African Journal of Biotechnology Vol. 5 (10), pp. 875-877, 16 May 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

*Full Length Research Paper*

# An endophytic Taxol-producing fungus BT2 isolated from *Taxus chinensis* var. *mairei*

**B.H. Guo[1], Y.C. Wang[1], X.W. Zhou[1], K. Hu[2], F. Tan[2], Z.Q. Miao[1] and K.X. Tang[1, 3*]**

[1]Shanghai Key Laboratory of Agrobiotechnology, Plant Biotechnology Research Center, School of Agriculture and Biology, Fudan-SJTU-Nottingham Plant Biotechnology R&D Center, Shanghai Jiao Tong University, Shanghai 200030, China.
[2]School of Life Science and Technology, Southwest Normal University, Chongqing 400715, P.R.China.
[3]State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan-SJTU-Nottingham Plant Biotechnology R&D Center, Morgan-Tan International Center for Life Sciences, Fudan University, Shanghai 200433, P. R. China.

Accepted 21 March, 2006

**BT2, a newly isolated endophytic fungus from *Taxus chinensis* var. *mairei*, was observed to produce Taxol. Besides Taxol, a potent anticancer drug, BT2 could also yield taxane baccatin III, which was an important intermediate for Taxol and semi-synthesis of Taxol in industry. The isolation of such a fungus may provide a promising alterative approach to produce Taxol, and BT2 can serve as a potential material for fungus engineering to improve Taxol production.**

**Key words:** Baccatin III, endophytic fungus, Taxol, *Taxus chinensis* var. *mairei*.

## INTRODUCTION

Taxol is a potent antimitotic agent with excellent activity against a range of cancers. It was first isolated from the bark of yew trees and approved by FDA (Food and Drug Administration) in 1992 (Wani et al., 1971; Kohler and Goldspiel, 1994). The supply of Taxol has been limited since the discovery of this natural product, and, with increasing applications in chemotherapy, the availability and cost of the drug will remain important issues. Since the first Taxol-producing fungus *Taxomyces andreanae* was isolated in 1993 (Stierle et al., 1993), there have been a few reports on the isolation of Taxol-producing endophytic fungi (Strobel et al., 1996; Li et al., 1996; Wang et al., 2000), demonstrating that organisms other than Taxus sp. could produce Taxol. Thus, fermentation processes using Taxol-producing microorganisms may be an alternative promising way to produce Taxol. Here we report another endophytic fungus BT2 which could produce Taxol and taxane baccatin III, an important intermediate for Taxol.

*Corresponding authors E-mail: kxtang1@yahoo.com.

## MATERIALS AND METHODS

### Isolation of endophytic fungi

To isolate endophytic fungi, twig and old inner bark were collected from trees of *Taxus chinensis* var. *mairei* growing in Jin Yun Mountain, China. After being cut into small pieces of about 0.25 $cm^2$ (0.5 cm x 0.5 cm), the bark pieces were treated with 70% (v/v) ethanol for 3 min followed by washing with sterilized water, and the outer bark was removed off with a sterilized sharp blade. Small pieces of inner bark were placed on the surface of potato dextrose agar (PDA) medium containing streptomycin (25 mg/l) and incubated at 25$^{o}$C in the darkness. After fungal emergence in the plates, some individual hyphal tips of the various fungi were transferred to new PDA medium and this was repeated three times for fungus purity.

