

第七屆中國醫藥大學暨亞洲大學生物科技研討會
The 7th China Medical University & Asia University
Joint Conference on Biotechnology

中國醫藥大學醫學檢驗生物技術學系
Department on Medical Laboratory Science and Biotechnology, CMU
中國醫藥大學生物科技學系
Department of Biological Science & Technology, China Medical University
亞洲大學生物科技學系
Department of Biotechnology, Asia University
亞洲大學保健營養生技學系
Department of Health and Nutrition Biotechnology, Asia University

103年05月14日 (星期三)
May 14 (Wednesday)

立夫教學大樓B1國際會議廳
LiFu Teaching Building B1 International Conference Hall

大會議程

時間	2014 年 5 月 14 日 (星期三)	備註
08:00~09:00	報到	
09:00~09:10	開幕式	
	貴賓致詞	
09:10~09:55	Keynote Speech I	
	主持人：鄭如茜 教授 (中國醫藥大學 醫學檢驗生物技術學系)	
	演講者：阮麗蓉 博士 (中央研究院基因體研究中心副研究員) Epigenetic Alterations Leading to Cancer	
09:55~10:40	Keynote Speech II	
	主持人：鄭如茜 教授 (中國醫藥大學 醫學檢驗生物技術學系)	
	演講者：林伶 總經理 (生寶臍帶血銀行) 兩岸三地醫療檢驗產業的現況及未來發展	
10:40~11:00	Coffee Break	
11:10~11:45	Keynote Speech III	壁報論文 評分 (單數號) 10:40~12:00
	主持人：范宗宸 主任 (亞洲大學 生物科技學系)	
	演講者：林旭陽 科長 (衛生福利部食品藥物管理署) 分子生物在食品檢驗的應用	
11:45~12:10	Conference Speech I	
	主持人：陳昭賢 主任 (中國醫藥大學 醫學檢驗生物技術學系)	
	演講者：石志榮 副教授 (中國醫藥大學 醫學檢驗生物技術學系) The role of androgen receptor in regulating the growth, migration and invasion of urothelial carcinoma cells	

12:10~13:10	午餐時間	
13:10~13:35	Conference Speech II	壁報論文 評分 (雙數號) 12:30~14:00
	<p>主持人：范宗宸 主任/教授 (亞洲大學 生物科技學系)</p> <p>演講者：蔡政芳 副教授 (亞洲大學 生物科技學系)</p> <p>Antioxidant Properties and Antioxidant Compounds of Various Extracts from the Edible Basidiomycete Grifola Frondosa (Maitake)</p>	
13:35~14:00	Conference Speech III	
	<p>主持人：翁靖如 教授 (中國醫藥大學 生物科技學系)</p> <p>演講者：魏宗德 教授 (中國醫藥大學 生物科技學系)</p> <p>Emodin represses TWIST1-induced epithelial- mesenchymal transitions in head and neck squamous cell carcinoma cells by inhibiting the β-catenin and Akt pathways</p>	
14:00~14:25	Conference Speech IV	
	<p>主持人：黃珮珍 主任 (亞洲大學保健營養生技學系)</p> <p>演講者：鄧正賢 助理教授 (亞洲大學保健營養生技學系)</p> <p>A New Process for Developing Massa Medicata Fermentata</p>	
14:25~15:45	學生口頭論文競賽	
	<p>(共 8 位,每位 10 分鐘)</p> <ol style="list-style-type: none"> 1. Marcelia Sugata (亞洲大學) 2. 詹辰馨(中國醫藥大學) 3. 陳岱妤(亞洲大學) 4. 蕭宜馨(中國醫藥大學) 5. 蔡宜哲(亞洲大學) 6. 蘇融廷(亞洲大學) 7. 劉祐瑄(中國醫藥大學) 8. 練偉忠(中國醫藥大學) 	
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K01 阮麗蓉 副研究員

中央研究院 基因體研究中心

Epigenetic Alterations Leading to Cancer

K02 林伶 總經理

生寶臍帶血銀行

兩岸三地醫療檢驗產業的現況及未來發展

K03 林旭陽 科長

衛生福利部 食品藥物管理署 檢驗研究組 食品生物科

分子生物在食品檢驗的應用

Conference Speech:

C01 石志榮 副教授

中國醫藥大學 醫學檢驗生物技術系

The role of androgen receptor in regulating the growth, migration and invasion of urothelial carcinoma cells

C02 蔡政芳 副教授

亞洲大學生物科技系

Antioxidant Properties and Antioxidant Compounds of Various Extracts from the Edible Basidiomycete Grifola Frondosa (Maitake)

C03 魏宗德 教授

中國醫藥大學 生物科技系

Emodin represses TWIST1-induced epithelial-mesenchymal transitions in head and neck squamous cell carcinoma cells by inhibiting the β -catenin and Akt pathways

C04 鄧正賢 助理教授

亞洲大學保健營養生物科技系

A New Process for Developing Massa Medicata Fermentata

Keynote Speech

Epigenetics and Epigenetic Alterations Leading to Cancer

Li-Jung Juan

Juan lab has been focusing on basic epigenetic mechanism and epigenetic alterations leading to cancer. Today I like to share the following two stories:

I. DNA methylation/demethylation and cancer. Tumor suppressor gene silencing by cytosine methylation is critical for cancer formation. Previously we showed that hNaa10p/hARD1 was essential for maintaining DNA methyltransferase 1 activity and recruitment to tumor suppressor genes for promoter hypermethylation-mediated gene silencing in human lung cancer (J Clin Invest, 2010). This study and studies from many others indicate that DNA methyltransferases are oncogenic. The burning issue remains in the field is whether DNA demethylation enzymes counteract this oncogenic effect. Our recent paper in Cell Reports (2012) demonstrate the first time that the dioxygenase TET1, a DNA demethylation enzyme which converts 5-methylcytosine to 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxylcytosine, is an important suppressor of tumor malignancy. We further uncovered that TET1 suppresses tumor development by activating the tissue inhibitors of metalloproteinases (TIMP).

II. Glycosylation and cancer epigenetics. Aberrant increase of protein O-GlcNAcylation by adding β -D-N-acetylglucosamine to serine or threonine residues of nuclear and cytoplasmic proteins correlates with cancer progression. However, the upstream regulatory mechanism and the downstream effectors are largely unknown. Protein O-GlcNAcylation is catalyzed by O-GlcNAc transferase (OGT) using uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) as the donor and removed by the glycosidase O-GlcNAcase (OGA). Both UDP-GlcNAc and OGT are known to be upregulated in cancer. Our recent finding (PNAS 2014) indicates that one of the OGT functions is to modify and stabilize the oncoprotein histone methyltransferase EZH2 and thus maintain its product histone 3 K27 trimethylation to silence a specific subset of potential tumor suppressor genes in breast cancer cells.

阮麗蓉 副研究員簡介

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現職：

Associate Research Fellow

Genomics Research Center

Academia Sinica

學歷：

-Ph.D. training with Jerry Workman, The Pennsylvania State University, 01/1992-08/1996

經歷：

-Postdoctoral Fellow (06/1997-10/2000) and Assistant Investigator (10/2000-03/2006), National Health Research Institutes, Taiwan,

-Assistant (04/2006-07/2009) and Associate Research Fellow (07/2009-present), Genomics Research Center, Academia Sinica, Taiwan

-Adjunct Assistant (08/2006-02/2010) and Associate Professor (02/2010-present), Institute of Molecular Medicine, College of Medicine, National Taiwan University, Taiwan

Honors

-1st Prize, Departmental Research Poster Award (1/76), The Penn State University, 1995

-1st NHRI Postdoctoral Fellowship Competition Award (3/40), 1998

-Asia-Pacific International Molecular Biology Network (A-IMBN) Feature Report, 2008

-Academia Sinica Major Discovery, 2008

-Academia Sinica Career Development Award, 2009

-4th TienTe Lee Biomedical Foundation Young Scientist Research Award, 2009

-Asia-Pacific International Molecular Biology Network (A-IMBN) Feature Report, 2010

-Best 5 Articles in Cell Reports in 2012

Publications in Last Five Years

(*corresponding author or co-corresponding author)

[O-GlcNAcylation regulates EZH2 protein stability and function.](#) Chu CS, Lo PW, Yeh YH, Hsu PH, Peng SH, Teng YC, Kang ML, Wong CH* and Juan LJ*. *PNAS*. 2014 Jan 28;111(4):1355-60. Epub 2014 Jan 13.

[Histone demethylase RBP2 promotes lung tumorigenesis and cancer metastasis.](#) Teng YC, Lee CF, Li YS, Chen YR, Hsiao PW, Chan MY, Lin FM, Huang HD, Chen YT, Jeng YM, Hsu CH, Yan Q, Tsai MD and Juan LJ*. *Cancer Res*, 2013 Aug 1;73(15):4711-4721. Epub 2013 May 30.

[TET1 suppresses cancer invasion by activating the tissue inhibitors of metalloproteinases.](#) Hsu CH, Peng KL, Kang ML, Chen YR, Yang YC, Tsai CH, Chu CS, Jeng YM, Chen YT, Lin FM, Huang HD, Lu YY, Teng YC, Lin ST, Lin RK, Tang FM, Lee SB, Hsu HM, Yu JC, Hsiao PW, Juan LJ*. *Cell Rep*. 2012 Sep 27;2(3):568-79. Epub 2012 Sep 20. (Best 5 Articles of Cell Reports in 2012)

[Breast cancer cells induce stromal fibroblasts to secrete ADAMTS1 for cancer invasion through an epigenetic change.](#) Tyan SW, Hsu CH, Peng KL, Chen CC, Kuo WH, Lee EY, Shew JY, Chang KJ, Juan LJ*, Lee WH. *PLoS One*. 2012;7(4):e35128. Epub 2012 Apr 13. (*co-corresponding author)

[The HPV E6 oncoprotein targets histone methyltransferases for modulating specific gene transcription.](#) Hsu CH, Peng KL, Jhang HC, Lin CH, Wu SY, Chiang CM, Lee SC, Yu WC, Juan LJ*. *Oncogene*. 2012 May 3;31(18):2335-49. Epub 2011 Oct 3.

[Protein kinase A-mediated serine 35 phosphorylation dissociates histone H1.4 from mitotic chromosome.](#) Chu CS, Hsu PH, Lo PW, Scheer E, Tora L, Tsai HJ, Tsai MD, Juan LJ*. *J Biol Chem*. 2011 Oct 14;286(41):35843-51. Epub 2011 Aug 18.

[Host-viral effects of chromatin assembly factor 1 interaction with HCMV IE2.](#) Lee SB, Lee CF, Ou DS, Dulal K, Chang LH, Ma CH, Huang CF, Zhu H, Lin YS, Juan LJ*. *Cell Res*. 2011 Aug;21(8):1230-47. Epub 2011 Mar 29.

[Transcriptional activation of endoplasmic reticulum chaperone GRP78 by HCMV IE1-72 protein.](#) Shi-Chen Ou D, Lee SB, Chu CS, Chang LH, Chung BC, Juan LJ*. *Cell Res*. 2011 Apr;21(4):642-53. Epub 2011 Jan 11.

[hNaa10p contributes to tumorigenesis by facilitating DNMT1-mediated tumor suppressor gene silencing.](#) Lee CF, Ou DS, Lee SB, Chang LH, Lin RK, Li YS, Upadhyay AK, Cheng X, Wang YC, Hsu HS, Hsiao M, Wu CW, Juan LJ*. *J Clin Invest*. 2010 Aug;120(8):2920-30. Epub 2010 Jul 1.

[Dysregulation of p53/Sp1 control leads to DNA methyltransferase-1 overexpression in lung cancer.](#) Lin RK, Wu CY, Chang JW, Juan LJ, Hsu HS, Chen CY, Lu YY, Tang YA, Yang YC,

Yang PC, Wang YC. *Cancer Res.* 2010 Jul 15;70(14):5807-17. Epub 2010 Jun 22.
[Gene-specific transcriptional activation mediated by the p150 subunit of the chromatin assembly factor 1.](#) Lee SB, Ou DS, Lee CF, Juan LJ*. *J Biol Chem.* 2009 May 22;284(21):14040-9. Epub 2009 Mar 26.
[The ARID domain of the H3K4 demethylase RBP2 binds to a DNA CCGCCC motif.](#)
Tu S, Teng YC, Yuan C, Wu YT, Chan MY, Cheng AN, Lin PH, Juan LJ*, Tsai MD. *Nat Struct Mol Biol.* 2008 Apr;15(4):419-21. Epub 2008 Feb 12. (*co-corresponding author)

Patent

Hsu, J.T.A., Hsieh, H.-P., Juan, L.-J., Chang, S.-Y. and Kuo, Y.-H. (2008) A combination of anti-viral activity. ROC Patent No. I293248

林伶 總經理簡介

姓名：林伶

現職：

生寶臍帶血銀行 總經理

學歷：

1982-1986 National Taiwan University, Taipei, Taiwan

Bachelor of Plant Pathology.

Major in Microbiology, and Biochemistry.

經歷：

- 11/2007 – 10/2012 China Country General Manager
Led China to achieve accelerated growth and performance, cultivating China into one of the fastest growing economies in BD. Business has grown from US\$ 101mm to 340 mm in 5 years.
- 10/2005- 11/2007 Asia Pacific Regional Business Director of Diabetes Care
Build sales/ marketing organization, and set clear marketing strategies to build a strong growth foundation for China and India. Also set the foundation for China growth in next 5 years: 45% to 50% from 2006 to 2010.
- 4/1998 - 9/2005 Country Manager of BD Taiwan. (Vietnam in 2000 to 2001)
Board member of AMCHAM Taiwan.
Chairman of AMCHAM Taiwan medical devices.
Board member of Taiwan Diabetes Foundation grew sales from USD 15 mm to USD 25 mm
- 11/97-3/98 Country Manager of Diagnostic Division,
Abbott Laboratory Taiwan Limited, In charge of an organization with 41 staff. The 1997 sales is USD 11.7 mm and targeting USD 13.8 mm sales in 1998, up 18% from a year earlier.
- 01/95-10/97 Regional Business Manager of Infusion System, BD Singapore
In charge of the regional sales and marketing of Infusion System business, covering China, Korea, Taiwan and Hong Kong.
- 07/90-12/94 Business Manager of Infusion System, BD Taiwan
In charge of the sales and marketing of Infusion System business of Taiwan. Infusion business grew business four times, and grew market share from 25% to 56%.

- 09/88-06/90 Product Manager of Medical Sector, BD Taiwan
 Responsible for sales and market development for all BD medical products by leveraging success experience from global business. Successful introduced new products to Taiwan, as the first country in entire Asia pac.
- 03/87-08/88 Product specialist of Medical Sector, BD Taiwan.
 Awarded the best medical sales person in BD Asia Pacific in 1987.

Additional professional activities

- Feb 2012 CEIBS: Globle CEO course
- July 2011 Honor : BD Transformation Hero
- May 2011 First one of the 25 Trainers of BD Global Marketing Power House for strategic marketing.
- July1995 Management training, Harvard University, business school
- May 1997 Finance program for non-finance background manager provided by Michigan University.

分子生物在食品檢驗的應用

林旭陽

衛生福利部食品藥物管理署研究檢驗組，為台灣食品藥物研究檢驗之國家實驗室，組內設六科，分別負責食品、西藥、管制藥品、醫療器材、化粧品之檢驗研究與科技發展，其中與食品有關者為第一科（食品化學科）與第二科（食品生物科）。本報告以食品生物科之部分業務為主，針對分子生物在食品檢驗應用的實務與面對未來挑戰重點介紹。

於食品生物檢驗領域，加強分子生物學檢驗技術的研究應用是過去十年之主要發展方向，將傳統以細胞層級之檢驗技術提升至分子生物層級，應用於基因改造食品、生物性食品攙偽、素食食品摻葷、含天然毒素之生物物種鑑別、食品中毒病原微生物及健康食品乳酸菌產品之菌種鑑別等檢驗。多年之研究已開發建立各種分子生物檢驗技術與方法、研發構築多種檢驗用參考質體標準品，並獲得多項國際及台灣之研發專利等。同時，藉由前述之多項檢驗技術，有效協助食品衛生管理之檢驗需求，如國際間未核准之基因改造作物流出、揭發素食產品摻葷、經濟摻偽、誤食有毒動植物、生蠔中諾羅病毒及豆干肉毒桿菌中毒等重大事件，顯示本組致力於食品分生檢驗技術之研發，對於台灣食品衛生安全之檢驗把關發揮極大功效，有效保障民眾健康。

面對無法預測的食品安全突發事件，本科亦積極規劃「引進高階分析技術」與「加強團隊合作」兩大主軸，持續創新食品生物檢驗技術。在引進高階分析技術方面，設置兼具快速、微量、精確的現代化分子生物分析儀器，如次世代定序技術、基質輔助雷射脫附游離飛行時間式質譜等，配合研發各種尖端檢測方法與技術，縮短檢測時間，全面提升分析效率。在加強團隊合作方面，因應食品中未知成分之危害風險，整合各類檢驗技術資源，加強跨領域檢驗技術合作，善用不同檢驗專才分工整合，以期以更有效率及更全方位的檢驗模式，及早發現、預警與解決各式各樣難以預料的食品安全隱藏問題。「檢驗並非萬能，但沒有檢驗卻是萬萬不能」，本科將更專注於食品檢驗技術的精進，並與國際同步，以精良檢驗能力輔助食品衛生行政管理，確保臺灣消費大眾飲食安全，提供民眾「食在安心」的飲食環境。

林旭陽 科長簡介

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現職：

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檢驗研究組 食品生物科 科長

學歷：

國立臺灣海洋大學

食品科學系學士，生物科技研究所碩士、博士

經歷：

單位 Department / Organization	職務 Title	時間 Period
衛生福利部食品藥物管理署 檢驗研究組 食品生物科	科長	2013 ~迄今
行政院衛生署食品藥物管理局 檢驗研究組 食品生物科	科長	2010 ~2013
行政院衛生署藥物食品檢驗局 食品微生物學組	薦任技正	2003 ~ 2009
行政院衛生署藥物食品檢驗局 食品微生物學組	薦任技士	1998 ~ 2003
行政院衛生署檢疫總所 基隆檢疫分所	薦任技士	1993 ~ 1998
行政院衛生署藥物食品檢驗局 食品微生物學組	技佐、技士	1989 ~ 1993
台灣省水產試驗所 水產加工系	技術員、技師	1987 ~ 1989

其他：農委會基因轉殖作物審議委員，經濟部標準檢驗局國家技術委員會委員

Research results list recent 5 years :

- ◆ CD Liao, YC Chen, HY Lin*, LC Chiueh, YC Shih. 2014. Incidence of citrinin in red yeast rice and various commercial *Monascus* products in Taiwan from 2009 to 2012. *Food Control*. 38:178-183. (SCI.)
- ◆ YC Chen, CD Liao, HY Lin*, LC Chiueh, YC Shih. 2013. Survey of aflatoxin contamination in peanut products in Taiwan from 1997 to 2011. *J. Food Drug Anal.* 21:247-252. (SCI.)
- ◆ YY Tang, HY Lin*, YC Chen, WT Su, SC Wang, LC Chiueh, YC Shin. 2013. Development of a Quantitative Multi-Mycotoxin Method in Rice, Maize, Wheat and Peanut Using UPLC-MS/MS. *Food Analytical Methods*. 6:727-736. (SCI.)
- ◆ CY Liao, WY Li, HF Lu, BN Huang, HY Lin*, LC Chiueh, YC Shin. 2013. Simultaneous Determination of Residues of Avermectin Antibiotics in Aquatic Animals by Liquid Chromatography-Electrospray Tandem Mass Spectrometry.

- Twn. J. Agr. Chem. Food Sci.* 51:69-79.
- ◆ WY Lee, CY Liao, HF Lu, BN Huang, HY Lin^{*}, LC Chiueh, YC Shin. 2013. Simultaneous Determination of Residues of Aminoglycosides Antibiotics in Foods of Animal Origin by Liquid Chromatography-Electrospray Tandem Mass Spectrometry. *Twn. J. Agr. Chem. Food Sci.* 51:59-68.
 - ◆ YY Tang, HF Lu, HY Lin^{*}, YC Shin, DF Hwang. 2012. Development of a Quantitative Multi-Class Method for 18 Antibiotics in Chicken, Pig, and Fish Muscle using UPLC-MS/MS. *Food Analytical Methods.* 5:1459-1468. (SCI.)
 - ◆ FP Lin, YH Ho, HY Lin, HJ Lin. 2012. Effect of C-terminal truncation on enzyme properties of recombinant amylopullulanase from *Thermoanaerobacter pseudoethanolicus*. *Extremophiles.* 16:395– 403. (SCI.)
 - ◆ YY Tang, HF Lu, HY Lin^{*}, YJ Chung-Wang, YC Shih, DF Hwang. 2012. Determination of Five Polyether Ionophore Antibiotics in Egg and Fresh Milk by Liquid Chromatography-Electrospray Tandem Mass Spectrometry. *Twn. J. Agr. Chem. Food Sci.* 50:78-87.
 - ◆ YY Tang, WY Lee, HF Lu, HY Lin^{*}, LC Chiueh, YC Shih. 2012. Simultaneous Determination of Residues of tetracycline antibiotics in Food by Liquid Chromatography-Electrospray Tandem Mass Spectrometry. *Twn. J. Agr. Chem. Food Sci.* 50:67-77.
 - ◆ YY Tang, HF Lu, HY Lin, DF Hwang. 2012. Multiclass analysis of 23 veterinary drugs in milk by ultraperformance liquid chromatography-electrospray tandem mass spectrometry *J. Chromatogra. B.* 881– 882 :12– 19. (SCI.)
 - ◆ CD LIAO, HY LIN^{*}, LC CHIUEH, YC SHIH. 2011. Simultaneous Quantification of Aflatoxins, Ochratoxin A and Zearalenone in Cereals by LC/-MS/MS *J. Food Drug Anal.* 19: 259-268. (SCI.)
 - ◆ HF Lu, YY Tang, HY Lin^{*}, DF Hwang, LC Chiueh, CF Lo. 2011. Simultaneous Determination of Multiclass Veterinary Drug Residues in Porcine Liver by Liquid Chromatography-Electrospray Tandem Mass Spectrometry. *Twn. J. Agr. Chem. Food Sci.* 49:64- 73.
 - ◆ YC CHEN, CD LIAO, SC WANG, HY LIN, LC CHIUEH, CF LO. 2011. Simultaneous Determination of Multi-Mycotoxin in Cereals Using LC-MS/MS *Ann. Rept. Food Drug Res.* 2 :164-171
 - ◆ YC CHEN, CD LIAO, HC CHO, SC WANG, HY LIN, LC CHIUEH, CF LO. 2011. A Survey of Aflatoxins in Peanut Products and Infant Foods in Taiwan *Ann. Rept. Food Drug Res.* 2 :172-177
 - ◆ HW TSUEI, YH CHANG, ZL JIA, CY LIN, HY LIN, LC CHIUEH. 2011. Survey of Animal-Derived Ingredients Adulteration in Commercial Vegetarian Foods *Ann. Rept. Food Drug Res.* 2 :214-222
 - ◆ FP Lin, KH Chang, HJ Lin, HY Lin 2010. Efficient Identification of *Vibrio cholerae* by PCR Using sodA Sequence and Serogroup RAPD Differentiation. *J. Fish. Soc. Taiwan*, 37(2):77-85.
 - ◆ CD LIAO, PC LIN, HY LIN, LC CHIUEH, YC SHIH. 2010. Survey on Citrinin in Commercial Monascus Products. *Ann. Rept. Food Drug Res.* 1 : 109-116
 - ◆ CD LIAO, LG KUO, HY LIN, LC CHIUEH, YC SHIH. 2010. Determination of Aflatoxins in Wines. *Ann. Rept. Food Drug Res.* 1 : 99-108
 - ◆ FP Lin, HH Chuang, YH Liu, CY Hsieh, PW Lin, HY Lin 2009. Effects of

C-Terminal Amino Acids Truncation on Enzyme Properties of *Aeromonas caviae* D1 Chitinase. *Arch Microbiol.* 191(3):265–273. (SCI.)

Patents :

闕麗卿、張源鑫、崔秀煒、林澤揚、林旭陽、吳宗熹、施養志、陳樹功 2010年. 產品中魚類成分之篩檢方法. 中華民國專利證書發明第325055號
 施養志、闕麗卿、張源鑫、林旭陽、林澤揚、吳宗熹 2010年. 食品中動物性成分之檢驗方法. 中華民國專利發明第I-333979號

Honors:

Year	Event	Sponsor	Article
2006	傑出研究獎優等	行政院	食品中動物性或植物性成分之鑑別與檢測
2005	傑出研究獎特優等	行政院	基因改造食品檢驗系統之建立
2004	傑出研究獎甲等	行政院	基因改造玉米等鑑別檢驗方法之探討與研究

Conference Speech

The role of androgen receptor in regulating the growth, migration and invasion of urothelial carcinoma cells

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Urothelial Carcinoma (UCa) arising from urothelium which lines the urethra, bladder, ureters, and renal pelvis is the most common malignancy of the urinary tract, comprising 90-95% of bladder cancer. Bladder cancer (BCa) is a major epidemiological problem whose incidence continues to rise each year and becomes a major economic burden on the health care system. BCa ranks as the ninth most common malignancy worldwide and the fourth among men in the United States, with estimated 72,570 new cases and 15,210 deaths in the United States in 2013, representing a heavy burden for patients and healthcare systems. In Taiwan, it is the tenth most common cancer. Upper urinary tract urothelial carcinomas (UUTUCs) are relatively uncommon tumors, accounting for about 5% of all urothelial tumors and 5–10% of all renal tumors, but cause severe morbidity and mortality.

Previous study has reported that male: female ratio of bladder cancer was 6.7 and it was similar for superficial and invasive tumors, indicating a greater risk of diagnosing bladder cancer in men than women for both non-muscle-invasive and muscle-invasive disease. And the deaths from bladder cancer in men are two fold higher than women. The etiology of this sex difference in incidence is proposed to link sex hormones. Sex hormones have been proposed to play a role in cancers which show gender difference in incidence and progression such as liver cancer, colorectal cancer and bladder cancer included. Androgens and androgen receptor (AR) have been demonstrated in BCa development and progression. Using carcinogen of N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) to induce BCa in mice, male mice lacking total AR or urothelial AR failed to develop BCa, and yet all wild type male mice developed BCa, suggesting AR might play key roles during BCa development. AR was also shown to affect BCa cell growth and invasion. UUTUC also afflicts more men than woman with a male-to-female ratio of 2~3 to 1, and females were associated with a better survival, indicating male hormones, androgens may play an important role in the progression of UUTUC. Our current studies have demonstrated that androgen receptor (AR), which mediates the actions of androgen, affects the BCa and UUTUC cell migration and invasion as well as the response to anti-cancer drugs.

Due to the AR's role on urothelial tumor progression, targeting AR signaling with anti-AR compounds on tumor cells to treat cancers could improve the efficacy of traditional toxic chemotherapy on UCa. This could provide a novel therapeutic strategy for UCa.

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Department of Urology, University of Rochester	Research Associate Assistant Professor	2002-2003	Pathology
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Cancer biology, Molecular cell biology, Molecular endocrinology

SIGNIFICANT PROJECT-RELATED PUBLICATION (Including periodical articles, technical reports, patents, or books related to the project in the recent 5 years)

1. **Shyr CR***, Chen CC, Hsieh TF, Chang CH, Ma WL, Yeh S, Messing E, Li TH, Li FY, Chang C 2013 The expression and actions of androgen receptor in upper urinary tract urothelial carcinoma (UUTUC) tissues and the primary cultured cells. *Endocrine* 43:191-199

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9. Chen CC, Hsieh TF, Chang CH, Ma WL, Hung XF, Tsai YR, Lin MH, Zhang C, Chang C, **Shyr CR*** 2013 Androgen receptor promotes the migration and invasion of upper urinary tract urothelial carcinoma cells through the upregulation of MMP-9 and COX-2. *Oncol Rep* 30:979-985
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12. Lin A M, **Shyr CR (co-first author)**, Lin J 2012 Bread containing type 3 resistant starch reduced glycemic index and glycemic response in healthy young adults. *Curr Top Nutraceutical Res* 10:143-150

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15. Xie S, Ni J, Lee YF, Liu S, Li G, **Shyr CR**, Chang C 2011 Increased acetylation in the DNA-binding domain of TR4 nuclear receptor by the coregulator ARA55 leads to suppression of TR4 transactivation. *J Biol Chem* 286:21129-21136
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Antioxidant Properties and Antioxidant Compounds of Various Extracts from the Edible Basidiomycete *Grifola Frondosa* (Maitake)

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Grifola frondosa is an edible mushroom currently available in Taiwan. Ethanolic, cold-water and hot-water extracts were prepared and their antioxidant properties were investigated. At 1 mg/mL, *G. frondosa* T1 and T2 cold-water extracts showed high reducing powers of 1.02 and 0.50, respectively. Chelating abilities on ferrous ions of *G. frondosa* T1 and T2 were higher for cold-water extracts than for ethanolic and hot-water extracts. For the scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, *G. frondosa* T1 and T2 extracts were effective in the following order: ethanolic > hot-water > cold-water. The *G. frondosa* hot-water extract showed high scavenging ability on superoxide anions. Total phenols, flavonoids, ascorbic acid and α -tocopherol are the major antioxidant components found in the various *G. frondosa* extracts. Based on EC₅₀ values (<20 mg/mL) obtained, the various extracts from *G. frondosa* investigated in this study display potent antioxidative properties.

