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Studies on *Ganoderma lucidum*

IV. Identification of strains by chemical compositions in mycelial extracts

Tsung-Che Tseng and Li-Lin Lay

Institute of Botany, Academia Sinica
Nankang, Taipei, Taiwan, Republic of China

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Abstract. Dry mycelia from three isolates of *Ganoderma lucidum* and one isolate of *G. tsugae* were analyzed for the chemical compositions consisted of carbohydrate, soluble protein, lipid and ash. Although there were no significant differences among the isolates, yet mycelia from *G. tsugae* contained less total carbohydrate, and the relative ratio of $C_{16:0}$ fatty acid in *G. tsugae* was two times higher than the isolates of *G. lucidum*.

Mycelial extracts from the isolates of *G. lucidum* and *G. tsugae* were separated based on polyacryamide disc gel electrophoresis, and then stained for soluble protein, esterase, peroxidase (PO), leucine aminopeptidase (LAP), and lactate dehydrogenase (LDH). By visible inspection, more bands were seen on gels of *G. tsugae* and *G. lucidum* NTU G001 than on the isolates of *G. lucidum* ATCC 32471 and 32472 in 2-week mycelial extracts. The enzyme gels of *G. lucidum* and *G. tsugae* were more easily distinguished based on esterase activity. Very little or no enzyme activity of PO, LAP, LDH were noted among the tested isolates. The essential amino acids from the mycelial extracts revealed that aspartic acid, glutamic acid, serine, glutamine, threonine plus glycine, and alanine are the predominant species in four isolates. Histidine, methionine, leucine and lysine are only detected in the mycelial extracts of *G. tsugae*. Based on our results we proposed that the chemical compositions in the mycelial extracts of the fungi should provide useful information for classification of the strains of *Ganoderma* species.

Key words: Essential amino acids; Esterase; Fatty acids; *Ganoderma lucidum; Ganoderma tsugae;* Soluble protein.

Introduction

Ganoderma lucidum (Fr.) Karst NTU G001, a wood rotting fungus and traditional Chinese medicine, was cultured in liquid media at various growth conditions. Preliminary study showed that the relative amounts of secondary fungal metabolites varied significantly during growth period was monitored by HPLC/TLC techniques. Gas chromatography reveals that $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ fatty acids are the predominant ones in the esterified forms (Tseng, *et al.*, 1984). Toxicity of ethanolic extracts of mycelia is quite low as followed by a mice LD_{50} test. Wistar rats fed with high doses of *G. lucidum* were found to have elevated total serum lipids

and cholesterol (Shiao, *et al.*, 1986). An endo-pectin methyl-*trans*-eliminase was also obtained from crude extract of mycelia cultured in polypropylene bags. The enzyme was able to macerate plant tissues (Tseng and Chang, 1987).

The identification and classification of strains of *G. lucidum* have been reported in Taiwan on the basis of cytological and physiological studies as well as mating test (Chang, 1983; Hsew and Wang, 1986). However, great difficulties have been encountered in many cases because it is insufficient to distinguish strains that genetically close each other. Recently, disc gel electrophoresis of proteins and enzymes has proved useful to evaluate the chemotaxonomic relationship of fungi (Chang, *et al.*, 1962: Stipes, 1970; Stipes, *et al.*, 1982; Shechter, *et al.*, 1973; Hseu and Wang, 1986). Serological techniques (Taguchi *et al.*, 1979) and DNA base composition are also reported (Normore, 1973; Kuninaga and Yokosawa, 1980).

The aims of this investigation were continuing to evaluate our subsequent research on correlations of growth condition and the productivity of effective ingredients of the fungus, to consider the applications of fatty acid profile, soluble protein and isozyme electrophoresis as well as essential amino acid composition in mycelial extracts as biochemical means to identify the strains of *G. lucidum*.

Materials and Methods

Chemicals

Ethanethiol, acrylamide, ammonium persulfate and Folin-Ciocalteu reagent were purchased from Wako Pure Chemicals (Osaka, Japan). Bisacrylamide, Bromophenol blue, Coomassie blue, o-phthalaldehyde, α-naphthy acetate, Blue R R salt, guaiacol, hydrogen peroxide, L-leucyl-β-naphthylamide-HCl, Fast black K salt, sodium lactate diphosphopyridine nucleotide (DPN), phenazine methosulfate, tetrazolium blue, and standard essential amino acids were obtained from Sigma Chemical Co. (St. Louis, USA). N, N, N′, N′-Tetramethylethy-lenediamine (TMED) from Matheson Coleman and Bell (New Jersey, USA). Organic solvents such as methanol, acetic acid and chloroform were purchased from ALPS Co. (Taipei, ROC). Ethanol and hydrochloride from E. Merk CO. (Darmatadt, W. Germany).

