國立嘉義大學生命科學院
110 年度學生學術研究成果優良海報競賽獲獎名單

學制別	姓名	指導老師	名次
食品科學系			
學士班	吳昀庭	羅至佑	1
	梁雅晴	張文昌	2
	李奕慧	呂英震	3
碩士班	王子瑄	張文昌	1
	江沂展	羅至佑	2
	劉中翔	張文昌	3
水生生物科學系			
學士班	邱淮祥	李安進	1
	林俐均	李安進	2
	謝睿宸	李安進	3
碩士班	曾郁仁	吳淑美	1
	林芝佑	黃承輝	2
	楊建銘	黃承輝、董哲煌	3
生物資源學系			
學士班	林翰昇	方引平	1
	陳心怡	方引平、林政道	2
	鄭子琪	陳宣汶	3
碩士班	陳俊佑	呂長澤	1
	蘇昱	林政道	2
	林唐禕	陳宣汶	3
生化科技學系			
學士班	劉禮玲	林芸薇	1
	簡晉誠	林芸薇	2
	黃奕翔	林芸薇	3
碩士班	林鼎展	陳瑞祥	1
	于仁豪	陳政男	2
	黃千芬	陳瑞祥	3
微生物免疫與生物藥學系			
學士班	吳宛庭	謝佳雯	1
	曾嚴慈	翁炳孫	2
	陳子瑞	吳進益	3
碩士班	李嘉雯	劉怡文	1
	謝文媛	翁博群	2
	楊子瑩	劉怡文	3
生命科學全英文碩士學位學程			
碩士班	KHALID AMJAD (安賀立)	賴弘智	1


生化科技學系

5-fluorouracil induced cytotoxicity via down-regulation radiation-sensitive 52 expression by inactivation p38 MAPK in human non-small cell lung squamous H1703 cells

[a]Department of Biochemical Science and Technology, National Chiayi University, Chiayi, Taiwan

Li-Ling Liu[a#], Yun-Wei Lin[*]

Abstract

5-fluorouracil (5-FU), an antimetabolite drug, is used to treat several cancers also including non-small cell lung cancer (NSCLC) cells through inhibition of thymidylate synthase (TS), blocking the DNA and RNA synthesis causing DNA damage. E2F1 is a transcription factor, which plays a crucial role in DNA repair and is highly expressed in the cancer cell. Radiation-sensitive 52 (Rad52) is important homologous recombination (HR) protein, which is a major mechanism of repair double-strand breaks (DSBs). In this study, I found that 5-FU reduces Rad52 mRNA and protein levels in squamous cell carcinoma H1703 cells. 5-FU induced cytotoxicity via decreased Rad52 expression in a p38 MAPK inactivation dependent manner. Moreover, I also found that E2F1 was associate with 5-FU-induced reduced Rad52 expression. Overall, 5-FU will have the potential to synergize with other drugs via p38 MAPK inactivation and Rad52 down-regulation.

Results

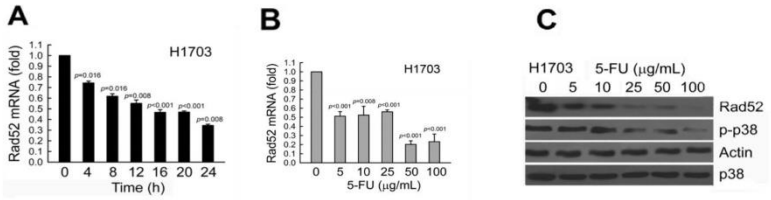


Fig. 1 5-FU reduced Rad52 mRNA and protein in a time-and dose-dependent manner.

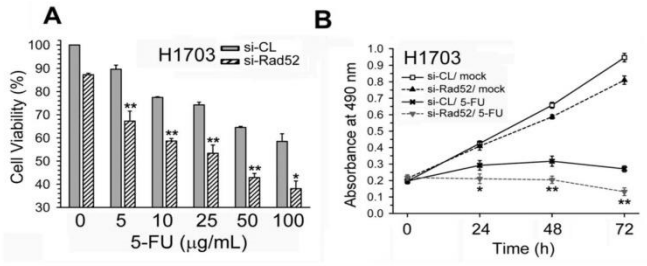


Fig. 2 Rad52 downregulation could enhance 5-FU-induced cytotoxicity and growth inhibition in NSCLC cells.

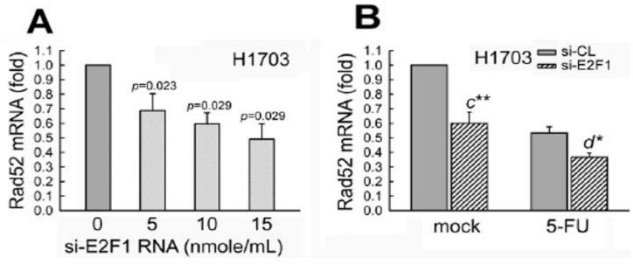


Fig. 3 E2F1 was involved in 5-FU-induced reduced Rad52 expression in H1703 cells.

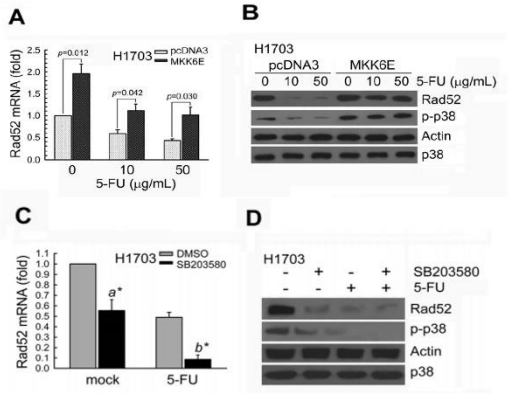


Fig.4 Reduction of Rad52 expression by 5-FU through MKK3/6-p38 MAPK inactivation in H1703 cells.

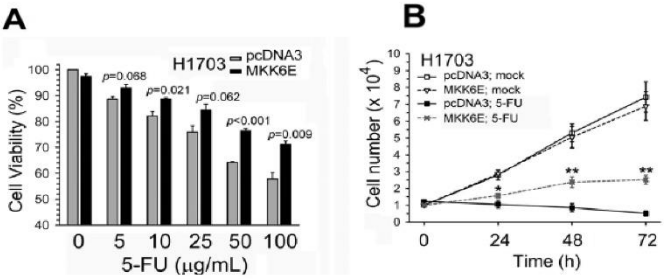


Fig.5 Enforce expression of the MKK6E vector rescued the 5-FU-induced decrease in H1703 cell viability and reversed the growth inhibition effect.

