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The Effect of Various Nutrients on the Sporulation of TRICHOPHYTON RUBRUM

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THE EFFECT OF VARIOUS NUTRIENTS ON THE SPORULATION OF TRICHOPHYTON RUBRUM

By

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Each person whose signature appears below certifies that he has read this thesis and that in his opinion it is adequate, in scope and quality, as a thesis for the degree Master of Science.

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Dedicated to my parents,

Mr. and Mrs. R. V. Austin

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INTRODUCTION

The term dermatophyte is commonly used to refer to a group of fungi which are limited in their pathology almost exclusively to the skin, hair, and nails. They do not invade the dermis under normal circumstances and are rarely found in other than the keratinized areas of the skin and its appendages.

As early as 1841, David Gruby cultured a fungus from a lesion on a patient with a skin infection called favus. To establish that the cultured microbial organism was, indeed, the cause of disease, he took the organism from the culture and reinoculated the normal skin. These same principles were used forty years later by Koch in his work with tuberculosis and have since been referred to as Koch's postulates.

In 1910 Sabouraud published a large volume, Les Teignes (The Tineas) which was devoted entirely to reports of fungus diseases of the skin. During the next two decades there was a great deal of confusion and many variants, of what is now considered to be a single species, were reported as separate species and the list of pathogens of human skin grew to several hundred "new species". Part of the confusion that existed at this time was due to the lack of training on the part of those reporting but there was also the need of a standard growth medium to minimize the great variation that is common when a single species is grown under slightly different conditions. Hopkins and Benham organized a mycology laboratory in connection with Columbia University and instituted an almost complete reversal in the splitting tendency which had led to the enormous number of species based on the insignificant variation that occurs normally in all of the dermatophytes, (Wilson and Plunkett, 1965).

Basic to all the work that followed, was the organization and classification by Emmons (1934). Using the principles of a botanical classification, which was based primarily upon the characteristics of the macroconidia, he listed all of the dermatophytes under three genera. This classification did much to clear up the confusion experienced by previous investigators and was accepted by most of the expert mycologists in the United States, England, and South America.

Almost all of the dermatophytes have been considered Deuteromycetes (Fungi Imperfecti) until the discovery of the ascigerous state in Keratinomyces ajelloi by Dawson and Gentles (1961). Since then several other dermatophytes have also developed the ascigerous state when grown by the hair-bait technique or by the use of the special media developed by Weitzman (1967). See table I for a complete listing of the dermatophytes in which the perfect state has been observed. These findings has placed these species, with a perfect state, in the Class Ascomycetes. However, the macroconidia, when present, are the most reliable aid in the identification because of their distinctive size, shape, and wall structure. Some of the dermatophytes do not commonly produce macroconidia when grown on a standard medium such as Sabouraud's glucose agar, therefore various means, some simple and some very complex, have been devised in an effort to stimulate the production of macroconidia.

It has already been known that thiamine is necessary for the induction of macroconidia by Trichophyton violaceum (Georg 1951). One of the strains studied showed an absolute requirement for histidine. This is the first report of a requirement for a specific amino acid by

TABLE I

DERMATOPHYTES IN WHICH THE PERFECT STATE HAS BEEN OBSERVED

Imperfect State (Deuteromycete)	Perfect State Ascomycete)	Investigator and Date
Microsporum cookei	Nannizzia cajetani	Ajello, 1961
Microsporum fulvum	Nannizzia	Stodkdale, 1963
Microsporum gypseum	Nannizzia incurvata Nannizzia gypsea	Stockdale, 1961 Stockdale, 1963
Microsporum nanum	Nannizzia obtusa	Dawson and Gentles, 1961
Microsporum persicolor	Nannizzia persicolor	Stockdale, 1967
Microsporum vanbreuseghemii	Nannizzia grubyia	Georg, Ajello, Friedman and Brinkman, 1962
Trichophyton mentagrophytes	Arthroderma simii	Stockdale, Mackenzie, and Austwick, 1965
Trichophyton ajelloi	Arthroderma uncinatum	Dawson and Gentles, 1961
Trichophyton georgiae	Arthroderma ciferrii	Varsavsky and Ajello, 1964
Trichophyton gloriae	Arthroderma gloriae	Ajello and Cheng, 1967
Trichophyton terrestre	Arthroderma quadrifidum	Dawson and Gentles, 1961
Trichophyton vanbreuseghemii	Arthroderma gertleri	Bohme, 1967

one of the dermatophytes. Yeast extract stimulates sporulation of Microsporum audouini (Hazen 1947) and tryptose is somewhat effective for Trichophyton rubrum (Benham 1948). Yeast extract and tryptose are, however, far from being chemically defined and so further studies are indicated.

Chin and Knight (1957) were able to induce macrospore production in four out of five strains of Trichophyton mentagrophytes and in six strains of Trichophyton rubrum by using increased carbon dioxide tension of from twelve to twenty-four per cent. These were not interpreted as mutations because when the fungi were grown again in the normal atmosphere, sporulation decreased or ceased as observed previously. It was suggested that the macrospore formation under increased carbon dioxide tension may be associated with the metabolism of pyrimidines and purines.

Georg and Camp (1957) proposed the use of nutritional tests for the identification of the dermatophytes. They used vitamin free casamino acids as a base media and added inositol, thiamine, nicotinic acid, and histidine separately or in combinations to demonstrate specific patterns of stimulation. Burack and Knight (1958) reported abundant macrospore production by six downy strains of Trichophyton rubrum and Trichophyton mentagrophytes when grown in submerged cultures of Sabouraud's glucose broth.

Stock and McPherson (1964), in doing respiratory studies, found that ammonia production and oxygen uptake was high when aspartic acid or alanine was the substrate. McBride and Stock (1966) compared the endogenous oxygen uptake of Microsporum gypseum macroconidia with the uptake of microconidia of Microsporum quinckeanum and found the rate

54 times higher in the macroconidia. Using (carbon 14) labeled D-glucose, oxidative assimilation studies were performed which showed that 75% of the assimilated glucose was associated with the macromolecular cell constituents. The remaining 25% was judged to be glucose and metabolic intermediates, because it was soluble in cold trichloroacetic acid.

