A. brassicicola* based on its morphological and ITS1-5.8S-ITS2 sequence analysis.

The endophytic fungus *A. brassicicola* was screened for paclitaxel production and the fungal extract was examined for the presence of taxol by chromatographic and spectroscopic analyses. The chromatographic properties (Rf values) of the extract showed similarities to the standard paclitaxel in solvent systems. Presence of taxol could be identified based on

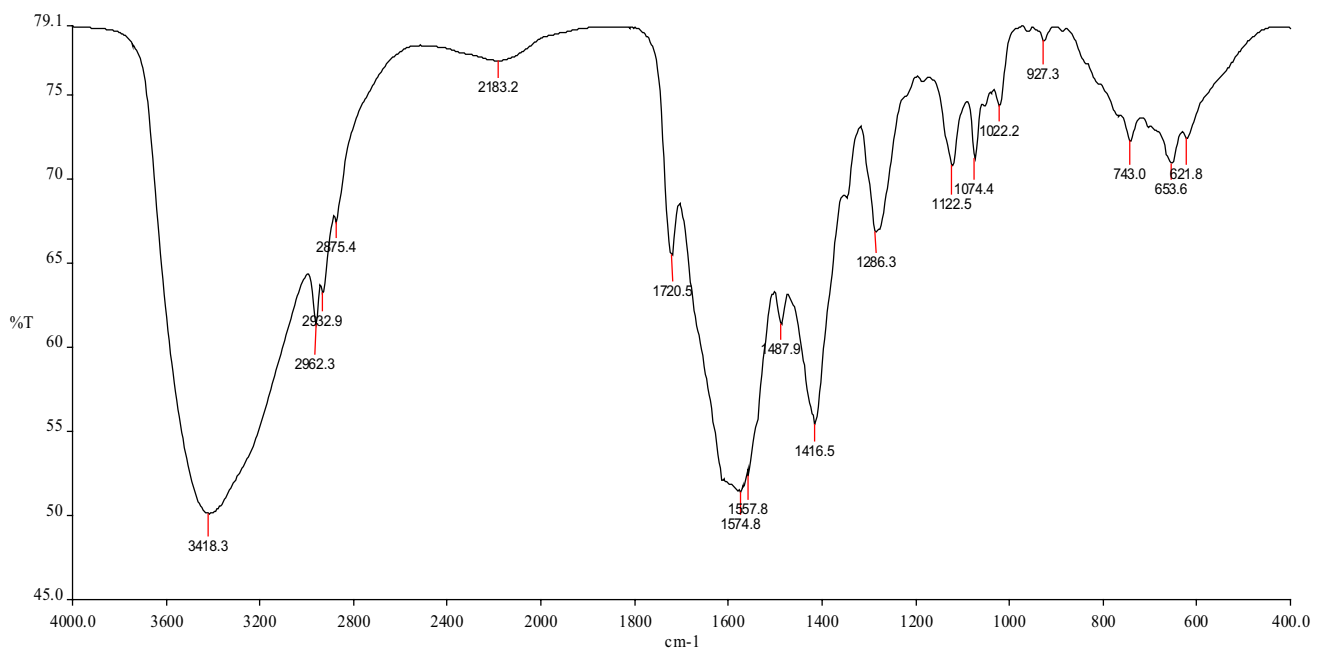


Fig. 4 FTIR spectrum of fungal taxol obtained from fraction 7 of the extract of *Alternaria brassicicola*

