

Note

Study of alcohol dehydrogenase involved in alcohol fermentation by Basidiomycetes.

Naomi TAKEMOTO¹, Yuka SAMEJIMA^{2, 4}, Tokumitsu MATSUI^{1, 3, 4}

Keywords

Alcohol dehydrogenase, Mushrooms, *Lepista nuda*, Alcoholic fermentation, Ethanol production

Summary

Production of alcoholic beverages using basidiomycetes has been reported. Alcoholic beverages produced by these basidiomycetes have also been reported to have various functionalities possessed by basidiomycetes. The capability of basidiomycetes to produce alcoholic beverages through fermentation may provide advantages. Alcoholic beverages with functionality derived from basidiomycetes can be given added value. However, the basidiomycetes used for the production of alcoholic beverages have no common feature of family and genus. The superior basidiomycete for producing alcoholic beverages is unknown. In this study, a wide range of basidiomycetes with ADH suitable for alcoholic fermentation were screened using various mushroom mycelia. The results indicate that basidiomycetes have both ADH involved in ethanol synthesis and decomposition, and that mushrooms with only ADH involved in ethanol production may be suitable for alcoholic beverage production. *L. nuda* had a high specific activity of ADH involved in ethanol synthesis, and Native-PAGE showed only the active band of ADH involved in ethanol synthesis. From the perspective of ethanol production, ADH that consume ethanol are to be avoided, thus mushrooms with ADH primarily involved in ethanol synthesis are suitable targets for alcoholic beverage production.

Basidiomycetes are known to have the ability to alcohol fermentation, and have been reported to produce ethanol as biomass or produce alcoholic beverages (1, 2). Wine production makes use of *Schizophyllum commune*, and of significant importance in Japanese sake production is *Agaricus blazei Murrill* (3, 4). Wine production

makes use of *S. commune* as a member of the *Pleurotaceae* family, sake production makes use of *A. blazei Murrill* as a member of the *Agaricaceae* family. Additionally, white-rot fungi that produce bioethanol from cellulose have been reported (5). These basidiomycete families involved in alcoholic fermentation have nothing in common. In addi-

1 Food Science and Nutrition Major Graduate School of Human Environmental Sciences Mukogawa Women's University

2 Hagoromo University of International Studies, Human life studies

3 Mukogawa Women's University, Food Sciences and Nutrition

4 Mukogawa Joshi Daigaku Eiyo Kagaku Kenkyujo, Research Institute for Nutrition Sciences

Corresponding Author

Tokumitsu MATSUI

6-46 Ikebiraki Nishinomiya, Hyogo, JP 663-8558

Mail: tokamura@mukogawa-u.ac.jp

tion, fruit body are also recognized as an excellent source of nutrition as they contain high levels of vitamins B and D, and dietary fiber (6-8). Basidiomycetes are β -D-glucan-producing fungi (9) that have important antioxidant properties (10). Alcoholic beverages produced by these basidiomycetes have also been reported to have β -D-glucan addition and antioxidant activity (11). The capability of basidiomycetes to produce alcoholic beverages through fermentation may provide advantages. To explore this, the enzymatic activity of these basidiomycetes requires evaluation. An essential enzyme for alcohol production is alcohol dehydrogenase (ADH). Moreover, the alcohol concentration repeatedly increases and decreases when alcoholic fermentation is performed using *Flammulina filiformis* (12). This is because ADH in *F. filiformis* has the function of both ethanol production and decomposition. Its ADH activity can generally be measured spectrophotometrically using ethanol as substrate. This reaction interconverts ethanol with acetaldehyde in alcohol metabolism. For alcoholic fermentation, it is important to know the ADH activity in producing ethanol from acetaldehyde. In this study, a wide range of basidiomycetes with ADH suitable for alcoholic fermentation were screened using various mycelia.

