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ภาคผนวก

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ภาคผนวกที่ 1

การเตรียมสารละลาย1. การเตรียมโพลีอะคริลาไมด์เจลชนิดแผ่น (Polyacrylamide slab gel)

(Laemmli, 1970)

(ขนาดประมาณ 14 x 16 ซม. ทน 1.5 มม.)

Resolving gel (7 % acrylamide) ประกอบด้วย

Tris-chloride buffer, pH 8.9	5	ml
Acrylamide sol.	10	ml
D.W.	25	ml
Ammonium persulfate sol.	175	ul

Stacking gel (3 % acrylamide) ประกอบด้วย

Tris-chloride buffer, pH 6.7	2.5	ml
Acrylamide sol.	2.0	ml
D.W.	15.2	ml
Ammonium persulfate sol.	150	ul

2. สารละลายสีซ้อมแอคติวิตีของเอนไซม์ esterase (Toyomasu and Zennyosi, 1981)

0.2 % Fast Blue RR Salt in 0.06 M Phosphate buffer, pH 5.4

0.5 % α -Naphthyl acetate in 50 % acetone3. สารละลายสีซ้อมแอคติวิตีของเอนไซม์ laccase (ดัดแปลงจากวิธีของ Leatham and Stahmann, 1981)

1.0 mM o-Tolidine in 0.1 M Sodium acetate buffer, pH 4.5

4. สารละลายสีย้อมแอสตีวิตีของเอนไซม์ glutamate dehydrogenase

(Darnall and Klotz, 1972)

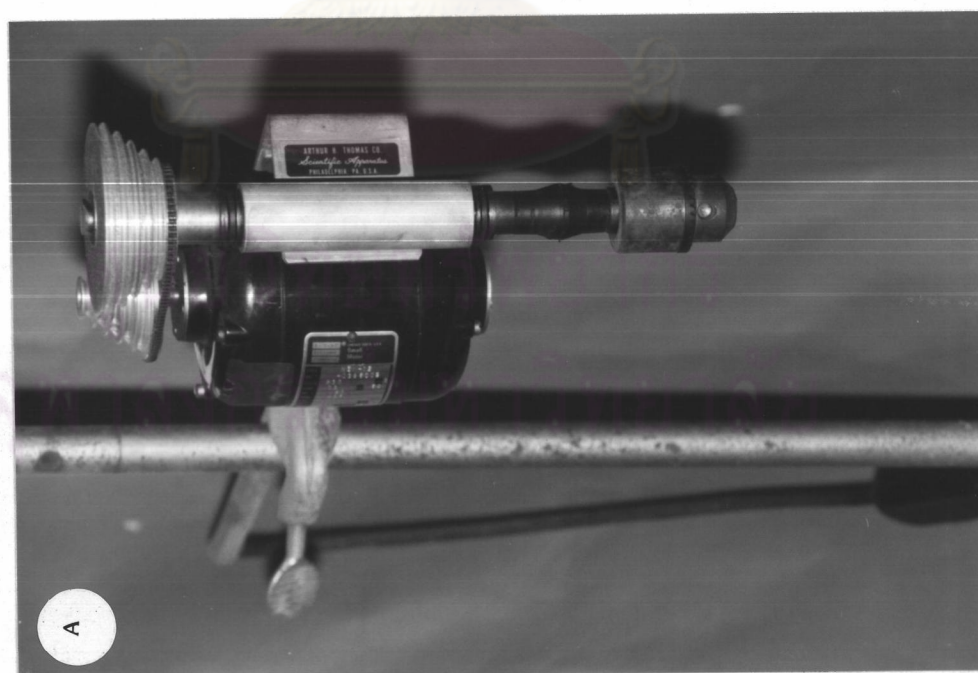
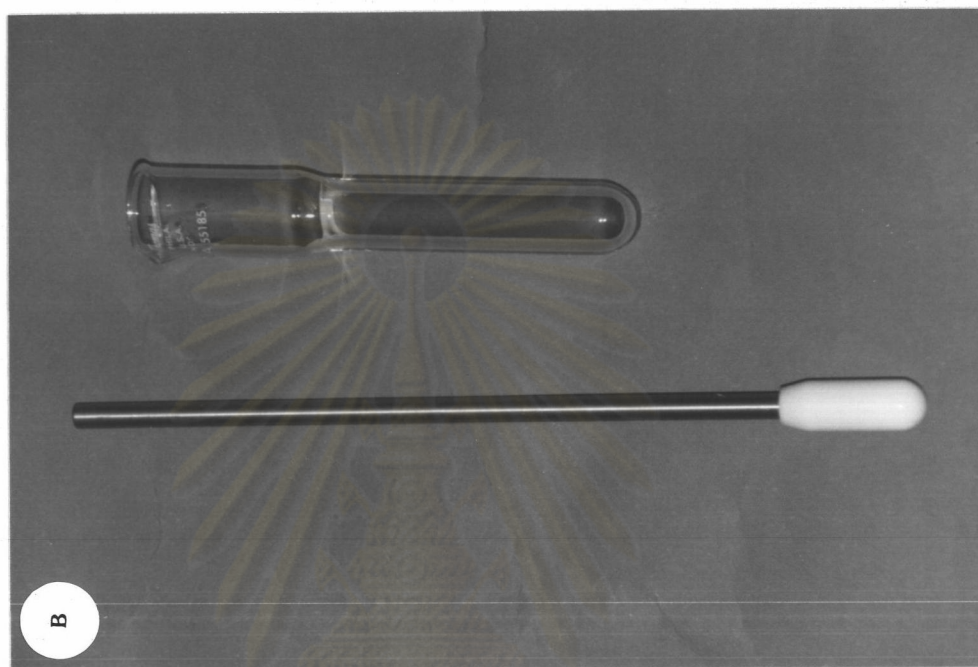
L-glutamic acid	3.68	g
NAD	99.51	mg
PMS	4.99	mg
NBT	35.16	mg
Sod. phosphate (dibasic)	1.95	g
D.W.	100	ml

