APPENDIX A

Area and population of Selangor from 2008 to 2010

District	Area (km^2)	Population (2008)	Population (2009)	Population (2010)
Petaling	484.32	1,514,100	1,547,100	1,782,375
Hulu Langat	849.48	1,142,500	1,170,900	1,141,880
Klang	626.78	825,100	840,700	848,149
Gombak	650.08	675,600	688,700	682,996
Kuala Langat	857.75	239,700	244,100	222,261
Kuala Selangor	1,194.55	198,100	201,600	210,406
Hulu Selangor	1,740.47	193,700	198,000	205,049
Sepang	555.51	146,400	150,500	212,050
Sabak Bernam	997.14	135,900	138,000	106,158
Total	**7,956.08**	**5,071,100**	**5,179,600**	**5,411,324**

APPENDIX B1

Dichloran Rose Bengal Chloramphenicol (DRBC) Agar

Content	g / L
Agar	15.0
Chloramphenicol	0.1
Dichloran	0.002
Glucose	10.0
Magnesium sulphate	0.5
Peptone	5.0
Potassium dihydrogen phosphate	1.0
Rose bengal	0.025

1. DRBC agar was prepared according to contents tabulated above.
2. The agar can also be obtained commercially.
3. 32 g of commercial DRBC was dissolved in 1 L of distilled or deionised water and autoclaved at 121°C at 15 psi.
4. DRBC is a light sensitive media.
5. Exposure to direct light will alter the media's chemical constituent to toxin that may inhibit fungal growth.

APPENDIX B2

Czapek Dox (CD) Agar

Content	g / L
Agar	12.0
Chloramphenicol	0.1
Ferrous sulphate	0.01
Magnesium glycerophosphate	0.5
Potassium chloride	0.5
Potassium sulphate	0.35
Sodium nitrate	2.0
Sucrose	30.0

1. CD agar was prepared according to contents tabulated above.
2. CD agar can also be obtained commercially.
3. 45.4 g of commercial CD agar was dissolved in 1 L of distilled or deionised water and autoclaved at 121°C at 15 psi.
4. Commercial CD agar was not added with chloramphenicol (antibiotic to inhibit bacterial growth).
5. Chloramphenicol was added prior to autoclaving (chloramphenicol is a heat-stabile substance, and therefore will not denature under hot temperature).

APPENDIX C

Wet-mount Slide Preparation

1. A drop of 95% ethyl alcohol was deposited in the middle of a clean microscope slide to disperse the abundance of fungal spores.
2. A very small fragment of young fungal culture to be examined was placed onto the alcohol drop.
3. Old fungal fragment tend to rupture, giving incomplete structural images.
4. Under a dissecting microscope, the fungal fragment was gently teased out using a pair of steel needles into a flat and even preparation.
5. Ethyl alcohol drop was left to evaporate before a drop of Acid Fuschin staining solution was added over the teased preparation.
6. A cover slip was then placed over the preparation carefully and gently at an angle, with one edge touching the slide first and was slowly lowered to spread out the staining solution and avoid air bubbles.
7. Trapped air bubbles were removed by gently pressing the cover slip to move them to the edge.
8. Excess staining solution around the edges was removed by blotting paper.
9. After a few minutes interval for stain penetration, the slide was ready for microscopic observation.

APPENDIX D1

PBS/Methanol (20 mM)

1. 90% water / methanol (v/v) was prepared.
2. 0.55 g NaH_2PO_4, 2.85 g Na_2HPO_4 and 9 g NaCl were mixed and filled up to 1 L with 90% water / methanol.
3. This buffer was used to rinse immunoaffinity column for ochratoxin-A clean-up.

APPENDIX D2

Sodium hydrogen carbonate buffer

1. $NaHCO_3$ was obtained commercially, in pellets form.
2. 84 g pellets in 1 L distilled or deionised water will give a 1 M buffer solution.
3. In this experiment, 10.92 g $NaHCO_3$ pellets were dissolved in 1 L distilled water to give 0.13 M buffer solution.
4. The final pH was approximately 8.1.
5. This buffer solution was used to dilute methanolic eluate containing ochratoxin-A after immunoaffinity column procedure.

APPENDIX D3

PBS/Tween (10 mM)

1. Washing buffer powder was provided with ochratoxin-A ELISA well test set.
2. It contains 0.05% tween 20.
3. The content was dissolved in 1 L distilled water.
4. The ready to use washing buffer expires after approximately 4 - 6 weeks at 4°C.
5. This buffer was used as washing solution in washing procedure during the ELISA test.

