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學生學術研究成果優良海報評選獲獎名單

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Comparison of *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine- and N-butyl-N-(4-hydroxybutyl)nitrosamine plus arsenic-induced bladder carcinogenesis

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Introduction

Bladder cancer

- Bladder cancer is reported to be the ninth most common type of cancer worldwide and 90% of these cases are a transitional cell (urothelial) carcinoma (TCC). According to their distinct genetic alternations, the initial development of TCC can be divided into two pathways: non-invasive papillary carcinoma and carcinoma in situ. (J. Urol. Nephrol. Suppl. 12-20, 2008 , Arch. Pathol. Lab. Med. 130, 844–852, 2006)
- Approximately 80% of TCCs are non-invasive papillary carcinomas which are low-grade intraurothelial neoplasias with a high recurrence rate. The remaining 20% of TCCs are initiated from carcinoma in situ and are at a high risk of progressing to muscle invasive disease with a substantial further risk of developing a distant metastasis. (Urol. Oncol. 28, 409-428, 2010 , Urol. Oncol. 28, 429–440, 2010)

Bladder cancer and bladder carcinogenesis

- Carcinogenesis can be induced by exposure to a single carcinogen, such as *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) which induces bladder tumor in rodents. (J. Cancer Res. 78, 840–845, 1987 , Science 231, 843–845, 1986)
- Humans may expose to more than one carcinogen in normal environment and carcinogenesis is usually therefore induced by the simultaneous effects of more than one factor. In rodent models, some compounds have been reported to promote BBN-induced bladder tumor formation including dimethylarsinic acid (DMA). (Cancer Res. 43, 4454–4457, 1983, Cancer Res. 43, 4454–4457, 1983 , Science 231, 843–845, 1986 , Carcinogenesis 17, 2435–2439, 1996)
- A 50 ppm DMA dose also has been reported to promote bladder tumor formation induced by five carcinogens in rats although the rats treated with DMA alone did not show pre-neoplastic bladder damage. (Cancer Res. 55, 1271–1276, 1995)

Arsenic acts as an carcinogen

- Epidemiological studies have provided compelling evidence that inorganic arsenic is carcinogenic to humans. Chronic ingestion of arsenic is known to increase the population risk of developing skin, lung, urinary bladder, and liver cancers. (IARC Scientific (Pub), Lyon, France, pp. 100– 106. 1987)
- Exposure to inorganic arsenic in humans is clearly carcinogenic. (Toxicol. and Appl. Pharmacol. 172, 249– 261. 2001)
- DMA^V, albeit at relatively high doses, is a multi-organ tumor promoter in rodents and a complete bladder carcinogen in rats. (Toxicol. and Appl. Pharmacol. 198, 366– 376. 2004)

Aim

N-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) has been used in rodent models of bladder tumor formation. We want to identify whether arsenic promotes BBN-induce bladder carcinogenesis.

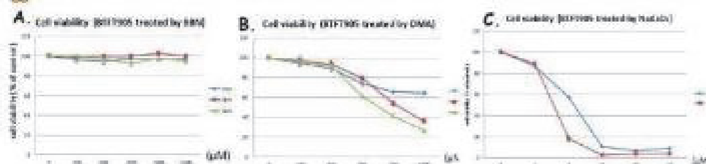
Materials and methods

RT4 cells / BFTC905 cells → Treated with BBN and/or DMA for short and long time → MTT assay; Measurement of intracellular ROS; immunofluorescence; Western blot

C57BL/6JNarl female mouse → control; BBN 300ppm; DMA 10ppm; $NaAsO_2$ 10ppm; BBN 300ppm+ DMA 10ppm; BBN 300ppm+ $NaAsO_2$ 10ppm → Treated by drinking water for 20 weeks → Sacrifice and bladder tissue collection → Western blot; H&E stain; Immunohistochemistry (IHC)

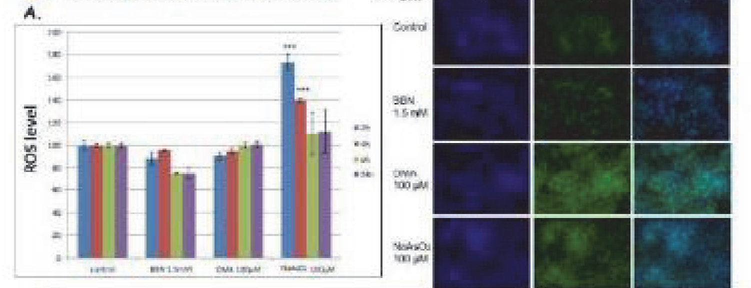
Results

Result 1 Effect of BBN, DMA and sodium arsenite on cell viability



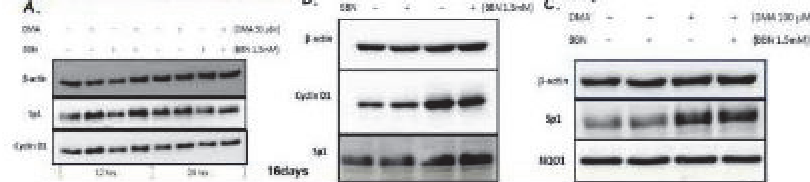
- Results are expressed as mean ± SE, n = 4.
- Cell toxicity : sodium arsenite > DMA > > BBN
- IC_{50} at 48 hour: BBN > 1.5 mM, DMA = 750 μM, sodium arsenite = 4 μM

Result 2 Up-regulation of ROS level and 8-OH-dG in BFTC905 cells after treating with sodium arsenite



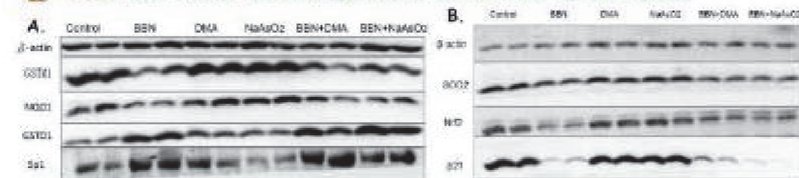
A. Relative ROS levels in BFTC905 cells expressed as fold of control. Data are expressed as mean ± SE, n = 3. ***p < 0.001 versus the control group.
B. Immunofluorescent staining of BFTC905 cells using anti- 8-OH-dG antibody (green). Nuclear staining with Hoechst 33342 (blue).

Result 3 Long time DMA exposure induces Sp1 and cyclin D1 protein expression in RT4 Cells.