Trichophyton schoenleinii is one of the dermatophytes in which macrospore production is very scarce and only rudimentary spores have been described but Grappel, Fethiere, and Blank (1971) were able to induce macroconidia by treating the inocula with rabbit antiserum.

Silva (1958) suggested that the response of the dermatophyte probably depends upon the presence of an unidentified substance in the active substrates. This explanation would account for the many varied and at times, apparently contradictory reports. Another factor that may contribute to this problem is the spontaneous development of pleomorphism by some of the dermatophytes.

Objective of the Study

The purpose of this study was to determine the effect of various nutrients on the macroconidia production of Trichophyton rubrum. Purines and pyrimidines were included in the nutrients tested because of the suggestion by Chin and Knight (1957) that metabolism of these substances were thought to be associated with the formation of macrospores when incubated with increased carbon dioxide concentrations.

The tryptose used by Benham (1947) and the carbon dioxide used by Chin and Knight (1957) though somewhat effective have not been generally accepted in the clinical laboratory as a practical diagnostic tool. In comparison, the nutritional tests of Georg and Camp (1957) have been

used widely and the Trichophyton agars available in dehydrated form from the Difco company are based upon their excellent work.

It was hoped that the information gained from this study would contribute to the development of a chemically defined media suitable for the induction of sporulation in this species.

MATERIALS AND METHODS

Organism

Trichophyton rubrum strain # 28, a granular variety, was obtained from the Department of Health, Education and Welfare, Public Health Service, National Communicable Disease Center, Atlanta, Georgia. Preliminary tests with this strain revealed typical colony morphology with moderate production of macroconidia when grown on Sabouraud's dextrose agar.

To minimize the development of pleomorphism, the stock culture was preserved by covering growth of a two-week old slant with sterile mineral oil and storing at room temperature.

Preparation of Media

The base media used was the same as Czapek's with the exception that glucose was substituted for the saccharose and it was made up in double strength to allow for the addition of an equal volume of the individual nutrients that were tested. The base media ingredients are shown in table II.

TABLE II

CZAPEK SOLUTION AGAR (modified)

Glucose	20 g	
Sodium nitrate	2 g	
Dipotassium phosphate	1 g	
Magnesium sulfate	0.5 g	
Potassium chloride	0.5 g	
Ferrous sulfate	0.01 g	
Distilled water	500 ml	(for double strength)

The nutrients employed were diverse and for convenience are arranged in four groups in table III.

TABLE III

NUTRIENTS EMPLOYED

Nutrient	Low concentration	High concentration
Amino acids:	50 mg/100 ml	250 mg/100 ml
Alanine		
Arginine HCl		
Asparagine		
Aspartic acid		
Cysteine, free base		
Cystine		
Glutamine		
Glutamic acid		
Histidine HCl		
Hydroxyproline		
Isoleucine		
Leucine		
Lysine HCl		
Methionine		
Phenylalanine		
Proline		
Serine		
Threonine		
Tryptophan		
Tyrosine		
Valine		
Purines and Pyrimidines:	50 mg/100 ml	250 mg/100 ml
Adenine		
Cytosine		
Guanine		
Hypoxanthine		
5-Methyl Cytosine HCl		
Thymine		
Uracil		
Xanthine, free base		
Complex mixtures		250 mg/100 ml
Peptone		
Yeast extract		
Miscellaneous	50 mg/100 ml	250 mg/100 ml
Lactate		
Malonate		
Pyruvate		

All of the media was sterilized by autoclaving for 15 minutes at 121° C. and then cooled in a 50° waterbath. A portion of each nutrient was then mixed with an equal volume of base media, the pH checked with a Corning Model 12 pH meter and adjusted to neutrality, (plus or minus 0.5 pH units) with 0.1 normal hydrochloric acid or sodium hydroxide, and 20 ml was dispensed into each of the petri dishes (15 X 100 millimeters in diameter). Five plates were prepared of each nutrient, one low concentration and four of the high concentration. Plates of the base media was prepared by mixing the double strength base media with an equal volume of sterile distilled water before dispensing into petri dishes.

Preparation of Inocula

When new test media was to be inoculated, material was transferred from the stock culture under oil to fresh Sabouraud's glucose agar slants and grown for two generations to check for typical growth characteristics. If macroscopic appearance was typical and the microscopic examination showed good sporulation, the growth of a second generation slant was covered with 5 milliliters of sterile distilled water and the surface was gently rubbed with a sterile loop to dislodge as many spores as possible. This suspension was transferred to sterile tubes, centrifuged, and washed 3 times with sterile water, to minimize the carry-over of nutrients to the test media. After the last wash the sediment was resuspended in 5 milliliters of fresh distilled water and a drop of the suspension was placed in the center of each of the prepared plates.

Incubation of Plates

As soon as the excess moisture from the inocula was absorbed into the agar, the plates were inverted and placed in the incubator at 25° C. Colonies on this chemically defined media took approximately twice as long to mature as when grown on Sabouraud's glucose agar, therefore, spore counts were made on all cultures after 3 and 4 weeks of incubation in order to encompass the period of maximum sporulation.

Spore Count Technique

The samples for microscopic examination were prepared by cutting a section of growth 2 millimeters wide along the entire radius of the colony. This material was placed on a microslide with a few drops of lactophenol cotton blue and teased apart so as to give maximum visualization of the structures. Three samples were taken from each plate. The slides were labeled with a diamond point pencil and a cover glass was placed on each mount to facilitate examination and to yield a semi-permanent preparation. The entire area of each mount was systematically examined and the number of macroconidia recorded.

RESULTS

Colony Size as Related to Nutrient Employed

Although the main purpose was not to study the effect of nutrients on growth in terms of colony size it was noted that the complex mixtures gave the most growth as shown in table IV. Peptone produced colonies 47 millimeters in diameter and yeast extract was a close second with colonies 45 mm in diameter. Of the chemically defined additives, proline produced the greatest growth stimulation with colonies 28 mm in diameter. Glutamine, alanine, arginine, and hypoxanthine were slightly stimulatory, and had colonies 25 mm in diameter as compared to the base media colonies which were 18 mm in diameter. Some of the nutrients appeared to be definitely inhibitory in both the high and low concentrations. For example adenine, methionine, glutamic acid, leucine, threonine, hydroxyproline, histidine, and cysteine all gave colonies of less than 14 mm. Malonate however, showed no growth stimulation at the high concentration but gave good colony growth at the low concentration, (0.25 mg/ml). Aspartic acid was strongly inhibitory in high concentration with colonies less than one-half those of the base media yet was slightly stimulatory in low concentration. See tables IV and V for a complete comparison.