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Publications in Last Five Years

1. Huang, B. R., Chang, P. C., Yeh, W. L., Lee, C. H., **Tsai, C. F.**, Lin, C., Lin, H. Y., Liu, Y. S., Wu, C. Y. J., Ko, P. Y., Huang, S. S., Hsu, H. C. and Lu, D. Y. 2014. Anti-Neuroinflammatory Effects of the Calcium Channel Blocker Nicardipine on Microglial Cells: Implications for Neuroprotection. PloS One (In press).
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 9. Chung, Y. C., Yeh, J. Y. and **Tsai, C. F.*** 2011. Antibacterial characteristics and activity of water-soluble chitosan derivatives prepared by the Maillard Reaction. *Molecules* 16: 8504-8514. (SCI)
 10. Yeh, J. Y., Hsieh, L. H., Wu, K. T. and **Tsai, C. F.*** 2011. Antioxidant Properties and Antioxidant Compounds of Various Extracts from the Edible Basidiomycete *Grifola Frondosa* (Maitake). *Molecules* 16: 3197-3211. (SCI)
 11. Chen, J. H., Huang, S. M., Chen, C. C., **Tsai, C. F.**, Yeh, W. L., Chou, S. C., Hsieh, W. T. and Lu, D.Y. 2011. Ghrelin induces cell migration through GHS-R, CaMKII, AMPK and NF-kappaB signaling pathway in glioma cells. *Journal of Cellular Biochemistry* 112: 2931-2941. (SCI)
 12. Hsiao, Y. L., Chen, P. F., **Tsai, C. F.**, Liu, H. C. and Chang, P. C. 2011. Choquet Integral Algorithm for T-cell Epitope Prediction Based on Fuzzy Measure. The 8th International Conference on Fuzzy Systems and Knowledge Discovery, p1639-p1642. (EI).

Emodin represses TWIST1-induced epithelial-mesenchymal transitions in head and neck squamous cell carcinoma cells by inhibiting the β -catenin and Akt pathways

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Head and neck squamous cell carcinoma (HNSCC) is one of the leading causes of cancer deaths worldwide. In recent studies, a crucial link has been discovered between the acquisition of metastatic traits and tumour-initiating abilities in cancer cells during the epithelial-mesenchymal transition (EMT). Herein, we demonstrated that the ectopic expression of *TWIST1*, the EMT regulator, in HNSCC FaDu cells triggered EMT and resulted in the acquisition of a mesenchymal phenotype. Moreover, FaDu-pFLAG-*TWIST1* cancer cell populations that were induced to EMT displayed an increased proportion of cells with the CD44 marker, which is associated with tumour initiation. Interestingly, we found that emodin treatment reduced the tumour-initiating abilities and inhibited cell migration and invasion in FaDu-pFLAG-*TWIST1* cells. Emodin directly inhibited TWIST1 expression, upregulated E-cadherin mRNA and protein expression, and downregulated vimentin mRNA and protein expression. Moreover, we found that emodin inhibited TWIST1 binding to the *E-cadherin* promoter and repressed *E-cadherin* transcription activity. We also found that emodin inhibited *TWIST1*-induced EMT by inhibiting the β -catenin and Akt pathways. More interestingly, emodin significantly inhibited *TWIST1*-induced invasion *in vivo*. Therefore, emodin might be applicable to anticancer therapy and could be a potential new therapeutic drug for HNSCC.

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Publications in Last Five Years

1. **Way TD**, Tsai SJ, Wang CM, Ho CT, Chou CH. (2013) Rhododendron formosanum and its constituents show pronounced growth inhibitory effect on non-small cell lung carcinoma cells. *Journal of agricultural and food chemistry* (in press) (Rank in Category: **1/57**, AGRICULTURE, MULTIDISCIPLINARY; Impact Factor: **2.906**)
2. Chen WC, Lai YA, Lin YC, Ma JW, Huang LF, Yang NS, Ho CT, Kuo SC, **Way TD**.* (2013) Curcumin Suppresses Doxorubicin-Induced Epithelial-Mesenchymal Transition via the Inhibition of TGF- β and PI3K/AKT Signaling Pathways in Triple-Negative Breast Cancer Cells. *Journal of agricultural and food chemistry* **61**, 11817-11824 (Rank in Category: **1/57**, AGRICULTURE, MULTIDISCIPLINARY; Impact Factor: **2.906**)
3. Chang HY, Hou SC, **Way TD**, Wong CH, Wang IF. Heat-shock protein dysregulation is associated with functional and pathological TDP-43 aggregation. *Nature Communication* **4**, 2757. (Rank in Category: **3/56**, MULTIDISCIPLINARY SCIENCES; Impact Factor: **10.015**)
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6. Tsai JH, Hsu LS, Lin CL, Hong HM, Pan MH, **Way TD**, Chen WJ. (2013) 3,5,4'-Trimethoxystilbene, a natural methoxylated analog of resveratrol, inhibits breast cancer cell invasiveness by downregulation of PI3K/Akt and Wnt/ β -catenin signaling cascades and reversal of epithelial-mesenchymal transition. *Toxicol Appl Pharmacol.* **272**, 746-756. (Rank in Category: **12/85**, TOXICOLOGY; Impact Factor: **3.975**)
7. Lee JC, Chou LC, Lien JC, Wu JC, Huang CH, Chung CH, Lee FY, Huang LJ, Kuo SC, **Way TD**.* (2013) CLC604 preferentially inhibits the growth of HER2-overexpressing cancer cells and sensitizes these cells to the inhibitory effect of Taxol in vitro and in vivo. *Oncol Rep.* **30**, 1762-1772.(Rank in Category: **111/196**, ONCOLOGY; Impact Factor: **2.297**)
8. Shieh JM, Chen YC, Lin YC, Lin JN, Chen WC, Chen YY, Ho CT, **Way TD**.* (2013) Demethoxycurcumin Inhibits Energy Metabolic and Oncogenic Signaling Pathways through AMPK Activation in Triple-Negative Breast Cancer Cells. *Journal of agricultural and food chemistry* **61**, 6366-6375. (Rank in Category: **1/57**, AGRICULTURE, MULTIDISCIPLINARY; Impact Factor: **2.906**)

9. Yang HL, Lin KY, Juan YC, Kumar KJ, **Way TD**, Shen PC, Chen SC, Hseu YC. (2013) The anti-cancer activity of *Antrodia camphorata* against human ovarian carcinoma (SKOV-3) cells via modulation of HER-2/neu signaling pathway. *J Ethnopharmacol.* **148**, 254-265. (Rank in Category: 3/21, INTEGRATIVE & COMPLEMENTARY MEDICINE; Impact Factor: 2.755)
10. Liu LC, Tsao TC, Hsu SR, Wang HC, Tsai TC, Kao JY, **Way TD**.* (2012) EGCG inhibits transforming growth factor- β -mediated epithelial-to-mesenchymal transition via the inhibition of Smad2 and Erk1/2 signaling pathways in nonsmall cell lung cancer cells. *Journal of agricultural and food chemistry* **60**, 9863-9873. (Rank in Category: 1/57, AGRICULTURE, MULTIDISCIPLINARY; Impact Factor: 2.906)
11. Hung CM, Su YH, Lin HY, Lin JN, Liu LC, Ho CT, **Way TD**.* (2012) Demethoxycurcumin Modulates Prostate Cancer Cell Proliferation via AMPK-Induced Down-regulation of HSP70 and EGFR. *Journal of agricultural and food chemistry*, **60**, 8427~8434. (Rank in Category: 1/57, AGRICULTURE, MULTIDISCIPLINARY; Impact Factor: 2.906)
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13. Lin VC, Tsai YC, Lin JN, Fan LL, Pan MH, Ho CT, Wu JY, **Way TD**.* (2012) Activation of AMPK by pterostilbene suppresses lipogenesis and cell-cycle progression in p53 positive and negative human prostate cancer cells. *Journal of agricultural and food chemistry* **60**, 6399-6407. (Rank in Category: 1/57, AGRICULTURE, MULTIDISCIPLINARY; Impact Factor: 2.906)
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18. Hung CM, Kuo DH, Chou CH, Su YC, Ho CT, **Way TD***. (2011) Osthole suppresses hepatocyte growth factor (HGF)-induced epithelial-mesenchymal transition via repression of the c-Met/Akt/mTOR pathway in human breast cancer cells. *Journal of agricultural and food chemistry* **59**, 9683-9690. (Rank in Category: 1/57, AGRICULTURE, MULTIDISCIPLINARY; Impact Factor: 2.906)
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Patent

專利名稱	專利號碼	類別	專利期間	發明者群
Novel hydrophilic derivatives of 2-aryl-4-quinolones as anticancer agents	2007328034	發明	2007.12.7 ~ 2027.12.7	張誌祥、孫仲銘、楊家欣、李正常、李國雄、鍾景光、潘秀玲、郭盛助、魏宗德、陳建廷、黃士鳴、鄧哲明、周立琛、吳天賞、黃麗嬌、黃敬哲
Novel hydrophilic derivatives of 2-aryl-4-quinolones as anticancer agents	Russia No 2424245	發明	2007.12.7 ~ 2027.12.7	郭盛助、鄧哲明、李國雄、黃麗嬌、周立琛、張誌祥、孫仲銘、吳天賞、潘秀玲、魏宗德、李正常、鍾景光、楊家欣、陳建廷、黃敬哲、黃士鳴
NOVEL HYDROPHILIC DERIVATIVES OF 2-ARYL-4-QUINOLONES AS ANTICANCER AGENTS	577130	發明	2007.12.7 ~ 2027.12.6	郭盛助、鄧哲明、李國雄、黃麗嬌、周立琛、張誌祥、孫仲銘、吳天賞、潘秀玲、魏宗德、李正常、鍾景光、楊家欣、陳建廷、黃敬哲、黃士鳴

Novel hydrophilic derivatives of 2-aryl-4-quinolones as anticancer agents	2009/03694	發明	2007.12.7 ~ 2027.12.7	郭盛助、鄧哲明、李國雄、黃麗嬌、周立琛、張誌祥、孫仲銘、吳天賞、潘秀玲、魏宗德、李正常、鍾景光、楊家欣、陳建廷、黃敬哲、黃士鳴
Novel hydrophilic derivatives of 2-aryl-4-quinolones as anticancer agents	2670292	發明	2012.1.31 ~ 2027.12.7	郭盛助、鄧哲明、李國雄、黃麗嬌、周立琛、張誌祥、孫仲銘、吳天賞、潘秀玲、魏宗德、李正常、鍾景光、楊家欣、陳建廷、黃敬哲、黃士鳴
抗癌劑としての2-アリアルール-4-キノロンの新規な親水性誘導體	特許第5102843號	發明	2012.10.5 ~ 2027.12.7	郭盛助、鄧哲明、李國雄、黃麗嬌、周立琛、張誌祥、孫仲銘、吳天賞、潘秀玲、魏宗德、李正常、鍾景光、楊家欣、陳建廷、黃敬哲、黃士鳴
Novel hydrophilic derivatives of 2-aryl-4-quinolones as anticancer agents	EP 2096924 B1	發明	2013.2.13 ~ 2027.12.7	郭盛助、鄧哲明、李國雄、黃麗嬌、周立琛、張誌祥、孫仲銘、吳天賞、潘秀玲、魏宗德、李正常、鍾景光、楊家欣、陳建廷、黃敬哲、黃士鳴

Tech transfer

技術轉移名稱	被授權單位	合約期間	發明者群
2-芳香基-4-喹啉酮的新穎親水性衍生物及其作為抗癌及其他疾病治療用途	醫睿醫藥科技股份有限公司 (技轉金額: 43,000,000)	2010.2 ~ 2016.10	郭盛助、鄧哲明、李國雄、黃麗嬌、周立琛、張誌祥、孫仲銘、吳天賞、潘秀玲、魏宗德、李正常、鍾景光、楊家欣、陳建廷、黃敬哲、黃士鳴
紫檀芪新衍生物及其作為抗癌及其他疾病治療用途	康富生技中心股份有限公司 (技轉金額: 26,000,000)	2013.4 ~ 2033.4	郭盛助、楊家欣、謝閔滄、吳天賞、李國雄、黃麗嬌、陳惠文、洪欣儀、魏宗德、張玲菊

A New Process for Developing Massa Medicata Fermentata

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Massa medicata fermentata (Shenqu) consists of the mixture of fermented powders of wheat flour, Semen Armeniacae Amarum, sprout of Semen Phaseoli, Herba Artemisiae Annuae, Fructus Xanthii and Herba Polygoni Hydropiperis. Shenqu has sweet, pungent and warm properties, and attributive to the spleen and stomach meridians. The actions of Shenqu is to promote digestion and harmonize the stomach.

Within the past two projects, we proceeded the above mycotoxin project to establish the method of analysis of Ochratoxin A in Chinese medicines. The method was applied to about 58 samples collected from retail shops in China and Taiwan, Ochratoxin A was detected in 52 samples of Massa Medicata Fermentata, measurable at 0.15-45.2 $\mu\text{g}/\text{kg}$. Evidence has suggested that mycotoxins contaminated in Shenqu produced in traditional processing, related to exposure fungal in fermented preparation. Shenqu was naturally contaminated by fungi that may become toxic for the human organism if the total amount ingested through consumption exceeds a certain tolerable dose. The quality management of Shenqu materials is very important for protecting consumer health. Therefore, a new process for developing Massa Medicata Fermentata was recommended in order to assess the optimal conditions for microbial fermentation of Shenqu.

The duration of this research project is approximately one years and divided into three parts.

1. The sub-subject I: To culture and determine the kinds of beneficial microorganism in Shenqu fermentation process.

The first part comprises the basic research dealing with identifying and classifying the microorganisms in Shenqu fermentation process. Beside, total plate count test were performed and yeast and mold testing represents the total fungi in sample. The results showed an enormous difference among the different batches of samples in the count of microorganisms. Twenty six of Shenqu were analysed in order to determine the microbial contamination level. The total viable aerobic count of Shenqu varied from 1.7×10^2 - 9.0×10^7 CFU/g and in 11 samples out of 26 was equal to or higher than 107 CFU/g. Rate of unqualified of the total viable aerobic count of Shenqu was 42.3 %. Total yeasts and molds count of Shenqu varied from 1×10^1 - 1.1×10^7 CFU/g and in 8 samples out of 26 was equal to or higher than 104 CFU/g. Rate of unqualified of the total yeasts and molds count of Shenqu was 30.7%. Microorganisms in the SQTCM Shenqu (No. 21) Sample collected from Sichuan sources were separated and identified. Three strains of microorganisms were isolated .2. The sub-subject II: To evaluate the effect of isolated unidentified strains and pure inoculation fermentation

The objective of sub-subject II was to evaluate the effect of isolated unidentified strains on promoting digestion. The active ingredients evaluated were lipase activity, protease activity and amylase activity, and screen out the microorganisms for the presence of aflatoxins.

In this survey, 26 samples were purchased in Mainland, Taiwan and Japan in order to investigate the aflatoxins contamination and evaluate the enzyme activity in Shenqu. The samples were analyzed of aflatoxins using LC with Post-Column Photochemical Derivatization. The method was applied to 26 samples and aflatoxins were detected in 12 samples, measurable at 1.5-76.8 $\mu\text{g}/\text{kg}$, 5 samples exceed standard requested of the total aflatoxin 15 $\mu\text{g}/\text{kg}$.

For selecting better fermentation strains, the quality of various Shenqu was evaluated by lipase activity, protease activity and amylase activity. The enzyme activities were found in 24 samples, measurable at 2.2-89.5 U/g (Amylase), 0.8-49.8 mU/g (Protease) and 7.3-331.2 (mU/g) (lipase). With regard to amylase activity and lipase, the SQTTCM Shenqu (No. 21) demonstrated higher activity than the others.

3. The sub-subject III: DNA sequencing analysis for the species identification of isolated microorganisms

Identification of Shenqu important microorganisms by ITS sequencing, especially using the ITS region, is reliable and can be used as an accurate alternative to conventional identification methods. Three strains of microorganisms isolated from the SQTTCM Shenqu (No. 21), they are *Bacillus safensis* (Bacillaceae) and *Lichtheimia ramosa* (Mucoraceae).

Keywords : Massa Medicata Fermentata ,Shenqu, aflatoxins, lipase, protease,
amylase

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鄧正賢，專長為層析分析分離技術及保健食品開發，十一年來在教學研究工作上積極投入，帶領學生從事台灣本土植物資源暨天然保健食品之生物活性篩選建立平台，連續三年獲得亞洲大學學術卓越研究獎及亞洲大學 102 年度學術成果豐碩獎，持續發表並進行台灣本土植物之活性研究。此外並獲得99年度亞洲大學「獎助創新教材獎」、98年度亞洲大學「獎助創新教材獎」、102年度亞洲大學產學合作獎。

Publications in Last Five Years

Jeng-Shyan Deng, Shyh-Shyun Huang, Tsung-Hui Lin, Min-Min Lee, Ching-Chuan Kuo, Ping-Jyun Sung, Wen-Chi Hou, Guan-Jhong Huang, Yueh-Hsiung Kuo. The analgesic and anti-inflammatory bioactivities of eburicoic acid and dehydroeburicoic acid isolated from *Antrodia camphorata* on the inflammatory mediator expression in mice. *Journal of Agricultural and Food Chemistry*, 2013; DOI: 10.1021/jf303820k. Publication Date (Web): March 15, 2013. (SCI)

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Oral Presentation

紫番薯青花素萃取物之抗發炎及抗癌功效
**Anti-inflammation and anti-cancer properties of anthocyanin
extracted from Purple Fleshed Sweet Potato (PFSP)**

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Purple fleshed sweet potato (PFSP) (*Ipomoea batatas* L. Lam) has been known to possess high amount of anthocyanin. Besides its antioxidant activity, anthocyanin is often associated with health preventive effects, such as reduce risk of inflammation and cancer. In this study, anthocyanin from steamed PFSP “Tainung 73”, which is locally grown in Taiwan, was extracted using acidified ethanol pH 3.5 under 80°C. The extract was evaluated for anti-inflammation and anti-cancer activities. Cell viability assay (MTT) showed that anthocyanin extracted from PFSP was not toxic to RAW 264.7 cells. The concentration of 4 mg/ml PFSP extract could suppress more than 50% NO production in LPS-induced RAW 264.7 cells. Furthermore, MTT study on MCF-7 (breast cancer), WiDr (colon adenocarcinoma), and SNU-1 (gastric cancer) cell lines displayed that PFSP extract was able to reduce cancer cell viability. These results suggested that PFSP extract has potential anti-inflammation and anti-cancer properties. In the future, PFSP TNG 73 extract can be developed or be applied in nutraceutical or drug industries.

Keywords: purple fleshed sweet potato, *Ipomoea batatas*, anthocyanin, anti-inflammation, anti-cancer

日本腦炎套膜蛋白突變對於病毒的附著和進入的影響
Effects of mutations in the envelope protein of Japanese encephalitis
virus on virus attachment and entry

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The envelope (E) protein has been demonstrated to play an important role in the binding and entry of Japanese encephalitis virus (JEV) into cells. In order to identify the molecular determinants were required for cell attachment and entry of JEV, we used a cytomegalovirus promoter-driven reverse genetics system to generate viruses with mutations in the E gene, which include eleven glycosaminoglycan (GAG)-binding motif mutants (K279M, H284Q, K286T, R288S, K290M, K290Q, K293Q, K297T, K389Q, K412M, and R416T), four arginine-glycine-aspartic acid (RGD) motif mutants (R387E, G388A, D389G, and D389H), three hinge region mutants (E52K, E138K, and S275P), domain I mutants (E52K, E138A, and E138K), and domain III mutant (S331R). We found that E138A, E138K, S275P, H284Q, R288S, K290M, K290Q, K293Q, D389G, E138K+D389H, K398Q, and R416T mutants exhibited smaller plaque size than its parental virulent strain RP-9X. S275P and R416T showed delays in the production of infectious virions from the infected cells. The results from the virus-binding assay revealed that D389G and D389H mutations affected the cell surface binding of the virus. Synchronized infectivity assay found that S275P, H284Q, K290Q, K290M, R387E, D389G, K398T, and R416T mutants showed delayed growth kinetics in the infected cells. Using the digestion analysis of protease K, we showed that growth kinetics of Q52K, E138A, S227P, S275P, K279M, H284Q, R288S, K297T, R387E, G388A and E138K+D389H mutants were lower than RP-9X. These results suggest that residue 389 in the E protein is an important determinant for the interaction between the virion and cell surface. Residues 52, 138, 227, 275, 279, 284, 288, 290, 297, 387, 398 and 416 may contribute to the entry of virus.

Keywords: Japanese encephalitis virus、Envelope (E)、Glycosaminoglycan binding motifs (GAGs)、RGD motif、Binding ability、Molecular determinants

海藻酸鈉保鮮劑對小黃瓜品質之影響
**Effect of Sodium Alginate Preservatives on the Quality of Cucumber
during Storage**

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Sodium alginate film can effectively improve the barrier of water vapor and can't be used by microorganisms, so that will be a good material to make edible preservative. In this experiment, we divided cucumber into ten groups storage at room temperature to understand the effects of different concentrations of alginate and calcium chloride content on the pH value, moisture content, weight loss, L.a.b value and respiration rate of cucumber. The concentration of alginate rang was 0.015 ~ 0.035 g / mL and calcium chloride rang was 0.01 ~ 0.06 g / mL. The results showed that the control group in the absence of preservative spray coating, moisture content, weights loss and respiration rate has a very high significant difference to experiment groups. However concentration of alginate 0.035 g / mL and calcium chloride 0.01 g / mL experiment group had lowest value in moisture content, weights loss and respiration rate. So that will be a good formula to made edible preservative. The analysis of different concentrations of calcium chloride did not affect the pH value of cucumber. However, as time went on cucumber pH value decreased gradually.

Keywords: Sodium Alginate, Quality, Preservatives

利用益生菌的生物轉換作為中草藥製造天然食用紅色色素的方法
**A biotransformation method for producing natural edible red
colorant from traditional Chinese medicine by the probiotic**

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The natural edible colorants are widely used as food colorants in Japan and Asia. Recently, the concept of food safety has been increased by days. The requirement of natural food colorants is getting more and more in industries. In order to produce natural food colorants, we develop a biotransformation system to produce natural edible red colorant by traditional Chinese medicine *Gardenia*, which has been used to produce yellow and blue colorants depending on various reaction conditions. As described by the Food and Agriculture Association of the United Nations and World Health Organization, probiotics are a group of live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. *Lactobacillus* and *Bifidobacteria* are the two most common types of microbes which are extensively used as probiotics. They are broadly used in lots of fermented food to alter the composition, nutrition, taste or smell. It also can be used as biotransformation systems to convert traditional Chinese medicine into effective compounds. In previous study, we used the probiotic *Lactobacillus rhamnosus* strain JB3 to produce natural edible colorant gardenia blue. We then modified the reaction condition to prepare gardenia red. The colorants stability was examined by the ration of wave length of red light (OD₄₅₀) and blue light (OD₅₃₀). Using probiotics to produce natural edible colorant could increase the safety of colorants and enhance the application of *Gardenia*.

Key words: natural edible red colors, *Gardenia*, probiotic

利用兩種動物模式確認近視的發展進程
To determine the progression of myopia by two different animal models

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Myopia is a common eye disease, and the highest prevalence is observed among Singapore, China, Taiwan and Japan. In addition to the associated substantial visual loss and economic losses, high myopia will lead to degenerative diseases such as myopic macular degeneration, retinal detachment, posterior staphyloma and blindness. Axial elongation of the posterior chamber of the eye is the phenotypic hallmark of myopia causing images to focus in front of the retina. Fortunately, the nonselective muscarinic acetylcholine receptor (mAChR) antagonist, atropine, is an effective drug that stops myopia progression; however, the mechanisms behind are not well known. We used two different myopia animal models which are lens-induced myopia (LIM) and form-deprivation myopia (FDM) to explore the myopia pathway. -10D lens and cotton cloth were used in LIM and FDM groups, respectively. The optometry was tested before and after study for all animals. Both LIM and FDM models were successfully induced myopia, and atropine was effectively recovered the ametropia in these two models. The animals were sacrificed after 21 days, and the eye tissues were collected for immunohistochemistry (IHC) study. We had tested several markers such as MMP-2, collagen-1, TNF- α , c-FOS and Id-1 to elucidate the mechanism involving in myopia. We found that MMP-2 expression was increased and collagen-1 expression was decreased at myopic eye, and the expression patterns were reversed after atropine treated. cFos, one kind of inflammatory-related transcription factors, was also increased at myopic eye and was reversed after atropine treated. But the protein expressions of other makers, such as TNF- α , Id1, M3 receptor, shown no difference between myopia model and control groups. In conclusion, our finding showed that the two different models of myopia were related with inflammation, but the mechanism still awaits further investigation.

Keywords: Myopia, Atropine, LIM, FDM, MMP-2, cFos

竹蓀栽培基質配方之研究

Studies on Culture Substrate of Formulae *Dictyophora indusiata*

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Dictyophora indusiata belonging to Basidiomycota, Agaromycetes, Phallales, Phallaceae and *Dictyophora*, is an edible mushroom widely used in Asian countries, especially in China. It has cosmopolitan distribution in tropical areas such as Indonesia, Malaysia, India, Southern China, Japan, and Taiwan. It was called “veiled lady mushroom” or “queen of the mushrooms” due to its beautiful appearance, delicious taste and health function. However, *D. indusiata* are not only appreciated as a highly tasty and nutritional food, but also as an important source of biologically active compounds containing pronounced medicinal value. Polysaccharides, the main ingredient of *D. indusiata*, exhibited several potential bioactivities such as anticancer, antitumor, immunomodulation activity, antioxidant activities, and so forth. Many studies suggested that *D. indusiata* contained polysaccharides β -d-glucan. In vivo and in vitro testing indicated this natural polysaccharide possessed excellent antioxidant activity. The anti-tumor activity of polysaccharides from *D. indusiata* might be partially associated with the activation of the immune system. The objective of this study was to select the suitable substrate formulations for mycelial growth of *D. indusiata*, and understood the effect of alternative substrates paddy straw or bamboo shavings on *D. indusiata*. Preliminary results showed that substrate formulation of sawdust(78%), wheat middlings(20%), CaSO₄(1%) and Sucrose(1%) have the highest mycelial growth 96.31±0.33(mm) in 40 days and sawdust(19.5%), bamboo shavings(58.5%), wheat middlings(20%), CaSO₄(1%) and Sucrose(1%) have the highest mycelial growth 104.61±1.30 in 40 days.

Keywords: *Dictyophora indusiata*, Microbial growth, substrate formula, sawdust

樟芝三萜類成份抗血癌計算分析研究
**Study of computational analysis of *Antrodia camphorate*
triterpenoid's anti-leukemia**

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Antrodia camphorate(AC) is one of the traditional Chinese folk medicine, often used in anti-inflammatory, to treat skin itching, solution alcoholism, etc. Some reports have suggested that it has many ingredients: polysaccharides, triterpenes, steroids, phenols, adenosine⁽¹⁾, triterpenoids with anti-leukemia, breast cancer, lung cancer⁽²⁾ effect, so the study of the use of *Antrodia camphorate* triterpenoids to treat leukemia remains to be elucidated. Acute promyelocytic leukemia (APL) is the result of reciprocal translocation between chromosomes 15(q22) and 17(q11-12), making the PML, RAR α gene fusion, so promyelocytic abnormal proliferation and accumulation in the bone marrow and blood, resulting in coagulation disorders, low white blood cells, and the mortality rate is very high⁽³⁾. AC in Zhankuic acid A triterpene composition, through TNF-related apoptosis-inducing ligand (TRAIL) induced the expression of death receptor 5 (DR5), the DR5 expression, resulting in NF κ B activation, Bax/Bcl-2 increase expression, induced HL-60 cells apoptosis⁽⁴⁾. AC in triterpenes mainly ergostan and lanostane, mainly ergostan is a 29 carbon atoms skeleton, 24-exo-methylene-26-oic acid side-chain, C-7, C-8, C-9, C-11 has a conjugated double bond; lanostane in eburicane as the skeleton, 24-exo-methylene-21-oic acid side-chain, C-7, C-8, C-9, C-11 has a double bond⁽⁶⁾. This study compared numbers of different medicinal triterpenoids, such as: *Antrodia*, Ginseng, Licorice, *Bupleurum*, *Poria*, using the Discovery Studio[®] computational analysis, different kinds of medicines triterpenoids' analysis and Tumor necrosis factor (TNF)-receptor 1-associated death domain protein (TRADD) docking situations and docking scores, and explored whether AC triterpenoids is the most important ingredient in its anti-leukemia effect. Preliminary results showed that AC triterpenoid structure is relatively basic, small molecules, and also contains some double built above, OH group and the additional hydrogen atoms connected, more likely to have hydrogen bonding festival and protein bonding, therefore, the more firmly bonded docking score is higher, after bonding the protein triterpenes, starts DR4 (Death receptor 4) and DR5 (Death receptor 5) performance of the enzyme caspase 8 activation, resulting in Bid, Bad / Bax produce further activation of Cytochrome-c, caspase 9, caspase 3, leading to DNA fragmentation and apoptosis.