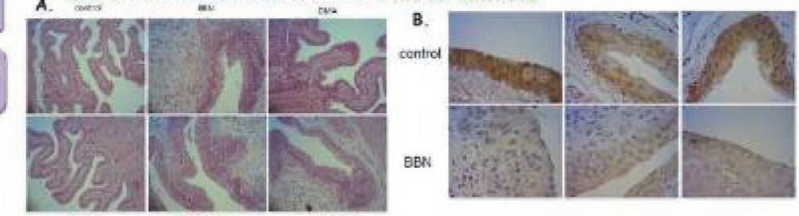
- RT4 cells were treated with BBN or/and DMA for one day, specificity protein 1 (Sp1) protein and cyclin D1 protein levels did not change.
- When RT4 cells were treated with DMA for 16 days, both Sp1 and cyclin D1 protein levels were up-regulated. In the combination of DMA and BBN, no significant change comparing with DMA alone.

Result 4 Effects of BBN and/or arsenic on the NQO-1, Sp1, GSTO1, GSTM1, SOD2, Nrf2 and p21 protein expression levels in the mouse bladder



- After drinking BBN alone and BBN combined with arsenic for 20 weeks, the proteins of Sp1 and GSTO1 were up-regulated while GSTM1, NQO1, Nrf2, SOD2 and p21 were down-regulated.
- In comparison of BBN alone and BBN combined with arsenic, Sp1 protein was slightly increased in the combination group.

Result 5 Effects of BBN and/or arsenic on the histological changes in the mouse bladder tissues. Down-regulation of GSTM1 expression in the mouse bladder tissues after treated with BBN for 20weeks.



- A . Histological changes in the mouse bladder tissues of BBN and/or arsenic treatment groups. Magnification : 100x , B. Immunostaining results using anti-GSTM1 antibodies. Magnification : 400x
- No significant difference between BBN alone and BBN combined with arsenic in HE stain.
- Down-regulation of GSTM1 protein in IHC stain.

Conclusion and Discussion

1. In cell assay, DMA but not BBN increases Sp1 and cyclin D1 protein expression. The inability of BBN on Sp1 elevation may due to the deficiency of liver metabolism in bladder cells.
2. In mouse model, BBN down-regulates GSTM1, NQO1 and SOD2 protein expression, which may caused by BBN- decreased Nrf2 expression. In addition, BBN also decreases p21 protein expression, that may increase cell proliferation.
3. In the results of H&E stain, BBN with/without arsenic induces urothelial hyperplasia in mouse.
4. Arsenic enhances BBN-increased Sp1 protein expression in mouse bladder epithelial cells, which may promote bladder cancinogensis.

The Gene Expression of Glutathione S-Transferase Mu Family Induced by A DNA Demethylating Agent in 7 Human Bladder Cancer Cell Lines

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介紹

穀胱甘肽S-轉移酶Mu家族

- 穀胱甘肽*S*-轉移酶 (glutathione *S*-transferase, GST) Mu家族，大多存在於細胞質中，屬於phase II解毒酵素，而GST Mu家族又可分5個亞型，分別從M1到M5。 Drug metabolism reviews 2011;13:179-193
- 穀胱甘肽*S*-轉移酶Mu家族其酵素功能為透過與穀胱甘肽的結合，造成親電子性化合物的去毒性作用，其中這些親電子化合物包括一些致癌物質、治療藥物、環境毒素和氧化壓力的產物。Nat Rev Urol 2009;6:281-289
- 不同的GSTM亞型酵素分別具有不同的組織分佈及代謝物質選擇性，而穀胱甘肽*S*-轉移酶Mu家族基因在DNA編碼上的分布均位在一號染色體上且位置相近，此位置也被認為具高度多型性。 BMC genetics 2012;13:89. ; Pharmacology 2000;61:154-166.

5-aza-2'-deoxycytidine藥物

- 5-aza-2'-deoxycytidine藥物主要是作用於抑制DNA甲基化轉移酶，使基因無法甲基化並活化具有轉錄活性的基因，可以重新啟動無法正常表達的基因，能活化癌細胞中抗增殖、細胞凋亡和誘導細胞分裂的相關基因，目前使用於治療骨髓性白血病和骨髓造血不良症候群。 *Oncogene* 2000;21:5483-5495.

GSTM基因DNA甲基化與膀胱癌間關係之研究

- 由於DNA甲基化與癌症的形成密切相關，因此偵測特定的基因DNA甲基化程度可作為癌症之診斷與預後訊息。*DAPK*、*RARB*、*e-cadherin*和P16這些基因已經被證實在早期膀胱癌中具有高度甲基化的情形，該研究同時測量膀胱檢體及尿液檢體作DNA甲基化研究，發現在尿液檢體中檢出的敏感度達90.9%。 Clin Cancer Res 2002;8:464-470.
- 近年來有許多文獻指出在不同族群中，GSTM基因多型性的表現與癌症有很大的關連，像是大腸直腸癌、肺癌與乳癌等。而當個體中存在GSTM1-null或GSTT1-null基因型，會使個體無法產生GSTM1或GSTT1蛋白，產生較低的酵素活性，當這些人暴露於特定的化學物質時，會使得這些個體容易罹患泌尿系統相關癌症，例如:膀胱癌、腎癌等。 Cancer Epidemiol Biomarkers Prev 1997;6:733-743. ; Nat Rev Urol 2009;6:281-289

實驗目的

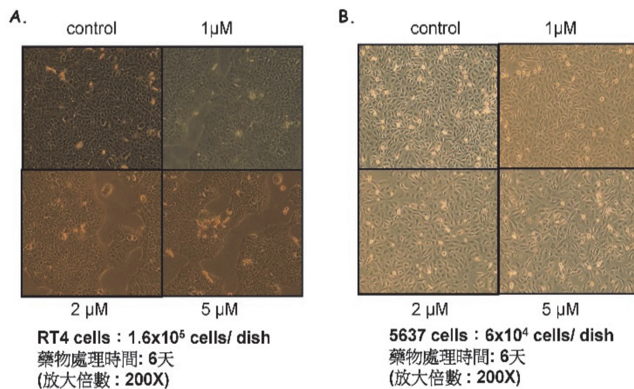
本篇研究目的是為了調查在7株不同癌化程度的人類膀胱癌細胞株中，分析其GSTM1基因甲基化情形，檢測其GSTM1基因是否具有DNA甲基化的現象，此外，GSTM家族具有五個亞型，皆位於一號染色體相連的位置上，因此一起併入分析，觀察這些穀胱甘肽轉移酶Mu家族的基因表現情形，探討其DNA甲基化在膀胱癌發展中的影響，並評估其做為診斷膀胱癌的生物標誌之可行性。

材料與方法

利用 5-aza-dC 藥物處理膀胱癌上皮細胞株 → RT4 cells, 5637 cells, TSGH 8301 cells, BFTC 905 cells, HT1376 cells, T24 cells, J82 cells → RT-PCR

