PATHOGENICITY AND NUTRITIONAL STUDIES OF SPOROTRICHUM SCHENCKII (MATRUCHOT 1903) AND VARIANTS OBTAINED BY ULTRAVIOLET LIGHT IRRADIATION

Thesis for the Degree of Ph. D.
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Seth S. Mizuba
1956

This is to certify that the

thesis entitled

Pathogenicity and Nutritional Studies of *Sporotrichum schenckii* (Matruchot 1905) and Variants Obtained by Ultraviolet Light Irradiation.

presented by

Seth S. Mizuba

has been accepted towards fulfillment of the requirements for

PhD degree in Botany (Mycology)

Everett D. Benedict
Major professor

Date January 20, 1956

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By

Seth S. Mizuba

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies
of Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements
for the degree of
DOCTOR OF PHILOSOPHY
Department of Botany and Plant Pathology

1956

Approved Everett S. Bennett

ABSTRACT

Morphological, pathogenetic, and nutritional studies of three strains of pathogenic Sporotrichum schenckii, their mutants and variants and of Sporotrichum epigeum, a non-pathogenic species, were conducted. The variant and mutant strains were obtained by irradiation of the fungous strains under ultraviolet light. Several yeast and pleomorphic forms of the parent strains were obtained which ordinarily exist only in the living animal or human host or on special enriched media at 37°C. Those strains that survived continual transfer without change were used in the morphological, pathogenetic, and nutritional studies along with several mycelial variants.

On Sabouraud dextrose agar plus animal and human bloods at 37°C. variations in morphological types from mycelium to mycelial-yeast or yeast forms and from pleomorphic to yeast forms were obtained. Anti-fungal factors in several animal bloods for specific strains were noted.

In the pathogenetic tests, the mycelial strains were found to infect mice more readily than the yeast or pleomorphic strains.

Carbon source studies showed that no one carbohydrate source was able to promote excellent growth of all strains of fungi and in most of the cases, the mutant strains showed inability to grow as vigorously as the parent strain.

The nitrogen source studies indicated that potassium nitrate, ammonium nitrate and dl-isoleucine were utilized poorly by the various strains. No one nitrogen source was able to support excellent growth of all strains of these fungi and as in the carbon source study, the mutant strains were unable to grow as vigorously as the parent strains. Microkjeldahl tests did not show that strains used in the nitrogen studies were able to fix nitrogen.

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INTRODUCTION

Eleven species of Sporotrichum, most of which were derived from decaying wood, were described by Link (1809). An organism resembling one described by Link causing a human disease was reported by Schenck (1899) in 1899. This organism was named Sporotrichum schenckii in 1900 by Helston and Perkins (1900) who isolated a fungus from a patient and found it to be identical with that of Schenck's. Since 1900, this organism has been reported as the cause of sporotrichosis in man and animals, such as the rat, mouse, rabbit, guinea pig, pig, horse, cow, sheep, and goat (Saunders 1948). De Beurmann and Ramond (1903) in France described the disease in 1903 and the fungus was named Sporotrichum beurmanni by Natruchot and Ramond (1905) in 1905. S. beurmanni and other pathogenic sporotricha described since are now thought to be variants of Schenck's fungus and are considered to be synonyms of S. schenckii. (Dodge.1936). The disease was brought into world-wide prominence in 1947 when it reached epidemic proportions among workers handling infected timber in South African gold mines. (Brown et al 1947). Sporotrichosis has been reported from all continents and seems to be most prevalent in the North Central portion of the United States and in France among the European countries (Conant et al 1944).

Lurie (1950, 1951) investigated the carbohydrate and

nitrogen metabolism of the pathogenic sporotricha. She found that the differences in utilization of carbohydrate and nitrogen were useless as a means of identifying and classifying pathogenic sporotricha.

The phenomenon of filamentous growth in nature or in laboratory media and growth of the yeast form in living animals or human beings or on special enriched media at 37°C has intrigued many investigators. Kurung and Yejian (1954) reported growing Sporotrichum schenckii, Blastomyces dermatitides and brasiliensis, and Histoplasma capsulatum in the yeast phase at room temperature and their maintenance for lengthy periods using a potato-flour-egg mixture medium.

The purpose of this paper is to present the results of studies on Sporotrichum schenckii strains and their mutants and variants obtained by ultraviolet light irradiation. Emphasis is placed on the pleomorphic (mixture of yeast and mycelium) and yeast phases obtained through ultraviolet light irradiation. Changes in morphology, pathogenicity, and in the ability to utilize different nitrogen and carbohydrate sources are investigated.

ULTRAVIOLET LIGHT IRRADIATION

INTRODUCTION

Ultraviolet light has been known to have destructive effects on microorganisms in lethal doses and variational and mutational effects in sublethal doses.

Lacasgne (1930) found that Saccharomyces ellipsoides exposed to radiations of wave-lengths between 2800 and 3800 Angstroms units fell into three classes according to their susceptibility to the rays. Those in the first class died instantly. Those in the second class died upon continued periods of irradiation, while those in the third class underwent a temporary stationary period of no cell division but ultimately recovered their power of reproduction. Among those that survived the exposure to the rays, Oster (1934-1935) in his work with Saccharomyces cerevisae observed certain abnormalities in cell growth and reproduction. He observed cells of giant size, cells more spherical in shape, cells showing a long filament-like process constricted at intervals along the length, and colonies which had their sizes increased at a rate roughly proportional to the increase in incident energy.

Emmons and Hollaender (1939) studies the production of a wide variety of mutants in fungi by ultraviolet irradiation. Horowitz et al (1946) caused mutations in fungi by the use of mustard gas, Meyers and Hanson (1945) by the use of neutrons and Hollaender and Zimmer (1945) by the use of

X-rays. The wide variety of mutants and variants in fungi caused by the above mentioned methods has brought into focus the question of a fungus being a new species or merely a variant.

Hollaender and Emmons (1946) found some of the Trichophyton mentagrophytes mutants closely resembling naturally occurring species. They found other mutants to be of types which could be classified as new "species" if one did not know that they had originated from specific fungous cultures. A mutant of Trichophyton interdigitale and T. violaceum.

Another question discussed by Hollaender and Emmons (1946) is the possibility of producing mutants by irradiation with sufficiently intense wave lengths, such as are present in the ultraviolet spectrum of sunlight.

Emmons (1932), in a report of spontaneously occurring mutations of Microsporum gypseum, hypothesized that many of the dermatophytes now known as species are only varieties of a single unstable species. This is probably the situation in the numerous so-called species of pathogenic sporotricha.

METHOD AND MATERIALS

The following is a history of cultures used in irradiation of spores:

(1) Sporotrichum schenckii. Duke University. Isolated February 1934.

(2) Sporotrichum schenckii. Kalamazoo, Michigan. Dr. H. R. Prentice. Isolated August 1953.

(3) Sporotrichum schenckii. Michigan Department of Health. Lansing, Michigan. Dr. J. Roberts. Isolated 1953.

Spores of a standard Sporotrichum schenckii culture grown for 14 days on Sabouraud agar plates were washed off with a physiological saline solution containing 1 to 100,000 parts of aerosol (diamyl sodium sulfo succinate), and filtered through non-absorbent cotton. Spore counts were made with a haemacytometer and the sport number was adjusted to 50,000,000 spores per cc. Twenty cubic centimeters of spore suspension were placed in a flat-bottom Petri dish which was mounted 6 inches from a General Electric Lamp emitting 80 to 95 per cent ultraviolet light of 2527 A°. The suspension was stirred with a glass rod and 1 cc. of spore suspension was withdrawn from the dish at 2, 4, 6, 8, 10, 12, 16, and 20 minutes after initial exposure. A non-irradiated 1 cc. control sample was taken previous to irradiation to check the possibility of spontaneous variants and mutants occurring among the spores. These 1 cc. spore suspensions were diluted serially from 1-10 to 1-10,000,000 at the shorter exposures and 1-10 to 1-100,000 at the longer exposures. The diluted spore suspensions were plated into Sabouraud dextrose agar plates and incubated at room temperatures. Morphologically different colonies were chosen and cultured for observation for stability during the year.

RESULTS AND DISCUSSION

Irradiation caused the following variations and muta-

tions to occur. From the Duke strain 13 morphologically different strains developed. When the Kalamazoo strain was exposed to ultraviolet light irradiation, 43 morphologically different forms resulted while from the Michigan Department of Health strain, 12 variants and mutants resulted.

From the Duke strain, 6 yeast forms were obtained at room temperature on Sabouraud agar. However, in the course of continued semi-weekly transfers, they lost their reproductive powers and only the mycelial forms survived. From the Kalamazoo strain, two yeast forms were obtained. These survived but later developed pleomorphic*. These pleomorphic forms were used in later nutritional and pathogenetic tests. From the Michigan Department of Health strain, one yeast form was obtained as a result of irradiation. This, too, was used in later nutritional and pathogenicity tests.

Initially, it was observed that all irradiated spores required a longer period before germination and growth into a colony. The variants and mutants were observed to take even longer. However, after six months of continued transfer, the variants and mutants were observed to have regained most of their ability to reproduce at the more normal rate.

The following is a list of organisms and code numbers which were assigned and which were used in all later patho-

---

*Pleomorphic denotes a mixture of yeast and filamentous forms

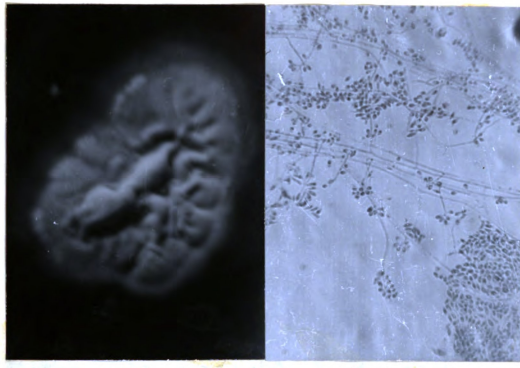
genetic and nutritional studies.

CODE NUMBERS	STRAINS	IRRADIATION TIME
2	Duke University. Mycelial form.	2 minutes
12	Duke University. Mycelial form.	4 minutes
13	Duke University. Mycelial form.	4 minutes
14	Duke University. (Original) Mycelial form.	0 Minutes
15	Kalamazoo. (Original) Mycelial form	0 minutes
24	Kalamazoo. Pleomorphic form.	6 minutes
34	Kalamazoo. Mycelial form.	12 minutes
41	Kalamazoo. Pleomorphic form.	6 minutes
58	Kalamazoo. Mycelial form.	4 minutes
60	Michigan Department of Health. Yeast form.	4 minutes
61	Michigan Department of Health Mycelial form.	4 minutes
64	Michigan Department of Health (Original) Mycelial form.	0 minutes
SP	Sporotrichum epigeum. American Type Culture Association	0 minutes

Photographs of the colonies (after 20 days) and mycelia (after seven days) of the above strains, grown on Sabouraud dextrose agar, appear in Plate I. Strain 41 shows the yeast phase. Both strains 24 and 41 shortly after transfer grew as yeast phases with the mycelial phase gradually developing with the advancing age of the colony. The yeast-like appearance of the somatic structure may readily be seen in strain 60. Strains 12 and 15 had a clustering of hypae as in a

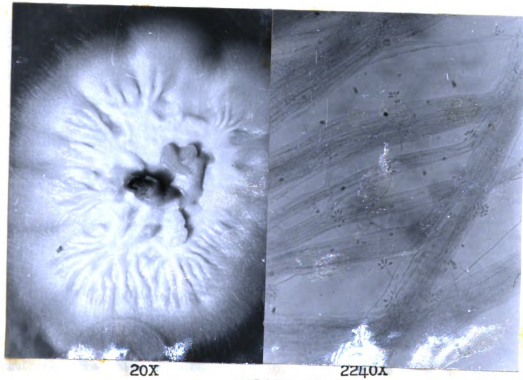
PLATE I

GROWTH ON SABOURAUD DEXTROSE AGAR OF DIFFERENT STRAINS OF SPOROTRICHUM SCHENCKII AND SPOROTRICHUM EPIGEUM
COLONIES: 20 DAYS OLD MYCELIA: 7 DAYS OLD



20X 224OX

STRAIN 2

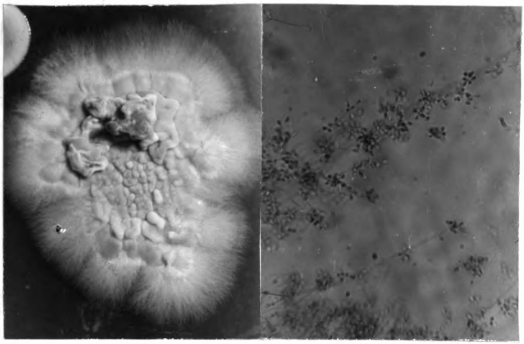


20X 224OX

STRAIN 12

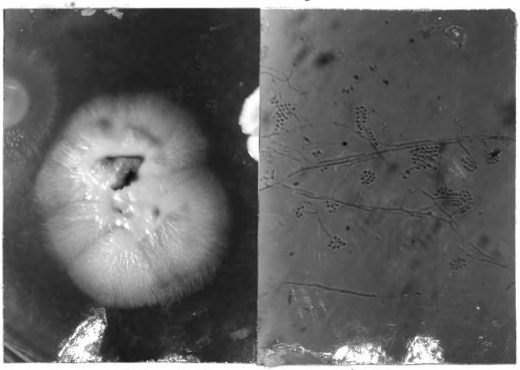
PLATE I (continued)

GROWTH ON SABOURAUD DEXTROSE AGAR OF DIFFERENT STRAINS OF SPOROTRICHUM SCHENCKII AND SPOROTRICHUM EPIGEUM



20X 224OX

STRAIN 13

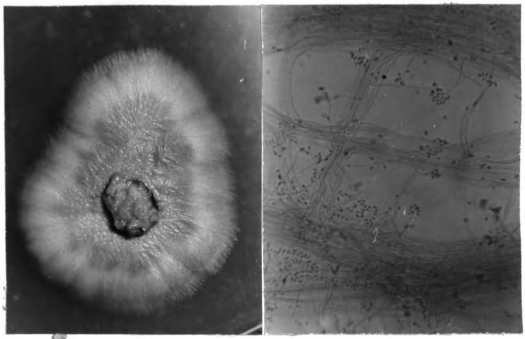


20X 224OX

STRAIN 14

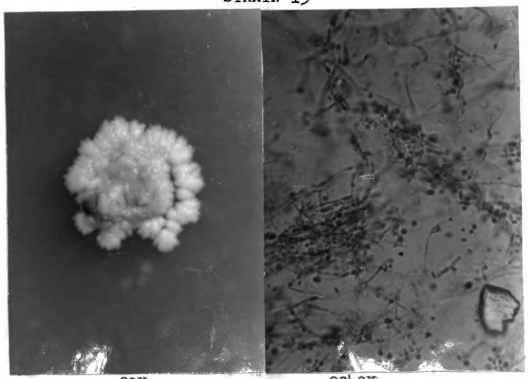
PLATE I (continued)

