

Cellulase activity of *Aspergilli* distributed in South Korea (1)

—Isolation and identification of *Aspergilli*—

LEE, Yung Nok, Nak Jung KIM, *Hang Won SUH

(Dept. of Biology, Korea Univ., *Pacific Chemical Co.)

한국산 *Aspergilli*의 셀룰라아제 활성에 관한 연구 (第1報)

—*Aspergilli*의分離 및 同定—

李永祿·金洛政·*徐恒源

(高麗大學校 生物學科·*太平洋化學)

ABSTRACT

410 strains which belong to the genus *Aspergillus* were isolated from specimens collected throughout South Korea, and 17 species and 1 variety through the 13 species groups were identified according to Raper-Fennell's classification key.

Among them the species and variety unrecorded in Korea are as follows: *A. giganteus*, *A. pseudoglaucus*, *A. spinulosus*, *A. ficuum*, *A. japonicus*, *A. flavus* var. *columnaris*, *A. flavipes*.

INTRODUCTION

The genus *Aspergillus* dates from Micheli's "Nova Plantarum Genera" (1729), which was the first to distinguish stalks and spore heads, but it was not until the middle of the 19th century that these fungi began to be recognized as active agents in decay processes, as causes of human and animal disease, and as fermenting agents capable of producing valuable metabolic products.

With such cognizance came the first adequately described and illustrated species in the works of Fresenius, Van Tieghem, and De Bary, although Link (1809) had earlier introduced a few species names that are still recognized. The genus began to be taken definite form with the work of Wehmer. He began reporting his biochemical studies in 1891, and led to the publication of his monograph on the genus *Aspergillus*

in 1901. Meanwhile, Tom & Church had published a series of reports on different groups of *Aspergillus* important in food microbiology that culminated in their taxonomic monograph, *The Aspergillus* (1926).

By the early 1940's the *Aspergillus* were being studied intensively in many laboratories. The need for a guide to facilitate the identification of the *Aspergillus* led Tom and Raper to publish "A manual of the *Aspergilli*" in 1945. This book was based primarily upon the morphology, 77 species, 8 varieties and 4 mutations were arranged into groups which showed related physiological or biochemical activities.

In the year since 1945, scores of new species of *Aspergillus* have been described newly discovered, necessitating the substantial enlargement of some of the groups then established and the recognition of five new groups. Finally in 19

65, Raper & Fennell published "The genus *Aspergillus*" which containing 132 species and 18 varieties through the 18 species groups.

In Korea, Lee and Chang (1964) isolated the strains of *A. flavus-oryzae* species group from Meju and soil. Kim, et. al., (1965) isolated the strains of some species groups. Lee and Kim (1968) isolated 63 strains from bean paste and soybean sauce, and identified *A. flavus*, *A. niger*, *A. nidulans*, *A. clavatus*, *A. fumigatus* and *A. ochraceus*. Kim (1971) isolated the strains of *Aspergilli* from various substratum and identified them according to their morphological and physiological characters. Kim (1972) identified some species but contents of description were unsatisfactory. Moon and Lee (1974) attempted the way of serological classification. Recently Lee and Lee (1976) identified 8 species including *A. thomii*, *A. candidus* which were unrecorded in Korea. In the present study, we isolated 410 strains from various specimens and identified them according to the key of Raper-Fennell (1965).

MATERIALS AND METHOD

1. Experimental materials

The experimental materials used in the present study were collected throughout South Korea from June to September 1974. Substrates from which *Aspergilli* strains were isolated were bread, Korean rice cakes, fruits, vegetables, cereals, beanpod, bean paste, soybean sauce, etc.

2. Isolation and maintenance of strains

10-15cc of Pfeffer's solution was poured into vial containing samples and cultured for 5 to 8 days, and then conidia

were inoculated on Czapek's agar medium.

After incubation at room temperature for 10-12 days the strains of *Aspergilli* were isolated on the basis of their morphological characters. Further pure culture was attained by two or more successive subcultures of single colony from the agar medium, and was maintained on the slant agar medium at 5°C and subcultured every two months.

3. Identification of species

The isolated 410 strains of *Aspergilli* were incubated on Czapek's solution agar at 24-26°C in 12 days to two weeks. Each strains were observed colony morphology and characters and were investigated, under the microscope, shape, size and color of conidial heads, conidiophore, conidium, vesicle and sterigmata, and then were examined whether or not of formation, shape, size and color of cleistothecium, ascospore, ascus, sclerotium and hülle cell by the classification key of Raper-Fennell (1965).