結果

結果 1 RT4及5637人類膀胱癌細胞株經5-aza-dC藥物處理後之細胞生長情形

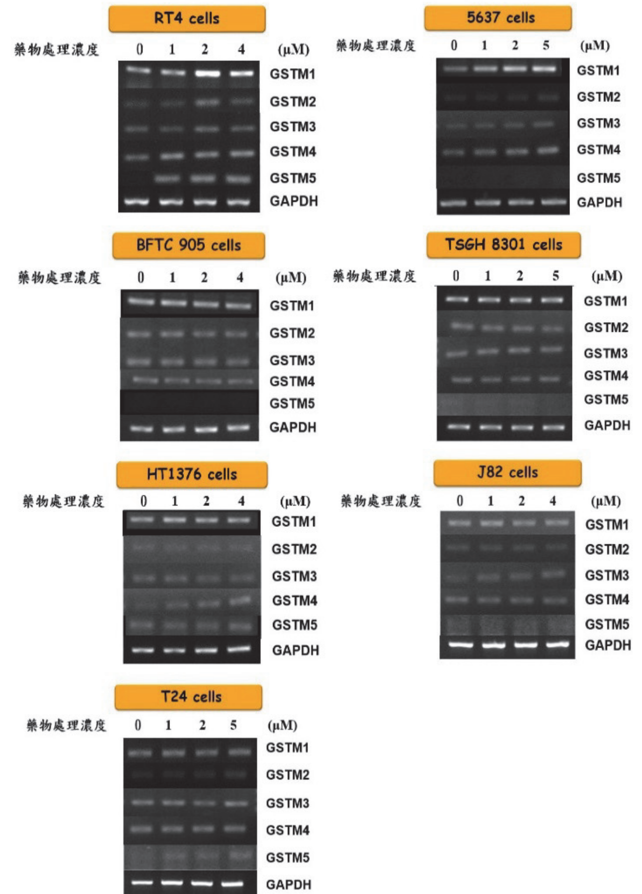


RT4 cells：1.6x10⁵ cells/ dish
藥物處理時間: 6天
(放大倍數：200X)

5637 cells：6x10⁴ cells/ dish
藥物處理時間: 6天
(放大倍數：200X)

- (A). 人類膀胱上皮癌細胞株RT4在5-aza-dC藥物處理下，細胞有隨著藥物濃度增加而減緩細胞生長和浮細胞增加的情形。
- (B). 人類膀胱上皮癌細胞株5637在5-aza-dC藥物處理下，細胞隨著藥物濃度增加而減緩細胞生長和浮細胞增加的情形比RT4細胞株更為明顯。

結果 2 7株不同癌化程度的人類膀胱癌細胞株中穀胱甘肽*S*-轉移酶Mu家族基因表現情形



- (A). 在膀胱上皮癌細胞RT4和5637中有看到GSTM家族基因因處理DNA甲基化抑制劑而表現量上升的趨勢。
- (B). 藥物濃度的調整依不同細胞株的生長情形做微調，最高處理到5μM。
- (C). 膀胱上皮癌細胞株依癌化程度(Grade)由上至下排列。RT4細胞株為Grade I，5637、TSGH8301、BFTC905細胞株為Grade II， J82、T24 和1376細胞株為Grade III，結果發現在較早期的膀胱癌細胞株其GSTM家族基因DNA甲基化現象發生率較高。

結果與討論

- 結果發現在癌化程度較低的RT4細胞株和5637細胞株中，其GSTM家族5個基因表達皆有上升的趨勢，而在其他癌化程度較高的另5株細胞株(TSGH8301、BFTC905、J82、T24和1376)則沒有此增加情形。此結果指出GSTM家族的基因表現，可能在人類膀胱癌發展的早期會被DNA甲基化關閉。
- 關於DNA被甲基化的確切位點，必須再利用焦磷酸測序技術，針對CpG island每個甲基化位點進行定性及定量檢測，找出具高度甲基化的DNA CpG位點。若能找出GSTM家族基因中具高度甲基化頻率的DNA CpG位點後，可進行Methylation Specific Primer的設計，方便往後大量檢體篩檢之用。另一方面，在BBN誘導小鼠形成膀胱癌的過程中，膀胱上皮細胞之GSTM1基因promoter確實之CpG甲基化位點也必須再利用焦磷酸測序技術檢測得知。

結論

- 在這些受DNA CpG甲基化而下調的穀胱甘肽*S*-轉移酶Mu家族M1~M5中，可挑選出作為檢測尿液細胞DNA之運用，將來也許可以開發為診斷膀胱癌及預防監測的一項生物標誌參考。

分析鴨疫雷氏桿菌生物膜形成之相關基因

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[1]Department of Microbiology, Immunology and Biopharmaceuticals, National Chiayi University

摘要

背景：鴨疫雷氏桿菌（*Riemerella anatipestifer*, *R. anatipesifer*）會引起水禽雷氏桿菌症，是一種嚴重的傳染性疾病，屬於黃桿菌科，目前已有21種血清型被分離出來。根據文獻，莢膜生合成的能力在*Streptococcus pneumonia*已被確認會影響生物膜生成之群落階段。而*R. anatipesifer*生物膜形成有關的基因，也曾被推測可能與其致病或存留於環境中有關。然而，參與*R. anatipesifer*生物膜形成相關基因仍缺乏進一步的探討。**材料與方法**：本實驗藉由體外生物膜的形成作為毒性指標，分離株形成生物膜的能力則分別以結晶紫染色法以及濾膜秤重法進行分析。另一方面，利用以細菌為食的秀麗隱桿線蟲（*Caenorhabditis elegans*, *C. elegans*）作為感染模式生物，使其食入不同基因型capsule genes之*R. anatipesifer*後分析生存曲線的變化，以判斷capsule genes對宿主毒力的重要性。透過隨機轉座子（transposon）突變，建立*R. anatipesifer*突變菌株庫，鑑定參與*R. anatipesifer*生物膜生成的基因。**結果與討論**：由結晶染色法與濾膜稱重法得知*R. anatipesifer* RA03的生物膜形成量最高，而*R. anatipesifer* RA04的生物膜形成量最低。進一步分析莢膜基因分型對生物膜形成之關聯，發現所增幅之PCR產物經比對後屬於同個基因型。然而，不同生物膜形成量之*R. anatipesifer*對秀麗隱桿線蟲的毒力卻有所差異。由此猜測本研究分析之莢膜基因並非主要影響生物膜形成之基因。透過建立*R. anatipesifer*突變菌株庫，進一步找出與生物膜有關之候選基因。

材料與方法

菌種

*R. anatipestifer*菌株為2006 年到2008 年間分離自台灣病鴨（國立嘉義大學獸醫學系 陳秋麟教授研究室分讓）。透過抗原抗體血清凝集法鑑定血清型2之*R. anatipestifer*。

