On several factors which affect the conidial characteristics of *Fusarium solani* (MART.) APP. et WR. I On the concentration of constituents of media, temperature and duration of incubation.

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Contents

Introduction

In culturing fungi, their characteristics are influenced greatly by cultural conditions. Studies regarding to the variations of fungal characteristics affected by altering cultural conditions, however, are relatively scanty. So, the writer observed how much degree the variations of fungal characteristics were revealed, culturing *Fusarium solani* (*Mart.*) APP. et WR. under various conditions. At the same time, he wanted to determine the cultural conditions convenient for identification of this fungus.

As for cultural conditions, a great many factors may be considered, i.e. kinds, concentration, constituents and hydrogen-ion concentration of media, temperature and duration of incubation, etc. In this paper, the writer wanted to report the influence upon the conidial characteristics affected by altering the concentrations of carbon and nitrogen sources in media, temperature and duration of incubation.

In pursuing this study, the writer expresses his hearty thanks to Prof. T. INOKUMA for his encouragement and to Dr. K. ITO for the advantages he kindly afforded in presentation of many *Fusarium* strains.

Material and methods

a). Material used.

Fusarium solani (MART.) APP. et WR., used in this study, was presented to

the writer from Dr. K. Itô, which was presented to the latter from Faculty of Agriculture, University of Kyûshû.

This fungus forms conidia abundantly on usual potato decoction agar. Microconidia are elliptical and macroconidia are usually 0-3-septate, relatively thick walled. Septa are clear. Curvature of conidia is scarce or not, and a foot cell is obscure. Both ends of conidia are roundish.

In this study, this fungus was cultured from mono-sporous origin and the stock culture was grown on potato decoction agar by mass transferring from mono-sporous culture above mentioned in turn, and left in room temperature.

b). Experimental methods.

Brown's "synthetic potato dextrose" agar[1] (dextrose, 2 gm., potato starch, 10 gm., asparagine, 2 gm., K_3PO_4, 1.25 gm., $MgSO_4$, 0.75 gm., agar, 17 gm., distilled water, 1000 ml.) was used as the standard medium throughout this study.

Experiment A.

1. Glucose-series: Variation of macroconidial characteristics affected by altering glucose percentage in media.

According to the results of preliminary experiment, only glucose concentrations in media were prepared at the grades of 0, 0.2, 0.5, 2.0, 5.0 and 10.0 per cent, respectively, while the other constituents were fixed to the standard. On these media that were poured into Petri dishes and autoclaved, the inocula grown from mono-sporous origin were inoculated and kept at 20°C. On the 15th and 30th day after inoculation, a part of fungal colonies was taken and stained by ruthenium red.[2)3)] Microscopic observations were carried out and measured the conidial sizes on these stained samples.

At each time, the samples were taken at about the same place of each plate.

2. Asparagine-series: Variations of macroconidial characteristics affected by altering asparagine percentage in media.

According to the results of preliminary experiments, only asparagine concentrations in media was prepared at the grades of 0, 0.2, 0.5 and 2.0 per cent, respectively, while the other constituents were fixed to the standard. On these media that were poured into Petri-dishes and autoclaved, the inocula grown from monnsporous origin were inoculated and incubated at 20°C. On the 15th and 30th day

after inoculation, a part of fungal colonies was taken, and treated in the same procedures as in glucose-series.

3. Temperature-series: Variations of macroconidial characteristics affected by altering temperatures of incubation.

Assuming that temperature of incubation may affect conidial characteristics, the writer pursued following experiments.

Fusarium solani, which was previously cultured by means of single spore culture, was inoculated on the standard medium and incubated at 13°C., 20°C. and 30°C., respectively. Then a part of fungal colonies was taken after the 15th and 30th day. and treated in the same procedures as in glucose-series.

Experiment B.

In these series in experiment A, various conidial characteristics appear as shown later in the results. In order to clarify the facts that these variations of size and shape of conidia are attributed to alteration of the cultural conditions, the suspensions of conidia produced on the media of 0 and 10 per cent in glucose-series, 0 and 2 per cent in asparagine-series, and at 13° and 30°C. in temperature-series were made and every loop of these suspensions was inoculated on the standard medium and incubated at 20°C.

