Mutagenic and Anti-mutagenic Effects of Methanol Extract of *Tremella flava* Chen Fermented Soymilk (TFS)

I-Chuan Sheih
Department of Food and Beverage Management, Ta Hwa University of Science and Technology, Hsinchu, Taiwan, ROC
Email: sic@tust.edu.tw

Tai-I Chen and Tony J. Fang
Department of Food Science and Biotechnology, National Chung Hsing University, Taichung, Taiwan, ROC
Email: tjfang@nchu.edu.tw

***Abstract*—*Tremella flava* Chen, a novel yellow jelly and edible mushroom, was isolated in Taiwan. Our preliminary study have shown the antioxidative, anti-inflammation and anti-infection property of *T. flava* fermented soymilk (TFS), and the objective of this study is to determine the potential adverse effects and antimutagenic applicability. The Ames test employing histidine mutants of the *S. typhimurium* tester stains TA97, TA98, TA100, TA102 and TA1535 were used to examine the mutagenicity of TFS. No mutagenic activity was observed for either test strains at all used doses (0.25~20.0 mg/plate), with or without S9 activation. On the other hand, TFS also inhibited 4-nitroquinoline-1-oxide (4NQO), mitomycin C and sodium azide-induced mutations in a dose-dependent manner. These results demonstrated that TFS might be a multifunctional and safety dietary supplement with antioxidative, anti-inflammation, anti-infection, and antimutagenic activities in the future.**

***Index Terms*—Tremella, soymilk, antimutagenic, Ames test, mutation**

I. Introduction

It has been found that the fermented solution, various extracts, or the polysaccharide from Tremella have significant bioactivity, such as immunomodulators [1], [2], hypoglycemic [3], anti-tumor [4], antioxidant and anti-inflammatory by *T. fuciformis* polysaccharides [5], hypocholesterolemic by *T. fuciformis* Berk (TFB) dietary fiber [6], [7] and the hot water extract of *T. fuciformi* is neuroprotective [8]. However, *Tremella flava* Chen, a novel fungus isolated in Taiwan, was used to ferment the soymilk that was used as beverage in our study. Our preliminary study have shown the antioxidative, anti-inflammation and anti-infection property of *T. flava* fermented soymilk (TFS) [2], and the objective of this study is to determine the potential adverse effects and antimutagenic applicability for dietary supplement.

Manuscript received July 21, 2017; revised September 20, 2017.

II. Materials and Methods

A. Microbes

Tremella flava is a novel, yellow and edible mushroom that belongs to the Tremellaceae family; it was isolated by Dr. Chee-Jen Chen in Taiwan. The genus Tremella belongs to the family of the Tremellaceae, which is a heterobasidiomycetous fungus.

B. Soymilk Fermentation

The soybeans were dipped in water and homogenized at 1:10 (w/v) ratio and filtered with a filtration fabric. The soymilk was sterilized at 121 ℃ for 15 min. One percent of the *T. flava* culture was added to the sterilized soymilk, and then incubated at 25 ℃ and 100 rpm for 2 d. The fermented soymilk (TFS) was lyophilized.

C. Toxicity Test

0.1 mL of fresh *S. typhimurium* cultures (TA97, TA98, TA100, TA102 and TA1535) were added to 500 μL of 0.2 M sodium phosphate buffer (pH 7.4) and 100 μL of TFS (0, 0.25, 0.5, 1.0, 5.0, 10.0, 20.0 mg/plate) without metabolic activation (S9 mix). The mixtures were incubated at 37 ℃ for 20 min. 1 mL of the diluent was transferred into empty sterile plates and added with molten nutrient agar. The plates were incubated at 37 ℃ for 48 hours, and the colonies are counted. Negative control plates are added with water instead of TFS.

D. Mutagenicity Test

0.1 mL of fresh *S. typhimurium* cultures (TA97, TA98, TA100, TA102 and TA1535) were added to 500μL of 0.2 M sodium phosphate buffer (pH 7.4) and 100μL of TFS (0, 0.25, 0.5, 1.0, 5.0, 10.0, 20.0 mg/plate) with and without metabolic activation (S9 mix). The mixtures were incubated at 37 ℃ for 20 min, after which 2.0 mL of top agar medium containing histidine/biotin were added at 45 ℃. The mixture is then poured onto minimal glucose plates and incubated at 37 ℃ for 48 hours before the histidine revertant colonies were counted. The

doi: 10.18178/ijfe.4.1.46-50

reversion rate was compared to that of negative control plates treated with the same amount of water instead of TFS. Positive control plates are added with diagnostic mutagens.