Keywords: *Antrodia camphorate*, leukemia, triterpenoid, Zhankuic acid A, death receptor 5, Discovery Studio.

應用蛋白質體學技術探討松杉靈芝乙醇萃取物對小鼠 3T3-L1 細胞
進行脂肪細胞分化作用之分子機轉

**A Proteomics Approach to the Molecular Mechanism of Adipogenic Effects
of *Ganoderma tsugae* Ethanolic Extracts in Murine 3T3-L1 Cells**

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Ganoderma or Lingzhi in oriental countries is a famous fungus with multiple medicinal properties. It has been used for promoting the health benefit and longevity in Far East countries for many centuries. It is also known that adipocyte differentiation is a key aspect of obesity development when homeostasis is unbalanced, and our preliminary studies have found that ethanolic extract of *Ganoderma tsugae* (GTEE), one of the most common species among the Lingzhi cultivated in Taiwan, has potential in affecting adipocyte differentiation in murine 3T3-L1 cells. However, the protein expression profile and molecular mechanism of GTEE on adipogenesis is yet to be explored. Therefore, a proteomics approach was employed to explore the protein expression profile of the GTEE-treated cells during adipogenesis. To further investigate the role of proteins and related molecular mechanism affected by GTEE during adipogenesis, we used the murine 3T3-L1 cells to study the adipogenic effect of GTEE. After adipocyte differentiation with or without GTEE treatment, the proteins of differentiated 3T3-L1 cells were collected and separated by 2-Dimension Gel Electrophoresis (2DGE). Subsequently, the protein expression profile was analyzed by gel image analysis software. Based on the proteomic approach analysis, the gel image analysis initially revealed that GTEE altered some proteins expression (up regulation and down regulation) during adipogenesis compared with the vehicle control and positive control. In addition, we will identify and realize the role and function of proteins by using MALDI-TOF-TOF and GeneGo MetaCore analysis. Taken together, our preliminary studies may help clarify the role and function of altered proteins and related molecular mechanism of adipogenic effect of GTEE in murine 3T3-L1 cells.

Keywords: *Ganoderma tsugae*, Proteomic approach, Adipogenesis, 3T3-L1, Lingzhi, 2DGE

Poster

CSC-3436 協同 Tamoxifen 抑制三陰性乳癌細胞之研究
CSC-3436 Synergizes with Tamoxifen against Triple-negative Breast Cancer Cells

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Breast cancer is the fourth leading cause of death of female in Taiwan, The current treatment of patients with breast cancer target estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor type II (HER-2) receptor type and other tumor markers for personalized medicine. However, triple negative breast cancer (TNBC) is a non-specific receptor expression and is therefore not suitable for use of hormone therapy and targeted therapy, currently there is no specific clinical treatment. Tamoxifen is one of the most widely used chemotherapeutic agents for the treatment of estrogen receptor (ER)-positive breast cancer patients. The main mechanism of tamoxifen has been demonstrated to induce apoptosis and reduce cell proliferation in tumor cells via inhibition of ER signaling. In this study, we used TNBC cell lines include MDA-MB-231, BT-20 and BT-549 cell lines. And used CSC-3436, a derivatives of 2-phenyl-naphthyridin-4-ones (2-PN) to address the hypothesis that the CSC-3436 will sensitize TNBC cells to tamoxifen. The results showed that CSC-3436 reduced cell viability and cell proliferation in TNBC, MDA-MB-231, BT-20 and BT-549 cell lines. CSC-3436 enhanced the anticancer effect of tamoxifen through inducing apoptosis in TNBC cells. We also found that CSC-3436 inhibited TNBC cells metastasis, Epithelial-Mesenchymal Transition (EMT), and reduced the transforming growth factor beta receptor-II (TGF β R-II), as well as downstream p-Samd signal transduction and other effects. CSC-3436 could be restored by ER α synergizes with tamoxifen against TNBC cells through the induction of apoptosis. Our analysis indicated that CSC-3436 synergized with tamoxifen against TNBC cells through the induction of apoptosis and that CSC-3436 could be used as a potential compound to improve tamoxifen sensitivity in ER-negative breast cancer cells. This combinatorial approach is worthy of continuing investigation.

Keywords: Triple negative breast cancer (TNBC) 、 estrogen receptor (ER) 、
Epithelial-Mesenchymal Transition (EMT) 、 apoptosis

食藥用菇類抑制人類結腸癌和胃癌細胞增生之研究
The studies for the inhibition of human colon cancer and gastric cancer cell proliferation by edible and medicinal mushrooms

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Cancer, especially colorectal cancer, is the most leading causes of death in Taiwan. Several edible mushrooms extracts have been known to have beneficial effects on human health. Their active compounds showed a potential inhibition on tumor cell growth. Therefore, in this study, we used both hot and cold water to extract four edible mushrooms including *Pleurotus eryngii*, *Agaricus blazei*, *Hericium erinaceus*, and *Flammulina velutipes*, and evaluated their inhibition effects on the growth of human colon cancer and gastric cancer cells. According to the results, hot and cold water crude extracts of *Agaricus blazei* could inhibit the growth of SUN-1 cell lines in dose-dependent manner. *Pleurotus eryngii* extracted in cold water showed strong cytostatic effect on two cell lines including WiDr and SUN-1. Furthermore, the crude extract of *Pleurotus eryngii* in the concentration of 2 mg/ml resulted in 2% cancer cell survival. Meanwhile, concentration of 2 mg/ml *Flammulina velutipes* matrix part extracted by cold water displayed the inhibition of cancer cell growth in a time-dependent manner and cancer cell viability was 51.92% after 48h treatment. These results indicated that edible part of *Flammulina velutipes* had better inhibitory activity on cancer cell lines compared to the matrix part. *Pleurotus eryngii*, *Agaricus blazei*, and *Flammulina velutipes* matrix were able to continue to further examine the mechanisms of their polysaccharide extracts to effectively inhibit cancer cell growth. In summary, mushroom extracts have highly potential to apply in the therapeutic or pharmaceutical development.

Key word: Mushrooms, Anticancer, Colon cancer, Gastric cancer

Doxorubicin-incorporated nanoparticles composed of chitosan and antitumor activity against MCF-7 cells in vitro

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Chitosan is Chitin Deacetylation of product. Chitosan has excellently biocompatible and biodegradable, bioactive, film- and gel-forming characteristics, and has polycationic nature. This study is to evaluate potential of chitosan nanoparticles as carriers for the anticancer drug. This novel delivery system is prepared using a simple and mild ionic-gelation method to which a tripolyphosphate pentasodium (TPP) solution was added to a low molecular-weight chitosan with different concentration. To investigate the molecular weight of chitosan on nanoparticles characteristics prepared by ionotropic gelation and its application on the controlled release of anticancer drugs.

As the concentration of chitosan solution and TPP decreased, the mean particle size of chitosan nanoparticles decreased. The particles sizes was about 13.6 nm~198.3 nm and 29.6 nm~268.3 nm. The particle size (268.3 nm) of nanoparticles prepared with high Mw (80 kDa) was larger than that (198.3 nm) of nanoparticles made of low Mw chitosan (40 kDa). The effects of DOX-loaded NPs on MCF-7 cells were studied using MTT assay to analyze the viability of MCF-7 cells, at a concentration of 0.3125 mg/ml, there was no cell survival at incubation 72 hours.

Keywords: chitosan, nanoparticles, controlled drug delivery system, Doxorubicin

Resistin promotes MMP-2 production and migration in human chondrosarcoma cells through AMPK/p38/mir 519d pathways

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Chondrosarcoma is a primary malignant bone cancer, with a potent capacity to invade locally and cause distant metastasis; it has a poor prognosis and shows a predilection for metastasis to the lungs. Resistin the most potent pro-inflammatory protein and plays a crucial role in migration and metastasis of human cancer cells. However, the effects of resistin on human chondrosarcoma cells are largely unknown. In this study, we found that treatment of human chondrosarcoma cells (JJ012) with resistin increased migration and expression of matrix metalloproteinase (MMP)-2. Resistin-mediated cell migration and MMP-2 expression were reduced by pretreatment with inhibitors of AMP-activated protein kinase (AMPK), and p38 mitogen-activated protein kinases (p38). **Results:** Resistin treatment induced phosphorylation of AMPK and p38. In addition, resistin reduced miRNA 519d expression. Transfection of 519d mimic also reduced resistin mediated migration and MMP2 expression. Furthermore, we analyzed samples from chondrosarcoma patients by immunohistochemical staining. The expression of resistin and MMP2 in chondrosarcoma patients was significantly higher than in normal cartilage. Taken together, these results suggest that resistin increased MMP-2 expression and migration in human chondrosarcoma cells.

山芙蓉根水萃取物對人類前列腺癌 PC-3 細胞凋亡之探討
The apoptotic effects of hot water extract of *Hibiscus taiwanensis*
roots on human prostate cancer PC-3 cells

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Hibiscus taiwanensis (HT), a native Taiwan plant, is a moderately tall shrub and widely distributed throughout Taiwan. The stem of HT has been used as anti-inflammatory, antifungal, antipyretic, and anthelmintic agents in the traditional Chinese medicine. However, little is known about the antiproliferative effects of HT on cancer cells. Prostate cancer, the most common cancer in adult men, is the second-leading cause of cancer death in western countries. Thus, the present study was investigate the anti-proliferative effects of the root of HT on human prostate cancer PC-3 cells. Our results showed that the hot water extracts of HT roots significantly inhibited PC-3 cell survival via the concentration-dependent manner, as demonstrated by MTT viability assay. To further confirm whether the antiproliferative effects of HT were resulted from inducing apoptosis in PC-3 cells, apoptosis-related proteins were examined. Through western blotting results, caspase-3 and PARP (poly ADP ribose polymerase) were activated upon HT stimulation. Meanwhile, Bax protein was increased through HT treatments in PC-3 cells, suggesting that Bcl-2 family proteins were also involved in HT-inducing apoptosis on PC-3 cells. Taken together, our results indicated that the hot water extracts of HT roots significantly induced human prostate cancer PC-3 cell apoptosis via caspase-3 and Bcl-2 family proteins

Keywords: *Hibiscus Taiwanensis*, apoptosis, prostate cancer, caspase-3, Bcl-2

Cucurbitane triterpenoid from *Momordica charantia* induces apoptosis and autophagy in breast cancer cells, in part, through peroxisome proliferator-activated receptor γ activation

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Although the anti-tumor activity of the crude extract of wild bitter gourd (*Momordica charantia* L.) has been reported, its bioactive constituents and the underlying mechanism remain undefined. Here, we report that 3 β ,7 β -dihydroxy-25-Methoxycucurbita-5,23-diene-19-al (DMC), a cucurbitane-type triterpene isolated from wild bitter gourd, induced apoptotic death in breast cancer cells through peroxisome proliferator-activated receptor (PPAR) γ activation. Luciferase reporter assays indicate the ability of DMC to activate PPAR γ , and pharmacological inhibition of PPAR γ protected cells from DMC's antiproliferative effect. Western blot analysis indicates that DMC suppressed the expression of many PPAR γ -targeted signaling effectors, including cyclin D1, CDK6, Bcl-2, XIAP, cyclooxygenase-2, and NF- κ B. Moreover, DMC inhibited mTOR-p70S6K signaling through Akt downregulation and AMPK activation. Together, the ability of DMC to modulate multiple PPAR γ -targeted signaling pathways provides a mechanistic basis to account for the antitumor activity of wild bitter gourd.

Keywords: *Momordica charantia*, PPARgamma, triterpene, autophagy, apoptosis

在人類鱗狀口腔癌細胞中 OSU-2S 誘導細胞凋亡及自噬作用
OSU-2S induces apoptosis and autophagy in human oral squamous carcinoma cells

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Abstract

Oral cancer has been one of the top 10 causes of death from cancer since 1991 in Taiwan and the death toll for oral cancer in males has been rising at a surprising rate.¹ OSU-2S is a novel protein kinase C delta-targeted antitumor agent, which is devoid of sphingosine-1-phosphate 1 receptor activity and is highly effective in suppressing liver cancer cells.²

In this study, we aimed to investigate its potential anti-tumor activity in oral cancer cells. The results showed that OSU-2S could inhibit the growth of the oral cancer cells with IC₅₀ value 4.7 and 3.7 μ M for SCC2095, and 3.7 and 2.6 μ M for SCC4 in 24h and 48h, respectively. Flow cytometric analysis indicated that OSU-2S suppressed the viability of SCC2095 cells by inducing apoptosis. Western blotting demonstrated that OSU-2S down regulated the expression of phosphorylated Akt and up regulated the expression of LC3B-II in a dose-dependent manner. The above data suggested that OSU-2S exerts anti-tumor effects by inducing apoptosis and autophagy in human oral squamous carcinoma cells.

Keyword: OSU-2S, oral cancer, apoptosis, autophagy

Dihydromyricetin inhibits melanogenesis by the attenuation of proteinase-activated receptor 2 through the PI3K and PKC pathways

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Abstract

Dihydromyricetin (DH) is a flavonoid component isolated from *Hovenia dulcis*. Previous reports showed that DH has several pharmacological characteristics, such as anti-intoxicant, anti-inflammatory, and anti-oxidative activities. The current study sought to evaluate the inhibitory effect of DH on pigmentation and identified the mechanisms involved by a melanocyte–keratinocyte coculture protocol, which was allowed testing of compounds for potential effects on pigmentation in a more physiologically relevant context. The preliminary results indicated that DH effectively suppressed murine tyrosinase activity and the amount of melanin. Through the Western blot results revealed that DH decrease the protein expression levels of tyrosinase, microphthalmia-associated transcriptional factor (MITF), and protease-activated receptor-2 (PAR-2). The PAR-2 is a keratinocyte receptor that regulates skin coloration. We found that the downmodulation of PAR-2 by DH was inhibited by the PKC inhibitor (GF109203X) or phosphatidylinositol-3-kinase (PI3K) inhibitor (LY294002). Our findings also revealed DH's function in the internalization of PAR-2. This study concluded that DH effectively inhibit the tyrosinase activity and reduce the amount of melanin in B16F10 cells. DH also attenuated the expression of PAR2 as well as induced PAR2 internalization in keratinocytes and thereby effectively downregulate melanogenic responses in the skin.

Keywords: Dihydromyricetin, MITF, tyrosinase, PAR 2, internalization.

**Diallyl trisulfide 經由標的 ROS 所調控的 HIF-1 α 和 IGFBP-3 的活性,
進而抑制高糖所引起的 H9c2 心肌纖維母細胞凋亡
Diallyl trisulfide (DATS) Suppresses HG-induced H9c2
Cardiomyoblast Apoptosis by Targeting ROS- mediated
HIF-1 α -IGFBP-3 Activation**

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Background:

Diabetes is one of the most common diseases to lead death in Taiwan and more than 80% patients are dead due to cardiovascular diseases. In our previous study, it is demonstrated that cardiac activation of HIF-1 α -IGFBP-3 signaling mediated by ROS-regulated PHD is involved in HG-induced apoptosis. Diallyl trisulfide (DATS) is the component in garlic oil with the strongest inhibitory effect on dilated cardiomyopathy. In this study, we will further investigate whether HIF-1 α -IGFBP-3 signaling governs the anti-apoptotic effect of DATS on high glucose-exposed H9c2 cardiomyoblast cells.

Methods and Results:

H9c2 cells were treated with 5.5 mM and 33mM glucose for 36 hr. It was observed that significant increased levels of cell apoptosis, ROS production, HIF-1 α , IGFBP-3 and down-regulated phosphorylated Akt phosphorylation induced by HG were reversed by the treatment of DATS in a dose-dependent manner. The results of co-immunoprecipitation (Co-IP) assay showed that DATS suppressed the extracellular association of IGF-1 with IGFBP-3 of H9c2 cardiomyoblast exposed to HG. The treatment of H₂O₂ and PHD siRNA increased HIF-1 α and IGFBP-3 protein levels which were decreased by DATS. Medium sample showed the similar results. The overexpressed HIF-1 α and IGFBP-3 reversed the level of cell apoptosis which was suppressed by the treatment of DATS in HG-exposed cells.

Conclusion:

Taken together, these findings indicate that the mediation of ROS-regulated PHD on HIF-1 α -IGFBP-3 signaling activation governs the anti-apoptotic effect of DATS on HG-exposed H9c2 cardiomyoblast cells.

Keywords: Diabetes, High glucose, Diallyl trisulfide, HIF-1 α , IGFBP-3, PHD

The Role of Cantharidin Induced Cell Apoptosis through G₂/M Phase Arrest in Nasopharyngeal Carcinoma Cells

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Abstract:

Nasopharyngeal carcinoma (NPC) is the most common cancer. NPC is caused by a combination of factors: viral, and environmental influences that is more common in certain regions of East Asia and Africa than elsewhere. The viral influence is associated with infection with Epstein-Barr virus (EBV). Nowadays, nasopharyngeal carcinoma can be treated by surgery, chemotherapy or radiotherapy.

Cantharidin is a chemical compound of terpenoid secreted by many species of blister beetle. In some references, cantharidin and its analogues may have activity against cancer cells. In our finding, Cantharidin has a potential ability to induce NPC cells death through G₂/M phase arrest and promote apoptosis by down-regulated Bcl-2, Bcl-X_L and up-regulated Bax, BID expression, as well as activated the expression of caspase-3 protein. Cantharidin treatment also caused the loss of mitochondrial membrane potential (MMP) in NPC cells. Further, we want to detailed analyze the apoptosis pathway by cantharidin and hope it would be a possible candidate for human cancer therapy.

Keywords: cantharidin, Nasopharyngeal carcinoma, Bcl-2 family, mitochondrial membrane potential (MMP)

Chinese herbal medicine Du-Huo (Radix Angelicae Pubescentis) has the function of anti-proliferation on A549

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Abstract

There are a lot of causes and risk factors of lung cancer, including smoking, passive smoking in the environment, occupational exposure, virus, and so on. The most common age of onset for lung cancer is over 40 years, especially between the ages of 50 and 70, in middle-aged people and the elderly. However, recent studies indicated that the age of onset for lung cancer has decreased to the people aged 20 to 30 years. According to 2012 statistics of the Ministry of Health and Welfare, Malignant neoplasms (Cancer) is the most deadly cause among the 10 leading causes of death in Taiwan, caused 28.4% of total deaths. Lung Cancer is the NO. 1 killer among all cancers with mortality rate 36.9% per 100,000 population.

Currently the treatments for lung cancer include surgery, chemotherapy and radiation therapy. Surgery is not suitable for all kinds of lung cancer. The side effects of chemotherapy usually make cancer patients very uncomfortable. Radiation therapy could make cancer cell develop the characteristics of stem cell. In sum of, we wonder if there are any Chinese herbal medicines can be used as an adjunctive therapy in lung cancer.

Previous studies reported that Du-Huo(Radix Angelicae Pubescentis) can be used to prevent degenerative joint disease, anti-inflammatory, and analgesia. In this study, we treated A549 cells with fifteen Chinese herbal medicines, using MTT assay to determine the survivability of Du-Huo. Finding that Du-Huo has ability to anti-proliferation of A549.

Key words: Du huo, A549 cell line

Sulforaphane induces cell death through the G2/M phase arrest and apoptosis by reactive oxygen species dependent pathway in human colon cancer HCT 116 cells

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Sulforaphane is a rather simple organic compound that is found in the largest concentrations in the cruciferous or mustard family of plants, including broccoli, cauliflower, and cabbage. Sulforaphane has been reported to exhibit a wide variety of biological activities including antioxidant, prevent and treat arthritis, protect against respiratory ailments and prevention of heart disease effects. In addition, it has been shown to inhibit the proliferation and to induce the apoptosis of a wide variety of tumor cells including breast, prostate and lung cancers. However, it is still unclear whether sulforaphane effectively induces apoptotic cell death of human colon cancer HCT 116 cells. Herein, HCT 116 cells were treated with different concentrations of sulforaphane for a specific time period and investigated for effects on apoptosis analyses. Our results indicated that HCT 116 cells after exposure to sulforaphane significantly decreased cell viability, induced cell morphological changes and induced G2/M phase arrest. In addition, sulforaphane was found to induce DNA damage and fragment that were determined by DNA gel electrophoresis. Western blotting results indicated that sulforaphane inhibit cyclin A and cdk1 expressions and promoted chkI and chk II levels that were caused G2/M phase arrest. Flow cytometry assay indicated that sulforaphane promoted calcium productions, increased percentage of early apoptotic cells and loss of mitochondrial membrane potential in HCT 116 cells. In the future, sulforaphane may be useful for developing new therapeutic regimens for the treatment of human colon cancer.

Keyword: Sulforaphane, human colon cancer, apoptosis, G2/M arrest

粉防己鹼影響 NPC-TW 076 細胞的內質網壓力導致細胞凋亡
Tetrandrine affects NPC-TW 076 cells on endoplasmic reticulum stress to induce apoptosis

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Tetrandrine is a kind of alkaloid which extracted from a China unique medicinal plant. It has been extensively reported to induce apoptosis in many human cancer cells such as leukemia, liver and colorectal cancer cells. Although many studies have been demonstrated that tetrandrine induces cell death in many human cancer cells, however, effects of tetrandrine on human nasopharyngeal carcinoma cells (NPC) still unknown. In the present studies, we use tetrandrine to inspect whether or not can induce apoptosis of NPC-TW 076 cells. Result indicated that tetrandrine induces time-dependant endoplasmic reticulum (ER) stress. And we also found that tetrandrine induces dose-dependant manner to cause cell cycle arrest in G0/G1 phase. Results also show that reactive oxygen species (ROS) release, ER stress occur and Ca^{2+} massive release in NPC-TW 076 cells after exposed to tetrandrine. Western blotting was used to inspect the protein expression which associated with apoptosis and that indicated inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6) were increased. We also used the scavenger of ROS (N-acetylcysteine ; NAC) exposed to cells then were treated with tetrandrine and results show that tetrandrine induce apoptosis was involved ROS production. Tetrandrine activated the activity of caspase in NPC-TW 076 cells. These results indicate that tetrandrine induces apoptosis through the endoplasmic reticulum stress in NPC-TW 076 cells.

Keyword: human nasopharyngeal carcinoma, endoplasmic reticulum stress, apoptosis, tetrandrine, inositol-requiring enzyme 1

去甲基斑蝥素經由外在路經誘導人類胃癌細胞AGS細胞凋亡
**Norcantharidin induced apoptosis of human gastric cancer AGS cells
through extrinsic pathway**

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Norcantharidin (NCTD), the demethylated analog of cantharidin, was isolated from natural blister beetles which have been used as a Chinese medicine in Chinese population for a long time. NCTD has been reported to induce cell death *in vitro* and *in vivo* of human gallbladder carcinoma and human liver cancer. However, there is no any available information to address the effect of NCTD on the apoptosis in gastric cancer cells. NCTD-induced apoptosis was determined by flow cytometric analysis, DAPI staining and comet assay. After that, we determined the mechanism of apoptosis in AGS cells by Western blotting. The results show NCTD reduced cell viability and induced cell death through the induction of apoptosis which was determined by PI staining and Annexin V assay. We also found NCTD-induced apoptosis was associated with enhanced ROS production, loss of mitochondria membrane potential ($\Delta\Psi_m$) and induction DNA damage. NCTD stimulated death receptor-mediated signaling transduction, such as TNF- α , TRAIL and DR5, item followed by AIF, cytochrome *c*, Endo G production. Taking together, our findings indicated that NCTD significantly induced apoptosis of AGS cells. The mechanism involved at least in part the down-regulation of cell survival and promoting of extrinsic associated signaling proteins.

Keyword: norcantharidin, gastric cancer, extrinsic pathway, DNA damage, apoptosis

**運動活化誘導老化大鼠海馬迴 IGF1/Akt 和 SIRT1 存活路徑並抑制凋亡
Exercise suppresses apoptosis by stimulating IGF1/Akt and AMPK/SIRT1
survival signaling in the hippocampus of induced aging rats**

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Aging is a natural phenomenon, the accumulation of molecular and cellular damage throughout life leads to age-related pathological condition. Extensive damage in the brain can cause neuronal dysfunction and trigger apoptosis. Exercise is presumed to delay the aging process and promote healthy since it seems to improve the function of most of the mechanisms involved in aging. Our purpose of this study was to evaluate the effects of exercise training on brain apoptotic and survival pathways in D-galactose-induced aging SD rats. Rats were allocated to the following groups : (1)young control group ; (2)young rats with swimming exercise group ; (3)aging control group, aging rats were induced and injected by the intraperitoneal injection of D-galactose. (4)aging rats with swimming exercise group. The Fas-dependent and mitochondrial dependent apoptotic pathway were all significantly increased in the induced-aging group relative to the control group whereas they were decreased in the aging-exercise group. The inflammation pathway marker were mass expression in D-galactose-induced aging brain, exercise significantly inhibited the inflammatory signaling activity. The components of brain survival pathway were all significantly decreased in the induced-aging group compared with the control group whereas they were increased in the aging-exercise group. Additionally, the anti-aging pathway markers were significantly reduced in D-galactose-induced aging group compared to those in the control group and obviously increased after exercise training. This study demonstrated that exercise training not only reduced aging-enhanced brain apoptosis and inflammatory signaling activity, but also enhanced the IGF-I/Akt and SIRT1 survival pathways in the hippocampus from aging-exercise rats, which provides one of the new beneficial effects for exercise training in aging brain.

Key words : D-galactose, exercise, brain, inflammatory, apoptotic, survival, SIRT1

WISP-1 在人類口腔鱗狀上皮細胞中調節血管內皮生長因子和血管新生的表現量

WISP-1 Increases Angiogenesis and VEGF-A Expression in Human Oral Squamous Cell Carcinomas

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Oral squamous cell carcinomas (OSCC) is most common metastasis to cervical lymph nodes by angiogenesis. Wnt-1 induced secreted protein 1 (WISP-1) is a cysteine-rich protein that belongs to the CCN family (CTGF / CYR61 / NOV). However, the effects of WISP-1 on angiogenesis and vascular endothelial growth factor- A (VEGF-A) expression in human OSCC are not clear. The aim of study is try to examine the effect of WISP-1 on angiogenesis and VEGF-A expression in human OSCC. The qPCR was used to examine the mRNA expression of VEGF. The FAK, c-Src, EGFR, ERK phosphorylation was examined by using Western blot method. A transient transfection protocol was used to examine HIF1- α activity. The chick chorioallantoic membrane (CAM) assay and matrix gel plug nude mice model *in vivo* used to examine expression of angiogenesis. We found that the expression of WISP-1 in human OSCC tissues were higher than in normal oral tissues. We also demonstrated that WISP-1 enhanced angiogenesis and VEGF-A up-regulation in human OSCC. WISP-1 induced VEGF-A expression and angiogenesis through $\alpha\beta 3$ integrin, FAK, c-Src, EGFR, and ERK pathway. Knockdown WISP-1 decreased VEGF-A expression and also abolished human OSCC condition medium-mediated angiogenesis *in vitro* as well as angiogenesis effects in the CAM and matrigel plug nude mice model *in vivo*. We characterized an important role for WISP-1 which regulates angiogenesis by increasing VEGF-A expression in human OSCC via the $\alpha\beta 3$ integrin, FAK, c-Src, EGFR, ERK, and HIF1- α signaling pathway.

Keyword : Oral squamous cell carcinomas, WISP-1, VEGF-A, Angiogenesis

山奈酚透過第一型類胰島素生長因子受體對
HT-29 人類結腸癌細胞的遷移之影響

**Effect of kaempferol on migration in HT-29 human colon cancer cells
via Insulin-Like Growth Factor 1 Receptor (IGF1R)**

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Insulin-Like Growth Factor-1 Receptor (IGF-1R) is highly expressed in human colon cancers and plays important roles in promoting malignancy. The IGF1R signaling pathways mainly involved in cell proliferation, differentiation, survival and migration. We constructed herein novel cell lines including constitutively activated IGF-1R which subsequently results in β subunit autophosphorylation and triggers downstream signaling that includes the PI-3K/Akt pathway. Kaempferol, a natural flavonoid, has been demonstrated to possess anti-proliferative and apoptosis-inducing activities in several cancer cell lines. On the other hand, to confirm the kaempferol can bind with IGF1R, we use computer-aided drug design software to predict the molecular docking of kaempferol in IGF1R. Cell viability and proliferation were measured using the MTT assay in four human colon cancer cell lines: HT-29, HT29-CD8, HT29-CD8-IGF1R and HT29-IGF1R with the treatment of kaempferol for either 24 or 48 hr. The metastasis ability of four colon cancer cells are characterizing by the wound healing assay. The long term goals of this study are to understand the underlying molecular mechanisms of kaempferol via IGF-1R signaling and to shed light on the therapy of colon cancer and tumor metastasis.

