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The Ability of Edible Mushrooms to Act as Biocatalysts: Preparation of Chiral Alcohols Using Basidiomycete Strains

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ABSTRACT

To examine the potential ability of edible mushrooms to act as biocatalysts, 19 basidiomycete strains were screened. Modified media (PG, O, and PGO medium) for liquid cultivation of these basidiomycete strains were designed and tested. Wet cells (>10 g) of 4 basidiomycete strains (*Pleurotus salmoneostramineus* H7, *P. salmoneostramineus* H13, *Ganoderma lucidum* NBRC31863, *Flammulina velutipes* NBRC31862) were harvested from PGO medium for 7 days. The stereoselective reduction of α-keto esters using the 4 strains was tested. It was found that each of these strains had a reducing activity toward 6 aliphatic α-keto esters. In the presence of L-alanine as an additive, the reduction of ethyl 2-oxobutanoate and ethyl 2-oxopentanoete by *P. salmoneostramineus* H7 produced the corresponding alcohol with a high conversion ratio and with excellent enantiomeric excess (>99% e.e. (*R*)). Furthermore, ethyl pyruvate, ethyl 2-oxobutanoate, and ethyl 2-oxopentanoate were predominantly reduced to the corresponding (*R*)-hydroxy ester (>99% e.e.) by *G. lucidum*. Thus, we found that these edible mushrooms have great potential to be used as biocatalysts for the stereoselective reduction of carbonyl compounds.

Keywords: Basidiomycete; Edible Mushroom; Biocatalyst; Chiral Alcohol

1. Introduction

A mushroom is the fleshy, spore-bearing fruiting body of a fungus, typically produced above ground on soil. The mushroom has been used as a food since ancient times, and more recently has again attracted attention as a functional food material. Edible mushrooms have been utilized as food and occasionally as medicines (medical mushrooms). The abilities of some mushrooms to inhibit tumor growth and to modulate the immune system have been studied [1-3]. Furthermore, the degradation of persistent organic substances and substances that are not readily degradable, such as DTT, dioxins, and PCBs, by use of certain mushrooms has been attempted and investigated [4-7]. Thus, the mushroom is one of the noteworthy microorganisms in food, medical, and environmental sciences; however, there are few reports about the applications of the mushrooms in other science fields.

In the 21st century it became impossible to disregard exhaustion of the earth's resources. Therefore, the effective use of resources is an important focus of research. In the interest of the effective use of resources and environmental protection, we investigated an environmentally friendly method of producing useful compounds, using microorganisms as the bio-resource. It has been demonstrated that microorganisms such as actinomycetes, micro green algae, and other bacteria are useful biocatalysts in the production of optically active alcohols (chiral building brocks) [8-14]. However, little information is known about the potential for edible mushrooms to act as biocatalysts for asymmetric organic syntheses.

This study describes the stereoselective reduction of α-keto esters by edible mushrooms as novel biocatalysts (**Figure 1**).

2. Material and Methods

2.1. Instruments and Chemicals

Gas chromatography was done using GL Science GC-353 gas chromatographs (DB-Wax, Agilent Technolo-

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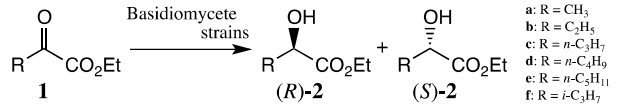


Figure 1. The reduction of *α*-keto esters (1a-f) by basidiomycete strains.

gies, Santa Clara, CA, USA, 0.25 mm × 30 m; TC-1, GL Science, Tokyo, Japan, 0.25 mm × 30 m; CP-Chirasil-DEX CB, Varian Inc., Lake Forest, CA, USA, 0.25 mm × 25 m; Gamma DEX 225, Sigma-Aldrich Co., St. Louise, MO, USA, 0.25 mm × 30 m). Ethyl pyruvate (**Figure 1**, **1a**), diatomaceous earth (granular), polypeptone, L-alanine, olive oil, pectin (from apple), and Daigo's potato dextrose agar (PDA) were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan. Difco™ potato dextrose broth (PDB) and Bacto™ yeast extract were purchased from Becton, Dickinson and Co., Franklin Lakes, NJ, USA. Arabic gum and ethyl 3-methyl-2-oxobutanoate (**1f**) were purchased from Sigma-Aldrich. Ethyl 2-oxobutanoate (**1b**), ethyl 2-oxopentanoate (**1c**), ethyl 2-oxohexanoate (**1d**), ethyl 2-oxoheptanoate (**1e**), and *α*-hydroxy esters (**2a-f**) were prepared according to the procedures in the literature [15]. All other chemicals used in this study were of analytical grade and comercially available.