Materials

An isolate of *G. lucidum* (Fr.) Karst NTU G001 was kindly provided by Dr. D. Chen, Department of Plant Pathology, National Taiwan University. Two isolates of *G. lucidum* ATCC 32471 and ATCC 32472 were purchased from American Type Cultural Center (ATCC), Washington D. C. An isolate of *Ganoderma tsugae*, originally isolated by a Japanese research worker, was donated by Mr. P. C. Chen, Departent of Plant Pathology, National Taiwan University. The stock cultures in this collection were maintained on potato dextrose agar slants and transferred at 2 weeks intervals.

Fourteen days' old mycelia of *G. lucidum* and *G. tsugae* grown in potato-dextrose (PD) broth were dried at 50 C and used for determination of chemical compositions in terms of carbohydrate, lipid and ash as previously described (Tseng, *et al.*, 1984).

Preparation of Cell Free Extract

Mycelia from the stock cultures were transferred to petri-dish containing PDA (potato 250 g; dextrose 20 g; agar 20 g/liter) and then incubated at 28 ± 2°C for 10 days. Four pieces of mycelial discs (approximately 1.5 cm^2), cut from the marging of colony, were then transferring into 100 ml of MDP broth (malt extract 3 g; dextrose 2 g; peptone 0.1 g/100 ml) in a 300 ml Erlenmeyer flask. Liquid cultures were incubated at 28 ± 2°C for various growth periods without shaking. The mycelia were harvested by filtration, using 4 layers cheese-

cloth, and the filtrates were stored at −4°C. The frozen mycelia were cut into small pieces and suspensed in buffer solution at a ratio of 1 g wet weight mycelia to 5 ml of 0.05 M phosphate buffer (pH 6.5) and then homogenized by using Sorvall Omni-Mixer (Du Pont Instruments, Instrument Products Division Sorvall Operations, Newtown, Conn). The homogenates were centrifuged at 20,000 xg for 20 min at 4°C. The supernatants were immediately used for analyses of soluble proteins, enzymes as well as essential amino acids.

Separation of Protein and Enzyme by Disc Gel Electrophoresis

Polyacrylamide disc gel electrophoresis of soluble proteins and enzymes were conducted by the method described by Davis (1964). Bromophenol blue was used as tracking dye to aid in determining the terminal electrophoresis. After separation, gels were removed and stained for soluble proteins with Commassie blue and then destained with solution of 7% acetic acid and 5% methanol. The protein content was estimated by Lowry method (Lowry, *et al.*, 1951). For detection of enzyme activities, the gels were immersed in the appropriate reaction mixtures as the following to detect sites of esterase, peroxidase, leucine aminopeptidase, and lactate dehydrogenase activities.

Esterase—0.22 g of α-naphthyl acetate in 200 ml of 0.08 M phosphate buffer (pH 7.0) and 0.4 g Blue RR salt, incubated at 30°C for 20 min.

Peroxidase—incubated at 0.25% guaiacol for 30 min then 0.3% hydrogen peroxide for 15 min at 30°C.

Leucine aminopeptidase—40 mg of L-leucyl-β-naphthylamide HCl in 100 ml of 0.2 M tris-maleate buffer (pH 0.6) and 50 mg Fast Black K salt, incubated at 30 C for 30 min.

Lactate dehydrogenase—12 g sodium lactate, 0.05 g diphosphopyridine nucleotide (DPN), 0.01 g phenazine methosulfate in 200 ml of 0.025 M tris-buffer (pH 7.5) and 0.05 g Tetrazolium blue, incubated at 30 C for 30 min.

Amino Acid Analysis

The analysis of essential amino acids from mycelial extracts was carried out using pre-column derivation of amino acids with o-phthaladehyde (OPA)/ethanethiol by the method of Hill *et al.*, (1979). Except, a 20 μl instead of a 5 μl aliquot of the derivated amino acid standards or 2-week old' mycelial extracts was analyzed by a high performance liquid chromatograph (Waters Associates, 34 Maple st., Milford, Mass) consisting of a Model 6000 A pump and Model 590 pump, a Model 660 Solvent Programmer and Wisp Model 710 B Sample Processor. The Model FS-970 spectrofluoro Monitor (Kratos/Schoeffel Instruments Corp., 24 Booker st., Westwood, NJ) with the following settings was used for detections: excitation monochromator set at 229 nm; the emission measured with a 470 nm cut-off filter; a time constant of 6 sec; a 1.0 μA full scale range setting, and a sensitivity dial setting of 418 units. A LiChrosorb RP-18 column (10 μm), 25 cm × 0.4 cm i.d., (Hibar pre-packed column RT 250-4, E. Merck Darmstadt F. R. Germany) was used. A flow rate of 2.0 ml/min was normally employed.

Results and Discussion

Mycelial Growth and Chemical Composition

Three isolates of *Ganoderma lucidum* ATCC 32471, ATCC 32472, NTU G001 and one isolate of *G. tsugae* can be cultured in potato dextrose broth and keep for maintaining vigorous growth in a certain period of time. The mycelial dry weight as shown in the Fig. 1 was still greatly increasing after 4 weeks.

