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The influence of nutrition on sporangial formation in *Araiospora streptandra*.

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UNIVERSITY OF LOUISVILLE

THE INFLUENCE OF NUTRITION ON SPORANGIAL FORMATION
IN ARAIOSPORA STREPTANDRA

A Dissertation

Submitted to the Faculty

Of the Graduate School of the University of Louisville

In Partial Fulfillment of the

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Of Master of Science

Department of Biology

By

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on Sporangial Formation in
Araspora streptandra

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The writer wishes to acknowledge his indebtedness to Dr. Harlow Bishop for his constant interest and **helpful** criticism.

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INTRODUCTION

The genus Araiospora presents an unusual complication of the morphology of its sporangia. It alone of the family Leptomitaceae possesses two types of sporangia, one with a smooth outer wall and the other with a conspicuously spiny outer wall. The question as to the possible significance of these two types of sporangia in the life history has been considered only by von Minden (1916) in his study of Araiospora spinosa. Von Minden concluded that smooth and spiny sporangia were formed under favorable nutritive conditions. This interpretation is singularly unconvincing since it is at variance with the widely accepted theories of Klebs, who believed that sporangia were formed under unfavorable nutritive conditions. The writer, on securing a pure culture of Araiospora streptandra, felt that an opportunity had presented itself by which a more complete understanding could be obtained of the functions of the two types of sporangia in the life history of this species of Araiospora.

HISTORY

The genus Araiospora, belonging to the family Leptomitaceae, was first established by Thaxter (1896) with his discovery and description of A. pulchra. The genus is distinguished by its two types of sporangia--one spiny and one smooth, the presence of an enlarged basal cell, and the formation of a layer of hexagonal periplasmic cells that surround the oospore. Thaxter (1896) transferred Cornu's Rhipidium spinosum (1872) to this new genus, naming it A. spinosa. Von Minden (1916) obtained a pure culture of A. spinosa. He described in detail the development of the fungus and the effect of various nutrient media on its growth. Linder (1926) described a new species from British Guiana. This species, A. coronata, was the first record for the genus from the tropics. Kervorkian (1934) established a new species, A. streptandra, found near Cambridge, Massachusetts, and at Kingston, Rhode Island.

The finding of A. streptandra in Jefferson County, Kentucky, is a new record for the genus. A. streptandra has hitherto been recorded only in Massachusetts and Rhode Island.

DESCRIPTION

DESCRIPTION OF A. STREPTANDRA (Kervorkian)

The mycelium of the plant consists of a large cylindrical basal cell with many branches arising from the apex. The hyphae is narrowed at frequent intervals into so-called constrictions. The sporangia are of two types, borne terminally or laterally, singly or in whorls of 2 to 6: (1) oblong to broadly oval and smooth, 25 to 100 X 25 to 40 microns (Plate I, figures 1-5); or (2) oval to pyriform, 50 to 125 X 35 to 50 microns, with numerous spines, 8 to 40 microns in length and sharply pointed to conical in shape (Plate II, figures 9-15). Antheridia are borne singly on short lateral branches, twisted around the stalk of the oogonium, and are irregular in outline. The oogonia are spherical, arising on short lateral branches near antheridial stalk in groups of 1 to 4 and are 50 to 70 microns in size. The oospores are spherical and surrounded by a single layer of hexagonal peripheral cells (Plate I, figures 6-8).

PLACE OF COLLECTION

Material which proved to contain Araiospora streptandra was gathered at Mantle's farm in Jefferson County, Kentucky, on April 23, 1939. Collection was made from a pond bordered by a dense woods; consequently, the water contained a heavy deposit of leaves and twigs. Previous experience has shown that such material was favorable for securing collections of water moulds. Material consisting of twigs and leaves was placed in Mason jars filled with water from the pond.

Examination of this material at the laboratory failed to reveal any fungal growth. Numerous zoospores were seen, however, and wheat grains were added as bait in an attempt to secure growth. The primary growth on the wheat proved to be a species of Pythium surrounded by many bacteria. After 7 days A. streptandra appeared. Contamination with bacteria was so bad in the gross culture that isolation into pure culture was made.

METHOD OF ISOLATION INTO PURE CULTURE

A grain of wheat from the gross culture was placed in a petri dish containing tap water. By examination with a binocular microscope, a single spiny sporangium of Araiospora streptandra was located. This sporangium was then placed in a petri dish containing S.A.1 agar.¹ After 24 hours examination by low power of the microscope showed that the sporangium was germinating by hyphal out-growth. Bacteria were still intermingled with the mycelium. Consequently, the petri dish was placed at a 70 degree slant, since experience has shown that bacteria do not grow well against gravity, while fungi do. Transfer of peripheral tips of mycelium was made to fresh S.A.1 agar. Six such transfers were made over a period of thirty days.

