KOR. JOUR. MICROBIOL. Vol. 11, 89~100 (1973)

*Rhizopus*의 아밀라아제에 관한연구*(第二報)
——한국 野生菌株의 덱스트리노제닉 및 삭카로제닉 아밀라아제 活性——

李永祿 · 尹京河
(高麗大學校 理工大 生物學科)

Studies on the Amylase of *Rhizopus** (II)
——Dextrinogenic and Saccharogenic Amylase Activities of Korean Wild Strains——

LEE, Yung Nok, and Kyung Ha YOON
(Department of Biology, Korea University)

ABSTRACT

Dextrinogenic and saccharogenic amylase activities of *Rhizopus* isolated from various substrates collected throughout South Korea are measured, and their amylase activities are surveyed from taxonomical, ecological, and physiological viewpoints.

Among the 151 strains of *Rhizopus*, strain number 49 exhibiting most prominent saccharogenic amylase activity is selected as a best strain, and strain number 17 is selected as a best strain for dextrinogenic amylase activity. Amylase activities of *Rhizopus* are so variable in different strains even in the same species. In general, however, *R. japonicus*, *R. liquefaciens*, and *R. shanghaiensis* show high saccharogenic amylase activities , and *R. chiuniang*, *R. achlamydosporus*, and *R. bahrnensis* show high dextrinogenic amylase activities, while *R. nigricans* exhibits lower activities for both amylase.

The strains isolated from kneaded cereals, such as "meju" and "kockja", exhibited very strong dextrinogenic and saccharogenic amylase activities as compared with the strains isolated from other substrates. The mean value of dextrinogenic amylase activities of the strains from southern inland region and saccharogenic amylase activities of the strains from middle coast region are, more or less, higher than those of the strains from the other regions.

In the culture at 30°C, those strains which exhibit better mycelial and air mycelial growth than sporangial formation show very high dextrinogenic and saccharogenic amylase activities.

INTRODUCTION

The series of studies on the bacterial amylase initiated by Fukumoto (1943, 1944) have been actively pursued and extended by many other Japanese workers (Fukueka, 1950; Yoshida, 1958; Oishi *et al.*, 1962). In the succeeding studies on the fungal amylase, Phillips and Caldwell(1951) and many other workers

*This work was supported by a grant from The Yonam Foundation

clarified that *Rhizopus* strains produce glucogenic amylase in addition to dextrinogenic amylase. Now, it is well known that the fungal strains are used in developing better fermentation process (Windish and Mhatre, 1965).

In our previous paper (Lee and Yoon, 1973), 151 strains of *Rhizopus* are isolated from the various samples which are collected throughout South Korea, identified, and maintained. In order to select the best strains having predominant amylase activity, dextrinogenic and saccharogenic amylase activities of these strains are measured, and analyzed based on the taxonomical, ecological and physiological characteristics in this study.

MATERIALS AND METHODS

1. Organisms

The 151 strains of *Rhizopus* isolated from various samples, which are collected throughout South Korea are used in this study. Collection areas of the samples are shown in Fig. 1. Original substrates from which *Rhizopus* strains are isolated and the regional distribution of the species are shown in table 1 and table 2, respectively.

