Studies on the Leaf Spot of the Rice Plant *

(6) Observations on the nuclear phenomena in the causal fungi (1)

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Already the physiological specialization in the causal of the present disease (Ophiobolus miyabeanus Ito et Kurib.) is studied by many investigators with the cultural behavior, but with the nuclear phenomena few observations are reported hitherto. In this paper, the number of nuclei contained in each cell is observed as the one of the characters of the present fungi, and some nuclear behaviors are compared with each other. And to count precisely the nnmber, it is necessary to follow the process of the nuclear division.

The writer wishes to express his grateful acknowledgement to Dr. Sakamoto for his valuable advises, and Dr. Itikawa who is kind enough to teach the Feulgen's method of nuclear staining with micro-organisms. His hearty gratitude to Mr. Hanaoka (one of the student of the Laboratory of Applied Botany) who assists the writer in the full course of those experiments.

Materials and Methods

1) Materials. The cultures of the present fungi are divided into two types, Conidial (C)-and Mycelial (M)-type. The former produces conidiospores abundantly on many media, the later non or very rare of them,

C_1-culture is sent from Phytopathological Laboratory of Agricultural Faculty of Kyoto University (its No. 13), and of this culture the descripttion is previously reported[1), 5)]. And the other cultures are isolated from the affected grains which are obtained in the Shonai district of Yamagata Pref.

The method for isolation is as follows; the conidiospore which produced on affected grains are wash out with the sterilised water, and they are suspended in the fused Richards' nutrient agar. After the suspended spores are poured into Petri dishes, those are incubated at 25°C for 1 or 2 days. Then the hyphae developed from single spore which ascertained with a microscope of low magnification, are piked up and transplanted in test-tube (Richards' nutrient agar slant). Thus a great many cultures (over 2 hundreds) are obtained, but at last, 2 C-type cultures and M-type ones are selected as material cultures. At the first time of this experiment of isolation, those isolates are M-type, but from one of M-type 2 C-type cultures are produced on the sterilised rice plant ear in test tubes.

Those cultures are classified into several groups by means of Tochinai and Sakamoto's method[7)], that is, those are cultured on rice-culm decoction—, potato decoction—,

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Saito's soy— and Richards' nutrient agar media. Seven days after incubation at 28°C, those are grouped according to external appearance of those.

In this experiment, only the clearly distinguishable cultures are classified into some groups. Its results are inserted in the following table (Tab. 1). C_3-culture is a saltant of C_2, its radial growth is very slow on Richards' nutrient and Saito's soy agar media (cf. Pl. 3-5, 6), but on Rice culm and potato decoction it is not so conspiquously different from C_1 and C_2. M_1-culture is more whitish in the mycelial color than V group. M_6-culture is very slow in the radial growth (cf. Pl. 2-9～12). A great part of the obtained isolates are belong to V group.

Table. 1. Groups of Material Fungi (s=saltant)

Gulture	C_1	C_2	C_3	M_1	M_2	M_3	M_4	M_6
Group	II	I	Is	s	V	V	V	s

2) Methods of culture. A large drop of Richards' nutrient liquid is placed on a slide glass kept in Petri-dish which is moisted with a wet filter-paper. In those drops the material fungi are inoculated and incubated at 28°C for required time.

3) Staining methods. To observe nuclear phenomena of the present fungi clearly, the Feulgen's reaction is very successful, in the all course of those observaions this method is applied, those material fungi are incubated for 4 days and so, these are mounted with Bouin solution for 10-15 minutes for fixation, and about 5 minutes with more younger materials After fixation these are washed with tap water. Then those are dehydrolysed with 1-N HCl (60°C) for 15 minutes, or 5-N HCl (room temperature) for 45-60 minutes[4]. When the dehydrolisis time is too long, the nuclei are destroyed, and too short these are not stained. The optimal time for dehydrolisis varies according to the material type too, with C-type it is shorter than with the other type; the external appearance of cell wall of the former seems to be softer. After washing with tap water, the Shiff's reagent is applied.

It is most important to determine the optimal pH of Shiff's reagent to stain the nuclei of the present fungi. As Itikawa reported[3], the optimal pH is higher than that of ordinally prepared reagent, in the present experiment it is 3.2-3.5. By the way, nuclei of many fungi, Aspergillus, Botrytis, etc, are clearly stained with this pH. When pH is lower than 3.0 these nuclei are colored faintly or entirely unstained, higher than 4.0 or so the cytoplasm of them is colored in red with Fuchsin, and so the descrimination of nuclei from the cytoplasm is almost impossible. In many cases, double staining with fast green (for 3-5 minutes) is used to contrast red color of nuclei to light blue of cytoplasm.