87 strains (Table 1) owned by our laboratory were used as test strains. Inoculum for the cultures in liquid medium were obtained from mycelium grown on potato-dextrose agar (PDA). The liquid medium contained 1.2g of potato-dextrose broth (PDB) in 50mL tap water. Glucose (2%) was added to PDB (pH 5.4) and 50ml of the PDB medium added to 100mL Erlenmeyer flasks which were then sterilized by autoclave at 121°C for 20min. These were then inoculated with five mycelial pieces (5 × 5mm², cut from the PDA with mycelial growth) over at 25°C with shaking at 100rpm. The culture period was 14 days to allow sufficient growth of mycelium. Mycelia were collected on 67µm mesh sheets and washed with

Table 1. List of basidiomycetes in this study

Familly	Genus	Species	Identification number
<i>Morchellaceae</i>	<i>Morchella</i>	<i>esculenta</i> var. <i>esculenta</i>	33132
<i>Sarcosomataceae</i>	<i>Urnula</i>	<i>U. craterium</i>	30137
<i>Pezizaceae</i>	<i>Peziza</i>	<i>P. vesiculosa</i>	30324
<i>Sarcoscyphaceae</i>	<i>Wynner</i>	<i>W. gigantea</i>	5928
<i>Clavicipitaceae</i>	<i>Cordyceps</i>	<i>C. militaris</i>	8826
<i>Auriculariaceae</i>	<i>Auricularia</i>	<i>polytricha</i>	W9*
<i>Tremellaceae</i>	<i>Tremella</i>	<i>T. fuciformis</i>	8990
<i>Hericiaceae</i>	<i>Hericium</i>	<i>H. ramosum</i>	Sn-1*
		<i>H. erinaceum</i>	Ym-2*
<i>Polyporaceae</i>	<i>Polyporus</i>	<i>P. arcularius</i>	W122*
	<i>Coriolus</i>	<i>C. versicolor</i>	4937
		<i>C. versicolor</i>	W18*
		<i>C. versicolor</i>	W17*
		<i>C. versicolor</i>	6516
	<i>Grifola</i>	<i>G. frondosa</i>	4911
		<i>G. frondosa</i>	Mi-3*
		<i>G. frondosa</i>	Mi-2*
		<i>G. frondosa</i>	Mi-1*
		<i>G. frondosa</i>	C9
	<i>Laetiporus</i>	<i>L. sulphureus</i> var. <i>miniatus</i>	W8*
	<i>Lenzites</i>	<i>L. betulina</i> (L.:Fr.) Fr.	4963
<i>Ganodermataceae</i>	<i>Ganoderma</i>	<i>G. lucidum</i>	31863
		<i>G. lucidum</i>	8346
<i>Ganodermataceae</i>	<i>Ganoderma</i>	<i>G. applanatum</i> (Pers.) Pat.	Napa*
<i>Hymenochaetaceae</i>	<i>Cyclomyces</i>	<i>C. fuscus</i> Fr.	9789
<i>Pleurotaceae</i>	<i>Schizophyllum</i>	<i>S. commune</i> Fr. : Fr	6504
		<i>S. commune</i> Fr. : Fr	6503
		<i>S. commune</i> Fr. : Fr	6505
		<i>S. commune</i> Fr. : Fr	30496
		<i>S. commune</i> Fr. : Fr	6502
		<i>S. commune</i> Fr. : Fr	30749
		<i>S. commune</i> Fr. : Fr	4929
<i>Bolbitaceae</i>	<i>Agrocybe</i>	<i>A. praecox</i>	30258