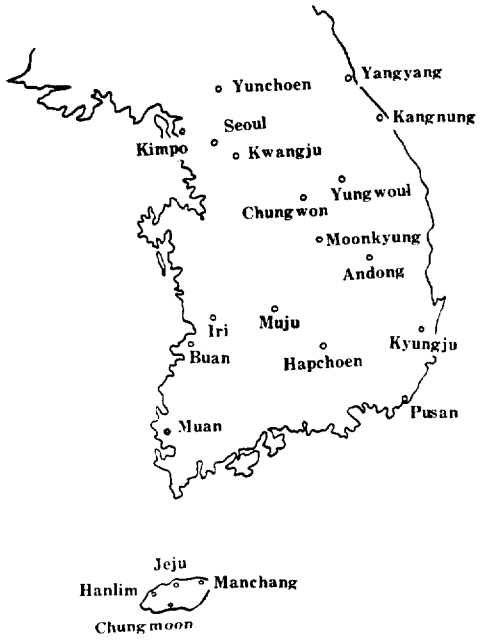

Fig. 1. Collection areas of the samples

2. Preparation of enzyme solution

In order to prepare enzyme solution, *Rhizopus* strains are inoculated in twenty milliliters of culture media added with 0.3gr sterilized calcium carbonate and incubated for 3 days at 30°C with shaking in 100m*l* flasks. Culture medium used in these experiments consisted of 50gr soluble starch, 5gr glucose, 5gr peptone, 1gr KH_2PO_4, 1gr K_2HPO_4, 1gr $MgSO_4 \cdot 7H_2O$, and 0.01gr $FeSO_4 \cdot 7H_2O$ per liter. After incubation for 3 days, culture medium was centrifuged at 6000 r.p.m. for 20 minutes and the supernatant was used as crude enzyme solution.

3. Determination of dextrinogenic amylase activity.

1M*l* enzyme solution was added to an equal volume of acetate buffer (0.5M, *p*H5.0) and incubated for 10 minutes at 37°C. After incubation, 2m*l* of 1% soluble starch solution was added to it, and then the mixture was further incubated for 30 minutes at 37°C. 5M*l* of 1N acetic acid was added to the reaction mixture in order to stop enzyme action. It was diluted with 50m*l* distilled water, and then 5m*l* of 0.1N I_2-KI solution was added. For the determination of dextrinogenic amylase activity, the optical density was measured at 660mμ and dextrinogenic amylase activity was expressed in RDP (relative dextrinizing power).

4. Determination of saccharogenic amylase activity.

The saccharogenic amylase activity was determined by the measurement of reducing sugar as glucose.

For determination of reducing sugar, Nelson's method(1956) was used. 1M*l* enzyme solution diluted one hundredfold was added to an equal volume of 1% soluble starch solution buffered by acetate (*p*H 4.8). After incubation for 5 minutes, at 40°C, 2m*l* of the low-alkalinity copper reagent was added to the reaction mixture, and heated for 10 minutes in boiling water bath. After cooling, 2m*l* of arsenomolybdate reagent was added. When all the cuprous oxide was dissolved after mixing, the solution was diluted to the 25m*l* mark on the test tube and then allowed to stand at least 15 minutes. Optical density was measured at 500mμ.

Table 1. Distribution of *Rhizopus* species in various substrates from which *Rhizopus* strains were isolated.

Species \ Substrates	Korean cakes & bread	Fruits & vegetable	Dried fishes	Cereals	Potatoes	Kneaded cereals	Wheat bran	Others	Total
R. nigricans	8	3	1	6	6	2	1	5	32
R. formosaensis				1	1		1		3
R. achlamydosporus	1				1	1			3
R. tritici	5	1		5	3	3			17
R. javanicus				2			1		3
R. chiuniang		1							1
R. acidus	1			2		4	2		9
R. chinensis	1								1
R. oryzae	5	1		3	2	1	2	1	15
R. delemar	1								1
R. chungkuoensis	4	1		3		2	3		13
R. hangchow						1			1
R. japonicus	1			2					3
R. bahrnensis	1		1	2		1		1	6
R. tonkinensis				2	1				3
R. arrhizus								1	1
R. niveus								2	2
R. shanghaiensis		1							1
R. liquefaciens	1			1		1			3
Unidentified strains	12	1		7	3	6	1	3	33
Total	41	9	2	36	17	22	11	13	151

RESULTS AND DISCUSSION

1. Selection of superior strains for amylase activity.

Dextrinogenic and saccharogenic amylase activities of *Rhizopus* strains isolated from various substrates in South Korea are shown in table 3, and the selected strains having predominant dextrinogenic and saccharogenic amylase activities are listed in table 4. Among the 151 strains of *Rhizopus*, strain number 49, 24, 79, 54, and 102 are selected for their prominent saccharogenic amylase activities, and strain number 17, 102, 130, 24, and 86 are selected for their dextrinogenic amylase activities. Strain number 49 exhibiting most prominent

Table 2. Regional distribution of Korean *Rhizopus* species.

