STUDIES ON THE HYPERTROPHIC DISEASE CAUSED BY TAPHRINA SPECIES (III). MYCELIAL GROWTH OF FUNGI ON SYNTHETIC MEDIUM

STUDIES ON THE HYPERTROPHIC DISEASE CAUSED BY TAPHRINA SPECIES (III). MYCELIAL GROWTH OF FUNGI ON SYNTHETIC MEDIUM

By

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Introduction

Taphrina fungus extends its mycelia into the intercellular space of the host tissue and grows vegetatively (1) (5). After the ascus formation, however, ascospore multiplies by budding like yeast. Thus, these fungi are of both the mycelium-type and conidium-type. Therefore, both types of growth must be included in the investigation on the physiology of these fungi.

As stated in the previous report (2), on the potato dextrose agar these fungi grew in mycelium type, but grew by budding on the synthetic medium (2) without distinction of liquid or solid. Therefore, it was presumed that the both types of growth of the *Taphrina* species were regulated by nutritional factors. We investigated to see if the organic nitrogen compounds and organic acid bring about a mycelium-type growth on the synthetic medium for this fungus. The results of these experiments will be mentioned in this paper.

Material and Method

The species used in this experiments is *Taphrina wiesneri* Mix. (*T. cerasi* Sadeb.).

The synthetic medium used as the basal medium is indicated below.
The composition of the synthetic medium:

$(NH_4)_2SO_4$	0.233 g
KH_2PO_4	0.1 g
$MgSO_4$	0.05 g
$FeCl_3$	trace

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glucose	5.0 g
agar	2.0 g
dist. water	100ml
pH 4–5	

Each medium was sterilized at 100°C for 10 min.. Then, 15ml of each medium was poured into petri dish of 9 cm in diameter and inoculated. After incubation at 25°C for 8 days they were examined for mycelial growth. Details of the method will be mentioned in every sections.

The observations of the mycelium-type growth are expressed in symbols as follows: – invisible, + slight, ++ good, +++ very good (This rank is comparable to the growth obtained on the potato dextrose agar), d: dense, r: rare, (density of mycelia).

The pH value of all media were adjusted to 4-5 with $NaHCO_3$.

Results

I. The mycelial growth on various media

Three nutrient media: potato dextose agar (P.D.A), the synthetic medium stated above and basal synthetic medium containing yeast extract were used.

As shown in Table 1, these fungi grew with a mycelium-type growth (Fig. 2, 4)

Table 1. The mycelium-type growth on various medium

Medium	
P.D.A	+++ d
B.M	–
B.M× 2	–
B.M + Y.Ex.	++ d

Note: Abbreviation
P.D.A.: Potato dextrose agar
B.M: Basal synthetic medium
B.M×2: Duplicate concentration of B.M
B.M.+Y.Ex: Yeast extract added B.M

on the P.D.A medium. While on the synthetic medium used for these experiments as a basal medium the fungus showed condium-type growth (Fig. 1, 3) and mycelium was not formed. On the synthetic medium yeast extract added, the fungus grew vigorously with a mycelium-type, but its growth was less than that of the P.D.A. medium.

II. Effect of vitamin

Vitamins used for this experiment were thiamin, riboflavin, pyridoxin, cobalamin, ascorbic acid, p-aminobenzoic acid, biotin and pantothenic acid. These vitamins were, singularly or in combination, added to the basal medium in

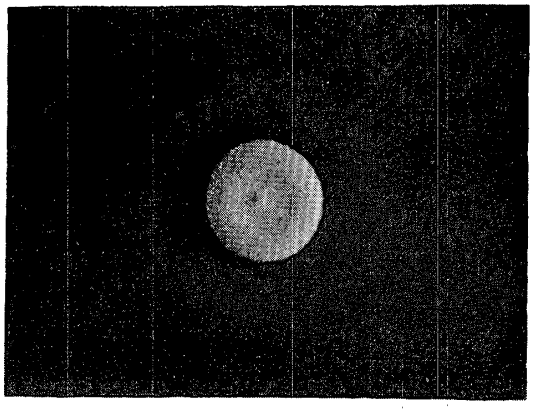

Fig. 1 Conidium-type growth on the synthetic medium. The fungus grows by budding like a yeast.

