JOURNAL OF BACTERIOLOGY
Vol. 88, No. 6, p. 1624-1628 December, 1964

CARBOHYDRATE NUTRITION AND SPORULATION OF *ALLESCHERIA BOYDII*[1]

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Received for publication 3 August 1964

ABSTRACT

CAZIN, JOHN, JR. (University of Iowa, Iowa City), AND DAVID W. DECKER. Carbohydrate nutrition and sporulation of *Allescheria boydii*. J. Bacteriol. 88:1624-1628. 1964.—The influence of various carbohydrates on the growth and sporulation of *Allescheria boydii* was studied by incorporating the test substance in a medium consisting of the inorganic salts mixture contained in Czapek Dox medium with 2% agar. The data indicate that a wide variety of carbohydrates serve adequately as the sole source of carbon for *A. boydii*, and for its imperfect form, *Monosporium apiospermum*. Of those compounds tested, only raffinose and inulin were not assimilated by any of the organisms; one strain differed from all others tested in not being able to utilize sucrose. Of the ascigerous strains tested, those maintained in stock culture for a long period of time were able to grow somewhat more profusely on the various carbohydrates; recently isolated strains produced ascocarps in greater abundance. In contrast to reports appearing in the literature, these organisms are capable of assimilating mannitol, maltose, and lactose.

In a study of mycetoma in North America, Boyd and Crutchfield (1921) reported isolation of an organism which possessed both a conidial and an ascigerous stage. This organism was submitted for identification to C. L. Shear who considered it a previously undescribed species, and who proposed the name *Allescheria boydii*. The following year, Shear (1922) published a complete description of the organism and suggested that it is related to the same general group of ascomycetes to which Eurotium belongs. Emmons (1944) demonstrated that *Monosporium apiospermum* (Saccardo, 1911), a member of the form family Moniliaceae of the Deuteromycetes, is the imperfect form of *A. boydii* (Shear, 1922).

[1] Reported in part at the 64th Annual Meeting of the American Society for Microbiology, Washington, D.C., 3-7 May 1964.

Though *A. boydii* is an infrequent infectious agent for human beings, it has been well established by the studies of Ajello (1956) that the organism is widespread in nature. Consequently, one might expect that sporadic infections by this organism are likely to occur. Although this organism has usually been associated with maduromycosis in man, we are now aware that it may also cause other types of infections.

Travis, Ulrich, and Phillips (1961), in an article on pulmonary allescheriasis, cited a number of references in which *A. boydii* was incriminated in otomycosis, septicemia, meningitis, and pulmonary infection. *M. apiospermum* was isolated from a corneal ulcer by Pautler, Roberts, and Beamer (1955), and characteristic hyphae and conidia were seen in tissue sections. Meyer and Herrold (1961) reported on repeated isolation of *A. boydii* from urine and prostatic secretions of a patient suffering from chronic prostatitis.

We have identified *A. boydii* isolated from tissue of human beings on two separate occasions. One isolation of the organism was made from tissue obtained from a cavity in the right upper lobe of a lung. A second isolate, which was submitted to us for identification, was from brain tissue of an 8-year-old child with meningitis. In confirming the identity of these two isolates of *A. boydii*, we noted that one of the organisms differed from our stock strains in its apparent inability to assimilate sucrose.

Relatively little is known about the nutrition of this organism, or about factors which influence its sporulation. Gordon (1957) reported that organic and inorganic nitrogen compounds serve equally well as a sole source of nitrogen for *A. boydii*, and that the organism is autotrophic for its growth factors. He also confirmed the results of Benham and Georg (1948) that rich sources of organic nitrogen stimulate the production of conidia, but inhibit the production of ascocarps.