Keywords: Kaempferol, IGF1R, migration, molecular docking

Effect of the butanol layer of the Si-Wu-Tang extract on prostate cancer cells

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Si-Wu-Tang (SWT) is one of the tonic Chinese prescriptions in Traditional Chinese Medicine and widely used in Asian countries. It is composed of 4 herbs, i.e., *Angelica sinensis* Radix, *Paeonia Alba* Radix, *Chuanxiong* Rhizoma and *Rehmannia* Radix. The studies show that SWT has blood-nourishing effects, promoting blood circulation effects, reducing blood stagnation, enhancing immunity and stimulating the reproduction of hematopoietic cells. However SWT is not only for women use, we discover that the STW also has impact on men's health. Prostate cancer ranks the seventh male cancer death and the most common male reproductive system cancers in Taiwan. In America, prostate cancer is the second common cause of cancer death and the leading cause of cancer death in males over 70 years old. This high mortality rate may result from late detection, since the five-year survival rate is about 87% when the cancer is diagnosed earlier. We found SWT extract (SWTE) inhibited the growth of human prostate cancer cell lines, but its mechanism of action still remained unclear yet. Therefore, this study aimed to explore the effect of the butanol layer of the SWT on the growth of prostate cancer cells and to identify its molecular mechanism of action. The results of this study will provide an alternative therapeutic way for the treatment of prostate cancer.

Keywords: Si-Wu-Tang, *Angelica sinensis* Radix, *Paeonia Alba* Radix, *Chuanxiong* Rhizoma, *Rehmannia* Radix, prostate cancer

Effect of the ethanolic extract from *Ganoderma tsugae* on prostate cancer cells

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Ganoderma tsugae (GT) is a traditional Chinese medicine and has been used in Asian countries for centuries. GT is known to protect the heart and cardiovascular and has anti-cancer effect. However, GT is no longer only used for women, because we have found that GT is also beneficial to men's health. Prostate cancer has the high mortality rate in males in the world including Taiwan. We have shown GT ethanolic extract (GTEE) inhibits the growth of human prostate cancer cells. Although its molecular mechanism of action remains unknown. Based on the literature review, we propose GTEE may influence the metabolism of the male sex hormone androgen which is currently under studying. Furthermore, we have also applied the partition fractionation approach to unraveling the active fraction of GTEE and its relevant molecular mechanism. The partition fractionation products of GTEE, including ethyl acetate layer, have been used to investigate their inhibition efficacy on prostate cancer. The results of this study will elucidate the active fraction of GTEE and provide an alternative adjuvant therapy against prostate cancer.

Keywords: *Ganoderma tsugae*, partition fraction, prostate cancer, androgen, adjuvant therapy

Malabaricone A 於口腔癌細胞之抗腫瘤機制研究
The Study of Molecular Mechanism Underlying the Anti-tumor
Effect of Malabaricone A in Oral Cancer Cells

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The mortality of oral squamous cell carcinoma (OSCC) is one of the ten leading causes of cancer deaths in Taiwan. Environmental carcinogens such as betel quid chewing, tobacco smoking and alcohol drinking have been identified as major risk factors for OSCC. In this study, we investigated the anti-cancer activity of Malabaricone A (Mal-A) and its molecular mechanisms in the oral squamous cell carcinoma cell line Ca922. Mal-A inhibited Ca922 cell viability in a dose- and time-dependent manner by MTT assay. Treatment with 25、50 and 75 μ M of Mal-A for 48 h led to DNA damage and apoptosis by DAPI staining. We also show that Mal-A reduced cell viability and induced cell death through the induction of apoptosis which was determined by PI and Annexin V assays. At first we used MTT assay screened from one of 34 kinds of compounds. Treatment with different dosage of Mal-A for 48h, Mal-A inhibited oral cancer cells viability in a dose- and time-dependent manner. Then we treatment with 50 μ M of Mal-A in 6、12、24 and 48h, result show that Mal-A inhibited oral cancer cells viability in a time-dependent manner. Our results suggested that Mal-A might exert cytotoxicity by induction of apoptosis and then leads to cell death in Ca922 cells.

Keywords: *Myristica malabarica*, Ca922, Anti-tumor

分析 SIAH1 和 SIAH2 在口腔鱗狀細胞癌中所扮演的機制

Analyzing SIAH1 and SIAH2 as oncogenic factors in oral squamous cell carcinoma

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The mortality of oral squamous cell carcinoma (OSCC) is one of the ten leading causes of cancer deaths in Taiwan. Environmental carcinogens such as betel quid chewing, tobacco smoking and alcohol drinking have been identified as major risk factors for OSCC. Our laboratory has found that the SIAH (seven in absentia homolog) family over-expressed due to gene amplification in OSCC. SIAH belongs to the N-terminus Ring-finger E3 family, and the involvement of the N-terminus Ring-finger E3s in crucial signaling pathways implicates in tumorigenesis. This study aimed at investigating the expression and functional significance of SIAH in OSCC. Real-time PCR were performed to examine the amplification of SIAH1 and SIAH2 gene in clinical specimens of OSCC tissues. Expression of SIAH1 and SIAH2 mRNA and protein was examined by quantitative RT-PCR and immunohistochemical assays respectively. Apoptosis was examined by annexin V staining. Levels of SIAH1 and SIAH2 DNA and mRNA were significantly greater in clinical OSCC specimens and in cultured OSCC cells. Knockdown of SIAH1 and SIAH2 led to growth suppression and apoptosis. These results revealed a tight correlation of SIAH1 and SIAH2 overexpression with OSCC and suggest an oncogenic role of SIAH1 and SIAH2 in oral cancer.

Keywords: SIAH1, SIAH2, oral cancer, N-terminus Ring-finger E3

**Berberine 經由 RAS/RAF/MEK/ERK 訊息途徑對黑色素癌 B16F10
細胞株之影響**

**Berberine effect on melanoma B16F10 cells via
RAS/RAF/MEK/ERK signaling pathway migration**

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Berberine is a primary component of the most functional extracts of *Coptidis rhizome* used in traditional Chinese medicine for centuries. In many papers, we know that Berberine inhibits metastasis and proliferation in breast cancer, stomach cancer, and cervical cancer. Melanoma is the most lethal and malignant in skin cancer. In the United States, although melanoma account for only 4% of skin cancer, cause about 80% of deaths. The overall 5-year survival rate for patients who melanoma is detected early before the tumor has spread to regional lymph nodes or other organs is better. The survival rate falls to 62 percent when the disease reaches the lymph nodes and 15 percent when the disease metastasizes to distant organs. We used B16F10 the melanoma cell line because it have high migration activity. Cell viability was tested by using MTT assay, B16F10 melanoma cells were treated with 15, 30 and 60 μ M Berberine. After 48 hours, the proliferation of B16F10 melanoma cells were suppressed, the IC₅₀ was 30 μ M. The migration activity of melanoma can be inhibited by Berberine via RAS/RAF/MEK/ERK metastasis pathway, and also confirmed that non-small-cell lung cancer (NSCLC) cells treated with Berberine. Nobody investigate B16F10 melanoma cells inhibits migration via RAS/RAF/MEK/ERK signal pathway. So, we investigate whether Berberine inhibits migration of B16F10 via RAS/RAF/MEK/ERK signal pathway or not. We hope that our research can make people know more about melanoma, and contribute to the treatment of melanoma skin tumors.

Keyword: B16-F10 melanoma cell, berberine, migration, RAS/RAF/MEK/ERK

miR-23b 抑制 SW620 和 HT-29 大腸癌細胞的轉移和增生
Mir-23b inhibits metastasis and proliferation of SW620 and HT-29
human colon cancer cells

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大腸癌是全球第四大致命性癌症，國人罹患大腸癌的機率正逐年增加中。大腸癌的死亡率和腫瘤的轉移與否有著很大的關係，而腫瘤的發生又和基因的突變導致細胞變異有關，現今仍有許多機制尚未明確。MicroRNAs 藉由負向調控下游基因的表現，使目標基因(target gene)的蛋白質於特定時間和特定細胞中維持著恆定的表現量。MicroRNAs 在生物體中參與細胞的許多功能，例如：細胞增生、自我吞噬、細胞凋亡與轉移。許多文獻提到 miR-23 抑制腫瘤基因的表現，參與細胞的增生、DNA 修補、細胞的分化、細胞週期停滯與細胞轉移。本實驗最主要目標就是藉由電腦軟體分析，搜尋 miR-23b 最有可能之標的基因 Myct1、Prkacb 和 Zeb1。將搜尋找到 miR-23b 的可能標的基因之結合位置選殖至 luciferase 報導載體，將基因重組 DNA 轉染到大腸癌細胞 SW620 和 HT-29，測試從軟體預測 miR-23b 最有可能之標的基因是否準確，接著使用西方點墨法進一步確認。最後作細胞增生與細胞轉移的功能性檢測實驗。希望本研究計畫的發現能運用在臨床的分析、檢驗與治療方面。

關鍵字：miR-23b、大腸癌細胞、細胞轉移和細胞增生

松杉靈芝萃取物對子宮內膜癌細胞的作用與機制
Effect and mechanism of *Ganoderma tsugae* extract on endometrial cancer cells

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Ganoderma, also named Lingzhi, is a traditional Chinese medicine that has been used for medicinal purposes in Oriental countries for several thousand years. It has a broad-spectrum of biological properties including immunomodulatory and anti-cancer activities. In this study, we study the effect and molecular mechanisms by which *Ganoderma tsugae* (GT), one of the most common species of *Ganoderma* cultivated in Taiwan, inhibits the proliferation of endometrial cancer cell lines, HEC-1-A, AN3 CA, and KLE. The MTT assay shows that *Ganoderma tsugae* extract (GTE) causes a marked reduction in the number of viable endometrial cancer cells. In addition, the trypan blue exclusion assay clearly indicates that the GTE shows growth inhibition effect on endometrial cancer cells. Our data also demonstrate that GTE induces cell cycle arrest by interfering with the expression of cell cycle regulator proteins such as cyclin E. In conclusion, our results may provide a GTE-mediated adjuvant therapeutic way in the treatment of endometrial cancer.

Keywords: *Ganoderma tsugae*, Lingzhi, endometrial cancer, HEC-1-A, AN3 CA, KLE

DNA 甲基化與口腔癌預後之相關

The association between DNA methylation and prognosis of oral cancer

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目的：口腔癌是全世界常見癌症之一。在台灣，好發於中年男性，100 年其癌症死亡率為男性排名的第四位。根據台灣國民健康署最新統計資料，台灣 96 至 100 年新診斷為口腔癌之五年期別存活率由第 0 期至第 4 期分別為：83.2%、76.9%、67.7%、54.9%及 33.3%，其存活率隨著期別增加而大幅下降，最大差距可到 50%。在許多口腔癌文獻中提到基因 DNA 甲基化與患者預後情形有關，所以本研究的目的，探討 DNA 甲基化是否可以成為預測口腔癌患者預後的生物標記 (biomarker)。

材料與方法：研究樣本來自中國醫藥大學附設醫院組織庫，樣本為 40 名接受口腔手術的患者的口腔內部頰黏膜之腫瘤組織切片，其患者開刀期間為 2003 年 9 月至 2009 年 1 月，自患者手術結束後開始追蹤至 2013 年 12 月止。檢測樣本的位點甲基化程度使用的平台為 Illumina GoldenGate Methylation Cancer Panel I，針對目前已知與癌症相關之 807 個基因的 1505 個 CpG 位點，測量各位點之甲基化程度(以 β 值呈現，其值範圍由 0~1，表示 CpG 位點甲基化程度的強度比例)，而 1505 個 CpG 位點刪除門檻的條件有二：第一條件為患者死亡組，若位點 β 值皆小於 0.2(預設)則刪除；在患者存活組，若位點 β 值皆大於 0.2(預設)，則刪除。第二條件為存活組的位點 β 值中位數大於死亡組則刪除。經 CpG 位點刪除門檻後所剩之位點再與患者之存活狀態進行 Log-rank test 檢定位點有無甲基化(預設 β 值 ≥ 0.2 為有甲基化狀態；反之 β 值 < 0.2 為無甲基化狀態)與患者存活狀態有無差異性，進而篩選出與預後狀態相關之基因甲基化。

結果：40 位患者從手術後開始追蹤五年後，死亡組共 18 位，其病理分期期別由 I 至 IV/ IVa 所佔比例分別為 17%、22%、22%及 39%；存活組共 22 位，其病理分期期別由 I 至 IV/ IVa 所佔比例分別為 23%、18%、32%及 27%。1505 個 CpG 位點依患者預後狀態及 CpG 位點甲基化的兩項刪除門檻條件篩選後剩餘 462 個位點，再將 462 個位點甲基化狀態與患者存活時間進行 Log-rank test 檢定其差異性並經錯誤發現率(False Discovery Rate)控制偽陽率後，其結果共 42 個位點甲基化狀態與患者存活時間具有顯著差異。

結論：

DNA 甲基化會影響口腔癌患者的存活情況，可作為預測口腔癌患者預後的生物標記。

關鍵字：oral cancer, methylation, biomarker

松杉靈芝乙醇萃取物對抗前列腺癌細胞的分子機制
Anti-cancer effects of *Ganoderma tsugae* extract on prostate cancer cells

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Prostate cancer is the most common malignancy in men and the second leading cause of cancer deaths among males in the Western world. The low survival rate of prostate cancer is caused by late diagnosis and its metastasis potential. *Ganoderma*, a traditional Chinese medicine (TCM), has been used for promoting human health and longevity in Asian countries for a long time. *Ganoderma tsugae* (GT), a species of *Ganoderma* which can be well-cultivated in Taiwan, has been shown to have multifacet effects, e.g., anti-tumor, immunomodulatory, and antioxidant activities. In this study, we attempt to demonstrate the molecular mechanism by which the ethanol extract of GT (GTE) inhibits the proliferation of prostate cancer cells. Our previous results showed that GTE significantly inhibited the growth of prostate cancer cells. Our data also indicated that the suppression of cell growth by GTE was through down-regulation of the PI3K/Akt pathway. In summary, this study suggests that GTE may be a potential adjuvant therapeutic agent in the treatment of prostate cancer.

Keywords: Traditional Chinese Medicine, *Ganoderma tsugae*, prostate cancer, PI3K/Akt pathway

山欖經由抑制在巨噬細胞中 miR-146a 表現有抗發炎和促進細胞凋亡的功能

Planchonella obovata has anti-inflammatory ability and induces apoptosis via inhibition of miR-146a expression in macrophage cell line RAW264.7

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發炎反應為人體抵抗外來病原菌入侵的一種機制，一般反應為紅腫熱痛，使血管擴張、組織胺滲出，產生痛覺。若發炎反應無法控制，將導致被破壞的組織無法修復、造成肌肉損傷、引起疾病。天然物中常含有許多抗炎和抗癌成分，我們發現實驗中先加入脂多糖(LPS, lipopolysaccharide)刺激老鼠巨噬細胞(RAW264.7)產生發炎反應，再加入天然物的山欖(*Planchonella obovata*)所萃取出成分，可以使 Nitric oxide (NO)的產生有明顯抑制。再利用 Luciferase 冷光報導基因及西方點墨法測試山欖萃取成分是否抑制發炎時誘發性環氧合酶 2 (COX-2, cyclooxygenase 2) 的表現。miR-146a 在發炎時有誘發表達，將使用山欖萃取成分測試是否抑制 miR-146a 誘發表現。同時，分析 miR-146a 發炎相關和細胞凋亡及細胞自噬之標的蛋白表現的情形。了解山欖在發炎反應和細胞凋亡與自噬中扮演的角色，進而研究出抗發炎的新藥物。

關鍵字：山欖、miR-146a、發炎及細胞凋亡

四物湯水萃乙酸乙酯層對前列腺癌細胞生長的影響

Effect of ethyl acetate fraction of Si-Wu-Tang on prostate cancer cells

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Si-Wu-Tang (SWT), also called Four-Agents-Decoction (FAD), is one of the typical Chinese prescriptions in traditional Chinese Medicine (TCM) and widely used in Asian countries. SWT has also been shown to nourishing blood, reducing blood stagnation, enhancing immunity, and promoting the reproduction of hematopoietic cells. Prostate cancer is the second most common cause of cancer death in American males and ranks the 7th of cancer death in Taiwan males. The low survival rate of prostate cancer may result from the late diagnosis although it is known that 87% of men will be treated in early diagnosis. In this study, we investigate the molecular mechanism by which the ethyl acetate fraction of SWT (SWT-EA) suppresses the growth of prostate cancer cells. Our results indicate that SWT-EA significantly inhibits the growth of prostate cancer cells. The data also show that the growth suppression effect of SWT-EA on prostate cancer cells is through disrupting the distribution of cell cycle. Furthermore, the induction of G1 phase arrest by SWT-EA is found to down-regulate the expression of cell cycle regulators, such as cyclins D1 and E. Taken together, this study suggests that SWT-EA may provide a useful alternative therapeutic way for the treatment of prostate cancer.

Keywords: Si-Wu-Tang, Traditional Chinese Medicine, ethyl acetate fraction of SWT, prostate cancer, cell cycle

Tetrandrine inhibits cell migration and invasion in Colon Cancer SW620 cells

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Abstract

Tetrandrine is a bisbenzylisoquinoline alkaloid isolated from the root of *Stephania tetrandra* which is a traditional Chinese medicine. Numerous evidences indicated that tetrandrine exhibits very broad pharmacological actions including anti-inflammatory, antioxidant effects, as well as anticancer.

In this study, we chose tetrandrine for examining the effects of cell migration and invasion of human highly metastatic colon cancer SW620 cells. At first, we use cell proliferation assay to measure the cell viability. Second, we investigate the inhibition effect of tetrandrine on the migration ability of SW620 cells which was measured by using wound healing assay and result show that tetrandrine inhibited cell migration. We also found similar effects of tetrandrine on invasion which was examined by using the Trans-Well Cell Invasion Assay and result show that tetrandrine inhibit the cell invasion of SW620. We found the adhesion ability was decreased in SW620 cells after exposed to tetrandrine which was examined by using adhesion ability assay. Western blotting assay, we detect several metastasis related proteins and result show that tetrandrine treatment leads to a decrease MMP-1,-2,-9 protein expression levels. To sum up, tetrandrine can down-regulate the invasion, migration, adhesion ability on colon cancer SW620 cells. Our findings suggest that tetrandrine may be a potential Chinese herbal medicine with the antimetastasis effect in colon cancer in future.

Keyword: Tetrandrine, colon cancer, migration, invasion

Synthesis and evaluation of anticancer activity of curcumin analogs

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Curcumin is an orange-yellow crystalline powder obtained from rhizome of *Curcuma longa* Linn (Zingiberaceae). Curcumin has a variety of biological activities. Within the past 30 years, cancer is a leading cause of death in Taiwan. Therefore, developing new anticancer drugs is in an urgent need. In the recent years, there are many studies reported that curcumin displayed anticancer activity. In this study, several curcumin analogs were synthesized and evaluated for anticancer activities. We expected to find a better anticancer agent than curcumin.

This study is focus on synthesis of two types of curcumin analogs, which are represented as WC and S series. WC series was used the catechol as a starting material, which was first cyclized with various ketones under P₂O₅ catalyst to obtain benzodioxole derivatives. The benzodioxoles were reacted with NBS, and then went forward the Grignard reaction to yield the corresponding aldehydes. Finally, the aldehydes were condensed with acetylacetone to give the corresponding curcumin analogs WC1~WC7. As synthetic curcumin analogs S series, bezaldehyde or piperonal was first reacted with acetone under strong base, and then reacted again with the substituted aldehydes to obtain the corresponding curcumin analogs S1~S7. The anticancer activities of these curcumin analogs WC1~WC7 and S1~S7 were evaluated on Hep3B, H460, A498 and COLO-205 human cancer cell lines. The preliminary results show that a part of S series exhibited better anticancer activities comparing with curcumin.

Keywords: anticancer activity, benzodioxole, curcumin analogs, synthesis

建立人造油體於斑馬成魚口部投藥系統平臺

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油體為一團中性脂肪分子，其外包覆著一層類似細胞膜成分的磷脂質，油體正是由磷脂質上鑲嵌的油體膜蛋白所建構而成。油體膜蛋白屬於一種結構性蛋白質，其主要生理功能為保持油體的穩定性，以利油體的長期儲存。本研究目的為如何創建一個良好的斑馬魚類投藥系統，考慮到油體包覆中藥，其味道並不受斑馬魚喜愛，因此本實驗以斑馬魚喜好之小型餌料作為材料，製備豐年蝦油，並測試最佳製備時間，來找尋橄欖油混合豐年蝦油的最佳重組油體穩定性條件以及測試其適口性。透過冷凍切片及螢光顯微鏡觀察油體在斑馬魚腸道內分解吸收情形，篩選出最適合的油體重組條件與比例。**實驗結果**：**1.**約於八小時左右豐年蝦油所具有的脂質含量最多，依不同比例的橄欖油與豐年蝦油進行油體重組，當橄欖油所含的比例愈高時，油體膜蛋白具有愈高的鑲嵌率。**2.**在橄欖油與豐年蝦油比例為 1：1 時，具有最大的懸浮穩定性。油體顆粒隨著橄欖油的增加有變大的趨勢，且電位也隨之上升；加入豐年蝦油後，則是隨其含量之增加，油體顆粒變小、表面電位變大。**3.**斑馬魚適口性試驗上以螢光染劑作為示範藥物進行包埋釋放定性及定量等實驗分析。完全使用橄欖油是具有最高的螢光染劑包覆率(95%)，隨著豐年蝦油比例的增加則有下降的趨勢。**4.**在釋放率的分析，隨豐年蝦油比例的增加，釋放率有增高的趨勢(橄欖油/豐年蝦油比例 1：4 其釋放率為 77.8%)，可以在斑馬魚排泄前有效的於腸道內釋放螢光染劑。**5.**在油餅崩解分析上，油餅於一小時內仍保有 90%的完整度。**6.**進食時間測量發現斑馬魚只花了 9 秒左右的時間就將含有豐年蝦油成分之油餅全部攝食，明顯比只含橄欖油的油餅快。**綜合以上論述**，經特殊處理過之油體確實可吸引斑馬魚前來覓食，其油體穩定適合作為斑馬成魚的投藥系統。

Keywords: 油體、斑馬魚、油體膜蛋白、橄欖油、豐年蝦油

MST3 透過 NKCC 調節離子平衡之研究

Investigation of MST3-regulated ion balance through NKCC

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背景介紹：MST3 (mammalian-Ste20-kinase3)為 Ste20 (sterile-20) kinase 家族，我們之前的研究發現 MST3 功能抑制細胞的轉移(cell migration)。最近發現 MST3 在老鼠腎臟集尿管表現，MST3 活性基因轉殖鼠促進鈉、鉀、氯排除。根據文獻指出其他 Ste-20 kinase，如：proline-alanine-rich Ste-20-related kinase (PASK)、oxidative stress response protein1 (OSR1)，會促進 Na-K-2Cl 離子通道(NKCC)打開，使離子進入，造成過多離子滯留於體內進而造成高血壓，目前研究方向主要是探討 MST3 是否也是經由 NKCC 來調節細胞的離子平衡。

研究方法：轉染 HA-MST3 plasmid 以及 HA-MST3 kinase dead (HA-MST3 失活) plasmid 進入 MDCK cell，形成 stable clones，而後利用高鹽(600 mM NaCl)與低氯(chloride free) 環境活化 NKCC 並使用 SBFI (sodium fluorescent indicator)、PBF1 (potassium fluorescent indicator)分別與細胞內的鈉離子與鉀離子結合，觀察在改變細胞外鈉離子以及氯離子濃度時，藉由偵測 SBFI 與 PBF1 的螢光變化來得知細胞內鈉離子和鉀離子的變化，並加入 Na-K-2Cl 離子通道抑制劑 (bumetanide)確認 MST3 調控 NKCC。

結果：我們獲得 MST3 過度表現細胞 (C-1、C-2)、MST3 失活細胞 (1-6、1-7)，在高鹽(600 mM NaCl)環境下，由 SBFI 偵測到的結果 MDCK (wild type)螢光變化值 0.7、C-1 螢光變化值 0.8、1-6 螢光變化值 0.7，由螢光變化值我們得知 C-1 細胞內鈉離子濃度提升最多，表示 MST3 過度表現細胞活化 NKCC 的程度較 MDCK 與 MST3 失活細胞大；低氯環境下，PBF1 偵測到 MDCK 螢光變化值為 0.6，之後比較 SBFI 以及 PBF1 螢光變化值是否都是 MST3 過度表現細胞活化 NKCC 程度較大，而後使用 bumetanide 來確認 MST3 是否經由 NKCC 來調節離子平衡。

Keywords: MST3, NKCC, Ste20 kinase, PASK, OSR1

Determination of lupeol in common medicinal plants in Taiwan by high-performance liquid chromatography

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A method first developed to determinated of lupeol (a novel anti-inflammatory and anti-cancer dietary triterpene) contained in common medicinal plants in Taiwan, including *Acanthopanax trifoliatum* (L.) Merr., *Berchemia racemosa magna*, *Rhynchochymum discolor* (Maxim.)Burt, *Ficus sarmentosa* Buch.-Ham. ex J. E. Sm. var. *henryi* (King. ex D. Oliver) Corner, *Clerodendrum kaempferi* (Jacq.) Siebold ex Steud., *Cyperus iria* Linn., *Cyclosorus parasiticus* (L.) Farw, *Hemerocallis lilioasphodelus* L. was established. The samples were analyzed by HPLC on a Lichrospher 100 RP-18e column and detected at 210 nm with acetonitrile water (99: 1, v/v) as the mobile phase at a flow rate of 1.0 mL/min. The regression equations of diosgenin was $Y=4.612X-9.118$ ($r=0.999$). The intraday and interday relative standard deviations of lupeol were at the levels of 1.34-3.87% and 0.47-2.38%, respectively. Detection limit was 50 μ g/mL based on a signal-to-noise ratio of 3:1. The method was applied to 40 samples and lupeol was detected in five samples, measurable at 192.4 mg/g contained in *Acanthopanax trifoliatum* (sequential extracts of n-hexane), 11.8 mg/g contained in *Berchemia racemosa* (sequential extracts of n-hexane), 35.5 mg/g contained in *Berchemia racemosa* (sequential extracts of ethyl acetate), 164.7 mg/g contained in *Ficus sarmentosa* (sequential extracts of n-hexane), 78.1 mg/g contained in *Ficus sarmentosa* (sequential extracts of n-hexane). In conclusion, this study had shown that the HPLC method could be applied successfully to analyze lupeol contained in common medicinal plants. Therefore, an easy and suitable method for the detection and assay of lupeol was established.

Keywords: lupeol, common medicinal plants, anti-cancer, HPLC

台灣常見高經濟價值螃蟹粒線體 DNA 中 *cytb* 基因序列之建立與比對

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本研究乃利用分子鑑定技術建立經濟性螃蟹粒線體 DNA 中完整 *cytb* 基因序列，圖譜資訊以作為生鮮暨加工製品之基因判別指標。所使用的樣本是收集自台中梧棲漁港，包括鋸緣青蟹(*Scylla serrata*)、台灣絨螯蟹(*Erimacrus isenbeckii*)、紅星梭子蟹(*Portunus sanguinolentus*)、遠海梭子蟹(*Portunus pelagicus*)、銹斑蟊(*Charybdis feriata*)、善泳蟊(*Charybdis natator*)、白紋方蟹(*Grapsus albolineatus*)、漢氏勞綿蟹(*Lauridromia dehaani*)、燦爛滑面蟹(*Etisus splendidus*)。

利用自行設計之 F202 及 R1013 引子組，搭配聚合 酶連鎖反應 polymerase chain reaction, PCR) 增幅螃蟹粒線體 DNA 中完整 *cytb* 基因序列，經定序比對確認後所得部分序列長度約為 750bp。並利用 GCG (Genetics Computer Group) 進行序列比對，比對結果顯示其基因序列差異度約在 16.82% 至 32.44% 之間。

本研究中所使用的 F202 及 R1013 此組引子對具有物種共通性，除建立高經濟價值甚至或有毒蟹類的粒線體 DNA 中 *cytb* 基因區塊之基因序列，更可利用差異度高之區塊設計作為物種判斷的基因條碼(DNA barcode)，並在此變異度高之序列區域設計特异性 TaqMan probe，達到有效開發加工品原料物種之鑑定方法，更可提供相關衛生單位作為檢測蟹類及其加工產品，預防摻假或誤食有毒蟹類的案件發生。

關鍵字: 螃蟹、分子鑑定技術、*cytb*、DNA barcode、TaqMan probe

建立台灣常見螺類粒線體 DNA 中 *cytb* 之基因序列

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本研究是利用分子鑑定技術建立常見經濟性螺類粒線體 DNA 中完整 *cytb* 之基因序列圖譜資訊以作為生鮮暨加工製品之基因判別指標，所使用的樣本收集自台中梧棲漁港採集的香螺(*Hemifusus tuba*)、漁舟蜆螺(*Nerita albicilla*)和福壽螺(*Pomacea canaliculata*)。

實驗結果顯示，本研究中使用 F-02 及 R-15 引子組配合聚合酶連鎖反應 (polymerase chain reaction, PCR) 增幅獲得香螺、漁舟蜆螺和福壽螺之粒線體 DNA 中部分 *cytb* 基因序列，經定序結果比對確認後所得序列長度皆約為 1100 bp。並利用 GCG (Genetics Computer Group) 將序列與 NCBI GenBank 基因資料庫中 *Haliotis diversicolor*(EU244334)、*Rapana venosa*(NC_011193)、*Babylonia areolata*(HQ416443)、*Thais clavigera*(DQ159954) 四種其他常見螺類粒線體 DNA 中 *cytb* 基因序列進行比對，結果顯示差異度約為 1.96%~39.07%

市售常見螺類加工產品如螺類罐頭、螺片真空包裝，容易發生內容不實或是摻雜其他廉價物種的現象發生，因此建立螺類物種之基因序列圖譜不僅可作為分子鑑定技術之參考依據，達到有效開發加工品原料物種之鑑定方法，更可提供相關衛生單位作為檢測螺類及其加工品快速檢測之用，預防摻假的現象發生。

關鍵字:螺類、*Haliotis diversicolor*、*Rapana venosa*、*Babylonia areolata*、*Thais clavigera*、*cytb*、分子鑑定技術

傳統與新興萃取法對杏鮑菇抗氧化能力之影響評估
**The evaluation for antioxidant abilities of *Pleurotus eryngii*
by traditional and emerging extraction methods**

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Pleurotus eryngii contains rich of natural substances especially for its antioxidant properties. The present study aimed to optimize the extraction method of *Pleurotus eryngii* fruiting body and to investigate the antioxidant properties. In this study, the extraction methods of *Pleurotus eryngii* fruiting body were divided to traditional extraction and emerging extraction. There are two kinds of traditional extraction methods: high temperature boiling and high temperature steaming. Each kind of traditional method consists of boiling by using oven-dried powder and boiling followed by cold water extraction and lyophilization. Meanwhile, emerging extraction method were divided to three different ways: microwave extraction, ultrasonic extraction and cold water extraction. Microwave and ultrasonic extractions included two different preparations: minced and diced of *Pleurotus eryngii* fruiting body. According to the results, the dry powder of *Pleurotus eryngii* extracted by high temperature steam presented the highest contents of total polyphenol (59.58 mg/g) and flavonoid (72.71 mg/g) respectively than other traditional methods. Within the emerging extraction methods, *Pleurotus eryngii* extracted by cold water showed the highest content of total polyphenol (232.08 mg/g) than others. Furthermore, minced preparation of *Pleurotus eryngii* extracted by microwave presented the highest contents of flavonoid (128.73 mg/g) among emerging extractions. In addition, the extract of *Pleurotus eryngii* by cold water exhibited the strongest scavenging activity of DPPH radicals (96.05%) among all extracts. The highest ferrous chelating ability (94.92%) was presented from the sample of high temperature steam extraction followed by cold water extraction and lyophilization. In summary, *Pleurotus eryngii* extracted by traditional extraction methods showed better chelating abilities, whereas extracted by emerging extraction methods presented stronger DPPH scavenging capacities. The extract of *Pleurotus eryngii* performed significant antioxidant activities, therefore it can be further developed into a variety of health food products in the future.