On the 15th and 30th day after inoculation, a part of fungal colonies was taken and treated in the same procedures as in experiment A.

In addition, the measurements of all experiments above mentioned were made on 3- and 1-septate conidia that have great important roles in identification of this fungus.

Results obtained

Experimental results will be shown briefly as follows:

Table I. Conidial sizes appeared in glucose-series. (μ)

Glucose %	3-septate conidia			1-septate conidia		
	Ranges appeared in length	Ranges appeared in width	Mean	Ranges appeared in length	Ranges appeared in width	Mean
0	39.2~28.0*	5.6~4.2	31.5×4.9	28.0~16.8	4.9~3.5	23.2×4.5
	40.6~26.6**	4.9~4.2	31.8×4.6	25.2~16.8	4.2~3.5	20.7×4.1
0.2	36.4~26.6	5.6~4.2	30.6×4.9	26.6~19.6	4.9~4.2	22.2×4.5
	39.2~25.2	5.6~4.2	29.7×4.5	25.2~16.8	4.2~3.5	20.4×4.2

0.5	39.2~26.6 37.8~22.4	5.6~4.2 4.9~4.2	30.2×4.6 31.4×4.5	25.2~16.2 25.2~16.8	4.2~3.5 4.9~3.5	21.4×4.1 20.9×4.2
2.0	35.0~25.2 39.2~25.2	5.6~4.2 4.9~4.2	29.3×4.9 30.6×4.3	23.8~15.4 23.8~15.4	4.2~3.5 4.2~3.5	19.9×4.1 19.0×4.1
5.0	No conidia produced.			26.6~15.4 28.0~15.4	5.6~4.2 5.6~3.5	22.0×4.9 21.1×4.2
10.0	No conidia produced.			25.2~15.4 21.0~12.6	6.3~4.2 4.2~3.5	18.9×5.5 16.9×3.6

* Value at 15th day. Fifty conidia were measured at random.

** Value at 30th day.

Table Ⅱ. Conidial sizes appeased in asparagine-series.

Asparagine %	3-septate conidia			1-septate conidia		
	Ranges appeared in length	Ranges appeared in width	Mean	Ranges appeared in length	Ranges appeared in width	Mean
0	36.4~26.6 40.6~26.6	5.6~4.2 4.9~4.2	30.8×4.9 32.6×4.5	22.8~15.4 25.2~15.4	4.9~3.5 4.2~3.5	19.7×4.1 19.9×3.9
0.2	36.4~26.6 39.2~25.2	5.6~4.2 5.6~4.2	31.1×4.8 30.5×4.5	28.0~16.8 25.2~15.4	4.9~3.5 4.2~3.5	22.3×4.0 19.9×3.8
0.5	39.2~29.4 No conidia produced.	6.3~4.9	32.8×5.3	28.0~19.6 25.2~15.4	4.9~3.5 4.2~3.5	23.0×4.4 20.4×4.0
2.0	No conidia produced.			No conidia produced. 28.0~15.4	4.9~3.5	21.8×4.0

Table Ⅲ. Conidial sizes appeared in temperature-series.

Temperature °C	3-septate conidia			1-septate conidia		
	Ranges appeared in length	Ranges appeared in width	Mean	Ranges appeared in length	Ranges appeared in widsh	Mean
13	No conidia produced.			33.6~19.6 30.8~18.2	4.9~3.5 4.9~4.2	27.0×4.0 22.5×4.3
20	35.0~26.6 35.0~23.8	4.9~3.5 4.9~4.2	29.5×4.4 29.8×4.6	26.6~18.2 25.2~15.4	4.9~3.5 4.2~2.8	21.4×3.7 21.1×3.8
30	37.8~28.0 36.4~28.0	4.9~3.5 4.9~4.2	32.1×4.2 31.9×4.6	25.2~18.2 29.4~16.8	4.2~2.8 4.2~3.5	20.7×3.5 21.3×3.9

Table Ⅳ. Conidial sizes appeared, when cultured conidia which produced under the various cultural conditions in experiment A, under the same condition.