Pigment Production

Pigment production is one of the more striking characteristics of some of the dermatophytes. Considerable variation exists within species so that this criteria is not without pitfalls. *Trichophyton rubrum* is characterized by the formation of red pigment. Some strains of the closely related *Trichophyton mentagrophytes* will, at times,

TABLE IV

COLONY CHARACTERISTICS
(high concentration)

Nutrient (1.25 mg/ml)	Type of growth	Color on reverse side of plate	Average diameter in mm	
Peptone	membranous-powdery	red	47	
Yeast extract	membranous-granular	dark red	45	
Proline	granular	brown-yellow	28	(25)
Glutamine	velvety-fuzzy	yellow	25	(28)
Alanine	velvety	light yellow-br.	25	(25)
Arginine HCl	velvety	red-yellow-brown	25	(25)
Hypoxanthine	memb. subsurface	none	25	(25)
Cystine	velvety-fuzzy	brown-yellow	20	(24)
Asparagine	velvety	yellow	20	(20)
Guanine	memb. subsurface	light pink	20	(20)
Xanthine, free base	memb. subsurface	cream	19	(20)
Isoleucine	velveth-powdery	red	18	(16)
Serine	velvety-granular	yellow-brown	18	(22)
Phenylalanine	granular	red	18	(18)
Pyruvate	memb. subsurface	none	17	(18)
Lysine HCl	velvety	yellow	16	(18)
Malonate	memb. subsurface	none	15	(30)
5-Methyl Cytosine	memb.-velvety	cream	15	(20)
Thymine	memb. subsurface	none	15	(15)
Lactate	memb. subsurface	none	15	(15)
Uracil	memb. subsurface	none	15	(15)
Cytosine	memb. subsurface	light pink	14	(20)
Tyrosine	velvety-powdery	dark red	14	(14)
Valine	granular-velvety	light red	14	(12)
Cysteine, free base	memb. velvety spots	none	12	(13)
Histidine HCl	membranous	yellow-brown	12	(12)
Hydroxyproline	membranous-powdery	cream	12	(12)
Threonine	memb. velvety spots	none	12	(12)
Leucine	velvety-fuzzy	none	12	(12)
Tryptophan	memb. velvety spots	yellow-brown	8	(14)
Methionine	membranous	none	7	(10)
Aspartic acid	memb. granular spots	red-brown	7	(24)
Glutamic acid	membranous	none	6	(10)
Adenine	memb. subsurface	none	6	(10)
Base media alone	membranous	none	18	

Note: The diameter of colonies with low concentration of nutrient (0.25 mg/ml) are given in parenthesis for comparison.

TABLE V
COLONY CHARACTERISTICS
(low concentration)

Nutrient (0.25 mg/ml)	Type of growth	Color on reverse side of plate	Diameter in mm	
Malonate	memb. subsurface	none	30	(15)
Glutamine	velvety	yellow-brown	28	(25)
Proline	granular	brown-yellow	25	(28)
Alanine	velvety	light yellow-br.	25	(25)
Arginine HCl	velvety	red-yellow	25	(25)
Hypoxanthine	memb. subsurface	cream	25	(25)
Aspartic acid	velvety-powdery	light brownish	24	(7)
Cystine	velvety-fuzzy	brownish pink	24	(20)
Serine	velvety	light yellow	22	(18)
5-Methyl Cytosine	memb.-velvety	cream	20	(15)
Asparagine	velvety-granular	yellow	20	(20)
Cytosine	memb. subsurface	light pink	20	(14)
Guanine	memb. subsurface	light pink	20	(20)
Xanthine, free base	memb. subsurface	cream	20	(19)
Phenylalanine	granular	red	18	(18)
Lysine HCl	velvety	brownish yellow	18	(16)
Pyruvate	memb. subsurface	none	18	(17)
Isoleucine	velvety-powdery	red	16	(18)
Thymine	memb. subsurface	cream	15	(15)
Uracil	memb. subsurface	cream	15	(15)
Lactate	memb. subsurface	none	15	(15)
Tyrosine	memb. -powdery	light red	14	(14)
Tryptophan	memb. velvety spots	none	14	(8)
Cysteine, free base	memb. velvety spots	none	13	(12)
Histidine HCl	membranous	light red	12	(12)
Hydroxyproline	membranous	light pink	12	(12)
Threonine	memb. granular spots	none	12	(12)
Leucine	velvety-fuzzy	red	12	(12)
Valine	granular-velvety	light red	12	(14)
Glutamic acid	memb.-velvety	red	10	(6)
Methionine	memb. granular spots	light pink	10	(7)
Adenine	memb. subsurface	none	10	(6)
Base media alone	membranous	none	18	

Note: The diameter of colonies with high concentration (1.25 mg/ml) are given in parenthesis for comparison.

produce a similar pigment, and because of this gross similarity, some authorities have recommended the use of 1% dextrose corn meal agar. Trichophyton rubrum is said to produce a typical red pigment while Trichophyton mentagrophytes gives no pigment. Haley (1964) states that about 10 per cent of Trichophyton rubrum strains will fail to produce characteristic pigment on this media so it is unwise to rely too strongly on this method of identification. She recommends the use of the in vitro hair culture instead.

There was great variation in pigment production among the nutrients studied; most of the colonies produced some pigment, either red, brown, yellow or cream. One-third of the colonies failed to produce any pigment at all.