a. Without S9 activation:

4-Nitroquinoline-N-oxide 0.5 μg/plate (TA97, TA98 and TA100)

Mitomycin C 0.5 μg/plate (TA102)

Sodium azide 4 μg/plate (TA1535)

b. With S9 activation:

Benzo[a]pyrene 1 μg/plate (TA97, TA98, TA100 and TA102)

2-Aminoanthracene 4 μg/plate (TA1535)

E. Antimutagenicity Test

The Ames test is a widely accepted method to detect chemicals that may induce gene mutation. The most common tester strains are TA98 and TA100, however, TA 97, TA102 and TA1535 is also used in this study to further ensure the results [7]. The antimutagenic effects of TFS were examined under the same conditions used in the mutagenicity test described above in D. Thus, after mixing the *S. typhimurium* strains with 0, 0.25, 0.5, 1.0, 5.0, 10.0 and 20.0 mg/plate of TFS, the antimutagenic potential of TFS was evaluated. Positive controls in absence of TFS were included for each strain. The inhibition of mutagenicity was calculated by using the following equation:

$$\% \text{ Inhibition} = (A - C) / (A - B) \times 100$$

A: number of revertants/plate induced by mutagen alone

B: number of spontaneous revertants (no mutagen, no sample)

C: number of revertants/plate induced by the extract and the mutagen

F. 28-day Gavage Toxicity Study

The mice were approximately 6-week-old upon reception. Animals were housed for a 14-day acclimation period. All animals were housed individually in clean, wire-mesh cages suspended above cage-board. All animals were housed throughout the acclimation period and during the study in an environmentally controlled room. Controls were set to maintain a temperature of 22 ± 3 ℃ and a 12-h light/12-h dark photoperiod. The basal diet used in this study is standard lab diet (chow diet MF-18). Reverse osmosis drinking water was provided ad libitum throughout the study period.

III. Results and Discussions

A. Toxicity Test Mutagenicity Test

A toxicity test is also necessary to confirm that the sample (*Tremella flava* fermented soymilk-TFS) is not toxic for the tester strains. At TFS concentrations 0.0 mg/mL~20.0mg/mL, the survival rates for *S. typhimurium* TA97, TA98, TA100, TA102 and TA1535 were ranged from 99.14 - 103.74%, 99.10 - 102.70%, 98.40 - 101.83%, 98.64 - 102.72% and 97.92 - 102.08%, respectively (Fig. 1). Results from statistical analysis showed no significant difference compared to that of the control group. The results indicated TFS used for this study is therefore not toxic for the used tester strains.

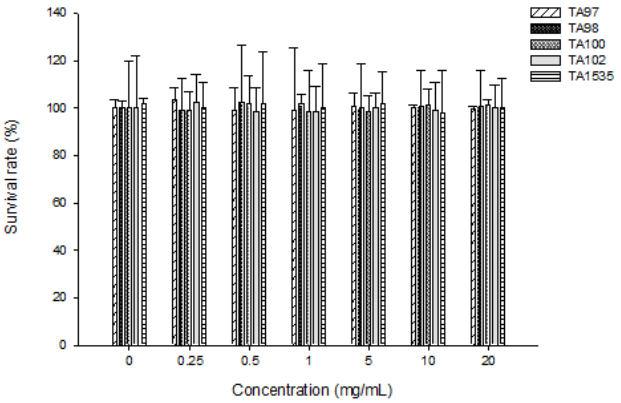
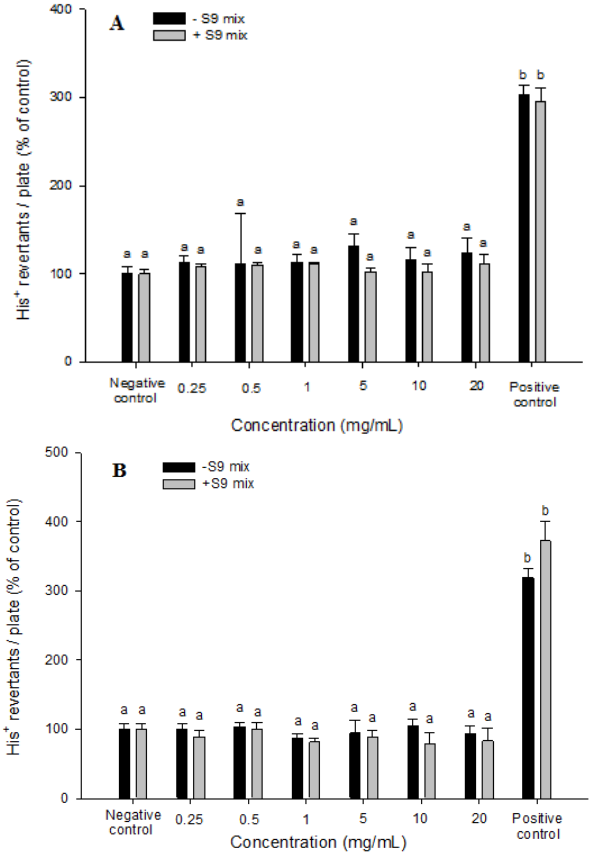