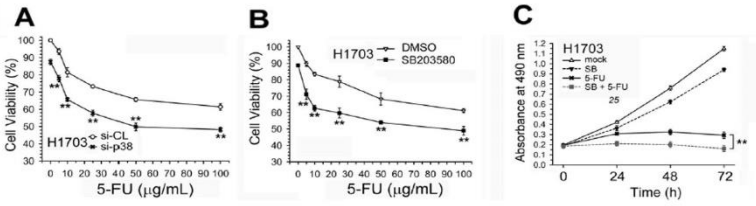
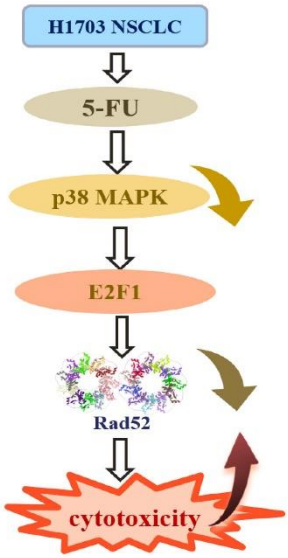


Fig. 6 Inhibition of p38 MAPK activation enhanced 5-FU-induced cytotoxicity.

Summary



Investigation of the inhibition of radiation-sensitive 52 by 5-fluorouracil through AKT pathway and enhance the cytotoxicity of erlotinib in lung adenocarcinoma A549 cells

Chin-Cheng Chien[a], Yun-Wei Lin[a*]

[a] Department of Biochemical Science and Technology, National Chiayi University, Chiayi, Taiwan

Abstract

Among all the diseases that humans suffered, lung cancer has become the most common malignant tumor around the world. About eighty percent of lung cancer patients are diagnosed with non-small cell lung cancer(NSCLC). A549 is a lung adenocarcinoma, which is one of the NSCLC. Radiation-sensitive52(Rad52) plays an important role in the homologous recombination in DNA repair. 5-Fluorouracil (5-FU) is a chemotherapeutic agent commonly used in solid malignancies. Erlotinib is an epidermal growth factor tyrosine kinase inhibitor. By using RT-qPCR, I found that the mRNA expression of Rad52 decreased when treating with chemotherapeutic agent 5-FU in A549 cells. Furthermore, I found that Rad52 mRNA down-regulation is in the AKT dependent manner. Under this circumstance, I noticed that the co-treatment of 5-FU and erlotinib decreased the Rad52 expression and improved cell cytotoxicity. Hence, I assume that 5-FU enhance the erlotinib induced cell cytotoxicity in lung adenocarcinoma A549 cells.

Results

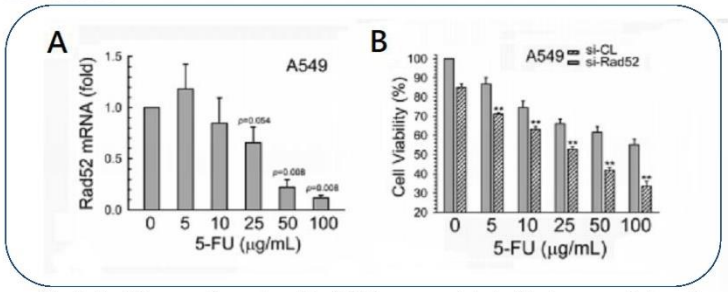


Fig. 1 Rad52 expression reduced by 5-FU was associated with down-regulation of cell viability in A549 cells.

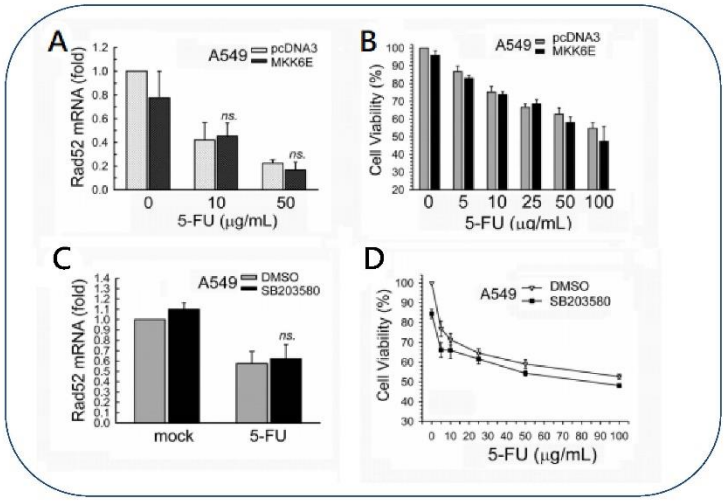


Fig. 2 Rad52 expression when treated with 5-FU was not related to p38 MAPK pathway.

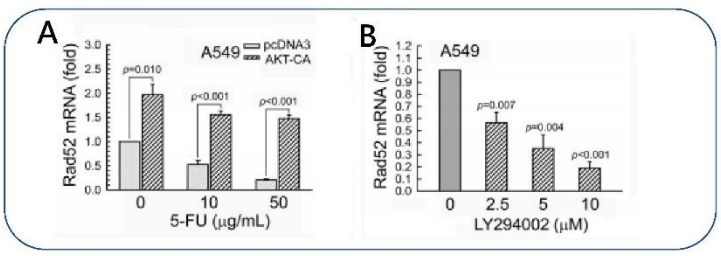


Fig. 3 Rad52 expression is related to AKT pathway when treated with AKT-CA transfection and LY294002.

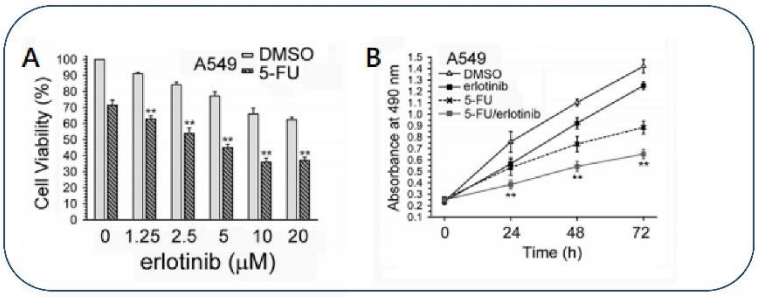


Fig. 4 Co-treatment of 5-FU and erlotinib synergistically enhanced cytotoxicity.