		<i>A. cylindracea</i>	Ya-1*
		<i>A. cylindracea</i>	Ya-2*
<i>Tricholomataceae</i>	<i>Flammulina</i>	<i>F. velutipes</i> Sing.	TR19*
		<i>F. velutipes</i> Sing.	SA-1*
		<i>F. velutipes</i> Sing.	30599
		<i>F. velutipes</i> Sing.	30494
		<i>F. velutipes</i> Sing.	7777
		<i>F. velutipes</i> Sing.	30602
		<i>F. velutipes</i> Sing.	30490
		<i>F. velutipes</i> Sing.	30905
		<i>F. velutipes</i> Sing.	30875
		<i>F. velutipes</i> Sing.	W4*
	<i>Flammulina</i>	<i>F. velutipes</i> Sing.	KAKI*
	<i>Oudemansiella</i>	<i>O. radicate</i> Sing	9785
	<i>Armillariella</i> Karst.	<i>A. mellea</i> Karst.	7037
		<i>A. mellea</i> Karst.	30879
	<i>Hypsizygus</i> Sing.	<i>H. marmoreus</i> Bigelow	Bn-1*
	<i>Panellus</i> Karst.	<i>P. serotinus</i>	30526
		<i>P. serotinus</i>	W2*
	<i>Lepista</i>	<i>L. nuda</i> Cooke	8104
<i>Agaricaceae</i>	<i>Agaricus</i> L.	<i>A. bisporus</i> var. <i>albidus</i>	7214
<i>Coprinaceae</i>	<i>Coprinus</i> Pers.	<i>C. disseminatus</i> S. F. Gray	7550
<i>Pleurotaceae</i>	<i>Pleurotus</i>	<i>P. eryngii</i>	Er-1*
		<i>P. eryngii</i>	Er-2*
		<i>P. cornucopiae</i> var. <i>citrinopileatus</i>	32796
		<i>P. cornucopiae</i> var. <i>citrinopileatus</i>	Ta-1*
<i>Pleurotaceae</i>	<i>Pleurotus</i>	<i>P. cystidiosus</i> subsp. <i>Abalonus</i>	Ka-1*
		<i>P. ostreatus</i>	Hr-24*
		<i>P. ostreatus</i>	Hr-21*
		<i>P. ostreatus</i>	Hr-23*
		<i>P. ostreatus</i>	30193
		<i>P. ostreatus</i>	7051
		<i>P. ostreatus</i>	30160
		<i>P. ostreatus</i>	W351-1*
		<i>P. ostreatus</i>	Hr-1*
		<i>P. ostreatus</i>	w351-2*
	<i>Lentius</i>	<i>L.s edodes</i> (Berk.)	Sh-1*
		<i>L.s edodes</i> (Berk.)	Sh-8*
		<i>L.s edodes</i> (Berk.)	Sh-18*
<i>Strophariaceae</i>	<i>Naematoloma</i> karst.	<i>N. sublateritium</i>	W53*
		<i>N.fasciculare</i>	30361
	<i>Stropharia</i>	<i>S.rugosoannulata</i> Farlow in Murr	30225
	<i>Pholiota</i> (Fr) Kummer	<i>P. nameko</i> (T.Ito) S. Ito et Imai in Imai	Na-1*
		<i>P. nameko</i> (T.Ito) S. Ito et Imai in Imai	Na-4*
		<i>P. nameko</i> (T.Ito) S. Ito et Imai in Imai	Na-6*
		<i>P. nameko</i> (T.Ito) S. Ito et Imai in Imai	Na-9*
		<i>P. adiposa</i> (Fr.) Kummer	Nm-1*
<i>Russulaceae</i>	<i>Lactarius</i>	<i>L.chrysorrhoeus</i> Fr.	8334
<i>Nidulariaceae</i>	<i>Cyathus</i>	<i>C. stercoreus</i> (Sche.) De Toni	9076
		Unidentified	C7*
		Unidentified	W133*
		Unidentified	W126*
		Unidentified	W12*
		Unidentified	W128*