Keywords: *Pleurotus eryngii* , Antioxidant, Extraction methods

五種食藥用菇類萃取物之活性成分分析
The studies for the active ingredients of
five edible and medicinal mushroom extracts

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Edible and medicinal mushrooms are rich in non-digestible polysaccharides, dietary fiber, vitamins (B1, B2, B6 and B12), calcium, phosphorus, iron and fat. Many studies have shown that edible and medicinal mushrooms have antioxidant and anti-tumor activities. Furthermore, they also can inhibit the increase of serum and liver cholesterol, promote blood circulation, prevent atherosclerosis and decrease blood pressure. However, some mushrooms still have less data regarding their active compound and activities. In this study, five mushrooms (*White Grifola frondosa*, *Agaricus blazei* Murill, *Pleurotus eryngii*, *Hericium erinaceus*, *Flammulina velutipes*) were investigated for their anti-inflammatory properties. Cell viability test results showed: White *G. frondosa* and *A. blazei* Murill extracts for mouse macrophages cell (RAW264.7) have cytotoxic, but *P. eryngii*、*H. erinaceus* and *F. velutipes* extracts not. Based on this result, choose *P. eryngii* and *F. velutipes* determine β -glucan and polysaccharide contents. The results showed that the polysaccharide contents in cold water extraction of *P. eryngii* had the highest value (294mg/g) > *F. velutipes* (matrix) (217mg/g) > *F. velutipes* (edible part) (189mg/g). In addition, hot water extraction of each sample presented the results of polysaccharide contents in the following orders: *F. velutipes* (edible part) (300mg/g) > *F. velutipes* (matrix) (244mg/g) > *P. eryngii* (142mg/g). The β -glucan contents in cold water extraction samples were as followings: *P. eryngii* (12.4%) > *F. velutipes* (matrix) (8.96%) > *F. velutipes* (edible part) (7.95%); and in hot water extraction samples: *P. eryngii* showed the highest among (22.1%) > *F. velutipes* (matrix) (18.96%) > *F. velutipes* (edible part) (17.97%). In summary, the results indicated that *Pleurotus eryngii* presented the best performance in five edible and medicinal mushroom extracts.

Keywords: Edible and medicinal mushroom, Polysaccharides, β -glucan, RAW264.7

利用田口方法設計用於舒壓的複方精油化學活性最佳化研究
Study of optimization chemical activity for Stress Relief type
Complex Essential Oil using Taguchi Method

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現代社會工作相對繁複，許多人精神受到極大的壓力，舒緩壓力是一重要議題，為尋求舒緩壓力，植物精油是一良好選擇。經過文獻調查得知在於芳香療法中常用於舒壓的精油種類有完全依蘭、穗甘松、真正薰衣草、佛手柑等，但是其複方比例與成效並無定見與證實。精油功效與其成分性質有極大的關係，所以我們先將複方精油以氣相色層分析-質譜儀進行成分分析，接著利用田口方法以四因子三水準L9直交表，進行化學活性的分析並找出最佳化因子與水準，化學活性分析項目包括自由基清除率與總酚含量測定。

Keywords: 田口方法、舒壓、複方精油、GC-MS、清除 DPPH 自由基、總酚含量

利用田口方法設計用於專注力的複方精油化學活性最佳化研究
Study of optimization chemical activity for Attention type Complex
Essential Oil using Taguchi Method

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外在環境、疲勞、睡太少、壓力、情緒焦慮等皆會影響工作時的專注力，專注力不佳會使學習或工作效率下降，如何使專注力提高是許多學童家長與工作者關心的議題。植物精油是植物的重要成份，運用植物所提出來的天然精油，透過皮膚吸收或從鼻子吸入進入人體，可以改善情緒並平撫壓力。為了使人們的專注力提高，使用精油來提升專注力是一種良好無副作用的選擇。為了解天然的複方精油在提升專注力的研究，我們利用魚骨圖因子分析與田口方法，以多因子實驗設計來找出複方精油最佳化的重要因子。精油成分與嗅覺受體連結後影響神經與腦部作用，所以精油的化學成分是重要因子。因此本研究由複方精油的清除DPPH自由基能力、總酚含量測定與成分分析為主。

由文獻可知常用於提升專注力的植物精油有快樂鼠尾草、廣藿香、胡椒薄荷、迷迭香等，所以我們選用四因子三水準L9直交表，利用回應圖與回應表找出此一複方精油影響化學活性的最佳化因子。

Keywords: 田口方法、專注力、複方精油、GC-MS、清除 DPPH 自由基能力、總酚含量

探討白舞菇液態深層培養之最佳條件及其多醣之抗氧化功效
**The studies for the optimal submerged culture conditions of
white *Grifola frondosa* and the antioxidant effects of its
Polysaccharides**

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Grifola frondosa is an edible and medicinal fungus with balanced nutrition. Its fruiting body is rich in minerals and trace metals. Many studies have reported that this fungus has many pharmacological functions such as antioxidant and anticancer. In this study, the optimal submerged culture conditions and the antioxidant capacity of its polysaccharides produced by white *Grifola frondosa* was evaluated. The submerged culture in shaking flasks was carried out under different number of days, pH, and carbon and nitrogen sources. Furthermore, phenol sulfuric acid method, reducing sugar and antioxidant experiments including scavenging DDPH free radical and scavenging ferrous ion chelating were performed. The best condition to obtain exopolysaccharide (EPS) of white *Grifola frondosa* from PDB culture was 7 days incubation under pH 6. The best carbon source was glucose; meanwhile the best nitrogen source was corn steep powder, followed by yeast extract. The best mixed source was corn steep powder mixed with PDB. Based on the results of antioxidant analysis, EPS produced by white *Grifola frondosa* from different culture conditions would have different antioxidant capacity. In summary, these results suggest that the EPS of white *Grifola frondosa* could be a suitable natural antioxidant for humans.

Keywords: *Grifola frondosa*, Submerged culture, Polysaccharide, Antioxidant

乳酸菌發酵萃取物之美白功效分析

The analysis of whitening effects of *Lactobacillus* extracts賴柏儒 Bo-Ru Lai^{1,#}、施養佳 Yang-Chia Shih^{1,*}¹Department of Biotechnology, Asia University, Wufeng, Taichung, Taiwan, R.O.C.

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Lactobacillus is one kind of probiotics and it can metabolize carbohydrate to produce over 50% of lactose. *Lactobacillus* is currently often applied in the food industry, feed additive and medical industries. However, only a few reports focused on skin care research field such as whitening effects. In this study, three strains of *Lactobacillus* fermented extracts were used to explore their inhibitory effects of tyrosinase activity. According to the results, all of our samples at the concentrations of 8mg/ml presented inhibition rate up to 80%, while the concentrations of 10mg/ml showed almost 100% inhibition effects. In addition, the results showed that *Lactobacillus* was able to be used as tyrosinase inhibitors. Therefore, NIH3T3 cells and B16F10 cell lines were examined to conduct viability tests and to explore the possibility of *Lactobacillus* applied in the Melanoma cell lines. The results indicated that two strains of cell lines after cultured with *Lactobacillus* fermented extracts presented non-toxic effects, and they even could increase the proliferations of NIH3T3 cells. In the future, we will further examine the mechanism of melanin reduction by *Lactobacillus* fermented extracts in B16-F10 cells to prove the *Lactobacillus* is a potential whitening material for the applications of skin care products.

Keywords: *Lactobacillus*, Tyrosinase, Whitening effects, B16-F10

添加不同基質牛樟芝菌絲體生長條件之探討
Effect of Supplying in Substrate on Mycelial Growth of *Antrodia cinnamomea*

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牛樟芝(*Antrodia cinnamomea*)又名牛樟菇、窟內菰、樟菇，臺灣特有是一種藥用真菌，野生牛樟芝生長在腐朽的牛樟木材內壁，牛樟芝在生物學分類為真菌界、擔子菌門、擔子菌亞門、多孔菌科、薄孔菌屬、樟芝種。本研究使用台灣特有的真菌牛樟芝，透過不同栽培方法對牛樟芝菌絲生長影響，探討利於牛樟芝菌絲生長最佳條件，使用人工栽培方式加速取得牛樟芝菌絲體，也可做為栽培菌種，另外添加不同穀物使得木屑栽培瓶菌絲體生長快速，替代牛樟木，減少生產成本也減少牛樟木的消耗，本實驗使用馬鈴薯洋菜培養基添加不同比例的牛樟木屑萃取液比較麥芽萃取物洋菜培養基、酵母葡萄糖洋菜培養基、酵母麥芽萃取物培養基的牛樟芝菌絲體生長，結果顯示馬鈴薯洋菜培養基添加 100%的牛樟木屑萃取液菌絲生長為最快，固態栽培的結果使用液態接種第 15 天觀察，牛樟芝菌絲體在糙米及薏仁基質菌絲有變色趨勢，在第 20 天觀察除了蕎麥基質的牛樟菌絲沒轉色，其餘糙米、薏仁、牛樟木皆有轉色，玻璃瓶木屑栽培部分，液態接種在第 42 天觀察，添加 75%的蕎麥基質牛樟芝菌絲生長為最佳，菌絲轉色且最厚。

關鍵字:牛樟木屑、牛樟芝、菌絲體、基質、馬鈴薯洋菜培養基。

舞菇功能性成份活性分析 *Grifola frondosa* Activity of functional ingredients

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舞菇(*Grifola frondosa*)，又稱灰樹花、舞茸，台灣又稱蓮花菌，是一種珍稀藥用真菌，文獻研究已證實其子實體及菌絲體均附有營養成分及生物活性也可以增加人體免疫力，提高對抗疾病的防禦能力具有抗氧化的成分。

本研究主要分析舞菇T2所含之生物活性成分，以95% Ethanol、99.8% Ethyl Acetate 分別進行超音波萃取乾燥粉末樣品。測試不同萃取層粗萃物之總多酚、總黃酮、DDPH 自由機清除能力、亞鐵離子螯合能力與利用細胞學實驗測試其活性有效成份。95% Ethanol萃取層中總多酚和DDPH 自由機清除能力活性有效成份比99.8% Ethyl Acetate萃取層多，99.8% Ethyl Acetate萃取層中總黃酮和亞鐵離子螯合能力活性有效成份比95% Ethanol萃取層多。99.8% Ethyl Acetate萃取層對Tyrosinase表現有增加的效果95%Ethanol之萃取層對Tyrosinase表現無顯著的效果，99.8% Ethyl Acetate萃取層和95%Ethanol之萃取層皆對MITF有抑制效果，但由實驗結果MITF和Tyrosinase兩者之間並非成正比之關係，是否有其他的機制路徑會影響Tyrosinase的表達量，將會是未來實驗展望之方向。

舞菇萃取物和純化物對酪胺酸 酶活性抑制研究
Inhibition of Tyrosinase Activity by Extract of *Grifola frondosa*

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本研究針對舞菇所含的功能性成分分析，其中以 95%EtOH 和 99.8%Ethyl Acetate 進行超音波萃取乾燥粉末樣品，檢測其總多酚，總黃酮的含量還有酪胺酸酶抑制黑色素活性的試驗，使用舞菇粗萃物和舞菇功能性成分的純化物當作抑制劑，本次抑制黑色素實驗中，算得 T2 粗萃物的 IC₅₀ 約為 60mg/ml，Gallic acid 的 IC₅₀ 約為 14mM，Syringaldehyde 的 IC₅₀ 約為 18mM，Syringic acid 的 IC₅₀ 約為 8.5mM，Hydroxybenzaldehyde 的 IC₅₀ 約為 9mM 皆可達到有效抑制的效果。另一方面，使用雙倒數圖與最高反應速率 V_{max} 做判斷，可得知 T2 粗萃物和 Gallic acid 屬於混合型抑制，Syringaldehyde 和 Syringic acid 屬於競爭型抑制 Hydroxybenzaldehyde 則屬於非競爭型抑制。

Keywords: 舞菇、Melanin、Tyrosinase、L-Dopamine

利用低溫萃取綬草中阿魏酸並探討其抗氧化及抑制酪胺酸 酶活性

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綬草 (*Spiranthes sinensis* (Pers.) Ames.) 為綬草屬之蘭科植物，其性甘、苦、平，有益氣養陰、清熱解毒的功效，已有多多年食用歷史，近年研究指出綬草中含有二氫菲類 (Phenanthrenes)、黃酮類化合物 (Flavones) 及阿魏酸 (Ferulic acid) 等物質，不同植物來源的黃酮類化合物及阿魏酸已被許多學者研究證實具有抗氧化、抗菌、抗發炎及抑制酪胺酸 酶活性等功效，但尚無文獻針對綬草中的黃酮類化合物及阿魏酸進行研究，為評估綬草萃取物是否具有開發成為化妝品原料的價值，因此本研究將建立最適化綬草萃取條件，並利用抗氧化試驗、美白試驗與抑菌試驗模組將綬草萃取物與已被證實具有抗氧化及美白功效的麥門冬萃取物做比較，以評估綬草萃取物的功效性，實驗結果顯示綬草的最佳化萃取條件為使用 75 % 乙醇搭配超音波輔助萃取，萃取出之總黃酮化合物含量達 4.28 mg/g、阿魏酸含量達 4.13 mg/g、萃取率為 11 %，此外當綬草萃取物濃度為 1,200 ppm 時具有清除 DPPH 自由基能力達到 95 %，而麥門冬萃取物僅有 15.5%，相較於綬草萃取物其清除超氧陰離子自由基能力為麥門冬萃取物的 1.23 倍，體外抑制酪胺酸 酶能力綬草萃取物抑制酪胺酸 酶活性是麥門冬萃取物的 10.4 倍，並且能達到麴酸抑制酪胺酸 酶活性的 30% 以上，由以上數據皆證明綬草較麥門冬更具有開發成化妝品原料的潛力。

關鍵字：綬草、阿魏酸、黃酮類、抗氧化、酪胺酸 酶

添加不同甘油濃度對茭白筍殼可食性膜物理性質之影響
**Effects of Glycerol Concentration on Physical Properties of Edible
Films Made from *Zizania Latifolia* Turcz Shell**

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There are abundant Cobs husk wastes per year in Taiwan. However in present mostly its applicability is limited to act as fertilizer or be discarded directly. We decide to utilize the Cobs husk to get the extract to develop the edible film product. In this experiment, we used Cobs husk as raw materials to made the edible film, then we used different concentrations (0.01, 0.03, 0.05, 0.07 and 0.09%) of glycerol as additive. The results showed that the concentration of glycerol is 0.05%, vapor permeation rate and elongation ratio had highest value. However, sensory evaluation of 0.01% glycerol film had higher scores on flavor, viscosity, and overall item.

Keywords: *Zizania Latifolia* Turcz, Glycerol, Edible Films,

利用木黴菌進行鳳梨皮纖維素水解條件最佳化之研究
The research of optimization the hydrolysis conditions of pineapple waste by using *Trichoderma reesei*.

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The average cultivation area of pineapple is 9,939 hectare per years in Taiwan. Estimate each hectare can produce about 22 ton pineapple waste. Therefore pineapple waste production is around 220,758 ton per year. The traditional treatment for those pineapple wastes is buried. However buried is easily to cause air pollution and increase fly population. Therefore use pineapple waste as materials to produce bioethanol,

to solve the increasingly serious energy problems and reuse agricultural waste is emergency. This experiment intends to use *Trichoderma reesei* to hydrolysis pineapple waste and product reducing sugar. The treatments include using wet grinding machine to treatment pineapple waste. Using anthrone sulfuric acid method to analysis total sugar content in pineapple wastes before and after treat with *Trichoderma reesei* . The total sugar and water soluble sugars are 7.92g/L and 8.17g/L glucose content. The best condition in reducing sugar production by using *Trichoderma reesei* to hydrolysis pineapple waste is pH5,72h and 300rpm. The reducing sugar is 25.04g/L and hydrolytic conversion efficiency is 15%.

Keywords: pineapple waste, *Trichoderma reesei*, reducing sugar ,hydrolysis condition

不同製備條件對龍鬚菜可食性薄膜理化性質之影響
**Effect of Different Processing Condition on the Physicochemical
Properties of Gracilaria. tenuistipitata edible film**

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In this study, we used Gracilaria. tenuistipitata to made edible film with different extraction time (1, 2 and 3 hours) and drying temperature (40, 50 and 60°C). at mean time we evaluated analysis the physicochemical properties as the future production important reference of Gracilaria. tenuistipitata edible film. The results showed that the extraction of one hour, drying temperature 40°C was the best conditions , their water vapor permeability was 0.41 WVP, Swelling percentage was 527.5%, tensile strength of 4.33 Mpa, penetration force was 1244 gf all had highest value.

Keywords: Gracilaria. tenuistipitata, edible film, Physicochemical Properties

Solid oil block of microalgae oil made into the bio-diesel

微藻油之固態油塊製成生質柴油之研究

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摘要:

微藻(Microalgae)因富含藻油在充分光合作用下產油率可高達百分之 50%以上，又因其中富含六個雙鍵的多元不飽和脂肪酸之二十二碳六烯酸(C₂₂H₃₂O₂) (Docosahexaenoic Acid, DHA)，在商業上常用微藻油提煉出 DHA。經醫學證明 DHA 為大腦及視網膜中含量最高的 ω-3 脂肪酸，佔大腦中多元不飽和脂肪酸的 40%，視網膜中多元不飽和脂肪酸的 60%，神經元的細胞膜中有 50%為 DHA。此外，DHA 能阻止血小板在血管壁上的沉積，預防或減輕動脈粥樣硬化和冠心病的發生。本研究最大特點是使用 *Aurantiochytrium* sp 微藻提煉精製藻油，做為保健產品或健康食品添加，其中所產生之固態副產品(占 20-25%)-藻油塊，這種藻油塊通常都被當作廢棄物或作為低價的動物飼料添加物。但本研究發現油塊中富含大量的棕櫚酸(Palmitic acid)高達百分之 67%以上，常溫下會形成固態。目前，在台灣生質柴油的來源，通常是生質柴油業主與速食業者結盟，回收廢食品炸油，但因廢油成分複雜，所製成的生質柴油也常遭外界質疑品質。然而，本研究所使用的藻油塊也同樣面臨相同的問題，既然稱為廢藻油塊，內含的雜質、游離性脂肪酸及微藻細胞壁會干擾轉酯化反應，是本研究的最大的困難點。因此，本研究針對固態微藻油塊發展出有別於目前商業常用程序，2 步驟轉酯化反應。本研究以獨特簡易 3 步驟，不需特殊昂貴裝置或昂貴催化劑，顛覆一般生產生質柴油的迷思，以製程設備簡單，生產成本低，又能兼顧整體產率的技術。所進行的轉酯化反應，第一步驟先進行皂化反應，固定脂肪酸組成，將雜質、甘油及游離性脂肪酸汰除，第二步驟進行還原反應，將需要的脂肪酸保存，最後一步驟是酸催化反應，讓十六，十八(烷)酸充分甲基酯化。整體而言，本研究能針對固態藻油塊發展出專門的生質柴油模式，成功的製成生質柴油。固態微藻油塊有別於世界各國或許多專家學者研究的生質能源，是將既有人類糧食及動物飼料拿來作生質能源，而本研究能針對微藻油之廢棄物，固態廢藻油塊轉化成優質生質能源，將廢棄物變成黃金的最佳呈現，也冀望本研究能拋磚引玉，緩和台灣面對世界能源危機之衝擊。

關鍵字: 微藻、棕櫚酸、固態微藻油塊、轉酯化反應、生質柴油

不同萃取條件對百香果果皮果膠質理化性質之影響
**Effect of extraction conditions on the quality characteristics of pectin
from passion fruit peel**

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Passion fruit is widely cultivated in Taitung and Nantou County in Taiwan. But there are abundant of wastes from the passion fruit peel per year, so we decide to utilize the passion fruit peel to separate the pectin. Pectin had very high viscosity, and it can be used as stabilizers and thickeners. In this study, we extracted pectin respectively from different pH value (pH1.5 and pH2.0) and different temperature (55°C, 65°C and 75°C). The results showed that the extraction ratio of pectin from passion fruit peel at 55°C, pH 1.5 had the highest value. When pectin extracted at 65°C had the highest viscosity. In the color analysis, pectin extracted under pH 1.5 had the highest a value and pectin extracted under pH2.0 had the highest b value. There were significant differences on spread ability and overall items of sensory evaluation of jam made from pectin.

Keywords: passion fruit, pectin, extraction

有效快速的方法避免熱反應性物質的熱失控反應
A swift and effective method for preventing thermal runaway
reaction accident of thermal reactive material

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Thermally reactive materials have caused many serious accidents involving storage and transportation, due to being thermally reactive. The safe storage and management of these materials still is a critical problem in many countries. We studied the thermal hazard of thermal reactive materials, such as a propellant, by employing differential scanning calorimetry (DSC) non-isothermal tests and isothermal tests, and then comparing the kinetic parameters by isothermal and non-isothermal of kinetics, avoiding the mistaken results of the single thermal analysis model. The chosen approach was to obtain reliable kinetics of thermal decomposition by safety and effective method, which acquired the safety parameters of storage condition that could be applied as thermal reactive materials' safer design during storage safety conditions and relevant operations.

Keywords: thermally reactive material, storage and transportation, kinetic parameter, safety and effective method, safer design

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銀耳多醣的抗氧化活性及其應用於保健飲品之開發
**Antioxidant activities of *Tremella fuciformis* polysaccharide
and its application to develop healthy drink**

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Tremella fuciformis has been recognized as a natural edible mushroom commercially available in Taiwan and Asian countries. Its polysaccharide is known to possess various physiological activities such as endothelial cell protection, hypoglycemic activity, anti-tumor, anti-radiation and enhancing immunity. This study was conducted to investigate the antioxidant activities of *Tremella* polysaccharide (TP) and to applicate it in the formulation of Tremella drink. Mixture design technique was performed to formulate the optimum proportion ingredients of Tremella drink. The main ingredients of drink were TP, mulberry and lemon extracts. In the beginning, TP was extracted from its fruiting body using hot water at temperature (75°C), time (5 h) and ratio of water to dried raw material (6:1). The extract was evaluated for antioxidant activities, dietary fiber and beta glucan contents. TP exhibited excellent chelating ability to ferrous ion (93%) and moderate scavenging activity to 1,1-diphenyl-2-picrylhydrazyl radicals (33%). Its flavonoid and total phenolic contents were 2.19 and 1.07 mg/g, respectively. TP also contained high dietary fiber and beta glucan. Furthermore, the formulations of drinks (8 formulas) were prepared according to the mixture design. The proportions of main ingredients were different for each formula. The results of antioxidant activities and sensory evaluation (color, taste and flavor) of formulas were then applied to the mathematical models. The models indicated that the optimum formula prepared using multiple ingredients has higher antioxidant activities and has higher preferences by panelists.

Keywords: *Tremella fuciformis*, polysaccharide, antioxidant, healthy drink

咖啡豆烘焙條件與感官品評及熱分解特性的關聯性
Coffee bean roasting conditions related to sensory evaluation and thermal decomposition characteristics

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This study concerns coffee bean roasting conditions, as they relate to sensory evaluation and thermal decomposition characteristics. The roasting process swells the beans and changes their color, taste, smell, and density, resulting in a special flavor of coffee. Most coffee beans are large-scale commercially roasted, but some coffee drinkers prefer to precisely control the freshness and the special flavor by their own roasting method. This study was conducted to develop a novel approach to thermal decomposition that includes the heat reactivity properties of coffee beans, such as the kinetics of reaction, pre-exponential factor ($\ln k_0$), reaction order (n), activation energy (E_a), heat of decomposition (H_d), isothermal time to maximum rate (TMR_{iso}) and total energy release (TER). The parameters and reactivity properties could be applied to design during roasting and storage conditions. Differential scanning calorimetric (DSC) experimental data were processed and then the kinetics was evaluated by simulation. Overall, we found the best coffee beans through various roasting conditions, along with relevant taste and pyrolysis characteristics in this study.

Keywords: coffee bean, roasting condition, sensory evaluation, simulation, pyrolysis

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白舞菇萃取物延緩老化之功效探討

The studies for the anti-aging activities of white *Grifola frondosa* extract

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Grifola frondosa contains bioactive compounds including polysaccharide, protein and antioxidant compounds. This study aimed to investigate the antioxidant activities and delay-aging effects of white *Grifola frondosa*. In this study, white *Grifola frondosa* was extracted by three kinds of methods: cold water (4°C), hot water and ethanol extractions. The results showed that the extracts from three kind of extractions contained total polyphenol 2.25 - 4.95 mg/g and flavonoid 0.28-2.14 mg/g. The antioxidant activities of extracts were measured by DPPH scavenging activity, ferrous chelating ability and reducing power methods. The ethanol extracts of white *Grifola frondosa* showed higher DPPH scavenging activity (96.81%) compared to cold water and hot water extracts; 47.7% and 58.11%, respectively. Whereas cold water extracts exhibited higher ferrous chelating ability (98.85%) compared to hot water (95.6%) and ethanol extracts (78.01%). The value of reducing power indicated that cold water extract (2.28) was stronger than hot water (2.17) and ethanol extracts (1.98). In addition, delay-aging assays showed that nitric oxide contents in hot water extract (1.67 mg/ml) were higher than cold water (0.89 mg/ml). Moreover, the results of hydrogen peroxide scavenging ability presented that cold water extract (85.31%) was higher than hot water (85.31%). MTT assay exhibited that both cold and hot water extracts of white *Grifola frondosa* were not toxic to NIH3T3 cells and furthermore they promoted the cell proliferations up to 100% by using 1 µg/ml concentration and incubation for 48 h. In summary, the above experimental results indicated that the extracts of white *Grifola frondosa* were able to promote fibroblast cells growth, anti-aging and antioxidant. The extracts of white *Grifola frondosa* have highly potential to develop the functional health food products in the future.

Keywords: *Grifola frondosa*, Antioxidant, Delay-aging

智慧技術用於估算滅火藥劑對液態過氧化物的抑制效能
Smart technology for evaluating fire extinguishing effect of
liquid peroxide

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Tert-butyl hydroperoxide (TBHP) 70 mass%, a solution of liquid peroxide, has been widely employed in the chemical industry as a polymerization initiator. We investigated the smart technology for predicting the mechanism of thermal decomposition and the inhibitive or hazardous reaction of TBHP by different calorimetric tests: using differential scanning calorimetry (DSC) non-isothermal tests versus DSC isothermal tests and vent sizing package 2 (VSP2) adiabatic tests versus DSC non-isothermal tests, respectively, for further understanding how to extinguish organic peroxide accidents under fire scenario or runaway reaction in a chemical plant. Meanwhile, we determined the thermal decomposition characteristics of TBHP mixed with inhibitive and hazardous materials, such as various protic acids to help prevent runaway reactions, fires or explosions in the fire system, process environment or storage condition in earlier stage of loss prevention. The results could be available to fire-related agencies as a reference application. The thermal hazard analysis method can also be used to assess and calculate the inhibitive and hazardous reaction for organic peroxides mixed with incompatible materials. The fire extinguishing system must be well designed for decreasing the degree of hazard. Disasters can be prevented in the first stage, and the results of this study are expected to aid process and storage safety for preventing an accident from occurring.