5. สารละลายวัดโปรตีน (Bradford, 1976)

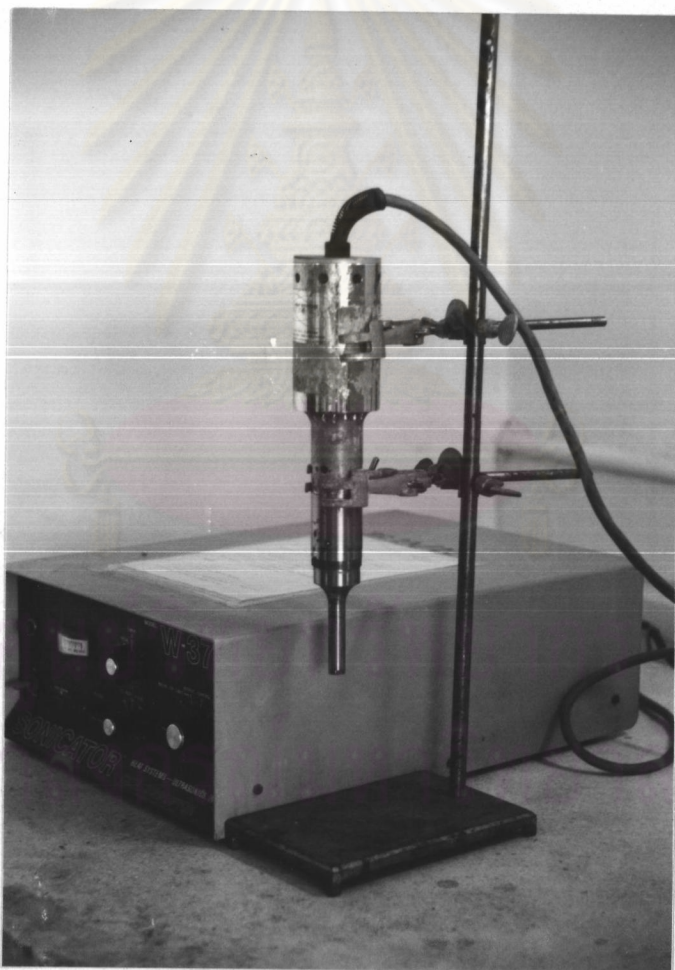
Coomassie Brilliant Blue G-250	0.1	g
95 % Ethanol	50	ml
85 % Phosphoric acid	100	ml
D.W. up to	1000	ml

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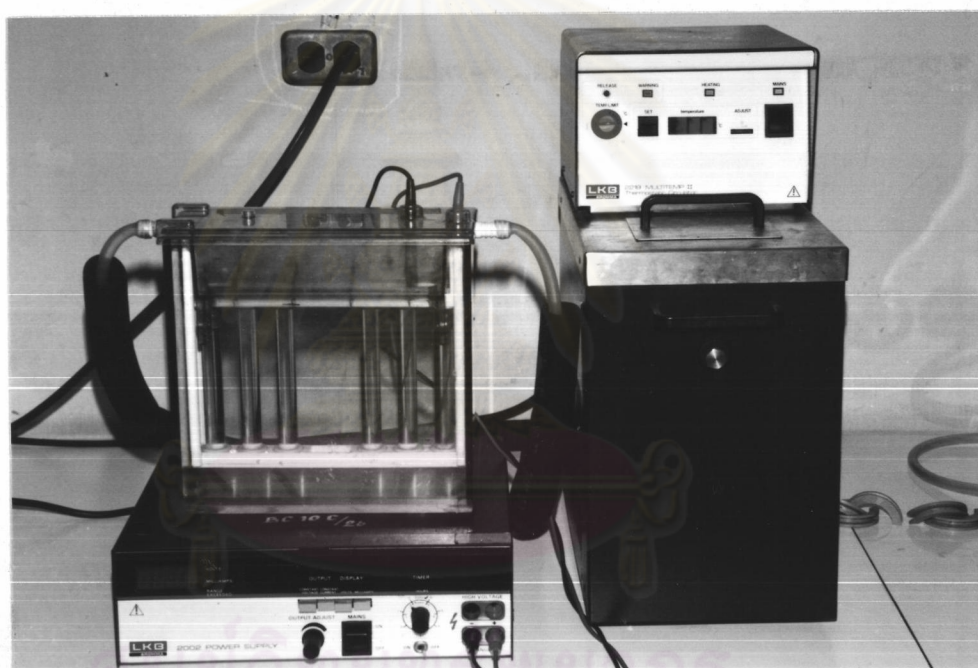
ภาพหน้าที่ 2 A. Thomas homogenizer apparatus
 B. Tissue grinding pestle and vessel



ภาพหมวดที่ 3 Sonicator (Heat system)



ภาพหน้าที่ 4 LKB Electrophoretic apparatus



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ภาคผนวกที่ 5 Protein content(mg/ml) in *L.edodes* mycelia after breaking with homogenizer and sonicator