Observations

1) The nuclear division. As the nuclei of the present fungi are very small, and the nuclear division is not always accompanied with the cell division (multinucleate),

it is difficult to precisely observe the steps of the phenomenon.

In thin mycelia slowly developed and in conidiospore, the resting stage is clearly distinguishable, but in the germinating conidia and vigorously developing mycelia this resting stage is not so distinct (cf. Pl. 1-C). In the later cases, the nuclei so rapidly divise that the telophasic changes suceed into the early prophase. Even in the resting conidia which are not germinated, these figures seemed to belong to the late telophase or early prophase are occasionally observed.

Table. 2-1 Observation of Nuclear Number in Each Cell

	Number of Cell (in actural number and in percentage)											
Culture	M_1-h		M_2-h		M_3-h		M_4-h		M_6-h		M_3-c	
Nuclear Number	act.	%	act.	%	act.	%	act.	%	act.	%	act.	%
1	14	1.7	30	3.8	13	2.1	15	0.9	6	0.7	0	0.0
2	86	10.2	206	26.6	186	24.4	102	12.4	59	7.1	20	2.8
3	135	16.1	213	27.5	224	29.4	149	18.2	137	16.6	47	6·6
4	350	41.7	221	28.5	301	39.6	294	35.8	330	40.0	119	16.7
5	134	16.0	49	6.3	21	2.8	129	15.7	138	16.7	98	13.8
6	69	8.2	31	4.0	12	1.6	59	7.2	87	10.5	122	17.2
7	22	2.6	18	2.3	1	0.1	33	4.0	33	4.0	86	12.1
8	15	1.8	7	0.9			17	2.1	25	3.0	91	12.8
9	6	0.7	0	0.0			13	1.6	7	0.9	55	7.7
10	2	0.2	1	0.1			6	0.7	2	0.2	30	4.2
11	3	0.4					2	0.2	2	0.2	13	1.8
12	3	0.4					2	0.2	1	0.1	9	1.3
13											13	1.8
14											5	0.7
15											1	0.1
16											2	0.3
17											0	0.0
18											0	0.0
19											1	0.1
Total	839		776		761		821		827		712	
Average	4.3±1.46		3.3±1.37		3.2±0.97		4.2±1.59		4.4±1.43		6.4±2.56	

Table. 2-2

	Number of Cell (in actural number and in percentage)											
Culture	C_1-h		C_2-h		C_3-h		C_1-c		C_2-c		C_3-c	
Nuclear Number	act.	%	act.	%	act.	%	act.	%	act.	%	act.	%
1	90	12.0	11	1.5	9	1.0	79	9.7	8	0.4	72	3.9
2	485	64.7	66	9.3	90	10.1	512	62.6	90	4.8	176	10.0
3	70	9.3	113	15.9	247	27.8	135	16.5	228	12.1	259	14.3
4	90	12.0	267	37.7	364	40.9	77	9.4	496	26.4	577	31.8
5	10	1.3	126	17.8	117	13.2	11	1.4	392	20.8	315	17.3
6	5	0.7	77	10.9	33	3.7	2	0.2	293	15.6	139	7.6
7			28	4.0	15	1.7	2	0.2	191	10.2	117	6.4
8			8	1.1	6	0.7			95	5.0	88	4.8
9			6	0.9	3	0.3			44	2.3	41	2.2
10			3	0.4	3	0.3			27	1.4	18	1.0
11			1	0.1	2	0.2			14	0.7	9	0.5
12			3	0.4	1	0.1			2	0.2	3	0.2
13									1	0.1		
Total	750		709		890		818		1881		1814	
Average	2.3±0.63		4.3±1.56		3.8±1.29		2.3±0.76		5.1±1.80		4.5±1.94	