RESULTS AND DISCUSSION

Among the 410 strains of *Aspergilli*, 276 strains were identified into 17 species and 1 variety according to Raper-Fennell's classification key, but the rest of them could not be determined the name of species.

Table 1-5 indicate the characteristics of the species according to the investigative items respectively. All of the description given in this paper are observed on the Czapek's solution agar at 24-26°C for 12 days to two weeks. Table 6 shows species distribution of the strains.

The following is the description of the

species and variety which is unrecorded in Korea.

Description of species

A. giganteus

Colonies on Czapek's solution agar growing fairly at 24-26°C, 3.5 to 5cm. in 12 days to two weeks, characterized by the development of abundant conidiophore; colonies at first white, becoming pale blue green as conidial heads mature; reverse dull tan, becoming brown in age; odor none to fetid; exudate inconspicuous.

Conidial heads split into divergent columns, pale blue green, 150 to 380 μ in diameter; conidiophore 2 to 4.2mm. in length, colorless, smooth walled, from the substratum; vesicle consisting of the expanded terminus of the conidiophores, small, 120 to 240 μ by 25 to 46 μ ; sterigmata in a single series, colorless, ranging from 2.5 to 3.5 μ by 2.3 to 2.7 μ at the base of the vesicle to 7 to 10 μ by 2.8 to 3.2 μ at the apex; conidia elliptical, smooth, thick walled, 3.2 to 4 μ by 2.2 to 2.8 μ .

A. pseudoglaucus

Colonies on Czapek's solution agar with 20 per cent of sucrose spreading rapidly at 24-26°C in 12 days to two weeks, plane, consisting of a felt of yellow-orange encrusted hyphae enmeshing abundant cleistothecia, yellow orange except the place where yellow green may be conspicuous from the presence of conidial heads admixed with cleistothecia in the mycelial felt; reverse orange brown; exudate abundant, clear; odor slightly pronounced.

Conidial heads few in number and generally submerged in the mycelial felt, small, radiate, dark yellow up to 100 μ in diameter; conidiophore smooth walled brownish, 120 to 250 in length, 5 to 15 μ in diameter; vesicle flask shaped, fertile over the half only, brownish. 10 to 20 μ in diameter; sterigmata in a single series, 5 to 8 μ by 2.5 to 3.8 μ ; conidia subglobose, spinulose, variable in size ranging from 4 to 7 μ in diameter; cleistothecia abundant, globose to subglobose, yellow, up to 100 μ , embedded in a felt of yellow orange mycelium; 10 to 13 μ in diameter;

Table 1. Characteristics of colony morphology.

Species	Items	diameter	texture	front color	reverse color	exudate	odor
<i>A. giganteus</i>		3.5-5cm	loosely velvety	blue green	brown	inconspicuous	none or fetid
<i>A. pseudoglaucus</i>		6-7cm	loosely velvety	yellow orange	orange brown	abundant	faintly pronounced
<i>A. spinulosus</i>		3-3.5cm	delicate floccose	olive green	olive brown	abundant	faintly pronounced
<i>A. ficuum</i>		5-6cm	velvety	black	colorless, faintly yellow	inconspicuous	not distinctive
<i>A. japonicus</i>		5-5.5cm	velvety	purple black	yellow green	lacking	not distinctive
<i>A. flavus var. columnaris</i>		3.4-4.5cm	velvety	yellow green	colorless	lacking	not pronounced
<i>A. flavipes</i>		2-3.5cm	velvety	pale yellow, white	yellow	abundant	not distinctive

Table 2. Characteristics of conidial head and conidiophore.

Species	Items	Conidial head			Conidiophore			Origin
		shape	color	size(μ)	marking	color	length & width (μ)	
<i>A. giganteus</i>	split	blue green	150-380	smooth	colorless	2000-4200/ 20-56	substratum	
<i>A. pseudo-glaucus</i>	radiate	green	up to 100	smooth	light brown	120-250/ 5-15	submerged hyphae	
<i>A. spinulosus</i>	radiate	olive green	70-170	smooth	brown	up to 480/ 3.5-6.5	substratum	
<i>A. ficuum</i>	split	brown black	200-700	smooth	brown	600-1700/ 10-20	substratum	
<i>A. japonicus</i>	radiate split	purple brown	120-800	smooth	colorless yellow brown	300-800/ up to 12	substratum	
<i>A. flavus var. columnaris</i>	columnar	green	350-500/ 40-60	rough	colorless	up to 560/ 4-11	substratum	
<i>A. flavipes</i>	radiate	white	40-60	smooth	yellow brown	400-3000/ up to 10	submerged aerial hyphae	

ascospore lenticular, 4.5 to 5.5 μ by 3.5 to 4.0 μ , smooth walled, with equatorial region rounded or flattened, without ridges.