Wolf, Bryden, and MacLaren (1950) were the first to demonstrate that *M. apiospermum* was

autotrophic for its vitamins; they also showed that the organism is able to utilize a wide variety of sugars, sugar alcohols, amino acids, and organic acids as carbon sources during a 2-week test period. Furthermore, they found that the organism was nonexacting in its nitrogen requirement, being able to utilize nitrate, ammonium, or amino nitrogen. The organism was shown to have an optimal pH of 7.0 to 7.6, and the optimal temperature for growth was 30 C.

For the past 2 years during our studies on *Allescheria* and *Monosporium* spp., we have maintained one set of our stock cultures on Sabouraud Dextrose Agar (Difco), and another set on Czapek Dox (Difco) agar. All of our stock strains exhibit typical gross morphology on the Sabouraud medium, and all except one strain exhibit typical morphology on the Czapek Dox medium. Because of the limited amount of information available concerning carbohydrate nutrition of these organisms, and the apparent inability of one of our strains to assimilate sucrose, all of our stock cultures were tested further to compare their ability to utilize a variety of different carbohydrate sources.

MATERIALS AND METHODS

The sources of our stock cultures used in this study are shown in Table 1. Although we recognize that the official name of these organisms is *A. boydii*, we have chosen to refer to strains 804 and 806 as *M. apiospermum*, because we have been unable to demonstrate the ascigerous stage of these particular isolates. This is fully in accord with Article 59, Section 4, Chapter V of the *International Code of Botanical Nomenclature*, (Kemink en Zoon, Utrecht, 338 p.).

In experiments designed to determine whether a specific carbohydrate can serve as the sole source of carbon, the test carbohydrate was included in a medium consisting of the inorganic salts mixture contained in Czapek Dox medium: carbohydrate, 15 g; $NaNO_3$, 3 g; K_2HPO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; agar (Agar Products Co.), 20 g; distilled water, 1,000 ml. This medium was sterilized in the autoclave at 121 C for 10 min, and on cooling allowed to solidify as slants. Similar media were prepared by adding sterile (filtered) carbohydrate solutions to Czapek Dox salts-agar, which had been autoclaved and cooled to 50 C.

The test media were inoculated with a spore suspension of the organisms prepared from a 2-week-old culture in the following manner. A sample (4 to 5 × 4 to 5 mm) of mycelial growth was cut from the surface of a Czapek Dox (3% sucrose) agar slant, and placed in a sterile test tube containing 2 ml of saline. The mycelial mat was broken into small pieces with a stiff wire needle, and the tube was shaken to dislodge spores. When grossly visible particles in the saline settled, a sample of the supernatant fluid was removed with a 5-mm bacteriological inoculating loop, and spread over the surface of medium to be tested. This procedure served as an adequate source of inoculum for all strains, including one strain which grew sparsely on the medium. All cultures were incubated at room temperature.

Comparative growth readings were made visually at the end of 4, 8, and 12 weeks, and lactophenol cotton blue slide mounts were prepared from each tube and examined microscopically for the presence of characteristic structures.

TABLE 1. *Source of cultures studied*

Organism	Source
Allescheria boydii 800	Mercy Hospital, Iowa City, Iowa. 1958.
A. boydii 801	V. A. Hospital, Iowa City, Iowa. Culture no. 53. (C.D.C. culture no. 471.) 1958.
A. boydii 802	V. A. Hospital, Iowa City, Iowa. Culture no. 54. (C.D.C. culture no. 191.) 1958.
A. boydii 803	V. A. Hospital, Iowa City, Iowa. Culture no. 55. 1958.
Monosporium apiospermum 804	V. A. Hospital, San Fernando, Calif. Culture no. 1501. 1958.
A. boydii 805	Isolated from human lung tissue. Iowa City, Iowa. 1960.
M. apiospermum 806	Isolated from human brain tissue. Iowa Methodist Hospital, Des Moines, Iowa. 1960.
A. boydii 807	Isolated from mouse spleen tissue. Iowa City, Iowa. 1961.

