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Microbial Production of a Plant Alkaloid, Camptothecin: A Promising Anticancer Agent

N. Salim[1], U.G. Chandrika[2], A.M. Abeysekera[3], S.M.C. Senanayeka

[1,4]Department of Botany, Faculty of Applied Science, [2] Department of Biochemistry, Faculty of Medical Science, [3] Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardeneura, Sri Lanka.
E-mail: udumalagala2002@yahoo.com

An endophytic fungus, isolated from the camptothecin (CPT)-producing plant *Ophiorrhiza mungos* L., was found to be capable of producing CPT under *in vitro* conditions. Objectives of the present study were to identify the fungus and optimize culture conditions for enhanced yield of the alkaloid. The CPT was extracted with hot ethanol (61%) from fungal mycelia grown for ten days in 100 ml potato dextrose broth medium (PDB) under shake-flask condition at 160 rpm. The samples were analyzed by HPLC-DAD using a reverse phase C18 column and the presence of CPT was detected at 256 nm. Identification of CPT was carried out using chromatographic behavior in HPLC (retention time), UV absorption spectra in the mobile phase obtained by the DAD detector and co-chromatography with added CPT standard. Quantification was carried out using external standard of CPT. Culture conditions were optimized for higher yield of CPT. The fungus was identified as ***Glomerella cingulata*** (isolate W128) by molecular tools. Detectable level of CPT was produced in 10 days old cultures while a highest yield, 17.8 ± 0.1 μg /g (mean ± standard error) of fresh weight (FW) of mycelia was obtained in cultures aged 18 days in PDB. Thereafter, the yield of CPT dropped with the age of the culture. Of the six common fungal media tested, potato sucrose broth (PSB) was superior and the fungus produced significantly higher CPT content 24.1 ± 0.2 μg/g FW than that of the other media tested. Modified PSB medium with 3% sucrose gave higher yields of CPT compared to that produced in standard PSB (1.5% sucrose). Addition of aqueous extracts of host plant into PSB had no effect in CPT yield while Al^{+3} and Ca^{+2} at 1 mg/100 ml in PSB enhanced the yield. However, a sharp attenuation in CPT production by the endophyte was observed through sub culturing of the fungus.