Figure 1. Toxicity test of TFS on *Salmonella typhimurium* strains TA97, TA98, TA100, TA102 and TA1535.

B. Mutagenicity Test

In this study, the ratio of *S. typhimurium* tester strain (TA97, TA98, TA100, TA102 and TA1535) revertants with and without the addition of S9 activation were 1.00 - 1.31 (-S9) and 0.99 - 1.11 (+S9) (Fig. 2A), 0.87 - 1.05 (-S9) and 0.78 - 1.01 (+S9) (Fig. 2B), 1.00 - 1.23 (-S9) and 1.00 - 1.14 (+S9) (Fig. 2C), 0.93 - 1.03 (-S9) and 0.75 - 1.02 (+S9) (Fig. 2D) and 0.89 - 1.31 (-S9) and 1.00 - 1.32 (+S9) (Fig. 2E). It is concluded that the test concentrations (0.25 mg/mL ~ 20 mg/mL) of TFS, with or without S9 activation, had no significant effect on colony numbers as compared to the control group, which indicates that TFS was not mutagenic for strains TA97, TA98, TA100, TA102 and TA1535.



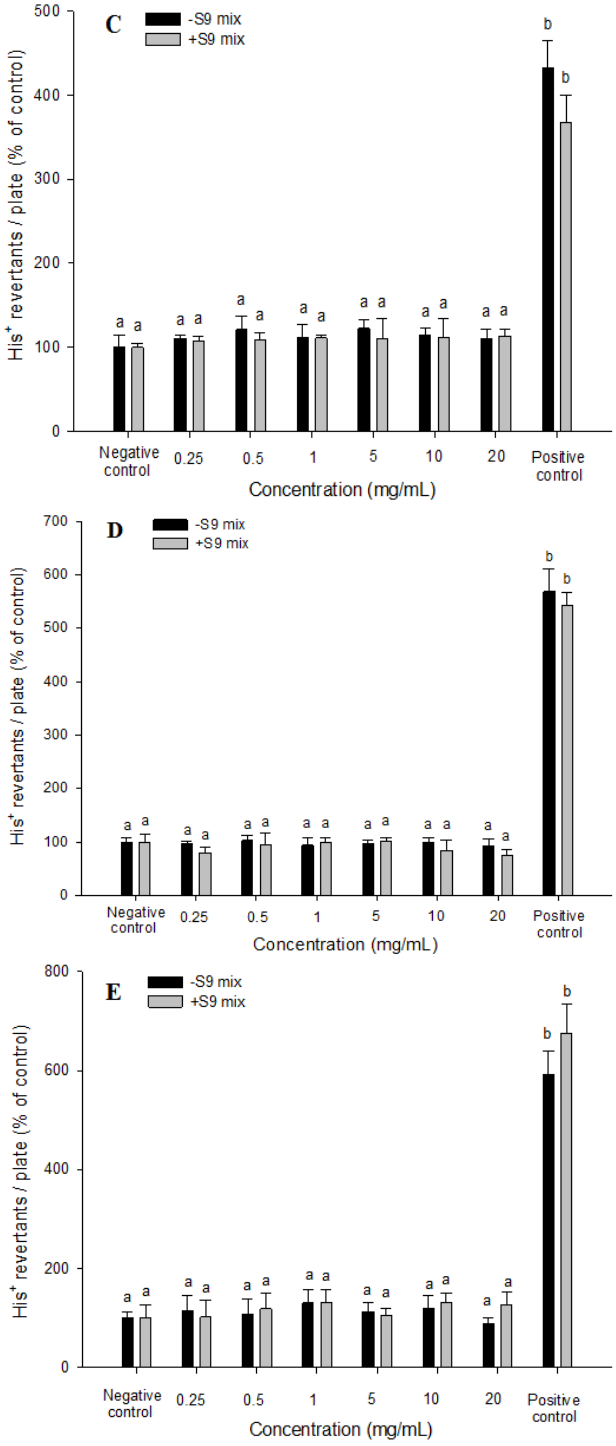


Figure 2. Effects of TFS concentrations on the ratio of *Salmonella typhimurium* tester strain revertants with and without the addition of S9 activation: (A) TA97, (B) TA98, (C) TA100, (D) TA102 and (E) TA1535.

