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Note

BIOTRANSFORMATION OF PODOPHYLLOTOXIN TO PICROPODOPHYLLIN BY MICROBES

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Biotransformation of podophyllotoxin (PT) by several microbial species has been investigated. Among the fungi tested, it was found that *Penicillium* strains can isomerize PT to picropodophyllin (PPT) in 8% yield and other strains also transform the substrate into the same product but with lower yield.

Keywords: Biotransformation; Podophyllotoxin; Picropodophyllin; *Penicillium melini*; *Penicillium citreo-viride*; *Syncephalastrum racemosum*; *Cunninghamella elegans*

Podophyllotoxin (PT) is a naturally occurring lignan which is extracted from a number of plant species such as *Podophyllum* spp. (Berberidaceae) [1], *Linum* spp. (Linaceae) [2], *Juniperus* spp. and *Callitrus* spp. (Cupressaceae) [3], and *Polygala* spp. (Polygalaceae) [4]. It specifically inhibited mitoses [5,6], and had significant antitumor activity [7,8]. But due to its strong toxicity and severe side effects [9] it cannot be directly used in clinics. Therefore its derivatives, etoposide (VP-16-213) and teniposide (VM-26),

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two well-known antitumor agents, were semisynthetically prepared by using PT as starting material and successfully employed in the treatment of small cell anaplastic lung carcinoma, Hodgkin's disease and non-Hodgkin's lymphoma [10–12]. Since many steps were involved for the chemical synthesis of etoposide from podophyllotoxin, scientists have devoted their efforts to employ microbial organisms or plant cell cultures for biotransformation. Although there was no successful report on the direct biotransformation of etoposide from PT or its derivatives, key intermediates or etoposide-related compounds were obtained [13–16]. In an attempt to synthesize etoposide or other biologically active compounds by microbial transformation, several microbes were examined to convert PT. It was found that *penicillium* strains could isomerize PT into PPT in 8% yield (Fig. 1). Table 1 shows the conversion rate of PT by each fungus.

To our knowledge, only one research group reported the hydroxylation of deoxypodophyllotoxin (DPT) by *Penicillium* species with 100% yield as well the conversion of DPT to deoxypicropodophyllin (DPP), an inactive antitumor agent, by other microbial strains tested [13], on which the authors concluded that the microbial conversion of lignans with cytotoxicity was difficult. Our experiments proved this point to some extent. But the

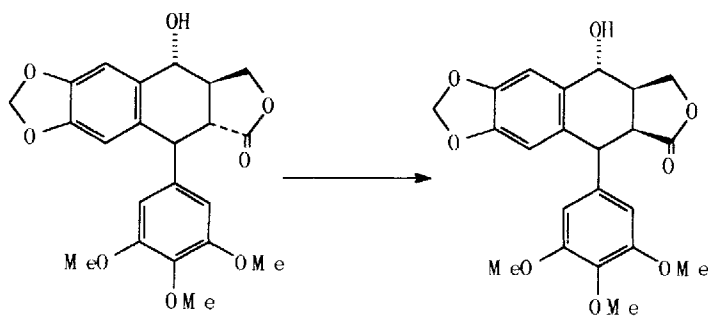


FIGURE 1 Biotransformation of podophyllotoxin to picropodophyllin by cultures of *Penicillium*, *Syncephalastrum* and *Cunninghamella* species.

TABLE 1 Conversion rate of PT to PPT by fungi

| Strain | Substrate (mg) | PPT (mg) | Conversion rate (%) |
|----------------------------------|----------------|----------|---------------------|
| <i>Cunninghamella elegans</i> | 150 | 8.4 | 5.6 |
| <i>Syncephalastrum racemosum</i> | 150 | 4.4 | 2.9 |
| <i>Penicillium melinii</i> | 150 | 12.6 | 8.4 |
| <i>Penicillium citreo-viride</i> | 150 | 9.8 | 6.5 |

results also suggested that some hydroxylases or other enzymes capable of transforming PT occur in *Penicillium* species. A number of *Penicillium* strains were therefore collected and their capability of converting PT and its derivatives is under investigation.

EXPERIMENTAL SECTION

PT was isolated and purified from the roots and rhizomes of *Podophyllum emodi* Wall. The fungi used for the microbial transformation were as follows: *Penicillium melini* strain 3.156 and *Penicillium citreo-viride* strain 3.4477, *Syncephalastrum racemosum* strain 3.226, *Cunninghamella elegans* strain 3.264. They were grown in standard potato medium or standard Czapeks medium (500 ml in a 1 L flask, totally 5 flasks) at 25°C on a rotary shaker (116 rpm). After two days of incubation, PT in ethanol (10 mg/ml) was added for three successive days (30 mg in each flask, totally 150 mg) and then the fermentation was allowed to continue for three more days. The filtrate of the culture broth was extracted with ethyl acetate and the extract was evaporated to dryness *in vacuo*. The residue was dissolved in methanol and developed on preparative TLC with CH₂Cl₂-EtOAc(4:1). By referring to the band of standard PT applied in parallel on the plate, the band corresponding to the transformed product was scraped off and eluted with Me₂CO. After recrystallization in Me₂CO, colorless needles were obtained. It was identified as PPT [17] based on its chemical and spectral data. PPT: mp 228–230°C, MS(m/z): 414(M⁺), 383(M–OCH₃); ¹HNMR(500 MHz, CDCl₃) δppm: 7.04(s, 1H, 5-H), 6.45(s, 2H, 2',6'-2H), 6.37(s, 1H, 8-H), 5.93(dd, 2H, *J* = 1.5/12.7 Hz, 6,7-OCH₂), 4.51(m, 2H, 11-H_α, 4-H), 4.44(dd, 1H, *J* = 6.5/9.5 Hz, 11-H_β), 4.11(d, 1H, *J* = 5.0 Hz, 1-H), 3.85(s, 3H, 4'-OCH₃), 3.82(s, 6H, 3',5'-2OCH₃), 3.24(dd, 1H, *J* = 7.5/9.5 Hz, 2-H), 2.74(m, 1H, 3-H).

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