Two different types of "purified" soluble starch were prepared from commercially available soluble starches. One of the "purified" starches was prepared by precipitation from solution with half-saturated ammonium sulfate as follows. To 300 ml of boiling distilled water, 15 g of soluble starch in the form of a thin paste were added while stirring, and the mixture was then cooled to 25 C. An equal volume of saturated ammonium sulfate solution (76.0 g of $(NH_4)_2SO_4$ per 100 ml of distilled water at 25 C) was added to the starch gel, and the mixture was allowed to stand for 30 min. The precipitated starch was removed from the supernatant fluid by centrifugation, washed repeatedly with distilled water until the washings gave a negative reaction with Nessler reagent, and dried under vacuum. A second form of "purified" soluble starch was prepared by continuous dialysis of commercially available soluble starch against distilled water for 1 week at 4 C, and by drying under vacuum.

Carbohydrates used in this study were obtained from the following sources: trehalose (3092F), General Biochemicals, Chagrin Falls, Ohio; soluble starch 1 (according to Lintner) (09901) Merck & Co., Inc., Rahway, N.J.; soluble starch 2 (suitable for iodometry) (74881) Merck & Co., Inc.; soluble starch 4 (according to Lintner) (1715), Mann Research Laboratories, Inc.; soluble starch 3 and all other carbohydrates, Difco.

RESULTS

Most of the carbohydrates tested in this study were found capable of serving as the sole source of carbon for *A. boydii* and *M. apiospermum* (Table 2). In general, only raffinose, inulin, and one of the three soluble starches tested were not able to support good growth of the organisms. Strain 806 differed strikingly from the other organisms tested in not being able to assimilate sucrose.

The two strains of *Allescheria* (Table 2) differed from one another somewhat in that strain 801 was able to grow more profusely on the various carbohydrates; strain 807 was able to produce mature ascocarps in greater abundance. Though strain 807 was able to produce mature ascocarps on the basal medium, even without added carbohydrate, these structures were never observed when galactose served as the sole carbohydrate source. That strain 807 was the more vigorous of the two *Allescheria* strains was reflected by the fact that an intense black pigmentation always

TABLE 2. *Carbohydrate assimilation and sporulation of Allescheria and Monosporium spp.*

Czapek Dox salts with carbohydrate	*A. boydii*		*M. apiospermum*	
	801	807	804	806
Glucose	4+* CAS†	4+ CAS	4+ C	4+ C K
Galactose	4+ C K	4+ C	4+ C	4+ C K
Fructose	4+ CAS	4+ CA	4+ C	4+ C K
Mannitol	3+ CASK	2+ CAS	3+ C	3+ C K
Arabinose	4+ CAS	2+ CAS	4+ C	4+ C K
Xylose	4+ CAS	4+ CAS	4+ C	4+ C
Raffinose	1+ C	1+ CAS	1+ C	1+ C
Maltose	4+ C	2+ CAS	4+ C	4+ C
Sucrose	4+ CAS	4+ CAS	4+ C	1+ C
Lactose	2+ CAS	2+ CAS	2+ C	2+ C K
Trehalose	4+ CAS	4+ CAS	4+ C	4+ C K
Inulin	1+ C	1+ CAS	1+ C	1+ C
Dextrin	4+ CAS	4+ CAS	4+ C	4+ C K
Starch (soluble) 1	1+ C	1+ CAS	1+ C	1+ C K
Starch (soluble) 2	4+ C	3+ CAS	4+ C	4+ C K
Starch (soluble) 3	4+ C	3+ CAS	4+ C	4+ C K
Starch (soluble) 4	3+ C	3+ CAS	3+ C	2+ C
Basal medium (no carbohydrate)	1+ C	1+ CAS	1+ C	1+ C

* Growth readings relative to basal salts with glucose.
† C = conidia; A = ascocarps; S = ascospores; K = coremia.

appeared in the medium whenever good growth occurred; the dark pigmentation regularly occurred with strain 801 only when the organism produced ascocarps.