2.2. Microorganisms and Cultivation

The *Pleurotus salmoneostramineus* H7 (Japanese name: tokiiro-hiratake), *P. salmoneostramineus* H13, and *Lepista sordida* (Japanese name: komurasaki-shimeji) were purchased from the Research Institute of Biotechnology, Fujiwara Techno-Art Co., Ltd., Japan. *Agaricus bisporus* NBRC30774, *Agaricus bisporus* NBRC30782, *Flammulina velutipes* NBRC31862 (Japanese name: enoki-take), *Ganoderma lucidum* NBRC31863 (Japanese name: mannen-take), *Grifola frondosa* NBRC30552 (Japanese name: mai-take), *Grifola frondosa* NBRC30661, *Grifola frondosa* NBRC32987, *Hericium erinaceum* NBRC-100328 (Japanese name: yamabushi-take), *Lentinula edodes* NBRC-30719, *Lentinula edodes* NBRC30720 (Japanese name: shii-take), *Lentinula edodes* NBRC-30723, *Lentinula edodes* NBRC30724, *Lyophyllum ulmarium* NBRC30775 (Japanese name: buna-shimeji), *Pholiota nameko* NBRC-30372 (Japanese name: nameko), *Tricholoma matsutake* NBRC30773 (Japanese name: matsu-take), and *Tricholoma giganteum* NBRC31860 (Japanese name: niou-shimeji) were purchased from the National Institute of Technology and Evaluation, Biological Resource Center, Japan (NBRC). These basidiomycete strains were maintained at 25˚C in PDA. The basidiomycete strains were grown in PDB and synthetic media (PG, O, or PGO medium) (200 mL) for 6 - 30 days at 25˚C with aerobic rotary shaking at 95 min^{-1} in a baffled 500-mL flask in the dark. The PG medium comprised 20 g glucose, 4 g polypepton, 1 g Bacto™ yeast extract, 0.46 g KH_2PO_4, 1 g K_2HPO_4, 2 g pectin, and 2 g arabic gum per 1 liter of distilled water (pH 5.5). The O medium comprised 20 g glucose, 4 g polypepton, 1 g Bacto™ yeast extract, 0.46 g KH_2PO_4, 1 g K_2HPO_4, and 10 g olive oil per 1 liter of distilled water (pH 5.5). The PGO medium comprised 20 g glucose, 4 g polypepton, 1 g Bacto™ yeast extract, 0.46 g KH_2PO_4, 1 g K_2HPO_4, 2 g pectin, 2 g arabic gum, and 10 g olive oil per 1 liter of distilled water (pH 5.5). The basidiomycete cells were harvested by filtration on a filter paper *in vacuo* and washed with saline (0.85% aqueous NaCl).

2.3. Reduction of *α*-Keto Esters with Basidiomycete Whole Cells

The saline-washed wet cells (0.5 g) were resuspended in a large test tube (ϕ30 mm × 200 mm) containing 20 mL of saline (or water), the substrate (0.15 mmol; corresponding substrate concentration was 7.5 mM) and additive (5.0 mmol) were added, and the culture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C. A portion of the reaction mixture was filtered using a diatomaceous earth short column (ϕ 10 mm × 30 mm), extracted with diethyl ether, and then concentrated under reduced pressure.