GROWTH ON SABOURAUD DEXTROSE AGAR OF DIFFERENT STRAINS OF SPOROTRICHUM SCHENCKII AND SPOROTRICHUM EPIGEUM



20X 2240X

STRAIN 15

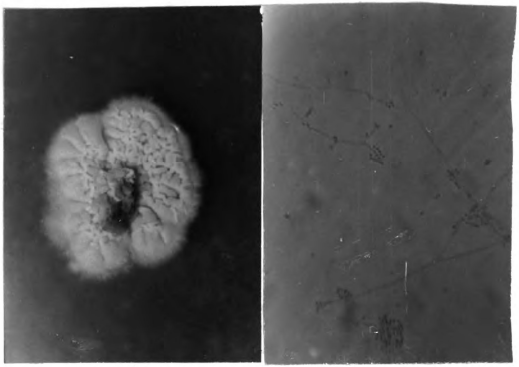


20X 2240X

STRAIN 24

PLATE I (continued)

GROWTH ON SABOURAUD DEXTROSE AGAR OF DIFFERENT STRAINS OF SPOROTRICHUM SCHENCKII AND SPOROTRICHUM EPIGEUM



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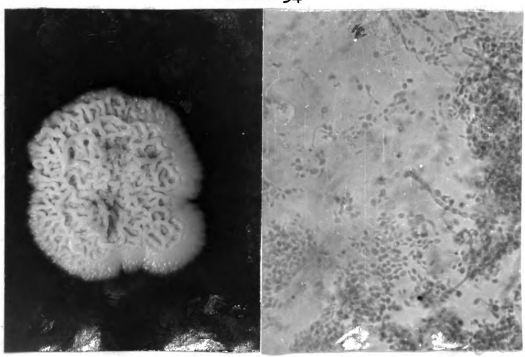
STRAIN 34



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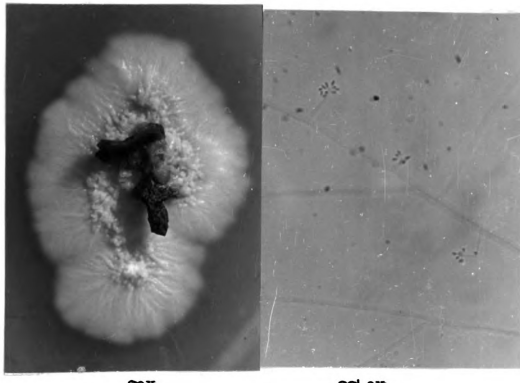
STRAIN 41

PLATE I (continued)

GROWTH ON SABOURAUD DEXTROSE AGAR OF DIFFERENT STRAINS OF SPOROTRICHUM SCHENCKII AND SPOROTRICHUM EPIGEUM



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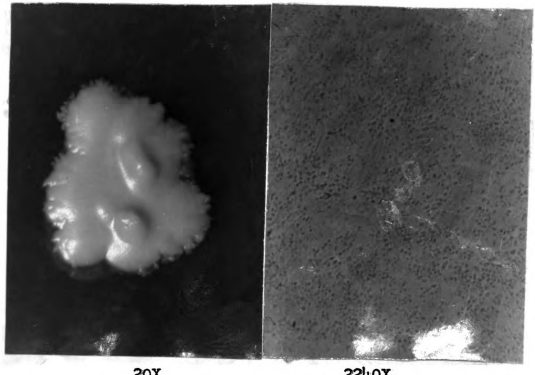
STRAIN 58



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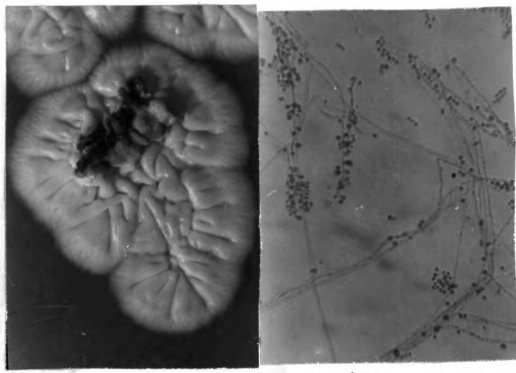
STRAIN 60

PLATE I (continued)

GROWTH ON SABOURAUD DEXTROSE AGAR OF DIFFERENT STRAINS OF SPOROTRICHUM SCHENCKII AND SPOROTRICHUM EPIGEUM



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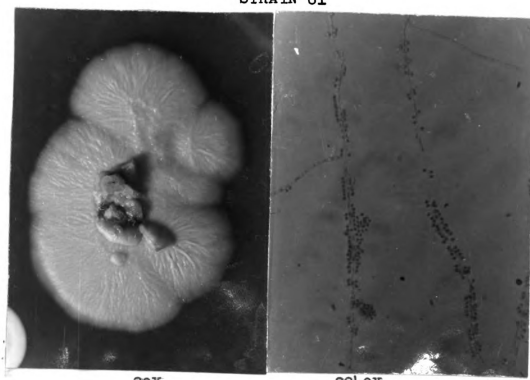
STRAIN 61



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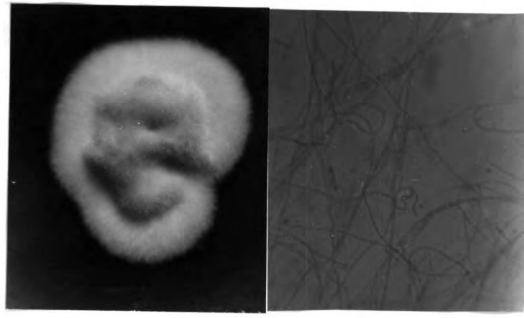
STRAIN 64

PLATE I (continued)

GROWTH ON SABOURAUD DEXTROSE AGAR OF DIFFERENT STRAINS OF SPOROTRICHUM SCHENCKII AND SPOROTRICHUM EPIGEUM



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STRAIN SP

coremia with relatively few spores which were not limited to the top of the structure. Also, strains 61 and 64 (original culture) produced mostly sessile conidia along the sides of the hyphae.

CONCLUSION

Strains morphologically different from the hyphal form of parent isolates ranging from true mycelial forms, pleomorphic forms, to true yeast forms of Sporotrichum schenckii and ordinarily present in the yeast form only in infected hosts or on specialized media at 37°C. were obtained by ultraviolet light irradiation.

STUDY OF MORPHOLOGY

INTRODUCTION

Sporotrichum schenckii when grown on Sabouraud dextrose agar at room temperature appears as a small white colony lacking aerial mycelium. With increase in growth, the surface of the colony becomes folded and leathery with the color varying from white to tan, or brown to black, depending on the strain and upon the media. When examined microscopically, delicate, branching, septate hyphae 1.5 to 2 microns in diameter bearing a cluster of pyriform conidia, 2 to 4 by 2 to 6 microns in size at the tips of the conidiophores, can be observed. In some strains, the conidia are sessile, being borne directly on the hyphae.

When cultured on cystine blood agar at 37°C., the growth remains soft and yeast-like, and is composed of fusiform to oval or oblong bodies and short mycelial fragments.

In the human or animal bodies, this organism exists as a fusiform single-cell in giant cells or polymorphonuclear cells in lesions or exudates.

In this phase of the investigation, a study was made of the colonies and the microscopic characteristics of the strains obtained by irradiation of spores when grown under the following conditions: (1) on Sabouraud dextrose agar at 25°C., and (2) on Sabouraud dextrose agar containing either 5 per cent animal or human bloods plus 20 units per cc. of penicillin and 40 units per cc. of streptomycin and

incubated at 37°C.

METHOD AND MATERIALS

Colonial morphology was studied by inoculation of Sabouraud glucose agar plates and Sabouraud glucose agar plates containing 5 per cent human or animal bloods. One set of the plain Sabouraud agar plates was incubated at 24°C. and the other set at 37°C. The blood agar plates were incubated at 37°C. and the presence or absence of hemolysis was noted.

Mycelial characteristics were studied by allowing the organisms to grow on blocks of Sabouraud glucose agar on sterile glass slides with a cover-slip on the agar block in a moist chamber. The mycelia were allowed to grow out on the slides and on the cover-slips. The mycelia on the cover-slips were mounted by removing the cover-slip, placing a drop of lactophenol-cotton blue on a clean slide, placing the cover-slip with mycelial growth on the drop, and sealing with fingernail polish.

RESULTS AND DISCUSSION

The S. schenckii strains along with S. epigeum are pictured in Plate 1. Strains 24 and 41 were yeast forms upon initial transfer to Sabouraud dextrose agar, but later developed into pleomorphic forms while strain 60 has remained a yeast form. The photographs show strains 41 and 60 as yeast forms, while strain 24 is shown as a pleomorphic

form. The irradiated forms from strains 14, strains 2, 12, and 13 were observed to be typical mycelial forms with pyriform conidial clusters. The same is true for strain 15 and its irradiated form, strain 34. Strain 64 is an atypical form of S. schenckii along with its irradiated form, strain 61 in which the conidia are sessile on the hyphae (Table I).

None of the strains grown on Sabouraud agar at 37°C. showed any change in microscopic characteristics from mycelial to yeast or yeast to mycelial growths, although the production of conidia was impaired and some of the mycelial colonies assumed a moist yeast-like growth.

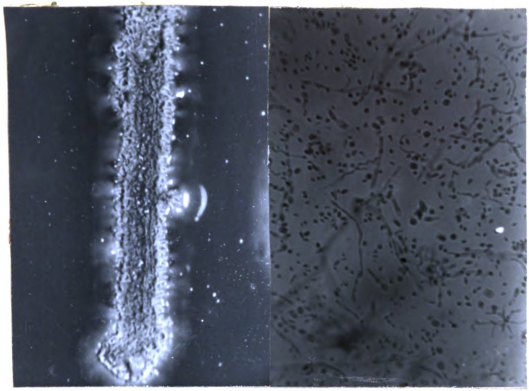
On horse blood, strains 2, 12, 13, 14, 24, 34, and 61 were mycelial and budding forms, whereas, strains 15, 24, 60, and 64 (Plate II) were pure yeast forms. Only strain 58 was a strict mycelial form.

On cow blood agar, both strains 13 and 15 were unable to reproduce. It is thought that an inhibitory substance, or substances were present in this particular blood since repeated inoculation upon blood agar containing blood from the same cow gave similar results. Strains 24, 41, and 60 were strict yeast types, whereas, strains 34, 58, 61, and 64 (Plate II) were pleomorphic types. Strain 2, 12, and 14 were mycelial types.

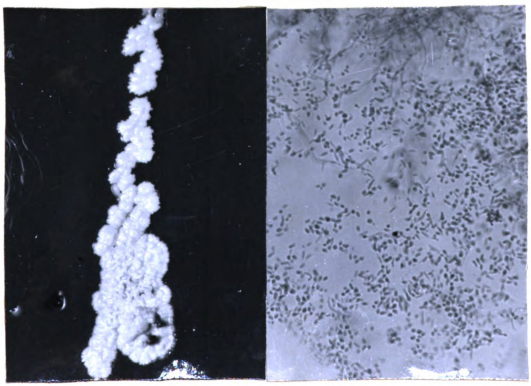
On chicken blood agar, strains 15, 24, and 60 were

PLATE II

GROWTH OF SPOROTRICHUM SCHENCKII STRAIN 64 ON SABOURAUD DEXTROSE BLOOD AGAR
COLONIES: 14 DAYS OLD SOMATIC STRUCTURES: 14 DAYS OLD



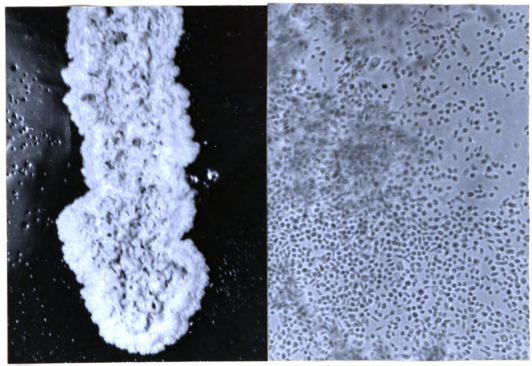
20X 2240X
GOAT BLOOD AGAR



20X 2240X
HUMAN BLOOD AGAR

PLATE II (continued)

GROWTH OF SPOROTRICHUM SCHENCKII STRAIN 64 ON SABOURAUD DEXTROSE BLOOD AGAR
COLONIES: 14 DAYS OLD SOMATIC STRUCTURES: 14 DAYS OLD



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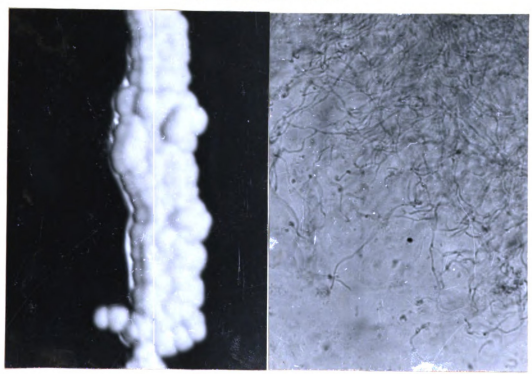
HORSE BLOOD AGAR



20X 2240X
COW BLOOD AGAR

TABLE I - (continued)

GROWTH STUDIES ON 5% SABOURAUD DEXTROSE BLOOD AGARS OF SPOROTRICHUM
Schenckii Strains and Sporotrichum Epigeum

(Y)=Yeast-like Colony

PIG			GOAT	
TYPE OF GROWTH	HEMOLYSIS	STRAIN (10 Days Old)	TYPE OF GROWTH	HEMOLYSIS
Yeast	-	2	Mycelium	+
Yeast	+	12	Pleomorphic	+
Mycelium	+	13	Mycelium (chlamy-spore)	-
Pleomorphic	+	14	Pleomorphic	+
Pleomorphic	-	15	Pleomorphic	+
Yeast	-	24	Yeast	+
Pleomorphic	-	34	Pleomorphic	+
Yeast	-	41	Pleomorphic	+
Pleomorphic	-	58	Pleomorphic	+
Yeast	+	60	Yeast	+
Pleomorphic	-	61	Pleomorphic	+
Pleomorphic	+	64	Pleomorphic	+
No growth	-	Sp	No growth	-

MINK			DOG	
TYPE OF GROWTH	HEMOLYSIS	STRAIN (10 Days Old)	TYPE OF GROWTH	HEMOLYSIS
No growth	-	2	No growth	-
" "	-	12	" "	-
" "	-	13	" "	-
" "	-	14	Pleomorphic	-
Mycelium (poor growth)	-	15	Yeast	-
No growth	-	24	No growth	-
" "	-	34	Pleomorphic	-
Pleomorphic	-	41	Yeast	-
Mycelium	-	58	Pleomorphic	-
Yeast	-	60	Yeast	-
Mycellium	-	61	Pleomorphic	-
Mycelium	-	64	Yeast	-
No growth	-	Sp	No growth	+

Sheep = No growth or hemolysis
Control = Cultures on Sabouraud blood agar at 37°C, with no change in original growth types.