體外生成生物膜定量分析系統—結晶紫分析法

將隔夜培養的菌液稀釋至OD_{600}=0.5，與酒精殺菌處理後之蓋玻片 (Marienfeld Laboratory Glassware, 18x18 mm) 共培養72小時。利用結晶紫染劑染色30分鐘後，以75%酒精震盪脫色10分鐘，測量OD_{595}的吸收光讀值。

體外生成生物膜定量分析系統—濾膜秤重分析法

將隔夜培養的菌液稀釋至OD_{600}=0.5，與cellulose membrane (Mixed Cellulose Filter, 0.8 μm x 13 mm dia White Grid, Millipore) 共培養72 小時。乾燥後，計算重量差。

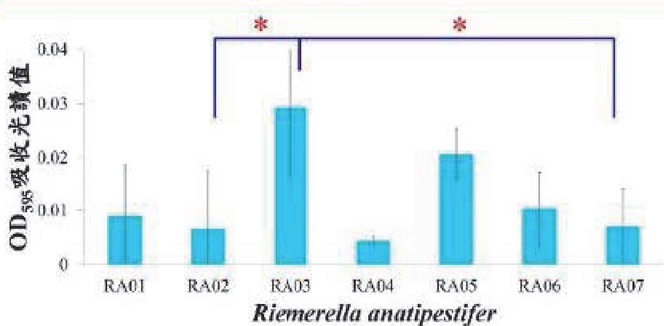
Capsule-related genes之定序分析

設計特異引子對，將*R. anatipestifer*莢膜相關基因以PCR分子技術增幅，進行核酸定序，將各菌株的contiguous sequence藉由MegAlign中Clustal V method分析其核苷酸序列或胺基酸序列上的差異。

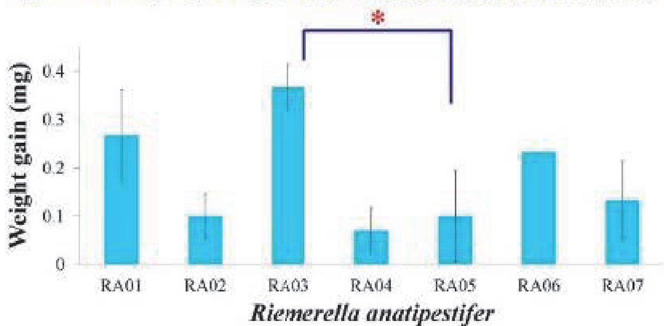
C. elegans 之毒力因子的分析

將Larvae 4 stage的線蟲各別餵以*E. coli* OP50做為對照組及*R. anatipestifer*做為實驗組。每24小時培養後，將存活線蟲利用滅菌白金絲挑至新感染平盤，以Prism5軟體 (GraphPad Software Inc.) 計算生存死亡率。

結果



圖一，*R. anatipestifer*血清型2菌株之生物膜生成指標-結晶紫分析法



圖二，*R. anatipestifer*血清型2菌株之生物膜生成指標-濾膜秤重分析法

體外生成生物膜定量分析系統

由圖一（結晶紫分析法）與圖二（濾膜秤重分析法）的結果得知，*R. anatipestifer* RA03的生物膜形成量最高；*R. anatipestifer* RA04的生物膜形成量最低。（*: $p<0.05$）

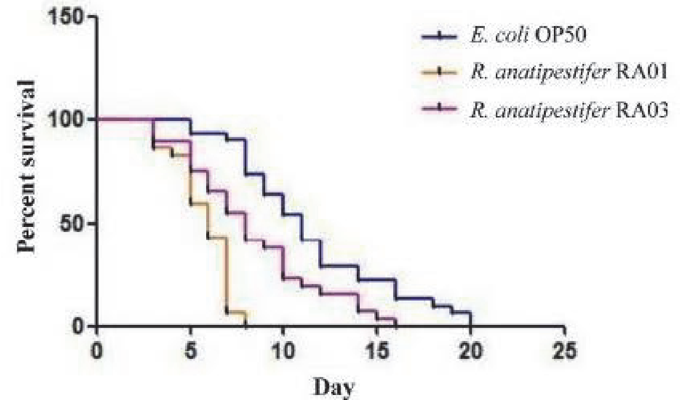
Capsule-related genes之定序分析

如圖三所示，本研究分析之莢膜基因capsule expression protein KpsF/GutQ (*kpsF/gutQ*) 以及poly-gamma-glutamate synthesis protein (*GSP*) 經定序分析後，*R. anatipestifer* RA01至*R. anatipestifer* RA07核苷酸序列無差異，皆屬於同個基因型。

圖三，*R. anatipestifer*血清型2菌株*kpsF/gutQ*基因片段之序列

C. elegans 之毒力因子的分析

圖四的結果，以*E. coli* OP50為對照組（藍綫），*R. anatipestifer* RA01（橘綫）以及*R. anatipestifer* RA03（紫綫）為實驗組，探討秀麗隱桿線蟲食入*R. anatipestifer*後生存曲綫的變化。本次實驗結果，*E. coli* OP50的median survival為11天；*R. anatipestifer* RA01為6天；*R. anatipestifer* RA03為8天。



圖四，*R. anatipestifer* RA01與*R. anatipestifer* RA03的感染試驗

結論

1. 血清型2之*R. anatipestifer*中，*R. anatipestifer* RA03的生物膜形成量最高；*R. anatipestifer* RA04的生物膜形成量最低。
2. 本研究分析之莢膜基因於血清型2之*R. anatipestifer*中皆屬於同個基因型，由此猜測該莢膜基因並非主要影響生物膜形成之基因。
3. 不同生物膜形成量之*R. anatipestifer*菌株對秀麗隱桿線蟲的毒力有所差異。
4. 透過建立*R. anatipesifer*突變菌株庫，進一步找出與生物膜有關之候選基因。

Analysis of Proinflammatory Response and Mechanism in Human Urinary Epithelial Cells Infected by *Candida albicans*

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Introduction

Candida albicans (*C. albicans*)

- *C. albicans* are normal constituents of the normal oral, urinary tract and gut flora in ~50% of the population and are also the most common fungal pathogen that can cause a variety of mucosal diseases with significant morbidity (Candida and Candidosis : Philadelphia Bailliere Tindal 1988).
- According to statistics, the majority of fugal infections are caused by the Candida species, and the most proportion is *C. albicans*, which accounting for 72.3% of the pathogenic Candida species (Mycopathologia 2011;172:131-139).
- *C. albicans* morphology mainly divided into three types: yeast form, pseudohyphae type, and hyphae and the first theory proposes that *C.albicans* must undergo morphogenic conversion from yeast to hyphal forms within the circulation which makes it more capable of invading the tissues (Infect. Immun 2000;68:3485-3490; Infect. Immun 2000;72:598-601).
- Through observing urine, blood, sputum and other common human body fluids, isolated Candida strains was mostly collected from urine, and up to 45.2% of Candida use urine as a source of nutrition (Diagnostic Microbiology and Infectious Disease 2013;76(2):182–186). The mortality rates after candidiasis infection is as high as 30-60% (Infectious Diseases Society of America 1995:20:1531-4).