Species \ Region	Seoul	Kyungki	Kangwon	Chungbuk	Kyungbuk	Kyungnam	Pusan	Jeonbuk	Jeonnam	Jeju	Total
R. nigricans	19	5	2		1		1	1		3	32
R. formosaensis	3										3
R. achlamydosporus	3										3
R. tritici	8			1	2	2	2		1	1	17
R. javanicus	2	1									3
R. chiuniang	1										1
R. acidus	6	1								2	9
R. chinensis	1										1
R. oryzae	12	1			1		1				15
R. delemar								1			1
R. chungkuoensis	11							1		1	13
R. hangchow	1										1
R. japonicus	1		1							1	3
R. bahrnensis	4				1					1	6
R. tonkinensis	2	1									3
R. arrhizus	1										1
R. niveus	2										2
R. shanghaiensis									1		1
R. liquefaciens	2				1						3
Unidentified strains	20	1	2		7			1		2	33
Total	99	10	5	1	13	2	4	4	2	11	151

saccharogenic amylase activity, 24.75 mg/m*l* is selected as the best strain for saccharogenic amylase activity. On the other hand, strain number 17 which exhibits most prominent dextrinogenic amylase activity, 4.53 relative dextrinizing power(RDP) is selected as the best strain for dextrinogenic amylase activity. The saccharogenic and dextrinogenic amylase activities of these strains are far exceeded that of *R. niveus* IAM 6035, which exhibits 17.08mg/m*l* and 1.36 RDP respectively, one of the most active amylase producing Japanese strains.

2. Amylase activity of *Rhizopus* among different species.

Dextrinogenic and saccharogenic activities of amylase from different species of the genus *Rhizopus* are shown in Fig. 2 and Fig. 3, respectively. The mean value of dextrinogenic amylase activity is higher, more or less, in *R. japonicus*, *R. liquefaciens*, *R. shanghaiensis*, *R. acidus*, and *R. bahrnensis* in decreasing order, and saccharogenic activity of amylase is higher in *R. chiuniang*, *R. achlamydosporus*, and *R. bahrnensis*, while both of amylase are lower in *R. nigricans*.

Inui and Takeda(1964) studied the amylase activities of *Rhizopus* from the taxonomical viewpoint and concluded that there was no relation between amylase activities and other morphological characteristics. On the other hand, Kim (1971) and Kitahara(1950) studied the amylase of *Aspergillus* and divided them into four subgroups based on dex-

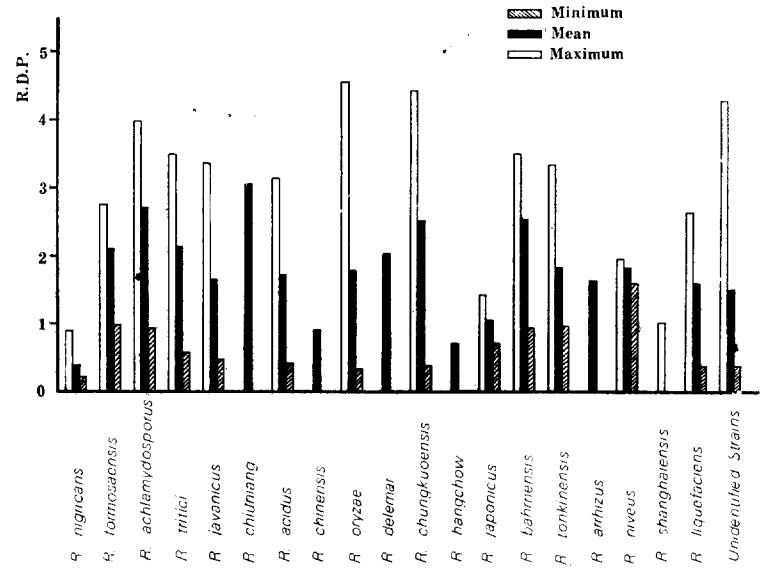

Fig. 2. Dextrinogenic amylase activity from different species of *Rhizopus*.