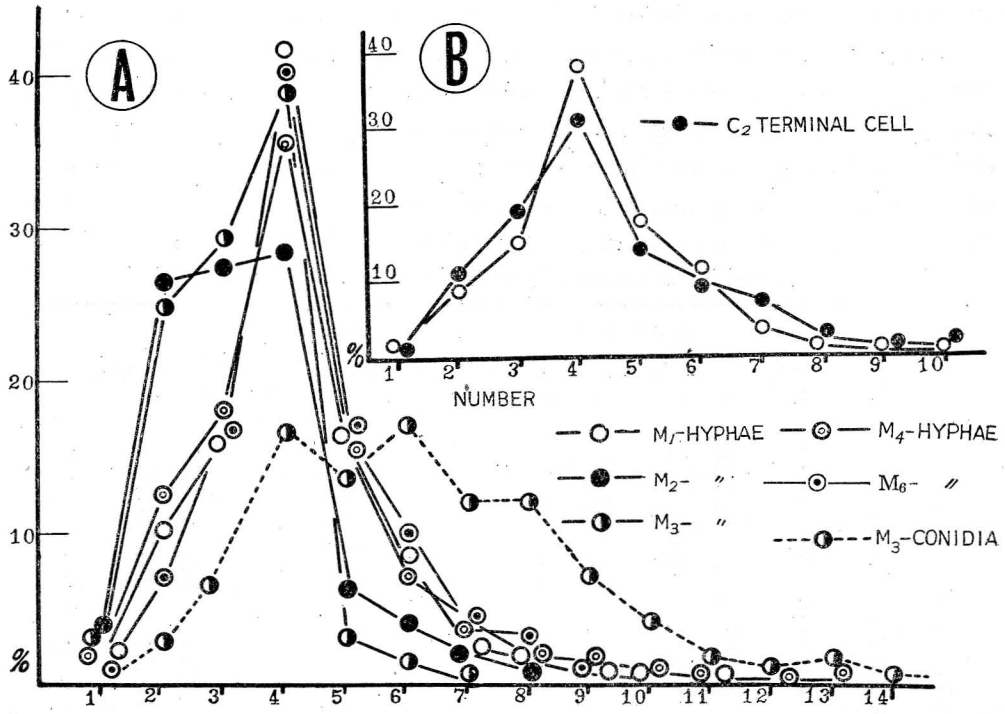

Fig. 1. Number of nuclei in one cell

Chromosomes are arranged on the equational plate in the metaphase, and according to the figures inserted as Fig. 3-A the chromosomal number is assumed to be n=2 in C_1 culture.

2) The nuclear number in mycelial cells. The number is counted with the thin mycelia cultured in Richards' nutrient, in those thin mycelia the nuclei are arranged in one line. The results of those observations are inserted in the following table and figures (Tab. 2 and Fig. 1, 2).

Two is the mode of nuclear number of C_1-culture which reaches at 60 in percentage. The percentage curve of nuclear number of C_1-culture falls rapidly to 15-8 % at 3, and again merely rises to 15-10 % at 4. The curves of the other have not so clearly distinguishable peaks as that of C_1, but those curves reach to the maximun at 4, namely 4 is the mode. The curves of M_1, M_2 and M_6-cultures are resemble each other in the lower percentage at 2 and 3 nuclei. and in the higher at 5 nuclei. The range of nuclear number is more narrow in C_1 than the others. The range is most wide in the conidiospore of M_3.

The distribution of nuclear number dose not vary according to the nutritional conditions (cf. Tab. 3). In the terminal cells the distribution of nuclear are somewhat different from the cell of the other parts (cf. Fig. 1-B and 2-B). C_1-culture greatly decrease in number of cells which contain 1 and increases cells contain 3 and es-