2.4. Analysis

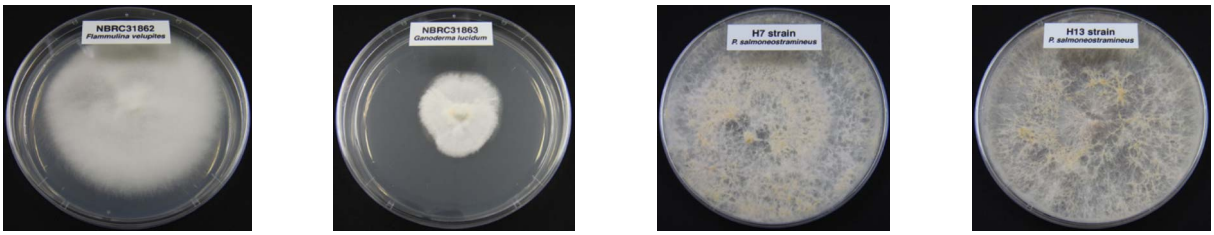
Conversions of produced alcohols (**Figure 1**, **2a-f**) were measured using a GLC with a DB-WAX capillary column (100 kPa He, 110˚C; **1a**, 3.78 min; **2a**, 4.75 min; **1b**, 4.73 min; **2b**, 5.92 min; **1f**, 4.54 min; **2f**, 6.41 min; 120˚C; **1c**, 4.84 min; **2c**, 6.45 min; 150˚C, **1d**, 3.83 min; **2d**, 4.68 min; **1e**, 4.78 min; **2e**, 6.07 min). The enantiomeric excesses (e.e.) of the products were measured using a GLC equipped with an optically active CP-Chirasil-DEX CB (**2a-e**) or Gamma DEX 225 capillary column (**2f**). The e.e. was calculated by the following formula; e.e.(%) = $\{(R - S)/(R + S)\} \times 100$. These *R* and *S* are the respective peak areas on GLC analyses. The absolute configurations of *α*-hydroxy esters (**2a-f**) were identified by comparing their retention times from the GLC analyses with those of authentic samples [15].

3. Results and Discussion

3.1. Screening of Basidiomycete Strains and Cultivation Media

The recommended medium for most basidiomycete strains tested in this study was PDA. However, all strains cultivated in PDB, with the exception of *Flammulina velutipes*, *Ganoderma lucidum*, and *Pleurotus salmoneostramineus*, resulted in 2 g or less of wet cells, even if

cultured for over 1 month in PDB (**Table 1**). To improve the cultivation rate in PDB, 3 new culture media (PG, O, and PGO medium) containing pectin, arabic gum, and olive oil were designed and tested for efficacy. We found that the cultivation in the PGO medium produced more wet cells than cultivation in other media (PDB, PG, and O medium) in a short time. Although cultivation of basidiomycete strains in the liquid medium requires a longer time compared to yeast strains and actinomycetes, the efficient cultivation of basidiomycetes was attained in the PGO medium we designed. In particular, over 10 g of wet cells from 4 basidiomycete strains (*F. velutipes* NBRC31862, *G. lucidum* NBRC31863, *P. salmoneostramineus* H7, and *P. salmoneostramineus* H13) were harvested after cultivation in PGO medium for 1 week. Therefore, we investigated the possibility that these 4 strains (see **Figure 2**) can act as biocatalysts for the asymmetric reduction of carbonyl compounds.



Flammulina velutipes NBRC31862 | *Ganoderma lucidum* NBRC31863 | *P. salmoneostramineus* H7 | *P. salmoneostramineus* H13

Figure 2. Pictures of basidiomycete strains used for the reduction of keto esters.

Table 1. The cultivation of basidiomycete strains in various culture media[1].

Strains		PDB		PG medium[2]		O medium[2]		PGO medium[2]	
		wet cells (g)	time (day)	wet cells (g)	time (day)	wet cells (g)	time (day)	wet cells (g)	time (day)
Pholita nameko	NBRC30372	0.5	30	7.0	17	7.0	14	6.0	10
Grifola frondosa	NBRC30552	1.0	30	1.2	18	1.2	16	2.0	10
Grifola frondosa	NBRC30661	0.5	25	0.7	14	0.6	12	1.0	10
Lentinula edodes	NBRC30719	1.5	30	1.0	14	1.2	12	2.0	10
Lentinula edodes	NBRC30720	1.0	30	1.0	14	0.8	12	1.0	10
Lentinula edodes	NBRC30723	0.5	30	0.8	14	1.0	1	1.5	10
Lentinula edodes	NBRC30724	1.0	30	1.1	14	1.3	12	2.5	10
Tricholoma matsutake	NBRC30773	0.1	30	0.1	30	0.1	30	0.1	20
Agaricus bisporus	NBRC30774	0.5	30	0.8	14	1.5	14	2.0	14
Lyophyllum ulmarium	NBRC30775	0.5	30	3.0	11	7.0	15	7.0	10
Agaricus bisporus	NBRC30782	0.5	20	1.0	14	1.5	14	1.8	14
Tricholoma giganteum	NBRC31680	1.2	15	1.0	12	1.8	14	2.0	10
Flammulina velutipes	NBRC31862	7.0	15	7.0	10	8.0	15	10.0	7
Ganoderma lucidum	NBRC31683	8.0	15	6.0	12	4.0	9	10.5	7
Grifola frondosa	NBRC32987	0.5	30	0.5	14	1.3	12	2.1	14
Hericium erinaceum	NBRC100328	2.5	30	1.5	14	1.5	12	2.0	10
Pleurotus salmoneostramineus	H7	18.0	15	13.5	12	12.0	10	15.0	7
Pleurotus salmoneostramineus	H13	20.0	15	15.0	12	11.0	10	14.0	7
Lepista sordida		0.5	32	0.5	30	1.0	20	1.5	14