A. Dikaryotic culture : parents (MU2,MU12) ; hybrids (C364,C366,C359,C373,C369,C377) and Monokaryotic culture (E6,E8,E1,E5,E9,N9,N10)

Age(days)	10			20			30			40			50		
Strains or isolates	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total
MU2	0.69	0.79	1.48	1.48	5.01	6.49	1.21	1.63	2.84	1.88	4.67	6.55	1.25	2.64	3.89
MU12	2.38	2.73	5.11	3.18	10.76	13.94	2.47	3.33	5.80	2.66	6.61	9.27	1.99	4.21	6.20
C364	5.93	6.80	12.73	8.60	29.09	37.69	3.93	5.31	9.24	2.26	5.61	7.87	1.77	3.74	5.51
C366	3.51	4.02	7.53	2.35	7.95	10.30	1.12	1.51	2.63	0.32	0.79	1.11	0.24	0.51	0.75
C359	2.14	2.45	4.59	3.51	11.87	15.38	2.69	3.63	6.32	2.51	6.24	8.75	0.42	0.89	1.31
C373	3.37	3.86	7.23	2.17	7.34	9.51	0.76	1.03	1.79	1.18	2.93	4.11	0.17	0.36	0.53
C369	0.40	0.46	0.86	3.02	10.21	13.23	0.60	0.81	1.41	1.55	3.85	5.40	0.62	1.31	1.93
C377	3.08	3.53	6.61	2.97	9.26	12.23	0.90	1.22	2.12	0.83	2.06	2.89	0.27	0.57	0.84
E6	0.39	0.07	0.46	0.36	0.15	0.51	0.23	0.11	0.34	0.17	0.19	0.36	0.13	0.21	0.34
E8	1.59	0.28	1.87	1.13	0.44	1.57	0.84	0.38	1.22	0.50	0.57	1.07	0.22	0.35	0.57
E1	4.39	0.77	5.16	4.75	1.86	6.61	4.15	1.88	6.03	2.95	3.35	6.30	2.14	3.44	5.58
E5	1.01	6.22	7.23	0.60	8.91	9.51	0.25	1.54	1.79	0.22	3.89	4.11	0.14	0.39	0.53
E9	4.83	0.85	5.68	5.50	2.16	7.66	3.95	1.79	5.74	3.12	3.54	6.66	2.35	3.78	6.13
N9	0.93	0.17	1.10	3.27	1.70	4.97	2.65	1.77	4.42	1.26	1.00	2.26	0.43	0.56	0.99
N10	2.84	0.53	3.37	3.80	1.97	5.77	3.43	2.29	5.72	2.35	1.87	4.22	0.30	0.39	0.69

ภาคผนวกที่ 5 (ต่อ)

B. Dikaryotic culture : parents (MU4,MU12) ; hybrids (C508,C507,C513,C502,C516,C504) and Monokaryotic culture (E6,E8,E1,E5,E9,N9,

Age(days)	10			20			30			40			50		
Strains or isolates	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total
MU4	2.38	2.73	5.11	0.47	1.44	1.91	0.71	0.96	1.67	0.95	2.36	3.31	0.20	0.42	0.62
MU12	2.02	2.31	4.33	2.54	8.59	11.13	2.39	3.23	5.62	2.28	5.66	7.94	0.96	2.03	2.99
C508	0.76	0.87	1.63	3.02	10.21	13.23	1.48	2.00	3.48	0.75	1.86	2.61	0.31	0.66	0.97
C507	3.71	4.25	7.96	4.29	14.51	18.80	3.23	4.57	7.80	3.25	8.07	11.32	1.41	2.14	3.55
C513	4.80	5.50	10.30	4.92	16.64	21.56	7.84	10.59	18.43	4.95	12.30	17.25	4.61	9.75	14.36
C502	3.38	3.87	7.25	3.22	10.89	14.11	4.01	5.41	9.42	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
C516	4.25	4.87	9.12	4.19	14.17	18.36	4.09	5.52	9.61	2.73	6.78	9.51	0.71	1.50	2.21
C504	2.73	3.26	5.99	6.83	23.10	29.93	1.30	1.76	3.06	2.07	5.14	7.21	0.38	0.80	1.18
FF10	2.39	1.00	3.39	2.12	1.88	4.00	0.88	1.20	2.08	1.08	1.21	2.29	1.21	0.95	2.16
FF9	0.62	0.26	0.88	2.52	2.24	4.76	2.03	1.50	3.53	0.79	0.89	1.68	1.44	1.13	2.57
FF7	1.13	0.48	1.61	0.96	0.85	1.81	0.36	0.27	0.63	0.47	0.53	1.00	0.65	0.51	1.16
FF2	0.29	0.12	0.41	0.29	0.26	0.55	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
FF5	1.11	0.47	1.58	0.84	0.75	1.59	1.02	0.76	1.78	0.21	0.24	0.45	1.76	1.38	3.14
N9	1.78	0.33	2.11	3.02	1.57	4.59	1.13	0.76	1.89	1.08	0.86	1.94	1.12	1.45	2.57
N10	1.67	0.31	1.98	2.14	1.11	3.25	3.25	2.17	5.42	1.05	0.84	1.89	2.10	2.71	4.81

ภาคผนวกที่ 6 Intracellular laccase activity (units/mg prot.) in *L.edodes* mycelia after breaking with homogenizer and sonicator

A. Dikaryotic culture : parents (MU2,MU12) ; hybrids (C364,C366,C359,C373,C369,C377) and Monokaryotic culture (E6,E8,E1,E5,E9,N9,N10)