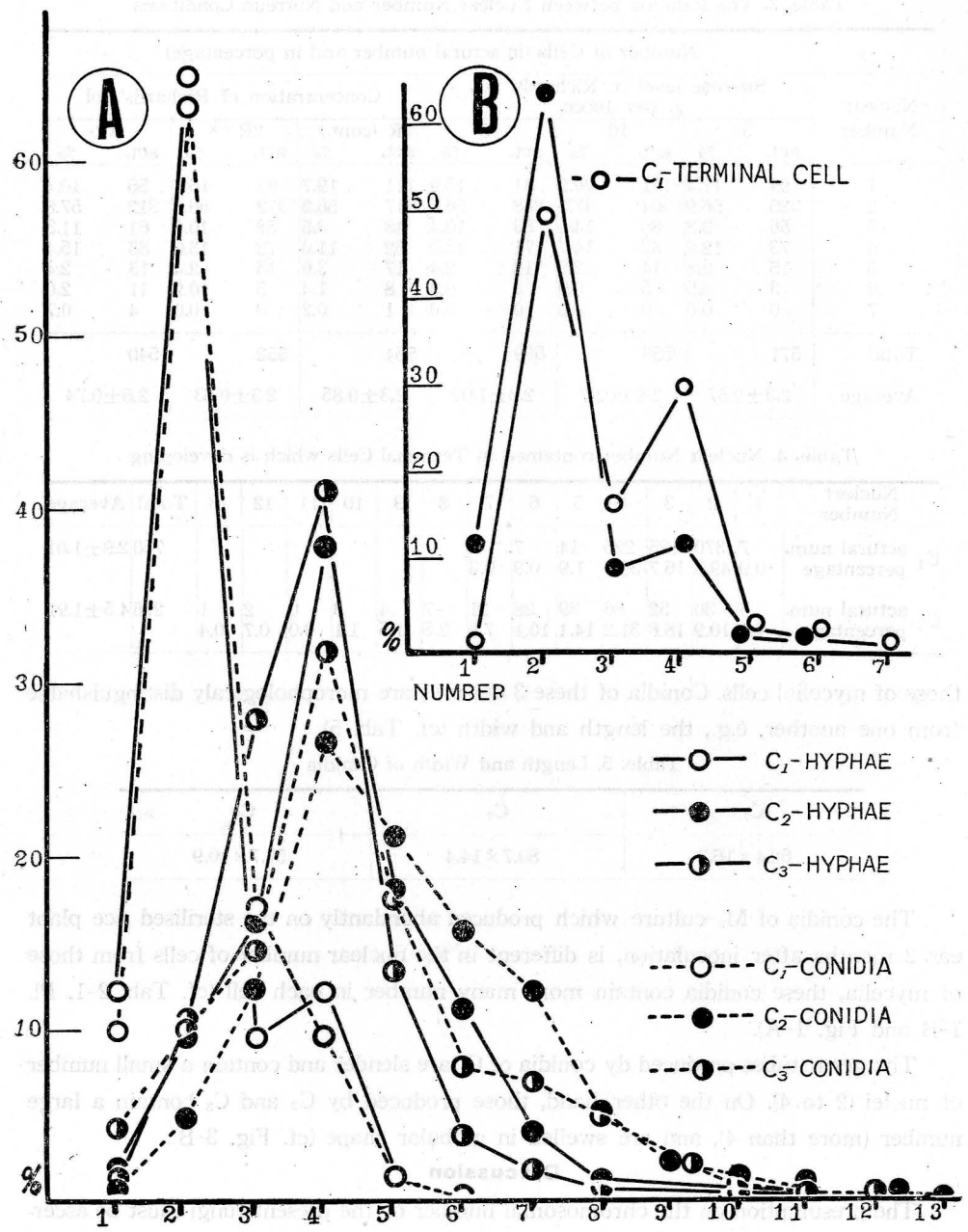

Fig. 2. Number of nuclei in one cell

pecially 4 in terminal cells. This trend is not so markedly observed with C_2 as C_1.

3) The nuclear number of conidia. With these cultures of Conidial types, the nuclear is observed, and those results are inserted in Tables and Figures (Tab. 2-1, 2 and Fig. 1, 2). The distribution curves of those number have a trend resembl to

Table. 3. The Relation between Nuclear Number and Nutreint Conditions

Nuclear Number	Number of Cells (in actural number and in percentage)											
	Sucrose level in Richards' sol. g. per 100cc.						Concentration of Richards' sol					
	5		10		15		R (cont.)		2R××		4R	
	act.	%	act.	%	act.	%	act.	%	act.	%	act.	%
1	99	17.4	111	20.0	81	15.9	111	19.7	92	16.7	56	10.4
2	325	56.9	264	47.5	288	56.6	317	56.2	312	56.5	312	57.8
3	56	9.8	80	14.4	53	10.4	48	8.5	58	10.5	61	11.3
4	73	12.8	82	14.7	71	13.9	62	11.0	72	13.0	83	15.4
5	15	2.8	14	2.5	12	2.4	17	3.0	13	2.4	13	2.4
6	3	0.5	5	0.9	4	0.8	8	1.4	5	0.9	11	2.0
7	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	4	0.7
Total	571		556		509		564		552		540	
Average	2.3±0.57		2.4±0.27		2.3±1.02		2.3±0.85		2.3±0.63		2.5±0.74	

