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Cellulase Activities of Aspergilli distributed in South Korea* Avicelase, CMCase and Salicinase Activities of the Strains Surveyed in Taxonomical Viewpoint.

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한국산 Aspergillus의 셀룰라아제 활성에 관한 硏究(第二報)
——菌株의 系統과 酵素活性——

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ABSTRACT

The 635 strains of Korean Aspergilli are isolated, cultured purely, and their Avicelase, CMCase and Salicinase activities are measured, in order to select the best strains exhibiting predominant cellulase activities, and to survey their cellulase activities in taxonomical and ecological viewpoints.

Strains No. 175, 255 and 254 are selected as the best Avicelase producing strains, strains No. 131, 151 and 116 are selected as the best CMCase producing strains, and strains No. 456, 457 and 253 are selected as the best Salicinase producing strains.

A. niger group and *A. clavatus* group exhibited the highest activities of Avcelase and Salicinase, respectively. *A. iniger* group and *A. clavatus* groups are showed the highest activites of CMCase. Among the different species tested, the activities of Avicelase, CMCase and Salicinase are highest in *A. phoenicis*, *A. clavatus*, and *A. japonicus* and *A. giganteus*, respectively.

Cellulase activities of Aspergilli from the inland regions of Korea are higher, more or less, than those of the strains from the other regions.

Avicelase and CMCase activities of Aspergilli isolated from bread and Korean cake are relatively higher, and Salicinase activities of the strains isolated from the cereals are higher than those of the strains from the other habitat substrate.

INTRODUCTION

The studies on cellulase had been initiated by Seielier's observation in 1907 which the juice of snail pancrease decomposed cotton fibre. Since then, the works were concentrated on cellulase study and it has been shown that cellulase, which decomposed natural cellulose, was produced in microorganisms.

Ikeda *et al.*(1973) investigated the physico-chemial properties on acid cellulase

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of *A. niger*. King and Simbert (1963) studied eight highly purified β-glucosidases from *A. niger* enzymatically, chemically and immunologically. Matsumura and Maejima(1963) reported the action of partially purified cellulase toward some substrate *i.e.*, swollen cellulose, sodium carboxymethyl cellulose and salicin. Imada *et al.*(1962) purified fungal cellulase from *Rhizopus* by fractionation with ammonium sulfate. Oote *et al.* (1964) investigated the distribution of cellulase activity and selected some high power strains in the *Rhizopus*. Cellulase activities of *Trichoderma* (Toyama, 1953), *Myrothecium*(Whitaker, 1953) and *Neurospora*(Kuroda and Teruga, 1967) have been also reported.

Reese *et al.* (1950) suggested that not less than two kinds of enzymes participated in decomposing of natural cellulose. However, Whitaker (1956) insisted on single hypothesis that a kind of enzyme participated. Recently, through the improvement of enzyme purifying technique, the varieties of cellulase in decomposing natural cellulose had been recognized. Cellulase has been utilizing in many fields such as fodder, medicine, food industry and pulp industry. Furthermore, the scope of its application is increased sharply day by day.

In this study, the 635 strains of Aspergilli isolated from South Korea are cultured purely and measured their Avicelase, CMCase and Salicinase activities in order to select the predominent strains and to survey their cellulase activities in taxonomical and ecological viewpoints.

MATERALIS AND METHODS

1. Experimental organisms

Total 635 strains of Korean Aspergilli including 410 strains, which were isolated and identified in our previous paper(Lee *et al.*, 1976) and newly isolated 225 strains (Lee *et. al.*, 1977) are used in this study.

2. Maintenance of the strains

Isolation and identification methods are described in details in our previous paper (Lee *et al.*, 1976). Stock cultures are maintained on the potato-glucose agar medium and subcultured every two months.

3. Preparation of enzyme solution

In order to prepare enzyme solution, a small portion of Aspergilli which had maintained at 5°C, are inoculated on Czapeck's agar medium and precultured at 30°C for 7 days. One loop of precultured Aspergilli are inoculated on 20ml of Czapeck's solution added 10g Wheat bran and 3g Chaffs in 250ml flask, and are cultured at 30°C for 5 days. After cultivation, 80 ml distilled water are added in these culture media, and then maintained at 5°C for 12 hours. Followed by these process, centrifugation are performed at 12,000g for 20 minutes. The filltrate from the supernatant are used as a crude enzyme solution.

4. Determination of enzyme activity

Avicelase: Avicelase activities are determined by Somogi-Nelson's Method. For determination of reducing sugar, Nelson's method (1944) is used. 0.5ml enzyme solution diluted ten folds is added to the mixture of 1 ml of 2% Avicel solution and 0.5 ml acetate buffer solution (*p*H 5.0). After incubation for 1 hour at 50 °C, 2ml of the low alkalinity copper reagent is added to the reaction mixture, and then heated for 30minutes in boiling water bath.