C. Antimutagenicity Test

In the absence of S9 activation, TFS was found to block 4-nitroquinoline-N-oxide, mitomycin C and sodium azide-induced mutations in TA97, TA98, TA100, TA102 and TA1535 in a dose-dependent manner (Table I). When TFS concentration was increased to 20 mg/plate, the histidine revertants for TA97, TA98, TA100, TA102 and TA1535 reduced to 182 ± 9, 162 ± 7, 309 ± 7, 801 ± 5 and 39 ± 8 CFU/plate, respectively. For all 5 TA strains, 20 mg/plate of TFS inhibited the mutagenic effects of mutagens on TA97, TA98, TA100, TA102 and TA1535 by 65.4 ± 4, 61.1 ± 6, 57.9 ± 2, 51.5 ± 4 and 70.8 ± 9%, respectively. The results showed that TFS possess anti-mutagenic effects against 4-nitroquinoline-N-oxide, mitomycin C and sodium azide-induced mutations on *S. typhimurium* TA strains by using the Ames test. Since TFS was antimutagenic in the absence of S9, it may exert this function by scavenging the free radicals induced by the mutagens used in this study.

TABLE I. INHIBITORY EFFECTS OF TFS ON THE MUTAGENICITY OF MUTAGENS ON *SALMONELLA TYPHIMURIUM* TA97, TA98, TA100, TA102 AND TA1535

A

TFS* (mg/plate)	His+ revertants/plate				
	TA97	TA98	TA100	TA102	TA1535
Spontaneous	115 ± 4	48 ± 8	140 ± 17	249 ± 13	13 ± 3
0.0**	372 ± 11[a]	367 ± 15[a]	541 ± 10[a]	1388 ± 11[a]	102 ± 10[a]
0.25	372 ± 17[a]	364 ± 13[a]	529 ± 3[a]	1292 ± 9[b]	100 ± 1[a]
0.5	368 ± 7[a]	351 ± 5[a]	503 ± 9[b]	1211 ± 6[c]	83 ± 8[b]
1	344 ± 2[b]	308 ± 18[b]	489 ± 2[c]	1104 ± 9[d]	81 ± 5[b]
5	296 ± 10[c]	255 ± 8[c]	406 ± 4[d]	1003 ± 5[e]	67 ± 5[c]
10	223 ± 13[d]	214 ± 16[d]	373 ± 2[e]	882 ± 8[f]	52 ± 6[d]
20	182 ± 9[e]	162 ± 7[e]	309 ± 7[f]	801 ± 5[g]	39 ± 8[e]

B

TFS* (mg/plate)	**Percent inhibition (%)**				
	TA97	**TA98**	**TA100**	**TA102**	**TA1535**
Spontaneous					
0.0**	0.0 ± 7[a]	0.0 ± 5[a]	0.0 ± 7[a]	0.0 ± 3[a]	0.0 ± 9[a]
0.25	0.0 ± 4[a]	0.9 ± 13[a]	3.0 ± 8[a]	8.4 ± 8[a]	2.2 ± 3[a]
0.5	1.6 ± 3[a]	5.0 ± 7[a]	9.5 ± 2[a]	15.6 ± 5[b]	21.3 ± 3[b]
1	10.9 ± 3[b]	18.5 ± 5[b]	13.0 ± 6[a]	25.0 ± 6[b]	23.6 ± 8[b]
5	25.0 ± 5[b]	35.1 ± 6[c]	33.7 ± 3[b]	33.9 ± 10[b]	39.3 ± 6[c]
10	33.5 ± 4[b]	48.0 ± 6[d]	41.9 ± 11[b]	44.4 ± 2[b]	56.2 ± 9[d]
20	65.4 ± 4[c]	61.1 ± 6[e]	57.9 ± 2[c]	51.5 ± 4[c]	70.8 ± 9[e]

*Each value represents the mean ± SD (n=3). Data bearing different superscript letters within a column are significantly different ($P < 0.05$), as determined by Duncan's multiple range test

**The positive controls without S9-mix were 4-nitroquinoline-N-oxide (0.5 μg/plate) for TA97, TA98, TA100; mitomycin C (0.5 μg/plate) for TA102; sodium azide (0.5 μg/plate) for TA1535.