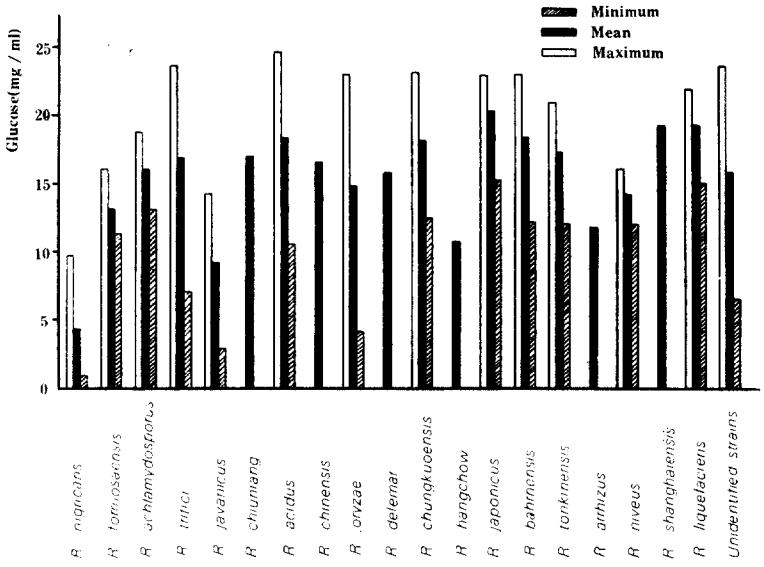

Fig. 3. Saccharogenic amylase activity from different species of *Rhizopus*.

trinogenic and saccharogenic activities of amylase. However, amylase activities of *Rhizopus* are so variable in different strains even in the same species, as shown in the Fig. 2 and Fig. 3, that it is not considered reasonable to use amylase activity as a criterion of classification key, at least in *Rhizopus*, although there are some differences in mean value among different species.

3. Amylase activity of wild strains in ecological viewpoint.

Dextrinogenic and saccharogenic amylase activities of Korean wild strains

from different regions are shown in table 5, and those of the strains based on the original substrates, from which *Rhizopus* strains are isolated, are shown in table 6. The results in table 5 indicate that the mean value of dextrinogenic amylase activities of the strains from southern inland region and saccharogenic amylase activities of the strains from middle coast region are, more or less, higher than those of the strains from the other regions. As shown in table 6, *Rhizopus* strains isolated from Korean kneaded cereals such as "meju" and "kockja" exhibited relatively high dextrinogenic and saccharogenic amylase activities as compared with those of the strains isolated from other substrates.

Table 2. Dextrinogenic and saccharogenic amylase activities of *Rhizopus* strains isolated from various substrates in South Korea.

Species	Strain no.	Amylase activity		Original substrates	Collection area
		dextrinogenic (RDP)	saccharogenic (mg/ml)		
R. nigricans	1	0.45	6.30	fruit	Seoul
	2	0.39	1.94	〃	〃
	4	0.23	5.50	meju	〃
	6	0.22	5.00	dried fish	Pusan
	13	0.42	1.44	meju	Seoul
	19	0.36	9.28	rice	〃
	31	0.35	4.85	waste	〃
	37	0.29	1.39	potato	Kyungju
	40	0.52	0.98	flower	Manchang
	41	0.41	4.54	straw	〃
	44	0.49	4.68	mushroom	Jeju
	45	0.42	3.68	korean cake	Seoul
	47	0.39	2.93	sweet potato	〃
	55	0.90	4.32	potato	Moonkyung
	56	0.36	6.24	〃	Mooju
	87	0.22	6.72	korean cake	Seoul
	90	0.37	5.23	bread	〃
	92	0.38	1.20	〃	〃
	98	0.53	5.80	wheat grain	Kwangju
	99	0.37	6.21	wheat bran	〃
	100	0.48	7.50	sweet potato	Seoul
	105	0.44	6.19	wheat grain	〃
	110	0.41	8.93	korean cake	〃
	115	0.88	6.00	fruit	〃
	126	0.30	6.75	bread	〃
	127	0.33	1.90	korean cake	〃
	134	0.22	2.52	bread	Yungwoul
	140	0.33	1.04	wheat grain	Kwangju
	142	0.21	1.76	cereal	〃
	143	0.43	1.20	〃	〃
	146	0.45	1.90	potato	Seoul
	151	0.35	2.00	useless medium	〃
	Mean	0.40	4.24		