Fig. 2 Mycelium-type growth on the synthetic medium. Margin of colony is mycelium-type growth and in center of the colony the mycelia transformed to conidia.

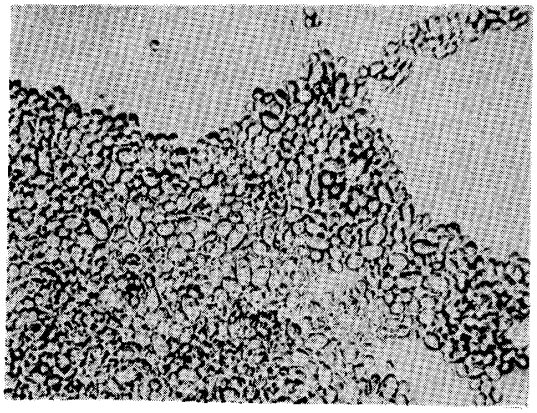

Fig. 3 Conidium-type growth. Conidia multiply by budding.

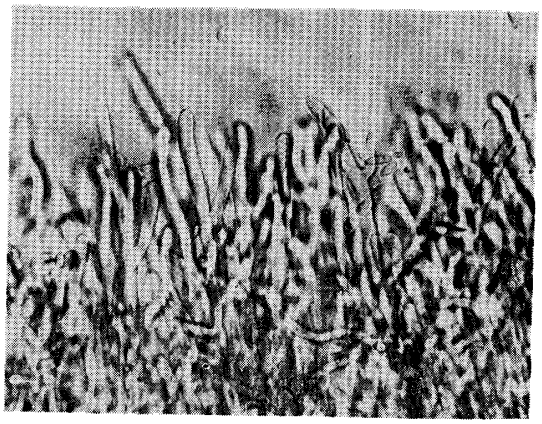

Fig. 4 Mycelium-type growth.

the following concentrations: B_1 100γ, B_2 20γ, B_5 200γ, B_{12} 20γ, nicotinic acid 20γ, p-aminobenzoic acid 20γ, biotin 5γ, pantothenic acid 20γ, and ascorbic acid 20γ per 100 ml.

The results are shown in Table 2: vitamins had no effect on the mycelium-type growth when they were added separately to the medium. When mixed some were slightly effective. Namely, short mycelia were observed when B_1 and biotin or B_1, B_6, biotin, p-aminboenzoic acid and panthothenic acid were simultaneously added. The degree of mycelium formation was almost the same in both media, so it was supposed that the coexistence of B_1 and biotin among various vitamins was effective on mycelium-type growth.

III. Effect of amino acid

1. Effect of various amino acids

The nineteen amino acids used were l-glutamic acid, l-cysteine, l-tyrosine,

Table 2. The effect of vitamin

Vitamin	
B_1	–
B_2	–
B_6	–
B_{12}	–
Ni	–
Pan	–
P	–
C	–
biotin	–
B_1 + biotin	+ d
B_1 + B_6 + Pan + P + Bi	+ d

Note: Abbreviation
Ni: nicotinic acid
Pan: pantothenic acid
P: p-aminobenzoic acid
Bi: biotin

glycine, l-aspartic acid, l-arginine, dl-α-alanine, l-tryptophane, l-leucine, dl-isoleucine, β-alanine, dl-phenylalanine, dl-serine, dl-threonine, l-proline, l-methionine, l-lysine, l-histidine, dl-valine.

One hundred mg of each amino acid was respectively added to 100 ml of the basal medium and observed if mycelia formed. In addition, the effect of various

Table 3. The effect of various amino acids

Amino acid	With vitamin	Without vitamin
l-Glutamic acid	–	–
l-Cysteine	–	–
l-Tyrosine	–	–
l-Aspartic acid	–	–
Glycine	–	–
l-Arginine	–	+ r
dl-α-Alanine	–	–
l-Tryptophane	–	–
l-Leucine	+ d	–
dl-isoleucine	+ d	–
β-Alanine	+ d	–
dl-Phenylalanine	–	–
dl-serine	+ d	+ d
dl-threonine	–	–
l-Proline	–	–
l-Methionine	–	–
l-Lysine	–	–
l-Histidine	+ r	+ r
dl-Valine	+ d	+ r

Note: thiamin (100γ) and biotin (5γ) per 100ml

amino acids was examined for the existence of thiamin (100γ/dl) and biotin (5γ/dl).