### Preparation of fungal extract

The isolated fungus was cultured in a liquid potato/dextrose medium at 25$^{o}$C in the darkness for three weeks. The crude mycelia pellets and the culture medium were collected, respectively, by centrifugation. The pellets were re-suspended by 100 ml ethanol and ultrasonicated for 20 min, then centrifuged to collect the supernatant. The culture medium was extracted with equal volume of methylene chloride twice, and the organic phase was finally

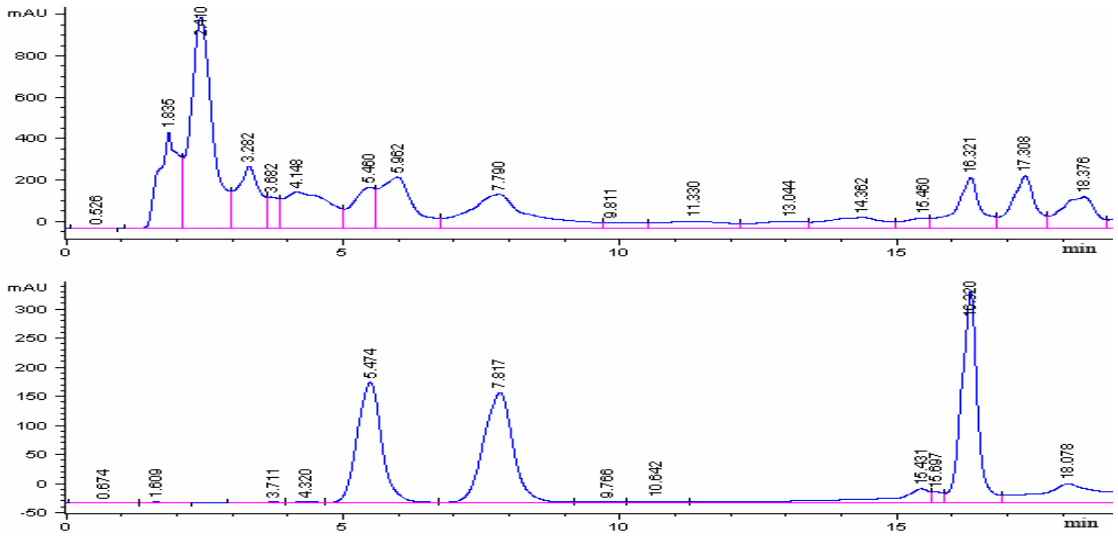



**Figure 1.** HPLC analysis of the fungus BT2 product (Upper spectrum) and of authentic baccatin III (retention time = 7.8 ± 0.1 min) and Taxol (retention time = 16.3 ± 0.1 min) (Lower spectrum).

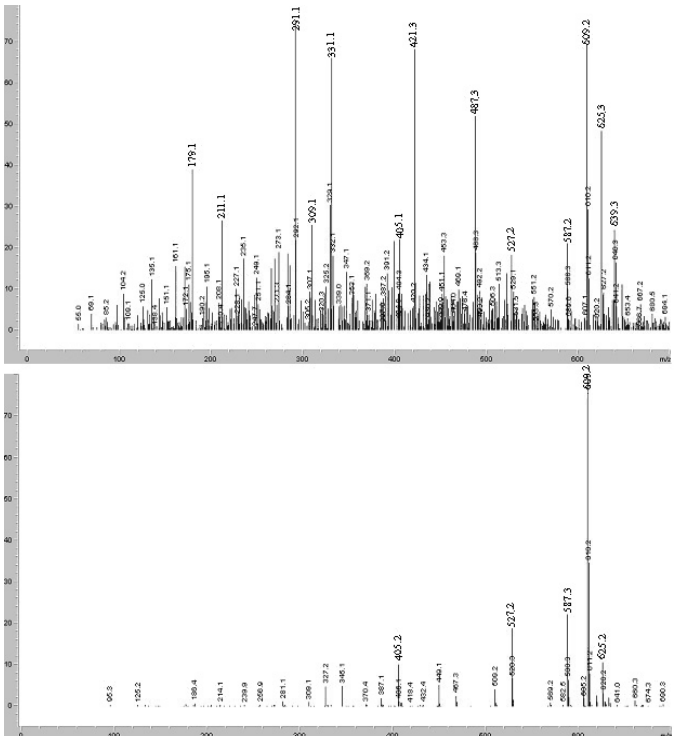


**Figure 2.** Combined reverse-phase HPLC-atmospheric pressure chemical ionization MS analysis of the fungus BT2 product (retention time = 7.8 ± 0.1 min; Upper spectrum) and of authentic baccatin III (retention time = 7.8 ± 0.1 min; Lower spectrum). The diagnostic mass spectral fragment ions are at m/z 587.2 $(M+H)^+$, 609.2 $(M+Na)^+$, and 625.2 $(M+K)^+$. Y-axis shows different ion's relative abundance (%).