Species	Strain no.	Amylase activity		Original substrates	Collection area
		dextrinogenic (RDP)	saccharogenic (mg/ml)		
R. formosaensis	16	2.78	11.44	rice	Seoul
	88	1.00	11.73	wheat bran	〃
	93	2.51	16.06	potato	〃
	Mean	2.10	13.08		
R. achlamydosporus	80	3.25	18.92	potato	Seul
	86	3.99	13.08	korean cake	〃
	131	0.91	16.10	kockja	Seoul
	Mean	2.72	16.02		
R. tritici	7	2.70	18.86	sweet potato	Hapchoen
	8	0.77	7.16	wheat grain	〃
	18	3.50	14.15	rice	Seoul
	29	1.34	11.02	〃	〃
	34	3.15	19.17	fruit	Buan
	46	3.42	11.46	cereal	Jeju
	59	3.04	15.75	sweet potato	Seoul
	67	0.56	20.95	potato	〃
	76	1.37	11.61	korean cake	Andong
	77	1.82	14.70	〃	〃
	79	0.80	23.73	bread	Kimpo
	81	1.97	17.54	korean cake	Seoul
	91	1.17	23.01	kockja	Chungwon
	133	2.09	14.85	〃	Seoul
	135	2.26	21.40	bread	〃
	144	3.40	21.38	meju	Pusan
	148	3.17	21.53	cereal	Seoul
	Mean	2.15	16.96		
R. javanicus	32	0.48	2.93	rice	Seoul
	97	3.38	14.49	bran	Kwangju
	128	1.16	10.45	wheat grain	Seoul
	Mean	1.67	9.29		
R. chiuniang	139	3.05	17.02	fruit	Seoul
R. acidus	49	1.83	24.75	cereal	Chungmoon
	50	2.61	15.25	bran	〃
	65	1.44	18.75	wheat bran	Seoul
	89	0.77	16.24	korean cake	〃
	106	0.82	20.10	wheat grain	〃
	111	0.41	17.31	meju	〃
	113	3.17	21.21	〃	〃
	120	2.53	10.66	〃	〃
	141	2.01	21.46	kockja	Kwangju
	Mean	1.73	18.44		
R. chinensis	96	0.93	16.63	korean cake	Seoul
R. oryzae	5	0.35	6.08	korean cake	Pusan
	9	2.21	13.05	bran	Seoul
	14	1.31	14.70	flower	〃

Species	Strain no.	Amylase activity: dextrinogenic (RDP)	Amylase activity: saccharogenic (mg/ml)	Original substrtes	Collection area
R. oryzae	17	4.53	17.58	rice	
	63	2.72	19.26	potato	〃
	69	2.79	18.96	sweet potato	〃
	84	1.95	13.04	Korean cake	〃
	94	1.26	11.27	〃	〃
	101	0.98	19.32	cereal	〃
	116	1.00	20.35	fruit	〃
	121	0.31	10.20	wheat bran	Kwangju
	123	1.20	15.58	korean cake	Seoul
	130	4.37	23.10	bread	〃
	137	1.01	15.45	kockja	〃
	149	1.01	4.20	cereal	Kyungbuk
	Mean	1.80	14.81		
R. delemar	73	2.04	15.85	korean cake	Iri
R. chungkuoensis	20	3.02	18.50	rice	Seoul
	42	0.92	12.60	korean cake	Jeju
	57	2.37	22.47	〃	Seoul
	60	1.95	21.40	〃	〃
	74	2.13	15.26	wheat bran	Iri
	78	1.65	22.22	cereal	Seoul
	85	2.43	15.58	kockja	〃
	102	4.41	23.31	bread	〃
	103	3.10	20.40	kockja	〃
	107	0.86	14.73	wheat bran	〃
	108	0.39	14.66	〃	〃
	119	0.96	17.94	fruit	〃
	125	1.89	17.10	wheat grain	〃
	Mean	2.01	18.17		
R. hangchow	104	0.72	10.76	kockja	Seoul
R. japomicus	27	0.72	22.24	rice	〃
	28	1.01	23.03	cereal	Kangnung
	43	1.41	15.40	korean cake	Jeju
	Mean	1.05	20.22		
R. bahrnensis	12	2.51	19.95	dried fish	Seoul
	15	3.15	20.28	flower	〃
	48	2.78	16.19	cereal	Jeju
	64	2.29	23.20	kockja	Seoul
	70	3.50	12.40	korean cake	〃
	150	0.94	17.48	cereal	〃
	Mean	2.53	18.25		
R. tonkinensis	62	0.97	12.30	potato	Seoul
	72	3.33	21.10	cereal	Yunchoen
	118	1.13	18.91	〃	Seoul
	Mean	1.81	17.44		
R. arrhizus	109	1.65	11.93	mushroom	Seoul