Age(days)	10			20			30			40			50		
Strains or isolates	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total
MU2	118	24	142	1572	63	1635	1327	35	1362	6235	272	6507	13445	1305	14750
MU12	422	87	509	2907	117	3024	9938	261	10199	14073	614	14687	11585	1125	12710
C364	107	21	128	481	19	500	1158	30	1188	2125	93	2218	1170	113	1283
C366	1222	251	1473	20360	12965	33325	132118	3471	135589	281860	12296	294156	237400	23049	260449
C359	6749	1386	8135	1003	40	1043	12004	315	12319	11396	497	11893	35036	3402	38438
C373	1664	342	2006	7169	288	7457	47215	1241	48456	13512	590	14102	416891	40477	457368
C369	904	185	1089	1992	80	2072	6010	158	6168	5796	253	6049	30052	2918	32970
C377	12856	2639	15495	23797	958	24755	205035	5387	210422	235860	10289	246149	449999	43692	493691
E6	12911	11696	24607	25089	45299	70388	50479	94162	144641	88306	46942	135248	115003	34831	149834
E8	1160	1050	2210	19487	35190	54677	43379	80916	124295	129934	69077	199011	325050	98468	423518
E1	75	68	143	123	222	345	281	525	806	229	122	351	138	42	180
E5	114	103	217	911	1645	2556	728	1359	2087	603	321	924	1454	441	1895
E9	304	275	579	76	138	214	173	322	495	211	112	323	441	133	574
N9	249	0	249	287	71	358	445	356	801	421	438	859	1466	1220	2686
N10	128	0	128	180	45	225	1126	900	2026	851	877	1728	1539	1281	2820

ภาคผนวกที่ 6 (ต่อ)

B. Dikaryotic culture : parents (MU4, MU12) ; hybrids (C508, C507, C513, C502, C516, C504) and Monokaryotic culture (E6, E8, E1, E5, E9, N9,

Age(days)	10			20			30			40			50		
	total	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated
MU4	512	105	617	20690	832	21522	67989	1786	69775	36526	1594	38120	17822	1730	19552
MU12	356	73	429	1169	47	1216	4282	113	4395	9739	425	10164	18545	1801	20346
C508	861	177	1038	1349	55	1404	83057	2182	85239	90186	3934	94120	257235	24975	282210
C507	330	67	397	653	27	680	11803	311	12114	2748	120	2868	25708	2496	28204
C513	105	21	126	480	20	500	301	8	309	308	13	321	597	58	655
C502	1022	209	1231	2062	83	2145	3164	83	3247	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
C516	308	64	372	35	1	36	260	6	266	195	8	203	119	12	131
C504	1003	206	1209	352	14	366	1538	41	1579	5435	237	5672	5841	567	6408
FF10	585	192	777	234	188	422	563	299	862	411	380	791	586	600	1186
FF9	1060	350	1410	960	775	1735	9935	5266	15201	5349	4949	10298	3570	3656	7226
FF7	1877	618	2495	125428	101302	226730	142845	75706	218551	93328	86322	179650	20730	21234	41964
FF2	228	35	263	2138	1728	3866	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
FF5	107	36	143	275	222	497	113	59	172	632	584	1216	15	15	30
N9	228	0	228	267	68	335	902	721	1623	421	439	860	472	392	864
N10	83	0	83	84	20	104	92	74	166	62	65	127	95	79	174

ภาคผนวกที่ 7 Acid phosphatase activity (units/mg prot. x 0.01) in *L.edodes* mycelia after breaking with homogenizer and sonicator

A. Dikaryotic culture : parents (MU2, MU12) ; hybrids (C364, C366, C359, C373, C369, C377) and Monokaryotic culture (E6, E8, E1, E5, E9, N9, N10)

Age(days)	10			20			30			40			50		
Strains or isolates	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total
MU2	5.09	3.68	8.77	7.31	0.39	7.70	7.17	2.66	9.83	5.13	1.87	7.00	4.54	1.17	5.71
MU12	4.12	2.98	7.10	3.88	1.36	5.24	3.74	1.38	5.12	4.47	1.90	6.37	3.61	0.93	4.54
C364	2.58	1.86	4.44	2.65	0.92	3.57	2.19	0.81	3.00	1.55	0.56	2.11	2.67	0.69	3.36
C366	1.81	1.30	3.11	3.63	1.28	4.91	11.53	4.27	15.80	11.63	4.24	15.87	12.36	3.17	15.53
C359	3.10	2.24	5.34	3.87	1.36	5.23	3.76	1.40	5.16	3.04	1.11	4.15	9.64	2.47	12.11
C373	3.23	2.33	5.56	5.98	2.10	8.08	18.53	6.86	25.39	3.75	1.37	5.12	69.79	17.93	87.72
C369	31.84	23.00	54.84	3.94	1.39	5.33	22.89	8.49	31.38	17.34	6.33	23.67	25.75	6.62	32.37
C377	3.64	2.62	6.26	4.14	1.46	5.60	6.24	2.31	8.55	7.77	2.84	10.61	22.08	5.67	27.75
E6	1.88	3.39	5.27	2.72	10.49	13.21	3.51	12.51	16.02	13.18	16.54	29.72	5.63	7.32	12.95
E8	2.95	5.35	8.30	3.39	13.10	16.49	6.24	22.27	28.51	8.24	10.32	18.56	18.87	24.53	43.40
E1	6.06	10.97	17.03	6.14	23.69	29.83	9.33	33.32	42.65	11.10	13.91	25.01	7.67	9.97	17.64
E5	4.03	7.30	11.33	5.94	22.92	28.86	17.62	62.93	80.55	19.27	24.15	43.42	28.80	37.45	66.25
E9	2.48	4.48	6.96	0.98	1.34	2.32	0.99	3.51	4.50	1.34	1.68	3.02	1.32	1.72	3.04
N9	5.12	1.84	6.96	5.76	4.13	9.89	4.56	3.86	8.42	6.67	7.81	14.48	12.57	9.54	22.11
N10	15.88	5.73	21.61	4.99	3.57	8.56	2.88	2.43	5.31	6.50	7.60	14.10	3.60	2.73	6.33