The numbers show NBRC No., * show Lab No.

distilled water. Then, 1g of 0.5mm low alkali glass beads (Yasui Instruments Co., Ltd.) and 1g of collected mycelium were combined in the Multi-beads shocker (Yasui Co., Ltd.), and the sample milled (on time 30sec, off time 30sec, total 1000 sec.). The sample was then centrifuged (15000 rpm, 4°C, 10min) and the supernatant liquid used as the crude enzyme solution.

Reaction progress of ADH specific activity, in all cases, was evaluated at 340nm by the decrease in NADH absorbance upon oxidation to NAD^+ , or by the increase in NADH absorbance as NAD^+ is reduced to NADH using a microplate reader (Tecan Trading AG) (13). ADH specific activity was calculated using the highest change in NADH absorbance per second. Protein content was measured using a Protein Assay BCA Kit (FUJIFILM Wako Pure Chemical Co.) with bovine serum albumin as the standard. The specific activity of ADH involved in ethanol-decomposition was determined by into one well of a 96-well microplate was added 44 μL of 0.2M Tris-HCl Buffer (pH 8.5), 44 μL of 80mM ethanol, and 62 μL of distilled water. This was then pre-incubated at 30°C for 10minutes. After this, 6 μL of crude enzyme solution was added, then using an injector, 15 μL of 20mM NAD^+ was injected, and the change in NADH absorbance measured over time. One unit of activity is defined as the amount of reduced NADH produced in 1mM NAD^+ by 1mg of crude enzyme solution in 1minute. The specific activity of ADH involved in ethanol-synthesis by into one well of a 96-well microplate, 44 μL of 0.2M Tris-HCl Buffer (pH 8.5), 44 μL of 80mM ethanol, and 62 μL of distilled water were combined and pre-incubated at 30°C for 10minutes. After that, 6 μL of crude enzyme solution was added and using an injector, 15 μL of 0.02mM NADH was injected with the changes in NADH absorption recorded over time. One unit of activity is defined as the amount of NADH oxidized to NAD^+ in a 1mM NADH solution by 1mg of crude enzyme solution in 1minute. ADH involved in ethanol-decomposi-

tion and ADH involved in ethanol-synthesis were measured three times, and the highest value was defined as each specific activity.

Molecular masses of ADH in the crude enzyme solution were determined by Native-PAGE (12). Different activity stain solutions were used to detect ADH involved in ethanol-consumption and ADH involved in ethanol-synthesis. As a substrate, 20mM ethanol was used with 0.5mM NAD^+ as the coenzyme in the activity staining solution for ADH involved in ethanol-decomposition. For activity staining of the ADH involved in ethanol-synthesis, 20mM acetaldehyde was used as the substrate with 0.5mM NADH as the coenzyme. 10 μL of the crude enzyme solution was applied. *S. commune* (NBRC 4929) has a high ADH activity, and a crude enzyme solution diluted 5-fold was used.

Table 2 shows the top 5 strains with the highest ADH specific activities involved in ethanol-decomposition and ethanol synthesis in 87 fungal strains. The specific activity of ADH involved in ethanol-decomposition was determined by measuring the amount of NAD^+ converted to NADH on the oxidation of ethanol to acetaldehyde. The ADH specific activity for ethanol-synthesis was determined by measuring the decrease in NADH, which is converted to NAD^+ during the reduction of acetaldehyde to ethanol (13). As these specific activities are measured using the crude enzyme solution, they are only apparent values and thus indicate the minimum unit of activity.

The basidiomycetes with the highest observed ethanol-decomposition specific activity was *Schizophyllum commune* (NBRC 30496). Of the top 5 ethanol-decomposition strains, four are *S. commune* strains (NBRC 30496, 6503, 30749, 6504) from the *Pleurotaceae* family. The other basidiomycetes in the top 5 is *Lenzites betulinus*, of the family *Polyporaceae* (Table 2(a)). The basidiomycete with the highest observed ethanol-synthesis specific activity is an *S. commune* (NBRC 4929) (Table 2(b)). In fact, three of the top 5

Table 2. ADH specific activity

(a)

Faimly	Species	Identification number	Specific activity (U/mg) NADH→NAD ⁺ (acetaldehyde → ethanol)
Pleurotaceae	<i>S. commune</i> Fr. : Fr	4929	120.32
Tricholomataceae	<i>L. nuda</i> Cooke	8104	51.76
Pleurotaceae	<i>S. commune</i> Fr. : Fr	6504	46.18
Hymenochaetaceae	<i>C. fuscus</i> Fr.	9789	42.59
Pleurotaceae	<i>L. edodes</i> (Berk.)	Sh-1*	38.44