Sucrose failed to serve as the sole carbon source for strain 806. Otherwise, the relative amount of growth exhibited by this organism on the different carbohydrates was essentially the same as for the other strains tested. We have maintained a culture of this organism growing in a very sparse fashion on Czapek Dox-sucrose agar for the past 2 years, and its ability to utilize the sugar has not changed. Strain 806 produced coremia in the presence of a number of the carbohydrates, and these coremia were always associated with colonies having a typical dark-gray to black surface, and an intense black pigmentation of the medium. Cultures containing coremia were usually apparent upon gross examination, although microscopic examination was always carried out to confirm their presence.

Growth and sporulation studies of four additional strains of *A. boydii* (800, 802, 803, and 805) revealed that, with a few minor exceptions, the relative amount of growth produced on the various carbohydrates was the same as that shown previously (Table 2). Ascocarps were not produced by strains 800 and 802 on any of the media; they were produced only sparsely by strain 805, and with some frequency by strain 803. In contrast to strain 806, all of these organisms were able to assimilate sucrose.

Growth and sporulation of the organisms on "purified" ammonium sulfate-precipitated soluble starches 1 and 2 were found to be comparable to that obtained when mannitol or arabinose served as the carbohydrate source. Dialysis of soluble starch 1, however, did not yield as suitable a product. Although good growth was obtained with the dialyzed starch, sporulation was somewhat inhibited.

No differences were observed between cultures grown on maltose sterilized either by filtration or in the autoclave.

All of the organisms tested were able to grow on the basal medium containing only agar as a carbon source (Table 2). This minimal amount of growth was designated as 1+. But in all instances where 1+ growth was recorded, only a barely discernible quantity of growth was present. No attempt was made to determine whether the organisms were utilizing agar as the sole source of carbon, or whether the minimal growth was attributed to contaminating substances present in the agar, which was known to be free from reducing sugars.

DISCUSSION

Several investigators reported that *A. boydii* is autotrophic for all of its growth factors, and that rich sources of organic nitrogen stimulate the production of mycelial growth and conidial production, but inhibit the production of ascocarps. Gordon (1957) also stated that variation in the ability of the organism to form ascocarps may be ascribed primarily to strain differences, although this property is influenced significantly by the history of the culture, and it may be altered completely by the type of medium employed for growth. No one has reported the effects of different carbon sources on sporulation by the organism.

We have shown in this study that the carbohydrate source supplied to the organism may markedly influence its growth, and perhaps, its sporulation also. However, there appears to be little or no relationship between the relative amount of growth produced, and the tendency for the organism to sporulate. Neither does there appear to be any distinct correlation between sporulation and the utilization of any particular carbohydrate. That history of the culture is also influential is exemplified by the fact that *A. boydii* 801, which is an old stock culture strain, was able to grow more profusely, but sporulate with less frequency, than strain 807 which is a fairly recent isolate (Cazin, McCulloch, and Braun, 1962). Apparently, strain 807 has not yet become as well adapted to the artificial test-tube environment as has strain 801. A similar comparison can be made between *M. apiospermum* 804 and 806.

Our findings differ from those of Wolf et al. (1950) who state that mannitol is not available to the organism as a source of carbon. All of our organisms assimilated this carbohydrate; moreover, when growing on this sugar alcohol, they sporulated abundantly and produced an intense black pigmentation in the medium. Although this difference might be attributed, in part, to the fact that Wolf and associates made their observations after a 2-week incubation period, we were able to observe relatively good growth at the same time interval.

The present study shows that strain 806, in contrast to all other strains tested, is unable to assimilate sucrose. None of the strains which we tested was able to assimilate raffinose; therefore, sucrose is probably utilized by an α-glucosidase. Because this enzyme is usually considered to be located intracellularly, it may be that strain 806 lacks a permease for sucrose. Wolf et al. (1950) tested only one strain of the organism in their study, and it may be that their single strain had a similar deficiency in its ability to utilize mannitol. As a result of our studies, we feel that these organisms sporulate so well on mannitol that we have begun a study to determine whether, by using this sole carbohydrate in the medium, we can rejuvenate some of our old stock strains which at present produce ascocarps only sparsely.