ภาคผนวกที่ 7 (ต่อ)

B. Dikaryotic culture : parents (MU4,MU12) ; hybrids (C508,C507,C513,C502,C516,C504) and Monokaryotic culture (E6,E8,E1,E5,E9,N9,

Age(days)	10			20			30			40			50		
Strains or isolates	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total	homogenized	sonicated	total
MU4	7.87	5.68	13.55	13.58	4.77	18.35	30.68	11.37	42.05	40.99	14.95	55.94	26.99	6.94	33.93
MU12	2.72	1.97	4.69	3.41	1.20	4.61	2.62	0.96	3.58	4.14	1.51	5.65	7.29	1.87	9.16
C508	7.94	5.73	13.67	4.22	1.48	5.70	6.31	2.34	8.65	7.34	10.88	18.22	17.90	16.83	34.73
C507	1.87	1.36	3.23	1.89	1.87	3.76	7.25	2.68	9.93	3.65	1.33	4.98	10.00	2.57	12.57
C513	2.72	1.96	4.68	0.82	0.16	0.98	0.91	0.33	1.24	1.11	0.40	1.51	3.14	0.77	3.91
C502	1.89	1.37	3.26	1.34	0.47	1.81	1.58	0.58	2.16	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
C516	3.24	2.35	5.59	2.91	1.02	3.93	5.01	1.86	6.87	6.27	2.29	8.56	14.73	3.79	18.52
C504	5.16	3.72	8.88	1.41	0.50	1.91	22.93	8.49	31.42	4.73	1.73	6.46	48.70	12.51	61.21
FF10	2.30	0.51	2.81	3.62	2.19	5.81	8.03	2.72	10.75	6.64	3.01	9.65	10.27	8.13	18.40
FF9	9.65	0.85	10.50	1.52	0.93	2.45	3.73	1.14	4.87	2.65	1.20	3.85	1.86	1.48	3.34
FF7	4.50	0.40	4.90	4.03	2.44	6.47	9.27	3.15	12.42	12.64	5.73	18.37	17.48	13.86	31.34
FF2	24.57	2.16	26.73	33.65	20.41	54.06	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
FF5	3.30	0.29	3.59	2.67	1.62	4.29	3.34	1.13	4.47	2.13	29.36	31.49	3.40	2.70	6.10
N9	4.93	1.78	6.71	5.77	4.12	9.89	10.40	8.81	19.21	7.42	10.84	18.26	4.71	3.57	8.28
N10	4.72	1.70	6.42	5.15	3.69	8.84	2.51	2.13	4.64	7.15	8.36	15.51	6.32	4.79	11.11

ภาคผนวกที่ 8

Comparison of extracellular laccase activities
(units/100 ml media x 1000)

A. Dikaryotic culture : parents (MU2, MU12) ;
hybrids (C364, C366, C359, C373, C369, C377)
and Monokaryotic culture (E6, E8, E1, E5, E9, N9, N10)

Strains or isolates	Age (days)	10	20	30	40	50
MU2		0.6	52.0	150.0	440.0	480.0
MU12		0.2	40.0	0.4	0.9	0.5
C364		0.0	7.2	21.4	24.0	133.5
C366		7.6	166.5	1216.5	1066.5	1333.0
C359		2.6	9.0	133.5	188.5	232.0
C373		3.7	71.0	320.0	176.0	760.0
C369		0.8	15.2	143.0	400.0	540.0
C377		17.0	180.0	1494.0	1500.0	813.5
E6		2.4	140.0	200.0	680.0	1120.0
E8		1.3	83.0	760.0	1750.0	3200.0
E1		0.0	0.0	0.0	0.0	0.0
E5		0.0	0.0	0.0	0.0	0.3
E9		0.0	0.0	0.0	0.4	0.5
N9		0.0	2.8	9.4	24.0	50.0
N10		0.0	2.1	20.0	37.8	56.0

B. Dikaryotic culture : parents (MU12, MU4) ;
hybrids (C508, C507, C513, C502, C516, C504)
and Monokaryotic culture (FF10, FF9, FF7, FF2, FF5,
N9, N10)

N10)

Strains or isolates	Age (days)	10	20	30	40	50
MU4		1.6	53.5	260.0	347.0	400.0
MU12		1.4	32.0	116.0	2200.0	880.0
C508		0.0	24.0	1440.0	853.0	640.0
C507		0.0	16.0	193.5	144.0	840.0
C513		0.0	2.6	10.0	22.0	10.1
C502		4.5	46.8	66.0	N.D.	N.D.
C516		0.0	0.0	0.6	1.0	9.6
C504		28.0	34.5	10.0	63.5	21.5
FF10		2.0	2.5	12.7	20.0	16.5
FF9		0.0	23.0	240.0	416.0	226.5
FF7		0.0	34.5	260.0	880.0	880.0
FF2		0.0	7300.0	N.D.	N.D.	N.D.
FF5		0.0	0.0	0.0	0.0	0.0
N9		0.0	1.4	4.5	11.0	20.0
N10		0.0	1.3	14.0	97.8	36.0