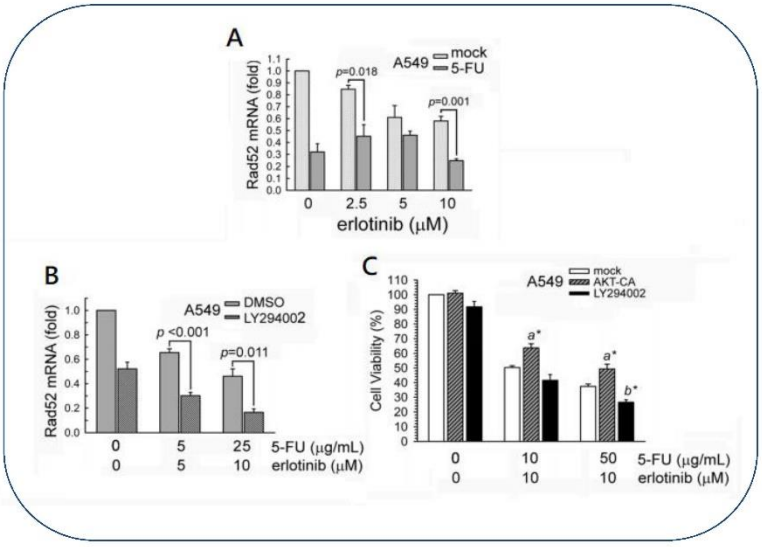
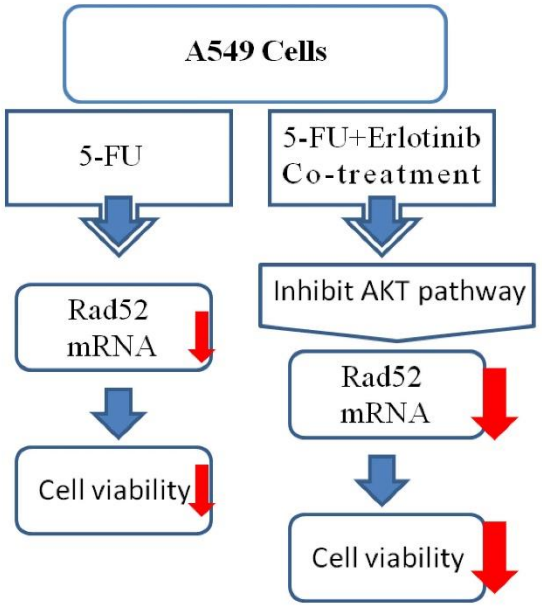


Fig. 5 Rad52 mRNA level with the co-treatment of both erlotinib and 5-FU thus decrease the cell viability.

Summary



5-fluorouracil potentiates the cytotoxic effect of erlotinib in human lung squamous carcinoma H520 cells by inhibiting p38 MAPK-mediated radiation-sensitive 52 expression

I-Hsiang Huang, Yun-Wei Lin *

Department of Biochemical Science and Technology, National Chiayi University, Chiayi, Taiwan

Abstract

5-fluorouracil (5-FU) is an anticancer agent that can inhibit new thymidylate synthase and fracture DNA strands. Radiation-sensitive 52 (Rad52) is a ring-shaped protein which can repair DNA damage. Previous study showed that the expression of Rad52 increased when DNA was damaged. Erlotinib is an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor. It can compete with the ATP binding site of EGFR to inhibit the downstream signal pathway. In the present study, 5-FU and gefitinib which was an EGFR tyrosine kinase inhibitor had a synergistic antiproliferative effect in human lung squamous cell carcinoma cells. However, the effect between 5-FU and erlotinib in H520 is underexplored. In this study, I found that 5-FU decreased the cell viability of H520 by inhibiting the expression of Rad52 via p38 MAPK inactivation. Furthermore, I tried to treat H520 with 5-FU and erlotinib to observe whether they would have synergistic cytotoxic effect in H520. The result shows that 5-FU and erlotinib can significantly decrease the cell viability and the expression of Rad52 mRNA comparing to treat with 5-FU or erlotinib alone. Moreover, I found that the cytotoxic effects of 5-FU and erlotinib was influenced by p38 MAPK pathway. In short, cotreating with 5-FU and erlotinib can decrease H520 viability by inhibiting Rad52 expression via p38 MAPK pathway.

Results

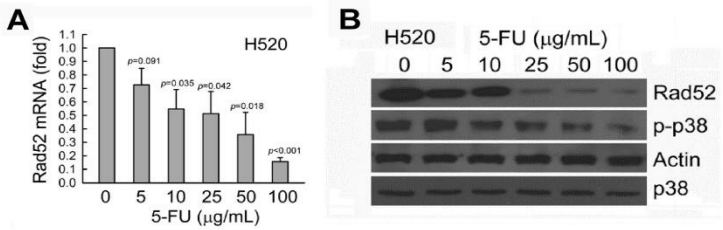


Fig. 1 5-FU downregulate Rad52 expression in a dose-dependent manner.

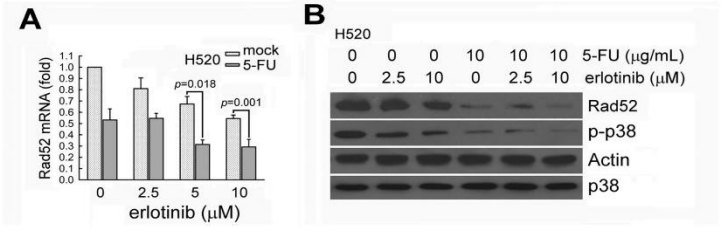


Fig. 2 5-FU decreased Rad52 protein and mRNA levels after erlotinib exposed.