Keywords: liquid peroxide; polymerization; smart technology; inhibitive; adiabatic

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食用龜板膠及鹿角膠食品對人體經絡體質改變的影響

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摘 要

龜板膠及鹿角膠食品由金吳記企業有限公司提供，為符合標準製作規範之 GMP 食品。每塊膠片淨重二錢半，成分含龜板膠、鹿角膠、人參、枸杞。本研究測量 40-50 歲（含男性 2 位、女性 8 位）的自願食用民眾。受測者每天早上食用 1 塊膠片，連續 5 天，每周停用 2 天，同時使用良導絡儀(安拓儀器 Co.)做身體經絡能量變化的測量。每周檢測二次受測者之身體十二經絡數值，試驗為期 4 周。由十二經絡測試結果發現，受測者在食用第三周時，明顯呈現上下焦氣血平衡、左右骨骼肌運作平衡、陰陽平衡機體代謝趨於正常、且體能狀況增強之現象，尤其十二經之不論虛實皆能在第三週趨向正常範圍。

龜板膠及鹿角膠食品為中國自古養生長壽之品，龜板為龜科動物的腹甲，可滋陰潛陽，益腎健骨、養血補心，鹿角膠為馬鹿或梅花鹿已骨化的角，可溫補肝腎，養精養血。將龜板及鹿角用水熬煮後，濃縮成固體膠塊可製成膠片。二者合服，一補陰、一補陽，對人體臟腑虛實可起自然調合作用。由上述結果認為，龜板膠及鹿角膠食品對人體健康為良好有益的補充性食品。

關鍵字：龜板膠、鹿角膠、十二經絡、陰陽、良導絡儀

Dwarf-castor oil made into the bio-diesel 矮種蓖麻籽油製成生質柴油之研究

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摘要:

本研究以矮種蓖麻油(dwarf-castor oil)製成生質柴油為研究主題。作為生質柴油第一要件，要不佔糧食耕種地或佔用陸耕地，第二要件，要能大規模產油及量產成生質柴油，而本研究之矮種蓖麻就是符合此二要件。種植蓖麻樹不會佔用可耕農地，且容易管理，因此可大量栽培，用於生產生質柴油。除此之外，矮種蓖麻尚能有效地改善空氣品質，及抑制地球暖化。本研究之矮種蓖麻油一年可三收，耐旱，抗病蟲害，少人工照護，因矮種可機械採收，是全世界目前為止，唯一可以機械採收的陸生植物生質油，種植成本低，一公頃成本約新台幣 10 萬元。本研究以獨特簡易 3 步驟，以製程設備簡單，生產成本低，又能兼顧整體產率的技術。所進行的轉酯化反應，首先進行皂化反應，將主體之蓖麻子油酸(12-hydroxy-9-octadecenoic acid)固定，用以提升轉酯化反應的產率，並將雜質、甘油及游離性脂肪酸剔除，接續進行還原反應，將轉化為生質柴油之脂肪酸保存，最後進行酸催化反應，讓蓖麻子油酸(Ricinoleic acid)充分與甲烷基酯化。整體而言，本研究能針對蓖麻籽油發展出專門的生產生質柴油模式，成功的製成生質柴油。未來，若可全面推廣休耕地種植矮種蓖麻，不但可解決農地休耕的問題，並可增加能源自產自給率及擴大就業，對於能源幾乎完全依靠進口的我國更是提供莫大的助益。

關鍵字: 矮種蓖麻油、陸生植物生質油、可機械採收、轉酯化反應、生質柴油

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冰滴咖啡以田口方法進行感官品評最佳化之研究 Study on Taguchi Method for Sensory Evaluation Optimization of Ice-dripping Coffee

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台灣近來飲用咖啡的習慣日漸風行，且一年中多處於亞熱帶氣候，也因此造就冰咖啡等相關飲品的市場需求。咖啡內含的咖啡因含量是影響人們選購冰咖啡的因素，但咖啡的香氣、顏色、味道也占了一定影響的因素。所以本研究由冰滴咖啡著手，目的在以不同製備方法由低溫咖啡利用感官品評法探討不同製備方法、泡製時間、咖啡濃度、咖啡豆種類、泡製溫度等對於咖啡整體風味的影響，希望能在找到將冰滴咖啡的咖啡因降低的同時，亦能顧及咖啡的風味，成為合適消費者飲用的良好咖啡。

感官品評是以「人」為工具，憑藉眼耳鼻口及雙手觸感等五種感覺系統，利用科學客觀的方法，結合心理與生理、物理化學和統計學等，對食品進行分析測量，藉由了解人類對這些產品的感受或喜歡程度從而測知產品本身品質的特性。

本研究的問卷設計採用 Likert 七點尺度量表方式評量，以 50 位經簡單訓練的大學學生作為品評員，受訪者回答選項從「非常不喜歡」到「非常喜歡」，分別依序給予 1 到 7 分數值代表，寫下對該樣品之直接飲用測試色澤、香氣、焦香味、酸味、苦味、整體風味，一共六個項目感官特性的接受程度，此量表中得分愈高者表示對此產品喜愛程度愈高。利用田口方法的四因子三水準 L9 表為基礎，其影響整體風味的因子為：咖啡豆類別 A3 (席拉多)、咖啡濃度 B2 (1:11)、浸泡時間 C3 (3 小時)、溫度為 D3 (12°C)，而且咖啡豆類別為主要影響因子。

關鍵字：冰咖啡、感官品評、Likert 七點尺度量表、整體風味

佳葉龍茶、名間烏龍茶和華剛烏龍茶抗氧化能力評估

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茶葉含有豐富的茶多酚，具有良好的消除自由基和抗氧化能力，本研究分別針對佳葉龍茶、名間烏龍茶和華剛烏龍茶進行抗氧化活性比較。茶葉萃取物之抗氧化能力評估則採用 DPPH 自由基清除試驗以及總酚類含量測定，在沖泡溫度 (90°C 和 30°C 萃取茶湯) 對 DPPH 自由基清除能力影響試驗，結果顯示 90°C 萃取茶湯自由基清除能力比 30°C 萃取茶湯好，其中名間烏龍效果最佳，而在沖泡時間對自由基清除力影響試驗中，發現時間長者自由基清除能力較佳，但總多酚含量測定試驗結果顯示沖泡時間對總多酚含量並無明顯差異。

關鍵字: 茶、抗氧化、DPPH 自由基清除試驗、總多酚含量

酸處理對虱目魚魚鱗水解液抗氧化能力及鈣結合力之影響
**The Antioxidant Activities and Calcium Binding Capacity of Milkfish
Scale Hydrolysate Extracted by Acid**

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In this study we used milkfish scale as raw material and hydrolysed by acid to develop liquid seasoning. We evaluated the antioxidant abilities and calcium binding capacity between different acid treated groups. The results showed that the proximate composition of the dry milk fish scale has the higher protein content (56.84%) and ash content (41.03%). The hydrochloric acid treated for 3 hours not only had the highest in the DPPH scavenging ability but also the strongest reducing power. Otherwise the ferrous ion chelating ability in acetic acid treated group was the strongest. The calcium binding capacity showed that the treated groups at different times have no significant, but the hydrochloric acid treated group had higher measured value (15 ppm).

Keywords: Milkfish Scale, Antioxidant Activities, Calcium Binding Capacity

霧峰香米取代對硬質餅乾品質之影響 Effect of TNG 71 Rice on Hard Biscuit Quality

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本研究以霧峰香米的白米及糙米為原料，嘗試開發低麩質的特色餅乾，並探討白米及糙米的添加對產品理化性質及感官評估的影響。研究結果顯示水含量以 75% 白米餅水分含量最高為 3.91%，在 50% 糙米餅乾水分含量最低值為 2.11%。在水活性方面以 75% 糙米餅乾最高，其測定值為 0.34，最小為 50% 白米餅乾，其測定值為 0.21。灰分方面皆以糙米餅乾有較高的含量，尤其以 100% 糙米餅乾測定值最高，其值為 1.59%。不論白米或糙米的餅乾，烘焙損失率、體積、比體積、硬度及脆度都因米粉的添加測定值有明顯的下降；在溶水性指標方面，以白米及糙米取代麵粉有助於溶水性的提升，尤其以糙米製作而成的餅乾有較高的溶水性其數值介於 13-16% 之間。在感官評估方面，香味、風味及總評皆以白米及糙米取代量 25% 得分最高，得分為分別為 5.5 分、5.93 分及 6.00 分。

Keywords: 香米、餅乾、理化性質、感官評估

霧峰香米取代對麵包品質之影響 Effect of TNG 71 Rice on Bread Quality

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本實驗以霧峰特產的香米及糙米為原料，嘗試開發低麩質的麵包，精白米米粉及糙米米粉取代麵粉的比例分別為 25、50、75 及 100%，同時藉由麵包產品的理化性質分析及感官評估來了解米粉取代對麵包品質的影響。結果顯示在水含量方面糙米 25% 取代麵包有最大值，數值為 28.22%，而在白米 100% 時有最小值，數值為 15.94%。在 L 值方面白米 100% 取代組分析值最高為 72.64，而空白組時有最小值為 49.61。在 a 值方面糙米 25% 有最大值為 16.59，而白米 100% 時有最小值，數值為 1.50。在 b 值方面白米 75% 有最大值，數值為 33.35，而白米 100% 時有最小值，數值為 23.83。在 WI 值方面空白組有最大值，數值為 60.90，而白米 100% 時有最小值，數值為 36.34。在麵包體積方面空白組有最大值，數值為 172g/ml，而白米 100% 時有最小值，數值為 48g/ml。在麵包硬度分析則顯示最大值為白米 100% 的 2128gf，而最小值為空白組的 576gf。灰分以糙米 100% 最多分析值為 1.48%，而白米 25% 的 0.65% 最小。而溶水性指標分析空白組最大為 4.29，而糙米 75% 最小為 2.73。在吸水性指標，白米 75% 最大為 6.58，而空白組最小為 4.41。感官評估方面，大致而言空白組、白米及糙米 25% 取代組在外觀、顏色、風味、口感及總評方面都有較高的得分。

Keywords: 香米、麵包、理化性質、感官評估

中國菜食譜配方之研究 Research of Chinese Recipes

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本實驗以市售及網路食譜建立中式食譜資料庫，探討國人飲食中主原料、副原料及調味料的使用頻率及組合搭配方式，由統計數字得知其主原料之肉類使用頻率依序為豬肉、雞肉、牛肉、甲殼類、魚類、貝類、頭足及軟體類、羊肉、鴨肉；而副原料的使用頻率依序為蔥、蒜、薑、辣椒、菇類、胡蘿蔔、蛋、番茄、香菜及青椒與甜椒…等，而調味料的使用頻率依序為鹽、糖、酒、醬油、香油、雞粉、白胡椒、黑胡椒、白醋及番茄醬…等。整體而言，在中國菜中最常使用之主食為豬肉，約占 27%，在副原料方面不論哪一種肉類都常使用蔥來進行料理，而調味料方面除搭配豬肉是以醬油排名第一外，其餘主原料皆以鹽為最常使用的調味料。

Keywords: 食譜、資料庫、中國菜

杏鮑菇素肉之研發 Development of Imitation Meat Using *Pleurotus eryngii*

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本研究以杏鮑菇為原料製作具獨特風味的素肉，並以素調理漿與素肉塊兩種市售素肉產品為對照組，探討各產品之理化性質並進行感官評估分析。結果顯示，在杏鮑菇素肉物性方面，硬度其值為 581.24 gf，杏鮑菇素肉之彈性值為 154.49 gf。在色澤方面，L、a 及 b 值分別為 56.58、3.92 及 19.52。杏鮑菇素肉之含水量測定值最低為 25.97%。水活性方面，以市售素肉塊與杏鮑菇素肉有較高的數值。杏鮑菇素肉之 pH 值為 6.82 與市售素調理漿和市售素肉塊有顯著性差異。感官評估之比較，除了市售素調理漿之外觀評分優於杏鮑菇素肉，在風味、口感與色澤方面，杏鮑菇素肉之得分與市售素調理漿在統計尚無顯著性差異，在總評方面杏鮑菇素肉與市售素調理漿皆優於市售素肉塊。

Keywords: 杏鮑菇、素肉、理化性質、感官評估

霧峰香米漢堡製作之研究 Development rice-burger using TNG 71 rice

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本實驗以霧峰香米的精白米為主要原料, 副原料為霧峰香米的糙米、黑糯糙米、台中私糯 2 號精白米及台稔糯 1 號精白米, 進而研發具有霧峰在地特色之香米漢堡。我們探討其最適加工流程, 同時分析米漢堡產品的理化性質、感官評估及影響產品因子之相關性。結果顯示在色澤分析方面, 搭配黑糯糙米之 C 組香米漢堡的 L 值為所有實驗組中最低, 而其 a 值為所有實驗組中最高。彈性與硬度數值以 C 組為最高, 水含量則並無顯著性差異, 水活性是搭配台中私糯 2 號精白米之 A 組為最高, 感官評估分數就統計上來看並無顯著性差異。A、B、C 三組米漢堡之相關性分析, A 組與 C 組的感官評估某些項目之間具有正相關性, 搭配台稔糯 1 號精白米之 B 組的硬度會影響品評員對感官評估的風味與餘味之評分; 從整體數據結果顯示品評員對三種米漢堡都有一定的接受度。

Keywords: 香米、漢堡、理化性質、感官評估

不同品種地瓜對地瓜圓理化性質之影響

Development Sweet Potato Cubes Using Different Varieties of Sweet Potato

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本研究將紅心、黃心及紫心等三種品種地瓜，分別製成地瓜圓點心食品，在 5°C 的冰箱中儲藏三天，並檢視地瓜圓儲存期間 pH 值、水含量、水活性、彈性、硬度及色差儀 L、a、b 值的變化，同時進行感官評估分析。結果顯示未經冷藏試驗的地瓜圓較為品評員所喜愛，其中又以紫心地瓜所製成的地瓜圓得分最高，在七分制的品評分數中獲得 6.6 分的高分；冷藏過後紫心地瓜圓，彈性從 729gf 上升至 927gf，硬度從 315gf 上升至 424gf，而感官評估的分數從 6.6 分下降至 4.0 分，這顯示冷藏地瓜圓物性的改變，會顯著影響品評員的喜好程度。

Keywords: 地瓜、地瓜圓、理化性質、感官評估

不同的澱粉對芋圓品質之影響

Effect of the Quality of Taro Balls Mixed With Different Starches

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本實驗以大甲的新鮮芋頭為原料，分別添加甘藷澱粉、樹薯澱粉、馬鈴薯澱粉、小麥澱粉及玉米澱粉製作成芋圓，並探討產品水活性、pH 值、L、a、b 值及感官評估之差異，此外將芋圓在 5°C 進行冷藏儲存試驗同時觀察其理化性質之變化。整體而言不同澱粉的芋圓，隨著冷藏的天數增加，芋圓的硬度及彈性遞增，添加樹薯澱粉之芋圓硬度和彈性測定值最小分別為 427gf 及 177gf，而其在感官評估各評分項目中色澤、香氣、彈性、嚼勁、風味及總評都有較高的得分，在七分制的品評分數中分別獲得 6.20、5.70、6.10、6.20、5.90 及 6.00 的高分，所以樹薯澱粉在常使用的澱粉中是最適合添加到芋圓產品中的澱粉。

Keywords: 芋圓、澱粉、理化性質、感官評估

不同油脂對沙茶醬品質的影響

Effect of Different Oils on the Quality of Taiwanese Style Barbecue Sauce

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本研究使用葵花油、花生油、芥花油及大豆油 4 種油品及花生仁、扁魚、蝦米、芝麻粉、大蒜頭、辣椒粉、芥末粉、五香粉、沙薑粉、白砂糖、鹽、醬油等副原料，經由焙炒製成沙茶醬。我們以市售第一品牌沙茶醬為對照組，同時分析原料油脂、沙茶醬成品之理化性質及進行感官評估分析。沙茶醬過氧化價以市售沙茶醬最高為 8.14meq/kg，以花生油沙茶醬測定值最低為 3.95meq/kg；酸價方面以花生油沙茶醬最高為 2.09mg/g，市售沙茶醬最低為 1.36mg/g。沙茶醬黏度以市售產品最高為 59.16cp、大豆油最低為 47.48cp，L、a、b 值方面沙茶醬 L 值芥花油較低為 58.19，其它四種沙茶醬則沒有顯著性差異，a 值以市售沙茶醬較高為 9.16，而自製四種沙茶醬較低，b 值以市售沙茶醬最高為 39.20，芥花油產品最低為 11.12。以沙茶醬單獨進行感官評估分析時，四種自製沙茶醬與對照組在外觀、香氣、嚐味、口感及總評等各項目得分皆沒有顯著性差異；而以沙茶醬拌麵進行感官評估分析時，在外觀上以葵花油最高為 5.70 分、市售沙茶醬最低為 4.70 分，在嚐味上葵花油最高為 5.90 分，市售沙茶醬最低為 4.50 分，總評方面四種自製沙茶醬得分約在 5.50~5.80 分之間，明顯高於市售沙茶醬之 4.60 分。

Keywords: 沙茶醬、油脂、理化性質、感官評估

有色米水稻之育種-以富含花青素及非糯品種為例 Colored rice breeding: A case study for breeding rice cultivars rich in anthocyanins and non-glutinous varieties

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近年來因為花青素強大的抗氧化能力而受大量的關注。有色稻（紫米）主要是因為種皮富含豐富的花青素，主要由矢車菊花青素-3-葡萄糖苷 cyanidin-3-glucoside (C3G) 和芍藥花青素-3-葡萄糖苷 peonidin-3-glucoside (P3G)所組成。除了抗氧化能力，在於心血管疾病、老年併發症、肥胖、抗血脂、血糖和癌症，在文獻上也多有記載；水稻種子約 90%由澱粉構成，米粒澱粉中是由直鏈澱粉(amylose)和支鏈澱粉(amylopectin)所組成，直鏈澱粉含量會影響米飯食用品質。而直鏈澱粉含量（amylose content，AC）因品種不同而有很大差異，有糯米(0%AC) 與非糯米及又有低(10-20%AC)、中(20-25%AC)及高含量(>25%AC)。低直鏈性澱粉性質之米粒，蒸煮後口感軟Q，放冷不易變硬是為國人所喜愛例如台農六十七號、台梗九號。本研究依照傳統育種方法，使用譜系育種法(pedigree breeding method)及回交育種法(backcross breeding method)進行雜交已期望育出具有紅色種皮及非糯性之水稻。由於私梗組合在 F1 容易發生半不捨的情況，因而由 F2 開始做選拔；使用材料為臺農六十七號（具適應性廣且產量高等特性）作為親本與亞洲大學紫私糯（具有高質量之花青素）雜交育種，經由回交方法每回交一次便會增加 25% 輪迴親本性狀，加快選拔速度差異，以期望快速得到具有花青素特性且具有臺農六十七號優良性狀之後代。由於臺農六十七號與亞洲大學紫私糯私梗差異親緣較遠，會容易產生不稔實等缺點，故在同時也使用臺中私十號做為親本與亞大紫私糯做雜交育種；每一期田間調查植株根莖葉具有紅色性狀，收穫考種挑選種皮為紅色，且為非糯性之種子繁殖；在自交六代後，人工選拔選出具有紅色性狀之後代且非糯性之後代。目前選拔出後代有台中私十號作為母本與亞洲大學紫私糯雜交後代-亞洲大學紫晶(已公布品種權公告)、台農六十七號作為父本與亞洲大學紫私糯雜交後代-亞洲大學紫圓。

關鍵字：育種、回交育種、

亞洲大學紫秈糯米米糠及其萃取物對麵條品質之影響
Development of noodles Products using Asia University selected
purple glutinous indica rice bran

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本研究利用亞洲大學紫秈糯米米糠及其萃取液，以添加不同比例到高筋麵粉中製成切麵條，研發具有特殊顏色、風味及抗氧化特性之麵條，並探討其理化性質及進行感官評估分析。研究結果顯示，在色澤分析方面，所有實驗組其L值皆低於對照組，且隨著添加量的增加遞減，特別是米糠粉末之麵條具有較低的L值。在黏彈性方面，所有實驗組皆低於對照組，並隨著添加量的增加遞減，而添加米糠粉末之麵條高於萃取液之實驗組。添加米糠粉末及萃取液之麵條烹煮後的水含量減少、烹煮損失率及烹煮增重率增加；整體而言感官評估的得分，添加米糠粉末及萃取液之麵條皆優於對照組。

Keywords: 紫秈糯米、米糠、麵條、理化性質、感官評估

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Keywords: 紫秈糯米、米糠、麵條、理化性質、感官評估

中醫典籍中腦血管疾病類藥材之藥性與功效相關性分析研究
Pharmaceutical property analysis of Chinese herbal medicines of
cardiovascular disease in ancient Pharmacopoeia

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本研究分析行政院衛生署中醫藥委員會於民國一百年六月出版的【中風中醫典籍彙編】(張賢哲, 2011)所收集之中醫古籍資料, 涵蓋中華醫典及中醫藥典籍檢索系統光碟中所有內容中, 與中風相關之方劑予以分析, 擷取其中共 783 方, 統計出各類藥物出現頻共次 7857 次, 並將其中出現頻次最高的前 60 種中藥, 再依「藥類」、「四氣」、「五味」、「歸經」、「功效」等欄位, 分別建立個別藥性資料, 運用 Excel 軟體整理成資料表單後並編碼, 之後再運用統計軟體 SPSS 分析「藥類」、「四氣」、「五味」、「歸經」、「功效」兩兩之間的相關性結果。由頻次統計結果可得知出現率最高的前三名依序是桂枝、防風、附子; 由「歸經」分析發現, 以歸肝、肺、脾經的藥物最多; 「五味」中則以辛味及甘味藥最常出現。

中藥的四氣、五味、歸經等藥性理論是中醫學對中藥功效作用的歸納和總結, 本研究以古代中醫藥文獻中對於中風治療方劑與中藥之使用結果為依據, 利用現代統計學予以歸納整理, 並分析其相關性, 期望研究結果可進一步提供現代研究治療中風之有效中藥材的科學性試驗研究方向, 並提供醫家們在臨床處方用藥時的參考。

關鍵字: 中風、中藥、藥性、歸經、功效、相關性統計

三種蓼科植物粗抽物清除 DPPH 自由基之探討

DPPH radical of three to explore the plant Polygonum crude extract Clear

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In recent years, the health consciousness is gradually accepted by consumers. Therefore, the development and application of the Chinese herbals have already become the world trend and more attractive to industries. In China it has been for a long time to use herbs for medical treatment of human diseases, however, effects on biological activity were not clearly proved for lack for scientific study. This study tested the cosmetic effect of Chinese herbal medicine, which documented in ancient books, in the scientific way in order to validate its biological activity.

Many papers report found that the antioxidant benefits of red wine because he has resveratrol, found in many literature fresh plants also contain resveratrol, such as peanuts, peanut outer membrane contains a lot of resveratrol. After that there found peanut bud peanut outer membrane more than 10 to 100 times resveratrol, and also seen in some of the reports have been found to have the presence of Polygonaceae resveratrol. That why I choice Rheum rhabarbarum, Fallopia multiflora and Polygonum cuspidatum in my experiment, and take these plants from three manufacturers's products, total 9 samples to test. In the test first get crude protoplasmic extract from plants, concentrated into powder, and re-dissolution after that do DPPH test. Search Results the Polygonum cuspidatum's efficiency is better than the other plants.

Keywords: Resveratrol, *Polygonum cuspidatum*, *Rheum rhabarbarum*,
Polygonum multiflorum Thunb, *Polygonaceae*,

利用水耕栽培提升根部吸收 Selenium(Se)之研究

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利用分子植物工廠中最基礎之概念模式，建構出最佳化之隔離環境，人工環控及養液供給系統，培育出富含功能性如有機 Selenium(Se)等之作物。Selenium 雖有慢性毒性，但目前的研究報告指出在很多動物系統中都有抗致癌作用 (anti-cancerigenic effect)。如抗皮膚癌、肝癌、腸癌、乳癌、胰臟癌等及提高免疫力之功用，雖然亦有報告指出太高劑量 Selenium(Se)可能有毒性，但目前大多數持正面看法，故在水耕中加入適當含量的 Selenium(Se)並監控植株內 Se 之含量。水耕栽培下，植物根部在養液中根部組織過度的浸泡在水或養液中，將會造成根部缺氧逆境影響植株，這些問題對於水耕栽培技術而言，是需要克服及考驗。本研究材料使用萵苣及白花椰菜以及使用自動監控系統 24 小時監控水中 pH 值及水中電導度，分別以氣霧與深水兩種不同氧氣供給方式，提供栽培植株氧氣之來源並比較其生長差異，另外在植株養分補給中分別進行 0mM、0.68mM、1.72mM Selenium(Se)的處理並觀察不同濃度下 Selenium(Se)對植株造成的影響，並在之後將收穫植株依不同的部位進行 ICP 檢測以了解 Selenium(Se)在植株中吸收之情形。

Keywords: Hydroponic , Selenium(Se) , INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETER (ICP-MS)

以纖維酵素優化萃取日本筋骨草的有效成分並評估降血糖之功效

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日本筋骨草含有大量的黃酮類、三萜類和其他活性化合物，具有治療糖尿病潛在的可能。本研究利用纖維水解酵素與超音波輔助進行日本筋骨草的萃取，以體外試驗評估萃取物降血糖的功效。結果顯示，日本筋骨草經纖維水解酵素於 40 °C 反應後，再以超音波輔助進行萃取，測得其總黃酮含量為 9.45 ± 0.14 mg/g，蛻皮甾酮含量為 0.973 ± 0.032 mg/g， α -glucosidase 活性抑制率為 $35.66 \pm 1.21\%$ ，且 HepG2 細胞對葡萄糖的攝取促進率為 $67.55 \pm 2.55\%$ 。與先前研究結果相比，日本筋骨草經纖維水解酵素處理後，再以超音波輔助進行萃取，可有效提升功效性成分含量，且體外降血糖試驗結果更具有調控血糖之效果。因此未來可將日本筋骨草的萃取物應用於治療糖尿病。

Keywords: 日本筋骨草、降血糖、纖維水解酵素、超音波

佛手柑、橙花、苦橙葉、依蘭依蘭精油對自律神經影響之研究
THE effects of aromatic essential oils of *Bergamot*, *Neroli*, *Petitgrain*
and *Ylang-Ylang* on autonomic nervous system

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近來利用精油做為媒介的芳香療法雖已廣為人們使用，並有許多實例證明其有效性，但關於芳療對人體影響的科學量測還是十分缺乏。為此本研究以噴霧吸入方式介入精油，並透過心律變異分析，來探討佛手柑、橙花、苦橙葉、依蘭依蘭精油對自律神經之影響研究。經研究結果顯示，佛手柑精油可在三分鐘內活化副交感神經，且抑制交感神經作用，且其抑制作用呈現持續性的進行，整體自律神經的平衡，也能快速的受佛手柑精油調節，達到放鬆之作用。橙花精油對交感神經和整體自律神經的調節較快，對副交感神經之調節作用則緩慢，與佛手柑精油相比，橙花精油對交感、副交感神經和自律神經的整體調節較不顯著。苦橙葉精油對交感神經的刺激較不明顯，反觀在提升副交感神經活性具有較顯著的作用，而在兩者的交互作用下，整體自律神經間的平衡也在五分鐘內表現出明顯的變化。最後在依蘭依蘭精油活化交感神經試驗中，發現其具有刺激交感神經活化之效能，但對副交感神經活及自律神經的調控上並無統計上的差異，推測因依蘭依蘭精油氣味較不受測試者喜愛，而受測人員對氣味的好惡是否影響其之生理變化，或是否需更長的時間才會有顯著性的反應，未來均需進一步的佐證。

關鍵字：植物精油、芳香療法、自律神經、心律變異

岩蘭草精油的萃取與香氣的分析 Vetiver essential oil extraction and analysis of aroma

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岩蘭草是一種具有特殊香氣之芳香植物，其精油具有寧神、鎮定、舒壓等功效，傳統精油萃取採用脂吸法、蒸餾法、溶劑萃取法等，這些方法不僅費工耗時，還需要消耗相當大的人力成本，然而隨著現代科技的發展，現在能夠使氣體呈現超臨界態這是一種新興且綠色環保的萃取方法，不會有殘留有毒物的有機溶劑等問題，本研究採用超臨界 CO₂ 流體萃取法萃取，栽種於無毒環境之岩蘭草，與使用水蒸氣蒸餾萃取法進行比較，發現不同萃取方法之精油純度與品質均不相同，超臨界萃取法，萃取物為浸膏狀的油狀物，純度相對較高，香味也較濃厚；水蒸氣蒸餾一為輕質油浮在水面上方，香氣聞起來與尚未萃取之岩蘭草氣味相近，不過卻少了類似土壤的味道與青草味；二為重質油密度較高沉降在瓶底，味道聞起來比較厚實味道較淡淡是較持久留香，且有些微的土壤味，依產量來說分離純化後所得之精油產量也只有 2.3 g，換算成產率的話也只有 2.3 %；而超臨界萃取之萃取物其產量約為 4 g 相較於水草物高出了 1.7 g，這邊證明了以超臨界二氧化碳流體萃取脂產物不僅純度高，同時也省去了精油複雜的提純過程，不僅省時省工，其萃取物可以很方便的調配成任意濃度的精油，因此非常適合做未來產品開發與應用。

Keywords: Vetiver, essential oil, extraction,

探討肥皂脂質與多醣添加對於皮膚保溼度之影響
Investigating the Effects of Skin Moisturizing in Soap Fortified with
Lipids and Polysaccharides

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油脂是肥皂的主要配方，不同油脂有不同的脂肪酸，不同脂肪酸的組合對肌膚的作用、起泡的特徵和硬度都會有所不同，本實驗選用三種不同油脂研究在何種比例上所完成的肥皂對肌膚的作用、起泡的特徵與硬度才會是最佳；文獻報告指出許多菇類含有豐富的活性物質，包含多醣、胺基酸、礦物質等，因而添加在保養品中會增加對皮膚的保溼度。本研究以直交設計法探討三種製皂常用油之最適化比例，並以整體接受度評比為最終的結果。實驗結果顯示，製皂之最適化油脂比例條件為椰子油:棕櫚油:橄欖油 = 3: 1: 1，而此條件所製備出的肥皂具有最佳的整體接受度。添加三種不同濃度多醣比較，對皮膚保溼度上並無顯著性差異，但整體接受度而言添加 0.5% 的多醣肥皂在起泡性、清潔力及硬度上都有很好的表現。所以考量油脂的特性、比例，添加物的適當濃度及功效和整體接受度高低，本研究可提供肥皂製備參考數值。

關鍵字：肥皂、脂質、多醣、添加、保溼度

含銀活性碳敷料應用於感染性傷口癒合之評估 Evaluation of Silver-Activated Carbon Fiber on Infected Wound Healing

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細菌感染是延長傷口的發炎期導致傷口癒合延遲的原因之一，銀離子具有廣效性抑菌效果，含銀活性碳纖維廣泛應用於傷口癒合和傷口感染治療，本研究室和科云生醫科技股份有限公司共同探討含銀活性碳敷料對感染性傷口癒合之影響。體外試驗得知，活性碳敷料較紗布比較可有效吸附細菌，並有效減少傷口處細菌量。同時，大鼠傷口癒合實驗中，科云生醫的活性碳敷料和含銀活性碳敷料可有顯著促進感染性傷口的面積修復率，進而增加傷口處膠原蛋白的生成。

Bacterial infection frequently leads to delayed wound healing due to the prolonged inflammatory phase. Silver has been applied on wound care because of its wide-spectrum antimicrobial activity and rare incidence of resistant bacteria developed. Silver containing dressings have been widely used for controlling wound infection. In this study, we investigated the efficacy of polyacrylonitrile-based activated carbon fiber on infected wound. The *in-vitro* examination indicated the fiber retained over 50% of the bacterial broth inside the dressing. And, the silver-containing activated carbon fiber can efficiently eliminate bacteria from the bacterial infection rat skin wound site. Therefore, in rat model of skin wound healing and dramatically promoted healing of infected wounds and promoted the production of collagen fiber.