Two-week old cultures of the tested isolates in potato dextrose broth were chosen for the chemical analysis, because it is in logarithmic

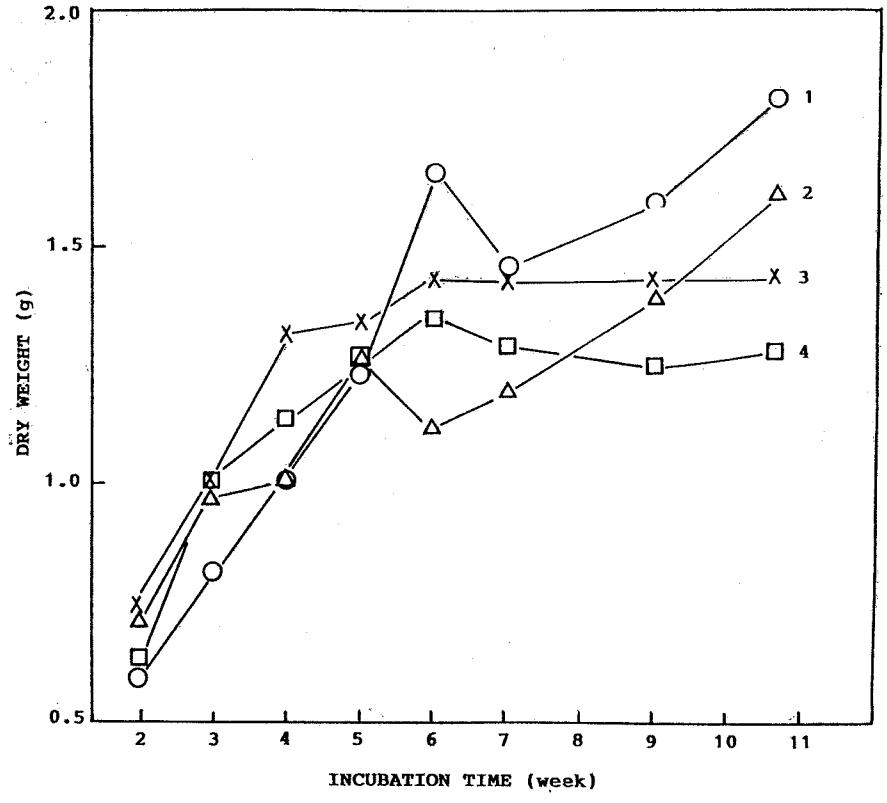

Fig. 1. Growth curves of three isolates of *Ganoderma lucidum* and one isolate of *Ganoderma tsugae*. ○, *G. tsugae*; △, *G. lucidum* NTU 32471; ×, *G. lucidum* NTU G001; □, *G. lucidum* ATCC 32472.

growth phase that represents a steady system in which the relative concentrations of metabolites and enzymes within the cell are constant, offering an ideal system for the study of various biochemical problems. Contents of total carbohydrates, lipids, soluble proteins and ash were showed in Table 1. Although there are no significant differences of the chemical composition in terms of percentage in dry mycelia basis, yet mycelia from *G. tsugae* contained slightly less total carbohydrate. Further analyses of fatty acids in the crude lipid portion of dry mycelia were conducted by gas chromatography. As specified in Table 2,

Table 1. *Chemical compositions of* ***Ganoderma lucidum*** *and* ***Ganoderma tsugae*** *isolates*

Isolates	Percentage as total dry weight of mycelium*			
	Total carbohydrate	Lipid	Soluble protein	Ash
G. lucidum ATCC 32471	74.2	4.3	8.9	3.5
G. lucidum ATCC 32472	74.0	4.2	8.5	3.3
G. lucidum NTU G001	75.0	5.9	8.3	3.7
G. tsugae	72.0	4.6	9.0	3.6

* Each value is the average of tripicate determinations.

Table 2. *Fatty acid compositions of saponifiable crude lipids in* ***Ganoderma lucidum*** *and* ***Ganoderma tsugae*** *isolates*

Isolates	Fatty acid composition (% of total)**											
	$C_{12:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$ $_{18:2}$	$C_{19:0}$	$C_{20:0}$	$C_{21:0}$	$C_{22:0}$	$C_{24:0}$
G. lucidum ATCC 32471	Trace	0.5	0.9	24.1	1.4	9.8	59.1	trace	1.3	trace	0.4	1.3
G. lucidum ATCC 32472	Trace	0.4	1.2	24.4	1.1	10.2	58.7	trace	1.2	trace	0.2	1.1
G. lucidum NTU G001	Trace	0.6	1.0	23.3	1.1	10.1	59.4	trace	1.5	trace	0.8	1.2
G. tsugae	Trace	0.7	0.4	57.7	2.1	16.2	20.1	0.1	0.9	trace	0.1	0.9

* Determination based on gas chromatographic method (Tseng, *et al.*, 1984).
** Each value is average of triplicate determinations.

the methyl esters of fatty acids obtained from saponification and methylation revealed that $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$ fatty acids were the predominant species in the four tested isolates. The relative ratio of $C_{16:0}$ fatty acid in *G. tsugae* is about two times higher than the three isolates of *G. lucidum*. The presence of $C_{18:1}$ and $C_{18:2}$ fatty acids in *G. tsugae* was in a lower ratio (20.1%). These results illustrated that the chemical compositions of the isolates of *G. lucidum* and *G. tsugae* in terms of fatty acid pattern, are no similarlity. Thus we consider it can provide an useful information to classify *Ganoderma* species.