APPENDIX E

Fungal count (CFU/g) of red rice at cold and room temperatures

Sample	CFU/g	Temp.
1	1.6×10^6	Cold
2	5.7×10^5	Cold
7	2.1×10^6	Cold
8	1.6×10^5	Cold
9	1.0×10^5	Cold
10	5.7×10^4	Cold
11	5.3×10^4	Cold
19	2.5×10^5	Cold
20	2.2×10^5	Cold
21	6.0×10^4	Cold
23	7.3×10^5	Cold
25	1.7×10^5	Cold
27	1.0×10^5	Cold
30	7.0×10^4	Cold
31	5.3×10^4	Cold
33	1.3×10^5	Cold
34	1.4×10^5	Cold
35	4.7×10^4	Cold
37	8.3×10^4	Cold
38	9.0×10^4	Cold
39	1.2×10^5	Cold
40	6.3×10^4	Cold
41	1.2×10^5	Cold
42	9.0×10^4	Cold
43	5.3×10^4	Cold
45	2.1×10^5	Cold
47	1.7×10^5	Cold
48	1.1×10^5	Cold
49	9.7×10^5	Cold

Sample	CFU/g	Temp.
3	1.1×10^5	Room
4	2.5×10^4	Room
5	8.0×10^4	Room
6	1.6×10^5	Room
12	1.1×10^5	Room
13	2.5×10^5	Room
14	1.4×10^4	Room
15	4.0×10^4	Room
16	2.3×10^5	Room
17	1.1×10^5	Room
18	3.4×10^4	Room
22	1.9×10^4	Room
24	6.7×10^4	Room
26	1.3×10^5	Room
28	1.6×10^4	Room
29	2.8×10^5	Room
32	5.0×10^4	Room
36	1.3×10^5	Room
44	3.3×10^4	Room
46	1.8×10^5	Room
50	5.3×10^4	Room

APPENDIX F1

Key in characterisation of *Monascus* species

CHARACTERISTICS	
I. Cleistothecial walls and aleurioconidia becoming brownish at maturity; no pigments after 7 days ……..	***Monascus ruber***
II. Cleistothecial walls and aleurioconidia remaining hyaline; orange or reddish pigments after 7 days	
A. Colonies not exceeding 28 mm diam., hyphae usually abundant with crystalline encrustation, ascospore broad 6.0 - 7.0 by 4.5 - 5.0 µm ……..…	***Monascus purpureus***
B. Colonies exceeding 28 mm diam., hyphae usually lacking crystalline encrustation, ascospore narrow 5.0 - 7.0 by 3.0 - 3.5 µm ……	***Monascus pilosus***

APPENDIX F2

Key in characterisation of *Aspergillus flavus* group species

I.	**Conidial heads in pale to intense yellow or yellow green shades when young**	
	A. Colonies not shifting to brown on Czapek's agar; conidia definitely echinulate	
	1. Sterigmata single or double (predominant); heads radiate or very loosely columnar ……	*A. flavus*
	2. Sterigmata typically single	
	a. Heads columnar …….	*A. flavus* **var.** *columnaris*
	b. Heads radiate …….	*A. parasiticus*
	B. Colonies shifting to light brownish green in age on Czapek's agar; conidia irregularly roughened or smooth	
	1. Conidia large, mostly 4.5 to 7.0µm, but can up to 8.0 to 10.0µm, elliptical at first, then globose to subglobose, smooth to irregularly roughened	
	a. Conidiophore borne from substrate …	*A. oryzae*
	b. Conidiophore borne from aerial hyphae	*A. oryzae* **var.** *effuses*
	2. Conidia small, oval to elliptical, mostly 3.0 to 3.5µm, by 2.4 to 3.0µm, smooth or nearly so	
	a. Growth negligible on Czapek's agar; conidial structures abundant, zonately arranged on Malt agar; conidiophores smooth or nearly so ……...	*A. zonatus*
	b. Growth spreading on both Czapek's and Malt agar; conidial structures often forming coremiform clusters; conidiophores conspicuously roughened	*A. clavato-flavus*
II.	**Conidial heads in deep yellow-green to olive-brown shades when young; conidia conspicuously verruculose**	
	A. Conidial heads at first deep yellow-green, shifting to brownish green or brown on Czapek's agar …….....	*A. tamarii*
	B. Conidial heads shift from olive-brown to dark-brown ……...	*A. flavo-furcatis*
III.	**Conidial heads in pale yellow-olive to grey-olive shades; conidia smooth or nearly so**	
	A. Conidiophores conspicuously echinulate ……	A. subolivaceus
	B. Conidiophores smooth or nearly so …….	A. avenaceus

APPENDIX F3

Key in characterisation of *Aspergillus niger* group species

Characteristics are described for colonies grown on Czapek agar:

I	**BISERIATE STERIGMATA**	
A.	Colonies (conidial heads) carbon black	
1.	Conidia 6 -10 µm diam. at maturity ……..	***A. carbonarius***
2.	Conidia 5 µm diam. or less at maturity	
a.	Conidiophore not exceeding 4 mm	
(1)	Colonies spreading rapidly …….....	***A. ficuum***
(2)	Colonies growing slowly	
(a)	Conidia at maturity flattened, mostly 3 - 3.5 µm diam., with longitudinal striation ……...	***A. phoenicis***
(b)	Conidia at maturity globose mostly 4 - 5 µm diam., irregularly roughened with conspicuous ridges and echinulation not arranged in longitudinal striation …….	***A. niger***
b.	Conidiophore exceeding 5 mm - 1 cm ……...	***A. pulverulentus***
B.	Colonies (conidial heads) greyish, olive, brownish, reddish	
1.	Conidial heads dark brown or reddish brown	
a.	Conidia under 5 µm diam., flattened	
(1)	Conidial heads dark brown, reverse uncoloured, conidiophores 2 - 3 mm, conidia 3 - 3.5 µm diam...	***A. tubigensis***
(2)	Conidial heads reddish brown, reverse in similar shade, conidiophores 1 - 1.5 mm, Conidia 4 - 4.5 µm diam. ……	***A. awamori***
b.	Conidia 6 - 8 µm diam., globose to subglobose, coarsely Tuberculate …….	***A. flavo-furcatis***
2.	Conidial heads greyish brown or olive brown	
a.	Conidia elliptical, conspicuously echinulate, 5 - 5.5 µm by 3.3 - 3.8 µm ……..	***A. ellipticus***
b.	Conidia globose	
(1)	Conspicuously spinulous …….	***A. heteromorphus***
(2)	Irregular and finely roughened	
(a)	Conidial heads small, split into numerous compact divergent columns	***A. foetidus***
(b)	Conidial heads large, columns few	
(1')	Basal mycelium on malt agar uncoloured or faint yellow	***A. foetidus* var. *pallidus***
(2')	Basal mycelium on malt agar bright golden yellow	***A. foetidus* var. *acidus***
II	**UNISERIATE STERIGMATA**	
A.	Conidia globose to subglobose, conspicuously echinulate, vesicles 20 - 35 µm (normally 15 - 45 µm) ……	***A. japonicus***
B.	Conidia subglobose to elliptical, conspicuously echinulate, vesicles 60 - 80 µm (normally 35 - 100 µm) ……	***A. aculeatus***

APPENDIX F4

Key in characterisation of *Penicillium chrysogenum* series species

Initially, there were four species placed under this series as recognised by Raper and Thom (1949). But subsequent re-examinations have reduced the other three species (*Penicillium meleagrinum* Biourge, *Penicillium cyaneo-fulvum* Biourge, and *Penicillium notatum* Westling) as synonymies of the current single representative of the series (*Penicillium chrysogenum* Thom).

PENICILLIUM	**MONOVERTICILATTA**		***P. thomii* Series**
			***P. frequentans* Series**
			***P. lividum* Series**
			***P. implicatum* Series**
			***P. decumbens* Series**
			***P. restrictum* Series**
			***P. adametzii* Series**
	ASSYMETRICA	*divaricata*	***P. raistrickii* Series**
			***P. lilacinum* Series**
			***P. janthinellum* Series**
			***P. godlewskii* Series**
			***P. canescens* Series**
			***P. nigricans* Series**
			***P. brasilianum* Series**
		velutina	***P. citrinum* Series**
			***P. chrysogenum* Series**
			***P. oxalicum* Series**
			***P. digitatum* Series**
			***P. roqueforti* Series**
			***P. brevicompactum* Series**
		lanata	***P. camemberti* Series**
			***P. commune* Series**
		funiculosa	***P. terrestre* Series**
			***P. pallidum* Series**
		fasciculata	
	BIVERTICILLATA	*symmetrica*	***P. herquei* Series**
			***P. funiculosum* Series**
			***P. duclauxii* Series**
			***P. purpurogenum* Series**
			***P. rugulosum* Series**
	POLYVERTICILLATA		

APPENDIX G1

Relationship between storage temperature and fungal count

(Independent Sample t-Test)

Group Statistics					
	TEMPERATURE	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**
CFU	**1**	**29**	2.9962E5	4.83682E5	89817.40390
	2	**21**	1.0100E5	80361.68241	17536.35679