(b)

Faimly	Species	Identification number	Specific activity (U/mg) NAD ⁺ →NADH (ethanol → acetaldehyde)
Pleurotaceae	<i>S. commune</i> Fr. : Fr	30496	6.6
Pleurotaceae	<i>S. commune</i> Fr. : Fr	6503	4.13
Pleurotaceae	<i>S. commune</i> Fr. : Fr	30749	3.45
Pleurotaceae	<i>S. commune</i> Fr. : Fr	6504	2.39
Polyporaceae	<i>L. betulina</i> (L.;Fr.)Fr.	4963	1.8

ethanol-synthesis activities occurred in *Pleurotaceae* strains, with two strains in particular, *S. commune* (NBRC 4929, 6504) showing high activity. The second highest ethanol-synthesis specific activity was observed for *Lepista nuda* Cooke (NBRC 8104) reaching as high as 52 units. However, the ADH specific activity of *L. nuda* in ethanol-decomposition remains was not determine. The activity of the ADH involved in ethanol-decomposition and ethanol-synthesis was generally high for *S. commune*. The data indicates the presence of two type alcohol dehydrogenases, and highlights strain-specific differences in enzymatic activity. This demonstrates that the ADH presence and activity is affected by strain specificity, and varies significantly within basidiomycete.

Next, we performed active staining for ADH by Native-PAGE. The basidiomycetes used for Native-PAGE were two types each with the highest ADH specific activity for ethanol-decomposition and ethanol-synthesis, and the second highest. In this study, an active-stained band of *S. commune* was confirmed around 135 kDa to 180 kDa (Figure 1(a)). This band indicates the ADH involved in the decomposition of ethanol. Then, to detect ADH involved in ethanol synthesis, the substrate and coenzyme of the active stain were changed. This identifies the active ADH enzymes as a

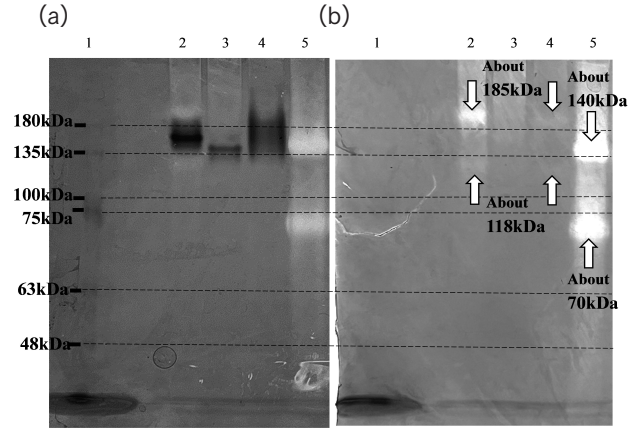


Figure 1 Native-PAGE of ADH in crude basidiomycete enzyme solution.

The activity-stained band shown in 1-a is ADH, which is involved in the decomposition of ethyl alcohol. The white band shown in 1-b is ADH involved in the production of ethyl alcohol.

Lane1: protein molecular weight marker; lane2: *S. commune* (NBRC 30496); lane3: *S. commune* (NBRC 6503); lane4: *S. commune* (NBRC 4929); lane5: *L. nuda* (NBRC: 8104)

white band (Figure 1(b)). The NADH hydrogen is transferred to the NBTH via the phenazine methosulphate and nitroblue tetrazolium chloride reaction, generating the blue formazan dye and staining the gel. In the presence of the ADH involved in ethanol-synthesis, two hydrogen ions (NADH + H⁺) and acetaldehyde combine to form ethanol. This then consumes the NADH, preventing its participation in the formazan dye reaction, thus disrupting the gel staining, giving rise to the indicative formazan-free white band. The ADH involved in the ethanol-decomposition of *S. commune* (NBRC 30496) is around 155kDa, while the ADH involved in alcohol-synthesis are around 185kDa and 118kDa. This highlights that different ADH types are involved in the decomposition and synthesis of ethanol. Furthermore, even within *S. commune*, variance in ADHs involved in ethanol-decomposition were observed depending on the strain. In *S. commune* (NBRC 6503) it was observed to weigh around 135kDa, while in *S. commune* (NBRC 4929) around 130 to 180kDa sized proteins observed. And while no ADH band for ethanol synthesis was observed involved in *S. commune* (NBRC 6503), *S. commune* (NBRC 30496) produced two bands of 185 and 118kDa, and so did *S. commune* (NBRC 4929). *S. com-*