C. albicans-induced Proinflammatory Response in Epithelial Cells

- Mucosal epithelia possess distinct mechanisms that enable discrimination between harmless commensal organisms and disease-causing pathogenic organisms. This process results in either nonresponsiveness or homeostasis (commensals) or activation of an immune response (pathogens) (Cell Host & Microbe 2010;8:225-235).
- Under suitable predisposing conditions, *C. albicans* is able to cause a variety of mucosal diseases with significant morbidity and potentially fatal disseminated infections in immunocompromised individuals and patients on immunosuppressive regimens (Candida and Candidosis: Philadelphia Bailliere Tindal 1988 ; Crit. Rev. Microbiol 2010;36:1-53).
- Glycosylation of *C. albicans* cell wall proteins is critical for induction of innate immune responses and IL-6, IL-8 or other proinflammatory cytokines activation and apoptosis of epithelial. (Infect. Immun Dec 2011;4902-4911; PLOS ONE 2012;7(11): e50518; Cell Host Microbe 2010;8:225-235).

Aim

This study aimed to identify the proinflammatory mediators in *C. albicans*–infected human bladder epithelial cells. We want to investigate proinflammatory mechanism of bladder epithelial cell infected by *C. albicans*. We hope to supply some alternative methods that can help patients with urinary tract infected by *C. albicans*.

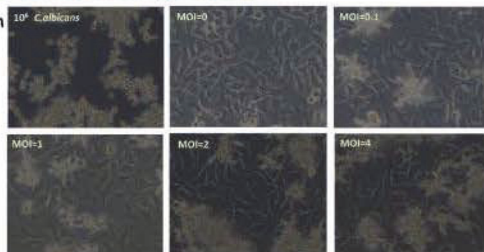
Materials and methods

Urinary epithelial cells co-incubated with *C. albicans* → TSGH 8301 cells → Microscopy / LDH assay / RT-PCR / Western blot / PGE_2 assay

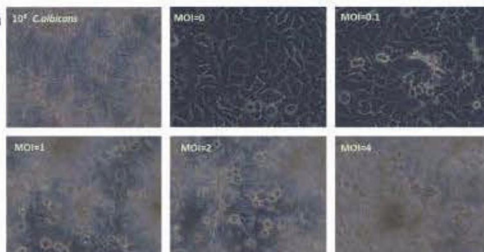
Results

Figure 1 Morphology of human bladder epithelial cells TSGH 8301 co-incubated with *C. albicans* for fungal burden

A. co-incubation time: 4 h
Magnification: 400X

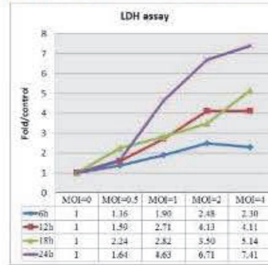


B. co-incubation time: 24 h
Magnification: 400X



1. After infection for 4 h, major form of *C. albicans* are germ tube and yeast type, and at 24 h, the major form shifts to hyphae and pseudohypha.
2. Compare to multiplicity of infection (MOI) of 0, the bladder epithelial cells morphology had change and damage after of *C. albicans* infection for 24 h.

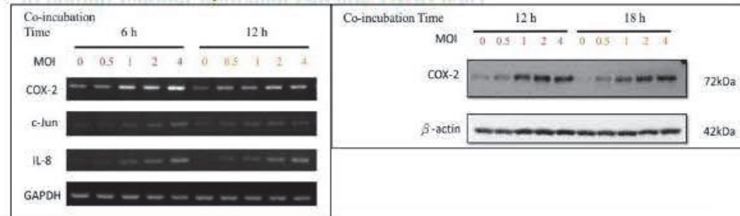
Figure 2 Induction of epithelial cell damage by *C. albicans* infection



	MOI=0	MOI=0.5	MOI=1	MOI=2	MOI=4
6h	1	1.36	1.90	2.48	2.30
12h	1	1.59	2.71	4.13	4.11
18h	1	2.24	2.82	3.50	5.14
24h	1	1.64	4.63	6.71	7.41

1. At different infection time, the higher the number of *C. albicans*, the more damage of TSGH8301 cells.
2. In MOI of 4, TSGH8301 cells are significant damage after infection for 24 h.

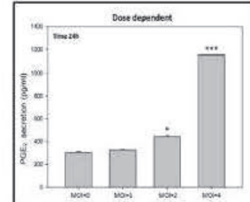
Figure 3 Gene and protein expression induced by *C. albicans* Infection in human bladder epithelial cell line TSGH 8301



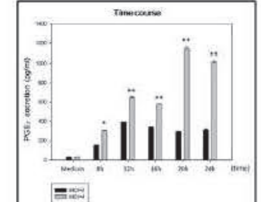
The induction of IL-8 and COX-2 gene or protein are significant expression in TSGH8301 cells after *C. albicans* infection at 6 h, 12 h and 18 h.

Figure 4 Culture medium prostaglandin E_2 (PGE_2) accumulation after TSGH 8301 cells infected by *C. albicans*

A. Dose-dependent at 24 h



B. Time-course in MOI=4

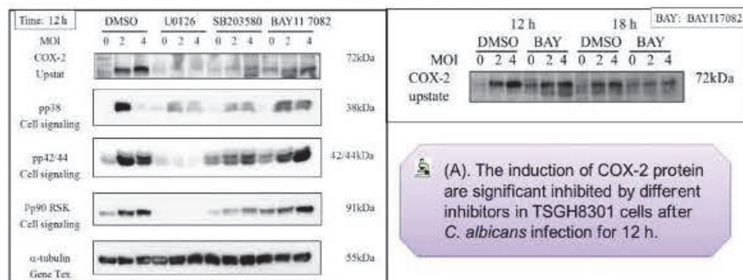


(A). After infection for 24 h, culture medium PGE_2 accumulation are evident in MOI of 2 and MOI of 4.

(B). In MOI of 4, culture medium PGE_2 accumulation are evident after TSGH 8301 cells infected by *C. albicans* for 8-24 h.

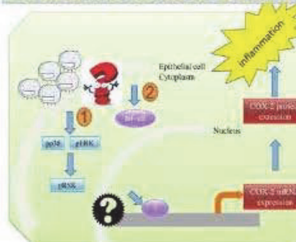
$^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ (MOI=0 versus MOI=4).

Figure 5 *C. albicans*-induced COX-2 expression is suppressed by inhibitors of MAPK and NF-κB signal pathways



(A). The induction of COX-2 protein are significant inhibited by different inhibitors in TSGH8301 cells after *C. albicans* infection for 12 h.