Species	Strain no.	Amylase activity		Orginal substrates	Collection area
		dextrinogenic (RDP)	saccharogenic (mg/ml)		
R. niveus	10	1.69	16.20	waste	Seoul
	35	1.97	12.21	〃	〃
	Mean	1.83	14.21		
R. shanghaiensis	23	1.11	19.40	fruit	Muan
R.liquefaciens	21	1.79	21.19	rice	Seoul
	25	2.65	15.11	kockja	Kyungju
	83	0.37	22.04	korean cake	Seoul
	Mean	1.60	19.44		
Unidentified strain	3	0.76	19.60	bread	Seoul
	11	1.04	16.59	useless medium	〃
	22	1.74	18.23	cereal	Kyungju
	24	4.29	23.80	kockja	〃
	26	2.70	9.68	bran	〃
	30	2.63	11.93	bread	Yangyang
	33	0.50	12.12	miyuck(alge)	Seoul
	36	0.86	15.01	bread	Kangnung
	38	2.12	16.17	mushroom	Chungmoon
	39	1.12	19.17	cereal	Hanlim
	51	2.02	17.08	bread	Moonkyung
	52	1.22	17.91	〃	〃
	53	1.01	21.56	〃	〃
	54	0.75	23.54	〃	〃
	58	0.77	9.41	korean cake	Seoul
	61	0.75	12.67	potato	〃
	66	1.82	16.98	〃	〃
	68	2.52	18.70	〃	〃
	71	1.52	17.81	cereal	Yunchoen
	75	1.50	16.58	kockja	Iri
	82	1.02	12.61	〃	Seoul
	95	1.72	6.75	korean cake	〃
	112	1.58	16.28	cereal	〃
	114	0.40	15.65	wheat grain	〃
	117	1.74	16.10	korean cake	〃
	122	0.38	20.20	cereal	〃
	124	0.67	10.64	meju	〃
	129	2.94	17.30	〃	〃
	132	0.47	10.80	vegetable	〃
	136	0.91	12.62	bread	〃
	138	0.43	9.79	cereal	〃
	145	2.76	17.48	meju	〃
	147	3.17	21.53	korean cake	〃
	Mean	1.51	15.91		

Table 4. Selected strains having predominant amylase activities.

(1) Selected strains for dextrinogenic amylase.

Strain no.	Species	Amylase activity	
		dextrinogenic (RDP)	saccharogenic (mg/m*l*)
17	*R. oryzae*	4.53	17.58
102	*R. chungkuoensis*	4.41	23.31
130	*R. oryzae*	4.37	23.10
24	Unidentified strain	4.29	23.80
86	*R. achlamydosporus*	3.99	13.08

(2) Selected strains for saccharogenic amylase.

Strain no.	Species	Amylase activity	
		dextrinogenic (RDP)	saccharogenic (mg/m*l*)
49	*R. acidus*	1.83	24.75
24	Unidentified strain	4.29	23.80
79	*R. tritici*	0.80	23.73
54	Unidentified strain	0.75	23.54
102	*R. chungkuoensis*	4.41	23.31
IAM 6035	*R. niveus*	1.36	17.08

Table 5. Amylase activities of Korean wild strains from different region.

Region	Number of strains	Amylase activity					
		dextrinogenic (RDP)			saccharogenic (mg/m*l*)		
		mean	max.	min.	mean	max.	min.
Middle Inland	118	1.48	4.53	0.22	13.35	23.20	1.04
Middle Coast	4	1.32	2.63	0.80	18.42	23.73	11.93
Southern Inland	13	1.77	4.29	0.36	13.05	23.80	1.30
Soutern Coast	5	1.64	3.15	0.22	14.20	21.38	6.08
Jeju	11	1.60	3.42	0.41	12.38	24.75	0.98

Table 6. Amylase activities of Korean wild strains based on the original substrates from which strains *Rhizopus* were isolated.