A. spinulosus

Colonies on Czapek's agar growing more or less restrictively 3 to 3.5cm. at 24-26°C in 12 days to two weeks, plane, composed of a white felt bearing cleistothecia beneath a layer of olive conidial heads, becoming wrinkled in age; reverse olive brown; exudate abundant, clear to pale amber; odor slightly pronounced.

Conidial heads arising from the substrate mycelium, small radiate, olive green, 70 to 170 μ in diameter; conidiophore smooth, brownish, up to 480 μ by 3.5 to 6.3 μ ; vesicle spatulate, small, fertile over the upper only slightly brownish 7 to 20 μ in diameter; sterigmata in a single series, slightly pigmented, 6 to 12 μ by 3.0 to 4.8 μ by 2.7 to 3.5 μ ; cleistothecia globose, 60 to 180 μ in diameter, composed of loosely interwoven hyphae, and surrounded by a weft of

drab olive-encrusted hyphae; asci eight spored, subglobose 22 to 36 μ ; ascospore elliptical thick walled, conspicuously spiny, with up to 1.2 in length, uncolored, 7 to 13 μ by 6 to 10 μ .

A. ficuum

Colonies on Czapek's solution agar growing rapidly, 5 to 6cm. at 24-26°C in 12 days to two weeks, plane, closely velutinous, thin white basal mycelium almost completely submerged, conidial structures abundant throughout most of the colony but thinning at the margin; reverse uncolored or slightly yellowish; odor not distinctive, exudate inconspicuous, clear, in very small droplets.

Conidial heads mostly splitting into several well-defined columns, 200 to 700 μ in diameter, brown black to purple black; conidiophore erect, mostly pigmented in brown shades, with walls commonly 2 to 3 μ thick, 600 to 1700 μ in length, diameter throughout most of their length 15 to 20 μ but reduced just below the vesicle 10 to 15 μ ; vesicle globose, fertile

Table 3. Characteristics of vesicle and conidium.

Species	Items	Vesicle				Conidium		
		shape	fertile area	color	size(μ)	marking	shape	size(μ)
<i>A. giganteus</i>		clavate	whole	colorless	120-240/ 25-46	smooth	elliptical	3.2-4/ 2.2-2.8
<i>A. pseudo-glaucus</i>		flask	half	brown	10-20	spinulose	subglobose	4.2-7
<i>A. spinulosus</i>		spathulate	upper only	brown	7-20	rough	elliptical	3-4.8/ 2.7-3.5
<i>A. ficuum</i>		globose	whole	faintly brown	22-70	faintly rough	horizontally flattened	3.2-4
<i>A. japonicus</i>		globose	whole	brown	up to 50	echinulate	globose, subglobose	3-3.5
<i>A. flavus var. columnaris</i>		subglobose	half	colorless	12-30	rough	globose subglobose	2.5-5.5
<i>A. flavipes</i>		subglobose elongate	whole	faintly yellow	10-22	smooth	globose, subglobose	2-3

Table 4. Characteristics of sterigmata.

Species	Items	arrangement	color	primary sterigmata		secondary sterigmata	
				length (μ)	width (μ)	length (μ)	width (μ)
<i>A. giganteus</i>		single	colorless	2.5-10	2.3-3.2		
<i>A. pseudoglaucus</i>		single	faintly colored	5-8	2.5-3.8		
<i>A. spinulosus</i>		single	faintly colored	6-12	3-3.5		
<i>A. ficuum</i>		double	brown	12-27	4-6.5	5-8.5	2.2-3
<i>A. japonicus</i>		single	brown	5-8	2.5-4.2		
<i>A. flavus var. columnaris</i>		single	colorless	6-10	3-4		
<i>A. flavipes</i>		double	colorless	5-7	2-3	4.5-6.5	1.5-2.3

Table 5. Characteristics of cleistothecium, ascus, ascospore and sclerotium.