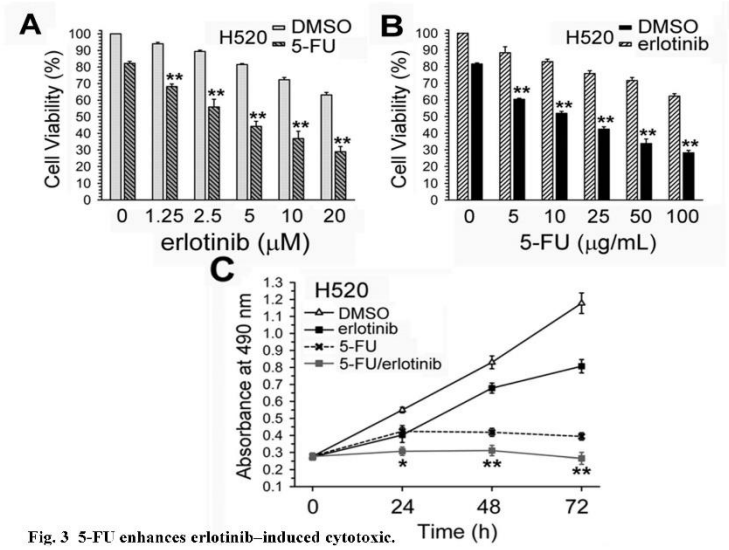


Fig. 3 5-FU enhances erlotinib–induced cytotoxic.

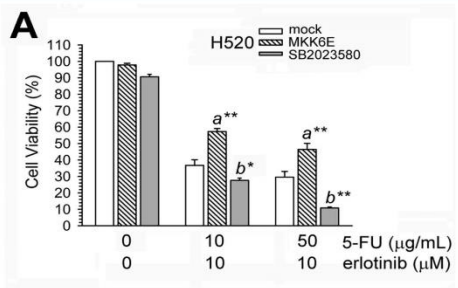


Fig.4 The cytotoxic of 5-FU and erlotinib combination was influenced by p38 MAPK.

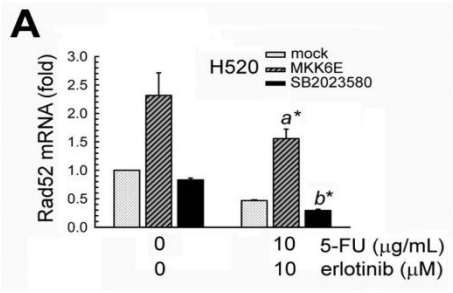


Fig. 5 The expression of Rad52 mRNA was influenced by p38 MAPK after 5-FU and erotinib exposed.

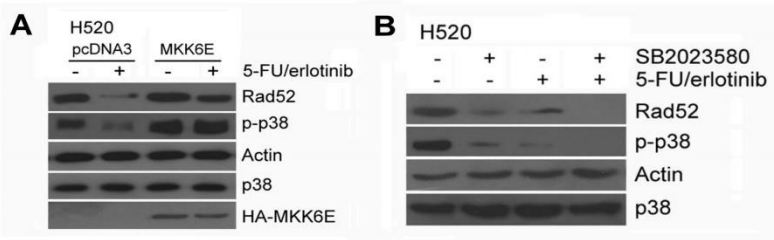
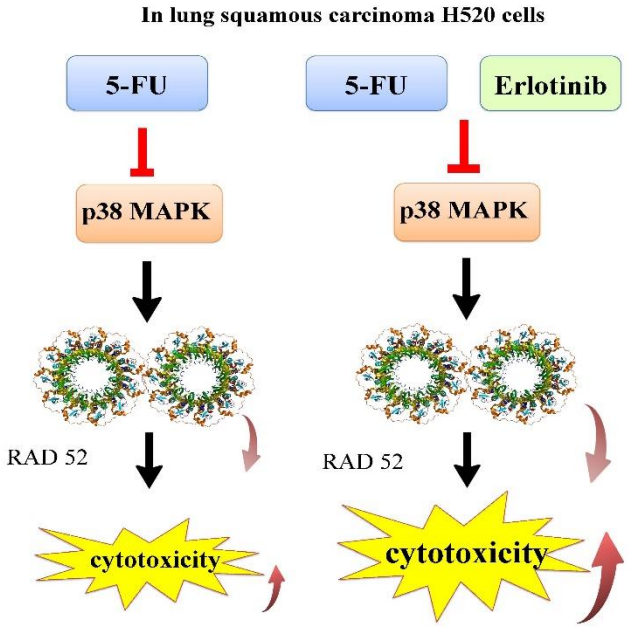


Fig. 6 The expression of Rad52 protein was influenced by p38 MAPK after 5-FU and erotinib exposed.

Summary



Study on Antimicrobial Mechanism of *Trichoderma* spp. through Comparative Genome Analysis

林鼎展[1],陳瑞祥[1],王廷方[2]

[1]國立嘉義大學生化科技學系
[2]中央研究院分子生物研究所

摘要

木黴菌是一種常見且有效的生物製劑，它能夠抑制植物病原真菌生長，並且也具有幫助植物生長及提高植物自身防禦反應的能力。本篇實驗比較了兩支台灣原生木黴菌*T. asperellum* FT-101及*T.virens* FT-333與國外生物控制劑標準菌株*T.virens* Gv29-8、*T. atroviride* P1以及生產工業纖維水解酶菌株*T. reesei* CBS1-2等五支木黴菌株的代謝產物對植物病原真菌的抑制能力，隨著三代定序的完備因此希望透過基因體分析並經由實驗找出不同木黴菌抗菌能力的差異及不同菌種有趣之處。

首先由玻璃紙抗生的結果顯示五支木黴菌皆具有廣泛的抑制植物病原真菌的能力，接著透過硫酸銨沉澱及LC-MS-MS分析不同木黴菌之間分泌的胞外蛋白的差異，並結合全基因體資料分析處理後發現，*T.virens* FT-333與Gv29.8中皆產生能抑制植物病原真菌Gliotoxin毒素合成的酵素--GliT 蛋白。與前人研究不同的是在*A. fumigatus*中指出GliT protein是胞內氧化還原活性的蛋白並且在其蛋白質N端沒有signal peptide，然而在木黴菌FT-333與Gv29.8中GliT protein 被分泌到胞外並且具有signal peptide。而在*T. reesei*中CBS1-2中發現具有抑制真菌生長Sorbicillinoid的合成酵素--Sor7 蛋白，而在sor相關合成基因突變株抗生實驗指出，在*T. reesei*抗生能力中Sorbicillionid這類化合物是重要的抑制病原真菌的化合物。且在TLC薄片分析中發現*T. reesei* sor4基因剃除菌絲內發現黃色代謝物，前人研究多數研究胞外代謝物卻未研究細胞內物質，在這裡我們懷疑其是一個未被發現的Sorbicillionid並且木黴菌Sorbicillinoid相關合成路徑並未研究透徹，最後我們希望透過酵母菌來表達木黴菌GliT 及Sor7重組蛋白，試著找出GliT protein 在*T.virens*與*A. fumigatus*其功能上的差異及Sor7 蛋白在合成Sorbicillinoid的功能。