mixed with the mycelia supernatant. The mixture was then evaporated at room temperature and the residue was re-suspended in 1 ml methanol and filtered through a 0.2 µm polymeric filter prior to HPLC analysis.

### Assays of Taxol and taxane baccatin III from fungi

Taxol and taxane baccatin III were first assayed by HPLC and mass spectrometry. HPLC-MS spectrum was obtained in a Perkin–Elmer HPLC ISS 200 system combined with a Hewlett–Packard Series 1100 MSD system. The sample was loaded onto an AlltechEconosil C18 column, eluted with 0.7 ml per min with a starting gradient from 50:50 (v/v) $H_2O$:methanol for 10 min, then eluted with 100% methanol for 20 min, and finally with 50:50 (v/v) $H_2O$:methanol for 10 min.

To verify the above two products, Taxane Immunoassay Kits (TA02, specific for Taxol; TA03, specific for baccatin III. Hawaii Biotech Inc) were used under the manufacturer's instruction based on the indirect competitive inhibition enzyme immunoassay (CIEIA).

### Fungus taxonomy

The endophytic fungus was classified according to the fungal identification method (Deng, 1963; Wei, 1979).

## RESULTS AND DISCUSSION

From a total of 21 isolated fungi, one fungus was observed to produce Taxol and taxane baccatin III by HPLC and mass spectrum (Figures 1 - 3), with a production of 4 - 7 µg and 12 - 18 µg per litre culture, respectively. Furthermore, the CIEIA test gave positive results, confirming the presence of Taxol and baccatin III in the fungal extract. Based on the morphology, pigmentation and growth rate of the colony, as well as the characteristics of the mycelia and conidia, the endophytic fungus was identified as one of *ozonium* species by the fungal identification method (Deng, 1963; Wei, 1979) and was named BT2 (Figure 4).

The biggest problem of using fungi fermentation to produce Taxol is its very low yield and unstable production. The Taxol yield of such reported fungi varies

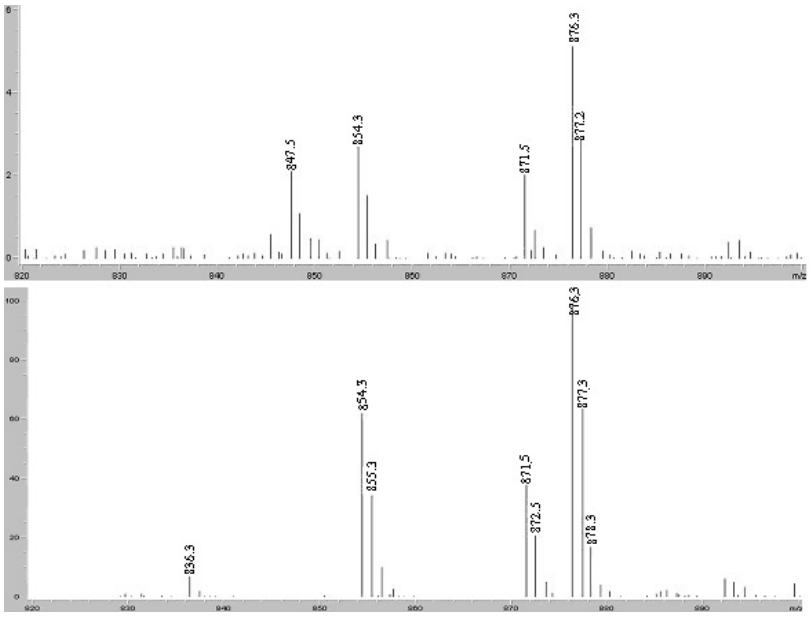
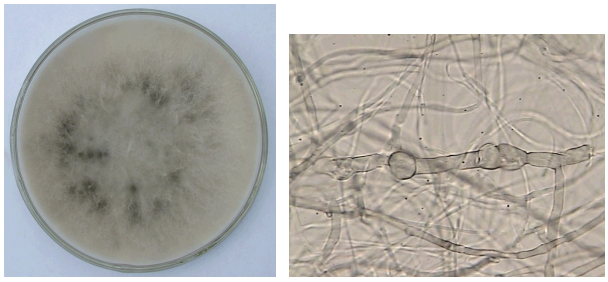