items	species	<i>A. pseudoglaucus</i>	<i>A. spinulosus</i>	<i>A. japonicus</i>
Cleistothecium	shape	subglobose	globose	—
	size (μ)	up to 100	60-180	—
Ascus	size (μ)	10-13	22-30	—
Ascospore	marking	smooth	spinulous	—
	shape	lenticular	elliptical	—
	color	yellow brown	colorless	—
	size (μ)	4.5-5.5/3.5-4.0	7-13/6-10	—
Sclerotium	shape	—	—	globose
	color	—	—	cream
	size (μ)	—	—	up to 400

over the whole surface, heavy walled, faintly brownish, variable from 22 to 70 μ in diameter; sterigmata in double series, brownish, primaries 12 to 27 μ by 4 to 4.5 μ , secondaries 5 to 8.5 μ by 2.2 to 3.0 μ ; conidia more or less roughened, horizontally flattened and longitudinally striate at maturity, mostly 3.2 to 4 μ .

A. japonicus

Colonies on Czapek's solution agar growing rapidly, attaining a diameter of 5 to 5.5cm. at 24-26°C in 12 days to two weeks, consisting of a thin, pale yellow, plane basal mycelium, producing cream colored globose sclerotia at marginal areas; reverse at first uncolored but becoming slightly yellow shades;

Table 6. Experimental results of identification

Species group	Species	Number
<i>A. clavatus</i>	<i>A. giganteus</i>	4
	<i>A. clavatus</i>	5
	unidentified strains	6
<i>A. glaucus</i>	<i>A. pseudoglaucus</i>	1
	unidentified strains	1
<i>A. ornatus</i>	<i>A. spinulosus</i>	1
<i>A. fumigatus</i>	<i>A. fumigatus</i>	12
<i>A. ochraceus</i>	<i>A. sulphureus</i>	1
	<i>A. ochraceus</i>	16
	unidentified strains	10
<i>A. niger</i>	<i>A. phoenicis</i>	33
	<i>A. ficuum</i>	4
	<i>A. japonicus</i>	1
	unidentified strains	67
<i>A. candidus</i>	<i>A. candidus</i>	6
	unidentified strains	4
<i>A. flavus</i>	<i>A. flavus</i>	162
	<i>A. flavus var. columnaris</i>	1
	unidentified strains	36
<i>A. wentii</i>	<i>A. thomii</i>	12
	unidentified strains	4
<i>A. versicolor</i>	<i>A. versicolor</i>	8
	<i>A. sydowi</i>	5
	unidentified strains	5
<i>A. nidulans</i>	<i>A. nidulans</i>	2
<i>A. ustus</i>	unidentified strais	1
<i>A. flavipes</i>	<i>A. flavipes</i>	2
Total		410

exudate not distinctive.

Conidial heads variable, small, radiate or split into few indistinctive columns rarely exceeding 300 μ in diameter but in some heads split into two divergent columns in age up to 800 μ long; conidiophore smooth, colorless of lightly yellow brown pigmented particularly just below the vesicle; mostly 300 to 800 μ by up to 12 μ ; vesicle yellowish brown shades, often somewhat elongate but in older or larger heads more nearly globose, mostly up to 50 μ in diameter, in normal heads fertile over most of their surface but small heads at the apex only; sterigmata in a single series, mostly 5 to 8 μ by 2.5 to 4.2 μ ; conidia strongly echinulate, with echines discret and regularly spaced, commonly 0.5 μ long, globose to subglobose, mostly 3 to 3.5 μ ; sclerotia produced tardily, cream colored, globose, up to 400 μ in diameter.

A. flavus var. columnaris

Colonies on Czapek's solution agar growing fairly, attaining 3.5 to 4.5cm. in diameter at 24-26°C in 12 days to two weeks, uniformly close textured and velvety, green; reverse uncolored; exudate lacking; odor not pronounced.

Conidial heads completely columnar and up to 350 to 500 long by 40 to 60 μ in diameter; conidiophore less roughened than those of the species, rarely over 500 μ long by 4 to 11 μ in diameter, uncolored; vesicle clavate when young becoming subglobose mostly 12 to 30 μ in diameter, commonly fertile over the upper one-half of their surface; sterigmata entirely in single series, colorless, 6 to 10 μ by 3 to 4 μ ; conidia globose to subglobose, variable from 2.5 to 5.5 μ in diameter.

A. flavipes

Colonies on Czapek's solution agar growing rather slowly, attaining 2 to 3.5cm. in diameter at 24-26°C in 12 days to two weeks, comparatively deep, sporulating abundantly from the basal mycelium and from aerial hyphae, very pale yellowish white; exudate usually abundant forming large droplets often embedded within pale yellowish mycelium; reverse usually in yellow shades, odor not distinctive.