ภาคผนวกที่ 9

Relative mobility (Rf) of laccase isozymes from *L.edodes mycelia*
 Dikaryotic culture : parents (MU2,MU12) ; hybrids (C364,C366,C359,C373,C369,C377)
 and Monokaryotic culture (E6,E8,E1,E5,E9,N9,N10)

At 30 days of growth period

band no.	intracellular laccase											extracellular laccase											
	MU2	MU12	C364	C366	C359	C373	C369	C377	E6	E8	E1	E9	N9	N10	MU2	MU12	C366	C359	C373	C369	C377	E6	E8
1																							
2										0.77													
3																							
4																							
5		0.73																					
6		0.72																					
7	0.71		0.71	0.71	0.71	0.71		0.71		0.71		0.71											
8																					0.70		
9																							
10	0.68	0.68	0.68	0.68	0.68	0.68		0.68	0.68						0.68								
11	0.66																						
12	0.63		0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63		0.63	0.63	0.63		0.63	0.63	0.63
13																							
14		0.61																					
15			0.55	0.55		0.55	0.55	0.55	0.55	0.55		0.55	0.55	0.55			0.55	0.55		0.55		0.55	
16												0.54	0.54										
17																							
18	0.45								0.45	0.45		0.45											
19	0.42		0.42			0.42			0.42	0.42		0.42											
20																							
21	0.38		0.38			0.38			0.38	0.38													
22																							
23																							
24																							