Conclusion and Discussion



In this study, we found that *C. albicans* caused the bladder epithelial cells morphology change, cell damage and inflammatory response including cyclooxygenase-2 (COX-2) and interleulin-8 (IL-8) expression and PGE_2 accumulation. The COX-2 gene was induced majorly though MAPK and partially NF-κB pathway.

In the future, we will identify the transcription factor(s) and promoter binding site(s) of *C. albicans*-induced COX-2 gene in bladder epithelial cells.

Synthesis of New Lipophilic Substituent Baicalein Derivatives and Biological Evaluation as Potent Antitumor Agents

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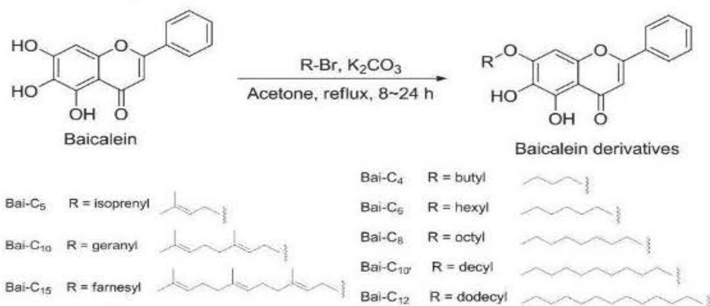
INTRODUCTION

Colon cancer is one of the most common malignant diseases worldwide. It always accompany poor prognosis and the more person are diagnosed in recent years in Taiwan. Baicalein, a flavonoid from *Scatellariae baicalensis* Georgi (*S. baicalensis*), has many pharmacological effect and demonstrates cytotoxicity in a variety of tumor cell line. But, baicalein is become over 90% converted to baicalein-7-*O*-glucuronide (baicalin) in the intestinal. In hepatic metabolic study, baicalein is more rapid glucuronidation and sulfation in 7th position of hydroxy group that in the intestinal. In the study, we modified baicalein by lipophilic substitution at the 7th position hydroxy group of A ring to terpenyl derivatives, including isoprenyl, geranyl, farnesyl groups and alkyl derivatives, including butyl, hexyl, octyl, decyl, dodecyl groups. The cytotoxic effects treated with baicalein or 7-*O*-farnesylbaicalein (**Bai-C_{15}**) in human colon cancer cell lines, human hepatocellular carcinoma and normal embryonic murine BNL CL.2 cell lines, cell growth inhibition was evaluated by MTT assay. Using flow cytometry examined cell cycle distribution by PI staining and apoptosis by annexin V-FITC/PI dual staining methods on human colon cancer SW480 cell lines. There from among those small-molecule compounds, 7-*O*-farnesylbaicalein (**Bai-C_{15}**) is significant potential anticancer activity in human colon cancer cell lines (HT-29, SW480 and DLD-1). **Bai-C_{15}** significantly inhibits the growth of human colon cancer cell lines, a little or no cytotoxicity in human hepatocellular carcinoma HepG2 cell lines and normal embryonic murine BNL CL.2 cell lines. In cell cycle analysis, **Bai-C_{15}** induces SW480 that arrested cell cycle at S phase and induced apoptosis. In wound-healing assay, **Bai-C_{15}** had a strong effect in inhibition cell migration. In summary, these preliminary results showed that **Bai-C_{15}** has a variety of function to inhibition in human colon cancer cells. That could to develop a new potential drug in human colon cancer therapy.

MATERIALS AND METHODS

Chemistry

Scheme 1: Synthesis of 7-*O*-substituted baicalein derivatives.



RESULTS

Table 1. Cytotoxicity of baicalein and its derivatives on human cancer cell line for 48 h

	R	IC_{50} (μM)				
		SW480 Colon	HT-29 Colon	DLD – 1 Colon	HepG2 Liver	BNL CL.2 Normal cells
Baicalein	H	18.18 ± 0.89	30.61 ± 0.46	27.88 ± 0.31	28.09 ± 0.51	> 40
Bai-C_1	Methyl	29.41 ± 0.46	> 40	30.93 ± 0.65	> 40	> 40
Bai-C_5	Isoprenyl	8.60 ± 0.37	> 20	> 20	> 20	> 20
Bai-C_{10}	Geranyl	2.84 ± 0.43	17.02 ± 0.25	9.77 ± 0.93	> 20	> 20
Bai-C_{15}	Farnesyl	**1.15 ± 0.15**	14. 95 ± 0.63	6.97 ± 0.15	> 20	> 20
Bai-C_4	Butyl	> 20	19.48 ± 0.35	> 20	> 20	> 20
Bai-C_6	Hexyl	9.48 ± 0.47	17.65 ± 0.16	19.01 ± 0.52	> 20	> 20
Bai-C_8	Octyl	3.03 ± 0.46	16.25 ± 0.62	15.41 ± 0.76	> 20	> 20
Bai-$C_{10'}$	Decyl	1.99 ± 0.38	> 20	15.52 ± 0.47	> 20	> 20
Bai-C_{12}	Dodecyl	**1.57 ± 0.20**	> 20	9.26 ± 0.10	> 20	> 20
Wogonin	H	35.06 ± 3.84	39.55 ± 0.23	36.87 ± 0.65	37.89 ± 2.26	> 40
Wo-C_1	Methyl	> 20	> 20	> 20	2.72 ± 0.84	> 20
Wo-C_5	Isoprenyl	> 20	> 20	> 20	> 20	> 20
Wo-C_{10}	Geranyl	> 20	> 20	> 20	> 20	> 20
Wo-C_{15}	Farnesyl	> 20	> 20	> 20	> 20	> 20
Chrysin	H	31.08 ± 2.96	19.49 ± 0.38	18.62 ± 0.81	16.50 ± 0.36	> 40
C-C_1	Methyl	> 20	> 20	> 20	> 20	> 20
C-C_5	Isoprenyl	> 20	> 20	> 20	> 20	> 20
C-C_{10}	Geranyl	> 20	> 20	> 20	> 20	> 20
C-C_{15}	Farnesyl	> 20	> 20	> 20	> 20	> 20