After cooling, 1 ml of arsenomolybdate reagent is added, and then allowed to stand at least 20 minutes. When all the cuprous oxide is perfectly dissolved, the solution is diluted with 20 ml of distilled water. The optical density is measured at 500 mμ. Avicelase activity is defined as the amounts of glucose produced by 1 ml enzyme solution from the Avicel per one hour.

CMCase and Salicinase: CMCase and Salicinase activities are determined by the same method of Avicelase, only except that reaction time of enzyme with substrate is for 30 minutes at 50°C. CMCase and Salicinase activties are defined as the amount of glucose produced by 1ml enzyme solution from the CMC and Salicin per 30 minutes, respectively.

RESULTS AND DISCUSSION

1. Selection of the strains

The activities of Avicelase, CMCase and Salicinase of Aspergilli are measured and three strains having the predominant enzyme activities are selected for Avicelase, CMCase and Salicinase, respectively. The results of selected strains are shown in Table 1. As shown in Table 1, strains No. 175 and 131 exhibiting most prominent Avicelase and CMCase activities of 4.35mg/ml and 6.35mg/ml are selected as the best strains for Avicelase and CMCase activities, respectively. Strain

Table 1. Selected strains having predominant cellulase activities.

1) Selected strains for Avicel

strain No.	Species group	Species	Habitat substrate	Collection area	Activity (mg/ml)
175	*A. niger*	*A. phoenicis*	Korean cake	Incheon	4.35
255	*A. niger*	unidentified	Korean cake	Gwangju	3.91
254	*A. niger*	unidentified	Korean cake	Jeonju	3.66

2) Selected strains for C.M.C.

Strain No.	Species group	Species	Habitat substrate	Collection area	Activity (mg/ml)
131	*A. niger*	*A. phoenicis*	Bread	Chungju	6.35
151	*A. clavatus*	*A. clavatus*	Bread	Chungju	5.93
116	*A. niger*	unidentified	Bread	Seoul	5.21

3) Selected strain for salicin

Strain No.	Species group	Species	Habitat substrate	Collection area	Activity (mg/ml)
253	*A. niger*	*A. japonicus*	Korean cake	Gwangju	47.65
456	*A. niger*	unidentified	Bread	Gunsan	49.88
457	*A. niger*	unidentified	Meju	Gunsan	49.41

Table 2. Distribution of Aspergilli species group in each region.

Species group \ Region	Middle inland	Southern inland	Western coast	Southern coast	Jeju island	Total
A. flavus	67	37	78	128	1	311
A. niger	47	25	32	47	0	151
A. clavatus	11	2	3	1	0	17
A. fumigatus	2	5	12	14	0	33
A. ochraceus	10	2	6	9	0	27
A. versicolor	2	3	1	18	0	24
A. wentii	4	3	11	7	0	25
A. candidus	6	0	8	6	0	20
A. nidulans	0	0	8	6	0	14
A. glaucus	0	1	0	1	0	2
A. ornatus	0	1	0	0	0	1
A. ustus	0	0	0	1	0	1
A. flavipes	0	1	0	0	0	1
A. cremeus	0	0	0	1	0	1
unidentified	0	1	2	4	0	7
Total	149	81	161	243	1	635

No. 456 which exhibits most prominent Salicinase activity, 49.88mg/ml, is selected as the best strain for Salicinase activity.

2. Cellulase activities of the strains from different regions

Table 3. Cellulase activities of Korean wild strains of *Aspergilli* from different regions.

Region	No. of strains	Cellulase activities (mg/ml) Avicel	C.M.C.	Salicin
Middle inland	149	0.77	2.45	12.68
Southern inland	81	0.82	1.96	13.37
Western coast	161	0.61	1.26	10.81
Southern coast	243	0.63	1.31	10.25
Jeju	1	0.59	2.61	21.18
Mean	(total) 635	0.68	1.65	11.38

Regional distribution of the species groups and cellulase activities of the strains from different regions are shown in Table 2 and Table 3, respectively. Relative distribution of *A. flavus* to *A. niger* is higher in southern coast than inland regions. Relative distribution of *A. niger* to *A. flavus* is higher in inland regions

Table 4. Cellulase activities of Korean wild strains of *Aspergilli* based on habitat substrates from which the strains isolated

Habitat substrate	No. of strains	Cellulase activities (mg/ml) for Avicel	C.M.C.	Salicin
Korean Bread	233	0.75	2.07	12.33
Meju	169	0.60	1.20	10.51
Cereals	49	0.60	1.73	13.05
Fruit	30	0.55	1.49	12.21
Others	124	0.69	1.38	9.65
Mean	(total) 635	0.68	1.65	11.38