Concentration & Temperature in Experiment A	3-septate conidia			1-spetate conidia		
	Ranges appeared in length	Ranges appeared in width	Mean	Ranges appeared in length	Ranges appeared in width	Mean
Glucose 0%	36.4~25.2 35.0~26.6	5.6~4.2 5.6~4.2	29.7×4.7 30.0×4.8	26.6~16.8 25.2~15.4	4.9~3.5 4.9~3.5	21.0×4.2 20.9×4.2
Glucose 10%	35.0~25.2 37.8~26.6	5.6~4.2 5.6~4.2	29.1×4.7 29.8×4.9	25.2~16.8 26.6~15.4	4.9~3.5 4.9~3.5	20.9×4.2 20.3×4.2

Asparagine 0 %	36.4～25.2 39.2～26.6	4.9～4.2 5.6～4.2	30.2×4.8 30.4×4.7	25.2～18.2 25.2～16.8	4.2～3.5 4.9～3.5	21.1×4.2 21.0×4.2
Asparagine 2 %	37.8～26.6 37.8～26.6	5.6～4.2 5.6～4.2	30.7×5.2 30.5×4.9	26.6～16.8 25.2～15.4	4.9～3.5 4.9～3.5	21.4×4.6 21.3×4.4
Temperature 13°C.	36.4～25.2 36.4～25.2	5.6～4.2 5.6～4.2	30.2×4.9 30.3×4.9	25.2～16.8 25.2～15.4	4.2 4.9～3.5	21.3×4.2 20.5×4.4
Temperature 30°C.	36.4～25.2 36.4～25.2	5.6～4.2 5.6～4.2	30.2×5.1 30.4×5.0	25.2～18.2 23.8～15.4	4.9～4.2 4.9～3.5	21.4×4.3 20.5×4.3

According to these tables, following figures will be given:

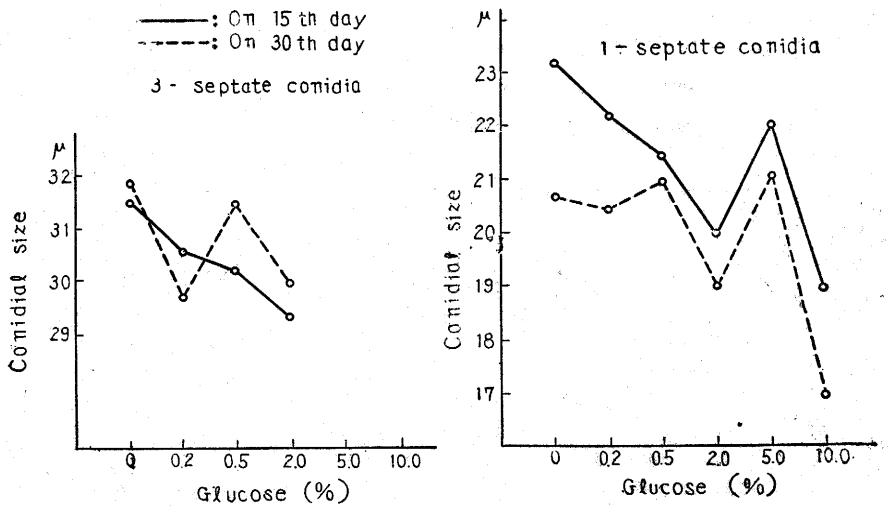

Fig. 1 The meae values of conidial sizes in glucose-series.