[1]The basidiomycete strains were grown in the medium at 25˚C with aerobic rotary shaking (110 min^{-1}) in baffled 500-mL flask in the dark condition; [2]Composition of each culture medium was described in materials and method section.

3.2. Ability to Reduce *α*-Keto Esters

Four basidiomycete strains were tested for their ability to reduce *α*-keto esters (**Figure 1**). The results of the α-keto ester reductions (**1a-f**) are summarized in **Tables 2-5**. The reduction of substrates by the *P. salmoneostramineus* H7 strain cultivated in the PGO medium exhibited a higher conversion ratio compared to reduction by the same strain cultivated in the PDB medium (**Table 2**). Furthermore, when the concentration of the reaction solvent, saline, was diluted from 0.85% to 0.085%, the time to reach the maximum conversion was shortened, while the stereoselectivity of the produced hydroxy esters decreased. An additive was introduced into the reaction mixture to improve the stereoselectivity. As a result, the reduction of ethyl 2-oxobutanoate (**1b**), ethyl 2-oxopentanoate (**1c**), and ethyl 2-oxoheptanoate (**1e**) in the presence of L-alanine produced the corresponding alcohols in excellent e.e. (**2b**, >99% e.e. (*R*); **2c**, >99% e.e. (*R*); **2e**, >99% e.e. (*S*), respectively). The effects of other additives (L-glycine, L-glutamate, L-aspartate, ascorbate, $CaCl_2$, $MnCl_2$, allyl alcohol, methyl vinyl ketone, quercetin, etc.) were tested; however, the stereoselectivity of the produced alcohols did not increase (data not shown).

As shown in **Table 3**, the reduction of substrates by the PGO-cultivated *P. salmoneostramineus* H13 strain was faster (**2a-d**, conversion >99% after 24 h) compared with that of the PDB-cultivated H13 strain. However, improvement in the enantioselectivity of the hydroxy

Table 2. The reduction of *α*-keto esters (1a-f) to the corresponding *α*-hydroxy esters (2a-f) by *P. salmoneostramineus* H7.

Product	PDB/0.85% NaCl[1]				PGO/0.85% NaCl[2]				PGO/0.085% NaCl[3]				PGO/0.85% NaCl/L-Ala[4]			
	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)
2a	>99	78	*S*	48	>99	85	*S*	48	>99	88	*S*	12	>99	92	*S*	24
2b	85	76	*S*	48	>99	53	*S*	48	>99	44	*S*	24	>99	>99	*R*	24
2c	>99	68	*S*	24	>99	60	*S*	24	>99	42	*S*	12	>99	>99	*R*	24
2d	98	94	*S*	48	>99	92	*S*	48	>99	77	*S*	12	>99	76	*R*	24
2e	42	>99	*S*	48	38	>99	*S*	48	56	77	*S*	24	86	>99	*S*	24
2f	>99	85	*S*	24	>99	67	*S*	24	>99	43	*S*	12	>99	86	*S*	24

[1]Substrate (0.15 mmol) and 0.85% NaCl aq. (20 ml) were added to the wet cells (0.5 g) cultivated in PDB, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [2]Substrate (0.15 mmol) and 0.85% NaCl aq. (20 ml) were added to the wet cells (0.5 g) cultivated in PGO-medium, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [3]Substrate (0.15 mmol) and 0.085% NaCl aq. (20 ml) were added to the wet cells (0.5 g) cultivated in PGO-medium, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [4]Substrate (0.15 mmol), L-alanine (5.0 mmol), and 0.085% NaCl aq. (20 ml) were added to the wet cells (0.5 g) cultivated in PGO-medium, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [5]Conversion was measured by a GLC analysis; [6]Enantiomeric excess (e.e.) and configuration (*R*/*S*) were determined by GLC analyses with optically active capillary columns.