Soluble Proteins:

The soluble proteins in mycelial extracts of the four isolates of *Ganoderma* species were examined by polyacrylamide disc gel electrophoresis. A maximum of about 20 protein bands was discernible on gel stained with Coomassie blue (Fig. 2, Table 3). Although the gels were not densitometrically scanned, a distinctive interspecific pattern did exist. More bands were seen on gels of *G. lucidum* NTU G001 and *G. tsugae* than on the isolates of *G. lucidum* ATCC 32471 and ATCC 32472 in 2-week mycelial extracts (Fig. 2-A). When the cultures kept grow to 3 and 4 weeks, less protein bands were observed and some major bands changed among the different stages of the same culture (Fig. 2-B, C, Table 3). These results revealed that disc gel electrophoresis is an effective tool in

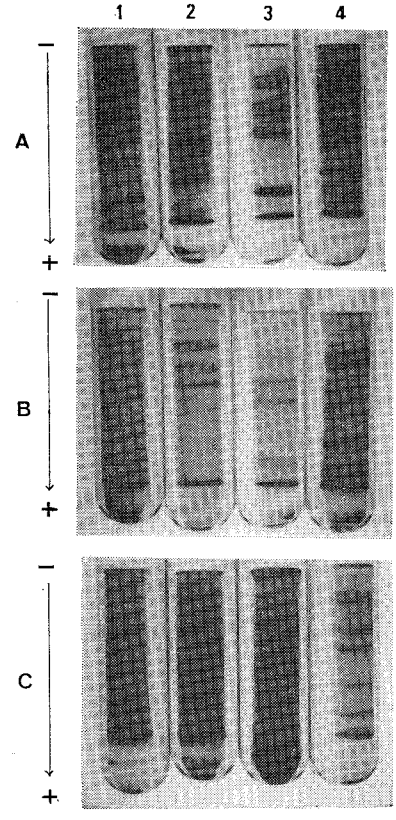

Fig. 2. Polyacrylamide gel patterns of soluble proteins from various stages of mycelial extracts of *Ganoderma lucidum* and *Ganoderma tsugae*.
A→2 weeks; B→3 weeks; C→4 weeks
1→*G. lucidum* NTU G001; 2→*G. tsugae*;
3→*G. lucidum* ATCC 23471;
4→*G. lucidum* ATCC 32472.

Table 3. *Polyacrylamide disc gel electrophoretic analysis of soluble proteins and marker enzymes of* ***Ganoderma lucidum*** *and* ***Ganoderma tsugae*** *mycelial extracts*

Isolates	Soluble proteins			Marker enzymes*											
	2	3 (weeks)	4	P.O.			LDG			LAP			Esterase		
				2	3	4	2	3	4	2	3	4	2	3	4
G. lucidum NTU G001	19**	13	9	0	0	0	0	0	0	0	0	0	7	4	0
G. lucidum ATCC 32471	16	12	8	0	0	0	0	0	0	0	0	0	7	4	0
G. lucidum ATCC 32472	15	13	7	0	0	0	0	0	0	0	0	0	7	4	0
G. tsugae	20	13	10	0	0	0	0	0	0	1	1	1	4	3	0

* P.O.→peroxidase; LDG→lactate dehydrogenase; LAP→leucine aminopeptidase;.
** Number of band in gel.

fractionating the complement of soluble mycelial proteins among *Ganoderma* species.

Marker Enzymes

One of our purposes of the current study is to determine the enzymes which may show wide variations among the strains of *Ganoderma* species. Electrophoretic patterns of four enzymes, i.e. peroxidase, lactate dehydrogenase, leucine aminopeptidase and esterase were identified.

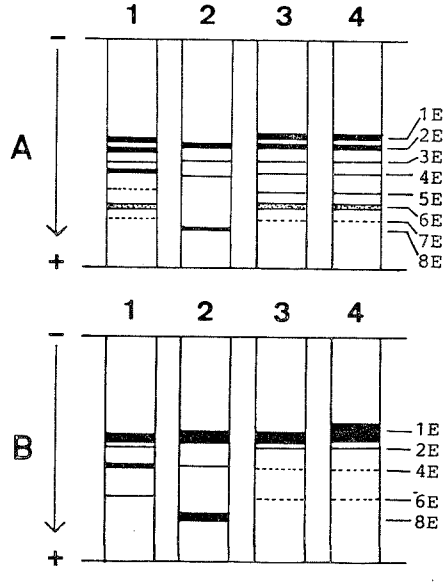

Fig. 3 Diagrammatic illustration of zymograms of esterase in 2 week (A) and 3 weeks (B) mycelial extracts of *Ganoderma lucidum* and *Ganoderma tsugae*.
1→*G. lucidum* NTU G001; 2→*G. tsugae*;
3→*G. lucidum* ATCC 32471;
4→*G. lucidum* ATCC 32472.

