

Screening of Lactate Dehydrogenase and Curd Rennet of Microorganisms

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Twenty strains of bacteria and 5 strains of basidiomycetes cultured under aerobic conditions and 9 strains of bacteria cultured under anaerobic conditions demonstrated high lactate dehydrogenase activity. One strain of basidiomycete cultured under aerobic conditions and 7 strains of yeasts cultured under anaerobic conditions demonstrated high curd rennet activity. The pHs cultured under aerobic and anaerobic conditions were between 6.1 and 7.1.

Introduction

It is reported that microorganisms have various physiological activity substances. The substance is related to our health and is contained in our foods. The fermented foods(miso, shoyu, natto, yoghurt and cheese)which contain the substance, are made by microorganisms. Preparation of yoghurt¹⁾ and cheese²⁾, have been used to regular microorganisms such as *Bifidobacterium*, *Lactobacillus*. However, there is no report regarding other microorganisms.

Therefore, we did a screening of lactate dehydrogenase and curd rennet of various microorganisms.

Materials and Methods

Organisms

Sixty-eight strains of bacteria, 34 strains of yeasts, 11 strains of molds and 19 strains of basidiomycetes were used.

Medium and culture conditions

Bacteria were grown in 300ml Erlenmyer flasks with 100ml of the medium(pH 7.0)containing 0.5% meat extract, 0.5% NaCl and

1.0% peptone, at 28°C for 24hr. Lactic bacteria were grown in 300ml Erlenmyer flasks with 100ml of the medium(pH 7.2)containing 0.24% meat extract, 0.5% yeast extract, 0.05% soluble starch, 1.0% protease peptone, 0.4% Na₂HPO₄, 0.05% glucose and 0.44% liver extract, at 37°C for 24hr. Yeasts, molds and basidiomycetes were grown in 300ml Erlenmyer flasks with 100ml of the medium(pH 5.5~6.0) containing 2% malt extract at 28°C for 24hr. Molds and basidiomycetes were grown in the medium at 25°C for 2 weeks. Cultivation was carried out under aerobic conditions on a rotary shaker(100rpm)and under anaerobic conditions. After cultivation, the culture broth was centrifuged(4°C, 20min, 8000rpm)to collect the cells. Molds and basidiomycetes were filtered with mesh. The cells were washed twice with 0.85% NaCl. The cells were stored at -20°C.

Preparation of cell free extract

The cells in 10mM potassium phosphate buffer(pH 7.0)were subjected to sonication with an ultrasonic oscillator(BRANSON, SONIFIER 250)4 times in 15sec(bacteria, yeasts, molds)and 8~12 times in 15sec(basidiomycetes)below

0~8°C. The undestroyed cells and debris were removed by centrifugation at 15,000rpm for 10min at 4°C. The supernatant solution obtained was used as the cell free extract.

Electrophoresis

Gel electrophoresis of the native enzyme was carried out with 7.5% polyacrylamide gel by the Davis³⁾ method. Twenty micrograms of LDH mixed with 20 μ l of 10mM potassium phosphate buffer(pH 7.0) or 20 μ l of cell free extract were electrophoresized at 20mA per gel until the dye front reached the bottom of the gel. After electrophoresis, the gel was stained with enzymatic activity staining mixture containing 0.2M Tris-HCl buffer(pH 8.5), 15mM NAD⁺, 80mM L-lactate, 6.5mM phenazine methosulfate and

12mM nitro blue tetrazolium.

Measurement of curd rennet activity

The reaction mixture contained milk and cell free extract. The cell free extract was replaced by milk in a blank. Incubation was carried out at 28°C for 18hr with a test tube 12mm in diameter. The reaction mixture was observed with a deposit, and pH was measured.

Results and Discussion

Polyacrylamide gel electrophoresis of the native enzyme followed by activity staining of lactate dehydrogenase is shown in Fig. 1. The activity staining of lactate dehydrogenase and curd rennet activities of various microorganisms are summarized in Table 1, 2, 3 and 4.