Since there was no pigment on the base media, it was quite clear that pigment production is altered greatly by the nature of the nutrients in the media. The pigment production by arginine was rather intense and crystals of, what appeared to be, pigment were found microscopically in the slide mounts, (figure 1). The pigment production of the dermatophytes has been studied rather extensively and varies with both the nitrogen and carbohydrate source. Silva (1958) found maximum pigment production in Trichophyton mentagrophytes by ornithine followed by, proline, phenylalanine, beta-alanine, serine, histidine, arginine, and lysine. Wirth (1957) extracted the pigment and attempted to identify the various components that go to make up the coloring complex. The colors observed in this study are listed in tables IV and V, and are shown in the colored plates in figures 2, 3, and 4.

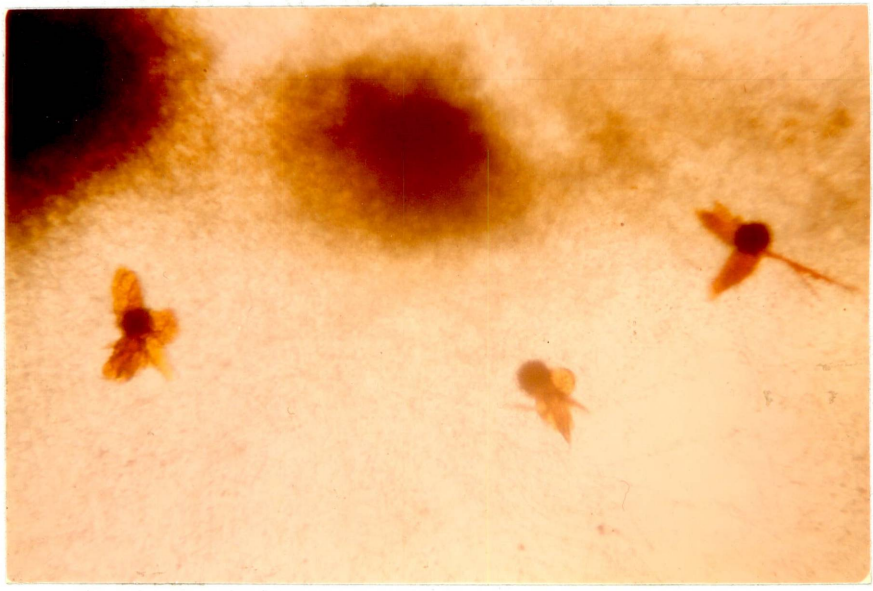


Figure 1. Reddish-orange crystals from the highly pigmented cultures with arginine.

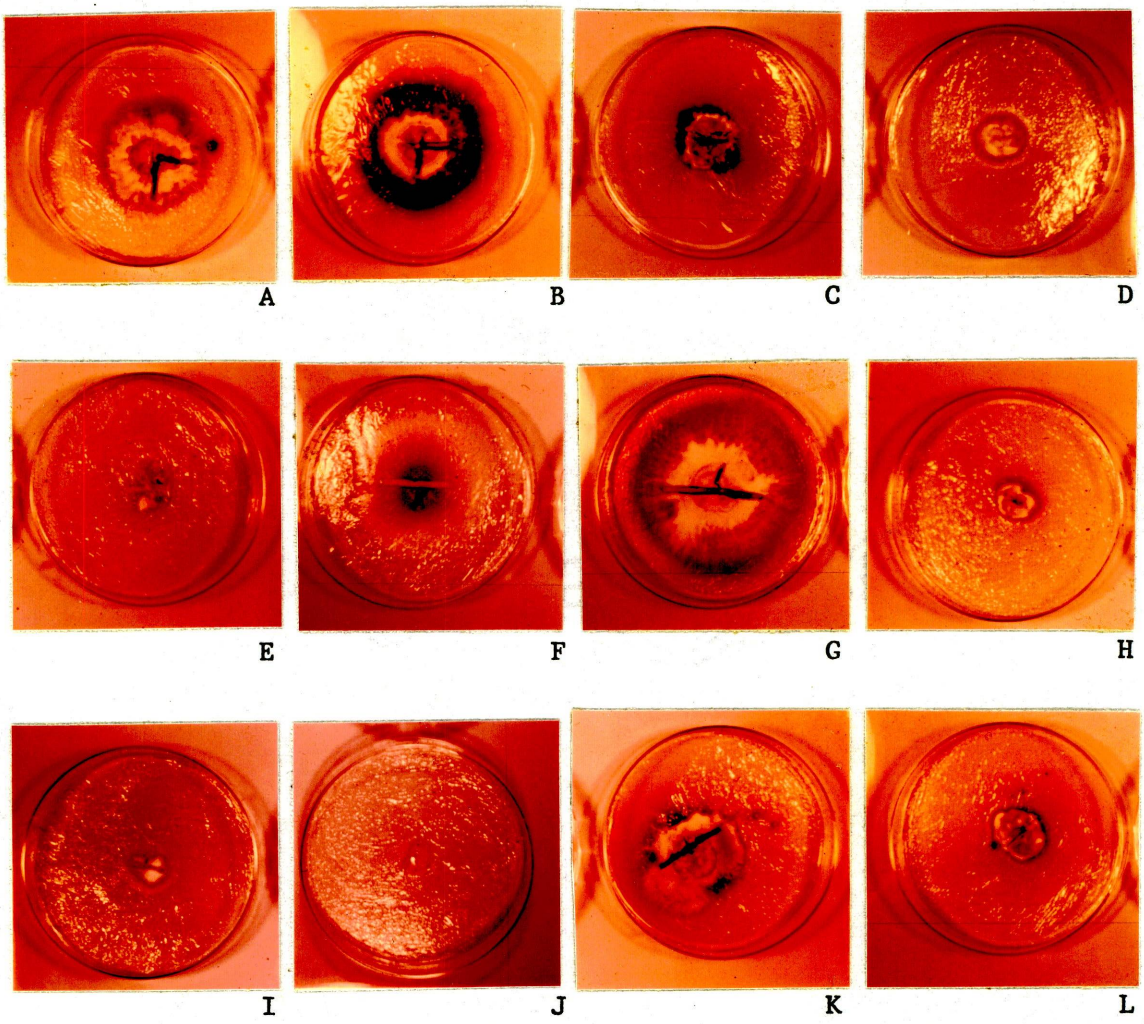


Figure 2. A. Alanine. B. Arginine HCl. C. Asparagine. D. Aspartic acid. E. Cysteine, free base. F. Cystine. G. Glutamine. H. Glutamic acid. I. Histidine HCl. J. Hydroxyproline. K. Isoleucine. L. Leucine.