mune (NBRC 4929) had a high specific activity involved in ethanol-synthesis, and no specific activity involved in ethanol-decomposition was detected. However, in the ADH activity-stained band by Native-PAGE, both ADH involved in ethanol-synthesis and ADH involved in ethanol-decomposition could be confirmed. The sample in this study used a crude enzyme solution. Therefore, both ADH involved in ethanol-synthesis and ethanol-decomposition are included. The ADH specific activity is calculated using the initial velocity, and is obtained from the increase/decrease in NADH for 1 second, which is the largest difference. Either the decomposition or synthesis of ethanol by ADH in 1 second during specific activity measurement, whichever is stronger, is occurred as the amount of NADH. *S. commune* (NBRC 4929) is strong in both ethanol synthesis and decomposition reactions, but the ethanol synthesis reaction was stronger. Therefore, it was inferred that the ADH specific activity based on the initial velocity showed a strong decrease in the amount of NADH, which means the production of ethanol. In Native-PAGE, *S. commune* (NBRC 4929) showed a strong blue activity-stained band, which means decomposition of ethanol. This is also due to the fact that both ADH involved in ethanol-synthesis and ADH involved in ethanol-decomposition work due to the use of the crude enzyme solution. The active-stained band of ADH involved in ethanol-decomposition is the result of formation of blue formazan at the location where ADH involved in ethanol-decomposition exists. The band involved in ethanol-synthesis is a band indicated by not causing activity staining, and it is considered that the active staining band formed formazan, which is ADH involved in ethanol-decomposition, was more likely to appear. As with ADH specific activity variations, differences in mycelia were also observed. Sameshima *et al.* reported that the ADH of TR19 (*F. velutipes*) is about 180kDa was involved in ethanol-decomposition and about 135kDa was involved in ethanol-

synthesis. *F. velutipes* belongs to the Tricholomataceae family and *S. commune* belongs to the Pleurotaceae family. It was suggested that the tendency of the molecular weight of ADH involved in the decomposition and synthesis of ethanol may differ depending on the family. Moreover, *L. nuda* (NBRC: 8104) showed no ethanol-decomposition activity but exhibited a high ethanol-synthesis activity. In Native-PAGE, only ADH bands involved in ethanol-synthesis were confirmed at 140kDa and 70kDa. This indicates that *L. nuda* synthesizes ethanol, but does not consume it. One yeast that produces ethyl alcohol is *Saccharomyces cerevisiae*. The Yeast ADH (YOL086C) is reported to be approximately 150 kDa (14), and the molecular weights of *S. commune* and *L. nuda*, which were subjected to ADH activity staining in this study, were different. The characteristics of ADH is considered to differ between yeast, which obtains energy by producing alcohol under anaerobic conditions, and basidiomycetes, which are capable of aerobically fermenting alcohol. Mushroom ADH are reported to be class III (15) and are characterized by a large Km, with a diminished ethanol generation capacity compared to ADH of class I and II. Where reactions occur in both directions of ethanol synthesis and decomposition, in order to actively produce alcohol, utilization of basidiomycetes is considered to be effective that only ADH activity involved in ethanol-synthesis like *L. nuda*. Importantly, for alcohol fermentation using mushrooms, ADH involved in synthesis and decomposition of ethanol must be considered for produce greater volumes of alcohol.