Then a portion of mycelia was placed in a 2 percent peptone solution, since bacteria grow well in this solution and give it a characteristic, cloudy appearance. After 24 hours examination showed that a pure culture had been obtained. Stock cultures were made from portions of the pure culture using test tube slants of S.A.1 agar.

1. Bishop (1937). S.A.1 agar formula: Levulose - 2 percent, Dextrose - 2 percent, Proteose peptone - 1 percent, Knop's solution - 0.1 percent, Agar - 1 percent.

CULTURAL METHODS

GROWTH ON WHEAT GRAINS

Several sterile wheat grains were placed in a petri dish containing 45 cc. of sterile distilled water. A portion of mycelium from the stock culture was placed in the petri dish with the wheat.

The first period of growth on the wheat was vegetative. Smooth sporangia appeared after 4 days and continued to form for 2 days. Spiny sporangia were observed after 6 days and continued to form for 7 days. Oogonia and oospores appeared 13 days after beginning of growth.

GROWTH ON CHERRY TWIGS

A sterile twig of Prunus serotina (1 year old) about one-half inch long was placed in a petri dish containing 45 cc. of sterile distilled water. Inoculum from the stock culture was placed in the petri dish.

Vegetative growth lasted 4 days followed by the appearance of smooth sporangia. This type of sporangia continued to form for 7 days. The first spiny sporangia formed after 11 days and continued to form for 7 days. Oogonia and oospores appeared 18 days after beginning of growth.

GROWTH ON NUTRIENT AGAR MEDIA

Corn meal agar proved to be unsatisfactory, since growth was entirely vegetative. Bishop (1937), in his work on Sapromyces, used S.A.1 agar and succeeded in inducing mycelial, sporangial and oogonial development on this medium. Because of the close taxonomic relationship between Araiospora and Sapromyces, S.A.1 agar was used in the culture of A. streptandra. Spiny sporangia formed in abundance, but no smooth sporangia or oospores were formed. This species of Araiospora has a tendency to heap up in its growth on solid media, making direct examination difficult, because of the denseness of mycelial growth. This difficulty was not encountered in using a liquid medium, since the mycelium tended to spread out thinly, making possible thorough and complete observation of all branches of the mycelium. An additional reason for the use of a liquid medium was that it more closely approximated the conditions under which the fungus lives in nature. For these two reasons, (namely, ease of examination and nearest semblance to natural conditions) the writer made extensive use of liquid medium for pursuing his study of developmental stages of A. streptandra.

GROWTH IN PEPTONE

Von Minden (1916) employed various concentrations of peptone in cultivating A. spinosa. Using his procedure, the fungus was grown, in each case in a 10 cc. volume of peptone which was placed in 125 cc. Erlenmeyer flasks. The following results were obtained:

In 10 percent peptone there was heavy mycelial growth for 6 days. From the 6th to the 14th day, the hyphae developed exceptionally dense cytoplasm and increased in diameter. No sporangia were formed and all growth ceased after 14 days.

In 5 percent peptone there was vegetative growth for 4 days. After 7 days spiny sporangia developed in small numbers. No change was observed after an additional 7 days.

In 3 percent peptone there was vegetative growth for the first 3 days. After 5 days many spiny sporangia were formed. There was no change observed after an additional 7 days.

In 2 percent peptone there was vegetative growth for the first 3 days. After 5 days numerous spiny sporangia developed. No change was observed after 7 more days.

In 1 percent peptone there was vegetative growth for one day. Numerous sporangia developed after 2 days. No appreciable change was observed after 12 days.

In 0.5 percent peptone there was no appreciable lapse of time between appearance of vegetative growth and the formation of spiny sporangia. There were fewer spiny sporangia formed than in 1 percent peptone. No change was observed after a lapse of 14 days.

In 0.25 percent peptone results were the same as those obtained in 0.5 percent peptone.

In 0.125 percent peptone spiny sporangia appeared after 24 hours. After 14 days growth, an average of 25 spiny sporangia were present.

In 0.06 percent peptone spiny sporangia appeared in 24 hours. After 14 days growth, an average of 10 spiny sporangia were present.

In 0.015 to 0.03 percent peptone only vegetative growth occurred. The hyphae were smaller than normal, and there was an absence of the characteristic constrictions of the hyphae.