Conidial heads radiate, persistently yellow or very pale buff shades resulting from the yellow brownish conidiophore,

mostly 40 to 60 μ in diameter; conidiophore mostly up to 700 μ by 10 μ but commonly up to 3.0mm. length when produced at margin of older colonies with walls relatively thick walled, smooth, appearing yellow to light brown under the microscope; vesicle subglobose to elongate faintly yellowish mostly 10 to 22 μ in diameter; sterigmata in double series, primaries 5 to 7 μ by 2 to 3 μ , secondaries 4.5 to 6.5 μ by 1.5 to 2.5 μ ; conidia smooth walled, colorless, globose to subglobose, 2 to 3 μ in diameter; hülle cell not observed.

摘 要

Aspergillus 屬에 屬하는 410균주를 우리나라 중부 이남 지역에서 수집한 표본에서 분리하여 순수배양하고 Raper-Fennell의 분류방법에 따라 동정한 결과 13균종군에 걸쳐 17종 1변종을 확인할 수 있었는데 그들 중 한국 미기록종과 변종은 다음과 같다.

A. giganteus, *A. pseudoglaucus*, *A. spinulosus*, *A. ficuum*, *A. japonicus*,
A. flavus var. *columnaris*, *A. flavipes*.

REFERENCES

- Alexopoulos. C.T., 1962. Introductory mycology. John Wiley and Sons. Inc. 217-240.
- Iizuka, H., 1953. Study on the morphology and classification of the violet-black *Aspergilli* (Part 1 and Part 2). *Jour. Agr. Chem Japan*. 27, 801-809.
- Iizuka, H., 1955. The electron microscopic investigation on classification of conidia of the genus *Aspergillus*. *J. Gen. Appl. Microbiol.*, 1, 10.
- Kim Jong-Hyup, Chang Kun-Hyung and Choi Chun-Eun, 1965. Studies on the antifungal action of leather in Korea (Part 1). On the isolation and identification of strains of *Aspergillus oryzae*, *A. niger* and *A. glaucus* from Korean-leather. *Kor. Jour. Microbiol.*, 3, 15-17.
- Kim Myung-Chan, 1972. Microbiological studies on Korean Jang (Part 2 and Part 2-2) On the microflora. *Jour. of the Institute for Agr. Resource Utilization*. 6, 1-17.
- Kim Sang-Jae, 1971. Taxonomical studies of Korean *Aspergilli*. *Kor. Jour. Microbiol.*, 9, 1-26.
- Lee Bae-Ham, Kim Sang-Jae and Lee Ho-Won, 1968. The taxonomical studies of Korean *Aspergilli*. *Kor. Jour. Microbiol.*, 6, 6-11.
- Lee Ke-Ho and Chang Kun-Hyung, 1964. Studies on Koji for optimum conditions of growth and identification of *Aspergillus* spp. (Part 2). *Kor. Jour. Microbiol.*, 2, 17-18.
- Lee Yung-Nok and Lee Sung-Kap, 1976. On the *Aspergillus* in Samchunpo. *Jour.*

- Education*. Korea univ., 5, 73-85. Wilkins Co., Baltimore.
10. Moon Hi-Joo and Lee Bae-Ham, 1974. Studies on the serological classification for Korean *Aspergilli*. *Kor. Jour. Microbiol.*, 12, 180-187.
11. Raper, K.B., and D.I. Fennell, 1965. The genus *Aspergillus*. The Williams and Wilkins Co., Baltimore.
12. The Handbook of Microbiology, 1953. Ki Bo Dang. 670-672.
13. Thom, C., and K.B. Raper, 1945. A manual of *Aspergilli*. The Williams and Wilkins Co., Baltimore.

Explanation of Plates.

Fig. 1-9: Conidial head of various species of *Aspergilli*.

- Fig. 1: *A. phoenicis* Fig. 2: *A. ficuum*
 Fig. 3: *A. japonicus* Fig. 4: *A. giganteus*
 Fig. 5: *A. sulphureus* Fig. 6: *A. clavatus*
 Fig. 7: *A. nidulans* Fig. 8: *A. sydowi*
 Fig. 9: *candidus*

Fig. 10: Conidiophore of *A. thomii*

Fig. 11: Conidiophore of *A. ochraceus*

Fig. 12-17: Conidial head of various species of *Aspergilli*

- Fig. 12: *A. flavus* var. *columnaris* Fig. 13: *A. pseudoglaucus*
 Fig. 14: *A. flavipes* Fig. 15: *A. spinulosus*
 Fig. 16: *A. versicolor* Fig. 17: *A. fumigatus*.