Substrates	Number of strains	Amylase activity					
		dextrinogenic (RDP)			saccharogenic (mg/m*l*)		
		mean	max.	min.	mean	max.	min.
Korean cake	41	1.47	4.41	0.22	14.13	23.73	1.20
Fruit	9	1.28	3.15	0.39	13.21	20.35	1.94
Dried fish	2	1.36	2.51	0.22	12.48	19.95	5.00
Cereal	36	1.44	4.53	0.21	14.82	24.75	1.04
Potato	17	1.56	3.25	0.29	12.56	20.95	1.30
Kneaded cereal	22	1.90	4.29	0.23	15.91	23.80	1.44
Bran	11	1.58	3.38	0.31	13.09	15.26	6.21
Others	13	1.19	3.15	0.35	10.56	20.28	0.98

Table 7. Amylase activities of *Rhizopus* strains based on the cultural characteristics.

Item	Temp.	Characteristics	Number of strains	Amylase activity					
				dextrinogenic (RDP)			saccharogenic (mg/ml)		
				mean	max.	min.	mean	max.	min.
Mycelial growth	30°C	+	83	1.95	4.53	0.31	16.51	24.75	2.93
		−	35	0.54	2.65	0.21	5.55	22.04	0.98
	37°C	+	81	1.96	4.53	0.31	16.53	24.75	2.93
		−	37	0.55	2.65	0.21	6.09	22.04	0.98
	41°C	+	10	1.86	3.17	0.41	18.30	24.75	10.66
		−	108	1.83	4.53	0.21	12.79	23.73	0.98
Fruiting formation	30°C	+	111	1.51	4.53	0.21	13.03	24.75	0.98
		−	7	1.60	2.65	0.37	16.87	22.04	11.93
	37°C	+	62	2.02	4.41	0.39	17.12	24.75	2.93
		−	56	0.96	4.53	0.21	8.98	23.10	0.98
Air mycelium	30°C	+	76	1.92	4.53	0.31	16.65	24.75	2.93
		−	42	0.79	3.99	0.21	7.10	22.04	0.98
	37°C	+	74	1.94	4.53	0.31	16.69	24.75	2.93
		−	44	0.81	3.99	0.21	7.49	22.04	0.98
	41°C	+	10	1.86	3.17	0.41	14.89	24.75	10.66
		−	108	1.49	4.53	0.21	13.10	23.73	0.98

Symbols: +, good; −, poor or none

4. Relations between amylase activities and cultural characteristics.

Amylase activities of *Rhizopus* strains based on the cultural characteristics are shown in table 7. Among the 151 strains of Korean *Rhizopus*, the 118 strains which were identified were used in this survey. Dextrinogenic and saccharogenic amylase acitivities of the strains exhibiting good mycelial growth and air mycelium at 30—37°C were higher than two- or threefolds as compared with those of the strains exhibiting poor or none mycelial growth and air mycelium formation at the same culturing temperature. On the contrary, amylase activities of the strains exhibiting good fruiting formation at 30°C were more or less lower than those of the strains which exhibit poor or none fruiting formation at 30°C. Therefore, it is concluded that those strains which exhibit better mycelial growth and air mycelium formation than sporangial formation at 30°C show very strong dextrinogenic and saccharogenic amylase activities. However, there are no relations between the amylase activities of *Rhizopus* strains and fermentation ability of carbohydrates such as inulin and galactose, although this is not represented in those data.

摘 要

韓國産 *Rhizopus* 菌株의 덱스트리노제닉 및 삭카로제닉 아밀라아제 활성을 측정하여 이를 분류학적 생태학적 및 생리학적 측면에서 검토하고 우량주를 선정하였다.

균주번호 R—49는 가장 우수한 삭카로제닉 아밀라아제 생성균주로서 선정 되었고 균주번호 R—17은

가장 우수한 벡스트리노제닉 아밀라아제 생성균주로 선정되었다.

