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In vitro TAXOL production, by *Pestalotiopsis breviseta* – A first report

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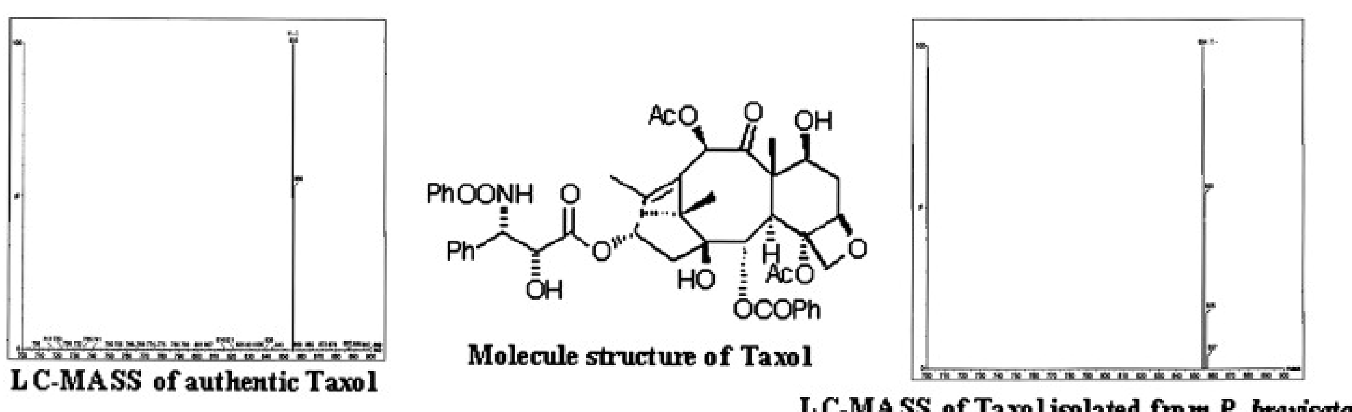
Abstract

Coelomycetous fungi were screened for the production of TAXOL. TAXOL production of Pestalotiopsis *breviseta* fungi is confirmed by Ultra Violet (UV) spectroscopic analysis, Infra Red (IR) analysis, high performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) and LC–MASS spectroscopy. TAXOL isolated from the *P. breviseta* fungus was identical with authentic TAXOL and produces 0.064mg/L (0.128% dry weight of fungal mat).

Graphical abstract

Taxol was produced by Coelomycetous fungi *Pestalotiopsis breviseta.* The TAXOL product was compared with standard TAXOL, it was identical.



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Introduction

Taxol, a diterpene was originally isolated from the bark of Pacific Yew tree (*Taxus brevifolia*) more than two decades ago and has proved to possess anticancer activity [1]. Its mode of action is unique, in that it inhibits mitosis through enhancement of polymerisation of tubulin and consequent stabilisation of microtubules during the process of cell division [2], [12]. However, a complete treatment for the patient requires approximately 2 g of TAXOL administered several times over many months. To obtain 1 kg of TAXOL it requires about 10,000 kg of bark [3] and several thousand trees must be cut to procure this quantity of bark. This scarcity of TAXOL and the ecological impact of harvesting it encouraged scientists to find alternative methods using microorganisms. A hyphomycetous fungus namely *Taxomyces andreanae* on *Taxus* sp. could produce TAXOL [4], [5]. A coelomycetous fungus, *Pestalotiopsis microspora*, an endophyte from the inner bark of *Taxus wallichiana* produced TAXOL in culture [6]. Keeping this in mind, an attempt has been made to examine the production of TAXOL by some other coelomycetous fungi as well. The TAXOL isolated from these fungi is biologically active against cancer cell lines and is also spectroscopically identical to authentic TAXOL. In order to lower the price of TAXOL and make it more available, a fermentation process involving a microorganism would be the most desirable means of supply. It was first discovered by Strobel et al. [4] that the fungus *T. andreanae* could produce TAXOL, though the yield was low. Strobel et al. [6] showed that *P. microspora* isolated from the bark of *T. wallachiana* produced TAXOL in mycelial cultures. This work prompted us to continue the search for TAXOL production from fungal sources. In the present study coelomycetous fungi were screened for the production of TAXOL.

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Materials and methods

The fungus was isolated from infected leaf of *Ervatamia divaricata*. The general laboratory techniques followed in the course of the present investigation were as outlined by Booth [7]. The test fungus used in the present study was grown in 2l Erlenmeyer flasks containing 500ml MID medium supplemented with 1g of soytone L^{-1} [8] for TAXOL production. Three mycelial agar plugs (0.5cm) were used as inoculum. The organism was grown at 24±2°C statically for 3–4weeks [6].

Results and discussion

Altogether 20 different coelomycetes were screened for the production of TAXOL. Positive results were obtained only for *P. breviseta*.

High performance liquid chromatography (HPLC)

The presence of TAXOL was further confirmed by using HPLC. The HPLC column was a C_{18}. The sample solutions of *P. breviseta* for HPLC analysis were filtered through a 0.2µm membrane before injection. The mobile phase consisted of methanol:water, 80:20v/v. The flow rate was $1 ml min^{-1}$. The quantification of TAXOL was based on an external standard of pacliTAXOL (Sigma) (Fig.3a and b).

Nuclear magnetic resonance (NMR)

Though the structure of TAXOL is complex [9], its proton NMR spectrum is relatively simple and can easily be assigned (Fig.4a and b).

A peak at δ 7.4 shows the presence of aromatic ring compounds (benzenoid derivatives, ie. peak due to aromatic ring hydrogen.

In addition to this, three peaks appear at δ 3.5, δ 2.1 and δ 1.5 which predict the existence of 3 types of hydrogen.

The peak at δ 1.5 is due to CH_3 group hydrogens. The peak at δ 2.1 is due to –C=C- hydrogen (methylene hydrogen as well as

MASS–spectrum

M+ peak at 856 is due to the molecular ions. This is observed in both samples and reference.

The more intense peak at 855 is due to (M−1) peak. When R–C–N–H for immediately loses H from N and this correspond to M−1 peak. Relative less intense peak at 857 and 858 may be due to M+1 and M+2 ions.

The method followed for TAXOL screening was described by Strobel et al. [6]. TAXOL was isolated from the culture filtrates of *P. brevesita*. The TAXOL isolated was quantified spectrophotometrically. The

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Hyper-production of taxol from Aspergillus fumigatus, an endophytic fungus isolated from Taxus sp. of the Northern Himalayan region

2019, Biotechnology Reports

> *Citation Excerpt :*
> …HPLC analysis recorded a peak with a specific retention time of 2.05, which is identical to standard paclitaxel and confirmed the presence of taxol in test samples (Fig. 11; a, b). Srinivasan and Kathiravan [84,94] also reported taxol yield of 92 μg/L and 0.064 mg/L from P. funereal and P. breviseta fungus and quantified with HPLC with a similar retention time of 2.822 and 2.210, respectively as standard taxol. Even Metarhizium anisopliae and Cladosporium cladosporioides MD2 fungal strains are very promising taxol producers with up to 800 mg/L yield quantified by HPLC [95].…

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