TABLE I

GROWTH STUDIES ON 56 SABOURAUD DEXTROSE BLOOD AGARS OF SPOROTRICHUM Schenckii Strains and Sporotrichum Epigeum

(Y)=Yeast-like Colony

HUMAN		STRAIN (10 Days old)	HORSE	
TYPE OF GROWTH	HEMOLYSIS		TYPE OF GROWTH	HEMOLYSIS
Mycelium	+	2	Mycelium	-
Mycelium	+	12	Pleomorphic	-
Mycelium	±	13	Mycelium (Y)	-
Mycelium (Y)	+	14	Mycelium (Y)	-
Mycelium (Y)	+	15	Yeast	-
Yeast	-	24	Yeast	-
Pleomorphic	-	34	Mycelium (Y)	-
Yeast	+	41	Mycelium (Y)	-
Mycelium (Y)	+	58	Mycelium	-
Yeast	-	60	Yeast	-
Mycelium	±	61	Pleomorphic	-
Yeast	+	64	Yeast	-
No growth	±	SP	No growth	-

COW			CHICKEN	
TYPE OF GROWTH	HEMOLYSIS		TYPE OF GROWTH	HEMOLYSIS
Mycelium	-	2	Mycelium	-
Mycelium	+	126	Pleomorphic	-
No growth	-	13	Mycelium	-
Mycelium	-	14	Mycelium	-
No growth	-	15	Yeast	-
Yeast	-	24	"	-
Pleomorphic	-	34	Mycelium	-
Yeast	-	41	Pleomorphic	-
Pleomorphic	-	58	Mycelium	-
Yeast (Growth Microscopic)	-	60	Yeast	-
Mycelium (Y)	-	61	Mycelium	-
Mycelium (Y)	-	64	"	-
No growth	-	Sp	No growth	-

of the yeast type, whereas, strains 14, 34, 58, 61, and 64 were pleomorphic types. Strain 13 was the only mycelial type.

On goat blood agar, strains 24 and 60 were yeast types, whereas, strains 12, 14, 15, 34, 41, 58, 61, and 64 (Plate II) were pleomorphic types. Of the two mycelial forms, strain 13 was observed to have more chlamydospore-like growth, while strain 2 had none.

On mink blood agar, strains 2, 13, 14, 14, 24, and 34 were unable to grow. Only strain 60 was a yeast type, whereas, strain 41 was a pleomorphic type. In the mycelial types, strains 15 and 58 were able to grow only very poorly while strains 61 and 64 showed relatively good growths.

On human blood agar, strains 24, 41, 60, and 64 (Plate II) grew as yeast forms while the others grew as mycelial forms.

On dog blood agar, strains 2, 12, 13, and 24 were unable to grow. Strains 41 and 60 were yeast types, while strains 14, 34, 58, and 61 were pleomorphic types. There was no strict mycelial form.

Usually sheep blood agar supported growth of the organism, but this particular sheep blood agar did not support the growth of any of the strains. There was no correlation between the ability of the strains to hemolyze the different types of bloods and the types of growth. Human and goat bloods were hemolyzed by most of the strains. Pig blood was hemolyzed by a few strains, notably by the Duke University strains

and the Michigan Department of Health strains. Cow and dog bloods were hemolyzed only by a single strain. It was interesting to note that human and dog bloods were hemolyzed by strain SP (Plate 3) in spite of the fact that no mycelial growth could be observed.

CONCLUSION

Dog, mink, and cow bloods were found to contain a certain substance or substances which inhibited the growth of certain strains of *S. schenckii*.

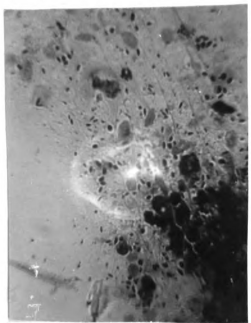
On the different blood agars where growth did occur, the strains varied from mycelial to pleomorphic to yeast forms with only strain 60 remaining consistently, a yeast form.

From this brief study, it can be seen that animal and human bloods contain certain factors which enable strains of *S. schenckii* to grow as yeast or pleomorphic forms at the appropriate temperature, just as in blood-cysteine or brain-heart infusion agars which are extremely rich in certain growth factors.

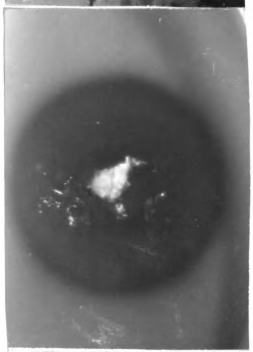
Although sporotrichosis has been reported in animals and humans and the ability of an organism to hemolyze blood is regarded as one of the factors in pathogenicity, only in the human, goat, and pig blood agars was hemolysis found to occur quite consistently.

PLATE III

CIGAR-SHAPED BODIES (SPOROTRICHUM SCHENCKII STRAIN 13) AS FOUND IN MICE AND ZONE OF HEMOLYSIS ON SABOURAUD DEXTROSE BLOOD AGAR CAUSED BY SPOROTRICHUM EPIGEUM



Cigar-shaped bodies
2240X



Zone of hemolysis
20X

PATHOGENICITY

INTRODUCTION

Sporotrichosis causes a subacute or chronic infection which is usually confined to the skin and subcutaneous tissues but may occur in internal organs. The organism is introduced by trauma and develops by causing a pustule, ulcer, or abscess, which fails to heal under ordinary treatment. Often, the regional lymphatics are invaded and cord-like thickening of the lymph vessels and subcutaneous abcesses result.

In the mouse or rat, intraperitoneal or intratesticular inoculation of spores results in abcesses in the pelvic region and mesenteric lymph nodes, and in the spleen and liver. The legs may swell, and ulceration may develop along the tail (Baker, 1947).

The purpose of this study was to determine the effects of Sporotrichum schenckii strains and Sporotrichum epigeum upon mice when introduced intraperitoneally.

METHOD AND MATERIALS

One-half milliliter of spore or yeast suspension (10 million cells per ml.) of 14-day old virulent cultures of sporotricha in saline solution was injected intraperitoneally into mice six to 12 months old. The avirulent strain SP was made into a suspension by macerating the washed mycelium from a 14-day old Petri dish culture. One-half milliliter of the dense milky-colored suspension was injected

intraperitoneally.

The mice were observed daily for deaths until the 28th day, at which time they were all sacrificed. At that time, the general external as well as internal conditions of the mice were recorded. The presence or absence of cigar-shaped bodies was noted by making Gram stains of smears from pus and nodules, and examining the stained smears microscopically.

RESULTS AND DISCUSSION

The results of mouse inoculation have been tabulated in Table II. Mice from the Michigan Department of Health when inoculated with the yeast or pleomorphic strains, 24, 41, and 60 remained non-infected. However, the same cultures infected one out of three inoculated mice which were obtained from the Zoology Department. Except for strain SP which was definitely known as a non-pathogen, the other strains caused infection in mice upon inoculation of the spores.

A difference in the morphological appearance of the organisms in the infected mice was noted in several of the strains. Normally, cigar-shaped bodies were observed upon Gram staining of the pus or crushed nodules, while a tendency toward formation of oval to round cells was observed in strain 2.

TABLE II (continued)

STUDY OF PATHOGENICITY OF SPOROTRICHUM SCHENCKII

STRAINS AND SPOROTRICHUM EPIGEUM IN MICHIGAN DEPARTMENT OF HEALTH MOUSE STRAIN
28 DAYS AFTER INOCULATION

Culture Inoculated	Sex of Mouse	External Condition	Infectivity Lesions	Infectivity Nodules	Micro.Exam. (Cigar Bodies)
2	Female	Normal	No pus	Numerous on mesent.	+
	Female	Normal	No pus	Numerous on mesent.	+
	Female	Normal	No pus	Num.on mesent.,liver	+
12	Female	Normal	No pus	Numerous on mesent.	+
	Female	Normal	No pus	Numerous on mesent.	+
	Female	Normal	No pus	None	-
13	Female	Emaciated	Pus in PC	Few on liver	+
	Female	Emaciated	Pus in PC	Few on liver,spleen	+
	Female	Normal	No pus	Few on mesentery	+
14	Female	Normal	No pus	Numerous on mesent.	+
	Female	Normal	No pus	Numerous on mesent.	+
	Female	Normal	No pus	Few on mesent.,liver	+
15	Female	Emaciated	Pus in PC	Num.on mesent.liver	+
	Female	Normal	No pus	Few on liver	+
	Female	Normal	No pus	Few on mesentery	+
24	Female	Normal	No pus	None	-
	Female	Normal	No pus	None	-
	Female	Dead; 7 days	No pus	None	-
34	Female	Normal	No pus	Few on mesent.spleen	+
	Female	Normal	No pus	Few on mesentery	+
	Female	Normal	No pus	Few on mesent.liver	+
41	Female	Normal	No pus	None	-
	Female	Normal	No pus	None	-
	Female	Normal	No pus	None	-
58	Female	Normal	No pus	Few on mesentery	+
	Female	Normal	No pus	Few on mesentery	+
	Female	Normal	No pus	Few on mesentery	+
60	Female	Normal	No pus	Few on mesent.liver	+
	Female	Normal	No pus	Few on mesent.liver	+
	Female	Normal	No pus	Few on mesent.liver	+
61	Female	Normal	No pus	Numerous on mesent.	+
	Female	Normal	No pus	Few on mesentery	+
64	Female	Normal	No pus	Num.on spleen,liver	+
	Female	Fur Rough	Pus in PC	Num.on mesent.liver	+
	Female	Fur Rough	Pus in PC	Num.on mesent.liver	+
Sp	Female	Normal	No pus	None	-
	Female	Normal	No pus	None	-
	Female	Normal	No pus	None	-
CN	Female	Normal	No pus	None	-
	Female	Normal	No pus	None	-
	Female	Normal	No pus	None	-

If the external appearance of the mice and the presence of cigar-shaped bodies were taken as indication of high virulence, strain 13 could be regarded as highly virulent since all the mice that were inoculated with it were emaciated with roughened fur after 28 days. Strain 64 also seemed quite virulent. However, differences in biological resistance to this strain were noted.

It was observed in making Gram stains of smears that crushed nodules invariably gave positive results as indicated by cigar-shaped bodies, but that pus smears more often gave negative than positive results.

CONCLUSION

Strains 24, 41, and 60 (pleomorphic and yeast forms) could infect none of the mice from the Michigan Department of Health, and only one out of three of the mice from the Michigan State University Zoology Department strains was infected.

Strains 13 and 64 were found to infect the greatest percentage of mice and to cause the appearance of the severest symptoms of sporotrichosis.

The tendency toward formation of oval to round cells instead of the regular cigar-shaped bodies in the host was noted for strain 2.

CARBOHYDRATE METABOLISM

INTRODUCTION

The carbohydrate fermentations of several pathogenic species of Sporotrichum have been reported. Schenck (1898) and Hektoen and Perkins (1900) reported on S. schenckii. Gougerot and Blanchetiere (1909) reported on the fermentation reaction differences of S. beurmanni and S. schenckii. Sucrose was fermented but not lactose by S. beurmanii while S. schenckii was found to hydrolyze and ferment lactose but not sucrose.

Meyer and Aird (1915) reported on 15 Sporotrichum species. They found that acid but not gas was produced by all strains in glucose media; lactose and mannose were not fermented and other sugars gave variable results. They were not able to differentiate species by carbohydrate fermentation.

Lurie (1950) studied nutritional requirements of seven pathogenic sporotricha and found that the organisms ferment glycerol, glucose, maltose, and fructose but not mannose or lactose to any appreciable extent. She confirmed Mayer and Aird's (1915) conclusion that the differentiation of species by carbohydrate fermentations is not possible.

This section on carbohydrate utilization was conducted to determine the change in pH, amount of growth with utilization of various carbon sources, and the possible correla-

tion to pathogenicity. Sporulation was also to be observed.

METHOD AND MATERIALS

Strains 2, 12, 13, 14, 15, 24, 41, 58, 60, 61, 64, and SP were used in this study. These strains were maintained on Sabouraud glucose agar slants and transferred bi-weekly. Five-day old cultures were used for the following inoculation of media.

The basal medium was of the following compositions:

BASAL MEDIUM

KH_2PO_4	1.0 gm.
$MgSO_4.7H_2O$	0.5 gm.
KCl	0.5 gm.
Trace elements	10 ml. stock solution
Vitamin mixture	10 ml. stock solution
Asparagine	2.0 gm.
Glass distilled H_2O	1000 ml.

Trace Elements (stock solution)

$CuSO_4.5H_2O$	0.039 gm.
$FeSO_4.7H_2O$	0.010 gm.
$McCl_2.4H_2O$	0.007 gm.
$ZnCl_2.7H_2O$	0.880 gm.
$Na_2B_4O_7.5H_2O$	0.026 gm.
$(NH_4)6Mo--C_{24}.4H_2O$	0.004 gm.
Glass distilled H_2O	1000 ml.