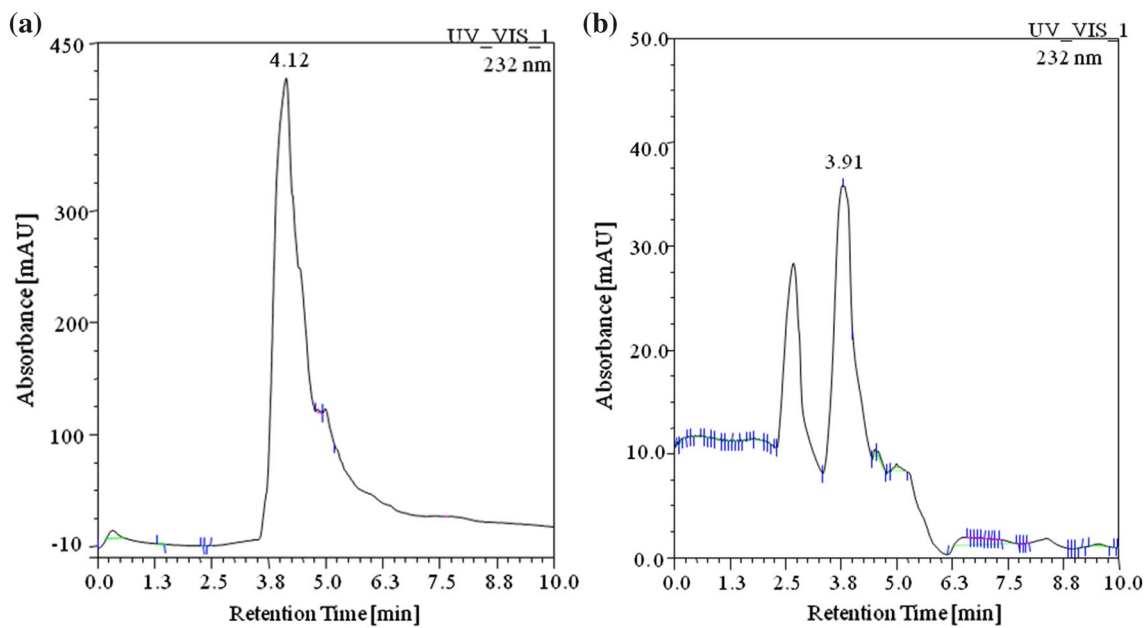


Fig. 5 High performance liquid chromatogram of authentic taxol and fungal taxol **a** elution profile of standard taxol (retention time: 4.12), **b** elution profile of fraction 7 extracted from *A. brassicicola* (retention time: 3.91)

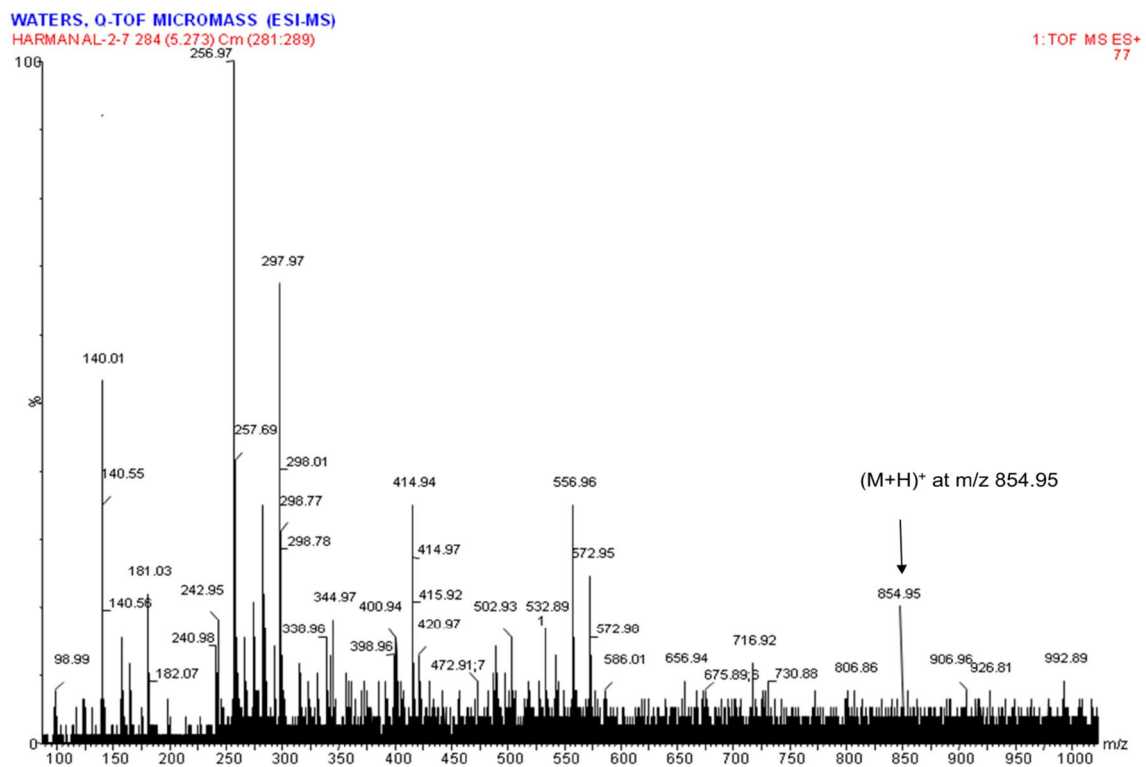


Fig. 6 Mass spectra of taxol obtained from fraction 7 of *A. brassicicola*. Characteristic ions at m/z 854.95 ($M+H$)⁺ were recorded in fraction 7 of *A. brassicicola*

its co-chromatographic mobilities with authentic taxol in a multitude of thin layer chromatographic systems (Stierle et al.