The results of growth in peptone established the following tentative hypotheses: (1) no sporangia develop in a high concentration (10 percent), or in low concentrations (0.015 to 0.03 percent) of peptone; and (2) spiny sporangia

appear consistently in concentrations of peptone of 0.06 to 5.0 percent. Optimum conditions for their formation appear in a concentration of 1 percent peptone.

GROWTH IN WATER FOLLOWING TRANSFERENCE FROM PEPTONE

In the following experiments, tufts of mycelia were transferred each time from varying percentages of peptone to petri dishes containing 45 cc. of sterile distilled water.

When the mycelium was transferred from 10 percent peptone to distilled water, no sporangia were formed. The plant had no appearance of viability.

After transference from 5 percent peptone to distilled water, no increase was observed in the number of spiny sporangia which had previously been formed in the 5 percent peptone. No emergence of zoospores occurred.

Upon transference to distilled water of material grown in 3 percent peptone, no emergence of zoospores was noted.

Following transference from 2 percent peptone to distilled water, no increase in the number of spiny sporangia previously formed in 2 percent peptone was observed. Zoospore emergence from a small

percentage of spiny sporangia took place after 3 days growth in water.

As a result of transference from 1 percent peptone to distilled water, emergence of zoospores from spiny sporangia occurred in 2 days. After 6 days numerous smooth sporangia developed. Discharge of zoospores from the smooth sporangia occurred in 24 hours.

Transference from 0.5 percent peptone to distilled water resulted in emergence of zoospores of spiny sporangia in 2 days. After 5 days growth of the mycelium, smooth sporangia developed. Zoospore emergence took place in 24 hours.

After transference from 0.25 percent peptone to distilled water, emergence of zoospores from spiny sporangia occurred in 2 days. After 4 days smooth sporangia developed. Discharge of zoospores from smooth sporangia occurred in 24 hours.

On transference from 0.125 percent peptone to distilled water, spiny sporangia discharged zoospores in 2 days. After 4 days smooth sporangia developed. Zoospore emergence from smooth sporangia occurred in less than 24 hours.

Following transference from 0.06 percent peptone to distilled water, spiny sporangia discharged zoospores after 2 days. Smooth sporangia began to

appear within 24 hours. Constant observation showed emergence of zoospores from the smooth sporangia in 12 to 24 hours.

The results of drastic reduction of nutrition brought out the following points: (1) zoospore emergence from spiny sporangia in material transferred to distilled water occurred only in concentrations of peptone from .06 to 2.0 percent; (2) zoospore emergence from spiny sporangia occurred in 48 to 72 hours; (3) smooth sporangia appeared for the first time after transfer to distilled water of mycelium grown in concentrations of peptone from 0.06 to 1.0 percent; and (4) zoospore emergence from smooth sporangia occurred in 12 to 24 hours.

GROWTH ON WHEAT IN WATER FOLLOWING TRANSFERENCE OF MATERIAL FROM PEPTONE

In the following experiments sterile grains of wheat were added to petri dished containing 45 cc. of sterile distilled water, and inoculation was made from different concentrations of peptone. Care was taken to prevent any direct contact between the wheat and the material used for inoculation.

When the material was transferred from 10 percent peptone to water and a grain of wheat was added, no growth appeared on the wheat after 22 days.

Transfer of material from concentrations of peptone from 3 to 5 percent brought about the same lack of change.

Following transference from 2 percent peptone to water containing wheat, no growth was established until after the sixth day.

After transfer from 1 percent peptone, growth appeared on the wheat in 4 days. A second grain of wheat was added after the appearance of smooth sporangia. Growth was established on the second grain in 2 days.

On transference from 0.5 percent peptone, growth appeared on the wheat in 2 days.

Growth appeared on the wheat in 2 days, also, upon transference from 0.25 percent peptone.

After transference from 0.125 percent peptone, growth again appeared on the wheat in 2 days.

Following transference from 0.06 percent peptone, growth appeared on the wheat in 36 hours.

The results of growth on wheat following transfer of material grown in various peptone concentrations to distilled water containing wheat

brought out the following factors: (1) no growth was established on wheat when added to a petri dish containing material transferred to distilled water from concentrations of peptone from 3 to 10 percent; (2) growth was established on wheat when added to a petri dish containing material transferred to water from concentrations of peptone from 0.06 to 2.0 percent; and (3) growth was established on wheat after 36 to 72 hours in the case of material transferred to water from peptone concentrations from 0.06 to 1.0 percent.

