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84. Tatsuzo Fujii : Biochemical Studies on Pathogenic Fungi. VI.* Nutritional Studies on *Trichophyton gypseum* with Special Reference to its Pleomorphism.

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Dermatophytes tend easily to change their culture form irreversibly under certain conditions *in vitro* and appear as sterile, downy growth, losing the initial characteristic morphology of each species. Factors such as long duration of cultivation, high temperature, and high sugar content in culture medium, etc. are known to accelerate this so-called "pleomorphism."[1,2]

With respect to physiological modifications accompanying such morphological alteration characteristic in dermatophytes, the only information available is the finding by Robbins and Ma[3] that the pleomorphic form (P-form) could utilize asparagine as the sole nitrogen source more readily than the original normal form (N-form) and furthermore gained the ability to use ammonium nitrogen which the N-form could not use at all. In the present work investigating carbon and nitrogen utilization of *Trichophyton gypseum*, results by the above workers were confirmed and some other differences in nutritional physiology between these two forms of the fungus were demonstrated.

Carbohydrate utilization as carbon source was first studied. The fungus was cultured on media containing one of mono-, di-, or polysaccharides besides proper nitrogen and mineral sources. After incubating at 28° for 14 days, the fungus pad was harvested and the dry weight was determined. Among five monosaccharides tested, glucose and mannose gave the best growth to both types of this fungus, which were followedby fructose, galactose, and sorbose, as indicated in Table I. On the other hand, dior polysaccharides were generally poor carbon sources. Table II shows that only

Table I.

Carbon source* in culture medium	Dry weight of fungus cells grown (mg.)	
	N-form	P-form
—	11.3	12.6
Glucose	54.4	80.6
Mannose	53.0	94.0
Fructose	48.6	59.0
Galactose	24.6	35.4
Sorbose	19.8	25.4

* 200 mg./10 cc. medium

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1) P. Tate: Biol. Rev., **4**, 41(1929).
2) P. H. Gregory: Biol. Rev., **10**. 208(1935).
3) W. J. Robbins, R. Ma: Amer. J. Bot., **32**, 509(1945).

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Table II.

Carbon source* in culture medium	Dry weight of fungus cells grown (mg.)	
	N-form	P-form
—	9.2	12.1
Maltose	34.9	50.1
Sucrose	12.1	15.7
Lactose	9.6	9.3
Starch	19.4	23.5
Inulin	5.6	10.9
Glucose	51.6	84.4

* 200 mg./10 cc. medium.

maltose and, to a lesser extent, starch supported some growth, though even that was far less marked than that given by glucose. Sucrose showed only a slight and lactose and inulin no growth stimulation at all.

As is clear from these tables, the P-form of *T. gypseum* always showed considerably better growth in the presence of respective sugar than the original N-form, suggesting the higher ability of the former to utilize these carbohydrates. Such increased sugar utilization by the P-form was also noted in its ability of glucose assimilation. When both forms of this fungus were grown on three kinds of glucose-peptone medium containing 1, 2, or 4% glucose and dry weight of the harvested mycelium and the amount of glucose consumed were determined at the time of the maximum growth, the economic coefficient thus obtained (dry weight of fungus cells divided by sugar consumed) was appreciably higher in the P-strain than in the N-form of *T. gypseum* in all the media as is apparent from Table III.

Table III.

Culture form of fungus	Glucose present before inoculation (g.)	Glucose remained after growth (g.)	Glucose consumed during growth (g.)	Dry weight of fungus cells grown (g.)	Economic coefficient*
N-Form	1.04	0.38	0.66	0.42	0.64
	2.18	0.82	1.36	0.56	0.41
	4.06	1.90	2.16	0.68	0.31
P-Form	0.99	0.17	0.82	0.56	0.68
	2.15	0.33	1.82	0.86	0.47
	4.08	1.54	2.54	1.15	0.45

* $\frac{\text{dry weight of cells (g.)}}{\text{glucose consumed (g.)}}$

Contrary to the only quantitative difference in carbohydrate utilization, clear-cut qualitative as well as quantitative differences were observed in nitrogen nutrition between these two forms of *T. gypseum*. When they were cultivated on synthetic media containing as the sole nitrogen source one of amino acids mentioned in Table IV,

Table IV.

Nitrogen source* in culture medium	Dry weight of fungus cells grown (mg.)	
	N-form	P-form
Glycine	±	176
DL-Alanine	±	220
DL-Valine	±	204
DL-Leucine	±	198
DL-Serine	—	232
L-Cysteine	—	—
L-Arginine	25	195
L-Glutamate	42	223
L-Aspartate	32	215
L-Phenylalanine	±	85
L-Proline	—	180

— no growth, ± very feeble growth, * 750 μmole/30 cc. medium.

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the P-form grew quite readily on every single amino acid except cysteine, while only arginine, glutamate, and aspartate supported slight but definite growth of the N-form, and others virtually no growth.

Furthermore, growth of *T. gypseum* on four kinds of medium containing different kinds of nitrogenous compounds was compared as described in Table V. The N-form did not grow on ammonium sulfate and produced only 1/3 to 1/4 dry weight of fungus mycelium on amino acid glutamate of that obtained on casein acid-hydrolysate plus two amino acid supplements or on peptone. On the contrary, the P-form showed some growth even on ammonium nitrogen and an appreciable growth on glutamate comparable to that obtained by a more complex nitrogen source, confirming the results by Robbins and Ma.[3]

Table V.