1993; Gangadevi and Muthumary 2009a). The chromatogram of HPLC analysis of the fungal extract and authentic taxol gave

a peak with similar retention time, when eluting from a reverse phase C18 column. UV spectral analysis further confirmed the presence of taxol in the extract as the spectrum of extract and authentic taxol superimposed. The IR spectral data of fungal taxol from *A. brassicicola* showed a similar spectra with that of authentic taxol as ascribed to different functional group stretches such as hydroxyl (–OH) and amide (–NH), esters and ketone (C=O) and aromatic ring (C=C) group stretches.

The amount of taxol produced by endophytic fungi associated with different non-*Taxus* plants varied from 70 to 795 µg/l (Gond et al. 2014 and references therein). The endophytic fungi, *Pestalotiopsis terminaliae* and *Chaetomella raphigera* associated with *T. arjuna* leaves produced taxol of 211.1 and 79.6 µg/l, respectively (Gangadevi and Muthumary 2009a, b). Reports regarding the production of taxol by *Alternaria* species are very scarce. Strobel et al. (1996b) reported the production of 0.16 µg/l taxol by *Alternaria* sp. Ja-69, while 84.5 µg/l by *A. Alternata* TPF6 (Tian et al. 2006). Ismaiel et al. (2017) reported 124.32 µg/l taxol by *A. tenuissima* isolated from *T. arjuna*. In the present study, the endophytic fungus *A. brassicicola* isolated from the leaves of *T. arjuna* produced about 140.8 µg/l taxol, where we are reporting for the first time the production of taxol by *A. brassicicola*.

The mass spectra analysis of *A. brassicicola* showed a parent ion peak of m/z 854.95. McClure and Schram (1992) observed major fragment ions in the MS of paclitaxel, which can be divided into three categories of the molecule. The peaks corresponding to taxol, exhibited m/z ratios corresponding to the molecular ions [M+H]⁺ of authentic taxol (854) confirming the taxol production by *A. brassicicola*. Similar results have been reported with other endophytic fungi isolated from different host plants (Gangadevi and Muthumary 2009a, b).

In conclusion, we are reporting for the first time the production of taxol by an endophytic fungus *A. brassicicola* isolated from *T. arjuna*. Further, optimization of conditions to enhance the production of taxol by *A. brassicicola* is essential.