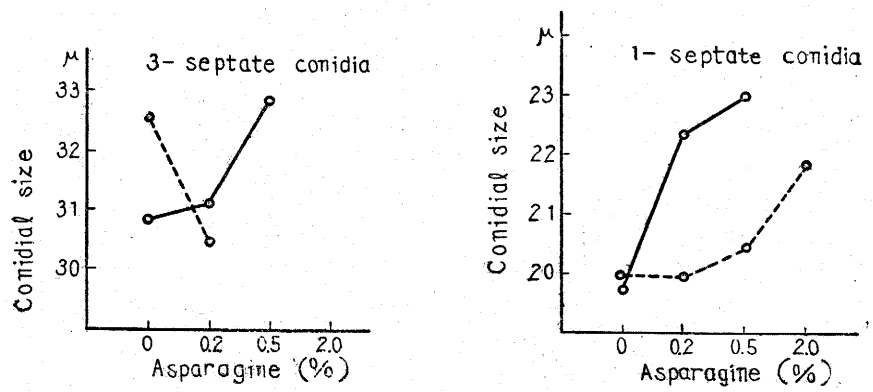

Fig. 2 The mean values of conidial sizes in asparagine-series.

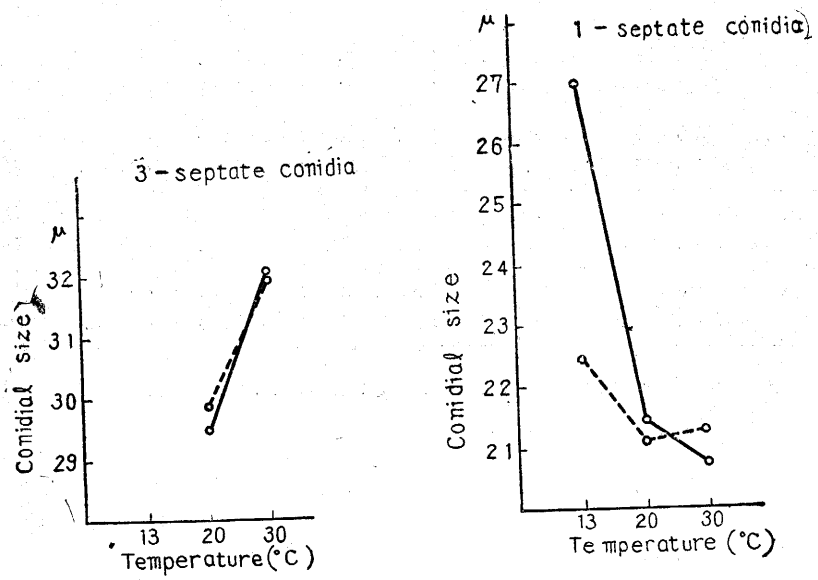

Fig. 3 The mean values of conidial sizes in temperature-series.

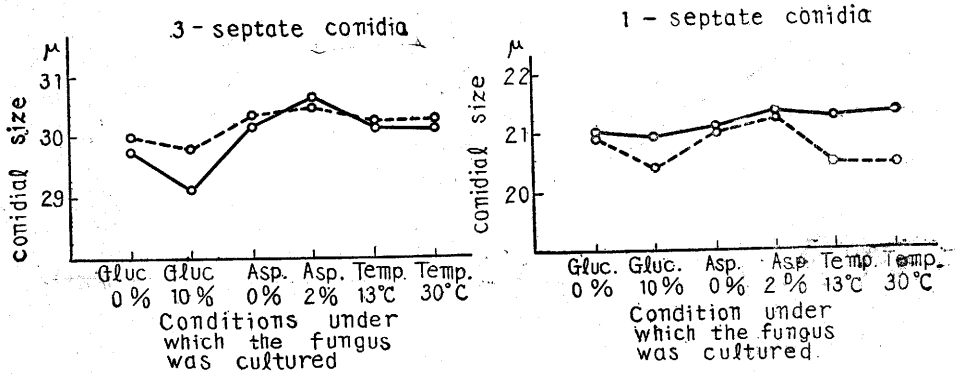

Fig. 4 The mean values of conidial sizes appeared under the same condition.

Discussion

Since carbon and nitrogen sources in media have great influences upon growth and sporulation of fungi, it is assumed naturally that conidial size and shape of Fusaria will be influenced by altering the concentrations of these sources. LILLY and BARNETT[4] investigated that a proper balance among the constiuents of media was quite important in growth and sporulation. WESTERGAARD and MITCHELL[5] ascertained that carbon-nitrogen ratio (C:N ratio) of media affected the formation of perithecia by *Neurospora crassa* and that high concentrations of glucose and potassium nitrate were unfavorable for the production of perithecia by this fungus.