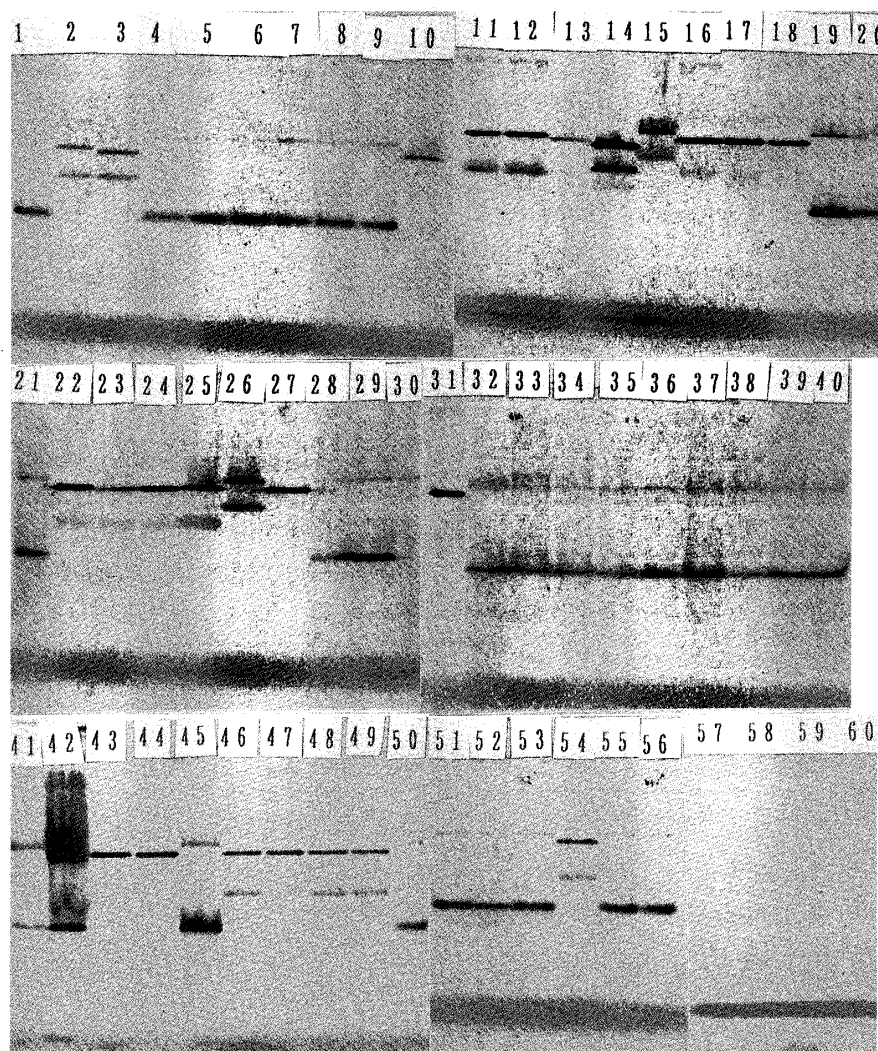


Fig. 1. Activity staining.