Eight sites of esterase activity were detected in 2-week mycelial extracts of the four tested isolates. Each site of the enzyme activity was designated as 1E, 2E, 3E...8E (Fig. 3). As the result showed in Fig. 3-A, esterase zymograms were grouped into two types. Seven sites of esterase activity belong to first type were observed among *G. lucidum* NTU G001, *G. lucidum* ATCC 32471 and ATCC 32472, yet *G. tsugae* showed 4 sites of the enzyme activity. Esterase isozyme patterns were also obtained from different stage of mycelial extract as shown in Fig. 3-B. Four sites (1E, 2E, 3E and 6E) of esterase activity were observed among three isolates of *G. lucidum,* however, *G. tsugae* showed three sites with a heavy band (8E). One site of leucine aminopeptidase was definited observed in *G. tsugae* from various stages of mycelial extracts (Table 3), yet *G. lucidum* ATCC 32471, ATCC 32472 and *Ganoderma lucidum* NTU G001 exhibited no leucine aminopeptidase activity. There are no peroxidase and lactate dehydrogenase activity being detected among the four isolates.

Few investigations have been made on the isozyme patterns in *Ganoderma* species and the reports are meager. Okunishi *et al.* (1979)

reported that esterase, tyrosinase, malate dehydrogenase and 6-phosphogluconate dehydrogenase appeared in the isozymes, and only esterase showed different patterns in mycelium, primordium, stipe and pilens of *Lentinus edoder.* Recently, Hseu and Wang (1986) have demonstratd that the isolates of *G. lucidum* were able to differentiated from other species by an enzyme kit (API-ZYM) for rapid identification. The enzymes used are α-and β-glucosidases, α-and β-galactosidaes, β-glucuronidase and N-acetyl-β-glucosaminidiase.

From the current studies, since esterase isozyme showed a wide range of variations in the mycelial extracts, thus we proposed it should be one of the marker enzymes useful for the identification of *Ganoderma* species.

Amino Acid Composition

Since o-phthalaldehyde/ethanethiol derivatives of amino acids in animal fluids such as plasma, urine, and tissue were analyzed with excellent separation and good sensitivity by reverse phase performance liquid chromatography (Davis *et al.,* 1979), attempts are therefore made to apply the rapid, efficient and sensitive method for the analysis of essential amino acids in mycelial extracts of *Ganoderma* species to set up a guideline for toxonomic evidence.

Two-week old mycelial extracts of *Ganoderma* isolates were served for this purpose. Figure 4-A showed a typical chromatogram of standard essential amino acids. The separation of standard amino acids is possible in less than an hour in picomole basis. Analyses of mycelial extracts from *Ganoderma* species using pre-column derivation with OPA have been demonstrated in Figs. 4-B and 5. The essential amino acids obtained from the mycelial extracts illustrated that aspartic acid, glutamic acid, serine, glutamine, threonine plus glycine, and alanine are the predominant species in the four isolates. Histidine, methionine, leucine and lysine are only detected in the mycelial extracts of *G. tsugae,* although small amounts of leucine and histidine also appeared in the *G. lucidum* ATCC 32472 mycelial extracts (Table 4), and the absence of arginine was confirmed in the current study. These results made us a great convince that analysis of amino acids in mycelial extracts using automated pre-column derivation with OPA should be accounted for an useful tool for application in chemotaxonomic evidence in the strains of *Ganoderma* species.

Based on our results we propose that the chemical compositions in terms of fatty acids, essential amino acids, soluble proteins and a marker enzyme (esterase) in the mycelial extracts of the fungi should provide useful information for classification of the strains of *Ganoderma* species. However, it should be noted that the ingredients obtained may be

Table 4. *Amino acid compositions of 2-week old mycelial extracts of* ***Ganoderma lucidum and Ganoderma tsugae***

Isolates	Essential amino acids (μmole)*															
	Asp	Glu	Asn	Ser	Gln	His	Thr + Gly	Ala	Arg	Tyr	Val	Met	Trp + Ile	Phe	Leu	Lys
G. luidcum NTU G001	28	30	9	26	42	—	53	57	—	7	20	—	12	—	—	—
G. tsugae	26	29	11	24	36	5	53	60	—	7	20	2	15	10	12	10
G. lucidum ATCC 32471	26	24	10	32	24	—	59	49	—	7	18	—	4	—	—	—
G. lucidum ATCC 32472	26	22	7	31	30	0.1	88	55	—	6	8	—	6	—	0.6	—

* Each value is estimated by comparing peak heighs against a calibration curve prepared using serial dilutions of a standard amino acid, by the method of high pressure liquid chromatography.