Keywords: Silver-activated carbon fiber, infected wound, collagen

不同品種米類及鮮奶油對米冰淇淋品質之影響
**Influence on Quality of Ice Cream Made from Different kinds of Rice
and Whipping Cream**

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In this experiment, we used japonica rice, indica rice and two kinds of waxy rice as raw materials to made ice cream without emulsifier. The results showed the ice cream made from indica rice has higher texture value and fastest melting rate than waxy rice ice cream. On the other hand, waxy rice ice cream showed high viscosity value resulted the slowest melting rate than the japonica rice ice cream. However, it showed lower mouth feel scores which resulted lower preferences to the panelists. Sensory evaluation of japonica rice ice cream has higher scores on smell, flavor, and mouth feel attributes. In this results waxy rice ice cream has higher viscosity value than other ice cream. It showed strange and novel texture we guessed its low melting rate thus decreased the ice cream's mouth feel characteristic. We concluded that japonica rice is a better raw material than indica rice to proper to make ice cream. There were no significant difference on ice cream quality between two groups of ice cream with different whipping cream.

Keywords: ice cream 、 quality 、 sensory evaluation 、 whipping cream

SCFBAC 抑制塵蟎所誘發的呼吸道發炎現象
SCFBAC inhibited allergic airway inflammation in *Dermatogoides pteronyssinus*- induced chronic asthmatic mice

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氣喘是全球性的重大公共衛生問題，近年來其罹患率與致死率持續增加，因此，氣喘在治療與預防上有其重要性。成熟之輔助性 T 淋巴球依其所分泌的淋巴激素不同，可分成 Th1 及 Th2 細胞。研究顯示氣喘患者有 Th2 細胞增多的趨勢，而 Th2 細胞在調節慢性發炎反應、維持呼吸道反應性和控制特異性 IgE 生成上扮演關鍵角色。研究顯示 Th1 和 Th2 細胞在體內是互相調節的，因此調節以 Th2 細胞為主的氣喘患者，使之趨向 Th1/Th2 平衡，是合理的治療策略，因此尋找能調節 Th1/Th2 平衡的口服藥物是現今之研究重點。牛樟菇 (Antrodia camphorata) 又名樟芝屬於無摺菌目多孔菌科多年生蕈菌類，為台灣特有種之珍貴藥材，牛樟菇已廣泛應用於傳統醫藥，對於肝病、高血壓和癌症有顯著療效，同時它也具有免疫調節、抗發炎和抗氧化的效果。但由於牛樟芝在自然界非常稀少、又生長緩慢且無法以人工方式栽培，因此造成供不應求，促使其價格變得非常昂貴。近年來利用人工培養方式，可以獲得大量的固態栽培樟芝。之前研究顯示，固態栽培樟芝也具有類似野生種樟芝的抗發炎效果，但沒有對固態栽培樟芝的免疫調節功能作探討。我們利用塵蟎誘發 BALB/c 氣喘動物模式，先給予小鼠口服 50 mg/kg 固態栽培樟芝粗萃物 (SCFBAC)，再利用塵蟎 (50 µg/mice) 刺激氣管，每周一次連續進行 5 周，並分析呼吸道阻力、發炎細胞浸潤肺臟以及周邊血清中的 total IgE、Der p-specific IgG1 和 Der p-specific IgG2a/2b。結果顯示 SCFBAC 確實能夠改善塵蟎誘發氣喘小鼠的呼吸道阻力，亦能減少呼吸道發炎細胞浸潤肺臟以及血清的 total IgE 和 Der p-specific IgG1 表現，但提高 Derp-specific IgG2a/2b 的表現。總結 SCFBAC 對於慢性氣喘小鼠具有抗發炎、減緩呼吸道過度反應和特異的免疫調節效果。

Keywords: 固態栽培樟芝、塵蟎、氣喘、Th1/Th2 平衡、細胞激素

Establishment of a drug selection system based on hepatitis C virus NS3/4A protease

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The prevalence of hepatitis C virus (HCV) infection is all around the world. Until now, over 170 million people were infected by HCV. The virus causes liver diseases such as chronic hepatitis, cirrhosis, and liver cancer. The standard therapy of HCV is pegylated-interferon (Peg-IFN) combined with ribavirin (RBV), but only 50% sustained viral response for HCV genotype 1(GT1). Boceprevir and Telaprevir, which are two linear ketoamide compounds, which covalently bind to the serine protease active-site of HCV NS3, have been approved by FDA to treat HCV infection in 2011. In clinical, these two inhibitors induce HCV genotype 1 virus RNA mutation rapidly within HCV NS3 protease. The aim of the study is to establish a HCV GT1 NS3/4A protease antiviral screening assay. The wild-type HCV genotype 1 NS3/4A protease and the mutants with common resistant sites of the NS3/4A protease in clinic were constructed and the expressions were determined by Western blotting. The activities of wild type and mutant NS3/4A protease were determined by cell-based luciferase assay. The NS3/4A proteinase inhibitors that exclude viral escape through common resistance sites in clinic are under screening by the high-throughput drug selection system.

Keywords : HCV, NS3/4A protease, drug selection

紅番薯葉萃取物對 3T3-L1 脂肪細胞分化的影響
Effects of purple sweet potato leaves extract (PSPLE) on
3T3-L1 adipocyte differentiation

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Obesity is a condition characterized by an increase of adipose tissue as a result of positive imbalance between food intake and energy expenditure. Adipocytes play a critical role in regulating lipid metabolism and energy balance and are associated with adipose tissue mass and obesity. It is a health hazard which is closely associated with metabolic syndromes which are manifested by many symptoms like elevated, glucose, cholesterol, triacylglycerol, increases in blood pressure, risk of cardiovascular disease, and heightened chances of being diagnosed with type 2 diabetes. Purple sweet potato leaves (PSPL) contain a high content of anti-oxidants which have been shown to exhibit a number of biological activities³. This study was investigated whether purple sweet potato leaves extracts (PSPLE) can affect differentiation of 3T3-L1 cells. Before differentiation, confluence of 3T3-L1 preadipocytes were treated for 1 days with PSPLE to investigate cell growth and cytotoxicity, then compared the proportion of differentiation of mature adipocytes. PSPLE significantly decreased 3T3-L1 preadipocytes proliferation assessed by MTT assay and inhibited differentiation by Oil red o staining analysis. After differentiation, 3T3-L1 adipocytes were treated for 3 days with PSPLE result in partial cell death. In addition, the amount of intracellular lipid reduced by treatment with PSPLE. Our results indicate the PSPLE inhibition of growth of preadipocytes population and adipogenesis of 3T3-L1 cells. We also found that PSPLE suppressed lipid droplet accumulation on 3T3-L1 adipocytes. PSPLE may have further alleviate effects on metabolic syndrome individuals.

Keywords : purple sweet potato leaves extract, 3T3-L1 cells, adipogenesis
preadipocytes proliferation

松杉靈芝萃取物對脂肪細胞分化及脂肪油滴形成之潛在影響
The Potential Effect of *Ganoderma Tsugae* Extracts on Adipogenesis
and
Lipid Droplet Formation

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Ganoderma, also known as Lingzhi, is a medicinal fungus and has long been used for medicinal purposes and longevity in Asian countries. It is also known that adipocyte differentiation is a key aspect of obesity development when homeostasis is unbalanced. Although some researches have indicated that *Ganoderma* has potential capability of affecting adipogenesis and lipid metabolism, many contradictory results are reported and remained to be fully elucidated. To further investigate the real role of *Ganoderma* on adipogenesis, we performed this study by using murine pre-adipocyte cell line, 3T3-L1. In this study, *Ganoderma tsugae* (GT), a popular species of Lingzhi locally cultivated in Taiwan, was obtained from the Luo-Kuei-Ying Fungi Agricultural Farm, Taoyuan, Taiwan. The ethanolic and water extracts of GTEE and GTWE were used in “*in vitro*” and “*in vivo*” experiments. In “*in vitro*” assay, the cell viability and toxicity was determined by MTT assay, and the lipid accumulation and lipid droplet size was observed by Oil red-O staining. Both GT extracts had no effects on cell viability of 3T3-L1 cells but contributed to adipocyte differentiation. Furthermore, GT extracts altered the lipid droplet formation size and regulated the adipogenic related markers. In “*in vivo*” assay, the histological analysis of fat-pad tissues seemed to be shown smaller adipocytes in GT extracts-fed mice than in HFD-fed mice, which were observed by Hematoxylin/Eosin staining approach. Our data indicate that GT extracts accelerate adipocyte differentiation, affect lipid accumulation and alter the lipid droplet formation size. In conclusion, both GTEE and GTWE may promote health benefits by modulating adipocyte differentiation and regulating the lipid droplet formation.

Keywords: *Ganoderma tsugae*, Lingzhi, health benefit, Adipogenesis, Lipid droplet, 3T3-L1

The suppression effect of *Ganoderma tsugae* on Gram-positive bacteria

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Bacterial infection has been a big problem to physicians especially getting a history of using antibiotics improperly. The drug resistance of bacteria is the major problem in clinical treatment nowadays. Discovering new anti-microbial agents is an important subject. *Ganoderma* (Lingzhi) has being seen as a top grade mushroom in traditional Chinese medicine. *Ganoderma lucidum* (*G. lucidum*) and *Ganoderma tsugae* (*G. tsugae*) are the two species being well-cultivated in Taiwan. A lot of studies proved that *G. lucidum* powder extract could play a role in anti-bacterial agent. However, there is no study focusing on the anti-microbial effect of *G. tsugae*. We investigated the anti-microbial effect of *G. tsugae* on both Gram-negative and Gram-positive bacteria. *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC BAA-47), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Streptococcus agalactiae* (ATCC 13813) were cultured in tryptic soy broth in 96-well microtiter tray to test the minimum inhibitory concentration (MIC) with the ethanol (GTEE) and aqueous (GTWE) extracts from *G. tsugae* fruiting body powder. The MIC test was performed with shaking 250 rpm at 37°C for 24 h. The cell viability was further confirmed with serial dilution and plate spreading. Tryptic soy agar plates were incubated at 37°C for 16-20 h, the colony formation was counted to determine the colony forming unit per mililiter (CFU). We found that GTEE has antimicrobial effect below 10 mg/ml of dosage to all the Gram-positive bacteria. However, its effect is lower on the Gram-negative bacteria we tested. The vehicle concentration was consisted at 5% to minimize the influences caused by ethanol. The dosage treated on *E. coli* and *P. aeruginosa* was the maximum with 5% vehicle. GTEE has antimicrobial effect on *B. subtilis*, *S. aureus* and *S. agalactiae*. However, to determine the MIC of GTEE in *E. coli* and *P. aeruginosa*, the vehicle concentration needed to be raised. The CFU was determined only on *P. aeruginosa* and our results show that although the MIC could not be determined at 5% of ethanol, the cell viability at 10 mg/ml decreases nearly to half of the vehicle only group. These two parts have to be confirmed and the relevant mechanisms are remained to be investigated.

Keywords: *Ganoderma tsugae*, anti-microbial effect, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*

標靶性奈米藥物載體於抑制胃幽門螺旋桿菌之研究應用 Targeted nanoparticles for anti-*Helicobacter pylori* study

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消化性潰瘍為一種普見的消化系統疾病，以胃幽門螺旋桿菌感染情況最嚴重。臨床上對抗胃幽門螺旋桿菌常用抗生素藥物，但抗生素藥物需長時間服用才可達到抑制胃幽門螺旋桿菌效果，並會產生一定副作用。本研究為結合標靶性奈米載體技術於探討包覆不同抗胃幽門螺旋桿菌的藥物，期待所形成岩藻醣-幾丁聚醣的奈米藥物載體可保護藥物不被胃酸破壞並主動標的幽門螺旋桿菌於達到藥物治療效果。

A variety of approaches have been proposed for overcoming the unpleasant side effects associated with antibiotics treatment of *Helicobacter pylori* infections. Berberine and epigallocatechin-3-gallate (EGCG) have been known to treat gastrointestinal infection and exert inhibitory effects on the proliferation of *Helicobacter pylori*. In our study, we developed fucose-conjugated targeted nanoparticles technology to protect berberine and EGCG from destruction by gastric acids and effectively control drug release to directly interact the intercellular space at the *H. pylori* infection site.

Keywords: Berberine, Epigallocatechin-3-gallate, *Helicobacter pylori*, Targeted nanoparticle

共病症對於 C 型肝炎患者發展成肝癌的影響
Comorbidity risk of hepatocellular carcinoma development in
chronic hepatitis C patients

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C 型肝炎病毒在過去稱為非 A /非 B 型肝炎，在 1989 年才首度被證實主要是一種 RNA 病毒所引起的病毒傳染疾病，由於病毒突變快速，尚未有預防疫苗，目前臨床上標準的治療組合方式是利用干擾素(interferon alfa) 加上雷巴威林(ribavirin)，但在第一次治療過後大約有 55%的病人是有效的，有 75%的病人會有一個以上的副作用導致停止治療或調整劑量，研究指出，C 型肝炎患者的並存疾病可能會影響治療的效用和結果。研究目的為了解共病症是否增加 C 型肝炎患者的負擔促使肝癌發生，及接受治療是否減緩肝癌的發生。

本研究使用回溯性世代研究法，資料來源是健保資料兩百萬抽樣檔中的門診檔案、門診醫令檔案，篩選 2004~2008 年至少有兩次 C 型肝炎診斷的新發個案，再排除年齡小於 18 歲、已有肝癌紀錄、以及在診斷後 90 天內罹患肝癌或者死亡，且追蹤至 2009 年至少有一年的追蹤期，最後納入 8775 名研究對象，使用 Cox 比例風險模式評估各危險因子與肝癌發生的風險比(hazard ratio, HR)。8775 名 C 型肝炎患者平均追蹤時間為 3.3 年，1826 名患者完成治療，追蹤期間累積 459 名患者發生肝癌，調整年齡、性別後，找出顯著影響肝癌發生的 17 個共病症，最後加上調整 C 型治療藥物效用，發現影響肝癌發生的共病症包含食道疾病(未包含食道靜脈曲張)(HR=0.55, P=0.039)、肝病(HR=1.85, P<0.001)，缺鐵性貧血(HR=1.09, P=0.044)，背部問題(HR=0.66, P=0.034)，糖尿病(HR=1.01, P=0.038)。另外，調整共病症後，完成肝炎治療的患者其肝癌發生的風險降低(HR=0.62, P=0.045)。

部分共病症會提高肝癌的發生，所以 C 型肝炎患者還需要關注共病症的問題並且有效完成治療，才能有效降低肝癌的發生。

Keywords: C 型肝炎，共病症，肝癌

黃芩苷與黃芩素用於治療幽門螺旋桿菌感染之功效
Study of baicalin and baicalein in treating *Helicobacter pylori* infection

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Helicobacter pylori infection is associated with chronic gastritis, peptic ulcers, and gastric cancer. About 50% of the population in the world is infected by *H. pylori*. Furthermore, 70% to 95% of *H. pylori*-infected patients are suffering from peptic ulcer. *Scutellaria baicalensis Georgi* is one of a common Chinese herb, and its major compounds are baicalin and baicalein. It has showed that both baicalein and baicalin harbored antibacterial activities. Baicalein, a primary metabolite of baicalin, differs from its mother compound merely by the 7-substituent (i.e. it possesses a phenolic hydroxyl (7-OH) instead of a glucuronic acid). In our study, baicalein exhibited stronger bactericidal activity than baicalin did. But baicalein also exhibited stronger cytotoxicity than baicalin did in AGS (human gastric cancer epithelial cell lines). It could also interfere the adhesion and invasion ability of *H. pylori* to epithelial cells and decrease *H. pylori*-induced IL-8 expression. Therefore, baicalein showed a better therapeutic ability than baicalin. Additionally, a mice infection model was established. Infected mice were treated with different amount of baicalin, baicalein and antibiotics for three days. Baicalin and baicalein could inhibit *H. pylori* growth in stomach and suppress VacA expression, which toxin causes progressive vacuolation as well as gastric injury. Moreover, *H. pylori* – specific IgM and IgA levels in mice treated with baicalein and antibiotics were decreased. Antibiotic treatment disturbed intestinal microflora, but baicalin and baicalein did not. These treatments could not only provide a new treatment of *H. pylori* infection and also overcome the problem that antibiotic treatment caused by disturbing the proper function of the intestinal flora.

Keywords: baicalin, baicalein, *Helicobacter pylori*

探討乳酸菌抗幽門螺旋桿菌感染之影響
Effects of *Lactobacillus rhamnosus* on treating *Helicobacter pylori* Infection

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幽門螺旋桿菌(*Helicobacter pylori*)為革蘭氏陰性、微需氧之細菌，許多研究指出許多胃部疾病，如胃潰瘍、胃癌等，都有可能因為幽門螺旋桿菌的感染引起。目前全球治療幽門螺旋桿菌感染最普遍使用的療法為三合一療法，使用兩種抗生素 - clarithromycin, amoxicillin/ metronidazole 的搭配加上氫離子幫浦阻斷劑來達到治療的效果，但近年來由於抗生素的濫用，導致幽門螺旋桿菌對抗生素的抗藥性逐年上升，加上抗生素的攝取也會導致腹瀉或是過敏等副作用的產生，促使相關研究人員尋找替代療法或是輔助治療。*Lactobacillus rhamnosus* 為乳酸菌的一種，屬革蘭氏陽性、兼性厭氧或微需氧之桿狀細菌，能在腸胃道及陰道中發現，是組成人類腸道內菌群的一小部分，其中，許多 *L. rhamnosus* 已被用來當作益生菌使用，其中有許多研究中，也證明益生菌能影響幽門螺旋桿菌之感染。因此在本研究的初步實驗中，使用了乳酸菌 *L. rhamnosus* 分離株- JB3，在不同 MOI(multiplicity of infection)下刺激幽門螺旋桿菌標準菌株 26695(ATCC 700392) 感染之人類胃癌上皮細胞株 AGS 細胞，六小時後，分析細胞發炎因子 (interleukin-8, IL-8)以及幽門螺旋桿菌貼附及侵入的能力(association ability)，發現兩者皆會受到不同程度之影響，然而此影響並不隨著 JB3 MOI 越高而影響程度越大，而是在某一特定的 MOI 下，對 IL-8 表現及 association ability 影響最劇，我們猜測此影響與乳酸菌的密度有關，而細菌的密度則由 quorum sensing 調控，因此本研究將進一步探討乳酸菌是否藉由 quorum sensing 的調控，影響幽門螺旋桿菌感染，以達到治療的目的。

Keywords : *H. pylori*, *L. rhamnosus*, quorum sensing

檳榔誘導單核球/巨噬細胞分化成蝕骨細胞之能力
The capability of Areca nut promote monocyte and macrophage differentiation

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Bone generation mainly relies on communication of osteoclasts and osteoblasts , through cell-cell balance in reconstruction and bone resorption. The preosteoclast cells which monocytes and macrophages have be stimulated by macrophage colony-stimulating factor(M-CSF), RANKL or some cytokines, express the surface receptor. Additionally, in this study we use RANKL as control. After the stimulation several uninuclear precursor cells combine to form a multinuclear cell. This process makes osteoclasts more mature and active in resorbed lacunae to refill it with new bone.

Areca nut extracts, traditional Chinese herbal medicine, is widespread in tropical Asia, and used to treat many symptoms including vermifuge, antidiarrheal, antipyretic, treating malaria in traditional Chinese medical treatment.

In this study, we treat with areca nut extracts on monocyte/macrophage lineage cell line-RAW264.7 in the different concentration. (20ug/ml,40ug/ml,60ug/ml,80ug/ml) Then we use tartrate-resistant acid phosphatase (TRAP) staining, osteoclast-specific stain, to identify whether cells are active osteoclasts .We find Raw264.7 coculture with 20 ug/ml significantly which compare to our control. So we figure out Areca nut is able to induce monocytes and macrophages differentiation. According to this study, we could know more about the relation between areca nut extracts and osteoclasts ,and the association to some diseases about bone of injury.

Keywords:areca nut extracts, Raw264.7, osteoclast, TRAP stain

The effects of type II arabinogalactan on osteogenesis of Human mesenchymal stem cells

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Backgrounds:

Human mesenchymal stem cells (hMSC) are self-renewing precursor cells that can be expanded in vitro and differentiated towards osteogenic, chondrogenic or adipogenic lineages. However, the limit number and inefficient hMSCs differentiation are the major barriers in hMSC-based therapy. It would be extremely beneficial if a naturally occurring agent could be identified that could induce hMSCs to undergo specific lineage differentiation. In the present study, we investigated the potential ability of type II arabinogalactan to enhance osteogenesis in hMSC cells and to determine the mechanism of action.

Materials and Methods:

The type II arabinogalactan (AGAF) consisting primarily of a (1→3)-β-D-galactan backbone with a (1→6)-β-D-galactan side chain was purified from *Anoectochilus formosanus*. Markers of hMSCs were measured using flow cytometry. hMSCs differentiated into adipocytes were analyzed by Oil-Red O staining. Osteogenic differentiation was identified by the mineralization of calcium deposits using Alizarin S staining. The mRNA expression of osteogenic marker genes (Runx2, type I collagen, Alkaline phosphatase and osteocalcin/OC) were detected using RT-PCR.

Results:

Our results showed that AGAF dose-dependently enhanced the gene expression of osteogenic markers and mineralization of hMSC cells. The p53 level was down-regulated after AGAF treatment. Depletion of p53 could inhibited the AGAF-induced osteogenic gene expression and mineralization in hMSC cells. Therefore, the p53 may play important roles in AGAF-induced osteogenesis in hMSC cells. In contrast, AGAF enhanced osteogenesis but does not promote adipogenesis of hMSC cells.

Conclusion:

These results showed that optimal AGAF induction could enhance the osteogenesis differentiation and maturation of hMSCs. Further studies to evaluate the underlying mechanism of p53 in the AGAF-induced osteogenesis in hMSCs are still needed.

Keywords: Mesenchymal stem cells, osteogenesis, arabinogalactan

日本腦炎病毒 DNA 複製子之構築及其抗病毒藥物快篩應用
Construction of Japanese encephalitis virus DNA replicon and its application on high-throughput screening anti-JEV agents

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Japanese encephalitis virus (JEV) is a member of mosquito-borne flaviviruses in the family *Flaviviridae*. JEV contains a single-strand, positive-sense RNA genome with approximately 11 kb in length that encodes a big ORF for polyproteins as three structure proteins (C, prM/M and E) and seven non-structure proteins (NS1, 2A, 2B, 3, 4A, 4B, and 5). This study intends to construct a DNA-based replicon using the full-length genome of JEV T1P1 strain and then setup as the high throughput screening platform. Firstly, the plasmid pBR322 was removed the fragment between EcoR1/BamH1, then inserted a linker containing five single restriction enzyme cutting sites for cloning the replicon. Secondly, CMV promoter, SV40pA, and Neomycin resistance gene were cloned into the modified pBR322. Thirdly, three big cDNA fragments (>4 kb) of JEV genome were cloned into modified pBR322. Sequencing of F1 cDNA fragment indicated nonsense mutations occurring at Nucleotides 690 and 809. The F1 mutant clone as the template was inserted EGFP-FMDV 2A between Nucleotides 119 and 2388, in-frame fused with F2 and F3 cDNA fragments (Nucleotides 2388-2477), and followed linking with 67 bp HDV ribozyme. The resultant replicon was transfected into BHK-21 and TE671 cells, and GFP fluorescence was observed 1 to 2 days post transfection. Expression of JEV sense and antisense genomes as well as viral proteins were detected using IFA, Western blotting and real time RT PCR. The replication of the DNA-based JEV replicon was easily measurable, indicating the replicon as high throughput platform for screening anti-JEV agents.

Key word: Japanese encephalitis virus, Replicon

NC-8 inhibits the osteoclast differentiation from Monocyte/ Macrophages Lineage precursor cell.