**Figure 3.** HPLC-MS analysis of the fungus BT2 product (retention time = 16.3 ± 0.1 min; Upper spectrum) and of authentic Taxol (retention time = 16.3 ± 0.1 min; Lower spectrum). The diagnostic mass spectral fragment ions are at m/z 854.3 $(M+H)^+$ and 876.3 $(M+Na)^+$. Y-axis shows different ion's relative abundance (%).



**A** **B**

**Figure 4.** Morphological observation of the fungus BT2 (A) and magnified micrograph of the fungal mycelium (B). Magnification: 400 X.

from 24 ng to 70 µg per litre culture (Stierle et al., 1993; Strobel et al., 1996). One strain of *Pestalotiopsis microspor* CP-4 (Li et al., 1996) produces Taxol varying from 50 to 1487 ng/l, indicating that it is genetically unstable.

Although the amount of Taxol produced by most endophytic fungi associated with taxus trees is relatively small when compared with that of the trees, the short generation time and high growth rate of fungi make it worth while to continue our investigation of these species.

The BT2 is a newly isolated fungus different from previous reported endophytic Taxol-producing fungi. Besides Taxol, BT2 could also yield taxane baccatin III, which is an important intermediate for Taxol and semi-synthesis of Taxol in industry. The isolation of Taxol- and taxane baccatin III-producing fungus will enable us to study Taxol biosynthetic pathway in fungi and to explore the possibility to improve Taxol or taxane baccatin III content by genetic engineering in the future.

## ACKNOWLEDGEMENTS

The authors would like to thank Professors Deqiang Xu and Yiping Xiao of Department of Microbiology at Fudan University for their assistance in fungus taxonomy. This work was funded by China National "863" High-tech Program, China Ministry of Education and Shanghai Science and Technology Committee.

### REFERENCES

Deng SQ (1963). Chinese fungi. Science Press. Beijing. pp. 757-758.

Kohler J, Goldspiel BR (1994). Evaluation of new drug Paclitaxel (Taxol). Pharmacotherapy 14: 3–34.

Li JY, Stroble G, Sidhu R, Hess WM, Ford EJ (1996). Endophytic Taxol-producing fungi from bald cypress, *Taxodium distichum*. Microbiology 142: 2223-2226.

Stierle A, Strobel G., Stierle D (1993). Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of pacific yew. Sci. 260: 214-216.

Strobel G, Yang XS, Sears J, Kramer R, Sidhu RS, Hess WM (1996). Taxol from *Pestalotiopsis microspora*, an endophytic fungus of *Taxus wallachiana*. Microbiol. 142: 435-440.

Wang JF, Li GL, Lu HY, Zheng ZH, Huang YJ, Su WJ (2000). Taxol from *Tubercularia sp.* strain TF5, an endophytic fungus of *Taxus mairei*. FEMS Microbiol. Lett. 193: 249-253.

Wani MC, Taylor HL, Wall ME, Coggon P, McPhail A (1971). Plant antitumor agents. VI. The isolation and structure of Taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J. Am. Chem. Soc. 93: 2325–2327.

Wei JC (1979). Handbook of fungal identification. Shanghai Scientific Press. Shanghai. p. 645.