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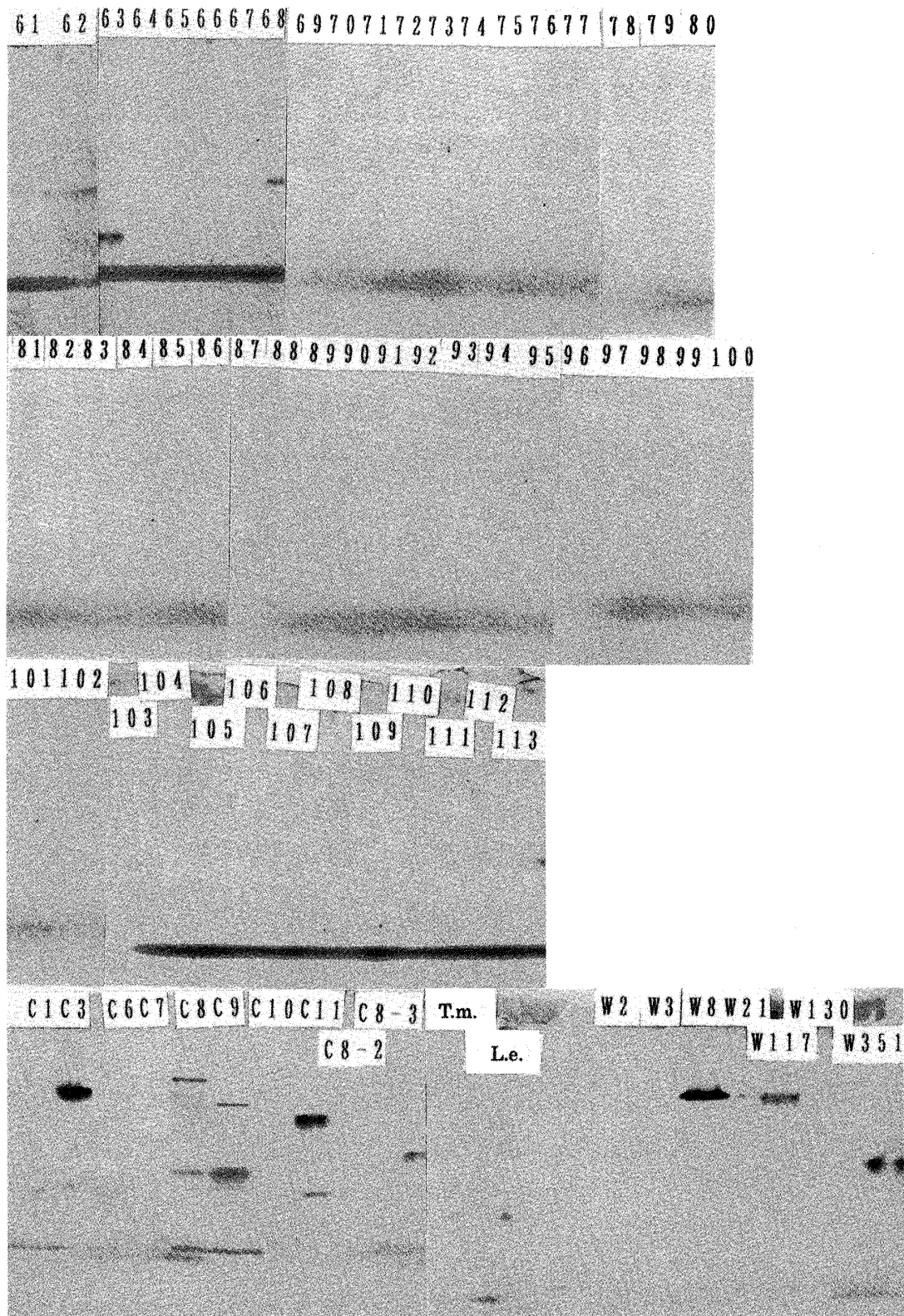


Fig. 1. Activity staining.

The activity staining of lactate dehydrogenase was examined by the method described in Materials and Methods. Number 1~113 and mark of C1~W351 are the same as Table 1.~4.