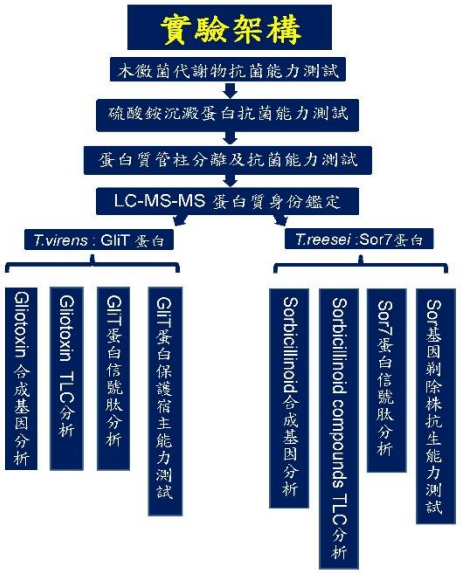
表一 木黴菌玻璃紙代謝物抗生抑制率

Plant fungal pathogens v.s. *Trichoderma* spp.	Phellinus noxius	Rhizoctonia solani	Sclerrotium rolfsii	Phytophthora spp.
P1	100%	100%	100%	100%
FT-101	100%	100%	99±1%	100%
FT-333	100%	100%	100%	100%
Gv29-8	100%	100%	100%	100%
CBS999.97(MAT1-2)	92±6%	79±2%	92±7%	100%

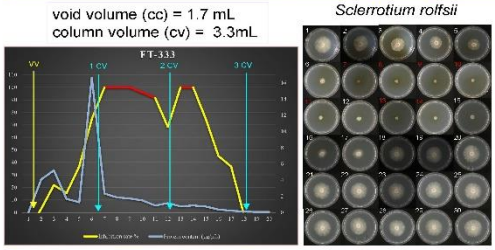
表二 木黴菌硫酸銨沉澱蛋白抗生抑制率

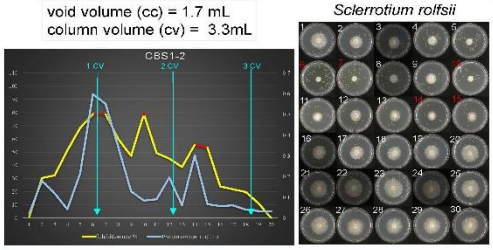
Plant fungal pathogens v.s. *Trichoderma* spp.	Phellinus noxius	Rhizoctonia solani	Sclerrotium rolfsii	Phytophthora spp.	Extracellular proteins
P1	36±2%	67±2%	19±3%	41±6%	7.0μg
FT-101	25±8%	4±2%	10±4%	9±14%	1.5μg
FT-333	100%	100%	100%	100%	216.6μg
Gv29-8	100%	100%	100%	100%	306.9μg
CBS999.97(MAT1-2)	72±2%	86±9%	76±8%	100%	274.2μg

實驗架構



圖一 FT-333及CBS1-2 polyacrylamide desalting column蛋白分離抗菌結果





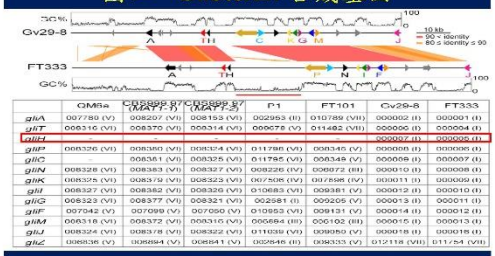
表三 FT-333 LC-MS-MS蛋白質分析結果

Protein ID	Name	Annotation
000004-T1	GliT	FAD/NAD(P)-binding domain
000543-T1		Cupin domain of unknown function DUF985
003844-T1		Xylulose 5-phosphate/Fructose 6-phosphate phosphoketolase
004999-T1		Cobalamin-independent methionine synthase MetE, C-terminal/archaeal
005263-T1	GPD1	Glyceraldehyde-3-phosphate dehydrogenase, type I
009396-T1		O-methyltransferase domain
009400-T1		FAD linked oxidase, N-terminal
010592-T1		FAD-binding domain

表四 CBS1-2 LC-MS-MS蛋白質分析結果

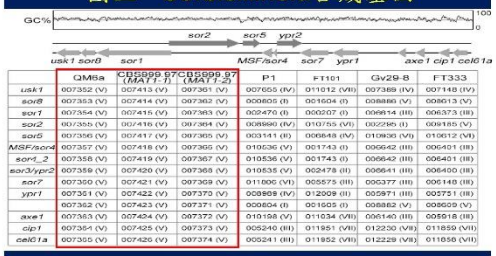
Protein ID	Name	Annotation
000609-T1		Glycoside hydrolase family 16
003497-T1		0
004300-T1		Glycoside hydrolase superfamily
004499-T1	HSPS1	Heat shock protein 70 family
005788-T1		Transaldolase/Fructose-6-phosphate aldolase
006949-T1		Glucanosyltransferase
007369-T1	SOR7	FAD linked oxidase, N-terminal
008943-T1		Peptidase M28

圖二 Gliotoxin 合成基因



	QM6a	CBS999.97 (MAT1-1)	CBS999.97 (MAT1-2)	P1	FT101	Cv29-8	FT333
gliA	007789 (VI)	008207 (VI)	008193 (VI)	002993 (II)	010789 (VII)	000002 (I)	000001 (I)
gliT	006316 (VI)	008370 (VI)	008314 (VI)	009678 (V)	011482 (VII)	000000 (I)	000004 (I)
gliH	-	-	-	-	-	000087 (I)	000005 (I)
gliP	008326 (VI)	008360 (VI)	008324 (VI)	011786 (VI)	008345 (V)	000008 (I)	000006 (I)
gliC		008361 (VI)	008325 (VI)	011785 (VI)	008348 (V)	000068 (I)	000007 (I)
gliN	008328 (VI)	008383 (VI)	008327 (VI)	008226 (IV)	008072 (III)	000010 (I)	000008 (I)
gliK	008335 (VI)	008379 (VI)	008323 (VI)	007595 (IV)	007898 (IV)	000011 (I)	000009 (I)
gliI	008327 (VI)	008382 (VI)	008326 (VI)	010683 (VI)	009381 (V)	000012 (I)	000010 (I)
gliG	008323 (VI)	008377 (VI)	008321 (VI)	002981 (II)	009205 (V)	000013 (I)	000011 (I)
gliF	007042 (V)	007099 (V)	007050 (V)	010853 (VI)	009131 (V)	000014 (I)	000012 (I)
gliM	006316 (VI)	008372 (VI)	008316 (VI)	006864 (III)	006102 (III)	000015 (I)	000013 (I)
gliJ	008324 (VI)	008378 (VI)	008322 (VI)	011039 (VI)	009850 (V)	000018 (I)	000018 (I)
gliZ	008336 (VI)	008384 (VI)	008561 (VI)	002946 (II)	008333 (V)	012118 (VII)	011754 (VII)

圖三 Sorbicillinoid合成基因

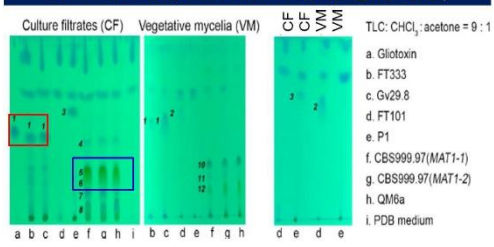


	QM6a	CBS999.97 (MAT1-1)	CBS999.97 (MAT1-2)	P1	FT101	Gv29-8	FT333
usk1	007352 (V)	007413 (V)	007361 (V)	007655 (IV)	011012 (VII)	007369 (IV)	007149 (IV)
sor0	007353 (V)	007414 (V)	007362 (V)	008605 (I)	001604 (I)	008869 (V)	008013 (V)
sor1	007354 (V)	007415 (V)	007363 (V)	002470 (I)	000207 (I)	006814 (III)	006373 (III)
sor2	007355 (V)	007416 (V)	007364 (V)	008880 (IV)	010755 (VI)	002295 (I)	009185 (V)
sor5	007356 (V)	007417 (V)	007365 (V)	003141 (II)	006848 (IV)	010936 (VI)	010612 (VI)
MSF/sor4	007357 (V)	007418 (V)	007366 (V)	010536 (V)	001743 (I)	006642 (III)	006401 (III)
sor4_2	007358 (V)	007419 (V)	007367 (V)	010536 (V)	001743 (I)	006642 (III)	006401 (III)
sor3/ypr2	007359 (V)	007420 (V)	007368 (V)	010535 (V)	002478 (II)	006641 (III)	006400 (III)
sor7	007360 (V)	007421 (V)	007369 (V)	011006 (VI)	005575 (III)	000377 (III)	006148 (III)
ypr1	007361 (V)	007422 (V)	007370 (V)	008969 (IV)	012009 (II)	005971 (III)	005751 (III)
	007362 (V)	007423 (V)	007371 (V)	006804 (I)	001605 (I)	008862 (V)	008609 (V)
axe1	007363 (V)	007424 (V)	007372 (V)	010198 (V)	011034 (VII)	006140 (III)	005918 (III)
cip1	007364 (V)	007425 (V)	007373 (V)	005240 (III)	011951 (VII)	012230 (VII)	011859 (VII)
cel61a	007365 (V)	007426 (V)	007374 (V)	000241 (III)	011952 (VII)	012229 (VII)	011856 (VII)

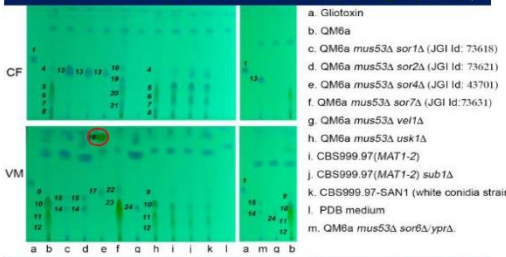
表五 *T.reesei* sor基因剔除株璃紙抗生抑制率

strain	Sclerrotium rolfsii
Wild type(QM6a)	100%
sor1Δ	59±5%
sor2Δ	72±3%
sor4Δ	69±19%
sor7Δ	93±2%
Vel1 Δ	57±4%
USK1 Δ	99±1%
CBS1-2	100%

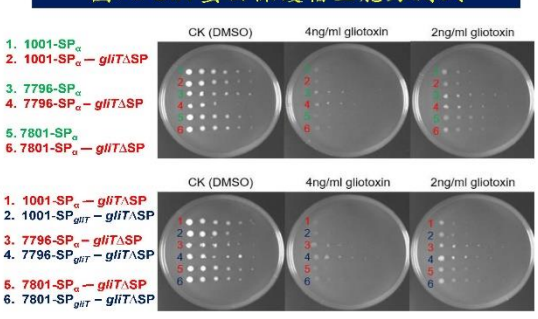
圖四 木黴菌Gliotoxin 薄層色層分析



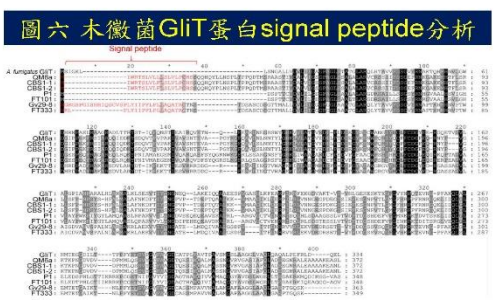
圖五 *T.reesei* sorbicillinoid薄層色層分析



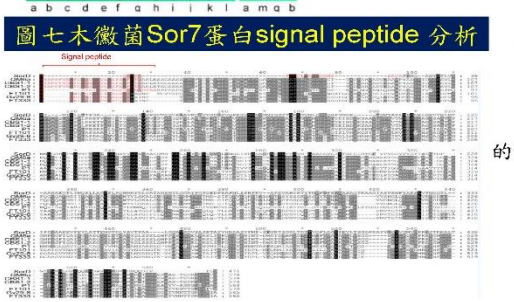
圖八 GliT蛋白保護宿主能力測試



圖六 木黴菌GliT蛋白signal peptide分析



圖七木黴菌Sor7蛋白signal peptide 分析



從圖八中可以看出不論是接上酵母菌本身的signal peptide或是接上FT-333本身的signal peptide的GliT蛋白接不具有保護酵母菌免於Gliotoxin毒素的能力

只有酵母菌本身的signal peptide
GliT蛋白接上酵母菌的signal peptide
GliT蛋白接上木黴菌FT-333的signal peptide

The effect of Probiotics on Resistin Induced ER stress and Epithelial-Mesenchymal Transition in Colorectal Cancer Cells

Jen-Hao Yu[1], Chia-Kung Yen[2], Chen-Nan Chen[1]

[1]Department of Biochemical Science and Technology, National Chiayi University, Chiayi, Taiwan
[2]Department of Food science, National Chiayi University, Chiayi, Taiwan

Abstract

Colorectal cancer (CRC) is the most commonly diagnosed and deadly cancer types in the world. Adipose is a powerful inflammatory substance that stimulates the growth of cancer cells. **Resistin** has been shown to be involved in many inflammation processes.The plasma concentration of resistin is higher in patients with CRC. **Epithelial-mesenchymal transition(EMT)** induces cancer cells to acquire stemness. EMT plays an important role in the migration and drug resistance of cancer cells. **ER stress** has been shown to regulate EMT in many tissues. **Probiotics** can activate the production of macrophages and lymphocytes in the intestinal tract, so it is believed to have the function of strengthening the immune system. Probiotics have anti-inflammatory effects, but it is still unknown whether resistin induces inflammation in CRC cells and whether it regulates ER stress and EMT.

Results

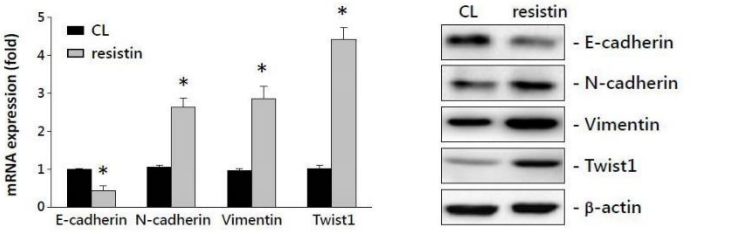


Figure 1. DLD-1 are stimulated by resistin to induce EMT

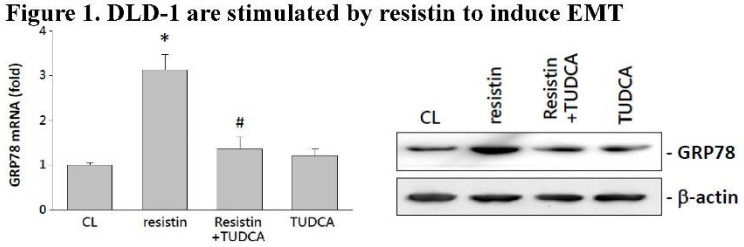


Figure 2. Expression of ER stress marker gene GRP78 in DLD-1 will be stimulated by resistin

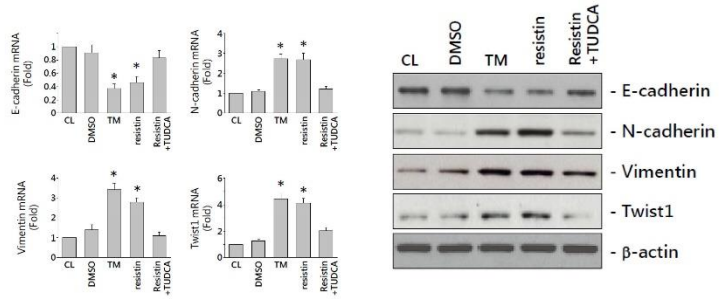


Figure 3. EMT gene expression induced by resistin in DLD-1 is regulated by ER stress

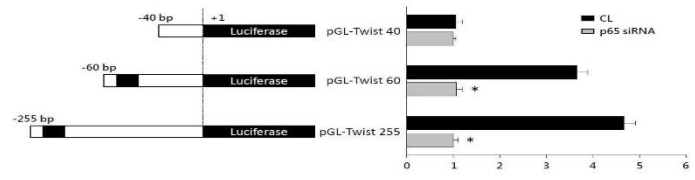


Figure 4. Regulating mechanism of resistin on related gene expression

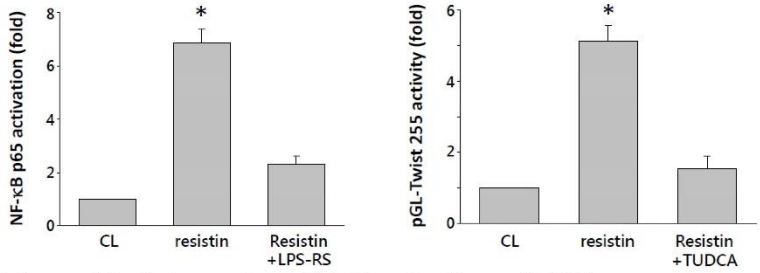


Figure 5. Resistin regulates NF-κB activation and EMT gene expression via TLR4 and downstream ER stress

Figure 6. TLR4 affects STAT3 phosphorylation

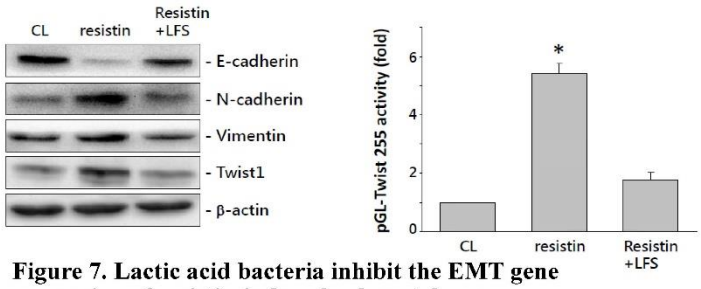
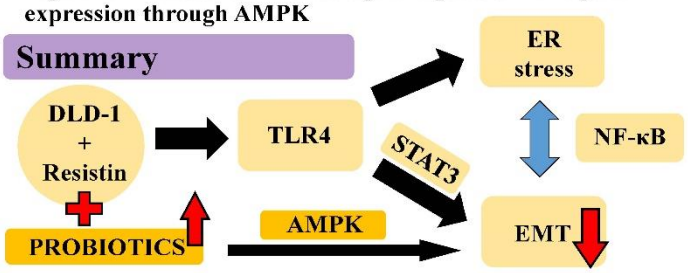