Table. 4. Nuclear Number contained in Terminal Cells which is developing

Nuclear Number		1	2	3	4	5	6	7	8	9	10	11	12	13	Total	Average
C_1	actural num.	7	370	125	225	14	7	2							750	2.9±1.01
	percentage	0.9	49.3	16.7	30.0	1.9	0.9	0.3								
C_2	actural num.	3	30	52	86	39	28	21	7	4	3	0	2	1	276	4.5±1.91
	percentage	1.1	10.9	18.8	31.2	14.1	10.1	7.6	2.5	1.5	1.1	0.0	0.7	0.4		

those of mycelial cells. Conidia of these 3 cultures are morophologicaly distinguishable from one another, e.g., the length and width (cf. Tab. 5).

Table. 5. Length and Width of Conidia

C_1	C_2	C_3
62.4×16.3	80.7×14.4	57.7×10.9

The conidia of M_3-culture which produced abundantly on the sterilised rice plant ear 2 months after inoculation, is different in the nuclear number of cells from those of mycelia, these conidia contain more many number in each cell (cf. Tab. 2-1, Pl. 1-B and Fig. 1-A).

The germ-tubes produced dy conidia of C_1 are slender and contain a small number of nuclei (2 to 4). On the other hand, those produced by C_2 and C_3 contain a large number (more than 4), and are swelled in globular shape (cf. Fig. 3-B).

Discussion

The presumption on the chromosomal number of the present fungi must be ascertained with more detailed observations. But with this experiment many those figures suggesting that the number is 2 are comparatively clearly observed.

Each mycelial and conidial cell usually contain from 2 to 4 nucIei, ranging from 1 to 13 (exceptionally reach to 19), and this number is somewhat greater than that of observed by Tachinai and Sakamoto[7], who reported as follows, "Each mycelial cell of Hel. Oryzae contained usually 2 to 3, ranging from 1 to 8".

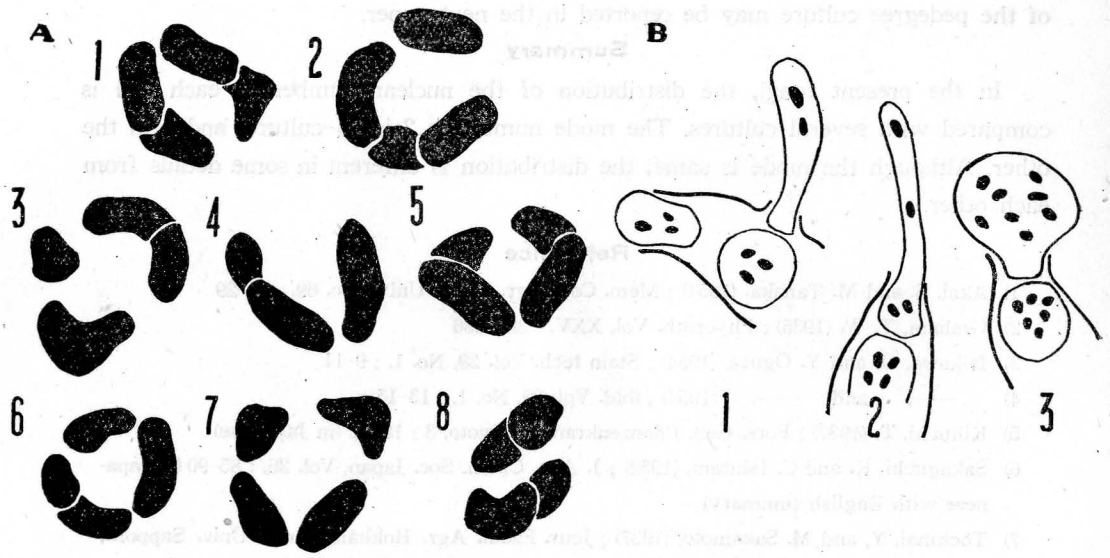

Fig. 3. Chromosomes and the type of germ-tube.
A, the figures suggesting 2 chromosomes (×ca. 15000)
B, 1~2 are germ-tubes of C_1 and 3 is of the others (×ca. 2000)

It is ascertained that C_1-cultre is different in nuclear number of each cell, and in the nuclear behavior in early stage of hyphal develpment from conidia. It is probable that the culture, which is different in nuclear number as C_1, specializes in some cultural behaviors. Previously Graham[2] assumed that the difference in number could easily account from some difference in characters of different cells. But the physiological speciaiization of the present fungi is not determined only by the nuclear number, that is, the cultures which are clearly distinguishable from each other on several media in cultural behaviors, have a resemble trend in nuclear number. On this point, Tochinai and Sakamoto observed that, "But at the same time there could not be observed any difference in the number of nuclei contained in the cells of mother strains and its saltant".