Fig. 18: Characteristically elongate and twisted hülle cells of *A. ustus* species group.

Fig. 19: Cleistothecia associated with hülle cells of *A. nidulans*

Fig. 20-23: Conidia of various species of *Aspergilli*

- Fig. 20: *A. fumigatus* Fig. 21: *A. versicolor*
 Fig. 22: *A. clavatus* Fig. 23: *A. flavus*

Fig. 24: Ascospores and ascus of *A. pseudoglaucus*

Fig. 25: Conidia of *A. thomii*

Fig. 26: Ascospores of *A. nidulans*

Fig. 27: Conidia of *A. japonicus*

Fig. 28: Ascospores of *A. spinulosus*

Plate 1

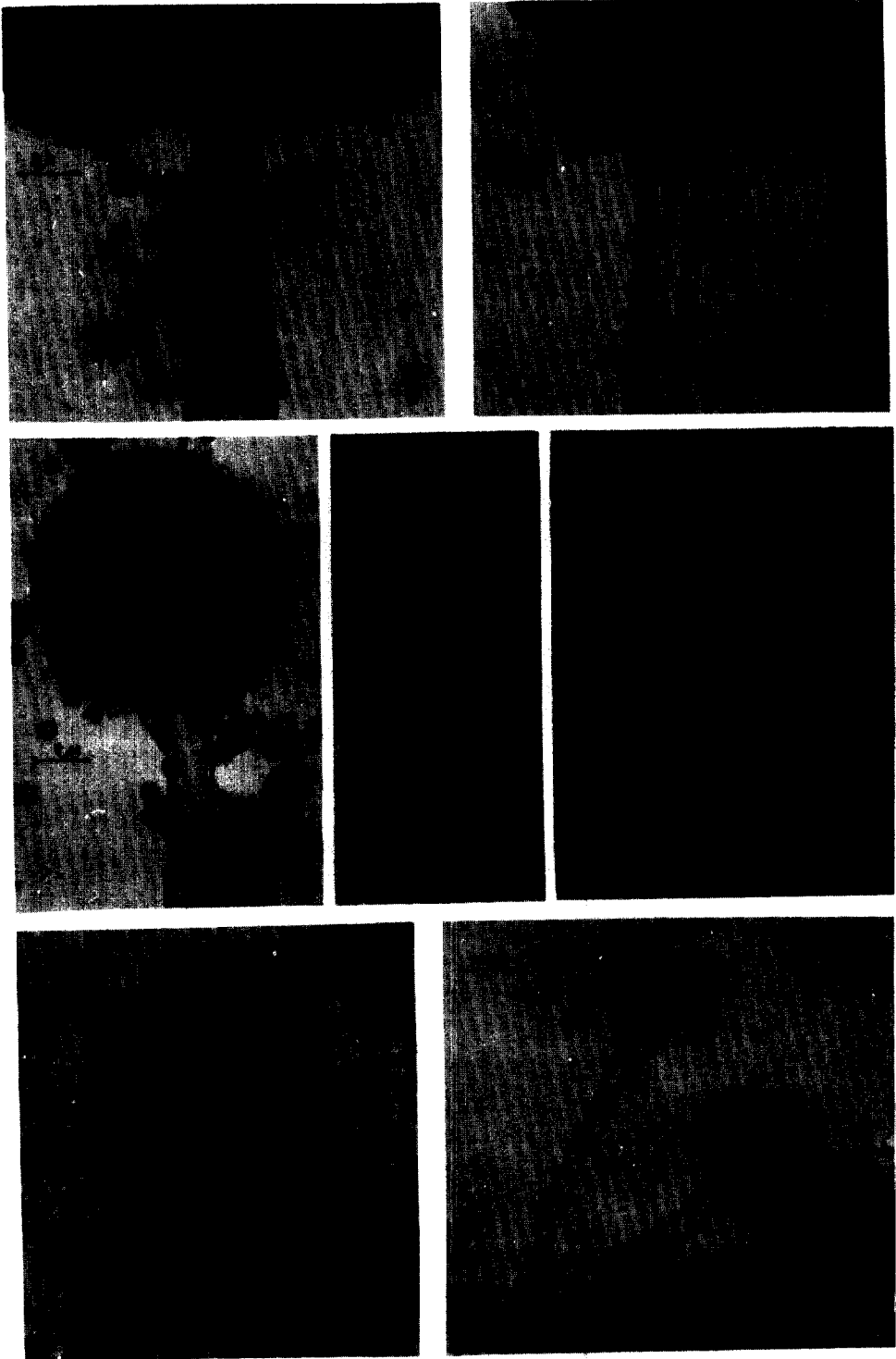


Plate 2

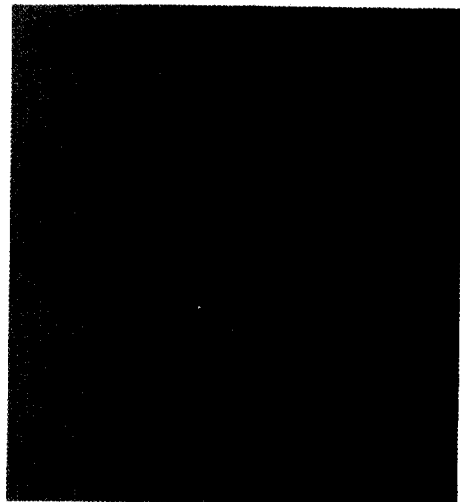
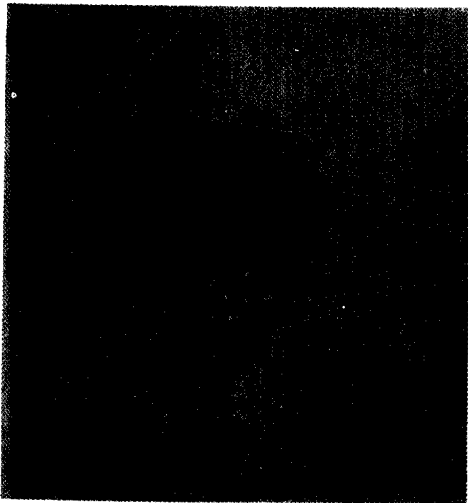
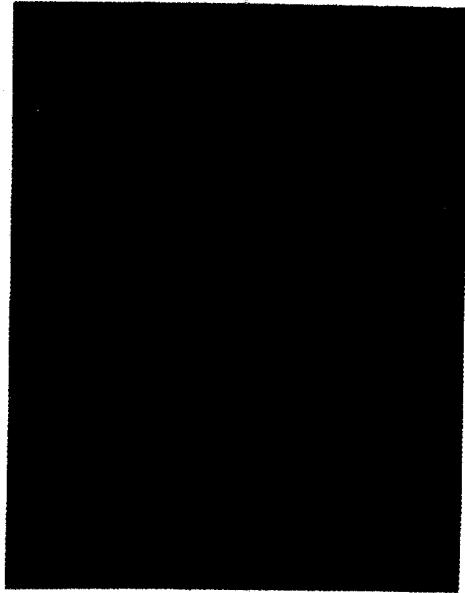
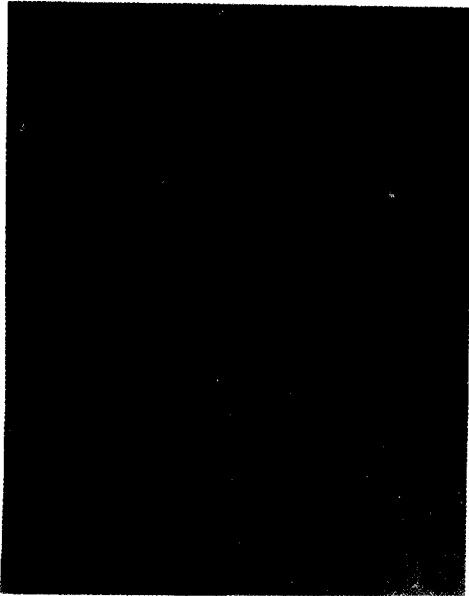
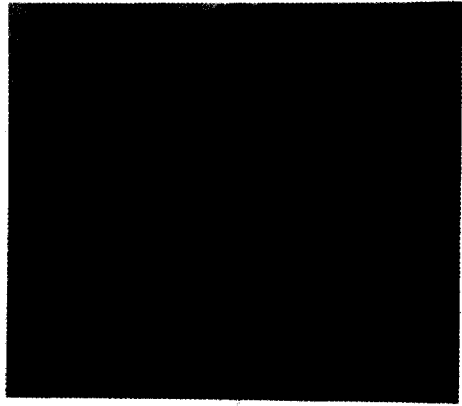
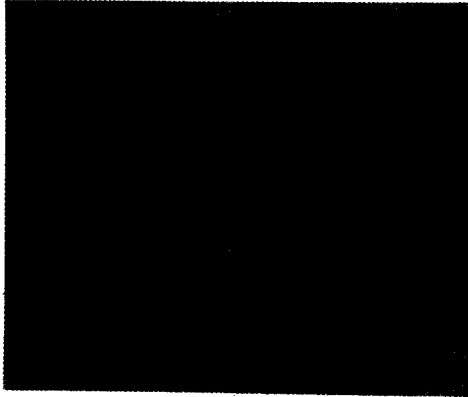