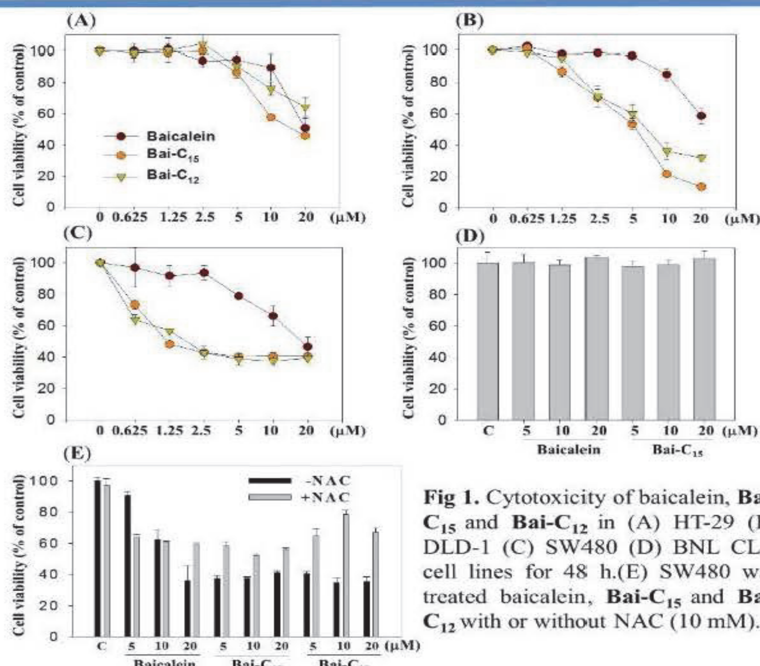


Fig 1. Cytotoxicity of baicalein, **Bai-C_{15}** and **Bai-C_{12}** in (A) HT-29 (B) DLD-1 (C) SW480 (D) BNL CL.2 cell lines for 48 h.(E) SW480 was treated baicalein, **Bai-C_{15}** and **Bai-C_{12}** with or without NAC (10 mM).

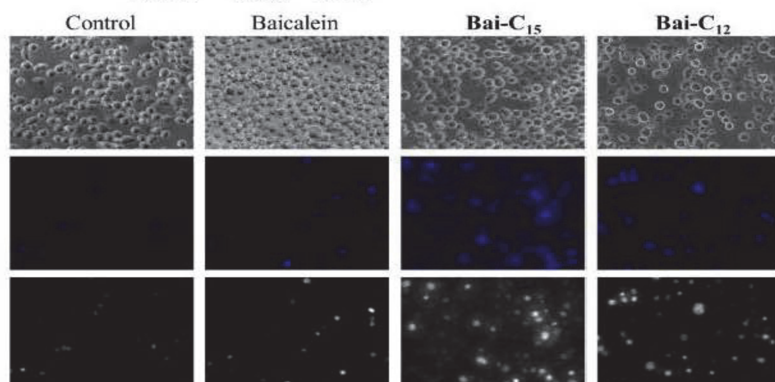


Fig 2. Effect of baicalein, **Bai-C_{15}** and **Bai-C_{12}** (20 μM) for 48 h, and then were stained with Hochest 33258 on the morphological (400×) in SW480.

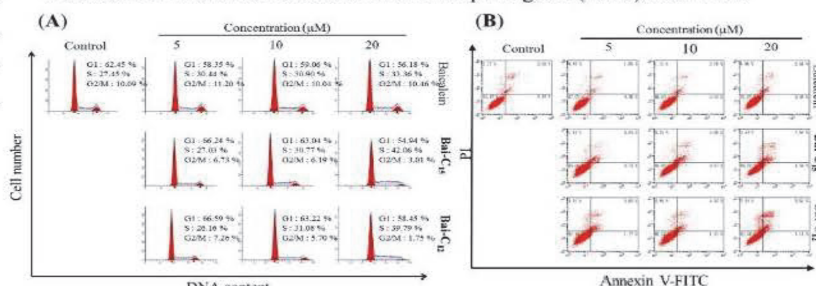


Fig 3. Cell cycle distribution (A) and apoptotic analysis (B) of SW480 cell lines treated with baicalein, **Bai-C_{15}** and **Bai-C_{12}** (5–20 μM) by flow cytometer.

CONCLUSIONS

In this study, we synthesized a series of lipophilic substitution flavonoid derivatives on 7th position hydroxyl group. In addition, **Bai-C_{15}** and **Bai-C_{12}** were better activity in human colon cancer cells. **Bai-C_{15}** compare with baicalein and it's derivatives that has the lowest IC_{50} value in SW480 cells and no cytotoxicity in BNL CL.2 (normal murine cell lines). However, NAC partly reduced the accumulation of ROS induced by **Bai-C_{15}**. Treatment with **Bai-C_{15}** increased cell shrinkage and DNA fragment in SW480 cells. The flow cytometric analysis, **Bai-C_{15}** mainly caused S phase arrest and increased apoptotic rate in SW480 cells. In conclusion, **Bai-C_{15}** can as a potential antitumor agent on human colon cancer cell lines in the further.

Molecular Epidemiology Studies of Invasive Infections Caused by *C. albicans* in a Hospital in North Taiwan from 2003 to 2011

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Background: *Candida albicans* is a commensal of humans but is also the main fungal pathogen of humans. As the changing of medical environment, candidemia is an increasing common cause of blood infection in hospitalized patients. Investigation of genetic evolution of *C. albicans* demonstrated the geographic clustering, and also hinted some association between antifungal resistance and genetic traits. **Methods:** Multilocus sequence typing (MLST), which based on sequencing 7 selected house-keeping genes and identifying polymorphic nucleotide sites, has been introduced to the characterization of *C. albicans* isolates for epidemiological purposes. **Results:** After analysis of allelic sequences of 330 *C. albicans* isolates from Chang-Gung Memorial Hospital Linkou (CGMHL) from 2003 to 2011, 125 isolates were assigned diploid sequence types (DSTs) but more than hundred isolates matched no DST in MLST database. Furthermore, by a phylogenetics algorithm eBURST analysis, we found that DST659 is possibly the most common clonal founder in CGMHL isolates during 2003 to 2011. With four much variable allele sequences of seven, UPGMA and neighbor joining methods were applied to create phenograms. Three cluster groups, DST659-like, DST669-like and DST69-like, were clearly separated. Interestingly, a subset of DST659-like group showed significantly higher biofilm formation than other groups. **Conclusions:** The association between biofilm formation and the cluster expansion of DST659 is worth further investigation.

Materials & Methods

Candida albicans isolates

All *C. albicans* isolates were collected from Chang-Gung Memorial Hospital Linkou (CGMHL) from 2003 to 2011. 288 isolates (87.3%) were collected from blood cultures obtained from invasive candidiasis patients, and 42 (12.7%) were isolated from candiuria patients without candiddemia. All of these isolates were identified by conventional methods, and the results were further confirmed with the API ID32C system (bioMerieux) or CHROMagar Candida (BD).