The statement by Emmons, Binford, and Utz (1963) that these organisms are not able to assimilate maltose or lactose also conflicts with our findings. We did observe, however, that growth was sparse on lactose, and that the most recently isolated strains of *A. boydii* grew only sparsely on maltose in contrast to the other strains that grew well on the carbohydrate. It seems unlikely that growth of these organisms on maltose can be attributed to the mechanical hydrolysis of maltose. Similar growth patterns were observed with medium containing maltose sterilized either by filtration or in the autoclave. It is possible, however, that a minimal amount of growth by these organisms may be due to a small amount of glucose which is known to be a natural contaminant of maltose.

The incongruity shown in our data concerning the assimilation of starch by these organisms has not been completely resolved. Other investigators report that these organisms do utilize starch as a sole source of carbon. The first sample tested (soluble starch 1) was a soluble starch prepared according to Lintner. When no growth was obtained with this material, two other samples of soluble starch (soluble starch 2 and 3), which were not Lintner-grade, were tested, and found to support good growth of the organisms. A sample of Lintner-grade starch obtained from a different source (soluble starch 4) was then tested, and also found to support good growth of the organisms.

Since these organisms grow so well on dextrin, we reasoned that the starches which provide good growth may be contaminated with dextrins. Preliminary results on a purified form of soluble starches 1 and 2, prepared by ammonium sulfate precipitation, suggest that such is not the case, because the organisms grow well on these materials. The inhibitory substance present in soluble starch 1, which is easily removed from the starch by ammonium sulfate precipitation, has not been characterized.

LITERATURE CITED

AJELLO, L. 1956. Soil as natural reservoir for human pathogenic fungi. Science **123**:876-879.

BENHAM, R. W., AND L. K. GEORG. 1948. *Allescheria boydii*, causative agent in a case of meningitis. J. Invest. Dermatol. **10**:99–110.

BOYD, M. F., AND E. D. CRUTCHFIELD. 1921. A contribution to the study of mycetoma in North America. Am. J. Trop. Med. **1**:215–289.

CAZIN, J., JR., W. F. MCCULLOCH, AND J. L. BRAUN. 1962. Isolation of *Histoplasma capsulatum*, *Allescheria boydii* and *Microsporum gypseum* from Iowa soil in an attempt to determine the probable point source of a case of histoplasmosis. J. Iowa Med. Soc. **52**:348–351.

EMMONS, C. W. 1944. *Allescheria boydii* and *Monosporium apiospermum*. Mycologia **36**: 188–193.

EMMONS, C. W., C. H. BINFORD, AND J. P. UTZ. 1963. Medical mycology. Lea & Febiger, Philadelphia.

GORDON, M. A. 1957. Nutrition and sporulation of *Allescheria boydii*. J. Bacteriol. **73**:199–205.

MEYER, E., AND R. D. HERROLD. 1961. *Allescheria boydii* isolated from a patient with chronic prostatitis. Am. J. Clin. Pathol. **35**:155–159.

PAUTLER, E. E., R. W. ROBERTS, AND P. R. BEAMER. 1955. Mycotic infection of the eye. *Monosporium apiospermum* associated with corneal ulcer. Arch. Ophthalmol. **53**:385–389.

SACCARDO, P. A. 1911. Notae mycologicae. Ann. Mycol. **9**:249–257.

SHEAR, C. L. 1922. Life history of an undescribed ascomycete isolated from a granular mycetoma of man. Mycologia **14**:239–243.

TRAVIS, R. E., E. W. ULRICH, AND S. PHILLIPS. 1961. Pulmonary allescheriasis. Ann. Internal Med. **54**:141–152.

WOLF, F. T., R. T. BRYDEN, AND J. A. MACLAREN. 1950. The nutrition of *Monosporium apiospermum*. Mycologia **42**:233–241.