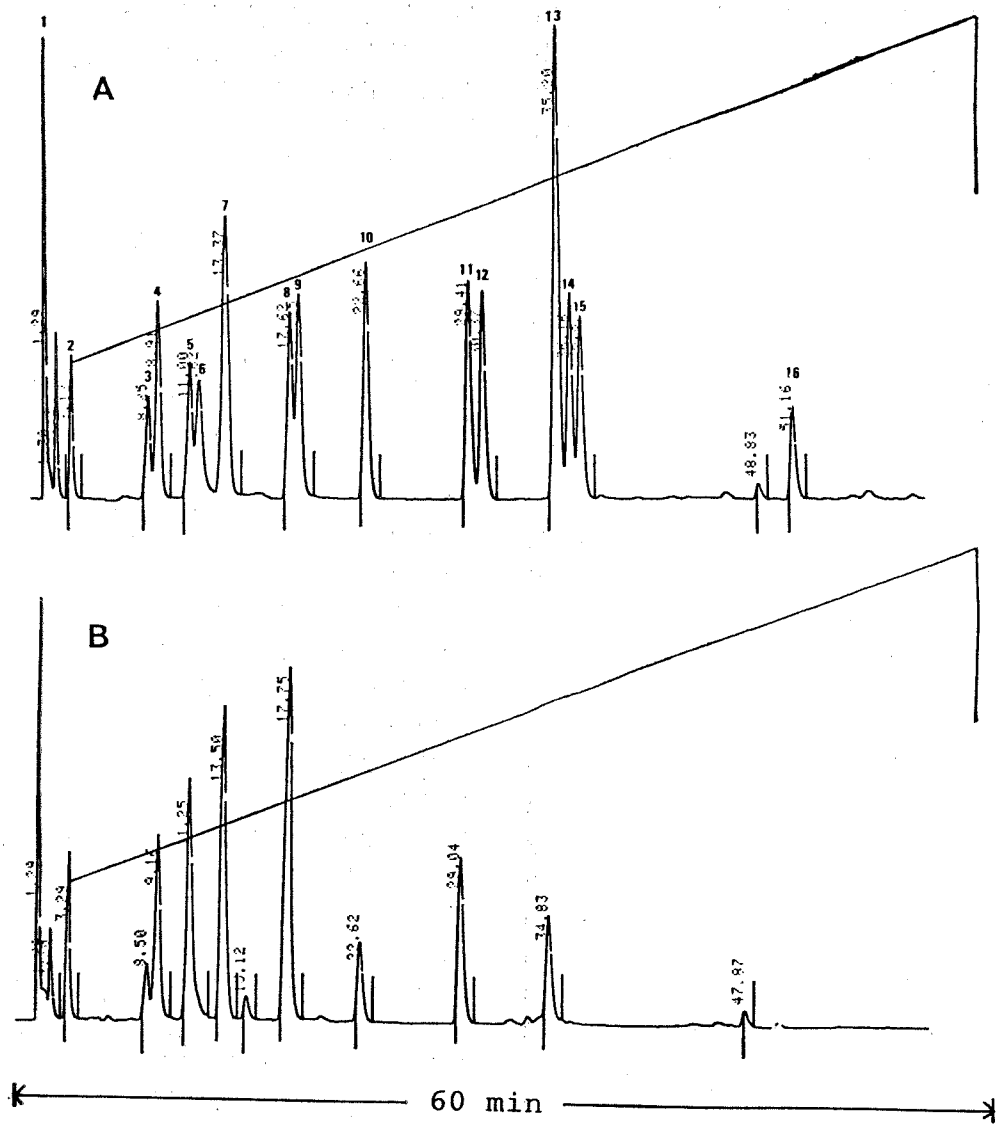

Fig. 4. Profile of essential amino acid standards (A), 30 pmoles each except tyrosine (28 pmoles) and lysine (60 pmoles), derivatized with 0- phthalaldehode/etanethiol (A). 20 μl of two weeks old mycelial extract of *G. lucidum* NTU G001, derivated with OPA was injected to the column(B).

Conditions:

Column: LiChrosorb RP-18 (10 μm), 25 cm$\times$0.4 cm i. d. Mobile phase: Gradient conditions, linear program from 20 to 80% (solvent B/solvent A), 60 min in duration. Solvent A: 0.0125M Na_2HPO_4, pH 7.2; Solvent B: CH_3CN.

Flow rate: 2 ml/min

Detector: Schoeffel FS-970 fluorescence HPLC detector, excitation wavelength 229 nm, emission wavelength 470 nm cut-off filter.

Amino acids: 1→glutamic acid (Glu); 2→aspartic acid (Asp); 3→asparagine (Asn); 4→serine (Ser); 5→glutamine (Gln); 6→histidine (His); 7→threonine+glycine (Thr+Gly); 8→alanine (Ala); 9→arginine (Arg); 10→tyrosine (Tyr); 11→valine (Val); 12→methionine (Met); 13→tryptophan+isoleucine (Trp+Ile); 14→phenylalanine (phe); 15→leucine (Leu); 16→lysine (Lys).