D. Subacute Toxicity (28-day) Gavage Study

Body weight change is a sensitive and direct sign of systemic toxicity in animals; physical changes such as body weight gain or organ weight change occur when chemicals or carcinogens enter the host. Compared to control group, no treatment-related biologically significant effects of TFS were noted on body weight or body weight gain at dose levels up to 2 g/kg BW/day for both genders (Fig. 3A and 3B). Body weights of both female and male groups fed with the highest dose (2 g/kg BW/day) were observed to be slightly, but not significantly lower than other given doses.

The results of feed consumption showed there were no treatment-related significant adverse effects of TFS on feed consumption or the feed efficiency ratio (Table II). The quantity of water consumed during the course of this study by TFS-treated animals was comparable to the control groups. These results showed that administration

of TFS at levels up to 2 g/kg BW/day to mice did not significantly affect feed and water consumption.

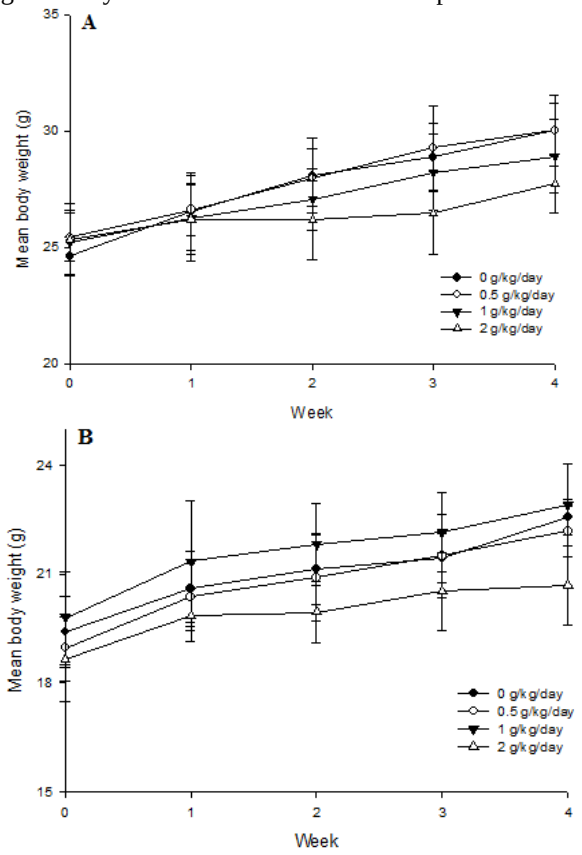


Figure 3. Effects of TFS on body weights in male (A) and female (B) BALB/c mice (n=4).

TABLE II. FEED CONSUMPTION (A) AND WATER INTAKE (B) BY BALB/C MICE DURING 28-DAY TOXICITY GAVAGE STUDY

A

	Time (week)	Feed consumption (g)*			
		Dose (g/kg BW/day)			
		0	0.5	1	2
Male	0	7.9[a]	13.4[a]	13.0[a]	10.7[a]
	1	16.4[a]	15.6[a]	14.8[a]	13.5[a]
	2	21.8[a]	19.4[a]	16.0[a]	16.2[a]
	3	17.8[a]	18.6[a]	16.9[a]	16.1[a]
	4	18.8[a]	17.6[a]	15.2[a]	16.9[a]
Female	0	14.7[a]	13.0[a]	13.6[a]	11.1[a]
	1	15.0[a]	13.0[a]	15.4[a]	12.5[a]
	2	16.3[a]	14.3[a]	15.9[a]	14.4[a]
	3	17.2[a]	16.3[a]	15.1[a]	13.5[a]
	4	18.4[a]	15.1[a]	14.9[a]	13.5[a]