Vitamin Mixture (stock solution)

Thiamine hydrochloride	0.10 gm.
Riboflavin	0.05 gm.
Pydridexine	0.05 gm.
Calcium penthothenate	0.02 gm.
Paraamino benzoic acid	0.05 gm.
Nicotinamide	0.02 gm.
Choline chloride	0.02 gm.
Inosite	0.04 gm.
Folic acid	4 micro gm.
Glass distilled H_2O	1000 ml.

To this basal medium was added a different carbohydrate source equivalent in carbon weight to 10 gm. of d-glucose as prescribed by Lilly and Barnett (1951). Carbohydrate sources used were d-ribose, d-xylose, d-glucose, d-fructose, l-sorbose, l-arabinose, d-mannose, l-galactose, maltose, sucrose, lactose, raffinose, rhamnose, dulcitol, glycerol, strach, and inulin.

The pH was adjusted to 6.4 by the use of 1 normal sodium hydroxide solution. This pH was chosen to avoid loss of nitrogen from the media in later nitrogen studies. The media were dispensed in 25 ml. amounts into acid-washed tubes (200 mm. x 25 mm.) which were twice rinsed in distilled water and once in glass distilled water. The media were heated for an hour on three successive days in flowing steam.

The method of inoculation of media in triplicate was as follows: The fungus growing as a mycelial mat was aseptically removed and placed in a Petri dish. Agar was scraped off the mycelial mat and the mat was chopped into approximately 1 mm. squares. These mycelial squares were then rinsed twice in sterile distilled water and a tube of liquid medium was inoculated with a single square. In preliminary tests conducted on the size of mycelial squares treated in the above manner and used as inoculum, if the squares were varied from 1 to 4 square millimeters, growth of the different squares

was very nearly of the same weights (Table III). since both l-asparagine and dl-asparagine were utilized equally well, l-asparagine was chosen as the nitrogen source. (Table IV). In the case of yeast cultures, a mass of organisms approximately equal to 1 cubic mm. was used as the inoculum. The inoculated tubes, containing 25 ml. of broth medium, were placed into wire baskets, slanted at a 15° angle and incubated at 24°C. for 25 days. (Figure 1). Number 1 Whatman filter paper, used in the harvest of mycelium, was prepared by drying in a 60°C. drying oven for 2 days, placing the paper in a dessicator containing calcium sulfate for 24 hours, and then weighed. The mycelial mat was harvested using a Buchner funnel containing the filter paper and applying slight suction. The yeast cells were filtered using double layers of filter paper in the Buchner funnel and without applying suction. Each filter paper was rinsed twice with distilled water, dried for two days in a 60°C. drying oven and then placed in a dessicator containing calcium sulfate for 24 hours. The weight of the mycelial growth and yeast cells was taken by subtracting from the total weight, the weight of the growths in the basal medium for each strain. During the filtration process, the pH of the filtrate of each culture was taken with a Beckman pH meter. The filtrate from each culture was saved for the carbohydrate analysis.

The amount of carbohydrate left in the medium after 25

TABLE III

GROWTH STUDY OF SPOROTRICHUM SCHENCKII

STRAIN 14 IN L-ASPARAGINE MEDIUM WITH VARIATIONS IN AMOUNT OF INOCULUM AFTER 25 DAYS AT 24°C.

Amount of Inoculum	Original PH	Final PH	Dry Wgt.of Mycelium in Mg.after 25 days
1x1 mm.	6.40	7.1	84.0
1x2 mm.	6.40	7.3	87.0
1x3 mm.	6.40	7.3	87.4
1x4 mm.	6.40	7.1	89.4

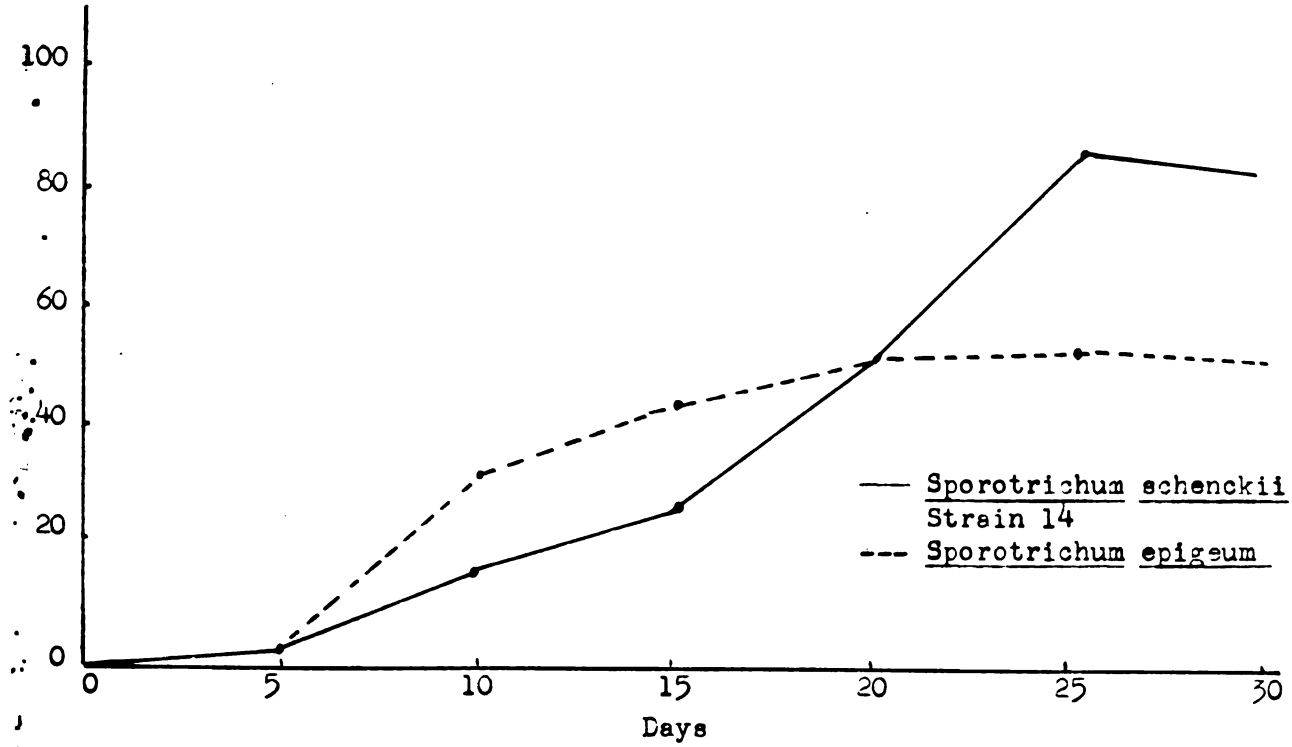
TABLE IV

COMPARISON OF DRY MYCELIAL GROWTHS IN L-ASPARAGINE AND DL-ASPARAGINE MEDIA OF SPOROTRICHUM SCHENCKII STRAIN 14 AND SPOROTRICHUM EPIGEUM INCUBATED AT 24°C FOR 25 DAYS
(Weights Represent Averages of Growths in Triplicate)

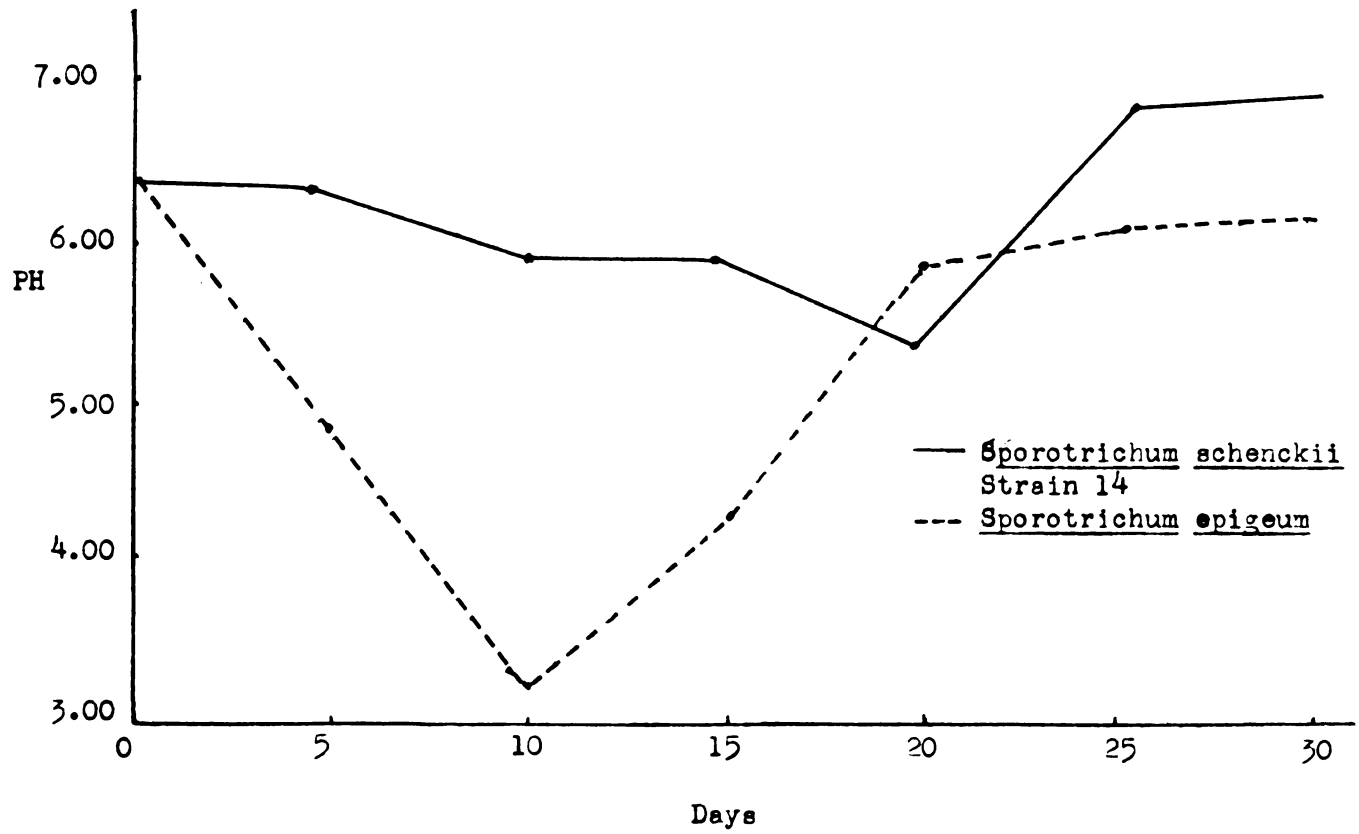
	L-Asparagine		DL-Asparagine	
Days	Strain 14	Strain Sp	Strain 14	Strain SP
0	0 Mg.	0 Mg.	0 Mg.	0 Mg.
5	1.8 "	2.1 "	0.5 "	0.9 "
10	3.5 "	35.1 "	1.70 "	5.5 "
15	26.9 "	45.0 "	24.0 "	20.9 "
20	48.9 "	48.0 "	55.9 "	48.8 "
15	84.3 "	54.3 "	79.0 "	46.6 "
30	83.0 "	52.0 "	80.1 "	49.9 "

FIGURE 1

GROWTH CURVES OF SPOROTRICHUM SCHENCKII STRAIN 14 AND SPOROTRICHUM EPIGEUM IN L-ASPARAGINE MEDIUM WHEN INCUBATED AT 24° C.



CHANGE OF PH BY SPOROTRICHUM SCHENCKII STRAIN 14 AND SPOROTRICHUM EPIGEUM IN L-ASPARAGINE MEDIUM WHEN INCUBATED AT 24° C.



days was determined in the manner prescribed by Lurie (1950) which is a modification of Benedict's and of the A. O. A. C. (1946) methods for monosaccharides, polysaccharides, and polyhydric alcohols.

Monosaccharide, polysaccharide, or polyhydric alcohol in the medium was calculated and the results appear in Table V. It must be pointed out that the carbohydrate titrated and present in the filtrate includes that actually left in the media of the original and whatever compounds that may have been produced by the fungi which are able to reduce alkaline copper reagents or are capable of being oxidized by potassium dichromate. Therefore, an unavoidable source of error exists and a lower utilization of carbohydrate would be indicated than actually occurred. However, this method of direct determination of carbohydrates before and after growth of the fungus should provide useful information as to their utilization.

RESULTS AND DISCUSSION

The results as they appear in Table V are the composite averages of each strain tested in triplicate. Carbohydrate sources found to favor the growth of equal or greater weight of mycelium than a medium with glucose as the carbohydrate source, and with l-asparagine as the nitrogen source, were dl-xylose, dl-fructose, l-galactose, l-sorbose, l-rhamnose, maltose and inulin. Strains 24, 34, 41, and 60 gave poor to fair growths with most carbohydrates employed which were utilized readily by other strains. Strain 41 grew well in

TABLE V

CARBOHYDRATE UTILIZATION AND CHANGE OF PH BY *SPOROTRICHUM SCHENCKII* STRAINS AND *SPOROTRICHUM EPIGEUM* AFTER 25 DAYS INCUBATION AT 25°C (Data Represents Averages of Triplicate Tubes)

STRAIN	PH After 25 Days	Dry Wgt. of Growth in Mg.	Carbohydrate Solution in Mg.	Carbohydrate Utilized in Mg.
		Basal Medium		
2	6.40	1.7	0	0
12	6.40	2.0	0	0
13	6.45	0.3	0	0
14	6.50	1.2	0	0
15	6.50	0.8	0	0
24	6.50	1.5	0	0
34	6.42	1.2	0	0
41	6.32	2.0	0	0
58	6.40	1.8	0	0
60	6.45	0.0	0	0
61	6.42	1.2	0	0
64	6.40	1.7	0	0
Sp	6.40	0.9	0	0
Control*	6.40	0.0	0	0
		L-Arabinose		
2	6.90	36.8	210.7	-39.6
12	6.90	46.83	216. 5	-33.8
13	6.89	45.0	221.1	-29.2
14	6.81	63.8	199.0	-51.3
15	6.80	24.5	5.8	-244.5
24	5.97	6.8	242.1	-8.2
34	6.11	7.3	250.3	-0.0
41	6.70	18.8	232.8	-17.5
58	6.91	26.8	224.6	-25.7
60	6.39	13.0	247.9	- 2.4
61	6.40	29.3	222.3	-28.0
64	6.78	27.9	236.3	-14.0
Sp	6.91	50.5	228.1	-22.2
Control	6.4	0	250.3	0

*Control for all sources of nitrogen indicates uninoculated tubes.