Table 1. Activities of lactate dehydrogenase and curd rennet of bacteria

Strain No.	Bacteria	LDH activity*		Curd rennet activity**			
		Aerobic	Anaerobic	Precipitation		pH	
				Aerobic	Anaerobic	Aerobic	Anaerobic
1	<i>Agrobacterium radiobacter</i> IAM 1526	+	+	—	—	6.7	6.3
2	<i>Agrobacterium tumefaciens</i> IAM B-26-1	+	+	—	—	6.6	6.2
3	<i>Alcaligenes faecalis</i> IAM B-141-1	+	+	—	—	6.7	6.5
4	<i>Alcaligenes polymorpha</i>	+	+	—	—	6.6	6.5
5	<i>Arthrobacter globiformus</i> IFO 12137	+	+	—	—	6.6	6.4
6	<i>Arthrobacter oxydans</i> IFO 12138	+	+	—	—	6.3	6.3
7	<i>Arthrobacter pascens</i> IFO 12139	+	+	—	—	6.7	6.3
8	<i>Arthrobacter simplex</i> IFO 3530	+	+	—	—	6.7	6.2
9	<i>Arthrobacter ureafaciens</i> IFO 12140	+	+	—	—	6.7	6.4
10	<i>Bacillus brevis</i> IFO 3331	+	+	—	—	6.7	6.5
11	<i>Bacillus cereus</i> IFO 3001	++	++	—	—	6.5	6.4
12	<i>Bacillus megaterrium</i> NI 8100 NTH B12	++	+	—	—	6.5	6.3
13	<i>Bacillus pumilus</i> IFO 3030	+	+	—	—	6.7	6.3
14	<i>Bacillus sphaericus</i> IFO 3525	++	+	—	—	6.5	6.3
15	<i>Bacillus brevis</i> IFO 3007	++	+	—	—	6.7	6.3
16	<i>Bacterium cadaveris</i> IFO 3731	+	+	—	—	6.4	6.3
17	<i>Bacterium ketoglutamicus</i> SO(1)Shionogi Co., Ltd	++	+	—	—	6.5	6.3
18	<i>Bacterium orleanense</i> IFO 3259	++	+	+	—	6.6	6.3
19	<i>Brevibacterium sp.</i> P145 Phage host	++	+	—	—	6.6	6.4
20	<i>Brevibacterium sp.</i> P145 N. Kato	+	+	—	—	6.7	6.3
21	<i>Corynebacterium fascians</i> IAM 1079	+	+	—	—	6.7	6.4
22	<i>Corynebacterium glutamicum</i> No.534 ATCC 13032	++	++	+	—	6.5	6.3
23	<i>Corynebacterium pseudodiphtherium</i>	+	+	—	—	6.6	6.2
24	<i>Enterobacter aerogenes</i> IFO 3320	++	+	+	—	6.5	6.5
25	<i>Enterobacter cloacae</i> IAM 1221	++	+	+	—	6.4	6.3
26	<i>Erwinia arvideae</i> IFO 3830	++	+	+	—	6.4	6.4
27	<i>Erwinia carotovora</i> IFO 3380	++	+	+	—	6.5	6.3
28	<i>Escherichia coli</i> K12 IFO 3208	+	+	—	—	6.6	6.2
29	<i>Escherichia freundii</i> S-96	++	+	—	—	6.5	6.4
30	<i>Escherichia intermedia</i> A-21	+	+	—	—	6.6	6.4
31	<i>Klebsiella pneumoniae</i> IFO 3317	++	+	+	—	6.4	6.6
32	<i>Klebsiella pneumoniae</i> IFO 12009	+	+	—	—	6.5	6.4