Table 5. Distribution of Aspergilli species in each species groups

Species group	No. of strains	Species	No. of strains
A. flavus	311	*A. flavus*	216
		A. flavus var. *columnaris*	2
		A. parasiticus	1
		unidentified	92
A. niger	151	*A. phoenicis*	64
		A. ficcum	4
		A. japonicus	1
		unidentified	82
A. clavatus	17	*A. clavatus*	7
		A. giganteus	4
		unidentified	6
A. fumigatus	33	*A. fumigatus*	33
A. ochraceus	27	*A. sulphureus*	1
		A. ochraceus	18
		unidentified	8
A. versicolor	24	*A. versicolor*	14
		A. sydowi	4
		unidentified	6
A. wentii	25	*A. thomii*	19
		unibentified	6
A. candidus	20	*A. candidus*	6
		unidentified	14
A. nidulans	14	*A. nidulans*	9
		A. unguis	1
		unidentified	4
A. glaucus	2	*A. pseudoglaucus*	1
		unidentified	1
A. ornatus	1	*A. spinulosus*	1
A. ustus	1	unidentified	1
A. flavipes	1	*A. flavipes*	1
A. cremeus	1	*A. itaconicus*	7
unidentified	7		
total	635		635

Table 6. Mean cellulase activities of Aspergilli species group

Species group	No. of strains	Cellulase activities (mg/ml) for		
		Avicel	C.M.C.	Salicin
A. flavus	311	0.57	1.34	13.06
A. niger	151	1.06	2.07	13.36
A. clavatus	17	0.65	21.66	19.63
A. fumigatus	33	0.83	2.06	2.47
A. ochraceus	27	0.51	1.07	2.29
A. versioclor	24	0.45	0.74	6.05
A. wentii	25	0.27	0.81	9.43
A. candidus	20	0.55	0.96	5.25
A. nidulans	14	0.49	0.64	5.43
A. glaucus	2	0.35	0.23	4.41
A. ornatus	1	0.02	0.52	5.76
A. ustus	1	0.26	0.05	5.65
A. cremeus	1	0.92	2.56	17.65
A. flavipes	1	0.29	0.85	2.82
unidentified	7	0.60	1.15	8.94
Mean	635	0.69	1.65	11.38

than southern coast region, although total numbers of *A. flavus* are higher than *A. niger* in inland regions.

Cellulase activties, such as Avicelase, CMCase and Salicinase of Aspergilli from different regions are not so much different, although CMCase activities of the strains from middle inland are more or less higher than the average value.

CMCase and Salicinase activites of the strain from Jeju island has shown relatively higher value, even though there is no statistical meaning. Generally, cellulase activites of Aspergilli isolated from inland regions in South Korea are, more or less, higher than those of the strains isolated from coastal regions.

3. Cellulase activities of the strains based on habitat substrates

Cellulase activities of the strains and their distribution according to the habitat substrates are shown in Table 4. The strains of Aspergilli isolated from bread and Korean cake exhibited, more or less, higher Avicelase and CMCase activities than the mean value, and the strains isolated from the cereals are shown relatively higher Salicinase activities. On the other hand, Avicelase, CMCase and Salicinase activities of the strains isolated from soybean malt are shown lower value than the average.

4. Cullulase activities of the strains surveyed in taxonomical viewpoints

The 635 strins of Aspergilli used in this study includes 21 species as follows; *A. flavus*, *A. flavus* var. *columnaris*, *A. parasiticus*, *A. phoenicis*, *A. ficcum*, *A. japonicus*, *A. clavatus*, *A. giganteus*, *A. funigatus*, *A. sulphureus*, *A. ochraceus*, *A. versicolor*, *A. sydowi*, *A. thomii*, *A. candius*, *A. nidulans*, *A, pseudoglaucus*, *A. flavipes*, *A. itaconicus*, *A. spinulosus*, and *A. unguis*. Their distribution in species and species groups are shown in Table 5.

Cellulase activities of the different species groups and species of the genus *Aspergillus* are shown in Table 6 and 7, respectively. Cellulase activities of the genus *Aspergillus* showed a great difference according to their species groups and speces, although their activities are so variable in different strains even though in the same species.