According to the writer's results, in high concentrations of glucose and aspa-

ragine, the fungus tends to become sterile. These facts agree with the results obtained by these authors.

HARTER[6] investigated that the conidial formation of *Fusarium solani* did not occur at 9.1°C., a few abnormal occurred at 14°C., many were produced at 18.4 °C. and typical occurred at 22.4°C.

In glucose-series, the higher the concentrations of glucose in media, the smaller the size of conidia. When the concentrations of glucose reach 5 per cent, 3-septate conidia cease to be formed. On the other hand, relatively large 1-septate conidia appear at that concentration. But at the concentration of 10 per cent, 3-septate conidia do not occur as in concentration of 5 per cent, and 1- snd 0-septate conidia become to smallest throughout this series. It is a considerable fact that the formation of 1-septate conidia at that concentration of glucose is scanty and almost all conidia have no septum, which are the smallest as mentioned above.

In conidial shape, there is no variation between the concentrations of 0 and 2 per cent of glucose, but conidial width tends to become broader against its length between 5 and 10 per cent. As the results, the conidial shape appears somewhat roundish.

Contents of conidia are generally rich in granular substances. When the concentrations of glucose reach about 5 per cent, volume and size of granules become larger and sometimes oil drops appear. At 10 per cent, contents of conidia are occupied by one to many large oil drops. Thus considerable differences in conidial characteristics occur between the conidia that have been produced on the two sorts of media, 0 and 10 per cent in the concentration of glucose.

On the other hand, at about 0.5 per cent in concentration of asparagine-series, 3-septate conidia cease to appear. This fact shows that asparagine affects stronger than glucose upon septal formation of conidia.

As has been shown in figures, the mean values of conidial size tends to become smaller in glucose-series, but this tendency cannot be recognized in asparagine-series, in accordance with the increase of concentrations.

It has been ascertained in preliminary experiments that temperature of incubation influences upon formation and size of conidia. In temperature-series, the shape of conidia is irregular at 13°C. as has been described by HARTER. It is thought

that this phenomenon is mainly attributed to the disturbance of formation of septa. Instead of absence of 3-septate conidia at that temperature, 1-septate conidia which have the size of nearly the same as 3-septate conidia, appear among 1-septate conidia that have usual size. These large sized 1-septate conidia are slightly smaller than normal 3-septate conidia, suggesting that the conidia, which may become 3-septate in normal condition, remain 1-septate, and if one measures normal 1-septate conidia only except these large abnormal ones, he will obtain the smallest value among any other conidia throughout this series. As the results, it may be acceptable that conidial size has a tendency to become larger with the increase of temperature of incubatien among the range of proper temperatures.

HORNE and MITTER[7] investigated that the degree of septation tended to decrease in accordance with increase of concentrations of glucose and asparagine, and granules in conidia became more numerous in propotion to the increase of concentrations of glucose. The writer's results agree almost exactly with theirs. At the same time, they stated, regarding to the periods of culture as time factor, that the degree of septation reached maximum on the 15th day, then decreased gradually. In writer's present experiment, it was unable to observe the effect of duration of culture except the case in higher concentrations of asparagine -series.

To ascertain whether the variations of conidial size and shape, appeared under various cultural conditions, are due to altering concentritions of constituents of media, the author practiced the experiment B. The cultures grown from these suspensions are very similar in growth and conidial formation, and no significant difference was observed in conidial size and shape.

From the results above mentioned, it is recognizable that the conidial characteristics are affected by the concentration of constituents of media and the temperature of incubation.

When compared the mean values of conidial size appeared in writer's experiments with those expressed by WOLLENWEBER and REINKING[8], the former is somewhat smaller than the latter, but the ranges of conidial size coincide with each other.

In addition, since this fungus forms 3- to 0-septate conidia, it is desirable to use media on which 3-, or less septate conidia, are produced abundantly. From

this point of view, in connection with the fact that the balance of carbon and nitrogen sources is one of the important cultural conditions, it may be concluded that 0.2 per cent ef glucose and asparagine as carbon and nitrogen sources should be used for culture of this fungus. Namely, the standard medium above mentioned is desirable to this fungus.