ศูนย์วิทยทรัพยากร
 จุฬาลงกรณ์มหาวิทยาลัย

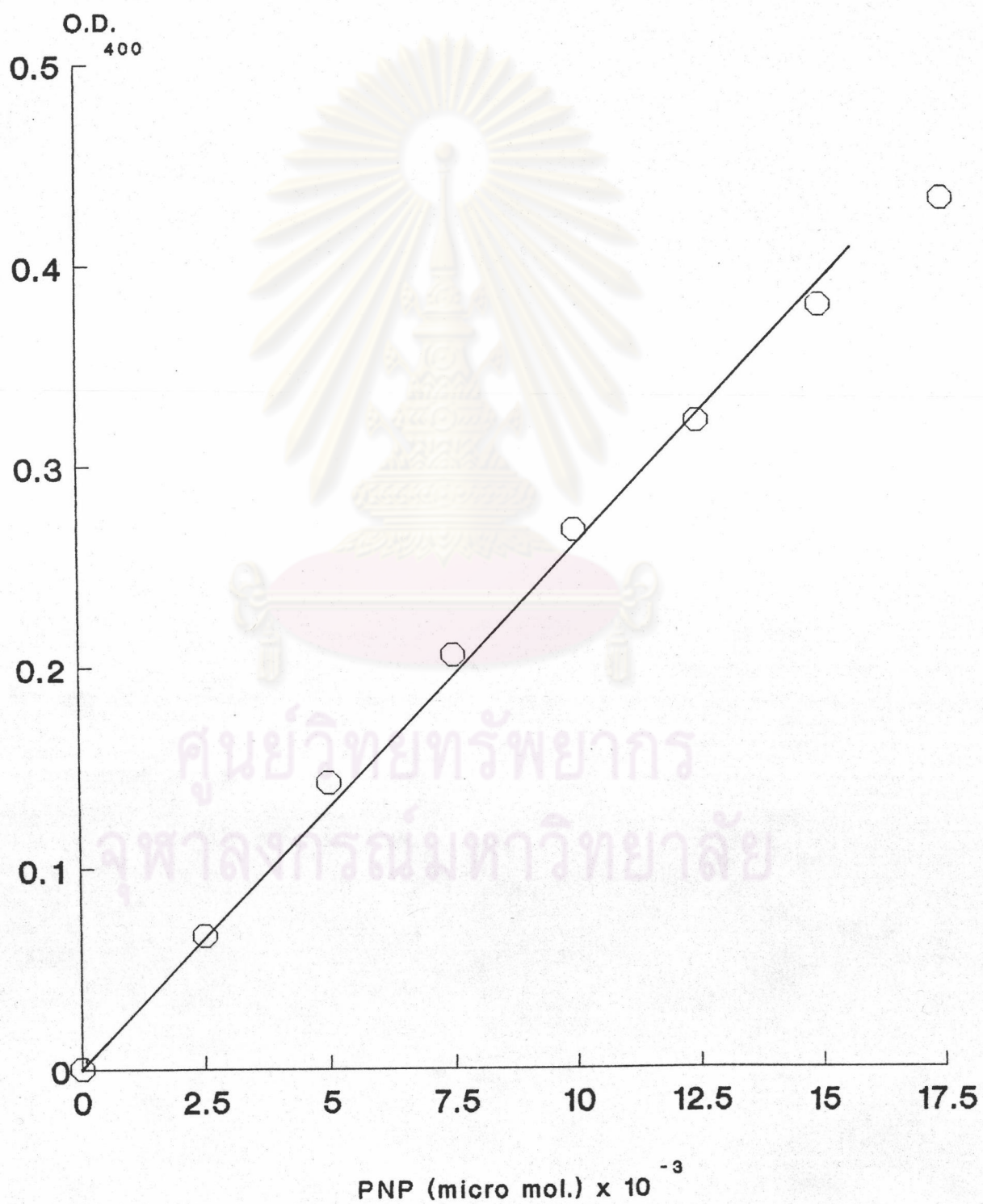
ภาคผนวกที่ 17

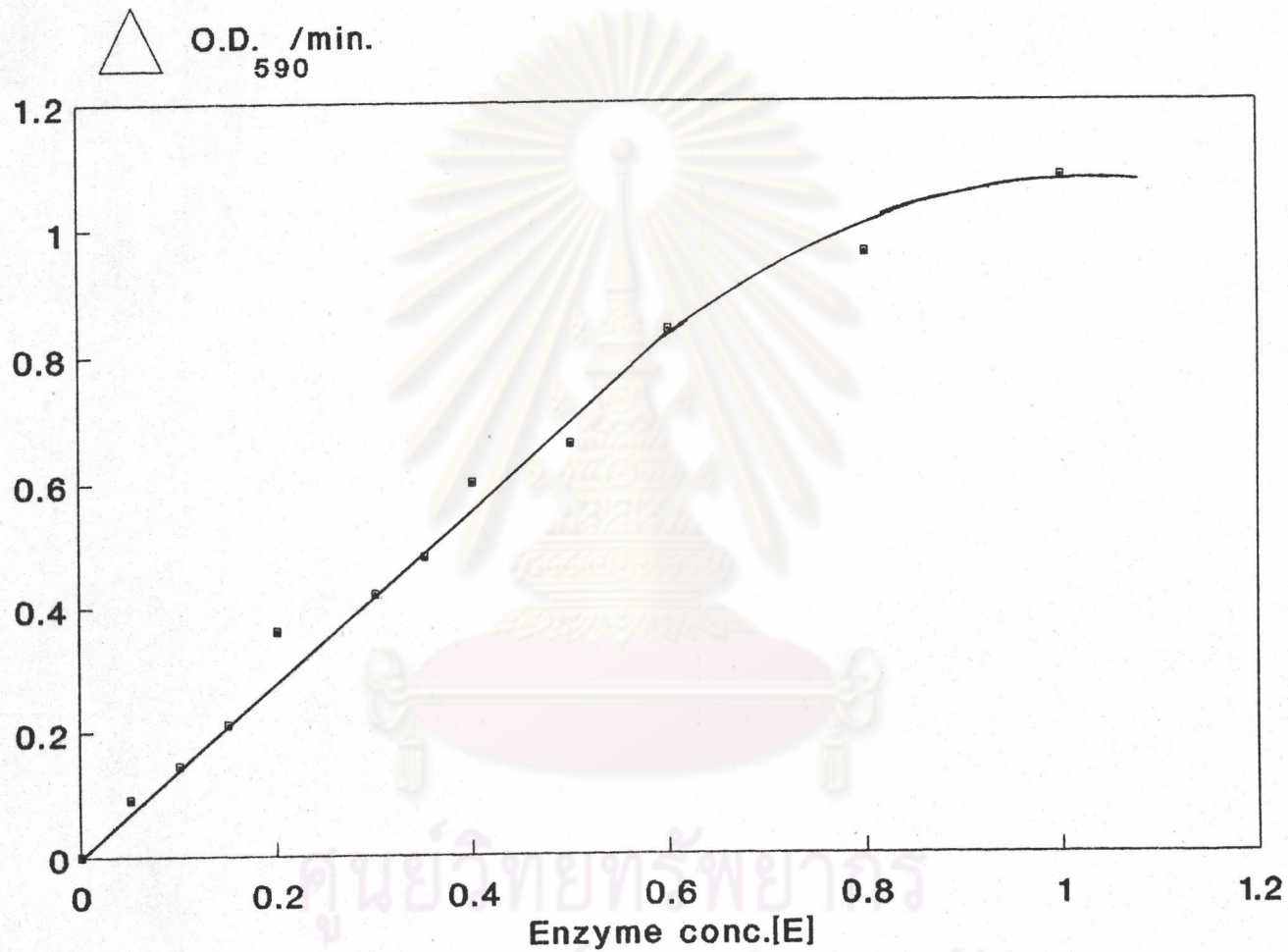
Protein standard curve using protein-dye binding method (Bradford,1976), bovine serum albumin was used as standard protein



ภาคผนวกที่ 18

PNP standard curve using p-Nitrophenol as standard





Changes of O.D. /min. In laccase enzyme assay according to various enzyme concentration

Rate of oxidation of substrates by a whole fresh mushroom homogenate
(Leatham and Stahmann, 1981)