Table 3. The reduction of *α*-keto esters (1a-f) to the corresponding *α*-hydroxy esters (2a-f) by *P. salmoneostramineus* H13.

Product	PDB/0.85% NaCl[1]				PGO/0.85% NaCl[2]				PGO/0.085% NaCl[3]				PGO/0.85% NaCl/L-Ala[4]			
	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)
2a	>99	83	*S*	48	>99	79	*S*	48	>99	87	*S*	12	>99	80	*S*	24
2b	>99	59	*S*	48	>99	65	*S*	48	>99	66	*S*	24	>99	>99	*R*	24
2c	>99	49	*S*	24	>99	76	*S*	24	>99	>99	*R*	24	96	86	*S*	24
2d	98	86	*S*	48	>99	96	*S*	24	>99	69	*S*	12	98	82	*R*	24
2e	46	76	*S*	48	94	>99	*R*	24	88	81	*S*	12	99	75	*S*	24
2f	>99	84	*S*	24	>99	84	*S*	48	>99	33	*S*	12	>99	27	*S*	24

[1]Substrate (0.15 mmol) and 0.85% NaCl aq. (20 ml) were added to the wet cells (0.5 g) cultivated in PDB, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [2]Substrate (0.15 mmol) and 0.85% NaCl aq. (20 ml) were added to the wet cells (0.5 g) cultivated in PGO-medium, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [3]Substrate (0.15 mmol) and 0.085% NaCl aq. (20 ml) were added to the wet cells (0.5 g) cultivated in PGO-medium, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [4]Substrate (0.15 mmol), L-alanine (5.0 mmol), and 0.085% NaCl aq. (20 ml) were added to the wet cells (0.5 g) cultivated in PGO-medium, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [5]Conversion was measured by a GLC analysis; [6]Enantiomeric excess (e.e.) and configuration (*R*/*S*) were determined by GLC analyses with optically active capillary columns.

Table 4. The reduction of *α*-keto esters (1a-f) to the corresponding *α*-hydroxy esters (2a-f) by *Ganoderma lucidum* NBRC-31863.

Product	PDB/0.85% NaCl[1]				PGO/0.85% NaCl[2]				PGO/0.085% NaCl[3]				PGO/0.85% NaCl/L-Ala[4]			
	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)
2a	60	54	*S*	48	>99	54	*S*	48	>99	29	*S*	12	>99	>99	*R*	24
2b	47	28	*S*	48	>99	85	*R*	48	>99	96	*R*	12	>99	>99	*R*	24
2c	75	50	*S*	48	>99	58	*S*	48	>99	17	*S*	12	>99	>99	*R*	24
2d	51	62	*S*	24	>99	86	*R*	48	>99	64	*S*	12	>99	69	*R*	12
2e	26	67	*S*	48	27	50	*S*	24	24	64	*S*	12	>99	56	*R*	24
2f	33	59	*S*	48	60	24	*S*	48	>99	65	*R*	48	>99	56	*R*	6

[1]Substrate (0.15 mmol) and 0.85% NaCl aq. (20 ml) were added to the wet cells (0.5 g) cultivated in PDB, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [2]Substrate (0.15 mmol) and 0.85% NaCl aq. (20 ml) were added to the wet cells (0.5 g) cultivated in PGO-medium, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [3]Substrate (0.15 mmol) and water (20 ml) were added to the wet cells (0.5 g) cultivated in PGO-medium, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [4]Substrate (0.15 mmol), L-alanine (5.0 mmol), and water (20 ml) were added to the wet cells (0.5 g) cultivated in PGO-medium, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [5]Conversion was measured by a GLC analysis; [6]Enantiomeric excess (e.e.) and configuration (*R*/*S*) were determined by GLC analyses with optically active capillary columns.

Table 5. The reduction of *α*-keto esters (1a-f) to the corresponding *α*-hydroxy esters (2a-f) by *Flammulina velutipes* NBRC-31862.