This study explored a wide range of basidiomycetes for ADH involved both in ethanol formation and consumption. The results indicate that basidiomycetes have both ADH involved in ethanol synthesis and decomposition, and that mushrooms with only ADH involved in ethanol production may be suitable for alcoholic beverage production. From the perspective of ethanol pro-

duction, ADH that consume ethanol are to be avoided, thus mushrooms with ADH primarily involved in ethanol-synthesis are suitable targets for alcoholic beverage production.

Reference list

- 1) Ryuichiro K. 2014. Investigation of the functions of mushrooms. *Nippon Kinoko Gakkaishi (Mushroom Sci. Biotech)* **21**: 155-64 (in Japanese).
- 2) Ichiro K, Kana U, Virginia A. 2020. Conservation of Xylose Fermentability in *Phlebia* Species and Direct Fermentation of Xylan by Selected Fungi. *Applied Biochem. Biotech.* **192**: 895-909.
- 3) Matsui T, Tomoko K, Shoko F. 2009. Characteristics of wine produced by mushroom fermentation using *Schizophyllum commune* NBRC 4929. *Mushroom Sci. Biotech.* **17**: 107-111.
- 4) Tokumitsu O, Tomoko O, Norie M, et al. 2000. Production of Sake by Mushroom Fermentation. *Mushroom Sci. Biotech.* **8**: 109-114.
- 5) Toshio M, Ojiro K, Akane M, Hirokazu K, et al. 2019. Effect on growth, sugar consumption, and aerobic ethanol fermentation of homologous expression of the sugar transporter gene *Pshxt1* in the white rot fungus *Phanerochaete sordida* YK-624. *Journal of Biosci. Bioengineer.* **128**: 537-543.
- 6) Mototeru Y, Tadashi Y. 1955. Changes in thiamine and riboflavin contents of vegetables and fruits in storage. *The Vitamin Society of Japan* **8**: 299-303.
- 7) Akira H, Hiroko N. 1958. On the riboflavin content of edible fungi. *The Vitamin Society of Japan*: 369-370.
- 8) Toshiko K. 1990. Determination of Ergosterol and Vitamin D₂ in Fungi. *Nippon Kasei Gakkaishi (Journal of Home Economics of Japan)*: 395-400 (in Japanese).
- 9) Susumi H, Sachiko N, Tsuneo A, et al. 1976. Structural Studies on the Anti-tumor Active Polysaccharides from *Coriolus versicolor* (Basidiomycetes). II. Structures of β -D-Glucan Moieties of Fractionated Polysaccharides. *Yakugaku Zasshi (YAKUGAKU ZASSHI)* **96**:419-424 (in Japanese).
- 10) Yukinori Y, Hiromi M, Ayako S, et al. 2012. Evaluation of antioxidant activity in edible mushrooms. *Nippon Kinoko Gakkaishi (Mushroom Sci. Biotech)* **20**: 89-92 (in Japanese).
- 11) Tokumitsu M. 2017. Development of functional foods by mushroom fermentation. *Kinoko Gakkaishi (Mushroom Sci. Biotech)* **24**: 169-175 (in Japanese).
- 12) Yuka S, Naomi T, Hiroko S, et al. 2022. Production of alcohol by *F. velutipes* TR-19. *Mukogawa Eiyō Kagaku Kenkyūsyozasshi (The Mukogawa Journal of Nut. Sci. R.)* **11**:17-22 (in Japanese).
- 13) Douglas B, J. G. Zeikus. 1994. Purification of acetaldehyde dehydrogenase and alcohol dehydrogenases from *Thermoanaerobacter ethanolicus* 39E and characterization of the secondary-alcohol dehydrogenase (2° Adh) as a bifunctional alcohol dehydrogenase-acetyl-CoA reductive thioesterase. *Biochem. J.* **302**: 163-170.
- 14) Olga S, James C P, Jacobus A. 2008. The alcohol dehydrogenases of *Saccharomyces cerevisiae*: a comprehensive review. *FEMS Yeast Res.* **8**: 967-78.
- 15) Annika N, Jawed S, Mustafa E, et al. 2004. Class III alcohol dehydrogenase: consistent pattern complemented with the mushroom enzyme. *FEBS Letters* **559**: 27-32.