In following every experiment, the effect of various organic substances was examined with and without these vitamins.

Table 3 shows the results. The mycelium-type growth was observed in arginine, α-alanine, leucine, isoleucine, β-alanine, serine, histidine, valine respectively. Those in α-alanine, leucine, isoleucine and serine showed mycelial growth only when vitamins were added. In glutamic acid, cysteine, tyrosine, glycine, aspartic acid, tryptophane, phenylalanine, threonine, proline, methionine, and lysine respectively no mycelial growth was observed even when vitamins added.

2. The mixed addition of various amino acids

One hundred mg of each amino acid: glutamic acid as monoaminodicarboxylic acid, glycine as monoaminocarboxylic acid, cysteine as sulfur containing amino acid, tyrosine as aromatic amino acid were added to 100 ml of the basal medium to make following composition.

I	glutamic acid glycine	II	glutamic acid glycine cysteine	III	glutamic acid glycine cysteine tyrosine

As appears in Table 4, this fungus grew slightly with mycelium-type only when the vitamins were added simultaneously. But the mycelium was very short and its growth was not vigorous.

Table 4. Mixed addition of various amino acids

Amino acid	With vitamin	Without vitamin
Gl + gly	+ d	–
Gl + gly + cyst	+ d	–
Gl + gly + cyst + ty	+ d	–

Note: Abbreviation
gl: glutamic acid, cyst: cysteine,
gly: glycine, ty: tyrosine,

Table 5. The concentration of amino acid

Glutamic acid	With vitamin	Without vitamin
100 mg	–	–
200 mg	+ r	+ r
500 mg	++ d	++ r

3. The concentration of amino acid

The effect of a concentration of amino acid on the mycelial growth was investigated. L-glutamic acid which is one of the suitable amino acid for the

conidium-type growth (2) was used.

One hundred mg, 200mg and 500mg of l-glutamic acid were added respectively to 100ml of the basal medium.

Table 5 shows the results. Mycelial growth was not observed in the 100mg, but in the 200 mg and 500mg solution. In the 200mg and 500mg solutions the mycelial growth was observed even without vitamins.

IV. The effect of organic acids

1. The effect of various organic acids

Seven organic acids: oxalic acid, tartaric acid, succinic acid, malic acid, fumaric acid, citric acid, malonic acid were used for this experiment. Organic acid was respectively added to the basal medium in a concentration of 20 mg and 40 mg per 100 ml.

As shown in Table 6, 7 the mycelium-type growth occured only on the oxalic acid added medium.

Table 6. The effect of various organic acids (20 mg)

Organic acid	With vitamin	Without vitamin
Oxalic acid	+ r	+ r
Tartaric acid	–	–
Succinic acid	–	–
Malic acid	–	–
Fumaric acid	–	–
Citric acid	–	–
Malonic acid	–	–

Note: The concentration of organic acids is 20mg per 100ml

Table 7. The effect of various organic acids (40 mg)

Organic acid	With vitamin	Without vitamin
Oxalic acid	+ r	+ r
Tartaric acid	–	–
Succinic acid	–	–
Malic acid	–	–
Fumaric acid	–	–
Citric acid	–	–
Malonic acid	–	–

Note: The concentration of organic acids is 40 mg per 100 ml

2. The mixed addition of organic acids

Various organic acids used in above experiment were added to the basal medium in the concentration of 20 mg per 100 ml to make following composition.

I	II	III
citric acid	citric acid	citric acid
malic acid	malic acid	malic acid
succinic acid	succinic acid	succinic acid
fumaric acid	fumaric acid	fumaric acid
	tartaric acid	tartaric acid
		oxalic acid

Table 8. Mixed addition of organic acids

Organic acid	With vitamin	Without vitamin
c + m + s + f	+ d	−
c + m + s + f + t	+ d	−
c + m + s + f + t + o	+ d	+ r

Note: Abbreviation
c: citric acid, m: malic acid,
s: succinic acid, f: fumaric acid,
t: tartaric acid, o: oxalic acid,

Table 8 shows the results. The fungus grew in the mycelium-type on every media when vitamins were added, but the mycelia were very short. In medium III, which contains oxalic acid, the mycelium-type growth was observed even with no vitamins.