33	<i>Klebsiella pneumoniae</i> IFO 12019	+	+	—	—	6.5	6.3
34	<i>Klebsiella pneumoniae</i> IFO 12932	+	+	—	—	6.5	6.3
35	<i>Klebsiella pneumoniae</i> IFO 13541	+	+	—	—	6.6	6.4
36	<i>Klebsiella pneumoniae</i> IFO 13703	++	+	—	—	6.5	6.4
37	<i>Micrococcus luteus</i> IFO 3763	++	+	—	—	6.5	6.3
38	<i>Micrococcus lysodeikticus</i> Fleming IFO 3333	+	+	—	—	6.4	6.5
39	<i>Micrococcus roseus</i> IFO 3764	+	+	—	—	6.5	6.4
40	<i>Micrococcus rubens</i> IFO 3768	+	+	—	—	6.5	6.4
41	<i>Proteus mirabilis</i> IFO 3849	+	+	—	—	6.6	6.4
42	<i>Proteus morgani</i> IFO 3168	++	++	—	—	6.8	6.6
43	<i>Proteus vulgaris</i> IFO 3988	++	++	+	—	6.4	6.4
44	<i>Pseudomonas fluorescens</i> IFO 3081	++	++	+	—	6.3	6.2
45	<i>Pseudomonas fragi</i> IFO 3458	++	+	+	—	6.5	6.4
46	<i>Pseudomonas graveolens</i> IFO 3460	+	+	+	—	6.4	6.4
47	<i>Pseudomonas iodium</i> IFO 3558	+	+	+	—	6.4	6.5
48	<i>Pseudomonas solanacearum</i> IFO 3509	+	+	+	—	6.4	6.5
49	<i>Pseudomonas striafaciens</i> IFO 3309	+	+	—	—	6.4	6.6
50	<i>Sarcina aurantiaca</i> IFO 3064	+	+	—	—	6.5	6.4
51	<i>Sarcina lutea</i> IFO 1099	+	+	—	—	6.5	6.4
52	<i>Serratia liquefaciens</i> IFO 12979	+	+	+	—	6.1	6.1
53	<i>Serratia marcescens</i> IFO 3054	+	++	+	—	6.5	6.5
54	<i>Serratia plymuthica</i> IFO 3055	+	++	—	—	6.4	6.4
55	<i>Staphylococcus aureus</i> IFO 3060	+	++	—	—	6.5	6.5
56	<i>Staphylococcus epidermidis</i> IFO 3762	+	++	—	—	6.6	6.4
57	<i>Bifidobacterium adolescentis</i> M101-4	—	—	—	—	6.6	6.4
58	<i>Bifidobacterium bifidum</i> A234-4	—	—	—	—	6.8	6.5
59	<i>Bifidobacterium breve</i> I-53-8	+	—	—	+	6.4	6.0
60	<i>Bifidobacterium infantis</i> I-10-5	—	—	—	—	6.6	6.4
61	<i>Bifidobacterium longum</i> M101-2	—	+	—	—	6.7	6.6
62	<i>Lactobacillus bulgaricus</i> IFO 13953	—	+	+	—	6.4	6.5
63	<i>Lactobacillus clausenii</i> IFO 3202	+	+	—	—	6.5	6.6
64	<i>Lactobacillus paracasei</i> IFO 3953	—	—	—	—	6.4	6.5
65	<i>Lactococcus cremoris</i> IFO 3427	—	—	—	—	6.4	6.4
66	<i>Lactococcus lactis</i> IFO 12007	—	—	—	—	6.4	6.4
67	<i>Pediococcus acidilactici</i> IFO 3888	—	—	—	—	—	6.5
68	<i>Streptococcus thermophilus</i> IFO 13957	+	+	—	—	6.5	6.2