*Rhizopus*의 아밀라아제 활성은 같은 종에 있어서도 균주에 따라 차이가 심하였다. 그러나 *R. japonicus*, *R. liquefaciens*, 및 *R. shanghaiensis*는 비교적 높은 삭카로제닉 아밀라아제 활성을 나타내었고, *R. chiuniang*, *R. achlamydosporus* 및 *R. bahrnensis*는 비교적 높은 벡스트리노제닉 아밀라아제 활성을 나타내었다. 한편 *R. nigricans*의 아밀라아제 활성은 양쪽 모두 현저하게 낮았다.

곡자나 배주에서 분리된 균주는 다른 기질에서 분리된 균주에 비해 강한 아밀라아제 활성을 나타내었고 南部內陸地方에서 분리된 균주와 中部海岸地方에서 분리된 菌株는 다른 地方에서 분리된 菌株에 비해 약간 높은 액화력과 당화력을 각각 나타내었다.

30°C의 배양에서 菌糸 또는 氣菌糸의 생장이 우수하고 포자낭의 형성능이 약한 균주의 아밀라아제 활성은 일반적으로 높았으나 탄수화물의 발효능과 아밀라아제 활성간에는 어떠한 相關關係도 찾아 볼 수 없었다.

REFERENCES

1. Fukueka, K., 1950. Bacterial amylase. 1. Isolation of powerful strain and culture conditions for amylase production. *J. Ferment. Technol.*, **28**, 241—248.
2. Fukumoto, J., 1943(a). Studies on bacterial amylase. Ⅰ. Isolation of bacteria excreting potent amylase and their distribution. *J. Agr. Chem. Soc. Japan*, **19**, 487—503.
3. Fukumoto, J., 1943(b). Studies on bacterial amylase. Ⅱ. Bacterial and physiological nature. *J. Agr. Chem. Soc. Japan*, **19**, 634—640.
4. Fukumoto, J., 1943(c). Studies on bacterial amylase. Ⅲ. Conditions of amylase production. (1). *J. Agr. Chem. Soc. Japan*, **19**, 689—692.
5. Fukumoto, J., 1943(d). Studies on bacterial amylase. Ⅳ. Conditions of amylase production. (2). *J. Agr. Chem. Soc. Japan*, **19**, 789—799.
6. Fukumoto, J., 1943(e). Studies on bacterial amylase. Ⅴ. Optimum *p*H and temperature and heat resistance. *J. Agr. Chem. Soc. Japan*, **19**, 833—861.
7. Fukumoto, J., 1944(a). Studies on bacterial amylase. Ⅵ. Liquefaction and saccharification. *J. Agr. Chem. Soc. Japan*, **20**, 23—26.
8. Fukumoto, J., 1944(b). Studies on bacterial amylase. Ⅶ. Discussion on the mechanism of starch decomposition. (1). *J. Agr. Chem. Soc. Japan*, **20**, 121—126.
9. Fukumoto, J., 1944(c). Studies on bacterial amylase. Ⅷ. Discussion on the mechanism of starch decomposition. (2). *J. Agr. Chem. Soc. Japan*, **20**, 309—314.
10. Inui, T., Y. Takeda, and Iizuke, H., 1964. Taxonomical studies on genus *Rhizopus*. *J. Gen. Appl. Microbiol.*, Ⅱ, Suppl. 1—121.
11. Kim, *et al.*, 1971. A study on dextrinogenic amylase in the *Aspergillus niger* group, *Kor. J. Microbiol.*, **9**, 155—162.
12. Kitahara, K., *et al.*, 1950. Studies on the composition of fungal diastase. *J. Ferment. Technol.*, **28**, 101.
13. Lee and Yoon., 1973. Studies on the amylase of *Rhizopus*(1). *Kor. J. Microbiol.* **11**, 31—50.
14. Nelson, N., 1944. *J. Biol. Chem.*, **153**, 375. in "Methods in carbohydrate chemistry 1" (Whistler, R.L., *et al.*, 1962).
15. Oishi, M., *et al.*, 1962. The synthesis of x-amylase by a cell-free system from *B. subtilis*. *Biochem. Biophys. Res. Commun*, **8**, 342—347.
16. Windish, W.W., and Mhatre, N.S., 1965. Microbial amylase. *Adv. in Appl. Microbiol.*, **7**, 273—304.
17. Yoshida, A., 1958. Mechanism of protein synthesis: bacterial α-amylase containing ethionine. *Biochem. Biophys. Acta*, **29**, 213—214.