1 : ***Cold***

2 : ***Room***

Independent Samples Test										
		Levene's Test for Equality of Variances		**t-test for Equality of Means**						
		F	**Sig.**	**t**	**df**	**Sig. (2-tailed)**	**Mean Diff.**	**Std. Error Diff.**	**95% Confidence Interval of the Difference**	
									Lower	**Upper**
CFU	Equal variances assumed	9.054	.004	1.858	48	**.069**	1.98621E5	1.06889E5	-16294.62492	4.13536E5
	Equal variances not assumed			2.170	30.114	.038	1.98621E5	91513.33156	11755.24068	3.85486E5

APPENDIX G2

Relationship between fungal count and Citrinin level

(Linear Regression)

Model Summary				
Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
1	.132[a]	.017	-.003	4629.34302
a. Predictors: (Constant), CFU				

ANOVA[b]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.811E7	1	1.811E7	.845	.036[a]
	Residual	1.029E9	48	2.143E7		
	Total	1.047E9	49			

a. Predictors: (Constant), CFU

b. Dependent Variable: CITRININ

APPENDIX G3

Relationship between Citrinin production by *Monascus purpureus* and *Monascus pilosus*

(Independent Sample t-Test)

Group Statistics					
	MONASCUS	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**
CITRININ	**1**	**29**	5.1791E3	5653.05911	1049.74672
	2	**21**	2.4329E3	1734.06370	378.40372

1 : ***Monascus purpureus***

2 : ***Monascus pilosus***

Independent Samples Test										
		Levene's Test for Equality of Variances		**t-test for Equality of Means**						
		F	**Sig.**	**t**	**df**	**Sig. (2-tailed)**	**Mean Diff.**	**Std. Error Diff.**	**95% Confidence Interval of the Difference**	
									Lower	**Upper**
Citrinin	Equal variances assumed	15.522	.000	2.149	48	**.037**	2746.16749	1278.03650	176.50288	5315.83210
	Equal variances not assumed			2.461	34.924	.019	2746.16749	1115.86628	480.66192	5011.67306

APPENDIX G4

Inhibition of *Aspergillus flavus* against citrinin concentration

Aspergillus flavus was present in 22 red rice samples (44%).

Citrinin Range	No. of Samples with Citrinin	No. of Samples with *A. flavus*	No. of *A. flavus* inhibition	Percentage *A. flavus* inhibition
0.23 – 4.99	38	15	23	23/38 = **60.53%**
5.00 – 9.99	7	4	3	3/7 = **42.86%**
10.00 – 14.99	2	2	0	0/2 = **0%**
15.00 – 20.65	3	1	2	2/3 = **66.67%**
TOTAL	**50 (all samples)**	**22 (44 %)**	**28**	**R^2 = 0.011**

** R^2 is very low by reason of presence of zero-valued figure.*

Inhibition pattern is not linear.

APPENDIX G5

Inhibition of *Aspergillus niger* against citrinin concentration

Aspergillus niger was present in 27 red rice samples (54%).

Citrinin Range	No. of Samples with Citrinin	No. of Samples with *A. niger*	No. of *A. niger* inhibition	Percentage *A. niger* inhibition
0.23 – 4.99	38	21	17	17/38 = **44.74%**
5.00 – 9.99	7	4	3	3/7 = **42.86%**
10.00 – 14.99	2	1	1	1/2 = **50%**
15.00 – 20.65	3	1	2	2/3 = **66.67%**
TOTAL	**50 (all samples)**	**27 (54 %)**	**23**	**R^2 = 0.7556**

BIODATA OF AUTHOR

Nik Iskandar Putra bin Samsudin, a Perak-born of a Kelantanese father and a Singaporean mother, was born in 1985. He received his primary education (1992 - 1997) in Sekolah Kebangsaan Tanah Merah (1), Kelantan, in which Science was his favourite subject. With outstanding examination result (5 A's), he was later admitted for secondary education in a government-sponsored residential school, Sekolah Menengah Sains Tengku Muhammad Faris Petra, also in Kelantan (1998-2002). He completed his secondary level with excellent result (8 A's, 1 B).

He had his pre-university education at Johor Matriculation College, Johor (2003-2004) majoring in Biological Sciences (3.40 / 4.00). He obtained his first degree, Bachelor of Science (Honours) Microbiology from Universiti Putra Malaysia, Serdang (2004-2007).

Of all the disciplines in Microbiology, he was always interested in Mycology (study of fungus). In his final year, he won the *Interscience Sdn. Bhd. Prize* for Best Undergraduate Final Year Project 2007 (departmental level) also in the field of Mycology. He graduated with 3.40 / 4.00.

Upon graduation, he was admitted to the tutorship under the Faculty of Food Science and Technology, UPM. There, he secured a government scholarship and embarked for Master of Science in Universiti Malaya, UM, Kuala Lumpur, in the field of Food Mycology (2009-2011).