Product	PDB/0.85% NaCl[1]				PGO/0.85% NaCl[2]				PGO/0.085% NaCl[3]				PGO/0.85% NaCl/L-Ala[4]			
	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)	conv. (%)[5]	e.e. (%)[6]	(*R*/*S*)[6]	time (h)
2a	>99	66	*S*	48	>99	71	*S*	24	>99	75	*S*	24	>99	71	*S*	24
2b	>99	14	*S*	48	92	>99	*R*	48	>99	20	*S*	12	>99	26	*S*	12
2c	>99	3	*R*	48	>99	22	*R*	48	>99	32	*R*	48	>99	44	*R*	48
2d	>99	2	*R*	48	86	8	*R*	48	>99	13	*R*	24	>99	25	*R*	24
2e	>99	37	*S*	48	75	34	*S*	24	98	17	*S*	12	98	12	*S*	12
2f	>99	9	*R*	48	84	8	*R*	48	>99	31	*R*	24	>99	85	*R*	24

[1]Substrate (0.15 mmol) and 0.85% NaCl aq. (20 ml) were added to the wet cells (0.5 g) cultivated in PDB, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [2]Substrate (0.15 mmol) and 0.85% NaCl aq. (20 ml) were added to the wet cells (0.5 g) cultivated in PGO-medium, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [3]Substrate (0.15 mmol) and water (20 ml) were added to the wet cells (0.5 g) cultivated in PGO-medium, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [4]Substrate (0.15 mmol), L-alanine (5.0 mmol), and water (20 ml) were added to the wet cells (0.5 g) cultivated in PGO-medium, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 min^{-1}) at 25˚C; [5]Conversion was measured by a GLC analysis; [6]Enantiomeric excess (e.e.) and configuration (*R*/*S*) were determined by GLC analyses with optically active capillary columns.

esters was not observed with the addition of L-alanine, except for the reduction of ethyl 2-oxobutanoate (**1b**).

The reduction of *α*-keto esters by the *G. lucidum* strain exhibited the same tendency as the *P. salmoneostramineus* H7 strain. The PGO-cultivated *G. lucidum* reduced the substrate to the corresponding hydroxy esters with high conversion ratios (**2a-d**, conversion >99%) compared to those of the PDB-cultivated *G. lucidum* (**Table 4**). Moreover, the reduction of substrates **1a-d** by the PGO-cultivated *G. lucidum* in the water was accelerated (from 48 h to 12 h). Furthermore, the introduction of L-alanine to the reaction mixture in water for substrates **1a-f** produced the corresponding alcohols with high conversion ratios (>99%). In particular, substrates **1a-c** were reduced to *α*-hydroxy esters **2a-c** in excellent e.e. (>99% e.e. (*R*)).

The PDB-cultivated *F. velutipes* reduced the substrates to the corresponding hydroxy esters with high conversion ratios (>99%) compared with those reduced by PGO-cultivated *F. velutipes*, as shown in **Table 5**. In the reduction catalyzed by *F. velutipes*, there was no the improvement in the products' stereoselectivity with the addition of L-alanine.

Thus, 6 aliphatic *α*-keto esters were converted to the corresponding *α*-hydroxy esters by 4 basidyomycete strains. In terms of the reduction conversion rates and the enantioselectivity of the products, these results suggest that the *P. salmoneostramineus* H7 strain and *G. lucidum* are

potential biocatalysts for the stereoselective reduction of keto esters to obtain chiral hydroxy esters.

4. Conclusion

In order to search for new applications of mushrooms, the ability of 19 edible mushrooms to act as biocatalysts were examined. These 19 strains were cultivated in the PDB liquid medium, but cell growth was slow. Therefore, we designed 3 modified culture media (PG, O, and PGO medium), and found that the PGO medium, which contains pectin, arabic gum, and olive oil, was suitable for the liquid cultivation of basidiomycete. In particular, over 10 g of wet cells were harvested after a short period of time when 4 basidiomycete strains were cultivated in PGO medium (*P. salmoneostramineus* H7, *salmoneostramineus* H13, *G. lucidum*, and *F. velutipes*). The reduction of α-keto esters using each of these 4 strains was tested. We found that these strains exhibited reducing activity toward 6 aliphatic α-keto esters. Furthermore, the stereospecific reductions of some α-keto esters to the corresponding (*R*)-alcohols, using *P. salmoneostramineus* H7 or *G. lucidum* in the presence of L-alanine, was accomplished with excellent e.e. (>99%). As mentioned above, we found that some edible mushroom could be used as biocatalysts for stereoselective reductions of α-keto esters, a similar finding to reports that studied bakers' yeast.

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