* Lactate dehydrogenase(LDH) ++, higher; +, normal; —, no activity.

**, Curd rennet activity +, settled; —, not settled.

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Table 2. Activities of lactate dehydrogenase and curd rennet of yeasts

Strain No.	Yeasts	LDH activity*		Curd rennet activity**			
		Aerobic	Anaerobic	Precipitation		pH	
				Aerobic	Anaerobic	Aerobic	Anaerobic
69	Awamori yeast (Sakamoto)	—	—	—	++	6.4	6.3
70	<i>Candida guilliermondii</i> IFO 0566	—	—	—	++	6.4	6.3
71	<i>Candida pelliculosa</i> IFO 0707	—	—	—	++	6.2	6.3
72	<i>Candida utilis</i> IFO 0619	—	—	—	++	6.3	6.3
73	<i>Cryptococcus albidus</i> IFO 0378	—	—	—	++	6.2	6.3
74	<i>Cryptococcus laurentii</i> IFO 0609	—	—	—	+	6.3	6.4
75	<i>Cryptococcus neoformans</i> IFO 0410	—	—	+	+	6.2	6.3
76	<i>Debaryomyces japonicus</i> IFO 0039	—	—	—	+	6.3	6.4
77	<i>Debaryomyces vini</i> Y.U.	+	—	—	+	6.3	6.4
78	<i>Endomycopsis capsularis</i> IFO 0672	—	—	—	+	6.3	6.4
79	<i>Endomyces decipiens</i> IFO 0102	—	—	—	+	6.3	6.4
80	<i>Hansenula miso</i> IFO 0146	—	—	—	+	6.3	6.4
81	<i>Hansenula suaveolens</i> IFO 0992	—	—	—	+	6.3	6.4
82	<i>Hansenula wingei</i> IFO 0976	—	—	—	+	6.3	6.4
83	<i>Kloeckera apiculata</i> IFO 0865	—	—	—	+	6.2	6.4
84	<i>Nadsonia fulvescens</i> IFO 0666	—	—	—	+	6.3	6.4
85	<i>Pichia anomala</i> IFO 0568	—	—	—	+	6.3	6.3
86	<i>Pichia orientalis</i> IFO 1279	—	—	—	+	6.2	6.4
87	<i>Pichia polymorpha</i> IFO 0195	—	—	—	+	6.3	6.4
88	<i>Pichia rhodanensis</i> IFO 1272	—	—	—	+	6.3	6.4
89	<i>Rhodotorula minuta</i> IFO 0387	—	—	—	—	6.3	6.6
90	<i>Rhodotorula rubra</i> IFO 0709	—	—	—	—	6.3	6.6
91	<i>Saccharomyces carlsbergensis</i> IFO 0641	—	+	—	+	6.3	6.5
92	<i>Saccharomyces rouxii</i> IFO 0487	—	—	—	—	6.3	6.5
93	<i>Saccharomyces sake</i> Kyoukai No.6	—	+	—	++	6.3	6.3
94	<i>Saccharomycodes ludwigii</i> IFO 1043	—	+	—	++	6.3	6.3
95	<i>Saccharomycopsis fibuligere</i> IFO 1744	—	+	—	+	6.3	6.4
96	<i>Torula rubra</i> var. <i>alpha</i>	—	—	—	+	6.3	6.4
97	<i>Torulasporea delbrueckii</i> IFO 0428	—	—	—	+	6.3	6.3
98	<i>Torulopsis aeria</i>	—	—	—	+	6.3	6.4
99	<i>Torulopsis candida</i> IFO 0768	—	—	—	—	6.2	6.6
100	<i>Trichosporon cutaneum</i> IFO 1198	—	—	—	—	6.3	6.6
101	Wine yeast	—	—	—	—	6.3	6.6
102	<i>Zygosaccharomyces rouxii</i> IFO 0505	—	—	—	—	6.3	6.6

*, Lactate dehydrogenase(LDH) ++, higher; +, normal; —, no activity.

**, Curd rennet activity +, settled; —, not settled.

Table 3. Activities of lactate dehydrogenase and curd rennet of molds

Strain No.	Molds	LDH activity*		Curd rennet activity**			
		Aerobic	Anaerobic	Precipitation		pH	
				Aerobic	Anaerobic	Aerobic	Anaerobic
103	<i>Aspergillus niger</i> IFO 4414	—	—	+	—	6.4	6.6
104	<i>Aspergillus oryzae</i> IFO 4176	—	—	+	—	6.2	6.7
105	<i>Aspergillus parasiticus</i> IFO 5241	—	—	+	—	6.3	6.7
106	<i>Monascus purpureus</i> IFO 4478	—	—	+	—	6.4	6.7
107	<i>Mucor circinelloides</i> f. <i>circinelloides</i> IFO 4554	—	—	+	—	6.5	6.7
108	<i>Neurospora sitophila</i> IFO 4596	—	—	+	—	6.4	6.7
109	<i>Penicillium camembertii</i> IFO 5855	—	+	—	—	—	6.6
110	<i>Penicillium crysogenum</i>	—	—	+	—	6.4	6.6
111	<i>Rhizomucor pusillus</i> IFO 4578	—	—	+	—	6.3	6.6
112	<i>Rhizopus javanicus</i> IFO 5441	—	+	+	—	6.2	6.7
113	<i>Rhizopus oryzae</i> IFO 4706	+	+	—	+	6.4	6.4

*, Lactate dehydrogenase(LDH) ++, higher; +, normal; —, no activity.