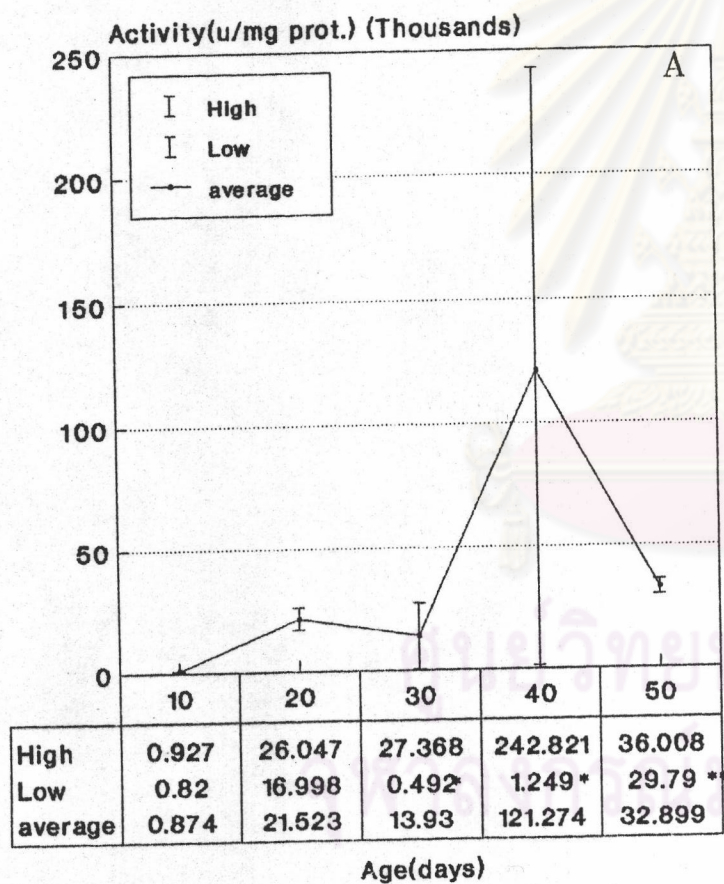
The oxidation rate are expressed as the mean steady-state rate of substrates (0.42 mM) for duplicate assays. Buffer were 21 mM-sodium acetate, pH 4.5, and 21 mM-sodium phosphate, pH 6.5. The assay wavelength was that of the maximal absorbance for the oxidized substrate. The molar absorption coefficients (ϵ) for the oxidized substrates were examined by sodium periodate oxidation.

Substrates	Oxidation rate ($\Delta A \text{ min}^{-1}$)		Assay wavelength (nm)	$10^{-3} \times \epsilon^{\circ}$ ($1 \text{ mol}^{-1} \text{ cm}^{-1}$)	
	pH 4.5	pH 6.5		pH 4.5	pH 6.5
<i>p</i> -Anisidine	0.026	0.000	460	-	-
<i>p</i> -Anisidine + 1 mM H_2O_2	0.026	0.000	460	-	-
Tyrosine	0.027	0.020	460	-	-
L-DOPA	0.075	0.070	460	3.85	3.96
Caffeic acid	0.064	0.024	390	-	-
Chlorogenic acid	0.054	0.000	390	-	-
<i>o</i> -Phenylenediamine	0.500	0.000	440	7.60	-
<i>p</i> -Phenylenediamine	0.706	0.000	460	-	-
Dimethylenediamine	0.640	0.090	510	-	-
Benzidine	0.943	0.000	600	8.87	-
Benzidine + 1 mM H_2O_2	0.950	0.000	600	8.87	-
3,3'-Dimethylbenzidine	1.640*	0.000	600	6.34	-
3,3'-Dimethoxybenzidine	2.836	2.040	375	not stable	
1,3-Dihydroxynaphthalene	0.036	0.000	440	-	-

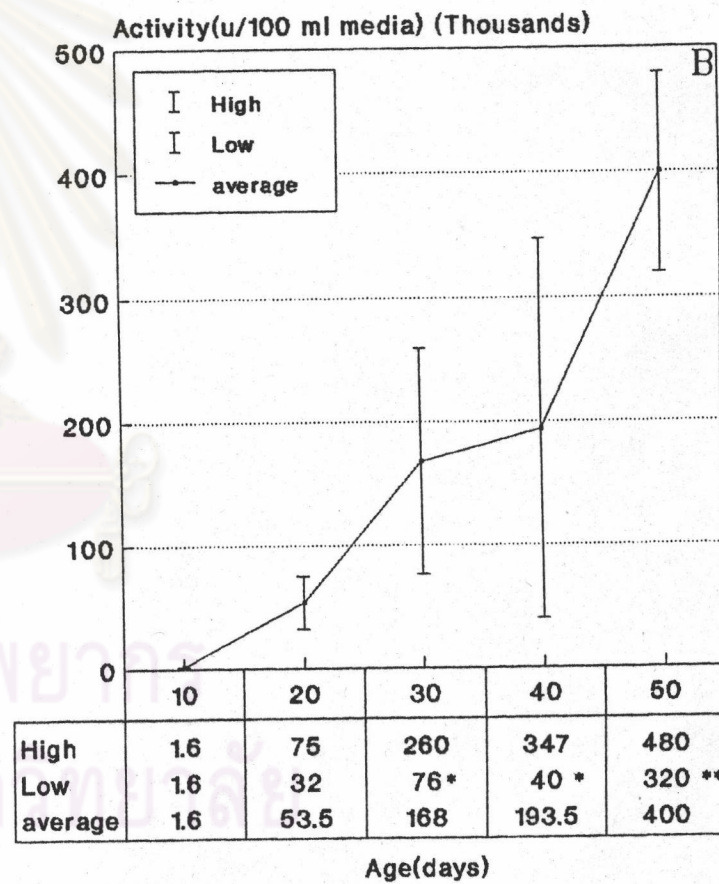
-. Not determined.

* Base on the oxidation of 3,3'-dimethylbenzidine at pH 4.5. the activity of laccase was 34.2 μmol substrate oxidized min (\times fresh weight tissue).

Fluctuation of laccase activity in some replication of *L. edodes* strain MU4, cultivated in PDYB medium (static culture), at 25 C. A. Intracellular B. Extracellular



* primodia formation



** mycelial maturation

ประวัติผู้เขียน

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