Among the different species groups tested, *A. niger* groups are shown relatively higher Avicelase activities and *A. clavatus* group showed relatively higher Salicinase activities than the mean value. On the other hand, both species group of

Table 7. Mean cellulase activities of Aspergilli species

Species group	strains	No. of strains	Cellulase activities (mg/ml) for Avicel	C.M.C.	Salicin
A. flavus	*A. flavus*	216	0.55	1.49	12.55
	A. flavus var. *columnaris*	2	0.47	1.26	8.59
	A. parasiticus	1	0.07	0.44	22.94
	unidentified	92	0.63	1.00	14.25
A. niger	*A. phoenicis*	64	0.94	2.51	13.54
	A. ficcum	4	0.75	2.30	7.86
	A. japonicus	1	2.23	3.64	47.65
	unidentified	82	1.15	2.86	13.07
A. clavatus	*A. clavatus*	7	0.59	3.58	21.76
	A. giganteus	4	0.52	3.14	24.00
	unidentified	6	0.82	1.27	14.24
A. fumigatus	*A. fumigatus*	33			
A. ochraceus	*A. sulphureus*	1	0	0.95	2.59
	A. ochraceus	18	0.50	1.03	2.42
	unidentified	8	0.61	1.19	1.95
A. versicolor	*A. versicolor*	14	0.45	0.73	6.11
	A. sydowi	4	0.37	0.42	4.97
	unidentified	6	0.52	1.08	6.64
A. wentii	*A. thomii*	19	0.29	0.84	10.27
	unidentified	6	0.20	0.71	6.77
A. candidus	*A. candidus*	6	0.75	1.00	6.02
	unidentified	14	0.35	0.93	2.29
A. nidulans	*A. nidulans*	9	0.43	0.62	6.27
	A. unguis	1	0.46	0.41	1.28
	unidentified	4	0.62	0.66	3.90
A. glaucus	*A. pseudoglaucus*	1	0.7	0.45	4.94
	unidentified	1	0	0	3.88
A. ornatus	*A. spinulosus*	1	0.02	0.52	5.76
A. ustus	unidetified	1	0.26	0.05	5.65
A. flavipes	*A. flavipes*	1	0.29	0.85	2.82
A. cremeus	*A. itaconicus*	1	0.92	2.56	17.65
unidentified		7	0.59	1.19	9.63
mean		635	0.68	1.65	11.38

Aspergilli, *A. niger* and *A. clavatus* are shown relatively higher CMCase activities. These results are in good agreement with King(1963) and Ikeda's(1967) reports.

Generally, *A. niger* and *A. clavatus* groups are showed relatively higher value compared with the other species groups in their cellulase activities.

Among the different species tested, the activities of Avicelase, CMCase and Salicinase are highest in *A. phoenicis*, *A. clavatus*, and *A. japonicus* and *A. giganteus*, respectively.

However, tested number of the strains of *A. clavatus* and *A. japonicus* are so fewer that it is not so meaningful. It is reasonable to conclude, therefore, that cellulase activities of *A. niger* and *A. clavatus* species groups are higher compared with the other species groups, since the tested number of strains of *A. niger* and *A. clavatus* species groups including *A. japonicus* and *A. clavatus* species, are meaningfully high respcetively.

摘 要

한국산 *Aspergillus* 635균주를 분리, 순수배양하고 그들의 Avicelase, CMCase 및 Salicinase 활성을 측정하여 우량균주를 선별하고 Aspergillus의 셀룰라아제 활성과 균주의 계통 및 생태학적 요인과의 상관관계를 조사하였다.

균주번호 175, 255 및 254등은 가장 우수한 Avicelase 생성균주로 선별되었고 균주번호 131, 151 및 116은 가장 우수한 CMCase 생성균주로 선별되었으며 균주번호 456, 457 및 253은 가장 우수한 Salicinase 생성균주로 선별되었다.

종군에 따른 *Aspergillus*의 Avicelase 및 Salicinase 활성은 *A. niger*군과 *A. clavatus*군이 각각 가장 높은 활성을 나타내었고 CMCase 활성은 *A. clavatus*군과 *A. niger*군이 공히 높은 활성을 보여 주었다. 종에 따른 *Aspergillus*의 Avicelase 활성은 *A. phoenicis*가 가장 높은 활성을 나타내었고 CMCase 활성은 *A. clavatus*가 가장 높은 활성을 보여 주었으며, *A. japonicus*, *A. giganteus*가 가장 높은 Salicinase 활성을 나타내었다.

지역적 분포에 따른 *Aspergillus*의 셀룰라아제 활성은 내륙지방에서 수집한 표품에서 분리된 균주가 비교적 높은 값을 나타내었다.

기질에 따른 *Aspergillus*의 Avicelase 및 CMCase 활성은 벽 및 빵에서 분리한 균주가 비교적 높은 값을 나타내었으며 Salicinase에 있어서는 곡물에서 분리한 균주가 비교적 높은 값을 나타내었다.

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