Summary

1. Conidial size tends to be small in accordance with the increase of concentions of glucose in media, and at 5 per cent in concentrations of glucose, 3-septate conidia cease to be formed. In addition, at high concentrations of glucose in media, granules appear abundantly and at 10 per cent, granules give place to oil drops.

2. 3-septate conidia cease to be formed when the concentrations of asparagine in media reach 0.5 per cent. Definite tendency of variation in conidial size and shape cannot be recognized.

3. Though irregularly large conidia appear at 13°C., it is recognized that the conidial size tends to increase in accordance with the rise of temperature of incubation.

4. Influence of duration of incubation upon conidial size and shape is obscure.

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フザリウム・ソラニー菌の分生胞子の性質に影響を及ぼす培養条件について

(摘　要)

(第Ⅰ報) 培地の組成の濃度，培養温度及び培養期間について

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菌類を培養する場合，菌類の現す性質は培養条件を様々に変へる事によつて著しい影響を受ける。菌類に対する培養条件としては非常に多くの因子が考へられるが，本報に於ては培地の炭素源及び窒素源の濃度の変化，培養温度及び培養期間の変化が *Fusarium solani* の conidia の性質，特に conidia の大さ及び内容に及ぼす影響に就て実験を行つた結果を簡単に述べる。

1. glucose-series として標準培養基中の glucose だけを様々の濃度に変化させた場合に現れる conidia の性質の変化を観察した。此の場合 glucose の濃度が高くなるにつれて conidia の大さは，小さくなる傾向を示し，濃度が 5 % に至ると 3-septate conidia の形成が止む。同時に元来顆粒質に富む conidia の内容は，濃度が 10 % になると，油滴が之に代つて現れて来る。

2. asparagine の濃度を 1. と同様に色々に変へた場合，0.5 % で 3-septate conidia の形成が止むが，濃度の増加と之に伴つて生ずる conidia の大さとの関係は明瞭でない。conidia の形成に対しては，asparagine は glucose よりも影響する所大である如くである。

3. 温度と conidia の大さとの関係は，温度の上昇と共に conidia が大きくなる傾向が見られる。

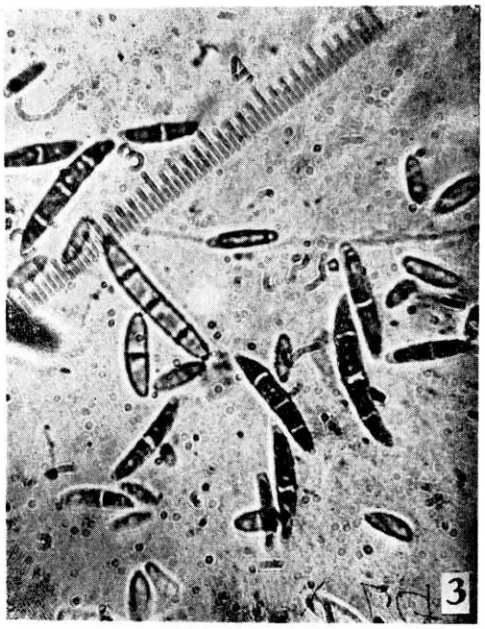
4. 培養期間の conidia の性質に及ぼす影響は明瞭でない。

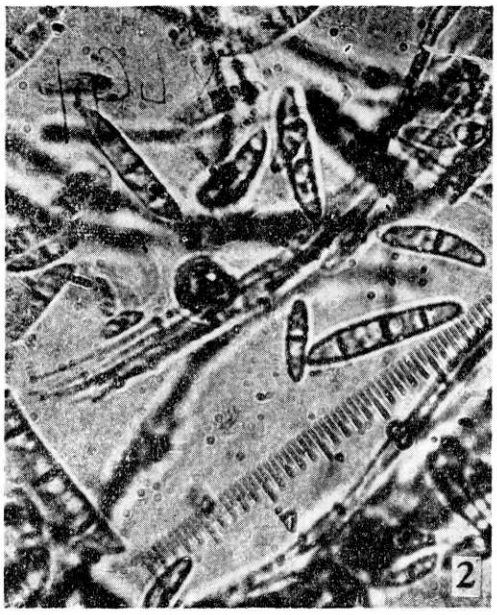
なほ，本研究の費用の一部は，文部省科学研究助成補助金によつた。記して謝意を表する。