V. The simultaneous addition of amino acid and organic acid

1. The effect of mixed addition of glutamic acid and organic acids

L-glutamic acid and four organic acids which compose the TCA cycle were simultaneously added to the basal medium. The concentration of *l*-glutamic acid was 100, 200 or 500 mg per 100 ml and that of each of the four organic acids was 20 mg.

Table 9. Mixed addition of l-glutamic acid and organic acid

Glutamic acid & organic acid	With vitamin	Without vitamin
100 mg + c + m + s + f	+ d	+ r
200 mg + c + m + s + f	++ d	++ r
500 mg + c + m + s + f	++ d	++ r

Note: See the note of Table 8.

As shown in Table 9, the fungus grew in mycelial form in all cases. Especially, in the medium containing 200 mg or 500 mg *l*-glutamic acid considerable good mycelial growth was observed.

The mycelial growth occured also without the vitamins, but its growth rate was less than those with vitamins.

2. The mixed addition of various amino acids and four organic acids

The amino acids used in this experiments were glutamic acid, glycine, cysteine

and tyrosine, and the organic acids used were citric acid, malic acid, succinic acid and fumaric acid. One hundred mg of each amino acid and 20 mg of each organic acid were respectively added to the basal medium to make the following composition.

I	II	III
glutamic acid	glutamic acid	glutamic acid
glycine	glycine	glycine
citric acid	cysteine	cysteine
malic acid	citric acid	tyrosine
succinic acid	malic acid	citric acid
fumaric acid	succinic acid	malic acid
	fumaric acid	succinic acid
		fumaric acid

Table 10. Mixed addition of amino acids and organic acids

Amino acid & organic acid	With vitamin	Without vitamin
Gl + gly + c + m + s + f	++ d	++ r
Gl + gly + cyst + c + m + s + f	+++ d	++ r
Gl + gly + cyst + ty + c + m + s + f	+++ d	+++ r

Note: Abbreviation
gl: glutamic acid, gly: glycine, cyst: cysteine,
ty: tyrosine, c: citric acid, m: malic acid,
s: succinic acid, f: fumaric acid,

The results appear in Table 10. The fungus grew vigorously in mycelial form in each medium. Especially, the mycelial growth on medium III was equivalent to those on the P.D.A medium. Also on medium III vigorous mycelium-type growth was observed without vitamins but the addition of thiamin and biotin promoted the growth of mycelia even more.

Discussion

This fungus grows with a mycelium and a conidium-type when parasited in the host tissue. It was also observed on a culture medium. Namely, on P.D.A. medium the fungi grew with a mycelium-type growth, but on a solid synthetized medium grew with a conidium-type. On a synthetized medium which contained yeast extract, mycelial growth was observed, and so it was presumed that vitamins or any organic substances were affecting the mycelial growth. But in these experiments the addition of vitamins was not so effective on the mycelial growth of this fungus if amino acids and organic acids were not added to the medium.

The increased concentration of amino acids caused mycelial growth solely. The increment of a concentration of inorganic nitrogen source was not effective. Therefore, it was concluded that the nutritional factor which causes mycelium-

type growth or condium-type growth is not based on a quantitative difference but on a qualitative difference.

Moreover mycelial growth was never observed in the liquid synthetic medium containing amino acids and organic acid, so in this case the other factors — the defficiency of oxygen or influence of another gas may inhibit the mycelial growth.

Although this fungus grew in mycelial form on the solid synthetized medium containing amino acids and organic acids, the mycelia transformed to conidia in the center of the colony where the mycelia aged. This phenomenon was observed also on the P.D.A. medium. Similar observations had been reported by Mix (4)(5). Therefore, it is supposed that such a conidia formation is a reaction to the defficiency of nutrients or the accumulation of metabolic products(3) in the medium.

Summary

Taphrina sp. has two types of growth, namely mycelium-type growth and conidium-type growth. One of the factor regulating such a growth is the nutritional factor.

On the solid culture medium, mixed addition of the amino acids and the organic acids: glutamic acid, glycine, cysteine, tyrosine, citric acid, fumaric acid, malic acid and succinic acid besides inorganic nutrients was effective for mycelial growth. But the myceial growth did not occured in the liquid medium in which the nutrients stated above were added.

Vitamins are not indispensable to mycelial growth, but these are effective when organic acids and amino acids are simultaneously added.

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