The most common type of the distribution of nuclear number is to be determined, but in this experiment it is impossible because of the insufficiency in cultures obtained from many other districts.

In the Koji molds, it is observed that[6], "The conidia of the constant strains have been found to contain 1–4 nuclei, the majiority two nuclei. The conidia of inconstant strains, however, are exceedingly multinucleate (8–20 or more),", and the following assumption is presented, "It might be assumed that the variavirity of the inconstant strains of the Koji molds chiefly depends upon the multinucleate property of the conidia.". In this experiment those exceedingly multinucleated cultures are found too.Although the pedegree cultures of those are not tested, the saltants do not so frequently appear in C_1-culture than in the other multinucleated ones. The details

of the pedegree culture may be reported in the next paper.

Summary

In the present fungi, the distribution of the nuclear number in each cell is compared with several cultures. The mode number is 2 in C_1-culture, and 4 in the other. Although the mode is same, the distribution is different in some details from each other.

Reference

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3) Itikawa, O. and Y. Ogura, (1954) ; Stain tech. Vol. 29, No. 1, : 9-11
4) ——, and ——, (1954) ; ibid. Vol. 29, No. 1, : 13-15
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摘　　要

後藤岩三郎：稲胡麻葉枯病の研究 (第6報)
病原菌の核現象について (1)

フオイルゲン染色法により，菌の核を観察した．その染色にはシツフ試薬の pHを 3.2～3.5 に保つことが重要であつた．

C_1においては核の数は2の場合が最も多く60%にも達した．その他では4が多かつたが，そのパーセンテージはそんなに高くはならなかつた．4をモードにする系統においても核数の分布に多少の差はみられたが，培養基の上では顕著にその外観が異つているものを含んでいる．

