The Biocontrol Mechanism of *Trichoderma harzianum*

研究內容：

1. *Trichoderma harzianum* 體內及體外蛋白質體

Trichoderma harzianum 是一土生性鞭狀真菌，在生物防治上能保護許多植物免於植物病源真菌如 *Rhizoctonia solani* 及 *Botrytis cinerea* 的感染，故能部份取代化學性的殺真菌劑，而減少對環境的衝擊。雖然 *Trichoderma* spp.在生物防治上成就顯著，但是對 *T. harzianum* 如何對 *R.solani* 及其他植物病源真菌發揮防制功能，其體內及體外蛋白質體的變化，尚無研究。本計畫將比較 *T. harzianum* 生長於經處過 *R. solan*i 及葡萄糖的不同環境下，分別取 *T. harzianum* 細胞內及細胞外蛋白質，透過二維電泳及 laser desorption/ionization time-of-flight mass spectrometry，及配合資料庫搜尋來鑑定蛋白質種類，另外再配合 N 端蛋白質定序來鑑定蛋白質，以了解 *T. harzianum* 遇到植物病源真菌時，菌體內外蛋白質表現的差異，進而了解生物防治的機制。

2. *Trichoderma harzianum* 代謝體

在於許多及微生物所分泌之代謝物中，其功能包括抗細菌、抗真菌、抗病毒、抗癌及促進植物生長等作用。本研究從*Trichoderma harzianum*菌株ETS 323 分離純化代謝物，希望能了解所分泌之代謝物對*T. harzianum*菌株ETS 323 在作物病害生物防治所扮演的角色。實驗計劃將*T. harzianum*菌株ETS 323 在PDA上培養 6 天後，以無菌水沖洗，以獲得孢子懸浮液 (10^6 spores/mL)，並將此孢子懸浮液接種於甘蔗渣中培養 8 天，再以乙酸乙酯 (EA)萃取後，經矽膠薄層色層分析 (silica gel TLC)得到分離條件，再以矽膠管柱層析 (silica gel column chromatography)純化，分別得到黃色粉末與橘黃色粉末，其結構將分別經X光單晶繞射 (x-ray diffraction)及 2D-核磁共振 (2D-NMR)鑑定。經分離純化之化合物分別用以測試是否可影響植物種子萌芽率與側根數目，另外亦檢測對*T. harziznum*菌株ETS 323、*T. virens*與植物病原菌 (*A. brassicicola*, *P. aphanidermatum*, *R. solani*, *P. capsici*及*E. carotovora*)之生長及孢子萌芽之影響。作物測試之結果經Ducan's test 分析以顯示效果之顯著性。往後應可更廣泛測試其他植物種子及土棲性病原菌之影響，以冀望能進一步了解其在生物防治的相關角色。

3. Development and Structure-Function Investigation of Protein Photcleavers and Their Antibiotic Applications

Reagents that can bind to proteins and cleave the protein backbone upon activation with light are dubbed photoprotease. Anthraquinone, 9,10-anthracenedione ($C_{14}H_8O_2$), is an aromatic organic compound and its derivatives exist in many herbal plants and microorganisms. They possess many biological functions, such as antiviral,

antibacterial, antifungal, and anticancer activities. We had isolated two anthraquinone derivatives, chrysophanol (1, 8-dihydroxy-3-methyl-anthraquinone, $C_{15}H_{10}O_4$) and pachybasin (1-hydroxy-3- methyl-anthraquinone, $C_{15}H_{10}O_3$), from the culture of filamentous fungus, *Trichoderma harzianum*. Preliminary studies indicate that both derivatives can degrade BSA and Lysozyme randomly under 365 nm UV radiation. Thus the use of these two compounds as scaffolds to develop metal-anthraquinone derivatives, $M(C_{15}H_9O_3)_n$: M = Cu^{2+}, Co^{3+}, Fe^{3+}, etc, and organic-anthraquinone derivatives as potential photoproteases having site-specific cleavage activity and hydrophilic property are the aims of this proposed research. To our knowledge, never has this type of metal complexes being utilized to cleave the protein backbone although the application of metal compounds as DNA cleaving agents has been broadly studied. Organic modified derivatives have been investigated but, nevertheless, in nonspecific cleavage way and very poor hydrophilic solubility. Also, the mechanism of the anthraquinone derivatives photoprotease activity is still an enigma but free radical was proposed. So, this mechanism will be further investigated by EPR and Synchrotron X-ray diffraction in this research.