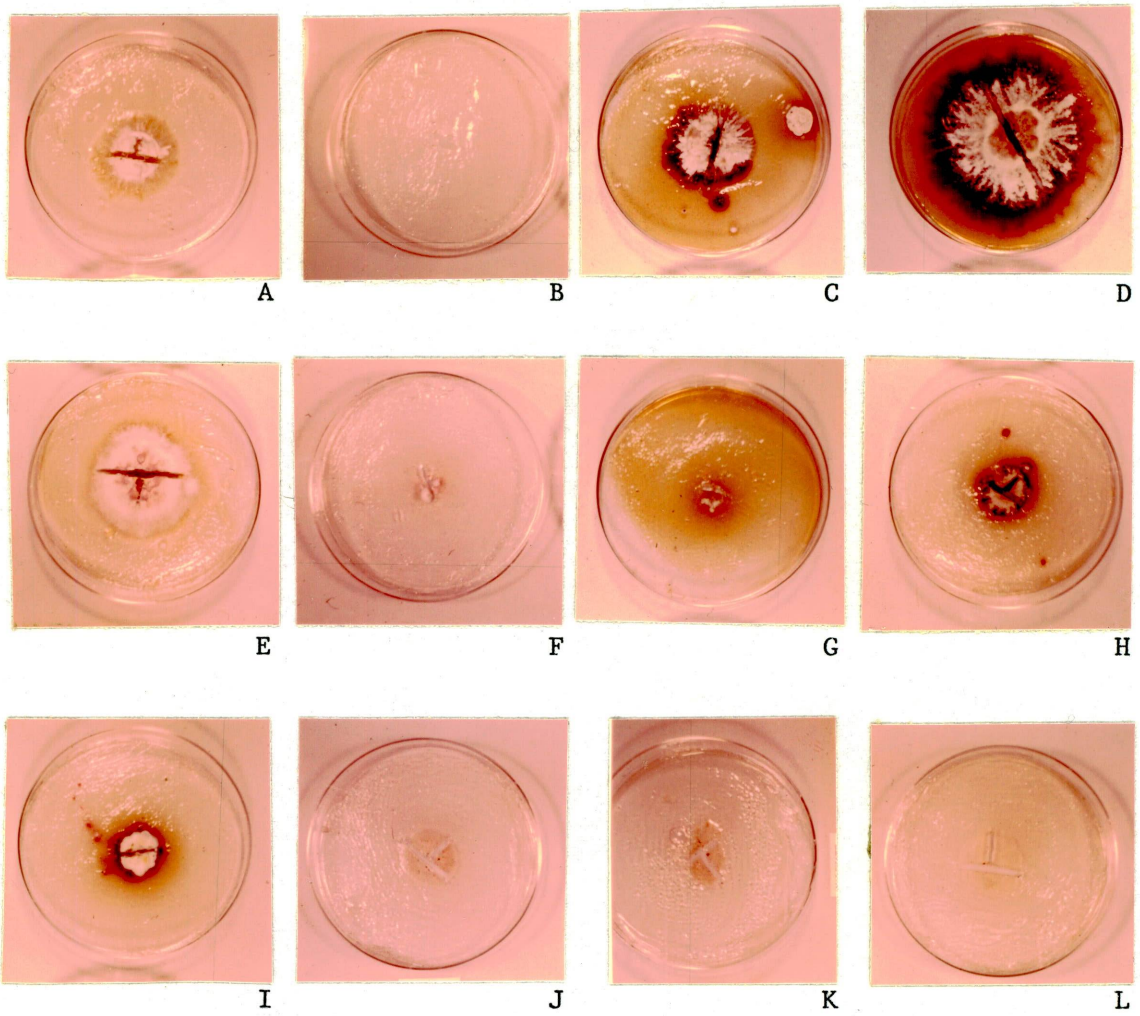


Figure 3. A. Lysine HCl. B. Methionine. C. Phenylalanine. D. Proline. E. Serine. F. Threonine. G. Tryptophan. H. Tyrosine. I. Valine. J. Lactate. K. Malonate. L. Pyruvate.