TABLE V (continued)

CARBOHYDRATE UTILIZATION AND CHANGE OF PH BY SPOROTRICHUM SCHENCKII STRAINS AND SPOROTRICHUM EPIGEUM AFTER 25 DAYS INCUBATION AT 25°C (Data Represents Averages of Triplicate Tubes)

Strain	PH After 25 Days	Dry Wgt. of Growth in Mg.	Carbohydrate Solution in Mg.	Carbohydrate Utilized in Mg.
		D-Xylose		
2	6.90	86.2	2.9	-247.4
12	6.79	75.2	21.3	-229.0
13	6.90	84.1	2.3	-248.0
14	6.70	69.7	28.3	-222.0
15	6.80	79.6	28.3	-222.0
24	6.39	1.5	220.7	- 23.6
34	6.40	10.5	725.6	- 23.6
41	6.40	25.0	196.2	- 24.7
58	6.22	69.1	112.6	- 54.1
60	6.40	17.0	225.6	-137.5
61	6.65	72.2	56.4	- 24.7
64	7.10	79.2	2.9	-193.9
Sp	5.80	34.1	225.6	-247.4
Control	6.40	0	250.3	0
		D-Fructose		
2	5.67	105.7	69.5	-180.8
12	5.72	102.4	85.5	-164.8
13	6.80	105.7	12.1	-238.2
14	5.60	88.4	66.6	-181.7
15	5.90	100.8	43.4	-206.9
24	6.00	2.9	224.5	- 25.6
34	5.60	7.3	224.5	- 25.8
41	5.90	23.3	223.7	- 26.6
58	5.50	67.3	184.3	- 66.0
60	6.20	14.6	235.3	- 15.0
61	6.00	36.4	96.4	-155.9
64	5.80	66.5	160.1	- 90.2
Sp	7.20	2.4	10.9	-239.4
Control	6.40	0	25.03	0

TABLE V (continued)

CARBOHYDRATE UTILIZATION AND CHANGE OF PH BY *SPOROTRICHUM SCHENCKII* STRAINS AND *SPOROTRICHUM EPIGEUM* AFTER 25 DAYS INCUBATION AT 25°C (Data Represents Averages of Triplicate Tubes)

	Glucose			
Strain	PH After 25 Days	Dry Wgt.of Growth in Mg.	Carbohydrate Solution in Mg.	Carbohydrate Utilized in Mg.
2	7.62	59.0	201.0	- 46.3
12	5.50	50.1	227.0	- 23.3
13	7.60	87.0	105.3	- 45.0
14	6.05	84.3	122.1	-128.2
15	6.20	63.8	198.0	- 52.3
24	6.35	4.6	247.3	- 3.0
34	6.01	18.1	230.0	- 20.3
41	6.40	1.4	248.0	- 12.3
58	5.80	13.0	243.3	- 5.3
60	6.80	7.5	222.1	- 7.0
61	5.10	26.4	211.1	- 28.2
64	6.00	43.6	200.8	- 39.2
Sp	5.81	56.4	250.3	- 49.5
Control	6.45	0	0	0

TABLE V (Continued)

CARBOHYDRATE UTILIZATION AND CHANGE OF PH BY SPOROTRICHUM SCHENCKII STRAINS AND SPOROTRICHUM EPIGEUM AFTER 25 DAYS INCUBATION AT 25°C (Data Represents Averages of Triplicate Tubes)

Strain	PH After 25 Days	Dry Wgt.of Growth in Mg.	Carbohydrate Solution in Mg.	Carbohydrate Utilized in Mg.
		L-Galactose		
2	6.90	59.3	201.1	- 49.2
12	7.10	63.3	230.1	- 20.2
13	6.20	47.6	83.8	-166.5
14	5.77	92.9	2.7	-247.6
15	7.10	51.3	222.3	- 28.0
24	6.90	3.2	248.0	- 2.3
34	6.32	9.1	242.4	- 7.9
41	6.60	14.6	224.5	- 25.8
58	6.82	21.7	232.4	- 17.9
60	6.60	4.2	229.0	- 21.3
61	5.95	29.3	231.1	- 19.0
64	6.90	33.0	226.8	- 23.5
Sp	6.80	77.9	2.7	-247.6
Control	6.40	0	250.3	0
		L-Sorbose		
2	7.30	104.7	208.1	- 42.2
12	6.59	105.1	148.3	-102.0
13	7.50	115.9	250.0	- 0.3
14	6.55	74.6	159.3	-101.0
15	6.80	84.9	217.1	- 33.2
24	6.45	4.3	209.5	- 40.8
34	6.50	16.8	212.3	- 38.0
41	6.61	79.6	151.7	- 98.6
58	6.72	80.3	182.3	- 68.0
60	6.50	16.0	210.6	- 39.7
61	6.52	33.9	199.3	- 51.0
64	6.65	70.3	176.6	- 73.7
Sp	7.10	8.3	214.0	- 36.3
Control	6.40	0	250.3	0

TABLE V (continued)

CARBOHYDRATE UTILIZATION AND CHANGE OF PH BY *SPOROTRICHUM SCHENCKII* STRAINS AND *SPOROTRICHUM EPIGEUM* AFTER 25 DAYS INCUBATION AT 25°C (Data Represents Averages of Triplicate Tubes)

STRAIN	PH After 25 Days	Dry Wgt.of Growth in Mg.	Carbohydrate Solution in Mg.	Carbohydrate Utilized in Mg.
		D-Mannose		
2	7.20	47.0	22.8	- 29.9
12	5.70	59.9	149.3	-101.0
13	6.96	59.5	190.7	- 59.6
14	7.20	39.6	200.6	- 49.7
15	5.80	35.2	228.7	- 21.6
24	6.40	3.4	250.3	0
34	6.05	5.0	246.9	- 3.4
41	5.10	36.9	162.5	- 87.8
58	4.68	66.4	7.5	242.8
60	6.16	9.5	163.0	- 87.3
61	4.90	64.1	149.3	-101.0
64	6.35	70.8	249.0	- 1.3
Sp	5.20	120.6	1.5	-248.8
Control	6.40	0	250.3	0
		L-Rhamnose		
2	6.57	129.6	230.4	- 22.7
12	5.92	51.0	97.5	-155.5
13	6.50	49.5	3.0	-250.0
14	5.08	46.2	73.1	179.9
15	6.12	37.0	188.9	- 64.1
24	5.90	3.7	240.8	- 12.2
34	5.42	24.9	207.2	- 45.8
41	6.60	9.2	79.2	-173.6
58	6.18	33.9	207.2	- 45.8
60	6.35	23.8	207.2	- 45.8
61	5.60	34.5	201.1	- 51.9
64	5.65	40.6	176.7	- 76.3
Sp	7.12	3.9	219.4	- 33.6
Control	6.42	0	253.0	0

TABLE V (continued)

CARBOHYDRATE UTILIZATION AND CHANGE OF PH BY SPOROTRICHUM SCHENCKII STRAINS AND SPOROTRICHUM EPIGEUM AFTER 25 DAYS INCUBATION AT 25°C (Data Represents Averages of Triplicate Tubes)

Strain	PH After	Dry Wgt.of Growth in Mg.	Carbohydrate Solution in Mg.	Carbohydrate Utilized in Mg.
		Glycerol		
2	5.70	29.2	52.1	-205.4
12	6.70	18.2	9.8	-247.7
13	5.85	24.2	40.3	-217.2
14	6.0	24.2	128.1	-129.4
15	6.2	13.5	177.3	-180.2
24	6.4	3.0	172.9	- 85.6
34	6.4	1.2	173.1	- 84.4
41	5.90	30.1	189.2	- 68.3
58	5.50	30.7	90.7	-166.8
60	6.30	6.1	93.5	-164.0
61	6.52	21.0	74.7	-182.8
64	6.30	8.2	68.1	-189.4
Sp	6.42	27.8	000	-257.5
Control	6.45	0	257.5	0
		Sucrose		
2	5.9	46.3	208.2	- 31.8
12	7.1	39.3	227.8	- 12.2
13	6.2	46.6	214.1	- 25.9
14	6.8	43.5	222.0	- 18.0
15	6.8	20.6	227.3	- 12.7
24	6.5	3.7	234.1	- 5.9
34	6.6	12.4	229.9	- 10.1
41	6.7	18.6	234.1	- 5.9
58	6.7	18.6	234.6	- 5.4
60	6.8	6.1	236.8	- 3.2
61	6.8	20.1	232.0	- 8.0
64	5.87	40.2	228.8	- 11.2
Sp	4.1	79.1	1.1	-238.9
Control	6.41	0	240.0	0

TABLE V (continued)

CARBOHYDRATE UTILIZATION AND CHANGE OF PH BY *SPOROTRICHUM SCHENCKII* STRAINS AND *SPOROTRICHUM EPIGEUM* AFTER 25 DAYS INCUBATION AT 25°C (Data Represents Averages of Triplicate Tubes)

Strain	PH After 25 Days	Dry Wgt.of Growth in Mg.	Carbohydrate Solution in Mg.	Carbohydrate Utilized in Mg.
		Lactose		
2	7.05	13.3	244.5	+ 4.5
12	7.37	14.2	193.1	-239.6
13	7.35	15.2	13.7	-226.3
14	7.15	17.3	253.7	+ 13.7
15	7.20	14.8	243.4	+ 3.4
24	6.42	5.7	238.2	- 1.8
34	7.00	12.7	178.2	- 61.8
41	7.25	13.6	192.5	- 47.5
58	7.25	23.8	246.7	- 3.3
60	7.17	14.4	239.4	- .6
61	7.10	20.1	185.7	- 54.3
64	7.10	13.7	235.4	- 4.6
Sp	7.35	19.0	191.4	- 48.6
Control	6.45	0	240.0	0
		Maltose		
2	6.5	100.5	trace	0
12	5.3	95.6	trace	0
13	6.72	101.1	trace	0
14	5.2	99.6	trace	0
15	7.0	39.8	159.4	- 80.6
24	4.84	5.7	319.8	+ 79.8
34	4.92	14.9	346.0	+106.0
41	6.8	24.4	321.3	+ 81.3
58	7.1	42.2	299.1	+ 59.1
60	7.1	24.7	318.2	+ 78.2
61	4.3	50.7	307.8	+ 67.8
64	7.0	31.2	241.8	+ 1.8
Sp	7.1	59.6	trace	0
Control	6.4	0	240.0	0

TABLE V (continued)

CARBOHYDRATE UTILIZATION AND CHANGE OF PH BY *SPOROTRICHUM SCHENCKII* STRAINS AND *SPOROTRICHUM EPIGEUM* AFTER 25 DAYS INCUBATION AT 25°C (Data Represents Averages of Triplicate Tubes)

Strain	PH After 25 Days	Dry Wgt. of Growth in Mg.	Carbohydrate Solution in Mg.	Carbohydrate Utilized in Mg.
		Starch		
2	6.78	25.0	216.9	- 10.1
12	5.99	67.7	144.2	- 82.8
13	6.9	17.9	194.9	- 32.1
14	5.79	32.0	223.3	- 3.7
15	6.70	29.3	216.1	- 10.9
24	6.18	11.7	236.7	+ 9.3
34	5.95	19.2	222.8	- 4.2
41	6.65	32.2	220.8	- 6.2
58	6.52	25.5	221.4	- 4.6
60	6.59	16.6	222.2	- 4.8
61	5.83	25.3	199.1	- 27.9
64	5.70	42.2	222.8	- 4.2
Sp	6.70	27.1	45.3	-118.1
Control	6.40	0	227.0	0
		Raffinose		
2	6.70	9.7	268.0	- 7.2
12	7.10	12.6	229.3	- 45.9
13	7.20	4.3	237.9	- 37.3
14	6.90	13.6	223.0	- 50.2
15	6.90	11.4	293.8	+ 17.6
24	6.42	3.5	288.0	+ 12.8
34	6.90	8.5	285.2	+ 10.0
41	7.10	5.9	293.6	+ 81.4
58	7.00	9.7	275.2	0.0
60	6.70	5.2	222.1	- 53.1
61	6.80	12.6	296.6	+ 21.4
64	7.10	10.2	286.6	+ 11.4
Sp	6.50	21.8	239.3	+ 35.9
Control	6.40	0	275.2	0

TABLE V (continued)

CARBOHYDRATE UTILIZATION AND CHANGE OF PH BY SPOROTRICHUM SCHENCKII STRAINS AND SPOROTRICHUM EPIGEUM AFTER 25 DAYS INCUBATION AT 25°C (Data Represents Averages of Triplicate Tubes)

Strain	PH After 25 Days	Dry Wgt.of Growth in Mg.	Carbohydrate Solution in Mg.	Carbohydrate Utilized in Mg.
		Dulcitol		
2	6.65	12.6	Could	Could
12	7.20	23.6		
13	7.25	10.7	not	not
14	6.95	21.9		
15	6.90	14.2	be	be
24	6.50	5.1		
34	6.90	8.6	determined	determined
41	6.85	5.3		
58	6.80	3.5	by	by
60	6.92	7.5		
61	6.80	11.3		
64	6.90	9.4	titration	titration
Sp	6.92	46.7	2.9	-250.1
Control	6.40	0	253.0	0
		Inulin		
2	6.00	65.6	122.2	-107.1
12	6.50	67.2	2.0	-227.3
13	6.00	82.9	1.0	-228.3
14	6.70	69.1	3.0	-226.3
15	6.02	84.0	1.0	-228.3
24	6.20	6.90	27.5	-201.8
34	6.50	27.1	39.7	-189.6
41	6.41	55.4	3.0	-226.3
58	6.73	37.0	122.2	-107.1
60	6.40	26.1	91.7	-137.6
61	6.72	119.7	106.9	-122.4
64	6.6	59.9	1.0	-228.3
Sp	7.2	43.9	91.7	-137.6
Control	6.4	0	229.3	0

sorbose, glycerol and starch.

In basal medium, which was lacking in any carbohydrate, there was very little growth (maximum yield of 2.10 mg.) with very little change in pH by the strains. The pathogenic sporotricha strains in media containing d-xylose, l-sorbose, lactose (except strain 24), raffinose, and dulcitol caused a shift toward alkalinity, whereas, media containing d-fructose, d-mannose (except strains 2, 13, and 14), glycerol (except strains 12 and 61), and l-rhamnose (except strains 2, 13, and 14) became more acid.