METHOD OF MAKING PERMANENT MOUNTS

Although much of the observation of material grown under various nutritive conditions was made directly, using low power of the microscope, it was found advisable to use prepared slides for detail. All slides made were stained with acid fuchin and nigrosin, dissolved in one-fifth strength lacto-phenol. This procedure yielded excellent results, since the acid fuchin stains the cytoplasm, and the nigrosin brings out the nuclei. The use of one-fifth strength lacto-phenol was adhered to because there was practically no distortion of the hyphae or reproductive organs.

All drawings from prepared slides were made with a Spencer camera lucida, using a 10 X ocular end and a 4 mm. objective. Each division of the scale equals 25 microns.

INTERPRETATION OF RESULTS

The influence of nutrition on the formation of sporangia was carefully worked out by Klebs (1899) on Saprolegnia mixta. He found that sporangia were formed as a result of reduction of nutrition. This reduction he brought about in two ways: (1) sporangia are formed when the mycelium is grown in a dilute solution which is further weakened as the fungus uses up the available nutrition; and (2) sporangia are formed following drastic reduction of nutrition as a result of transfer of well nourished mycelium to pure water.

The application of Klebs' principles to the genus Araiospora is complicated by the presence of two types of sporangia. Von Minden (1916) considered this problem in his research on Araiospora spinosa (Cornu) Thaxter. He concluded that exhaustion of nutrient matter was not the cause of sporangial formation, since both smooth and spiny sporangia appeared when the mycelium was well nourished.

As a result of the writer's work on Ariospora streptandra, the conditions favoring the formation of smooth sporangia appear to be in general harmony with Klebs' theory. The details of the process of sporangial formation are different in

A. streptandra and S. mixta. In S. mixta the the sporangia are produced either by reduction of concentration of nutrient or by transfer of well nourished mycelia to water. In A. streptandra reduction of concentration of nutrient does not induce the formation of smooth sporangia. Smooth sporangia are produced only by transfer of mycelium to distilled water.

The culture of A. streptandra on wheat grains in water presents a composite picture of developmental events. The following steps occur in a definite sequence: (1) vegetative growth, (2) production of spiny sporangia. The first period of growth of A. streptandra on wheat grains is vegetative. The hyphae are small in size and the cytoplasmic contents are not dense. This condition can probably be accounted for by the small amount of nutrition which has been made available by enzyme activity. This supposition is supported by the results of growth in peptone, since in weak concentrations (Table I) the hyphae were identical in appearance to those formed during vegetative growth on wheat. Smooth appear after 4 days, presumably because enzyme activity has made more nutrient matter available. Coupled with the formation of smooth sporangia, the hyphae have

become larger in diameter and more dense. Spiny sporangia finally appear after 6 days. By this time enzyme activity has probably made a greater amount of the nutrient matter available, as indicated by the extreme softness of the wheat grain.

The formation of spiny sporangia of A. streptandra cannot be readily explained by Klebs' theories. Spiny sporangia develop in solutions of peptone of widely varying concentrations (Table I). However, it will be noted that the relative number of sporangia developed in different solutions varies according to a definite pattern. There is an increase in number of spiny sporangia formed as the concentrations of peptone passes from .06 to 2.0 percent. The opposite phenomenon is observed as the concentration of peptone is further increased, since fewer spiny sporangia are formed in 5 percent than in 3 percent peptone. Evidently the spiny sporangia require a considerable amount of nutrition for their formation. As additional food is made available, the number of spiny sporangia is increased up to an optimal point. In higher concentrations of nutrient, there is a more and more pronounced tendency for potential sporangia to become vegetative outgrowths. Finally, in 10 percent peptone, only

vegetative growth occurs. It is, therefore, apparent that a more complicated relationship exists between nutrition and the formation of spiny sporangia of Araiospora than Klebs found in the case of the sporangia of S. mixta. There is an optimal set of conditions and two minimal sets of conditions for the formation of spiny sporangia.

The conditions for the discharge of zoospores from spiny sporangia are quite similar to those affecting the discharge from smooth sporangia. As a rule, when the material bearing spiny sporangia is transferred to water, discharge of zoospores takes place within 48 hours. A greater percentage of spiny sporangia are discharged when the mycelium has been previously grown in .06 percent peptone than when the mycelium was grown in 2 percent peptone. Moreover, there are consistent gradations between these two limits. Therefore, the discharge of zoospores from spiny sporangia is favored by the growth of the mycelia in the weaker concentrations of peptone. One outstanding fact is the exceptionally long time required for the opening of spiny sporangia in 2 percent peptone. The failure of spiny sporangia to discharge zoospores in concentrations of peptone above

2 percent is difficult to explain. Harper (1899) pointed out that vacuoles fuse together to form cleavage furrows in the zoospore formation. If we accept Harper's theory, we may suppose that the extreme density of the protoplasm in spiny sporangia formed in 3 percent peptone, or above, interferes with the formation of vacuoles necessary for sporangial cleavage. The exact role of spiny sporangia of A. streptandra is uncertain. They seem to be more resistant and are, perhaps, comparable to the gemmae, or resistant bodies, formed by other water moulds.

