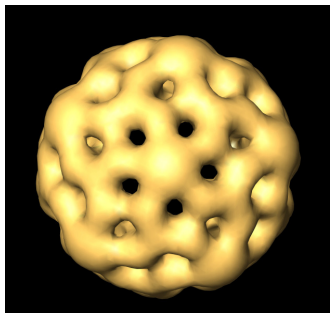
發明名稱

（以下內容一頁為限，不可揭露關鍵技術內容；填表完成後請刪除此行）



發明人：黃慶璨教授
單　位：國立臺灣大學生化科技學系
簡　歷：

市場及需求: 分子農場為新興市場，潛力無限。

技術摘要(含成果):
本技術包括穩定的菇類農桿菌媒介轉形系統及金針菇多基因表現平台。本技術建構一系列腸病毒 71 型類病毒顆粒表現載體，利用 2A 短鏈胜肽聯結腸病毒外鞘蛋白質 P1 及 3C 蛋白質截切酶等基因，經 2A 短鏈胜肽媒介截切作用於共轉譯時期分離 P1 和 3C 後，3C 蛋白質截切酶接著將病毒外鞘蛋白質分割成 VP1、VP2、VP3、VP4 等 4 個病毒次單元，可於胞內組裝成類病毒顆粒。為提升生產病毒次單元的效率，利用單點突變 3C 蛋白質截切酶，以避免宿主細胞 SUMO 蛋白質修飾化(sumoylation)而提高轉錄活性。挑取經農桿菌媒介轉形後可穩定繼代的金針菇轉形株，以南方氏墨點法分析顯示異源基因嵌入宿主染色體拷貝數，續以西方墨點法經專一性單株抗體辨識，可偵測病毒次單元 VP1 及 VP2 訊號；各病毒次單元之部分胺基酸序列亦可成功經液相層析質譜儀探測比對。進一步以蛋白質複合體熱穩定性分析法測試最適類病毒顆粒組裝緩衝液環境後，利用穿透式電子顯微鏡取得類病毒顆粒 2 維影像，並以電腦影像分析技術成功模擬類病毒顆粒之 3 維立體結構。上述結果證實本技術可於金針菇菌絲體內自行組裝成完整類病毒顆粒。

優勢: 金針菇可工業生產，密閉環控生產條件基因污染生態風險低。類病毒顆粒免疫刺激效果較次單元佳。

競爭產品: 目前無。

專利現況: 本研究團隊具有近二十年菇類轉形及異源基因表現研究經驗

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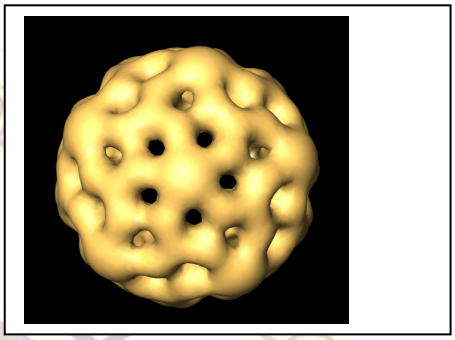
Title of Invention

(Below is limited to 1-page only; be careful not to disclose vital technology content. Please delete these words when the document is finished)



PI： Prof. Huang, Ching-Tsan
Department of Biochemical Science and Technology, National Taiwan U.

Experience:

Market Needs: Molecular pharming is an emerging market with tremendous potential.

Our Technology:

This invention include the mushroom *Agrobacterium*-mediated transformation and a polycistronic expression system in enoki mushroom *F. velutipes.* The genes of EV71 P1 structural protein and 3C protease were constructed in the same transcript linked by 2A peptides. After P1 and 3C were separated via 2A peptides mediated cleavage in co-translational level, 3C protease can proteolytically cleavage P1 into four subunits, VP1, VP2, VP3, and VP4. These four subunits automatically assemble to form VLPs within transgenic mycelia. To increase the efficiency of subunits production, a site-directed mutation of 3C protease was made to prevent sumoylation from host cells. After ATMT and *F. velutipes* transformants selection, the crude extraction of EV71 VLPs from transgenic mycelia were purified. The subunits VP1 and VP2 were detected using specific monoclonal antibodies. The partial peptide sequences of VP1 to VP4 were assigned by LC-MS/MS and mascot. Moreover, the 2D images of EV71 VLPs can be observed under TEM, and their 3D structure was reconstructed *de novo*. The above results showed this invention provide a novel method in production of EV71 VLPs in enoki mushrooms.

Strength: Enoki can be produced at industrial scale under closed and environment-controlled conditions to minimize the risk of gene pollution. Immunogenecity of virus-like particles is better than subunit viral proteins.

Competing Products: None at this moment.

Intellectual Properties: Almost 20 years of experience in mushroom transformation and heterologous gene expression.

Contact (do not need to fill out):

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