染色体数は更に検討を要するが n=2 ではないかと思われる．

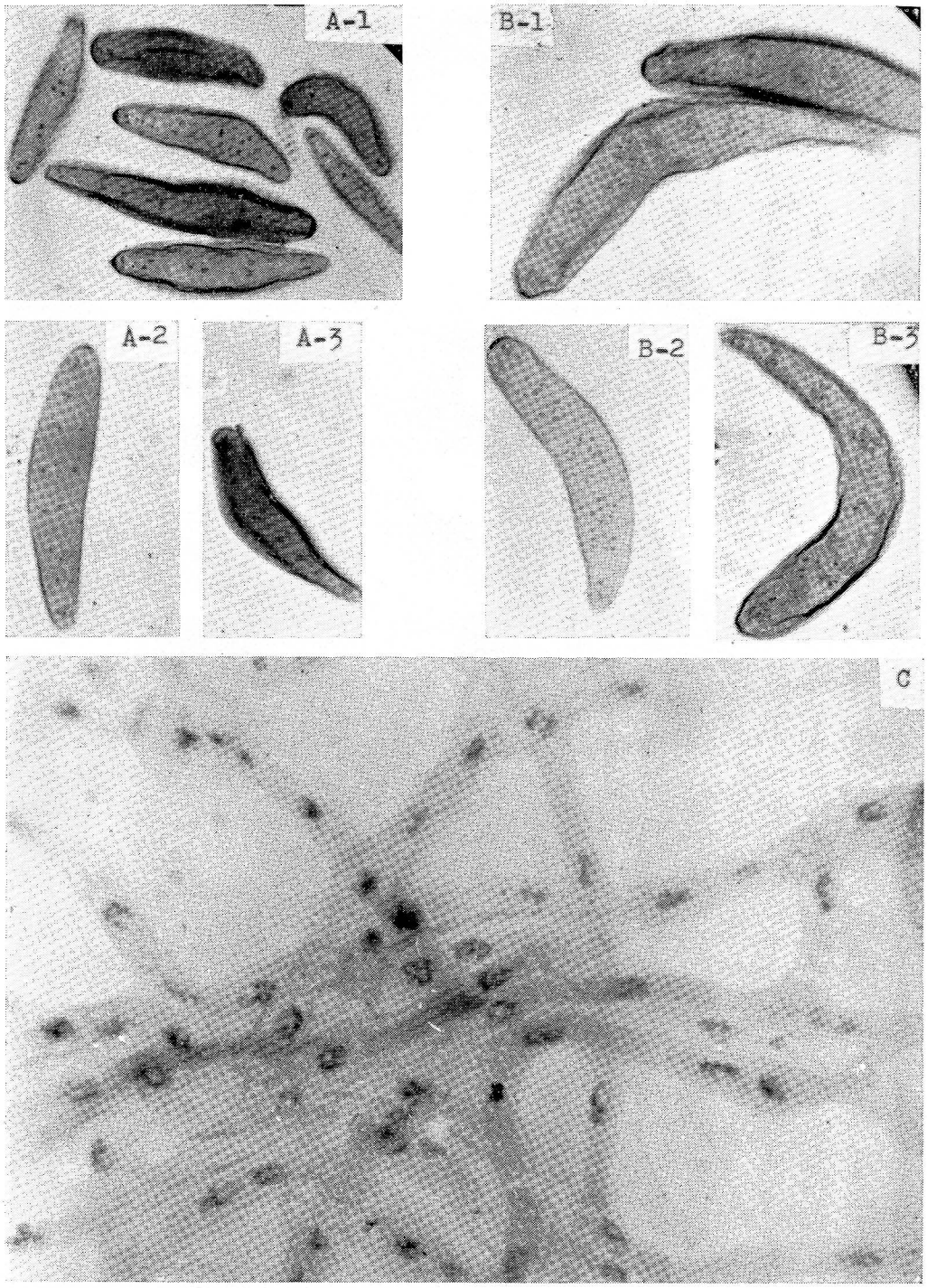

Plate-1

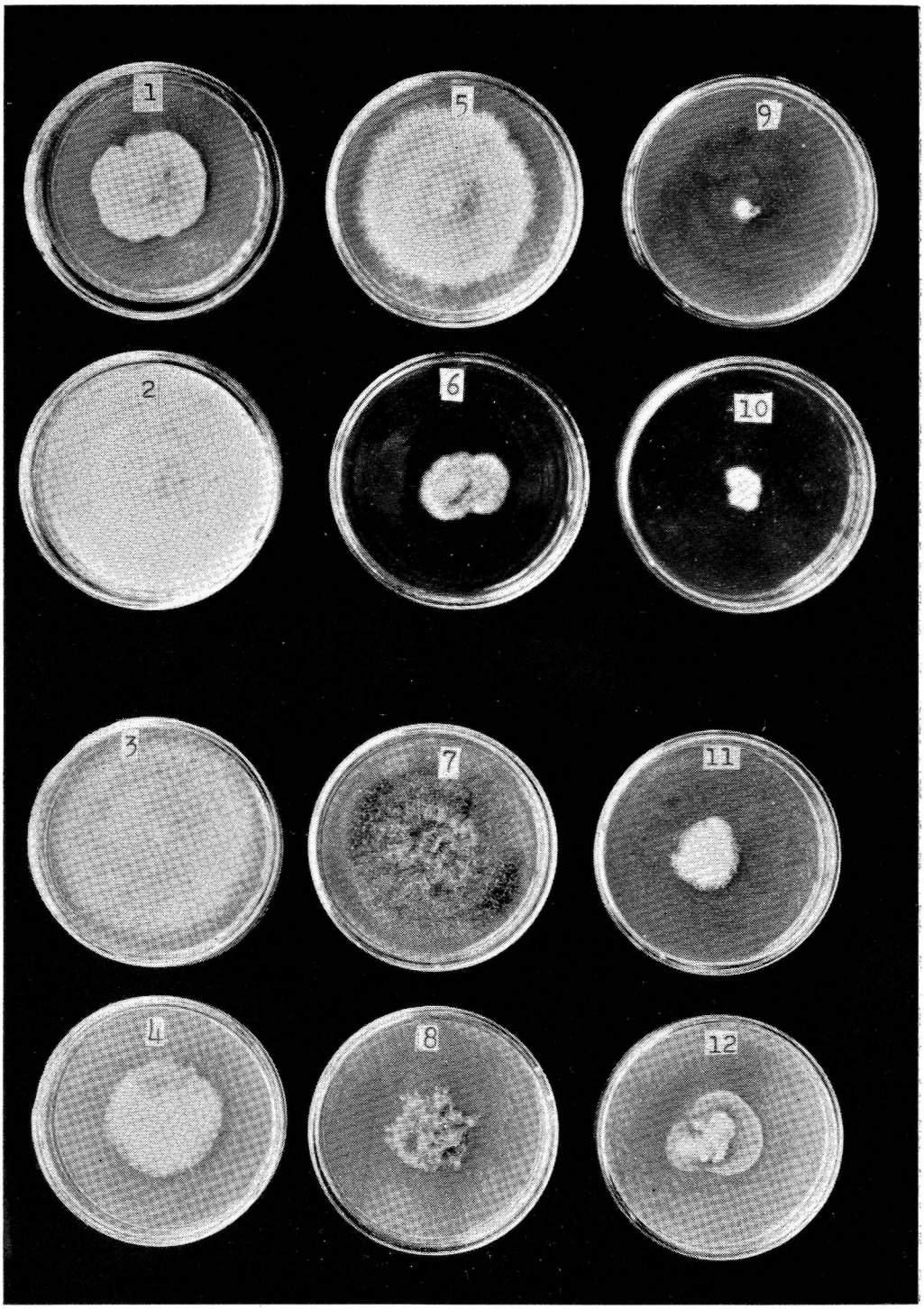

Plate-2

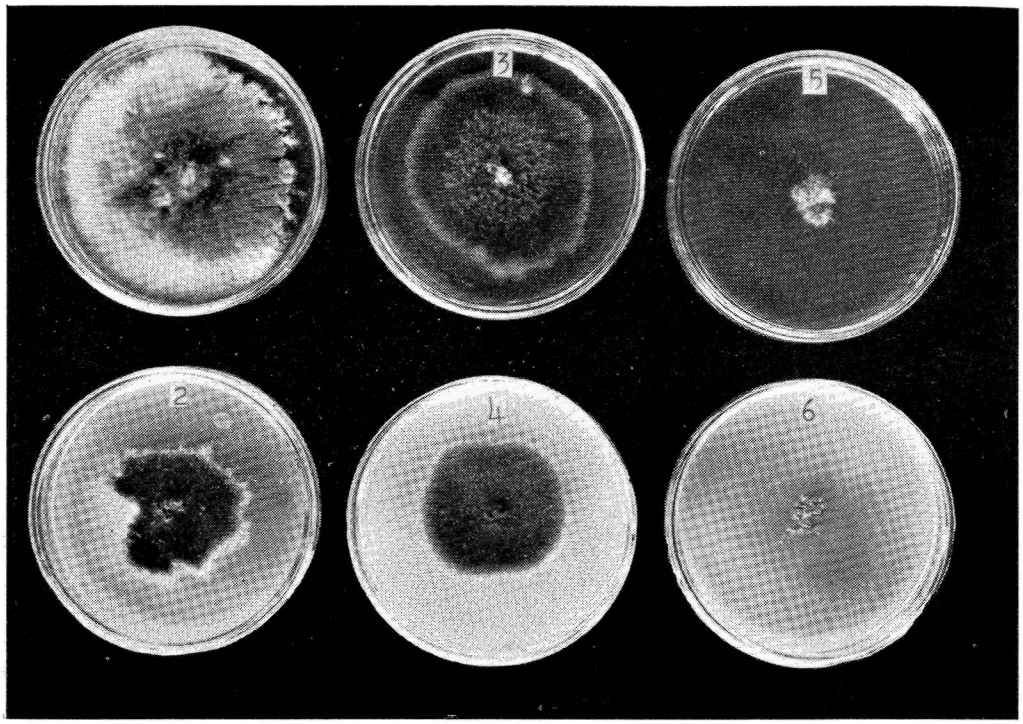

Plate-3

Explanation of Plate

Plate 1. A is conidiospore of C_1 and B is of M_3 (× ca. 700). C is hyphae of C_1, showing the various steps of nuclear division (× ca. 2500).

Plate 2. 1-4 are M_1; 5 is M_3, 6 is M_2 and 7-8 are M_4; 9-12 are M_6. First rank (1, 5, 9) is cultured on Richards' nutrient agar, 2nd on Saito's soy agar, 3rd on rice culm decoction agar, 4th on potato decoction agar.

Plate 3. 1-2 are C_1, 3-4 are C_2 and 5-6 are C_3. Upper rank is cultured on Richards' nutreint agar, under is on Saito's soy agar.