**, Curd rennet activity +, settled; —, not settled.

Table 4. Activities of lactate dehydrogenase and curd rennet of basidiomycetes

Strain No.	Basidiomycetes	LDH activity*	Curd rennet activity**	
			Precipitation	pH
C001	<i>Pleurotus ostreatus</i>	—	—	6.7
C003	<i>Flammulina velutipes</i>	++	—	6.8
C006	<i>Agaricus bisporus</i>	+	—	6.9
C007	<i>Agrocybe cylindracea</i>	—	—	6.8
C008-1	<i>Pleurotus cornucopiae</i>	++	—	6.9
C008-2	<i>Pleurotus cornucopiae</i>	—	—	7.0
C008-3	<i>Pleurotus cornucopiae</i>	—	—	7.1
C009	<i>Grifola frondosa</i>	++	—	6.8
C010	<i>Hypsizygus marmoreus</i>	—	—	6.9
C011	<i>Pleurotus sp.</i> (Awabitate)	++	—	6.7
	<i>Lentinus edodes</i>	+	—	6.9
	<i>Tricholoma matsutake</i>	—	—	6.9
W002	<i>Pleurotus ostreatus</i>	—	—	7.0
W003	<i>Collybia dryophila</i>	—	—	7.0
W008	<i>Laetitorus sulphureus</i>	++	—	7.0
W021	Mushroom Wild Type	—	—	7.0
W117	Mushroom Wild Type	+	+	6.8
W130	<i>Schizophyllum commune</i>	—	++	6.8
W351	<i>Pleurotus ostreatus</i>	+	—	6.9

*, Lactate dehydrogenase (LDH) ++, higher; +, normal; —, no activity.

***, Curd rennet activity +, settled; —, not settled.

Activity of lactate dehydrogenase and curd rennet of basidiomycetes were examined with cells obtained under aerobic conditions.

Twenty (*Bacillus cereus* IFO 3001, *Bacillus megaterrium* NI 8100 NTH B12, *Bacillus sphaericus* IFO 3525, *Bacillus brevis* IFO 3007, *Bacterium ketoglutamicus* SO(1) Shionogi Co., Ltd, *Bacterium orleanense* IFO 3259, *Brevibacterium sp.* P145 Phage host, *Corynebacterium glutamicum* No.534 ATCC 13032, *Enterobacter aerogenes* IFO 3320, *Enterobacter cloacae* IAM 1221, *Erwinia arvideae* IFO 3830, *Erwinia carotovora* IFO 3380, *Escherichia freundii* S-96, *Klebsiella pneumoniae* IFO 3317, *Klebsiella pneumoniae* IFO 13703, *Micrococcus luteus* IFO 3763, *Proteus morgani* IFO 3168, *Proteus vulgaris* IFO 3988, *Pseudomonas fluorescens* IFO 3081, *Pseudomonas fragi* IFO 3458) strains of bacteria and 5 (C003 *Flammulina velutipes*, C008-1 *Pleurotus cornucopiae*, C009 *Grifola frondosa*, C011 *Pleurotus sp.* (Awabitate), W008 *Laetitorus sulphureus*) strains of basidiomycetes cultured under aerobic conditions, and 9 (*Bacillus cereus* IFO 3001, *Corynebacterium glutamicum* No.534 ATCC 13032, *Proteus morgani* IFO 3168, *Proteus vulgaris* IFO 3988, *Pseudomonas fluorescens* IFO 3081, *Serratia marcescens* IFO 3054, *Serratia*

plymuthica IFO 3055, *Staphylococcus aureus* IFO 3060, *Staphylococcus epidermidis* IFO 3762) strains of bacteria cultured under anaerobic conditions demonstrated high lactate dehydrogenase activity.

One (W130 *Schizophyllum commune*) strain of basidiomycete cultured under aerobic conditions and 7 (Awamori yeast (Sakamoto), *Candida guilliermondii* IFO 0566, *Candida pelliculosa* IFO 0707, *Candida utilis* IFO 0619, *Cryptococcus albidus* IFO 0378, *Saccharomyces sake* Kyoukai No.6, *Saccharomyces ludwigii* IFO 1043) strains of yeasts cultured under anaerobic conditions demonstrated high curd rennet activity. The pHs under aerobic and anaerobic conditions were between 6.1 and 7.1.

References

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