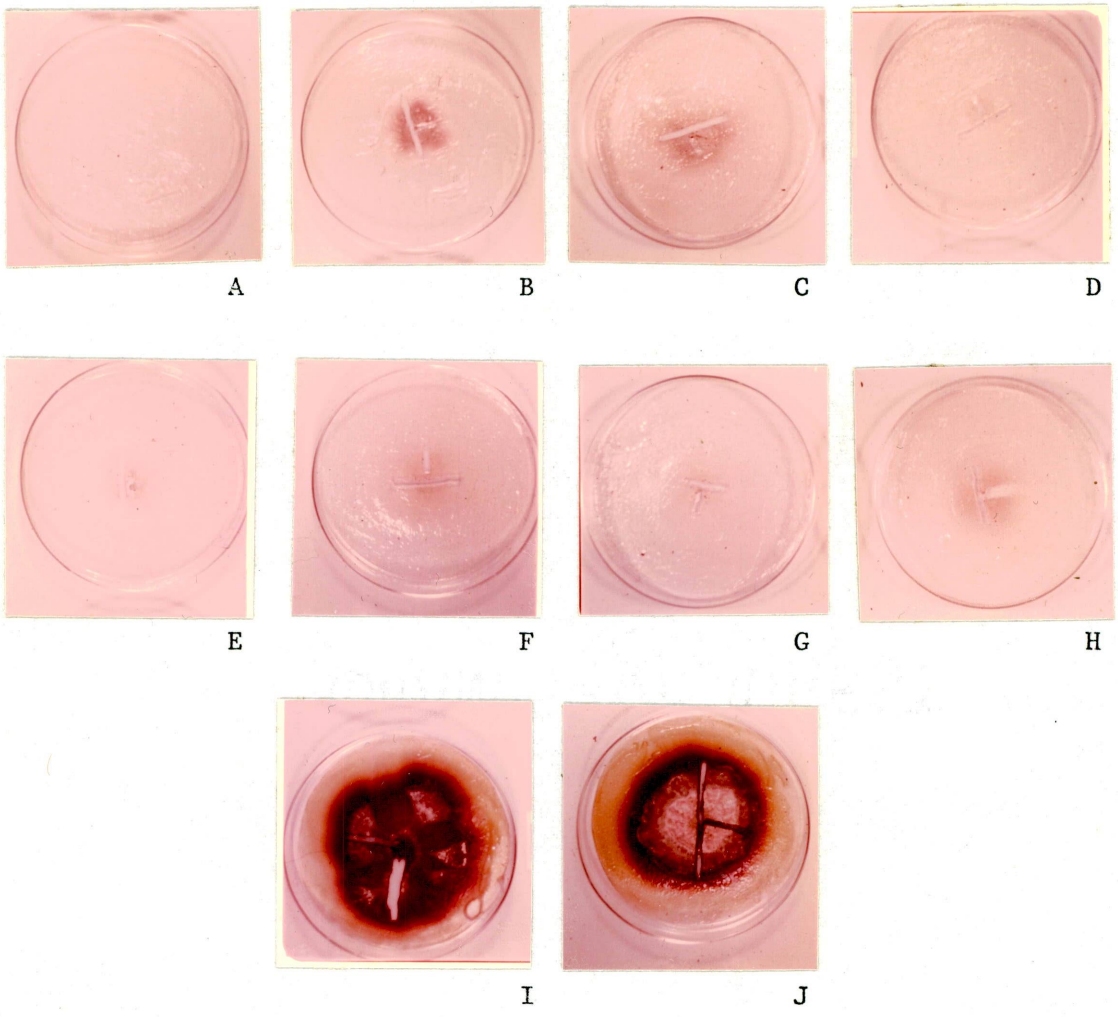


Figure 4. A. Adenine. B. Cytosine. C. Guanine. D. Hypoxanthine. E. 5-Methyl cytosine HCl. F. Thymine. G. Uracil. H. Xanthine, free base. I. Peptone. J. Yeast extract.

Macrospore Production

One of the most striking findings was the lack of correlation between colony size and the production of macroconidia. For example aspartic acid produced an average spore count of 244 from a colony diameter of 6-8 millimeters whereas tryptophan produced no spores on colonies of similar size. Proline was second in stimulatory action with a spore count of 151 and a colony diameter of 28-30 mm. The nutrients that produced the largest colonies was peptone and yeast extract, both complex substances. Only five of the individual amino acids were judged to give good colony size and at the same time produced good sporulation. These were asparagine, serine, isoleucine, phenylalanine and proline. The colony size varied from a minimum of 18 mm to a maximum of 30 mm. The spore counts for this group ranged from 98 to 151. There were 6 nutrients that were only fair in their ability to stimulate sporulation. They were lysine, alanine, tyrosine, leucine, glutamic acid, and malonate. This group had spore counts that varied from a high of 56 for valine, down to 28 for malonate. There were 5 nutrients that were more stimulatory in the low concentration. These were valine, leucine, methionine, cysteine, and histidine. Although the colony size for these were very similar to the corresponding colonies with the higher concentration, the number of macroconidia were approximately 2-10 times greater with the lower concentration, and ranged from 106 for valine down to 24 for cysteine. The other nutrients were only fair in their ability to stimulate macroconidia production. Tables VI and VII give a complete listing of the number of macroconidia for the high and low concentrations of each nutrient.

TABLE VI

MACROCONIDIA PRODUCTION

Nutrient (high concentration) (1.25 mg/ml)	Macroconidia per mount	
Aspartic acid	244	(6)
Proline	151	(27)
Phenylalanine	131	(53)
Peptone	130	
Isoleucine	125	(68)
Yeast extract	118	
Serine	105	(28)
Asparagine	98	(10)
Valine	56	(106)
Lysine HCl	48	(9)
Alanine	45	(24)
Tyrosine	44	(3)
Leucine	34	(70)
Glutamic acid	28	(16)
Malonate	28	(0)
Glutamine	14	(3)
Cystine	7	(0)
Methionine	7	(55)
Threonine	6	(2)
5-Methyl Cytosine HCl	6	(0)
Cysteine, free base	5	(24)
Histidine HCl	4	(49)
Hydroxyproline	4	(9)
Arginine HCl	4	(5)
Pyruvate	2	(0)
Tryptophan	0	(0)
Adenine	0	(0)
Cytosine	0	(0)
Guanine	0	(0)
Hypoxanthine	0	(0)
Thymine	0	(0)
Uracil	0	(0)
Xanthine, free base	0	(0)
Lactate	0	(0)
Base media alone	0	

Note: The values in parenthesis are the number of macroconidia per mount found with the low concentration of nutrient (0.25 mg/ml).

TABLE VII

MACROCONIDIA PRODUCTION

Nutrient (low concentration) (0.25 mg/ml)	Macroconidia per mount	
Valine	106	(56)
Leucine	70	(34)
Isoleucine	68	(125)
Methionine	55	(7)
Phenylalanine	53	(131)
Histidine HCl	49	(4)
Serine	28	(105)
Proline	27	(151)
Alanine	24	(45)
Cysteine, free base	24	(5)
Glutamic acid	16	(28)
Asparagine	10	(98)
Lysine HCl	9	(48)
Hydroxyproline	9	(4)
Aspartic acid	6	(244)
Arginine HCl	5	(4)
Tyrosine	3	(44)
Glutamine	3	(14)
Threonine	2	(6)
Malonate	0	(28)
5-Methyl Cytosine HCl	0	(6)
Pyruvate	0	(2)
Tryptophan	0	(0)
Adenine	0	(0)
Cytosine	0	(0)
Guanine	0	(0)
Hypoxanthine	0	(0)
Thymine	0	(0)
Uracil	0	(0)
Xanthine, free base	0	(0)
Lactate	0	(0)
Base media alone	0	

Note: The values in parenthesis are the number of macroconidia per mount found with the high concentration of nutrient (1.25 mg/ml).