Multilocus sequence typing (MLST) analysis

The *C. albicans* MLST is based on the method developed by Bougnoux et al.. *C. albicans* MLST typing is based on variations of 7 housekeeping gene loci, including AAT1a、ACC1、VPS13、MPIb、ADP1、ZWF1b and SYA1. The internal regions of these genes were PCR amplified and sequenced. Each sequence result was assigned with an allele number (http://calbicans.mlst.net/), and then the combination of the 7 allele numbers defined a unique diploid strain types (DSTs) representing its genotype. The numbered clades of *C. albicans* were described as previously. A dendrogram was constructed with the use of un-weighted pair group method with arithmetic Mean (UPGMA). Numbers at nodal points indicate bootstrap values for 1,000 replications.

eBURST analysis

Putative relationships between isolates were determined by eBURST (http://eburst.mlst.net/). The eBURST algorithm was used for not only placing all related isolates into clonal complexes but also predicting the ancestral DST of each complex. The results of eBURST displayed the most parsimonious patterns of each descent from the ancestral DST type.

Antifugal susceptibility testing

A commercially prepared dried colorimetric microdilution panel (Sensititre YeastOne, TREK Diagnostic Systems) was used for susceptibility testing of *Candida* to 5-flucytosine, amphotericin B, anidulafungim, caspofungin, fluconazole, itraconazole, micafungin, posaconazole, and voriconazole, in accordance with the manual provided. In brief, 24-h incubated yeast isolates were suspended into distilled water and the density was adjusted to 0.5 McFarland, followed by 1/550 dilution into 11 ml YeastOne medium (1.5-8 x 10^8 cfu/ml at final). Diluted solution and antifungal agents were added into 96-microtiter wells, which were then incubated at 35 ℃ without CO_2 for 24 h. The minimal inhibition concentration (MIC) was finally determined according to the guideline provided. MIC_{50} and MIC_{90} values of *C. albicans* isolates against each antifungal agent were also calculated.

In vitro biofilm weighting analysis

Sterile MF-Millipore membrane (0.8 μm pore size) (Millipore) were placed into 12-well culture wells, and incubated with 1-ml fetal bovine serum for 16 hr. The membranes were PBS washed and wet with Spider medium (10 g/L mannitol, 20 g/L Bacto peptone, 10 g/L Nutrient broth, 10 g/L yeast extract, 4 g/L potassium phosphate dibasic, pH7.2), following inoculation with *C. albicans* for 90 min at 37 ℃. Unbound *C. albicans* yeasts were removed by PBS washing, and then incubated with sterile Spider medium for 60 hours. Finally, the PBS washed filter membranes were air dried and weighted. Blank was performed with non-inoculation membrane in the same procedure, while a biofilm defective strain ΔTEC1 was used for negative control and its congenic parental wild type *C. albicans* SC5314 was used for positive control. The mean with 2 standard deviations of ΔTEC1 membrane weights were used for the threshold of biofilm formation. *C. albicans* SC5314 (a/a) and ΔTEC1 were performed with 9 different replicates independently.

Results

Table 1. The list of *C. albicans* isolates collected from different years in CGMHL

year	Numbers of *C. albicans*	ICU		Pediatrics	Candiduria
		Candidemia	Candidemia Candiduria		
2011	40	29	0	6	5
2010	38	18	6	7	7
2009	44	14	13	7	10
2008	45	20	11	5	9
2007	48	19	13	10	6
2006	34	21	3	9	1
2005	31	14	5	9	3
2004	30	21	0	8	1
2003	26	14	0	12	0
Total	330	164	51	73	42

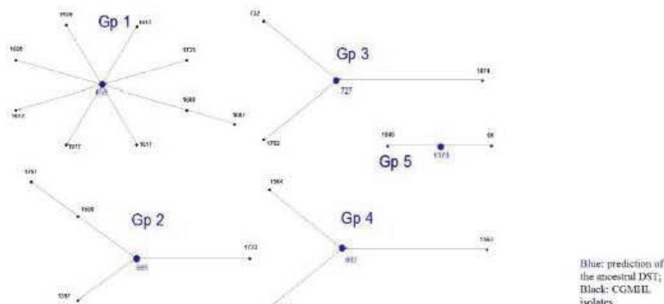

Figure 1. The diagram of eBURST analysis for *C. albicans* in CGMHL

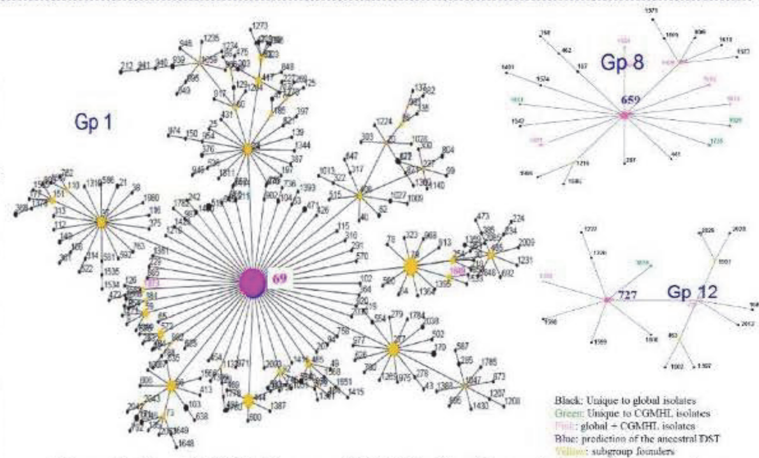

Figure 2. The eBURST diagram of CGMHL *C. albicans* isolates in comparison with global isolates

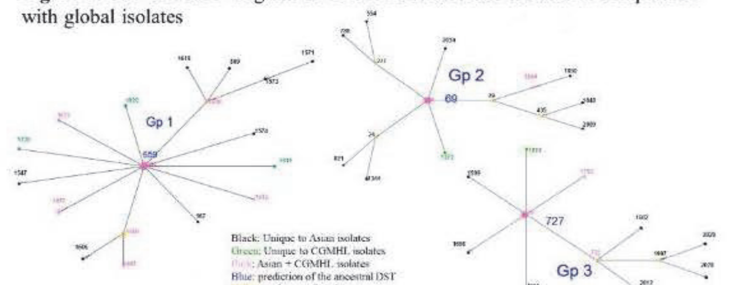

Figure 3. The eBURST diagram of CGMHL *C. albicans* isolates in comparison with Asian isolates

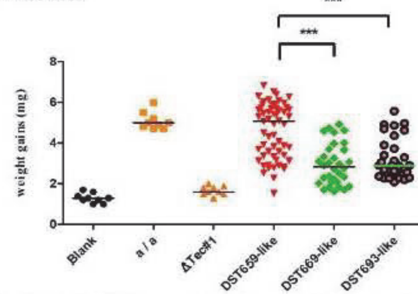

Figure 4. *Candida* biofilm analysis by weighting membrane. Note: a/a stands for positive control using *C. albicans* SC5314 strain; Tec#1 stands for negative control using *C. albicans* SC5314 *ΔTEC1*.

Conclusions

1. DST659 is the main cluster founder of *C. albicans* isolates in CGMHL by eBURST analysis.
2. DST659-like group showed significantly higher biofilm formation than other groups.