Nitrogen source in culture medium	Content per mg./100 cc. medium	Dry weight of fungus cells grown (mg.) N-form	P-form
$(NH_4)_2SO_4$	280	0	32
Monosodium L-glutamate	500	36	211
Casein acid-hydrolysate	42 (as amino-N)		
DL-Tryptophan	50	106	239
L-Cystine	20		
Peptone (Polypeptone Takeda)	51 (as amino-N)	133	292

These facts may suggest that, as a result of pleomorphic variation, *T. gypseum* acquired elevated metabolic activity in transforming simple nitrogen sources such as ammonium salt or single amino acid to all the necessary building stones of the fungus proteins, in addition to the increased ability in sugar utilization.

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Experimental

Organisms employed—O. P. H.-A 801 strain of *Trichophyton gypseum* Bodin, showing typical normal growth, isolated from a patient with tinea pedis by Dr. S. Ikeda of Osaka Prefectural Hospital, was employed. When grown on Sabouraud's glucose-agar, this strain gave light buff, powdery colonies, and produced numerous microconidia as well as some macroconidia and spirals. The N-form was kept throughout this study in freely sporulating condition *in vitro* by transferring to fresh media of low glucose content (2%) at short intervals not exceeding 10 days as suggested by Robbins and Ma.[3]

This normal strain was cultured on Sabouraud's 4% glucose-agar at 30° for 4 weeks. From the white colonies appearing locally, an inoculum was transferred to a fresh medium and cultured similarly. After subsequent 5 cultivations in the same manner, completely pleomorphic strain was isolated which had no spores, nor any other mycological elements and showed white, velvety appearance, which was used for the experiments.

Measurement of Growth on Carbon Source—To a basal medium described below, respective carbohydrate to be tested was added to make a 2% solution, and peptone as nitrogen source in 0.2%. About 2 mg. of cells in suspension from the stock culture was inoculated to a 50-cc. Erlenmeyer flask containing 10 cc. of this culture medium, previously sterilized 3 times by heating at 100° for 15 mins. After incubating at 28° for 14 days, fungus mycelial mat grown was collected, washed with water, drained, dried at 105~110° until of constant weight, and weighed.

In determining economic coefficient of glucose assimilation, the fungus was grown in a 500-cc. flask containing 100 cc. of culture medium of respective glucose concentration. The amount of glucose present in the medium before inoculation and after 14 days' cultivation were determined by Somogyi's colorimetric method.[4] The dry weight of the fungus mat was weighed as mentioned above.

4) M. Somogyi : J. Biol. Chem., **160**, 69(1945).

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Basal Medium	$MgSO_4 \cdot 7H_2O$	0.1 g.
	M/15 Phosphate buffer (pH 7.0)	100 cc.
	Trace element solution*	0.5 cc.
	Distilled water,	to make 1000 cc.

* Containing H_3BO_3, $CuSO_4$, $MnSO_4$, $ZnSO_4$, and iron alum.

Measurement of Growth on Nitrogen Source—To the basal medium, respective nitrogen source was added to give desired concentration and glucose as carbon source to 2%. Inoculation was made in a 100-cc. Erlenmeyer flask containing 30 cc. of such medium. Other details were the same as above.

Summary

Carbohydrate utilization and nitrogen requirement of *Trichophyton gypseum* were studied and differences in the nutritional physiology between the normal and pleomorphic forms of this fungus were demonstrated. The pleomorphic form showed better growth and more efficient assimilation on monosaccharides than the normal form. Di- or polysaccharides generally failed to support appreciable growth of either form. The pleomorphic form grew far more readily on single amino acid than the normal form, and reproduced even on ammonium nitrogen to some extent which the normal form could not utilize at all.

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85. Tatsuzo Fujii : Biochemical Studies on Pathogenic Fungi. VII.* The Effect of Synthetic Fungicides and Fatty Acids on the Respiration of *Trichophyton gypseum*.

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As a means of evaluating the *in vitro* activity of antibacterial compounds, attempts have been made by several workers to take the degree of their inhibitory action upon the respiration of some representative species.[1~3] This method was applied to dermatophytes by Nickerson.[4,5] According to him, such "metabolic assay method" is more advantageous than the prevailing growth- or germination-inhibition test, because by the former method the direct effect of the compound on respiration of the preformed "adult" fungus mycelium, which is apparently a critical index of the fungal metabolism, can be determined quantitatively.

Applying the findings obtained by the previous study[6] on the respiration of a dermatophyte, *Trichophyton gypseum*, inhibitory action of several antimycotic compounds on the rate of glucose respiration of this fungus was examined here. In dealing with this organism, particular precaution was taken as to the culture forms *in vitro*. Owing to the significant differences in some physiological behavior as well as in morphology

* This work is a part of series entitled "Biochemical Studies on Pathogenic Fungi" by Yuki Ito. Part VI : This Bulletin, **5**, 503(1957).

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5) W. J. Nickerson, J. B. Chadwick : Arch. Biochem., **10**, 81(1946).
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