With other carbohydrate sources, there was no consistency in a shift of pH either toward alkalinity or acidity. This is in agreement with the studies of other investigators on glycerol, d-glucose, and d-fructose media for the non-irradiated strain 14 (Duke University), but not for several of the other non-irradiated and irradiated strains. The results with maltose remain erratic.

Among the media showing consistent lowering of the carbohydrate, but with no consistency in the lowering of pH after the growth of the organism were sucrose, starch (except strain 24), inulin, l-arabinose, d-xylose, l-galactose, and l-sorbose.

All except two investigators have reported mannose not to be fermented by pathogenic sporotricha. However, the irradiated strains and also two non-irradiated strains, 15 and 64, fermented small amounts of mannose.

It was of interest to note that while there was reduction of

the carbohydrate in most media, in media containing maltose, or lactose, there was an actual increase in the amount of reducing substance, probably representative metabolic by-products of the fungus.

The ability of the organisms to grow and reproduce, seems to be the most important factor in relation to pathogenicity. These strains did not grow well in the majority of the carbohydrate media. Another one which was weakly pathogenic in mice was strain 2 which grew, however, as well as other more strongly pathogenic strains. Later nitrogen studies reveal that this strain was unable to grow well in some of the media containing nitrogen.

Of the carbohydrates tested, maltose, and sucrose came closest to being excellent promoters of sporulation of S. schenckii strains (Table VI). No carbohydrate allowed excellent sporulation in all strains tested, just as no carbohydrate showed luxuriant growth in all of the strains tested. The non-pathogenic strain, S. epigeum, which was non-sporulating on Sabouraud dextrose agar, showed no sporulation in any of the media containing carbohydrates.

The abundance of mycelium was not necessarily correlated with sporulation since growth in inulin was poor, while sporulation was good.

CONCLUSION

D-fructose and maltose were found to produce the most luxuriant growths of most of the strains tested.

TABLE VI

:GREE OF SPORULATION IN MEDIA CONTAINING CARBOHYDRATE FOR *SPOROTRICHUM SCHENCKII* STRAINS AND *SPOROTRICHUM EPIGEUM* AFTER 25 DAYS INCUBATION AT 24°C.

Strain	Basal Medium	D-Xylose	D-Arabinose	D-Fructose	L-Glucose	L-Galactose	L-Sorbose	D-Mannose	L-Rhamnose	Glycerol	Sucrose	Lactose	Maltose	Starch	Raffinose	Dulcitol	Inulin
2	-	++	++	++	++	+++	+++	+++	++	+	+++	++	+++	++	++	+++	+
12	-	++	++	++	++	+++	-	++	++	+++	+++	++	+++	++	++	++	+++
13	-	++	++	+++	++	+++	+++	+++	+++	+	++	++	+++	++	++	++	+++
14	-	++	++	++	++	+++	+	++	++	+++	+++	++	+++	++	++	++	++
15	-	++	++	++	++	+++	+	+++	++	+++	-	+	+++	++	++	++	++
24	-	+	+	+	+	+	+	-	+	-	++	-	+	+	+	+	+
34	-	+	+	++	+	+	+	+	++	-	-	++	+++	+	-	++	++
41	-	+	+	+	-	+	-	-	++	-	++	+	+	+	++	+	-
58	-	+	+	+	++	-	+	+	-	+	++	++	+++	+	++	+++	++
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
61	-	+	+	+	+	++	+	+	+	+	+	+	++	+	+	+++	+
64	-	++	++	++	++	++	+	++	++	+++	+++	+++	++	++	+++	+++	+++
Sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = no spores visible microscopically
· = 1/3 of surface covered with spores
++ = 1/2 of surface covered with spores
+++ = dense spore mass on surface

The strains were able to produce acid in glycerol, d-glucose and d-fructose, but not all strains were able to lower the pH in mannose.

There is a certain amount of correlation between pathogenicity and the ability of the cultures to grow in carbohydrate media. The mycelial forms which are able to grow well in most carbohydrate media were shown to be more virulent than the pleomorphic or yeast forms which grew poorly or only fairly well in most carbohydrate media.

Maltose and sucrose were found to be best for the sporulation of most strains.

Strains 24, 41, and 60 were consistently poor in growth, sporulation, and pathogenicity.

NITROGEN METABOLISM

INTRODUCTION

Studies by different investigators have revealed that various fungus species and even strains of the same species display great diversity as well as versatility in their ability to utilize various forms of nitrogen.

Robbins (1937) has codified information with respect to the ability of various fungi to utilize nitrogen, as shown in the following table.

	N Sources Utilized			
	N_2	NO_3	NH_3	Organic N
Group I	+	+	+	+
Group II	-	+	+	+
Group III	-	-	+	+
Group IV	-	-	-	+

Group IV, which includes organisms utilizing only organic nitrogen, is thought to represent a high degree of specialization and to have evolved from Group III. Group III which includes organisms able to utilize ammonia and organic nitrogen, is thought to have evolved from Group II. This group includes organisms able to utilize nitrates, ammonia, and organic nitrogen which in turn is thought to have evolved from Group I. This first group includes organisms which are able to utilize inorganic nitrogen as well as nitrates, ammonia, and organic nitrogen.

Nitrogen fixation has been reported by many investigators for both bacteria and fungi since the latter 1800's.

In regard to genera of bacteria such as Rhizobium, Azotobacter, and Clostridium (Foster, 1949), nitrogen fixation has been agreed upon by investigators, but regarding genera of fungi, there are many disagreements. The first recognized study is that of Duggar and Davis (1916) on nitrogen dixation by Phoma betae and Aspergillus niger. The organisms were grown in Kjeldahl flasks and the nitrogen content determined without removing either the mycelium or medium prior to digestion. Controls used were of two types; uninoculated flasks which had been stored under the same conditions as the inoculated flasks were analyzed for nitrogen at the end of the experiment, and a culture of Azotobacter vinlandii was used as a positive control. They showed that A. niger did not fix nitrogen but that P. betae and A. vinlandii did fix nitrogen with the nitrogen-fixing power of P. betae being very poor compared with A. vinlandii.

Many earlier claims, especially during extended incubation periods, have been met with considerable skepticism because of the lack of rigorous controls. Burris and Wilson (1947) reported that the principal sources of error are those involved in sampling, in the inadequacy of the Kjeldahl method for the measurement of certain types of fixed nitrogen and in the low sensitivity of the usual chemical methods of analysis. Foster

(1949) reported that an important source of error is the fact that the air contains a variety of different forms of fixed nitrogen. These are present in very minute quantities which are subject to concentration within the organisms which utilizes them as rapidly as they go into solution. Traces of ammonia and other volatile nitrogen compounds may not appear significant but in many cases where only a few milligrams of nitrogen have been fixed over relatively long periods of 20 to 60 days, it is thought that this amount of nitrogen could be concentrated by the fungus from the impurities in the atmosphere.

On the other hand, claims for nitrogen fixation by certain fungi cannot be rejected because results cannot be repeated. Factors such as strain specificity and the loss of nitrogen-fixing power, due to variation and selection when cultivated on the usual nitrogen-containing media, can often play a part.

Lurie (1951) reported the possibility of certain human pathogenic sporotricha species being capable of fixing atmospheric nitrogen. The fungi were grown in various liquid media for four weeks and the growths were then weighed and compared. Kjeldahl tests were used to determine the total nitrogen present in the mycelia and media after four weeks of growth at room temperature.

It has been reported by Mosher *et al* (1936) for *Trichophyton*

mentagrophytes and confirmed by Lurie (1951) on pathogenic sporotricha that a mixture of amino acids promotes growth better than a single amino acid. Lurie (1951) reported growth of pathogenic sporotricha with ammonium nitrate as the only source of nitrogen in the base medium. Foster (1949) reported that most fungi can utilize either nitrate nitrogen or ammonia nitrogen with ammonia nitrogen usually being utilized preferentially when both are present. Lurie (1951) further found that urea and various amino acids were available to the fungi with no single amino acid being indispensable. There was no apparent difference in the nitrogen metabolism of eight pathogenic sporotricha studied.

The purpose of this investigation was to determine the nitrogen requirements of non-irradiated and irradiated Sporotrichum schenckii strains and the possible correlation between nitrogen requirements and pathogenicity.

METHOD AND MATERIALS

The equipment and media used in the study of different sources of nitrogen were similar to that used in the study of different sources of carbon with only minor variations. D-glucose, although not giving the most luxuriant growth of strains tested, was chosen for the observation of the influence of a carbohydrate on various sources of nitrogen. The media were prepared in a manner similar to that media for carbohydrate studies. The amount of nitrogen in each

medium was standardized at 10.65 mg. per 25 ml. according to Lilly and Barnett's ratio (1951). The amino acids and inorganic salts and vitamins went into solution without difficulty by dissolving in hot water. However, the casamino acid dissolved after raising the pH of the water to 10 and then later adjusting the pH to 6.4. In adjusting the pH, four to five ml. of 1 N sodium hydroxide per liter were added to the medium. A pH of 6.4 was selected to prevent any free ammonia from escaping from a neutral to basic solution upon application of heat. Tubed media were sterilized by autoclaving at 121°C. for 20 minutes.

The sterilized media were inoculated, incubated, and harvested in a manner similar to that followed in the carbohydrate studies. However, in the harvest of the material, ordinary funnels instead of Buchner funnels were used to separate the mycelia from the media. The media were saved and analyzed for nitrogen content along with the weighed mycelia on filter papers by use of the microkjeldahl test technique. The weight of the mycelium, final pH of the medium, and degree of sporulation were recorded. Total maximum weight as recorded in Table VII was calculated by subtracting the weight of the mycelium that grew in base medium from the weight of the mycelium in the medium containing a specific nitrogen source. The weighed mycelium plus filter paper and filter paper alone were subjected to

the microkjeldahl test. The microkjeldahl tests to determine the amount of nitrogen in the mycelium are those obtained after the nitrogen content in the filer paper was subtracted from the nitrogen content of both the mycelium plus filter paper. From the weight of the mycelium, the percentage of nitrogen and protein in the dry fungus was calculated. The results as they appear in Table VII are averages of triplicate data.

RESULTS AND DISCUSSION

The data on mycelial weights as tabulated in Table VII show that the used l(+) glutamic acid followed by l-asparagine, dl-ornithine, and dl-alanine resulted in consistently greater amounts of growth than any of the nitrogen sources tested. The true yeast strain 60, and the pleomorphic strain 24 and 41 showed poor to fair amounts of growth in the majority of the various nitrogen-containing media with very few exceptions. Lurie (1950, 1951) and other authors reported that growth of pathogenic sporotricha strains in a mixture of amino acids was superior to a single amino acid. However, the S. schenckii strains investigated were able to grow as well in l(+) glutamic acid and l-asparagine as in casamino acid. The possible explanation may have been in the sodium hydroxide used to dissolve the casamino acid which may have been slightly antagonistic to the organisms. However, comparison with weights obtained by Lurie (1951)

NITROGEN SOURCE UTILIZATION AND CHANGE OF PH BY SPOROTRICHUM SCHENCKII STRAINS AND SPOROTRICHUM EPIGEUM AFTER 25 DAYS INCUBATION AT 25°C (Data Represents Averages of Triplicate Cultures)

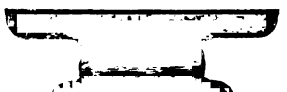
Strain	Wgt. of Growth in Mg.	% of N in Dry Fungus	% of Protein in Dry Fungus (NX6.25)	N Content of Fungus in Mg.	N in Filtered Medium in Mg.	Total N in Medium and Fungus in Mg.	Final PH
Basal Medium							
2	1.90	1.20	7.52	0.02	0.02	0.04	6.40
12	1.90	1.40	8.77	0.03	0.02	0.05	6.40
13	0.80	1.32	8.26	0.01	0.02	0.03	6.45
14	0.90	1.15	7.20	0.02	0.03	0.05	6.50
15	1.00	1.82	11.39	0.01	0.05	0.06	6.50
24	1.00	1.62	10.13	0.02	0.04	0.06	6.50
34	1.00	1.72	10.77	0.02	0.07	0.09	6.42
41	0.90	1.20	7.52	0.02	0.07	0.09	6.32
58	0.80	1.37	8.58	0.03	0.01	0.04	6.40
60	1.80	0	0	0	0.02	0.02	6.45
61	2.00	1.72	10.77	0.03	0.04	0.07	6.42
64	1.40	1.93	12.08	0.03	0.03	0.06	6.40
Sp	1.30	1.67	10.45	0.02	0.05	0.07	6.40
Control	0	0	0	0	0.01	0.01	6.40
Potassium Nitrate							
2	13.70	1.17	7.33	0.16	10.49	10.65	6.98
12	4.30	2.25	14.09	0.10	10.55	10.65	5.83
13	11.10	2.14	13.40	0.24	10.41	10.65	6.92
14	1.10	1.48	9.26	0.02	10.62	10.64	5.35
15	2.90	1.92	12.00	0.06	10.59	10.64	6.50
24	1.20	2.15	13.45	0.01	10.64	10.65	6.62
34	2.80	1.70	10.63	0.05	10.60	10.65	6.34
41	1.20	1.15	7.21	0.01	10.65	10.66	6.86
58	9.70	1.41	8.84	0.14	10.51	10.65	6.80
60	1.90	2.73	17.27	0.05	10.60	10.65	6.67
61	5.10	1.15	7.20	0.06	10.59	10.65	5.71
64	1.20	2.55	15.66	0.04	10.60	10.64	6.59
Sp	22.50	1.66	10.40	0.38	10.27	10.65	7.88
Control*	0	0	0	0	10.65	10.65	6.45

*Control for all sources of nitrogen indicate uninoculated tubes.