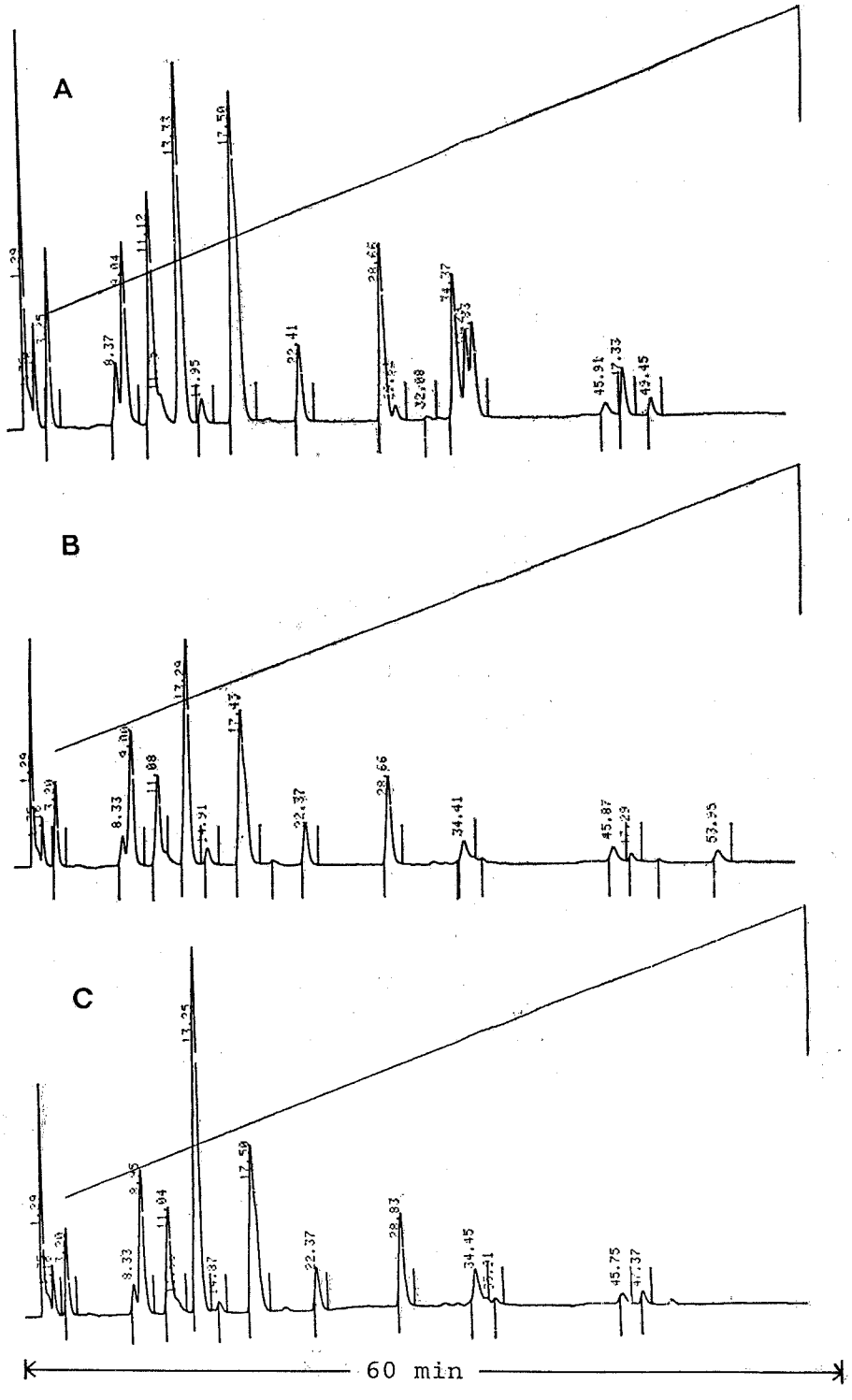

Fig. 5. Profiles of essential amino acids from 2-week old mycelial extracts of *G. tsugae* (A); *G. lucidum* ATCC 30471 (B); *G. lucidum* ATCC 32472 (C). Conditions same as Fig. 4.

influenced by a number of factors: composition of growth medium, temperature of incubation, age of culture and by the techniques employed to analyze the samples.

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Literature Cited

Chang, L. O., A. Srb, and F. S. Steward. 1962. Electrophoretic separation of soluble proteins of *Neurospora*. Nature **193**: 756-759.

Chang, T. T. 1983. Studies on biology of several species of *Ganoderma* in Taiwan. Research Institute of Plant patholgy and Entomology, National Taiwan University. Master thesis, 130 pp.