A definite role can be assigned to the smooth sporangia of A. streptandra on the basis of the writer's experiments. The extreme readiness with which they liberate zoospores, coupled with their formation in large numbers under conditions of low nutrition, indicates that they are the chief means of dispersal. The appearance and discharge of smooth sporangia under conditions unfavorable for active growth enables the fungus, by means of its zoospores, to reach new, favorable substrata.

SUMMARY

1. The discovery of Aralospora streptandra in Kentucky is a new record for the genus. Hitherto, it has been found only in Massachusetts and Rhode Island.

2. A pure culture of A. streptandra was obtained by selecting a single sporangia and eliminating contaminants by sub-cultures. Although A. spinosa has been cultivated by von Minden (1916), the first pure culture of A. streptandra (as far as available literature indicates) is here recorded.

3. Spiny sporangia are induced under favorable nutritive conditions (.06 to 5 percent peptone).

4. Smooth sporangia are induced as a result of drastic reduction of nutrition by transfer of mycelium grown in .06 to 1 percent peptone to water.

5. Zoospore discharge by the smooth sporangia is in all cases more rapid than that of the spiny sporangia.

6. The smooth sporangia are the chief means of dispersal to a more favorable substrata.

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TABLE I
Formation and Discharge of Sporangia
under Varying Nutritive Conditions

SPINY SPORANGIA					SMOOTH SPORANGIA			
In peptone					After transfer to water			
Concen- tration of pep- tone by percent	No. of days vega- tative growth	No. :formed:	No. :dis- :charged:	Time :required: :for dis- :charge	No. :formed:	No. :dis- :charged:	Time :required: :for dis- :charge	
0.03	: X	: 0	: 0	: 0	: 0	: 0	: 0	
0.06	: 1	: *	: ***	: 48 hrs.	: ***	: ***	: 12-24 hrs.	
0.125	: 1	: *	: ***	: "	: **	: **	: 24 hrs.	
0.25	: 1	: **	: **	: "	: **	: **	: "	
0.5	: 1	: **	: **	: "	: *	: *	: "	
1.0	: 1	: ***	: *	: "	: *	: *	: "	
2.0	: 3	: **	: *	: 72 hrs.	: 0	: 0	: 0	
3.0	: 3	: **	: 0	: 0	: 0	: 0	: 0	
5.0	: 4	: *	: 0	: 0	: 0	: 0	: 0	
10.0	: X	: 0	: 0	: 0	: 0	: 0	: 0	

Explanation of symbols:

X - continuous vegetative growth

0 - none

* - degree of response:

*** - most pronounced degree

** - intermediate degree

* - least degree

EXPLANATION OF PLATE I

- FIGURE 1. Smooth sporangium formed after transfer from .12 percent peptone. The spores have all emerged. Note the lateral position on hypha.
- FIGURE 2. Smooth sporangium formed after transfer from .06 percent peptone. Note the zoospore stuck in the exit tube.
- FIGURE 3. Smooth sporangia from material grown on wheat. Note the spore germinating through the exit tube.
- FIGURE 4. Smooth sporangia formed after transfer from .06 percent peptone. All zoospores have emerged.
- FIGURE 5. Smooth sporangia following growth on Prunus.
- FIGURE 6. Young stage in oogonial formation. Note the twisting of the antheridium (on the left) around the oogonial stalk
- FIGURE 7. Older stage in oospore formation. Note the beginning of the formation of the thick wall around the periphery of the oospore.
- FIGURE 8. Mature oospore. Note the thick wall around the oospore.

PLATE I

EXPLANATION OF PLATE II

- FIGURE 9. Spiny sporangium formed in 1 percent peptone.
- FIGURE 10. Spiny sporangium formed on wheat after 6 days growth.
- FIGURE 11. Spiny sporangium formed as a result of growth in 2 percent peptone.
- FIGURE 12. Spiny sporangium formed on wheat after 5 days growth. The zoospores have been discharged.
- FIGURE 13. Spiny sporangium formed on wheat after 4 days growth. Note maturing zoospores.
- FIGURE 14. Spiny sporangium formed in 1 percent peptone. Note its lateral position.
- FIGURE 15. Spiny sporangium formed on wheat 8 days growth. Note its lateral position.

PLATE II

100 μ