TABLE VII (continued)

NITROGEN SOURCE UTILIZATION AND CHANGE OF PH BY *SPOROTRICHUM SCHENCKII* STRAINS AND *SPOROTRICHUM EPIGEUM* AFTER 25 DAYS INCUBATION AT 25°C (Data Represent Averages of Triplicate Cultures)

Strain	Wgt. of Growth in Mg.	% of N in Dry Fungus	% of Protein in Dry Fungus (NX6.25)	N Content of Fungus in Mg.	N in Filtered Medium in Mg.	Total N in Medium and Fungus in Mg.	Final PH
Ammonium Nitrate							
2	3.2	1.62	10.12	.05	10.60	10.65	6.45
12	1.0	3.78	20.37	.38	10.27	10.65	6.45
13	4.8	1.96	12.29	.09	10.56	10.65	6.40
14	4.8	1.61	10.01	.08	10.57	10.65	6.45
15	2.2	1.96	12.30	.04	10.61	10.65	6.40
24	2.8	1.30	8.14	.08	10.57	10.65	6.60
34	5.2	1.89	11.90	.10	10.55	10.65	6.33
41	4.1	1.38	8.64	.06	10.59	10.65	6.50
58	5.0	1.21	7.58	.07	10.58	10.65	6.60
60	4.0	1.02	6.38	.04	10.60	10.64	6.55
61	4.8	1.65	11.32	.08	10.08	10.65	6.60
64	1.6	2.71	16.80	.04	10.62	10.66	6.60
Sp	2.0	1.19	7.45	.02	10.63	10.63	6.60
Control					10.65	10.65	6.40
Ammonium Sulfate							
2	28.2	1.80	11.28	.59	10.14	10.65	2.75
12	18.0	1.58	9.89	.23	10.42	10.65	2.80
13	37.7	1.76	11.01	.60	10.05	10.65	2.80
14	25.1	1.16	7.27	.29	10.36	10.65	2.80
15	22.7	1.26	7.89	.29	10.36	10.65	2.75
24	9.8	1.24	7.76	.22	10.53	10.65	4.68
34	9.9	2.14	13.39	.21	10.44	10.65	2.80
41	4.0	1.46	9.14	.58	10.07	10.65	2.80
58	24.5	1.31	8.20	.32	10.33	10.65	2.65
60	4.7	1.11	6.95	.52	10.13	10.65	2.20
61	16.7	1.43	8.95	.24	10.40	10.64	3.15
64	22.3	1.37	8.59	.38	10.28	10.66	2.95
Sp	44.2	1.02	6.39	.45	10.20	10.65	2.41
Control					10.65	10.65	6.40



NITROGEN SOURCE UTILIZATION AND CHANGE OF PH BY *SPOROTRICHUM SCHENCKII* STRAINS AND *SPOROTRICHUM EPIGEUM* AFTER 25 DAYS INCUBATION AT 25°C (Data Represent Averages of Triplicate Cultures)

Strain	Wgt. of Growth in Mg.	% of N in Dry Fungus	% of Protein in Dry Fungus (NX6.25)	N Content of Fungus in Mg.	N in Filtered Medium in Mg.	Total N in Medium and Fungus in Mg.	Final PH
Ammonium Tartrate							
2	47.10	1.43	8.95	0.67	9.99	10.66	4.70
12	43.60	1.22	7.64	0.53	10.13	10.64	5.10
13	52.70	1.06	6.63	0.56	10.09	10.65	5.03
14	24.70	1.03	6.45	0.25	10.40	10.65	6.01
15	46.90	1.74	10.39	0.82	9.83	10.65	5.10
24	3.70	1.31	8.20	0.05	10.60	10.65	6.36
34	6.90	1.03	6.45	0.07	10.57	10.64	5.40
41	7.90	2.06	12.90	0.16	10.49	10.65	5.10
58	42.60	1.41	8.82	0.60	10.05	10.65	6.30
60	7.00	2.76	17.30	0.19	10.46	10.65	5.00
61	45.70	1.18	7.39	0.54	10.11	10.65	4.40
64	51.90	1.02	6.39	0.53	10.12	10.65	4.50
Sp	48.80	1.04	6.51	0.51	10.14	10.65	4.99
Control	0	0	0	0	10.65	10.65	6.40
Glycine							
2	51.00	2.24	14.03	1.14	9.51	10.65	7.70
12	40.60	2.54	15.91	1.03	9.62	10.65	4.80
13	76.70	3.00	18.79	1.44	9.21	10.65	7.00
14	34.90	2.62	16.41	0.91	9.74	10.65	4.50
15	51.60	2.26	12.25	1.17	9.47	10.64	5.32
24	4.60	3.12	19.68	0.14	10.50	10.64	6.20
34	7.50	2.90	18.15	0.22	10.42	10.64	5.65
41	5.00	2.50	15.65	0.14	10.51	10.65	6.34
58	17.00	2.24	14.30	0.38	10.27	10.65	4.31
60	1.50	2.34	14.65	0.35	10.30	10.65	6.31
61	10.90	2.90	18.15	0.32	10.33	10.65	4.40
64	41.50	2.06	12.90	0.86	9.77	10.65	5.60
Sp	39.10	1.88	11.78	0.74	9.91	10.65	3.91
Control	0	0	0	0	10.65	10.65	6.43

NITROGEN SOURCE UTILIZATION AND CHANGE OF PH BY *SPOROTRICHUM SCHENCKII* STRAINS AND *SPOROTRICHUM EPIGEUM* AFTER 25 DAYS INCUBATION AT 25°C (Data Represent Averages of Triplicate Cultures)

Strain	Wgt. of Growth in Mg.	% of N in Dry Fungus	% of Protein in Dry Fungus (%NX6.25)	N Content of Fungus in Mg.	N in Filtered Medium in Mg.	Total N in Medium and Fungus in Mg.	Final PH
			L-Asparagine				
2	59.00	2.20	13.78	1.30	9.35	10.65	7.62
12	50.10	2.76	17.39	1.38	9.26	10.64	5.50
13	87.00	2.12	13.39	1.85	8.80	10.65	7.60
14	84.30	1.56	9.75	1.32	9.34	10.66	6.05
15	63.80	2.64	16.52	1.68	8.97	10.65	6.20
24	4.60	1.20	7.51	.04	10.61	10.65	6.35
34	18.10	1.96	12.23	.36	10.29	10.65	6.01
41	1.40	2.00	12.51	.03	10.62	10.65	6.40
58	13.00	1.96	12.28	.26	10.39	10.65	5.80
60	71.30	2.16	12.72	1.54	9.11	10.65	6.80
61	26.40	2.76	17.29	.73	9.92	10.65	5.10
64	43.60	2.60	16.29	1.13	9.52	10.65	6.00
Sp	56.40	1.86	11.63	1.05	9.60	10.65	5.81
Control	0	0	0	0	10.65	10.65	6.45
			DL-Ornithine				
2	62.80	2.04	12.78	1.29	9.35	10.64	3.90
12	35.20	1.82	11.39	0.64	10.01	10.65	6.25
13	55.60	2.14	13.39	1.18	9.47	10.65	4.83
14	29.80	2.88	18.08	0.83	9.82	10.65	6.25
15	42.40	2.84	17.79	1.23	9.42	10.65	6.12
24	3.40	2.00	12.52	0.07	10.58	10.65	5.90
34	33.50	3.80	23.39	1.27	9.38	10.65	4.10
41	51.30	2.10	13.14	1.78	8.88	10.65	4.05
58	33.40	2.48	14.90	0.83	9.82	10.65	6.00
60	47.10	3.62	22.65	1.66	9.00	10.65	4.55
61	40.00	2.90	18.15	1.16	9.49	10.65	6.02
64	47.00	2.14	13.40	1.01	9.64	10.65	4.10
Sp	50.00	1.75	10.96	0.88	9.77	10.65	3.30
Control	0	0	0	0	10.65	10.65	6.40

NITROGEN SOURCE UTILIZATION AND CHANGE OF PH BY SPOROTRICHUM SCHENCKII STRAINS AND SPOROTRICHUM EPIGEUM AFTER 25 DAYS INCUBATION AT 25°C (Data Represent Averages of Triplicate Cultures)

Strain	Wgt.of Growth in Mg.	% of N in Dry Fungus	% of Protein in Dry Fungus (%NX6.25)	N Content of Fungus in Mg.	N in Filtered Medium in Mg.	Total N in Medium and Fungus in Mg.	Final PH
DL-Proline							
2	1.60	2.20	13.73	0.35	10.30	10.65	6.60
12	44.60	2.02	12.64	0.90	9.75	10.65	5.90
13	109.10	2.00	13.51	2.18	8.47	10.65	6.69
14	54.20	1.71	11.50	0.93	9.72	10.65	5.60
15	28.70	2.58	17.43	0.74	9.91	10.65	6.30
24	4.10	1.65	10.32	0.68	9.97	10.65	6.37
34	4.80	1.66	10.39	0.80	9.85	10.65	6.17
41	18.60	1.56	9.76	2.90	7.75	10.65	6.40
58	14.10	1.57	9.83	2.26	8.39	10.64	6.15
60	1.10	3.02	18.91	0.33	10.31	10.65	6.45
61	2.60	1.54	9.64	0.40	10.25	10.65	6.15
64	6.70	1.09	6.82	0.73	9.92	10.65	6.40
Sp	69.40	1.45	9.08	0.99	9.66	10.65	3.40
Control	0	0	0	0	0	10.65	6.37
DL-Alanine							
2	42.60	1.18	7.39	0.50	10.15	10.65	7.32
12	58.90	1.16	7.26	0.68	9.97	10.65	5.10
13	81.80	1.09	6.82	0.89	9.77	10.65	6.68
14	48.00	1.18	7.39	0.48	10.17	10.65	4.81
15	21.70	1.57	9.82	0.35	10.30	10.65	6.20
24	63.10	1.28	8.01	0.81	9.84	10.65	6.60
34	3.00	2.83	17.70	0.09	10.56	10.65	6.02
41	24.70	1.02	6.39	0.25	10.40	10.65	5.40
58	64.50	1.30	8.15	0.84	9.81	10.65	5.91
60	47.00	1.29	8.09	0.61	10.04	10.65	5.30
61	23.00	1.56	9.76	0.37	10.28	10.65	4.92
64	27.70	1.25	7.82	0.35	10.30	10.65	6.01
Sp	54.60	3.62	22.32	1.98	8.67	10.65	5.25
Control	0	0	0	0	10.65	10.65	6.45

TABLE VII (continued)

NITROGEN SOURCE UTILIZATION AND CHANGE OF PH BY SPOROTRICHUM SCHENCKII STRAINS AND SPOROTRICHUM EPIGEUM AFTER 25 DAYS INCUBATION AT 25°C (Data Represent Averages of Triplicate Cultures)

Strain	Wgt. in Growth in Mg.	% of N in Dry Fungus	% of Protein in Dry Fungus (%NX6.25)	N Content of Fungus in Mg.	N in Filtered medium in Mg.	Total N in Medium and Fungus in Mg.	Final PH
Arginine							
2	11.30	1.80	11.27	0.20	10.44	10.64	6.10
12	13.10	1.46	9.14	0.19	10.44	10.65	5.50
13	75.10	1.35	8.45	0.10	10.55	10.65	6.00
14	30.00	1.75	10.93	0.53	10.12	10.65	4.80
15	25.20	1.46	9.14	0.37	10.28	10.65	6.19
24	8.20	1.02	6.39	0.83	9.82	10.65	6.21
34	7.40	1.57	9.33	0.12	10.53	10.66	6.10
41	35.70	1.45	9.08	0.52	10.13	10.65	4.50
58	43.80	1.45	9.08	0.52	10.13	10.65	5.80
60	16.70	1.55	9.70	0.26	10.39	10.65	6.12
61	4.10	2.64	16.52	0.10	10.55	10.65	5.98
64	12.90	1.46	9.14	0.19	10.46	10.65	6.30
Sp	48.70	1.52	9.51	0.74	9.91	10.65	3.90
Control	0	0	0	0	10.65	10.65	6.40
Methionine							
2	44.90	2.12	13.28	0.94	9.71	10.65	5.45
12	27.50	1.70	10.62	0.47	10.18	10.65	4.30
13	36.30	1.53	9.58	0.56	10.09	10.65	6.30
14	23.60	1.02	6.39	0.24	10.41	10.65	4.10
15	40.20	1.00	6.26	0.40	10.24	10.64	4.72
24	8.60	1.79	11.21	0.15	10.49	10.64	5.90
34	8.80	1.75	10.97	0.15	10.50	10.65	5.62
41	14.60	2.16	13.52	0.31	10.34	10.65	4.42
58	30.80	1.56	10.02	0.48	10.17	10.65	5.82
60	33.20	1.23	7.70	0.41	10.24	10.65	4.82
61	29.90	1.43	8.96	0.43	10.22	10.66	4.30
64	30.20	1.60	10.01	0.48	10.17	10.65	4.50
Sp	28.30	1.41	9.02	0.40	10.25	10.65	4.75
Control	0	0	0	0	10.65	10.65	6.62

TABLE VII (continued)

NITROGEN SOURCE UTILIZATION AND CHANGE OF PH BY SPOROTRICHUM SCHENCKII STRAINS AND SPOROTRICHUM EPIGEUM AFTER 25 DAYS INCUBATION AT 25°C (Data Represent Averages of Triplicate Cultures)

Strain	Wgt.of Growth in Mg.	% of N in Dry Fungus	% of Protein in Dry Fungus (%NX6.25)	N Content of Fungus in Mg.	N in Filtered Medium in Mg.	Total N in Medium and Fungus in Mg.	Final PH
L (+) Glutamic Acid							
2	71.90	1.37	8.58	0.99	9.65	10.64	7.15
12	64.00	2.46	15.40	1.57	10.08	10.65	4.96
13	88.70	1.62	10.14	1.43	9.22	10.65	7.25
14	38.00	2.17	13.60	0.83	9.82	10.65	3.92
15	73.30	1.93	12.01	1.42	9.24	10.66	7.39
24	4.10	1.42	8.89	0.06	10.59	10.65	3.50
34	22.90	1.53	9.67	0.35	10.30	10.65	3.70
41	29.60	2.32	14.52	0.69	9.90	10.65	3.67
58	76.00	2.12	13.55	1.61	9.03	10.64	5.67
60	10.60	2.43	15.30	0.26	10.40	10.66	2.50
61	29.80	2.79	17.45	0.83	10.82	10.65	3.80
64	78.30	1.11	6.95	0.87	9.78	10.65	7.32
Sp	55.20	1.38	8.64	0.76	9.89	10.65	4.02
Control	0	0	0	0	10.65	10.65	6.40
DL-Leucine							
2	25.40	1.70	10.62	0.44	10.20	10.64	5.30
12	25.50	1.23	7.70	0.31	10.34	10.65	4.40
13	76.10	2.00	12.51	0.15	10.51	10.66	6.92
14	17.20	2.18	13.64	0.38	10.27	10.65	4.40
15	30.40	1.15	7.20	0.35	10.30	10.65	4.40
24	4.80	1.36	8.52	0.07	10.58	10.65	5.90
34	9.10	1.92	12.00	0.18	10.46	10.64	4.80
41	17.30	1.32	8.26	9.23	10.43	10.66	4.80
58	43.30	1.29	8.08	0.56	10.09	10.65	5.80
60	17.00	1.60	10.01	0.27	10.37	10.64	4.60
61	7.30	2.30	14.40	0.17	10.48	10.65	4.45
64	27.80	1.41	8.84	0.39	10.27	10.66	4.60
Sp	53.10	1.09	8.08	0.53	10.12	10.65	7.60
Control	0	0	0	0	10.65	10.65	6.40