Davis, B. J. 1964. Disc elctrophoresis-II method and application to human serum proteins "Gel electrophoresis" Conference Chairman Fredrich, J. P., Ann. New York Acad. Sci. 121, Art 2: 404.

Davis, T. P., C. W. Genhrke, C.W. Gehrke, Jr., H.D. Cunningham, K. C. Kuo, K. O. Gerhardt, H. D. Johnson, and H. Charles. 1979. High-performance liquid chromatographic analysis of biogenic amines in biological materials as O-phthalaldehyde derivaties. J. Chromatogr. Biomed. Appl. **162**: 293-310.

Hill, D. W., F. H. Walters, T. D. Wilson, and J. D. Sturat. 1979. High performance liquid chromatographic determination of amino acids in picomole range. Anal. Chem. **5**: 1338-1341.

Hseu, R. S. and H. H. Wang. 1986. Application of hydrolytic enzyme in API-ZYM enzyme testing system to rapid identification of *Ganoderma* species. J. Chin. Agr. Chem. Soc. **24**: 101-109.

Lowry, O. H., J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin Phenol reagent. J. Biol. Chem. **193**: 265-275.

Normore, W. M. 1973. Guamine plus cytosine (GC) composition of the DNA of bacteria, fungi, algae and protozoa. *In* A. I. Laskin and H. A. Lechevalier Cleaveland (eds), CRC Handbook of Microbiology, vol. II. Microbial Composition. CRC Press, pp. 585-740.

Okunishi, M., K. Yamada, and K. Komagata. 1979. Electrophoretic comparison of enzymes from Basidomycetes in different stages of development. J. Gen. Appl. Microbiol. **25**: 329-334.

Shechter, Y., R. Hall, F. G. Oelshleg, Jr., M. A. Stahmann, R. D. Snider, R. G. Franke, R. W. Holton, M. M. Reddy, and R. D. Garber. 1973. Symposium on the use of electrophoresis in the taxonomy of algae and fungi. Bull. Torrey Botany Club **100**: 253-312.

Shiao, M. S., T. C. Tseng, Y. Y. Hao and Y. S. Shieh. 1986. Studies on *Ganoderma lucidum*. II. The effects of *G. lucidum* on lipid metabolism in rats. Bot. Bull. Academia Sinica **27**: 139-146.

Stipes, R. J. 1970. Comparative mycelia protein and enzyme patterns in four species of *Ceratocystis*. Mycologia **62**: 987-995.

Stipes, R. J., G. H. Emert, and R. D. Brown Jr. 1982. Differentiation of *Endothia gyrosa* and *Endothia parasitica* by disc electrophoresis of intramycelial enzymes and proteins. Mycologia **74**: 138-141.

Taguchi M., M. Tsukiji, and T. Tsuchiya. 1979. Rapid identification of yeast by serological method. A combined serological and biological method. Sabouraudia **17**: 185-192.

Tseng, T. C., M. S. Shiao, Y. S. Shieh, and Y. Y. Hao. 1984. Studies on *Ganoderma lucidum* 1. Liquid culture and chemical composition of mycelium. Bot. Bull. Academia Sinica **25**: 149-157.

Tseng, T. C. and L. S. Chang. 1988. Studies on *Ganoderma lucidum*. III. Production of pectolytic enzymes. Bot. Bull. Academia Sinica **29**: 23-32.

靈　芝　之　研　究

四、利用菌絲萃取物之化學成分鑑別菌種

曾聰徹　賴麗鈴

中央研究院植物研究所

分析靈芝菌種包括三株 *Ganoderma lucidum* 和一株 *Ganoderma tsugae* 之乾菌絲所得之碳水化合物，水溶性蛋白質，脂肪及灰份含量，發現並無顯著區別。但 *G. tsugae* 菌絲的碳水化合物含量較低，而且飽和脂肪酸 $C_{16:0}$ 的含量，高出其他菌株之2倍。

利用電泳方法，分析 *G. lucidum* 及 *G. tsugae* 菌絲萃取物中之水溶液蛋白質以及一些標記酶 (marker enzyme) 包括 Esterase, Peroxidase, Leucine aminopeptidase 和 Lactate dehydrogenase, 結果發現萃取液中以 *G. tsugae* 和 *G. lucidum* NTU G001 所含 的水溶性蛋白質帶，比 *G. lucidum* ATCC 32471 和 32472 兩菌株較多，且彼此可以區別；就 Esterase 酶之電泳圖譜而言，*G. lucidum* 和 *G. tsugae* 之間，具有明顯可區分之活性反應帶，至於其他酵素則闕如。

當檢試四種菌株之菌絲萃取物中之主要胺基酸時，發現以 Aspartic acid, Glutomic acid, Serine, Glutamine, Threonine, Glycine 和 Alanine 爲主，而 Histidine, Methionine, Leucine 和 Lysine 則只存在於 *G. tsugae*. 根據以上之結果顯示，利用菌絲萃取液之化學成分，可以作爲 *Ganoderma* species 之分類依據。