Acknowledgements We are thankful to TIFAC-CORE, Thapar Institute of Engineering & Technology, Patiala 147004, India for providing necessary facilities to carry out the work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Abdel-Azeem AM, Zaki SM, Khalil WF, Makhlof NA, Farghaly LM (2016) Anti-rheumatoid activity of secondary metabolites produced by endophytic *Chaetomium globosum*. *Front Microbiol* 7:1477
- de Andrade HF, de Araújo LCA, dos Santos BS, Paiva PMG, Napoleão TH, dos Santos Correia MT, de Oliveira MBM, de SouzaLima GM, Ximenes RM, da Silva TD, da Silva GR, da Silva MV (2018) Screening of endophytic fungi stored in a culture collection for taxol production. *Braz J Microbiol* 49:59–63
- Gangadevi V, Muthumary J (2009a) Taxol production by *Pestalotiopsis terminaliae*, an endophytic fungus of *Terminalia arjuna* (arjun tree). *Biotechnol Appl Biochem* 52:9–15
- Gangadevi V, Muthumary J (2009b) A novel endophytic taxol producing fungus *Chaetomella raphigera* isolated from a medicinal plant, *Terminalia arjuna*. *Appl Biochem Biotechnol* 158:675–684
- Gond SK, Kharwar RN, White JF Jr (2014) Will fungi be the new source of the blockbuster drug taxol? *Fungal Biol Rev* 28:77–84
- Gu W (2009) Bioactive metabolites from *Alternaria brassicicola* ML-P08, an endophytic fungus residing in *Malus halliana*. *World J Microbiol Biotechnol* 25:1677–1683
- Ismaiel AA, Ahmed AS, Hassan IA, El-Sayed ER, Karam El-Din AA (2017) Production of paclitaxel with anticancer activity by two local fungal endophytes, *Aspergillus fumigatus* and *Alternaria tenuissima*. *Appl Microbiol Biotechnol* 101:5831–5846
- Kharwar RN, Mishra A, Gond SK, Stierle A, Stierle D (2011) Anticancer compounds derived from fungal endophytes: their importance and future challenges. *Nat Prod Rep* 28:1208–1228
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874
- Li JY, Stroble G, Sidhu R, Hess WM, Ford EJ (1996) Endophytic taxol producing fungi from bald cypress, *Taxodium distichum*. *Microbiology* 142:2223–2226
- Lou J, Fu L, Peng Y, Zhou L (2013) Metabolites from *Alternaria* fungi and their bioactivities. *Molecules* 18:5891–5935
- McClure TD, Schram KH (1992) The mass spectrometry of taxol. *J Am Soc Mass Spectrom* 3:672–679
- Matsumoto M, Matsutani S, Shigeru S, Kenji Y, Hiroshi H, Hayashi F, Terui Y, Nakai H, Uotani N, Kawamura Y (1992) Depudecin: a novel compound inducing the flat phenotype of NIH3T3 cells doubly transformed by ras- and src- oncogene, produced by *Alternaria brassicicola*. *J Antibiot* 45:879–885
- Prabavathy D, Nachiyar CV (2011) Screening and characterisation of antimicrobial compound from endophytic *Aspergillus* sp. isolated from *Ficus carica*. *J Pharm Res* 4:1935–1936
- Sah B, Subban K, Chelliah J (2017) Cloning and sequence analysis of 10-deacetyl baccatin III-10-O-acetyl transferase gene and WRKY1 transcription factor from taxol-producing endophytic fungus *Lasiodiopodia theobroamea*. *FEMS Microbiol Lett* 364:24–29
- Stierle A, Stroble G, Stierle D (1993) Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. *Science* 260:214–216
- Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. *Microbiol Mol Biol Rev* 67:491–502
- Strobel GA, Yang X, Sears J, Kramer R, Sidhu RS, Hess WM (1996a) Taxol from *Pestalotiopsis microspora*, an endophytic fungus from *Taxus wallichiana*. *Microbiology* 142:435–440
- Strobel GA, Hess WM, Ford E, Sidhu RS, Yang X (1996b) Taxol from fungal endophytes and the issue of biodiversity. *J Ind Microbiol* 17:417–423
- Tan RX, Zhou WX (2001) Endophytes: a rich source of functional metabolites. *Nat Product Rep* 18:448–459
- Tian R, Yang Q, Zhou G, Tan J, Zhang L, Fang C (2006) Taxonomic study on a taxol producing fungus isolated from bark of *Taxus chinensis* var. *mairii*. *J Wuhan Bot Res* 24:541–545
- Vasundhara M, Baranwal M, Sivaramaiah N, Kumar A (2017) Isolation and characterization of trichalasin-producing endophytic fungus from *Taxus baccata*. *Ann Microbiol* 67:255–261
- Wang J, Li G, Lu H, Zheng Z, Huang Y, Su W (2000) Taxol from *Tubercularia* sp. strain TF5, an endophytic fungus of *Taxus mairii*. *FEMS Microbiol Lett* 193:249–253

- Wani M, Taylor H, Wall M, Coggon P, McPhail A (1971) Plant anti-tumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J Am Chem Soc* 93:2325–2327
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Snisky JJ, White TJ (eds) *PCR protocols*. Academic Press, London, pp 315–322
- Zhang P, Zhou PP, Jiang C, Yu H, Yu LJ (2008) Screening of taxol-producing fungi based on PCR amplification from *Taxus*. *Biotechnol Lett* 30:2119–2123
- Zhao K, Ping W, Li Q, Hao S, Zhao L, Gao T, Zhou D (2009) *Aspergillus niger* var. *taxi*, a new species variant of taxol-producing fungus isolated from *Taxus cuspidata* in China. *J Appl Microbiol* 107:1202–1207

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations