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The growth and production of hepatotoxic luteoskyrin by Penicillium islandicum

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*Penicillium islandicum*之生長與肝毒性黃米毒素之產生

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摘　要

本研究在探討影響*Penicillium islandicum* 之生長與黃米毒素產生活性之許多種因素,結果黃米毒素與相關色素之產量是在眞菌培養之後期爲最高。以多種培養基培養時，菌絲生長及黃米毒素之產量在麥芽糖抽出物培養液中最高。在適當受質中黃米毒素之產量在第18天時爲最高，另在靜置培養所得菌絲生長及黃米毒素產量較振盪培養爲高，菌絲生長之最適pH值介於微酸性至中性之間。碳源中之澱粉，蔗糖，果糖，麥芽糖與葡萄糖均有促進菌絲生長與黃米毒素及相關色素之產生，其中以澱粉促進菌絲生長與果糖促進黃米毒素產生之活性爲最強。乳糖雖能促進黃米毒素及相關色素之產生, 但抑制菌絲之生長。檸檬酸鹽，麩胺酸鹽，丙二酸鹽，琥珀酸鹽，延胡索酸鹽與乙酸鹽等均抑制菌絲生長。黃米毒素及相關色素之產生, 以乙酸鹽之抑制作用最強。氮源中麥芽抽出物，酵母抽出物，蛋白胨，酪蛋白氨基酸，天冬醯胺與麩胺醯胺等均可促進菌絲之生長，但是黃米毒素及相關色素產生方面，僅麥芽抽出物，天冬醯胺及麩胺醯胺等有促進作用。穀物中眞菌生長與黃米毒素產量依序爲糙米＞麥片＞小麥＞精白米＞黃皮玉米＞白皮玉米＞米穀。又精白米經不同方法處理時，烹煮者之產毒量高於乾炒，烘焙及油炸者，添加5%之蔗糖時不但增加米食中之含水量，也使其產毒量更高。

關鍵詞：碳源，氮源，pH值，含水量，黃米毒素，*Penicillium islandicum*。

前　言

米是許多真菌生長與產毒之良好受質。污染於糙米中之真菌毒素除致癌性之黃麴毒素(aflatoxins)外，最受注意的是具有肝毒活性及致癌活性而由*P. islandicum* 產生之黃變米毒素(yellowed rice toxin)中之黃米毒素(luteoskyrin), rugulosin，環萎黃毒素(cyclochlortine)等。*P. islandicum*係儲藏性真菌，它廣泛地分佈於自然界中。當受到污染之米貯藏在適當之環境，該菌極易產生黃變米毒素而造成米在貯藏中之黃變現象，甚至導致攝食者肝臟萎縮，肝硬化，肝腫瘤甚至肝癌[1,2]。1965年在日本之Nagano pref.之7個飼禽農場中發生因餵飼市售之米餅而導致13,610隻鷄中毒,其中有2,891隻因急性中毒而死亡,而當時成功地自每克米餅中分離15000株之*P. islandicum*，若以這些米餵飼小鼠,經3至6天後相繼出現抑鬱及黃疸病徵最後死亡,經組織學剖析結果發現肝臟中心葉壞死，進而基質崩壞，致出現典型的黃變米中毒症[2]。黃米毒素是黃變米毒素中具誘變性與致癌性且產量最多者[1]，它能導致實驗動物急性肝臟損害,肝臟形態改變，其病徵最先是擴大變黃，然後萎縮出現小紅斑，肝臟中心葉壞死及脂肪變性等[3]。1961年Uraguchi發現日本人若每人平均每年消耗150公斤以上的白米，若1000粒白米中僅有一粒受到*P. islandicum*之感染而有能力於每公斤的黴米內產生6mg之黃米毒素時，

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Table 1. Effect of the medium on the growth and luteoskyrin production by *Penicillium islandicum* THT-2[a]

Medium	Dry weight of mycelium (mg/ml)	Luteoskyrin (μg/ml)
Czapek solution agar	19.13 ± 0.68[b]	16.81 ± 2.15
Czapek solution broth	18.75 ± 1.07	16.02 ± 1.49
Malt extract agar	18.83 ± 1.03	21.79 ± 0.89
Malt extract broth	20.52 ± 1.54	23.13 ± 1.51
Potato dextrose agar	17.42 ± 0.91	17.29 ± 1.37
Potato dextrose broth	19.62 ± 1.16	17.43 ± 1.13
Glucose-lactose-phosphate	9.82 ± 0.59	9.02 ± 0.64
Nutrient broth	11.09 ± 0.82	9.31 ± 0.81
Nutrient agar	11.79 ± 0.79	10.85 ± 0.55

[a]. The medium was incubated at 25 ± 1℃ for 18 days.
[b]. Each value represents the mean ± SD, n=5.

則可使每人每年攝取之黃米毒素量達0.9g，若人之易感性與小鼠相當，則此量相當於對人單一口授半致死劑量(LD_{50})(220 mg/Kg)之十分之一[4]。

1956至1957年研究報告顯示，臺灣倉儲米已明顯受到黃變米毒素及產毒菌之污染[5]。於1979年Tzean等研究稻穀，糙米與白米上之真菌相與儲存條件對真菌族群變化之影響時，並未成功地分離到*P. islandicum*[6]，但於1987年Tseng與Tseng發現臺灣有極少數貯藏米受到黃米毒素產生菌之污染，亦發現極少量之市售米粒受到黃米毒素之污染[7]。

污染穀粒中真菌之生長與產毒，在收成前或貯藏時即已有顯著之影響，但各種真菌生長與產毒所需之條件均有差異。在穀物中真菌生長所需之有效水分以其含水量為決定因子，而其含水量亦可因種子之澱粉或油含量而有差異，因高含水量可能導致貯存穀物之自然加熱現象，使溫度升高，而造成真菌之快速生長及產毒，也因為長黴而使含水量之改變致使其更具易感性，尤其穀物未充分混合時為最[8]，另外穀物收成時若含許多尚未完全成熟者比均勻成熟者更易造成溫度之上升及長黴[9,10]。真菌生長與產毒之重要內在因素是受質種類與含水量，其中碳水化合物是最重要之受質。其它，如穀物pH值亦為重要因素之一，通常真菌生長及產毒係在微酸性至中性之環境中[10]。今就多種碳源與氮源，穀物之種類，穀物之含水量，及pH值等內在因子，探討黃米毒素產生菌-*P. islandicum*之生長與產毒,藉以謀求防治食米受污染之道。

材料與方法

一、菌種與培養方法

㈠分生孢子取得

黃米毒素產生菌*P. islandicum* THT-2[7]接種於馬鈴薯葡萄糖瓊脂培養基(potato dextrose agar)中，於25±1℃培養箱中培養14天，以生理食鹽水洗出分生孢子，經無菌尼龍絲網過濾後以系列倍數稀釋，分別取1 ml，以直接平皿培養計數孢子，及spectrophotometer在650nm波長下測定吸光度，比較其數值並繪出標準曲線，製備分生孢子濃度時均依此曲線，以取得濃度為約4×10^6 cfu/ml供每一實驗時之接種用。

㈡不同培養基中真菌生長與產毒之活性

取20 ml之Czapek solution agar (CZA), Czapek solution broth (CZB), Malt extract agar (MEA), Malt extract broth (MEB),Potato dextrose agar(PDA),Potato dextrose broth (PDB),Glucose-lactose-peptone (GLP), Nutrient broth (NB) 及Nutrient agar (NA)(Difco)，經滅菌接種後置於25±1℃培養箱中培養18天。另分取20 ml之CZA,以4%NaOH或2N HCl分別調整pH值為2至

Table 2. Effect of pH on the growth and luteoskyrin production by *Penicillium islandicum* THT-2 in Czapek solution agar[a]

pH	Incubation temperature (℃)						
	10	15	20	25	30	35	40
Dry weight of mycelium (mg/ml)							
2	−[b]	0.32 ± 0.03[c]	0.42 ± 0.01	0.64 ± 0.06	0.81 ± 0.04	0.79 ± 0.07	0.29 ± 0.14
3	0.41 ± 0.04	1.23 ± 0.13	2.27 ± 0.27	4.03 ± 0.29	4.03 ± 0.36	3.34 ± 0.34	1.34 ± 0.23
4	0.46 ± 0.02	2.39 ± 0.34	3.97 ± 0.32	13.14 ± 0.64	13.78 ± 1.14	5.72 ± 0.12	1.36 ± 0.31
5	0.61 ± 0.05	4.11 ± 0.32	6.13 ± 0.21	18.52 ± 1.26	18.12 ± 1.18	13.03 ± 0.73	1.53 ± 0.14
6	0.82 ± 0.08	4.32 ± 0.36	6.82 ± 0.80	19.53 ± 0.83	19.57 ± 1.27	14.77 ± 0.81	1.77 ± 0.16
7	1.44 ± 0.08	4.02 ± 0.32	6.01 ± 0.53	18.63 ± 0.98	18.05 ± 0.74	13.38 ± 1.56	2.13 ± 0.48
8	1.35 ± 0.14	3.81 ± 0.29	4.79 ± 0.44	12.01 ± 0.80	11.74 ± 1.36	5.15 ± 0.93	0.89 ± 0.44
9	−	−	0.66 ± 0.07	0.84 ± 0.01	1.18 ± 0.01	0.64 ± 0.01	0.43 ± 0.01
Luteoskyrin (μ g/ml)							
2	−	−	−	?[d]	?	−	−
3	−	1.03 ± 0.09	3.84 ± 0.22	3.99 ± 0.64	3.23 ± 0.10	1.93 ± 0.72	?
4	?	1.61 ± 0.17	4.52 ± 0.37	13.33 ± 1.28	8.45 ± 0.91	6.16 ± 0.55	?
5	?	2.86 ± 0.26	6.03 ± 0.23	14.47 ± 0.66	13.15 ± 0.69	10.93 ± 0.76	1.32 ± 0.19
6	0.46 ± 0.01	2.53 ± 0.14	8.34 ± 0.43	18.12 ± 0.72	16.57 ± 0.87	12.28 ± 0.51	1.42 ± 0.32
7	0.62 ± 0.03	2.56 ± 0.22	10.93 ± 0.62	18.71 ± 0.83	17.55 ± 1.35	13.54 ± 0.69	1.62 ± 0.36
8	0.53 ± 0.06	2.03 ± 0.31	4.73 ± 0.36	9.50 ± 0.80	8.72 ± 0.73	4.94 ± 0.82	ND
9	−	−	ND[e]	?	ND	ND	ND

a. The medium (pH 2～9) was incubated at various temperature for 18 days.
b. − : Mycelial growth (or luteoskyrin production) was not observed.
c. Each value represents the mean ± SD, n=5.
d. ? : Uncertain result.
e. ND: not detected.

9,經接種後置於10～40℃培養箱中培養18天，及50 ml之CZB與200 g之糙米(含水量18%)，置錐形瓶中經滅菌，接種後，分別置於溫度15, 20, 25, 30 及 35 各±1℃之培養箱中以振盪及靜置培養24天。 又取已經滅菌之600 g含水量13.5, 16.5, 18.8, 22.5, 26.2及30.0%之完整及碎裂之秈稻 (*Oryza sativa* var. *indica*) 及粳稻 (*Oryza sativa* var. *japonica*) 之精白米 (polished rice), 糙米(un-polished rice)以及穀粒 (unhulled rice), 接種後於25±1℃中培養30天。在特定時間內分別測定黃米毒素量及(或)菌絲乾重。

㈢添加碳源與氮源影響真菌生長與產毒之活性

分取250 ml之CZB置500 ml三角瓶中，分別再添加3%培養基量之碳源：澱粉 (starch)，蔗糖 (sucrose)，果糖(fructose)，麥芽糖(maltose)，乳糖 (lactose)，葡萄糖 (glucose)，檸檬酸鹽 (citrate)，麩胺酸鹽 (glutamate)，丙二酸鹽 (malonate)，琥珀酸鹽 (succinate)，延胡索酸鹽 (fumarate)，乙酸鹽 (acetate)；或0.2%培養基量之氮源：麥芽抽出物(malt extract)，酵母抽出物(yeast extract)，蛋白腖(peptone)，酪蛋白胺基酸 (casamino acid)，天冬醯胺 (asparagine)，麩胺醯胺(glutamine)，硝酸銨 (NH_4NO_3)，氯化銨 (NH_4Cl)，尿素 [$CO(NH_2)_2$] 與硫酸銨[$(NH_4)_2SO_4$b]，後四種氮源時則以

Table 3. Effect of cultural status on luteoskyrin production by *Penicillium islandicum* THT-2.

Temperature (℃)	Luteoskyrin (μ g/g)					
	CZB[a]			Unpolished rice[b]		
	12[c]	18	24	12	18	24
Stationary cultivation						
15	0.63 ± 0.12[d]	2.12 ± 0.38	3.41 ± 0.21	?[e]	2.63 ± 0.07	1.89 ± 0.17
20	8.32 ± 0.79	8.77 ± 0.92	9.02 ± 0.83	7.92 ± 0.34	6.57 ± 0.41	5.37 ± 0.36
25	10.44 ± 0.94	18.04 ± 1.01	17.99 ± 0.52	8.83 ± 0.33	11.39 ± 0.53	10.71 ± 0.48
30	9.94 ± 1.34	17.94 ± 0.77	17.78 ± 1.43	8.16 ± 0.28	8.34 ± 0.40	8.17 ± 0.34
35	4.18 ± 0.35	6.31 ± 0.58	7.08 ± 1.41	4.44 ± 0.44	5.54 ± 0.56	5.14 ± 0.46
Shake cultivation						
15	0.82 ± 0.07	2.13 ± 0.22	3.13 ± 0.26	1.18 ± 0.01	2.08 ± 0.04	1.37 ± 0.36
20	7.82 ± 0.92	8.02 ± 0.72	8.13 ± 0.53	7.68 ± 0.22	8.14 ± 0.31	5.02 ± 0.28
25	10.23 ± 1.02	15.32 ± 0.83	14.93 ± 0.66	8.42 ± 0.33	10.83 ± 0.37	9.99 ± 0.34
30	9.84 ± 0.63	15.04 ± 0.74	14.26 ± 0.99	7.97 ± 0.27	9.04 ± 0.34	7.88 ± 0.21
35	2.92 ± 0.33	4.50 ± 0.13	4.07 ± 0.22	2.83 ± 0.36	4.40 ± 0.13	3.77 ± 0.36

[a]. The volume of CZB : 50 ml/flask.
[b]. The volume of unpolished rice : 200 g/flask (18% water content).
[c]. Incubation period (days).
[d]. Each value represents the mean ± SD, n=5.
[e]. ?: Uncertain result.

0.2%量取代原培養基之硝酸鈉($NaNO_3$)。經高壓滅菌，接種，充分振搖後置於25±1℃培養18天後，測定菌絲乾重與培養基中色素與黃米毒素之量。分別7組CZB接種後培養於10～40℃30天，自第六天起每4天測定其pH值之變異情形。

㈣穀物中真菌生長與產毒之活性

取含水量分別為19.0, 18.6, 18.8, 18.8, 19.1, 18.6 與 19.0%之糙米(unpolished rice)，精白米(polished rice)，穀(unhulled rice) (粳稻)，小麥(wheat)，麥片 (oatmeal)，黃皮玉米(yellow corn)與白皮玉米 (white corn)各600 g，分別置於三角瓶中，經高壓滅菌，接種，後置於25±1℃ 培養30天，觀察真菌之生長與測定黃米毒素量。

另再分取精白米600 g，依米食之處理方式分別烹煮(boiled)，100℃乾炒 (fried) 8分鐘，80℃烘焙 (baked)12分鐘及適量玉米油160℃油炸(deep fried) 2分鐘後各分二組，其中一組加入5%之蔗糖。將二組分別接種1ml之分生孢子懸浮液，置於25±1℃中培養60天，並分別於特定時間內測定含水量及觀察真菌生長與測定黃米毒素量。

二、色素及黃米毒素之分析

㈠高效能薄層層析(HPTLC)板之製備

取層析板(Merck, Art. 13748, Damstadt, Germany)，經10%EDTA或Oxalic acid(Sigma)以水平展開後風乾，再以80℃活化2小時，取出置乾燥箱內備用。

㈡萃取

由瓊脂培養基中，挑取菌落，再以50 ml石油醚洗淨培養基，洗液 (含分生孢子)并入菌

Table 4. Effect of water content on luteoskyrin production in rice by *Penicillium islandicum* THT-2[a]

Water content (%)	Luteoskyrin (μ g/g)					
	Oryza sativa var. *indica*			*Oryza sativa* var. *japonica*		
	Polished rice	Unpolished rice	Unhulled rice	Polished rice	Unpolished rice	Unhulled rice
Whole rice						
13.5	ND[b]	ND	ND	ND	ND	ND
16.5	0.25 ± 0.03[c]	0.47 ± 0.06	ND	0.35 ± 0.09	0.63 ± 0.11	ND
18.8	1.64 ± 0.14	1.77 ± 0.14[d]	?[d]	1.61 ± 0.10	1.83 ± 0.26	0.25 ± 0.05
22.5	4.17 ± 0.23	5.73 ± 0.22	0.41 ± 0.08	4.57 ± 0.43	5.77 ± 0.61	0.43 ± 0.08
26.2	6.32 ± 0.40	7.48 ± 0.38	0.70 ± 0.05	6.48 ± 0.53	8.51 ± 0.60	0.73 ± 0.08
30.0	7.61 ± 0.30	8.97 ± 0.25	1.10 ± 0.14	8.62 ± 0.38	10.53 ± 0.38	1.72 ± 0.16
Cracked rice						
13.5	ND	ND	−[e]	ND	ND	−
16.5	0.45 ± 0.07	0.75 ± 0.13	−	0.68 ± 0.06	1.07 ± 0.19	−
18.8	2.25 ± 0.12	2.05 ± 0.17	−	1.95 ± 0.18	2.36 ± 0.22	−
22.5	4.05 ± 0.20	8.01 ± 0.01	−	4.92 ± 0.40	6.13 ± 0.49	−
26.2	7.77 ± 0.36	8.45 ± 0.26	−	7.17 ± 0.39	9.89 ± 0.38	−
30.0	8.29 ± 0.41	9.21 ± 0.45	−	9.49 ± 0.41	11.27 ± 0.79	−

[a]. The medium was incubated at 25 ± 1℃ for 30 days.
[b]. ND: not detected.
[c]. Each value represents the mean ± SD, n=5.
[d]. ?: Uncertian result.
[e]. −: Not determined.

落中，培養基以100 ml之石油醚(或正己烷)去油脂，再以二重複方式以150 ml丙酮(或乙醚)振搖萃取。惟菌落(含分生孢子) 則先以70℃烘乾10小時後研磨，再以90ml丙酮 (或乙醚)及依培養基之萃取方法萃取。若黴米時於充分研碎後，取50g以100ml之石油醚去油脂及干擾物質，再以二重複方式以150ml丙酮(或乙醚)振搖萃取[7,11]，萃取液減壓濃縮至乾,以20 ml之丙酮溶出, 再濃縮至2 ml供分析用。

㈢ 將萃取物經以活化之層析板及展開液(Toluene: ethyl acetate : 90% formic acid ; 6:3:1, v/v/v)展開，將層析板上之色素，分別溶出後定量之。分析黃米毒素時，將標準之黃米毒素(Sigma, USA)及10 μ l濃縮液點在經處理及活化之HPTLC層析板，以單向或雙向及多重之水平展開法展開後，以spectrodensitometer定性與定量之[11]。

三、菌絲乾重之測定

取濾紙置濾斗上，經70℃烘乾10小時後取出秤重，從固態培養基中挑起之菌落 (及以50 ml石油醚洗出孢子之洗液)或培養之液態培養基，以乾燥過之濾紙濾乾，濾紙(含菌落)再以相同之溫度與時間烘乾，取出置乾燥箱，俟溫度降至室溫時再秤重，所得之重量減去原重量即得菌絲乾重。

Table 5. Effect of carbon source on the growth and luteoskyrin production by *Penicillium islandicum* THT-2[a]

Carbon source[b]	Mycelium (mg/ml)	Pigments (mg/ml)	Luteoskyrin (μg/ml)
Control	9.03 ± 0.81[c]	0.64 ± 0.05	7.30 ± 0.57
Starch	15.89 ± 1.64	1.05 ± 0.08	14.21 ± 1.12
Sucrose	10.26 ± 1.30	0.85 ± 0.08	13.90 ± 1.07
Fructose	11.56 ± 1.11	1.47 ± 0.13	16.45 ± 1.23
Maltose	14.40 ± 1.42	1.00 ± 0.08	14.52 ± 1.17
Lactose	6.77 ± 0.62	1.04 ± 0.10	13.92 ± 1.09
Glucose	11.93 ± 1.62	1.08 ± 0.08	12.57 ± 0.97
Citrate	2.83 ± 0.45	0.31 ± 0.01	1.64 ± 0.16
Glutamate	4.60 ± 0.40	0.48 ± 0.01	2.22 ± 0.17
Malonate	2.70 ± 0.29	0.33 ± 0.01	1.84 ± 0.15
Succinate	2.00 ± 0.19	0.28 ± 0.01	1.45 ± 0.12
Fumarate	1.63 ± 0.14	0.42 ± 0.02	2.13 ± 0.20
Acetate	0.82 ± 0.07	0.06 ± 0.00	0.30 ± 0.03

[a]. The medium was incubated at 25 ± 1℃ for 18 days.
[b]. 3% each of the carbon source in Czapek solution broth.
[c]. Each value represents the mean ± SD, n=5.

四、含水量及pH值之測定

秤取6 g之樣品置入經140℃烘乾2小時，乾燥前後之重量差與樣品重量之百分率(%)表示含水量。至於pH值則以pH測定儀測定之。

結果與討論

P. islandicum THT-2 在培養基中之生長為MEB> PDB> CZA> MEA> CZB> PDA> NA> NB> GLP, 但luteoskyrin之產量為MEB> MEA> PDB> PDA> CZA> CZB> NA> NB> GLP，最高之菌絲乾重與毒素量分別為 20.52 mg/ml及23.13 μg/ml (表一)。該菌可在pH值3～8之範圍內生長，其最適之pH值在5～7之弱酸至中性之間，在此pH下溫度於25～30℃間，菌絲之生長與黃米毒素之產量達到最高，菌絲乾重為18.05～19.57 mg/ml，毒素量為13.15～18.71 μg/ml (表二)。若該菌接種於CZB及糙米中,經靜置與振盪培養，在最適溫(25～30℃)時之第18天該菌之產毒量前者分別為18.04 μg/ml及11.39 μg/g，後者分別15.32 μg/ml及10.83 μg/ml，此後毒素有減少之趨勢，其原因可能係因其產毒之活性已至後期，且培養基中積聚之毒素因受到環境因素之影響而造成減少之現象(表三)。

農產品中糙米是產生黃米毒素之良好基質，但是未脫殼之穀粒可能因外殼之保護作用,以及精白米因缺乏米糠之成分，致影響黃米毒素之產生。真菌在這些農產品中產毒之差異與農產品成分之差異，或影響酵素活性之金屬含量有關，有待深入探討。不同品種之糙米，精白米及穀經接種培養，其產毒量依序為糙米> 精白米> 穀，粳稻米> 秈稻米，且隨含水量之增加而增加，惟在培養時具相同含水量及品種中，碎米中之產毒量比完整米高(表四)。由此結果顯示黃米毒素之產量會受到稻米中之含水量之影響。真菌不易在完整米粒之內部生長與產毒，惟碎裂之米粒在貯存時可增加真菌生長與產毒之面積，甚至干擾穀粒內之通氣及含水量，以致於碎裂之米粒更易造成真菌菌落之形成與生長，尤其以青黴菌屬真菌為最甚。穀粒

Table 6. Effect of nitrogen source on the growth and luteoskyrin production by *Penicillium islandicum* THT-2[a]

Exp.[b]	Nitrogen source[c]	Mycelium (mg/ml)	Pigments (mg/ml)	Luteoskyrin (μ g/ml)
A	Control	9.54 ± 1.21[d]	0.64 ± 0.06	7.71 ± 1.39
	Malt extract	15.63 ± 1.46	1.12 ± 0.09	12.80 ± 1.43
	Yeast extract	19.43 ± 1.76	0.28 ± 0.02	1.64 ± 0.15
	Peptone	17.21 ± 1.76	0.29 ± 0.01	1.58 ± 0.13
	Casamino acid	16.16 ± 1.41	0.36 ± 0.01	3.29 ± 0.36
	Aspargine	12.44 ± 1.23	1.47 ± 0.11	12.21 ± 1.21
	Glutamine	10.91 ± 1.39	1.49 ± 0.12	12.54 ± 1.17
B	NH_4NO_3	4.21 ± 0.60	0.15 ± 0.01	2.20 ± 0.18
	NH_4Cl	6.57 ± 0.26	0.13 ± 0.01	1.94 ± 0.21
	$CO(NH_2)_2$	3.85 ± 0.63	0.20 ± 0.02	2.81 ± 0.25
	$(NH_4)_2SO_4$	3.95 ± 0.39	0.14 ± 0.02	2.09 ± 0.22

[a]. The medium was incubated at 25 ± 1℃ for 18 days.

[b]. In experiment A of Czapek solution broth was supplemented with the compounds listed;in experiment B, $NaNO_3$ in Czapek medium was replaced as cited in the table.

[c]. 0.2% each of the nitrogen source in Czapek solution broth.

[d]. Each value represents the mean ± SD, n=5.

Table 7. The change of pH value in Czapek solution broth during the growth of *Penicillium islandicum* THT-2[a]

Temperature (℃)	pH value							
	Incubation period (days)							
	0	6	10	14	18	22	26	30
10	7.3 ± 0.2[b]	6.8 ± 0.3	6.8 ± 0.3	6.6 ± 0.3	6.6 ± 0.4	6.5 ± 0.3	5.7 ± 0.1	5.8 ± 0.2
15	7.3 ± 0.1	6.6 ± 0.2	6.0 ± 0.3	5.8 ± 0.4	5.4 ± 0.2	5.3 ± 0.3	5.4 ± 0.3	5.4 ± 0.1
20	7.3 ± 0.2	5.1 ± 0.2	5.3 ± 0.2	4.6 ± 0.3	4.8 ± 0.2	5.2 ± 0.2	5.6 ± 0.1	5.6 ± 0.2
25	7.3 ± 0.1	5.2 ± 0.1	4.6 ± 0.4	4.1 ± 0.2	4.3 ± 0.2	4.6 ± 0.4	4.8 ± 0.2	5.2 ± 0.2
30	7.3 ± 0.1	5.3 ± 0.3	4.5 ± 0.1	4.2 ± 0.1	4.4 ± 0.1	4.8 ± 0.3	5.2 ± 0.3	5.3 ± 0.3
35	7.3 ± 0.2	5.8 ± 0.2	5.3 ± 0.2	4.8 ± 0.2	4.6 ± 0.2	5.0 ± 0.2	5.1 ± 0.2	5.4 ± 0.2
40	7.2 ± 0.2	6.4 ± 0.1	6.0 ± 0.0	5.5 ± 0.2	5.6 ± 0.2	5.8 ± 0.0	5.8 ± 0.5	5.8 ± 0.1

[a]. The volume of each culture medium: 20 ml.

[b]. Each value represents the mean ± SD, n=5 .

Table 8. The growth and luteoskyrin production of *Penicillium islandicum* THT-2 in different substrates[a]

Substrate	Water content (%)	Growth rate	Luteoskyrin (μ g/g substrate)
Unpolished rice	19.0	5+[b]	6.97 ± 0.48[c]
Polished rice	18.6	5+	5.74 ± 0.38
Unhulled rice	18.8	2+	0.95 ± 0.07
Wheat grains	18.8	5+	6.13 ± 0.54
Oatmeal	19.1	5+	6.51 ± 0.39
Yellowed corn	18.6	4+	3.88 ± 0.30
White corn	19.0	4+	3.45 ± 0.25

[a]. The medium was incubated at 25 ± 1℃ for 30 days.
[b]. 2+, 3+, 4+, 5+: the growth rate in medium.
[c]. Each value represents the mean ± SD, n=5.

Table 9. Effect of rice processing method on the growth of *Penicillium islandicum* THT-2 and the variation of water content of processed rice[a]

Medium[b]	Water content (%)						
	Incubation period (days)						
	0	10	20	30	40	50	60
R	15.2 ± 1.6/−[c]	15.3 ± 1.5/−	15.9 ± 1.5/−	16.9 ± 1.7/−	16.3 ± 1.7/−	14.0 ± 1.4/−	13.7 ± 1.3/−
R+S	15.2 ± 1.5/−	15.8 ± 1.4/−	16.9 ± 1.4/2+	20.2 ± 1.9/2+	19.4 ± 1.8/2+	17.3 ± 1.5/2+	15.8 ± 1.2/2+
BR	56.0 ± 4.7/−	52.4 ± 6.4/−	56.1 ± 6.0/4+	56.4 ± 8.7/5+	52.8 ± 6.2/5+	52.2 ± 5.8/5+	43.4 ± 3.4/5+
BR+S	56.1 ± 4.9/−	54.5 ± 5.6/3+	57.3 ± 5.8/4+	58.1 ± 6.6/5+	52.2 ± 6.5/5+	49.6 ± 6.0/5+	47.8 ± 3.8/5+
FR	6.9 ± 0.5/−	9.4 ± 0.8/−	12.3 ± 1.2/−	15.5 ± 2.1/−	14.9 ± 1.6/−	14.4 ± 1.5/−	12.5 ± 1.4/−
FR+S	7.1 ± 0.8/−	10.1 ± 1.3/−	12.8 ± 2.4/−	17.9 ± 2.0/+	17.2 ± 1.4/2+	16.3 ± 2.0/2+	13.8 ± 1.3/2+
BaR	6.7 ± 0.9/−	9.2 ± 1.1/−	13.4 ± 1.4/−	14.5 ± 1.3/−	14.4 ± 1.2/−	13.3 ± 1.4/−	12.3 ± 1.4/−
BaR+S	6.4 ± 0.6/−	10.9 ± 1.0/−	14.3 ± 1.3/−	17.4 ± 1.7/+	16.8 ± 1.3/2+	16.7 ± 1.2/2+	15.8 ± 1.4/2+
DFR	5.5 ± 0.6/−	6.3 ± 0.7/−	8.1 ± 0.7/−	10.4 ± 2.3/−	10.1 ± 0.9/−	8.8 ± 0.9/−	8.3 ± 0.8/−
DFR+S	5.4 ± 0.5/−	7.0 ± 0.7/−	9.5 ± 0.9/−	12.7 ± 1.3/−	12.7 ± 1.3/−	9.1 ± 0.9/−	8.8 ± 0.9/-

[a]. The medium was incubated at 25 ± 1℃.
[b]. R: rice, S: 5%sucrose, BR: Boiled rice, FR: Fried rice, BaR: Baked rice, DFR: Deep fried rice.
[c]. Each value represents the mean ± SD, n=5/−, +, 2+, 3+, 4+, 5+: the growth rate in medium.

中可非常明顯地觀察菌絲生長良好即為明顯之例証。

添加之碳源均能影響真菌生長，黃米毒素及相關色素之產生(表五)。具有促進真菌生長之碳水化合物，如澱粉，蔗糖，果糖，麥芽糖與葡萄糖等，其中以澱粉之活性最強，惟乳糖

Table 10. Effect of processing method on the production of luteoskyrin in rice by *Penicillium islandicum* THT-2[a]

Medium[b]	Luteoskyrin (μg/g) Incubation period (days)						
	0	10	20	30	40	50	60
R	–	–[c]	–	–	–	–	–
R+S	–	–	0.97 ± 0.09[d]	1.21 ± 0.11	1.14 ± 0.07	0.97 ± 0.07	0.85 ± 0.11
BR	–	4.53 ± 0.40	7.10 ± 0.52	9.05 ± 0.61	8.34 ± 0.61	7.33 ± 0.61	7.20 ± 0.64
BR+S	–	6.30 ± 0.54	12.40 ± 0.97	12.81 ± 0.85	12.51 ± 1.05	12.06 ± 1.00	11.66 ± 0.93
FR	–	–	–	–	–	–	–
FR+S	–	–	–	–	1.26 ± 0.09	1.22 ± 0.11	1.18 ± 0.08
BaR	–	–	–	–	–	–	–
BaR+S	–	–	–	–	1.40 ± 0.10	1.31 ± 0.11	1.20 ± 0.15
DFR	–	–	–	–	–	–	–
DFR+S	–	–	–	–	–	–	–

[a]. The medium was incubated at 25 ± 1℃.

[b]. R: rice, S: 5%sucrose, BR: Boiled rice, FR: Fried rice, BaR: Baked rice, DFR: Deep fried rice.

[c]. –: not detected.

[d]. Each value represents the mean ± SD, n=5.

能減緩真菌之生長。對於色素及毒素之產生，澱粉，蔗糖，果糖，麥芽糖，乳糖與葡萄糖等均有促進作用，其中以果糖之活性最大。惟檸檬酸鹽，麩胺酸鹽，丙二酸鹽，琥珀酸鹽，延胡索酸鹽與乙酸鹽等卻抑制真菌之生長與黃米毒素及相關色素之產生，其中以乙酸鹽之抑制能力最強。又乳糖雖對真菌生長無促進作用，但對於黃米毒素與相關色素之產生卻有明顯之促進作用。添加之氮源亦可影響真菌生長，黃米毒素與相關色素之產量(表六)。雖麥芽抽出物，酵母抽出物，蛋白腺，酪蛋白胺基酸，天冬醯胺，麩胺醯胺均能促進真菌之生長，但於相關色素及黃米毒素產生上，僅麥芽抽出物，天冬醯胺與麩胺醯胺等具有極強之促進作用，因此顯示此三種物質似與黃米毒素產生時之先軀物質有密切之相關性，麩胺醯胺之作用與Ueno及Ishikawa[12]研究結果相同，至於酵母抽出物，蛋白腺和酪蛋白胺基酸均可促進真菌之生長，但是卻會抑制色素與黃米毒素之產生,此現象可能是修飾了產毒真菌之生長特性或次生代謝活性所致，此有待進一步探討。另外硝酸銨，氯化銨，尿素與硫酸銨等對真菌生長，及黃米毒素與相關色素之產生均有抑制之作用。產毒菌生長時培養基之pH值明顯下降， 生長旺盛時尤甚，若該菌培養在25～30℃中自第六天開始即下降，至第14天可下降至4.1～4.2，然後再輕微上昇，至第30天則達5.2～5.3左右(表七)。在高經濟之農產品中菌絲之生長與黃米毒素之產量依序為糙米> 麥片> 小麥> 精白米> 黃皮玉米> 白皮玉米> 米穀(表八)。 其中之米，麥類之產毒量較高，此應與其中之成分有關。

精白米經乾炒，烘培或油炸，其含水量減低至8%以下，或因高溫而導致米中有利真菌生長與代謝所需成分之改變時，經60天之培養尚無法使樣品中之含水量達到適於真菌生長與產毒之需要，惟培養8天後雖然真菌無法生長， 但樣品因吸收空氣中濕氣之關係使米食內之含水量增加，至30天時其含水量達到最高，但此後又逐漸減少。若添加5%之蔗糖時含水

量增加量較高，俟含水量高達16%時即出現真菌生長與黃米毒素產生之現象(表九與表十)。含水量高達56%之烹煮米食，若添加5%之蔗糖時真菌生長與產毒量更高。精白米經過烹煮，乾炒，烘焙與油炸等手續處理，除烹煮者之含水量極高外，其餘含水量均極低。但由於濕度平衡之關係，因此含水量低之米食於真菌培養期間內，雖然真菌未見生長但會因米之吸收濕氣關係而使米食內之水份達到平衡狀態，此時亦可能誘導真菌之生長與黃米毒素之產生，惟經30日後或因蒸散關係，致使水份輕微散失而導致米食之含水量也略為降低。在培養過程中亦發現於真菌生長旺盛時間內，培養容器內壁常有水滴產生，此現象可能是因真菌生長旺盛時呼吸作用之加強而產生水汽之凝結，或是在培養基中菌絲分泌之含色素之桔棕色小液滴所致，尤其添加5%蔗糖者其含水量之增加更大，究其原因可能因為含5%蔗糖之米食經久置於室溫時具吸濕之能力所導致。由於*P. islandicum* THT-2具有此種能力因此農產品若污染該菌時，貯存期間可能造成其含水量之增加而使誘導真菌生長與產毒活性亦隨之增加。目前以米為原料之食品種類甚多，這些食品也往往會添加具誘導黃米毒素產生菌之生長及黃米毒素產生之蔗糖以增加風味與食慾，惟經常發現這些食品經短時間存放，均因吸濕關係不但影響風味，甚至還可能導致細菌與真菌之滋生，而導致該食品品質之降低與微生物或其毒素安全問題之產生,深值注意。

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The Growth and Production of Hepatotoxic Luteoskyrin by *Penicillium islandicum*

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ABSTRACT

The maximal production of luteoskyrin and related pigments by *Penicillium islandicum* was obtained during the late phase of cultivation. Maximum growth of mycelial was observed in potato dextrose broth and the highest productivity of luteoskyrin in malt extract broth. In an optimal substrate, luteoskyrin production was the highest in the 18th day. Mycelial growth and luteoskyrin production were greater in stationary incubation than when shaken. The optimal pH for the mycelial growth was between weak acidity and neutrality. Mycelial growth and the production of luteoskyrin and related pigments were increased by supplying a carbon source such as starch, sucrose, fructose, maltose or glucose. Starch and fructose maximized mycelial growth and luteoskyrin production respectively. Lactose also enhanced luteoskyrin production but inhibited mycelial growth.

The organic salts citrate,glutamate, malonate, succinate, fumarate and acetate inhibited mycelial growth and the production of luteoskyrin and related pigments. The acetate in particular displayed the strongest inhibitory effects in this test. Mycelial growth was enhanced by the following nitrogen sources: malt extract, yeast extract, peptone, casamino acid, asparagine and glutamine. However, the production of luteoskyrin and related pigments were only enhanced by malt extract, asparagine and glutamine. Potency of mycelial growth and luteoskyrin production in crops was found in the order of unpolished rice > oatmeal> wheat> polished rice> yellow corn> white corn>unhulled rice. A comparison of polished rice which had been subjected to various treatments showed boiled rice to be the best substrate for luteoskyrin production, especially in the presence of 5% sucrose.

Key words : Carbon source, nitrogen source, pH value, water content, luteoskyrin, *Penicillium islandicum*.