DISCUSSION

According to the first principle of Klebs, (Cochrane, 1958) reproduction is most likely to occur when a mycelium that is growing vigorously experiences a depletion of nutrients. This can occur either from transferal of the active mycelial to a medium low in nutrients or when a mycelium exhausts its nutrients. This principle would appear to apply to the colonies that had good growth, but fails to explain the excellent sporulation obtained with aspartic acid on colonies only 6-8 mm in diameter. Here we have something else acting as the triggering mechanism. It seems more likely that we may have produced a relative, rather than an absolute depletion of nutrients. A somewhat similar interaction was observed by Chin and Knight (1963). They observed no stimulation by increased tensions of carbon dioxide when glucose was omitted from the medium. They also reported the principle carbohydrate found in the macroconidia to be glucose which was present in a higher concentration than in the other structures of the fungus. Furthermore strains which gave a poor response to carbon dioxide stimulation, did not show an increase in the carbohydrate content.

From the above findings it would appear that the factors that favor sporulation are very complex and are influenced not only by specific nutrients but are also sensitive to the concentration of these substances, and their relationship to one another. From the data obtained in this study, it would appear that the source of nitrogen plays a major role in influencing the sporulation. The base media used contained no organic source of nitrogen and it is interesting to note that there was no sporulation observed on these plates. Other nutrients

such as histidine produced a membranous type of growth with very poor spore production. By comparison, aspartic acid produced small membranous colonies with granular spots. Associated with these areas of short aerial mycelium was found large numbers of macroconidia (figure 5). In the areas where the growth was subsurface or membranous, without any aerial growth, chlamydospores were the principle structures observed (figure 6). The color plates in figure 4 show these differences particularly well. Note the granular to powder-like surface of the peptone and yeast extract plates as compared to the membranous growth of the other nutrients.

Yeast extract and peptone are both accepted as being relatively efficient in stimulating sporulation but when the values for macrospore production per square millimeter are compared (table VIII), eleven of the amino acids gave values equal to or greater than either one. When these were submitted to statistical analysis using the square root transformation and the t values (Steel and Toorie, 1960) were calculated, five of the amino acids gave spore counts significantly greater than yeast extract with P values less than 0.05 (table IX).

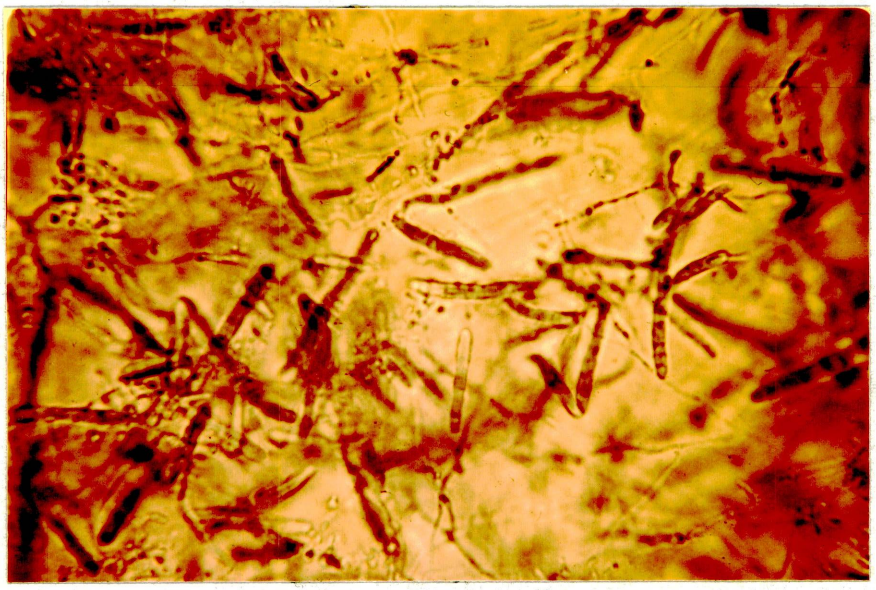
$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{S_p \quad 1/N_1 + 1/N_2}}$$

$$S_p = \sqrt{\frac{(N_1 - 1)S_1^2 + (N_2 - 1)S_2^2}{N_1 + N_2 - 2}}$$

Where $\bar{x}$ = mean

N = number of samples per nutrient

S^2 = variance



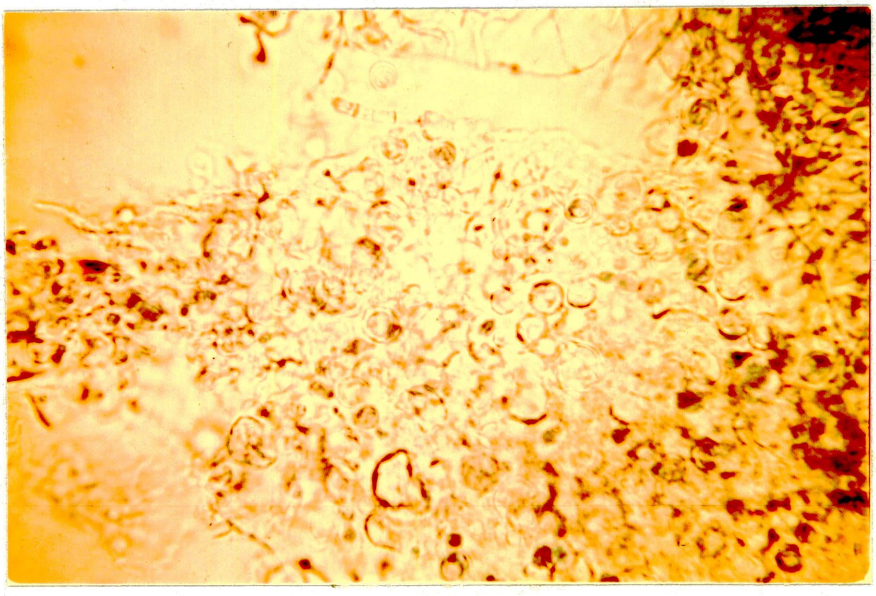


Figure 5. The enormous number of macroconidia produced on aspartic acid.

Figure 6. Many large chlamydospores produced on histidine.