Plate 3

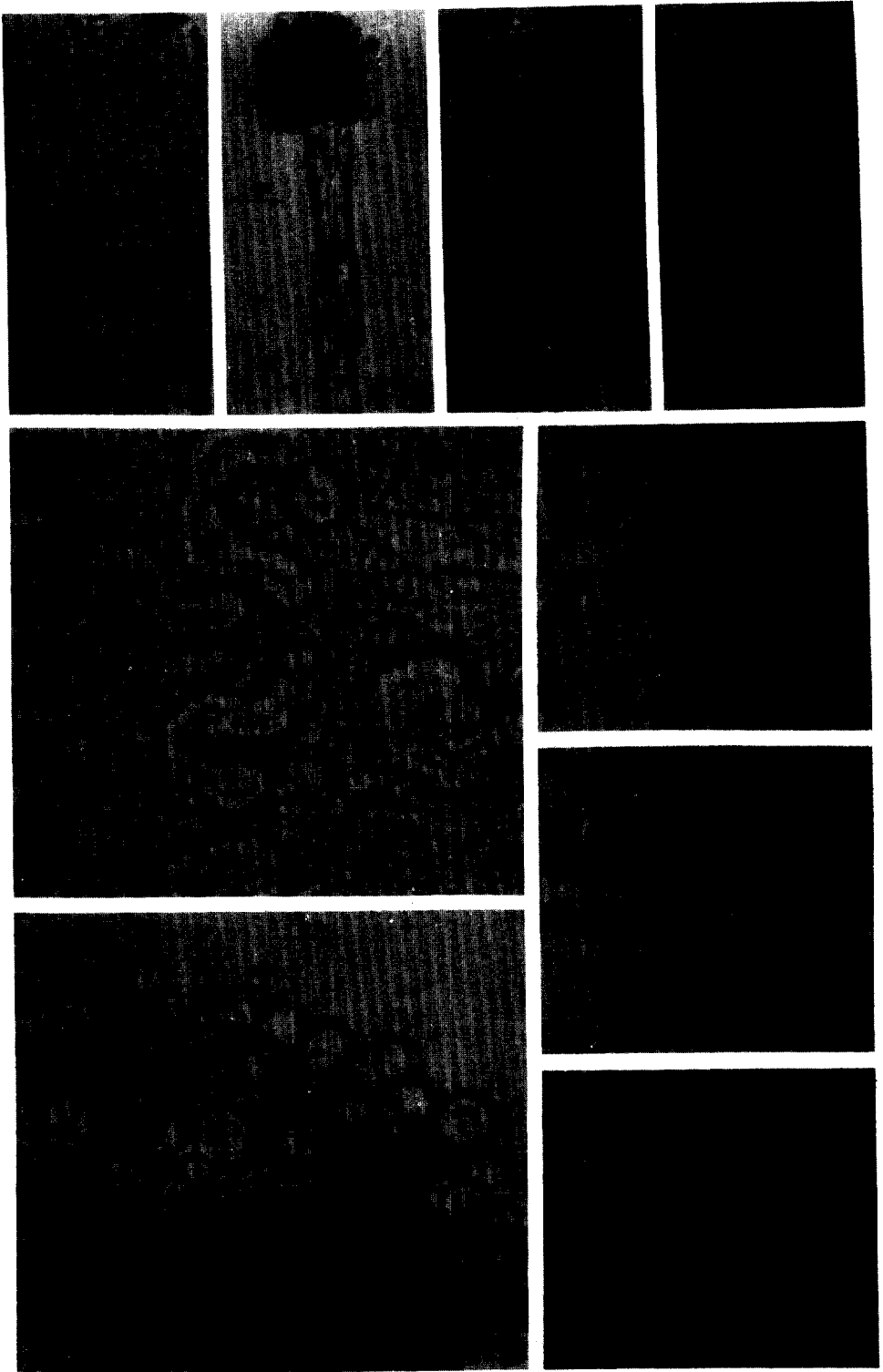


Plate 4