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摘要:

人類骨骼的形態與構成，涉及成骨細胞的骨基質合成和蝕骨細胞的骨骼吸收這兩者之間微妙的調控。蝕骨細胞是一個巨大並具有多核的細胞，主要由巨噬細胞和單核球的前驅細胞分化而來。當人體蝕骨細胞大於造骨細胞時就會造成骨質疏鬆症。異甜菊醇 (isosteviol) 是甜葉菊葉內成份之一甜菊糖 (stevioside) 經化學修飾合成新的衍生物；本研究主角 NC-8 (*ent-16-oxobeyeran-19-N-methylureido*) 異甜菊純衍生物在先前文獻中即具有良好的生物活性，例如可以抑制 B 型肝炎病毒 (HBV) 基因表現及病毒 DNA 複製機制。在這個研究中，我們探討 NC-8 是否會促進或抑制 RAW 264.7 細胞形成蝕骨細胞。我們利用 MTT Assay 觀察 NC-8 是否會造成 RAW 264.7 細胞毒性並使用抗酒石酸酸性磷酸酶 (TRAP) 染色，做為鑑定蝕骨細胞專一性染色法。實驗結果顯示，NC-8 對 RAW 264.7 細胞毒殺活性 IC_{50} 大約在 $40\mu\text{g/ml}$ ；進一步利用抗酒石酸酸性磷酸酶 (TRAP) 染色法則顯示 NC-8 會抑制 RAW 264.7 細胞分化成為蝕骨細胞。因此在本研究中，NC-8 不單單可以成為新型治療病毒性肝炎之潛力藥物，還可以進一步抑制單核球或者巨噬前驅細胞分化成為蝕骨細胞以間接避免骨質疏鬆的產生。

關鍵字: NC-8、Osteoclasts、RAW 264.7 murine cell、TRAP stain

EGCG 對日本腦炎病毒複製的影響 The effects of EGCG to JEV replication

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日本腦炎病毒(Japan encephalitis virus, JEV)是由蚊子傳播的 *Flaviviridae* 病毒，會導致嚴重的神經系統疾病及高致死率，倖存者也會留下永久性神經損傷之後遺症。主要治療方式依靠支持療法。Epigallocatechin-3-gallate (EGCG) 經研究證實具有抗腫瘤、抗病毒等功效，可抑制 HCV 病毒進入細胞感染。但鮮有 EGCG 對抗 JEV 相關的研究，因而引起我們的興趣。文獻報導 EGCG 為組蛋白去乙酰酶抑制劑(histone deacetylase inhibitors, HDACi)，影響細胞內訊息傳遞及相關蛋白之磷酸化、乙酰化和泛素化等修飾平衡。本論文將探討三種 HDACi 抑制 JEV 複製之功效並其相關機制。三種 HDACi 為 EGCG、trichostatin A (TSA) 及 valproic acid sodium salt (VPA)。MTT assay 得到 EGCG 對 BHK21 的 CC_{50} 為 39 μM 。欲知此三種藥物是否會抑制 JEV 產生，將 JEV(MOI=0.1) 感染幼倉鼠腎成纖維細胞 (BHK21)，分別處理不同劑量並觀察其細胞病變(CPE) 變化。EGCG 對病毒 CPE 抑制在 10 及 20 μM 較為明顯，並抑制 57% 及 94% 的感染細胞上清液中病毒產量。TSA 會隨著藥物濃度增加而減少 CPE，但抑制感染細胞上清液中病毒產量效果並不明顯。1-1000 μM VPA 則無抑制 JEV 複製之功效。病毒斑點減少實驗得到 EGCG 之抗 JEV IC_{50} 為 13.2 μM 。透過病毒貼附試驗得知 EGCG 會抑制 JEV 病毒貼附。流式細胞儀分析 BHK21 細胞處理不同濃度之 EGCG 並與控制組相比，發現隨著藥物濃度增加而減低病毒感染細胞之凋亡現象。西方墨點法顯示 JEV 感染細胞後會影響 p53 之磷酸化表現，EGCG 可提升感染細胞之 p53 磷酸化。免疫沉澱實驗中，細胞感染病毒會增加 p53 的泛素化，但加入 EGCG 則可抑制其泛素化表現。未來將繼續確認 EGCG 之抗 JEV 分子機制。期盼此為基礎可應用發展抗日本腦炎病毒藥物。

Keywords : JEV, EGCG, p53

地瓜葉對於 3T3-L1 脂肪細胞分化的影響
Effect of Sweet Potato Leaves on 3T3-L1 Adipocytes Differentiation

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As a result of changes in lifestyle and dietary pattern, the prevalence of obesity becomes gradually increased, which is related to many chronic diseases. Therefore, how to prevent the development of obesity has gained so much attention to recent research studies. In this study, we treated with purple sweet potato leaves (PSPL) and green sweet potato leaves (GSPL) PBS extract, and examined whether they affected the differentiation of 3T3-L1 adipocytes. The result showed that PSPL and GSPL could reduce accumulation of triglyceride, especially when treated with 10 mg/mL PSPL and GSPL. They reduced 80% and 87% ($p < 0.05$), respectively. PSPL and GSPL significantly inhibited C/EBP α of mRNA expression for 3 days ($p < 0.05$). In six days, PSPL and GSPL decreased PPAR γ , C/EBP α and SREBP-1c mRNA expression ($p < 0.05$). Collectively, PSPL and GSPL in addition to inhibit expression of fat synthesis-related genes can also be reduce lipogenesis and triglyceride accumulation, and then avchieving the anti-obesity effect, triglyceride accumulation, thus avchieving the anti-obesity effect. They have the potential to be developed into a health food.

Keywords: 3T3-L1, sweet potato leaves, differentiation

探討十字花科黑腐病菌中 *Pad* 基因之功能
Functional study of *Pad* gene in *Xanthomonas campestris* pv.
campestris

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Xanthomonas campestris pv. *campestris* (*Xcc*) is the causal agent of black rot disease which causes severe crop losses worldwide. Lysozyme is the major component of the innate immune system and represents the first line of defense against pathogens. The *pad* gene may contribute to *Xcc* virulence by providing protection against host lysozyme. In this study, we constructed a *pad* mutant of *Xcc*, and conducted extracellular enzymes assay, pathogenic testing, antibiotic resistance assay and anti-stress testing to study the biological functions of *pad* gene in *Xcc*. The current results showed that *Pad* gene does required for the pathogenicity in a laboratory infection system and there is no significant difference between the *pad* mutant and its parental strain in the other assays.

Keywords: *XCC*, *Pad*, lysozyme

探討十字花科黑腐病菌中 *smR* 基因之功能
Functional study of *smR* gene in *Xanthomonas campestris* pv.
campestris

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Xanthomonas campestris pv. *campestris* (簡稱 *Xcc*) 為革蘭氏陰性菌，形狀為桿狀。此疾病造成全球農作物嚴重的損失。此菌會感染十字花科植物會引起十字花科黑腐病 (black rot)，造成農業上重大的損失。植物感染此疾病後，典型症狀包括 V 形的黃色病變，從葉緣慢慢感染至的葉脈，沿著葉脈組織變黑而壞死。*smR* 基因主導生成含 small MutS-related (Smr) domain 的蛋白，MutS 為核酸內切酶，在 DNA 完整性維持上扮演重要功能。在 *Xcc*，*smR* 基因的相關研究未曾被報導，因此其細胞和分子功能仍不清楚，所以我們想知道這個基因的功能為何。本實驗目的研究 *smR* 的功能，因而構築 *smR* 突變株、*smR* 突變互補株與 *smR* 大量表現株。進行胞外酵素測試、致病性測試以及逆境的測試，目前的結果顯示：*smR* 基因經過突變之後，不影響致病能力；*smR* 之突變株、大量互補株及互補株與野生株在胞外酵素的分泌上均無顯著性差異；*smR* 大量表現株與互補株之存活率比野生株差，且互補株比大量表現株較差。

關鍵詞：*Xcc*，*smR*，革蘭氏陰性菌，十字花科，MutS，核酸內切酶

虎杖萃取物對小鼠免疫調節反應之影響
Effects of *Polygonum cuspidatum* extract on immune responses in normal mice

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Polygonum cuspidatum is a natural plants used as a traditional Chinese herbal medicine. Crude extract of *Polygonum cuspidatum* (CEPC) has biological effects but there have not been any studies on effects of CEPC on immune responses in normal mice. The purpose of the present study was to determine *in vivo* effects of CEPC on immune responses in normal mice. BALB/c mice were orally administered CEPC (0, 50, 100, 150 and 200 mg/kg) for 3 weeks after which time blood, liver, and spleen samples were collected. CEPC did not significantly affect body weights or tissue weights of liver and spleen when compared with control mice. CEPC increased the percentages of CD3 (T-cell maker), 11b (monocytes), Mac-3 (macrophages) cells and reduced the percentage of CD19 (B-cell maker) when compared with control mice. CEPC (100 mg/kg) stimulated macrophage phagocytosis of blood samples but did not affect macrophage phagocytosis from I.P Activity of splenic natural killer cells was increased by CEPC (50 mg/kg). CEPC inhibited T and B cell proliferation when cells were treated with Con A and LPS, respectively.

KeyWords: *Polygonum cuspidatum*, Balb/c mice, phagocytosis, macrophage, Natural killer cells.

Analysis of Antibacterial Effects from extract of *Tremella fuciformis*

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Abstract

In this study, ultrasonication was applied to extract bioactive components from different strains of *Tremella fuciformis* (i.e. LT13, LT15 and market-obtained) using 95% alcohol as the solvent. Antibacterial activity of ethanolic extracts were analyzed on G (+) bacteria including *Streptococcus mutans* (SM), *Staphylococcus aureus* (SA) and *Propionibacterium acnes* (PA) using solid media in the plates. Results showed that the market-obtained (MO) strain had antibacterial effect for SA after four hours to twelve hours. Strain LT13 showed antibacterial effect for SA after four hours to sixteen hours. In liquid broth study, strain MO showed antibacterial effect on SM after four hour to eight hour incubation. Strain LT13 showed antibacterial effect on SM after twelve hour to sixteen hour incubation. For PA, strain MO showed antibacterial effect after two hour to sixteen hour incubation. Strain LT13 showed antibacterial effect on PA after two hour to sixteen hour incubation. Further studies will be focused on the antibacterial effects of strain LT15 for SM, SA and PA. The bioactive components will also be tentatively identified.

Key words: *Tremella fuciformis*; Antibacterial activity; *Streptococcus mutans*; *Staphylococcus aureus*; *Propionibacterium acnes*

腸病毒 71 型毒力因子分析

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摘要

腸病毒 71 型 (Enterovirus 71) 為人類重要病原菌之一，主要感染嬰幼童，引起手足口症及中樞神經系統相關的疾病。臨床上受腸病毒 71 型感染患者可從輕度症狀情況進而引起嚴重神經系統併發症，例如腦炎，腦膜炎，脊髓灰質炎樣麻痺，甚至導致病患死亡，然而對於腸病毒 71 型危害問題目前並無有效的疫苗或抗病毒藥物，只能依賴支持性療法醫治患者，因此腸病毒 71 型成為近年來在公共衛生上迫切需要解決的重要議題。為了瞭解病毒基因體上之重要毒力因子決定位，本研究藉由重組方法導入基因突變，以加速病毒的演化。首先病毒全基因體 cDNA 經 PCR 方式進行隨機突變增幅後，以胞外轉錄方式產生基因體 RNA，並轉染到細胞中以產生突變株。收取轉染細胞上清液經由繼代培養後，以免疫螢光分析及 RT-PCR 可證明具感染性病毒顆粒的存在。為了篩選出可能的重要致病因子，上述所獲得之隨機突變病毒株，接著以病毒斑減少中和試驗篩選出病毒斑較大的病毒株 Hau1。生長曲線分析中顯示 Hau1 複製效率比野生株快，並且具有產生較高病毒力價的能力。為了釐清造成影響的分子機制，先利用吸附試驗確認出 Hau1 的吸附能力比野生株高，且在蛋白質表現分析中也顯示 Hau1 的表現較野生株來的快。進一步對全基因體定序結果顯示 Hau1 分別在殼體蛋白 VP2:142^{T→M} 和 3A 蛋白酶 53^{R→H} 發生胺基酸突變，導致病毒吸附及複製能力發生改變。未來我們將利用位點突變方法，逐一證明這 2 個位點何者才是最主要的影響因子。

關鍵字: 腸病毒 71 型、毒力因子、突變

發展簡易核酸萃取方法以應用於快速檢測番茄斑萎病毒屬病毒
Development of a convenient nucleic acid extraction method for prompt
detection of tospoviruses

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Antibody-based immunoassays and nucleic acid-based reverse transcription-polymerase chain reaction (RT-PCR) are commonly used for detecting virus infections in plants. RT-PCR is usually more sensitive and specific than immunoassays in diagnosis and identification of viruses. Nucleic acid extraction is essential for preparing templates of RT-PCR. However, the traditional nucleic acid preparation methods are time-consuming. Commercial kits can be used to save procedure time but expensive. Development of a low-cost rapid nucleic acid extraction method is worthy for plant virus detection in RT-PCR. Previously, a cheap and convenient nucleic acid extraction buffer, denoted TPS (Thomson and Dietzgen, 1995), had been reported to quickly release viral RNA and DNA from plant tissues for nucleic acid amplification. In this study, an alternative nucleic acid extraction buffer, denoted TSE, was developed and used to release the genomic RNA of tospoviruses belonging to the plant-infecting genus of the family *Bunyaviridae*. The procedure of TSE buffer-based method was optimized as incubation of plant tissue, after ground directly with buffer, at 70°C for 1 min. The TSE-extracted nucleic acids of the plant tissues individually infected with different tospoviruses, including *Tomato spotted wilt virus* (TSWV), *Watermelon silver mottle virus* (WSMoV) and *Melon yellow spot virus* (MYSV), can be successfully used in virus detection by one-step RT-PCR. The TPS buffer and a commercial kit were also used in nucleic acid extraction of the tospovirus-infected plant tissues for comparison. Our result showed that the TSE buffer is reliable for releasing tospoviral RNAs as well as TPS buffer and commercial kit to prompt virus detection by one-step RT-PCR amplification.

Keywords: *Tospovirus*, nucleic acid extraction, detection

Cloning and expression of two homologous cellulase genes from anaerobic fungus *Piromyces rhizinflata*: Homology modeling, structural comparison and characterization

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Abstract

Two homologous cellulase genes, *cel6B* and *cel6C*, were isolated from the cDNA library of ruminal fungus, *Piromyces rhizinflata* strain 2301. These genes shared 68% nucleotide identity and 60% amino acid identity in their catalytic domains. The deduced amino acid sequence of the catalytic domains of Cel6B and Cel6C showed homology with family 6 of glycosyl hydrolases. The catalytic domains of two cellulases were expressed in *Escherichia coli* and the purified proteins were used to characterize the enzymes. The optimal activity conditions with carboxymethyl cellulose (CMC) as the substrate were pH 5.5 to 6.5 and 37 to 50°C for Cel6B and pH 6.5 and 37°C for Cel6C. The thermal stabilities of the two enzymes, which were incubated at different temperatures for 10 min, differed substantially. At 45°C, Cel6B retained 100% activity, whereas Cel6C lost more than 60% of its enzyme activity. Homology modeling was performed to generate three-dimensional (3D) structures of Cel6B and Cel6C to demonstrate the factors that govern the thermostability of Cel6B. The structural properties of Cel6B were compared with those of Cel6C, indicating that the higher stability of Cel6B could be associated with increases in hydrophobicity and compactness.

Keywords: *Piromyces rhizinflata*; Cellulase; Homology modeling; Thermostability

經 γ -照射之蛹蟲草菌絲製備之多醣物理性質
**Physical properties of polysaccharides from *Cordyceps militaris*
mycelia with various doses γ -irradiation**

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Food irradiation can confirm hygienic quality and prolong the product shelf life. However, due to lack of international consensus, effective identification methods and detailed quality characterization are required for the general use of this technology. Polysaccharide was prepared by hot water extracted of *Cordyceps militaris* mycelia were irradiated at 0, 10 and 20 kGy. The rheological properties of polysaccharide were studied using viscosity, molecular weight (Mw) and thermal properties. The content of polysaccharide was in the range of 29.70 to 44.82 mg/g and then increased significantly with γ -irradiation (0-20 kGy). For *C. militaris* mycelia crude polysaccharide, the main molecular weight distributions were in 3.7×10^6 Da and 3.6×10^4 Da. Mw showed that increased irradiated dose (10 and 20 KGy) had high amount of the high-molecular weight (+33% and +45%) and had low amount of the low-molecular weight (-7% and -9%) than non-irradiated control. Increasing irradiation up to 20 kGy increased the apparent viscosity of polysaccharide. For *C. militaris* mycelia crude polysaccharide, the apparent viscosity were in 0.94-0.95 cp. Polysaccharides from *C. militaris* mycelia with 0, 10 and 20 KGy showed a melting endothermic peak at 246.25-264.66 °C and their onset and completion temperatures were 244.41-244.94 and 281.44-285.16 °C, respectively. In addition, the enthalpy (ΔH) were in the descending order: 10 KGy (112.80 J/g) > 20 KGy (98.10 J/g) > 0 KGy (86.86 J/g). The results of this study indicated that γ -irradiation treatment might result in the changes of crystalline regions in the polysaccharide structure. The notable changes in rheological properties of irradiated mycelia should be taken into consideration before using the radiation technology as a commercial tool for sterilization.

Keywords: *Cordyceps militaris*; Gamma irradiation; polysaccharide; viscosity; molecular weight; thermal analysis

不同萎凋時間對三裂葉蟛蜞菊抗氧化能力與抑菌作用之影響
Antioxidant and Antimicrobial Activities of *Wedelia trilobata* (L.)
Extracted by Different Withering Time

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本研究以三裂葉蟛蜞菊為原料，分別進行 0、3 及 6 小時的萎凋作用後以 RO 水萃取，分別測定其 DPPH 自由基清除能力、還原力活性、螯合亞鐵離子能力及抑菌活性。結果顯示 DPPH 自由基清除能力，在各萎凋時間皆有不錯的清除力，尤其在濃度 1.2mg/ml 時，清除能力達 65% 以上；還原力方面，以未經萎凋水萃液的蟛蜞菊在 1.2 mg/mL 的濃度時，還原力之 O.D. 值有較高的數值，其數值為 0.59，而在萎凋萃取 6 小時有較低的 O.D. 值，其數值為 0.42；亞鐵離子螯合能力方面，結果顯示蟛蜞菊經 6 小時萎凋水萃液有較高的螯合能力，在 0.6 及 1.2mg/ml 濃度時統計上有顯著的差異，其數值分別為 67.76 及 73.34%。在抑菌方面，結果顯示蟛蜞菊水萃取物對於 *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*, *Listeria monocytogenes* 及 *Bacillus cereus* 菌株皆無抑菌作用。

Keywords: 蟛蜞菊、抗氧化、抑菌作用、水萃

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探討十字花科黑腐病菌中 *Pcm* 基因之功能

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Xanthomonas campestris pv. *campestris* (Xcc) 能感染十字花科植物，導致黑腐病。本報告目的為利用同源重組技術，探討在 Xcc 中 *Pcm* 基因之功能性。分別在基因中插入外來抗藥基因，達到破壞基因的功能性，得到 *Pcm* 的突變株。將 *Pcm* 完整基因承接在 pRK415 上得到互補質體，再將互補質體送入突變株裡，得到 *Pcm* 基因之互補株，另外，將互補質體送入野生株裡，得到 *Pcm* 基因之大量表現株。進行逆境測試、致病性測試、胞外酵素測試，分析探討其功能。在逆境實驗顯示，在不同 pH 值、NaCl 濃度的 plate 上，各菌株之表現差異不顯著，在 0.05% 和 0.1% SDS 濃度的 plate 上，發現 *Pcm* 之突變株的表現較其他菌株差。在葉片感染實驗顯示，*Pcm* 之突變株可降低致病能力。在胞外酵素實驗顯示，*Pcm* 之野生株、突變株、大量表現株、互補株在胞外酵素的分泌上，均無顯著性差異。

關鍵字: *Xanthomonas campestris* pv. *campestris* , mutant , extracellular enzymes activity assay , adversity assay

探討十字花科黑腐病菌中 *snaR* 基因之功能**Study on the function of *snaR* in *Xanthomonas campestris* pv. *Campestris***陳怡秀 Yi-Shiou Chen[#]、胡若梅 Rouh-Mei Hu^{*}

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Xanthomonas campestris pv. *campestris* (*Xcc*) 能感染十字花科植物而導致黑腐病。本報告目的為探討在 *Xcc* 中 *snaR* 基因之功能性。利用同源重組技術分別在基因中插入外來抗藥基因達到破壞基因的功能性得到 *snaR* 的突變株，另外將 *snaR* 的突變株基因承接在 pRK415 上得到 *snaR* 基因的互補株，將 *snaR* 完整基因承接在 pRK415 上得到 *snaR* 基因的大量表現株。再以逆境試驗、致病性測試、胞外酵素分泌能力測試以及胞外多醣含量測試，分析探討其功能。目前的實驗結果顯示，在致病性測試方面 *snaR* 突變株的致病能力較差；在逆境測試部分於 SDS plate 上突變株的生長狀況也明顯較差。在胞外酵素分泌分析測試在纖維酶胞外酵素有較為顯著性的差異；在胞外多醣含量測試 *snaR* 之突變株的胞外多醣分泌含量也較少。

Keywords: *Xcc*, *snaR*, 十字花科植物, 黑腐病, *snaR*

探討十字花科黑腐病菌中 *surE* 基因之功能

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Xanthomonas campestris pv. *campestris* (*Xcc*) 為革蘭氏陰性菌，具單極性單鞭毛，形狀為桿狀。此菌會感染十字花科植物而導致黑腐病。此疾病造成全球農作物嚴重的損失。感染此疾病後，典型症狀包含 V 型的黃色病變，從葉緣慢慢感染至葉脈，沿著葉脈組織變黑而壞死。*surE* 基因是高度保守的原核生物，由文獻報導推論在其他菌種，*surE* 基因功能與細菌的生存(survival)有關，這也是此基因命名的由來。沒有人知道關於 *surE* 在 *Xcc* 的生化功能或生理作用。本研究首先構築 *surE* 突變株，利用了三種測試方法，(1)逆境測試(2)存活率(3)致病性，去測試 *surE* 突變株的生存能力。結果發現，在這些測試中 *surE* 突變沒有造成顯著的影響，因此 *Xcc* 的生理調控與其他菌種有所不同。

Keywords: *Xanthomonas campestris* pv. *Campestris* , *surE* , Brassicaceae ,
Pathogenicity assay

探討十字花科黑腐病菌中 *rpfN* 基因之功能

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Xanthomonas campestris pv. *campestris* (*Xcc*) 可以感染十字花科植物，導致黑腐病。本研究目的為利用同源重組技術構築 *Xcc rpfN* 突變株，同時利用質體大量表現 *rpfN*，構築 *rpfN* 大量表現株以及互補株。利用逆境反應、致病性及胞外酵素生成能力的比較，探討 *rpfN* 基因之功能性。就目前的實驗結果顯示，在逆境環境中 *rpfN* 突變株和野生株並無明顯差異，*rpfN* 互補株在逆境環境生長較差，然而在致病性測試中，結果顯示突變株致病能力較高，最後在胞外酵素測試中，皆無明顯的差異。

Keywords: *Xcc*, *rpfN*, 十字花科植物, 黑腐病,

探討十字花科黑腐病菌中 *cysD* 與 *nodQ* 基因之功能

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Xanthomonas campestris pv. *Campestris*(*Xcc*)能感染十字花科植物而導致黑腐病。由文獻得知，*cysD* 與 *nodQ* 會影響菌株的致病能力。本研究實驗目的在於探討在 *Xcc* 中 *cysD* 與 *nodQ* 基因的功能性，透過同源重組的技術分別在 *cysD* 與 *nodQ* 中間插入一段抗藥性基因，藉此獲得突變株，再由各項實驗分析其基因的功能性。實驗結果顯示：(1)當 *cysD* 與 *nodQ* 基因被破壞之後，感染能力會降低。(2) *cysD* 的生存能力比野生株(*Xc17*)略高，*nodQ* 則比野生株(*Xc17*)略低。

Keywords: 基因功能性, 同源重組技術, 十字花科黑腐病菌

紅鳳菜對於 RAW264.7 巨噬細胞發炎及 3T3-L1 脂肪細胞分化的影響**Effect of *Gynura bicolor* on RAW264.7 Macrophage inflammatory and 3T3-L1 Adipocytes Differentiation**

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紅鳳菜 (*Gynura bicolor*) 屬多年生的宿根草本植物，最大的特徵就是它全株呈現紫紅色，別名紅菜，可採摘作為食蔬。在民間的觀念中，紅鳳菜全草入藥可活血、止血、消腫、解毒、富含鐵質，在傳統食療裡是女性的補血聖品。目前已知發炎反應會促使誘發型一氧化氮合酶 (Inducible nitric oxide synthase, iNOS) 表現進而生成一氧化氮 (Nitric oxide, NO)，此外也會透過活化第二型環氧化酶 (Cyclooxygenase-2, COX-2) 表現進而生成促發炎產物-前列腺素 E2 (Prostaglandin E2, PGE2) 及抗發炎產物-前列腺素 D2 (Prostaglandin D2, PGD2) 的衍生物 15-deoxy- Δ 12,14-prostaglandin J2 (15d-PGJ2)，與多種發炎相關疾病之進展及組織損傷密切相關。第一型血基質氧化酶 (Heme oxygenase-1, HO-1) 是近年來被發現具有抗氧化、抗發炎及細胞保護作用。紅鳳菜在日常生活中是經常食用的蔬菜，然而是否具有抗發炎、抗氧化及抵抗脂質生成的功效是值得研究探討的。本研究進行成分分析，並以 RAW264.7 巨噬細胞及 3T3-L1 前驅脂肪細胞為模式，將紅鳳菜磨碎後以 75% 酒精浸泡進行粗萃，經減壓濃縮後完成 75% 酒精萃取物 (75% GBEE)，取得萃取物後進行 NO₂⁻ 生成試驗、油紅染色試驗以及西方墨點法分析。結果顯示，對於抑制發炎介質 NO₂⁻ 之生成並不顯著，具有些微活化 HO-1 蛋白質表現作用，在脂質生成的油紅染色試驗中 2.5mg/mL 及 3mg/mL 的濃度下具有顯著效果，而在 NBT 實驗分析中發現，當處理以 1 mg/mL 濃度之 75% GBEE，則可輕微抑制 NBT 之生成。綜合實驗結果，本研究推測紅鳳菜 75% 酒精萃取物在 RAW264.7 細胞經 LPS 刺激發炎的情況下，抑制發炎能力並不顯著；可些微提升 HO-1 蛋白質生成；在 3T3-L1 細胞分化後生成脂肪油滴，在油紅染色試驗中抑制脂肪生成可能來自於細胞死亡。整理而言，紅鳳菜可能有作為保健食品之潛力，但在抑制發炎及脂肪生成方面並不顯著。

關鍵字：紅鳳菜、抗發炎、抗脂質生成

Caffeic Acid as A Neuroinflammatory Inhibitor with Anti-oxidant Properties in Microglial Activation

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Backgrounds: Microglial activation has been widely demonstrated to mediate inflammatory processes that are crucial in several neurodegenerative disorders. Pharmaceuticals that can deliver direct inhibitory effect on microglia is therefore considered as a potential strategy to counterbalance neurodegenerative progression. Caffeic acid, a natural phenols in honeybee propolis, is known to possess antioxidant, and anti-inflammatory properties. Accordingly, the current study intended to probe the effects of caffeic acid on microglia activation by using in vitro and in vivo models.

Results: Western blots or Griess reagent assay revealed caffeic acid significantly inhibited the expressions of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and the production of nitric oxide (NO). Administration of caffeic acid resulted in increased expressions of heme oxygenase (HO)-1, erythropoietin (EPO) and suppressors of cytokine signaling (SOCS)-3 in microglia. The phosphorylated adenosine monophosphate-activated protein kinase- α (AMPK- α) was further found to regulate the anti-inflammatory effects of caffeic acid. In vivo results from immunohistochemistry (IHC) along with rotarod test also revealed the anti-neuroinflammatory effects of caffeic acid in microglia activation. **Conclusion:** The current study has evidenced several possible molecular determinants, EPO, AMPK, HO-1 and SOCS-3, in mediating anti-neuroinflammatory responses in microglial cells.

Keywords: Caffeic acid, (HO)-1, EPO , (SOCS)-3 , AMPK- α , anti-neuroinflammatory

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報名、行政及網頁

馬瑋笙/王曄淨/儲慧玫

註冊及報到組:

負責人:張益銓/吳怡瑩

組員:詹晨馨/曾煥鴻 /蘇致柔

接待及講台組:

負責人:陳昭賢/林振文

組員:楊婕/林玉苹

現場及放映組:

負責人:莊淨媛/石志榮

組員:王珮涵/曾瀟文/林儀辰

報名及餐點組:

負責人:儲慧玫/馬瑋笙

組員:史亭瑩/吳佳霈

壁報組：

負責人：黃蕙君/姜中人

組員：許博咨/黃柏翰

場佈、攝影及機動組：

負責人：劉國慶/陸自利

組員：林鈺峰/黃梅子/穆文翔

編輯組：

負責人：鄭如茜/林孟亮

組員：王玟權/賴君宜/陳育雯/楊 婕

研討會學術海報張貼注意事項

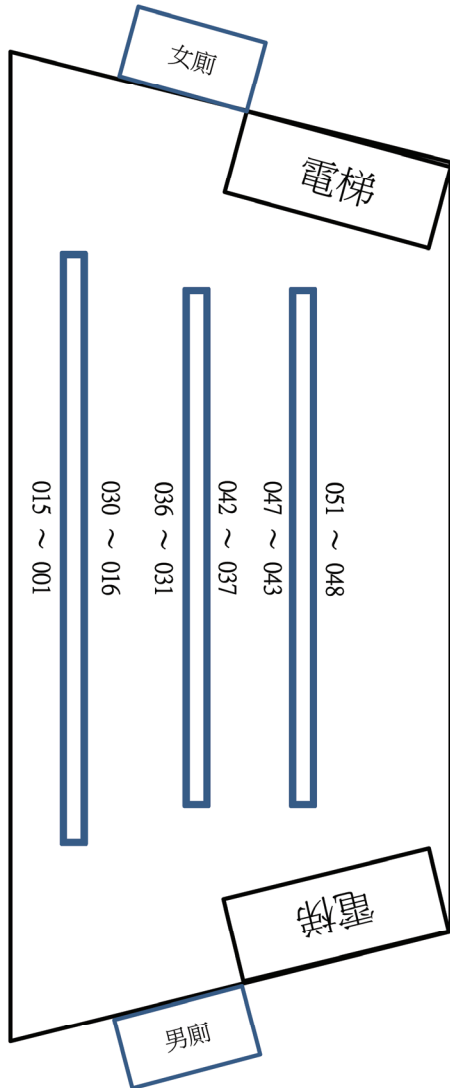
1. 請把您的海報張貼在指定的位置(請參考下一頁)，評審將於早上 10:40 至下午 14:00 為海報打分數。
2. 海報張貼於 B1 展場者，請使用報到時發放的黏土，不可破壞壁面；張貼於 2F 展場者，請使用現場供應的圖釘。
3. 海報張貼方式如下：



海報展示會場平面圖

展示會場(一)

立夫教學大樓二樓中庭



展示會場(二)

立夫教學大樓 B1