TABLE VIII

MACROCONIDIA PER SQUARE MILLIMETER

Nutrient	Number of samples	Total count	Average count	Average per mm^2	Standard deviation
Aspartic acid	12	2931	244	30.5	13.1
Phenylalanine	12	1577	131	7.3	3.3
Isoleucine	12	1504	125	6.9	5.3
Serine	12	1260	105	5.8	3.4
Proline	12	1810	151	5.2	3.8
Asparagine	12	1177	98	4.9	2.7
Glutamic acid	12	330	27	4.6	4.3
Valine	12	678	56	4.2	3.7
Lysine	12	583	49	3.2	3.0
Tyrosine	12	523	44	3.1	3.0
Leucine	12	409	34	2.8	2.8
Peptone	9	1175	130	2.8	5.8
Yeast extract	9	1060	118	2.6	2.2
Alanine	12	536	45	1.8	0.9
Malonate	6	334	28	1.7	1.4
Methionine	12	83	7	0.9	0.9
Glutamine	12	170	14	0.5	0.4
Threonine	12	77	6	0.5	0.6
Cysteine, free base	12	65	5	0.4	0.6
Histidine	12	47	4	0.4	0.4
Cystine	12	85	7	0.4	0.3
Hydroxyproline	12	53	4	0.4	0.2
5-Methyl cytosine	12	66	5	0.3	0.4
Arginine	12	42	3	0.1	0.1
Pyruvate	12	18	1.5	0.1	0.1
Adenine	12	6	0.5	0.1	0.08
Tryptophan	12	6	0.5	0.1	0.08
Lactate	12	5	0.4	0.03	0.04
Thymine	12	3	0.25	0.02	0.06
Hypoxanthine	12	4	0.33	0.01	0.03
Cytosine	12	1	0.08	0.01	0.3
Guanine	12	0	0	0	0
Uracil	12	0	0	0	0
Xanthine	12	0	0	0	0
Base media alone	12	0	0	0	0

Note: Four plates of each nutrient were set up except for peptone, yeast extract, and malonate. Three plates were used for peptone and yeast extract and two plates for malonate. Three mounts were made from each plate.

TABLE IX

"t" VALUES OF SINGLE AMINO ACIDS VS YEAST EXTRACT

Amino acid	t value	P
Aspartic acid	6.3	0.01
Phenylalanine	3.7	0.01
Serine	2.5	0.02
Isoleucine	2.3	0.05
Asparagine	2.1	0.05
Proline	1.96	0.1
Glutamic acid	1.3	0.2
Valine	1.2	0.3

Note: P is the probability of having t this large or larger by chance.

Proposal for further studies

Further studies are needed to test a large number of other strains to determine whether the reactions observed with strain # 28 are typical of the species.

Additional investigations using mixtures of the various amino acids should be performed to find the best combination for good growth and maximum sporulation.

A preliminary study was performed using a mixture of five amino acids. Valine, leucine, methionine, cysteine, and histidine were added to the base media so as to yield a final concentration of 0.25 mg/ml of each of the amino acids. The average colony size was 24 mm but the macroconidia production was poor (16 per mount or 0.67 per square millimeter).

SUMMARY

Trichophyton rubrum was grown on a base media without any organic source of nitrogen. There were no macroconidia produced on this media but when an amino acid, purine, pyrimidine or a complex mixture such as peptone or yeast extract was added, sporulation took place in varying degrees of intensity.

The most abundant sporulation was produced by aspartic acid which had four times more macroconidia per square mm than any of the other nutrients.

Yeast extract and peptone are both accepted as being relatively efficient in producing sporulation yet, eleven of the amino acids had spore densities equal to or greater than either one.

Five of the amino acids gave good sporulation as well as colony size and had t values significantly greater than yeast extract. The colony size varied from a minimum of 18 mm to a maximum of 30 mm, and had spore densities of 4.9 for asparagine to 7.3 per square mm for phenylalanine. These amino acids were asparagine, proline, serine, isoleucine, and phenylalanine.

Five of the nutrients were more stimulatory in the low concentration. They were valine, leucine, methionine, cysteine, and histidine. Although the colony size for these were very similar to the corresponding colonies with the higher concentration of nutrient, the number of macroconidia were approximately 2-10 fold greater.

Large reddish-orange crystals were observed in the microscopic mounts of the highly pigmented cultures with arginine and were judged to be crystals of the red pigment.

The gross appearance of the colonies that yielded good sporulation were not those with heavy aerial mycelium. On the contrary, sporulation was greatest when the growth was more submerged and there was only scanty granular to powdery surface growth.

When the growth was entirely membranous without aerial mycelium or was subsurface only, there was little or no macroconidia produced and the predominant structures were chlamydospores and a few microconidia.

Judging from the data obtained from this study, it seems very unlikely that purines or pyrimidines are instrumental in the production of sporulation of Trichophyton rubrum.

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LOMA LINDA UNIVERSITY

Graduate School

THE EFFECT OF VARIOUS NUTRIENTS ON THE SPORULATION OF *TRICHOPHYTON RUBRUM*

By

P. Jerry Austin

An Abstract of a Thesis in Partial Fulfillment of the

Requirements for the Degree

Master of Science in the Field of Microbiology

June 1972

ABSTRACT

Trichophyton rubrum was grown on a chemically defined media in an effort to find a substance that would give the maximum production of macroconidia. Twenty-one amino acids were tested and about one half of them were active in stimulating macroconidial formation. Eight purines and pyrimidines were also tested but none of them gave more than scanty sporulation. The base media used was Czapek solution agar (modified): the saccharose was replaced with an equal amount of glucose. The various nutrients were tested at two concentrations, 1.25 mg/ml and 0.25 mg/ml. Yeast extract and peptone were also included in the study since these complex mixtures are both known to increase growth and promote sporulation of some of the dermatophytes.

The color of the colonies varied greatly and ranged from colorless on the base media to a deep red. Some of the mounts had large reddish-orange crystals, believed to be crystalized pigment.

Five of the amino acids produced abundant sporulation, and had significantly higher counts of macroconidia than either yeast extract or peptone. Aspartic acid was outstandingly effecient; the number of macroconidia per square millimeter was more than four times greater than any of the other nutrients.

Additional investigations are proposed to compare the reaction of other strains and to find the best combination for good growth and maximum sporulation.