

## 人工固體栽培樟芝成分抽離暨自由基清除

### 抗變異活性探討

#### **Active components isolation and their antimutagenic activities from Solid-state cultivated *Taiwanofungus camphoratus* via hydroxy radical scavenging effect**

##### 中文摘要

樟芝為台灣本土獨特菌種，價格昂貴，民間傳為解毒、保肝、抗癌之機能性食品即食療補助用品；產學界多數以液體深層發酵培養為主，但無法令其自行合成具有作用的三萜類 (Triterpenoids)。固體栽培是新開發出來的栽培技術；運用此法培養出的樟芝，已有報告指出其成分與野生樟芝較為相似。

本研究利用固體栽培樟芝子實體作為研究材料，探討其抗突變之作用機轉。將樟芝粉末分別經乙醇和冷水萃取，其乙醇萃取液再經由分配萃取，初步分離成正己烷層、乙酸乙酯層、甲醇層等三部分，而水萃取液則經透析後再行酒精沈澱探討其多醣的生物活性。

本研究以安姆氏試驗法之 *Salmonella typhimurium* TA98 及 TA100 突變株，發現無論加或不加老鼠肝臟均質物 S9 mix 活化，其均呈現陰性反應，顯示樟芝對微生物不具致突變性。此外以 TA102 進行基因毒性試驗及自由基抗變異活性分析；結果發現樟芝不具基因毒性且其乙醇粗萃取物及水萃取物對清除過氧化氫自由基之抗變異效果達統計上顯著抑制效果。而進一步分離所得的三個部分，對清除過氧化氫自由基抗變異效果依序為：甲醇層>乙酸乙酯層>正己烷層。

因此再利用中壓液態層析分離抽取甲醇層，依序得出三個部分。抗變異能力測試結果，Fraction3 的效果最佳，其 SR50 為 0.005mg/ml，即具有較佳的抗變異效果。綜合以上實驗結果，顯示樟芝不具基因毒性及致突變性，此外還可藉由去變異 (Desmutation) 及抗變異 (Antimutation) 達到清除自由基之功效。

##### 英文摘要

*Taiwanofungus camphoratus* is an unique fungal species in Taiwan which is rare and expensive. It has been used as a functional food for drug intoxication, hepatoprotection and anti-tumor. This study used the fruiting body of *T. camphoratus* obtained from solid stage to further explore its anti-tumor mechanism. Fruiting body was extracted by ethanol and cold water. Ethanol extracts were further partitioned using n-Hexane, ethyl acetate, and methanol fraction. Cold water extracts were eluted through dialysis membrane (M.W. 3000) and precipitated in ethanol which were then used to analyze the bioactivation of *T. camphoratus*. This study also use the Ames test to evaluate the mutagenesis of *T. camphorates*. The *Salmonella typhimurium* TA98,

and TA100 mutant strains were used. Either alone or in the presence of S9 mix, *T. camphoratus* extracts were not mutagenic. The genotoxicity and antimutagenic effects of *T. camphoratus* extracts were examined using *S. typhimurium* strain TA102. Our results showed that *T. camphoratus* extracts were non-genotoxic as well as antimutagenic for TA102, which were pretreated with H<sub>2</sub>O<sub>2</sub>-generated ROS in the Ames test. The H<sub>2</sub>O<sub>2</sub> scavenging effect of the *T. camphoratus* ethanol extracts was highest in the methanol layer, followed by Ethyl acetate layer and n-Hexane layer. Therefore we further isolated three fractions seriesly from the methanol layer by Medium Pressure Liquid Chromatography. Through the antimutagenic assay, Fraction 3 had the highest antimutagenic ability (SR50 : 0.005mg/ml). In summary, we found that *T. camphoratus* is antigenotoxic and antimutagenic, which also has the ability of desmutation and antimutation to scavenge free radicals.