B

	Time (week)	Water intake (mL)*			
		Dose (g/kg BW/day)			
		0	0.5	1	2
Male	0	17.0[a]	22.0[a]	19.0[a]	12.0[a]
	1	23.0[a]	30.0[a]	23.0[a]	22.0[a]
	2	17.0[a]	17.0[a]	16.0[a]	15.0[a]
	3	16.0[a]	17.0[a]	15.0[a]	16.0[a]
	4	16.0[a]	13.0[a]	12.0[a]	14.0[a]
Female	0	24.0[a]	29.0[a]	22.0[a]	18.0[a]
	1	31.0[a]	30.0[a]	23.0[a]	22.0[a]
	2	16.0[a]	15.0[a]	15.0[a]	12.0[a]
	3	18.0[a]	20.0[a]	16.0[a]	13.0[a]
	4	16.0[a]	14.0[a]	13.0[a]	11.0[a]

IV. CONCLUSION

TFS (0.25 mg/mL - 20 mg/mL), with or without S9 activation, had no significant effect on colony numbers as compared to the control group, indicating that TFS was not mutagenic for strains *S. typhimurium* TA97, TA98, TA100, TA102 and TA1535. On the other hand, TFS was found to block 4-nitroquinoline-N-oxide, mitomycin C and sodium azide-induced mutations in strains TA97, TA98, TA100, TA102 and TA1535 in a dose-dependent manner without S9 activation. The highest percent inhibition was found in *S. typhimurium* TA97 (65%) and TA1535 (71%), so TFS is therefore suggested to possess anti-mutagenic properties.

For subacute toxicity (28-day) gavage study, results suggest that administration of TFS at levels up to 2 g/kg/day to mice for 28 days has no adverse effects on pre-clinical observations, body weights, feed consumption and water intake.

ACKNOWLEDGMENT

We thank National Science Council, ROC, project no. Most 105-2221-E-233-006-MY2 and 102-2313-B-005 -023-MY3 for financially supporting this research.

REFERENCES

[1] H. A. EI-Enshasy and R. Hatti-Kaul, "Mushroom immunomodulators: Unique molecules with unlimited applications," *Trends Biotechnol.* vol. 31, pp. 668–677, 2013.
[2] T. I. Chen, I. C. Sheih, H. Y. J. Jeng, and T. J. Fang, "Anti-Inflammation and anti-infection applicability of *Tremella flava* Chen Fermented Soymilk (TFS) in a BALB/c Mice Model," IPCBEE, vol. 77, pp. 6-10, 2014.
[3] P. Kalac, "A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms," *J. Sci. Food Agric.* vol. 93, pp. 209–218, 2013.
[4] C. K. Han, H. C Chiang, *et al*., "Comparison of immunomodulatory and anticancer activities in different strains of *Tremella fuciformis* Berk," *Am. J. Chin. Med*., vol. 43, pp. 1637-1655, 2015.
[5] H. Li, H. S. Lee, S. H. Kim, B. Moon, and C. Lee, "Antioxidant and anti-inflammatory activities of methanol extracts of *Tremella fuciformis* and its major phenolic acids," *J. Food Sci.* vol. 79, pp. C460-468, 2014.
[6] H. H. Cheng, W. C. Hou, and M. L. Lu, "Interactions of lipid metabolism and intestinal physiology with *Tremella fuciformis* Berk edible mushroom in rat fed a high-cholesterol diet with or without Nebacitin," *IPCBEE*, vol. 50, pp. 7438-7443, 2002.
[7] V. Caz, A. Gil-Ramirez, *et al*., "Modulation of cholesterol-related gene expression by dietary fiber fractions from edible mushrooms," *J Agric. Food Chem.*, 63, pp. 7371–7380, 2015.
[8] H. J. Parka, H. S. Shimb, *et al*., "*Tremella fuciformis* enhances the neurite outgrowth of PC12 cells and restores trimethyltin-induced impairment of memory in rats via activation of CREB transcription and cholinergic systems," *Behav. Brain Res.*, vol. 229, pp. 82–90, 2012.

I—Chuan Sheih has ever been as a RD for four years in the Resource Microbiology Institute and a researcher for six years in Food Industry Research Development Institute. She has completed her PhD in 2009 and done a postdoctoral research for two and a half years in National Chung Hsing University. She is the associate professor of department of Food and Beverage Management in Ta Hwa University of Science and Technology and a project director of Ministry of Science

and Technology. She has published many SCI papers in reputed journals at the same time.

Tony J. Fang is a professor of. Department of Food Science and Biotechnology in National Chung Hsing University. He focuses in food microbiology, fermentation biotechnology and health, nutrition and food chemistry. He has published many SCI papers in reputed journals. He is also a project director of Ministry of Science and Technology.