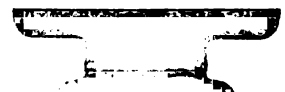
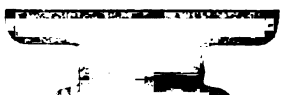


TABLE VII (continued)

NITROGEN SOURCE UTILIZATION AND CHANGE OF PH BY SPOROTRICHUM SCHENCKII STRAINS AND SPOROTRICHUM EPIGEUM AFTER 25 DAYS INCUBATION AT 25°C (Data Represent Averages of Triplicate Cultures)

Strain	Wgt. of Growth in Mg.	% of N in Dry Fungus	% of Protein in Dry Fungus (NX6.25)	N-Content of Fungus in Mg.	N in Filtered Medium in Mg.	Total N in Medium and Fungus in Mg.	Final PH
L-Trytophane							
2	4.60	1.63	10.20	0.08	10.57	10.65	6.65
12	35.50	1.18	7.39	0.42	10.27	10.65	6.63
13	55.10	1.53	9.58	0.84	10.81	10.65	6.10
14	25.30	1.90	11.89	0.49	10.16	10.65	5.80
15	38.50	1.19	7.45	0.46	10.18	10.64	5.50
24	2.60	2.32	14.50	0.06	10.59	10.65	6.50
34	3.00	2.95	18.45	0.09	10.57	10.66	6.10
41	7.20	2.15	13.45	0.15	10.49	10.65	6.80
58	6.90	1.69	10.58	0.17	10.48	10.65	6.30
60	12.70	1.84	11.50	0.23	10.43	10.66	6.40
61	38.30	1.23	7.70	0.47	10.18	10.65	5.40
64	42.90	1.17	7.32	0.50	10.14	10.64	6.45
Sp	45.40	1.19	7.45	0.54	10.11	10.65	7.25
Control	0	0	0	0	10.65	10.65	6.40
Threonine							
2	6.40	1.40	8.76	0.09	10.56	10.65	6.60
12	26.40	1.28	8.00	0.34	10.31	10.65	4.90
13	7.50	1.43	8.96	0.11	10.53	10.64	6.60
14	41.20	1.37	8.57	0.56	10.09	10.65	4.70
15	43.40	1.02	6.32	0.44	10.21	10.65	6.30
24	25.40	1.20	7.51	0.31	10.34	10.65	6.35
34	54.80	1.26	7.89	0.69	9.96	10.65	5.50
41	18.50	1.80	11.28	0.33	10.32	10.65	6.29
58	13.80	2.03	12.71	0.28	10.37	10.65	5.10
60	5.60	1.43	8.95	0.08	10.57	10.65	5.00
61	13.80	1.28	8.01	0.18	10.53	10.65	4.90
64	5.30	2.23	14.10	0.12	10.53	10.65	4.45
Sp	8.90	2.34	14.63	0.21	10.44	10.65	7.60
Control	0	0	0	0	10.65	10.65	6.41



NITROGEN SOURCE UTILIZATION AND CHANGE OF PH BY *SPOROTRICHUM SCHENCKII* STRAINS AND *SPOROTRICHUM EPIGEUM* AFTER 25 DAYS INCUBATION AT 25°C (Data Represent Averages of Triplicate Cultures)

Strain	Wgt. of Growth in Mg.	% of N in Dry Fungus	% of Protein in Dry Fungus (NX6.25)	N Content of Fungus in Mg.	N in Filtered Medium in Mg.	Total N in Medium and Fungus in Mg.	Final PH
L-Histidine							
2	23.80	2.68	16.78	0.64	10.01	10.65	5.90
12	39.00	2.65	16.00	1.03	9.62	10.65	6.20
13	67.90	2.57	17.43	1.75	9.89	10.64	7.05
14	54.40	1.33	8.34	0.72	9.93	10.65	6.70
15	20.20	1.15	7.20	0.23	10.43	10.66	6.78
24	1.00	2.61	17.71	0.01	10.63	10.64	6.70
34	30.30	1.46	9.14	0.44	10.20	10.64	6.55
41	4.90	1.43	8.95	0.70	9.95	10.65	6.10
58	26.30	1.99	13.50	0.50	10.15	10.65	6.20
60	9.50	1.27	7.95	0.12	10.54	10.66	6.60
61	13.70	1.09	6.83	0.14	10.51	10.65	6.12
64	29.10	1.04	6.52	0.30	10.36	10.66	6.60
Sp	23.50	1.14	7.14	0.27	10.38	10.65	7.32
Control	0	0	0	0	10.65	10.65	6.63
Casamino Acid							
2	16.00	2.70	16.90	0.44	10.21	10.65	6.12
12	59.40	3.00	18.38	1.48	9.17	10.65	5.62
13	29.40	2.19	13.70	0.86	9.79	10.65	6.20
14	65.00	2.99	18.70	1.94	8.71	10.65	5.50
15	24.80	3.14	19.65	0.78	9.87	10.65	6.20
24	14.00	2.26	14.15	0.32	10.33	10.65	6.40
34	21.80	3.41	19.65	0.78	9.87	10.65	6.10
41	19.76	2.97	18.60	0.58	10.07	10.65	6.20
58	66.60	2.80	17.51	1.88	8.77	10.65	5.50
60	26.80	3.04	19.29	0.82	9.83	10.65	6.21
61	50.80	2.23	13.95	1.16	9.49	10.65	3.12
64	60.00	2.37	14.82	1.42	9.23	10.65	6.32
Sp	96.10	3.29	20.30	2.16	8.49	10.65	6.00
Control	0	0	0	0	10.65	10.65	6.45

TABLE VII (continued)

NITROGEN SOURCE UTILIZATION AND CHANGE OF PH BY *SPOROTRICHUM SCHENCKII* STRAINS AND *SPOROTRICHUM EPIGEUM* AFTER 25 DAYS INCUBATION AT 25°C (Data Represent Averages of Triplicate Cultures)

	DL-isoleucine						
Strain	Wgt.of Growth in Mg.	% of N in Dry Fungus	% of Protein in Dry Fungus (NX6.25)	N-Content of Fungus in Mg.	N in Filtered Medium in Mg.	Total N in Medium and Fungus in Mg.	Final PH
2	4.80	1.70	10.63	0.08	10.57	10.65	6.60
12	22.50	1.90	11.90	0.43	10.22	10.65	5.45
13	33.40	1.79	11.20	0.60	10.05	10.65	6.69
14	24.40	2.29	14.32	0.56	10.26	10.64	4.72
15	20.00	1.89	11.82	0.38	10.55	10.65	6.40
24	5.40	1.89	11.82	0.10	10.58	10.66	6.55
34	6.90	1.12	7.01	0.08	10.57	10.66	6.30
41	8.50	1.06	6.63	0.09	10.56	10.65	6.45
58	36.20	1.80	11.27	0.65	10.00	10.65	5.22
60	7.00	1.90	11.89	0.13	10.51	10.64	5.50
61	45.60	1.09	6.82	0.50	10.14	10.64	4.82
64	3.80	2.46	15.39	0.09	10.56	10.65	6.26
Sp	51.60	1.70	10.63	0.88	9.77	10.65	7.20
Control	0	0	0	0	10.65	10.65	6.42

indicates that with a balanced medium containing the necessary trace elements and vitamins such as pyrimidine, the latter being shown by Drouhet and Mariat (1950) to be the sole growth factor essential to the development of S.schenckii and S. tropicale, single amino acids could be utilized as well as a mixture of amino acids. The variation caused by different irradiated strains was not the influencing factor. Original non-irradiated cultures showed proportional increase in weight indicating that the fungus is able to utilize a specific amino acid in the presence of required growth factors as efficiently as in the presence of more than one amino acid.

Inorganic nitrogen in the form of potassium nitrate, ammonium nitrate, ammonium tartrate, and ammonium sulfate were available to the fungous strains with the ammonium sulfate and ammonium tartrate being better utilized than the potassium nitrate and ammonium nitrate.

In certain media, yeast or pleomorphic forms showed abundant growth. In l-asparagine and methionine media, strains 24 and 41 gave only sparse growth, whereas, strain 60 grew quite abundantly. In dl-ornithine medium, strain 24 grew poorly, whereas, strains 34 and 60 grew quite well. Dl-alanine medium was utilized by strains 24, 41, and 60 but not by strain 34. Arginine was poorly utilized by strain 24,

fairly well by strains 60 and quite well by strain 41. Threonine was poorly utilized by strain 60 and fairly well by strains 24 and 41.

Generally, strains derived from 14 and 64 were able to utilize nitrogen sources better than strains derived from strain 15.

In most nitrogen source media, the non-pathogenic S. epigeum denoted as strain SP was able to grow well.

There was wide variation in the percentage of nitrogen in the dry fungus from 1.02 to 3.80. In comparing with Lurie's (1951) study on nitrogen metabolism, it is observed that the percentage is higher in some cases, but in most cases is very similar (Lurie's results range from 1.4 to 2.6 per cent for different strains.) This percentage is slightly lower for Aspergillus niger which was found by Steinberg (1942) to have from 4 to 6 per cent nitrogen.

The percentage of protein in dry fungus was calculated by multiplying the percentage of nitrogen by the factor of 6.26 used by Lurie (1951). These percentages ranged from a low of 6.51 to a high of 23.79 in comparison with Lurie's low of 8.7 and a high of 16.9.

The actual nitrogen in the mycelium when measured in milligrams ranged from a low of 0.03 mg. to 2.18 mg. The total utilizable nitrogen in all media was 10.65 mg.

The nitrogen content in the media directly calculated by the microkjeldahl method when added to the nitrogen in the mycelium should have given a factor amounting to 10.65 mg. of nitrogen if these fungous strains were only able to use nitrogen provided and not nitrogen from the air. Lurie (1951) reported the possibility of S. schenckii fixing nitrogen from the air. Under the conditions of this experiment there were a few cases in which the nitrogen in mycelium and medium totaled 1 mg. more than the original nitrogen in the media. However, there were cases too, when the total nitrogen in mycelium and medium did not add up to the nitrogen in the original media. Therefore, under these conditions of growth, it cannot be concluded that these fungous strains fix nitrogen and S. schenckii strains tested must tentatively be placed in Group II instead of Group I.

Sporulation of S. schenckii differed according to the strains as well as the medium used (Table VIII). In many cases, strains derived from 14 were able to sporulate fairly well to quite well, whereas, strains derived from 15 did not sporulate or did poorly to fairly well. On the other hand, strains derived from 64 either did not sporulate at all or sporulated very well. In comparing media containing different nitrogen sources, l (+) glutamic acid, l-tryptophane, casamino acid, and dl-ornithine seem to be best for sporulation.

TABLE VIII

DEGREE OF SPORULATION IN NITROGEN MEDIA FOR *SPOROTRICHUM SCHENCKII* STRAINS AND *SPOROTRICHUM EPIGEUM*
After 25 Days Incubation at 24°C

Strain	Base Medium	Potassium nitrate	Ammonium nitrate	Ammonium sulfate	Ammonium tartrate	Glycine	L-Asparagine	DL-Ornithine	DL-Proline	DL-Alanine	Arginine	Methionine	L(+)Glutamic acid	Leucine	L-Tryptophane	Threonine	L-Histidine	Casamino acid
2	-	+	+	++	++	++	+++	+++	+	+++	-	++	++	+	++	++	+++	+++
12	-	++	+	+	+	+	+	+++	+	+	+	+	+++	+	+++	+	+++	+++
13	-	++	++	++	+++	++	+++	+++	+++	+++	+++	++	+++	+	+++	+++	++	+++
14	-	+	++	+	+	+	+	++	++	+	+	+	+++	+	++	++	+	+++
15	-	+	++	+	++	++	+++	++	++	+++	+	+	+++	+++	+++	++	++	+++
24	-	+	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-
34	-	-	+	+	+	-	+	-	+	+	-	-	+	+	++	+	-	+
41	-	+	-	-	+	+	-	-	+	++	-	-	+	+	+	-	-	-
58	-	+	+	+	++	+	+	++	+	+	+	+	++	+	++	+	-	++
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
61	-	+	+	++	+	-	+	+	-	+	+	+	++	+	++	+	+	++
64	-	+	+	+	+	++	+	+	+	+	+	+	+++	+++	++	+	+++	+
Sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

-= no spores visible
+= 1/3 of surface covered with spores
++= 1/2 of surface covered with spores
+++= dense spore mass on surface

CONCLUSION

L(+) glutamic acid and l-asparagine were assimilated most easily by the fungous strains. Proline, found to be a "primary" amino acid for A. niger studies conducted by Steinberg (1942) gave relatively good growth but did not promote the most abundant growth.

The amino acid sources together with ammonium sulfate and ammonium tartrate were good sources of nitrogen but ammonium nitrate and potassium nitrate were found to be poor sources of nitrogen. Ammonium compounds have generally been regarded as being utilized before other nitrogen sources. In the case of ammonium nitrate, the possibility of antagonistic substances being present in the medium is very likely.

The percentage of nitrogen in the mycelium ranged from 1.0 to 3.8 depending upon the strain and nitrogen source.

Under these experimental conditions, S. schenckii strains and S. epigeum do not appear capable of fixing atmospheric nitrogens.

No single amino acid is indispensable when the organisms are provided with essential elements and vitamins.

No specific strain always produced mycelium abundantly in the nitrogen media used. However, strains 24, 41, and 60 were observed to produce sparse growth in many of the media containing different types of nitrogen.

Sporulation was observed to be dependent upon strain variation as well as on the type of nitrogen in the medium.

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