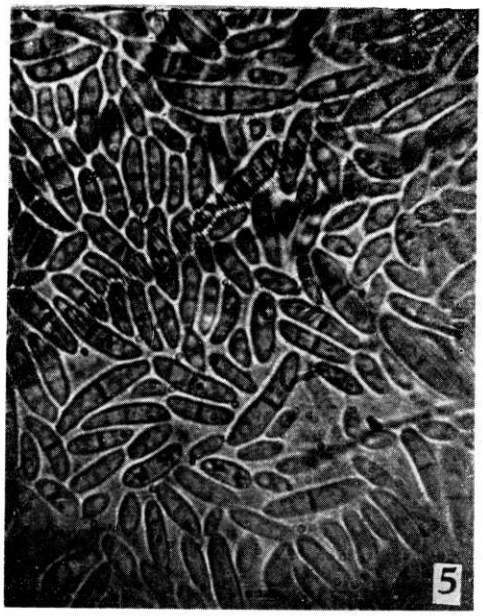
Explanation of the Plate Ⅱ, Ⅲ (×ca, 600)

1. Conidia produced on the standard medium.
2. Conidia produced on the medium containing no glucose.
3. Conidia produced on the medium containing 0.5 per cent of glucose.
4. Conidia produced on the medium containing 2.0 per cent of glucose.
5. Conidia produced on the medium containing 5.0 per cent of glucose.
6. Conidia produced on the medium containing 10.0 per cent of glucose.
7. Conidia produced on the medium containing no asparagine.
8. Conidia produced on the medium containing 0.5 per sent of asparagine.

Plate II



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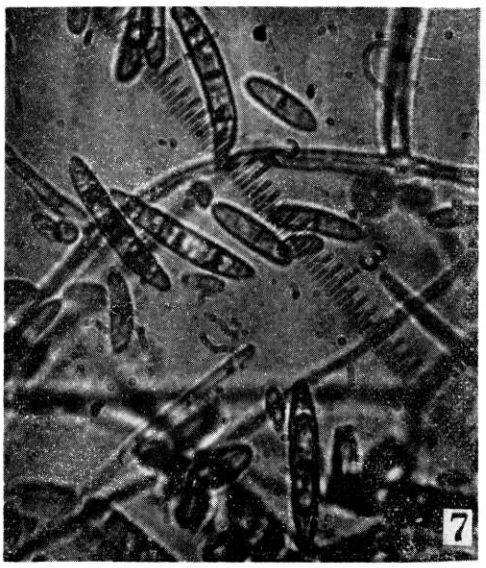
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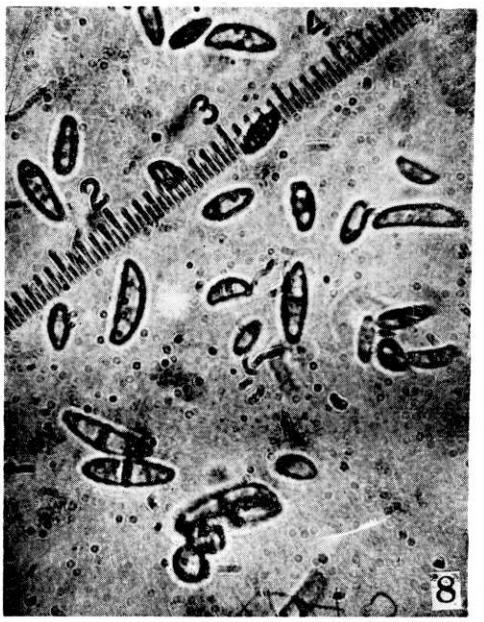
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