Figure 7. Lactic acid bacteria inhibit the EMT gene expression of resistin-induced colorectal cancer

Figure 8. Lactic acid bacteria liquid regulates EMT gene expression through AMPK

Summary



Conclusion

In conclusion, our findings indicate that the addition of resistin to CRC cells can increase the expression of the ER stress gene GRP78 and subsequently upregulation of EMT gene. It also confirmed that the EMT induced by resistin is mediated by the NF-kB and STAT3 activation. By adding Lactobacillus supernatant (LFS), it was found that the gene expression of EMT could be reduced.

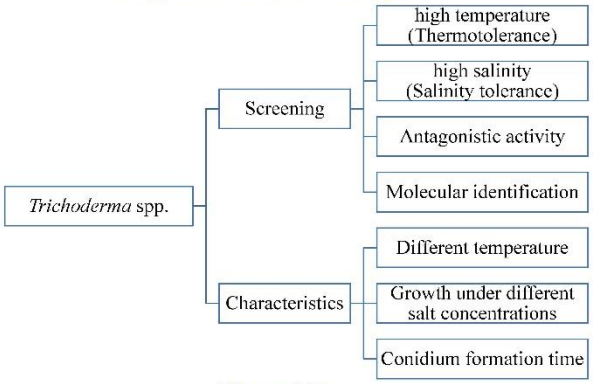
Screening and Characteristic Analysis of Stress-tolerant *Trichoderma* spp.

Chien-Fen Huang and Ruey-Shyang Chen
Department of Biochemical Science and Technology, National Chiayi University

Abstract

Trichoderma spp. are ascomycetes fungi widely found in soil which are often used as biocontrol agent and biological fertilizer to control plant pathogens and to promote plant growth. Under abiotic stress, physiological functions of microorganisms could be affected by adversity which will affect not only plant growth but also the efficiency of biological agents. In this study, 21 *Trichoderma* isolates were tested under high temperature and high salinity to screen stress-tolerant isolates. These isolates were also tested for their antagonistic activity against plant pathogen *Sclerotium rolfsii*. Among tested isolates, Tri-269, identified as *Trichoderma ghanense*, was the most tolerant isolate under high temperature and high salinity and was selected for further study. Growth rate and conidium formation time under different temperature and/or different salt concentration were evaluated.

Experimental Design



Results

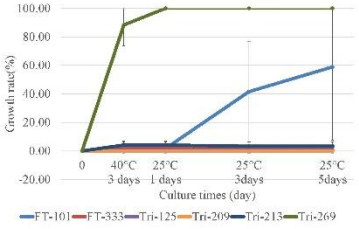


Fig. 1. Thermotolerant analysis of *Trichoderma* spp. treated at 40°C for 3 days and incubated at 25°C for 5 days.

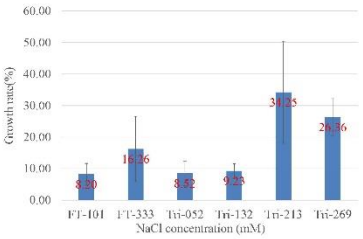


Fig. 2. Salinity tolerant analysis of *Trichoderma* spp. growth under 2000 mM NaCl.

Fig. 3. Phylogenetic tree of the *Trichoderma* based on the ITS-rDNA sequences.

Table. 1. Antagonistic activity of *Trichoderma* isolates against *Sclerotium rolfsii*.

Isolate	Growth inhibition rate (%)
FT-333	100%
FT-101	52%
Tri-213	0%
Tri-269	87%

Fig. 4. Growth of *T. ghanense* Tri-269 on PDA after 7 days incubation at 25 °C.

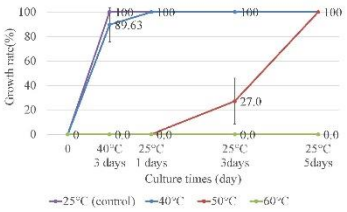


Fig. 5. Thermotolerance of Tri-269 under different temperature for 3 days.

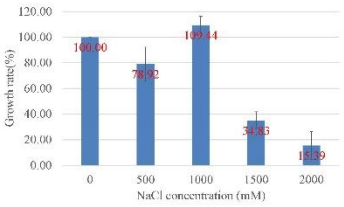


Fig. 6. Salinity tolerance of Tri-269 under different salt concentration.

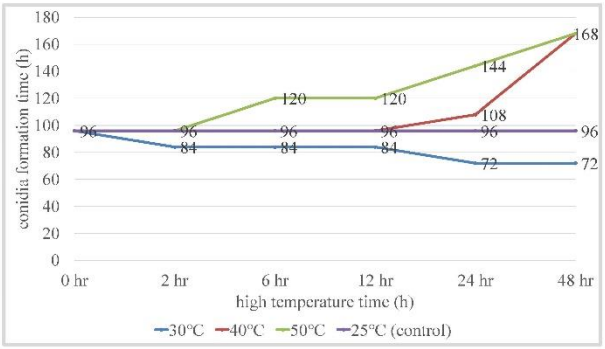


Fig. 7. Conidium formation time of Tri-269 treated at high temperature.

Summary

- A total of 21 *Trichoderma* isolates were screened under 40°C and 2000 mM NaCl for stress-tolerant activity.
- Tri-269 had a 89.63% growth rate 3 days after incubation at 40°C.
- Tri-213 and Tri-269 had a 34.25% and 26.36% growth rate, respectively, while incubated at 2000 mM NaCl PDB.
- Test stress tolerant strains' antagonistic activity to, Tri-213 and Tri-269 had 0% and 87% growth inhibition rate against *S. rolfsii* respectively.
- Tri-269 was identified as *Trichoderma ghanense*.
- Tri-269 was able to grow under 40°C and remain alive under 50°C.
- Tri-269 had the lowest conidium formation time at 30°C for 48 hours.