DESTRUCTION OF THIAMINE BY CERTAIN FUNGI

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(Received October 5, 1957)

Yonezawa and Aoki (1) examined the thiamine-decomposing activity of cell suspensions of 96 strains of yeast-like fungi isolated from the oral cavity and found that, while *Candida albicans*, of which there were 62 strains, failed to show any thiaminase activity, the C₄ strain forming abnormal colonies had a strong thiaminase activity. Another strain, A₉, was also found to have thiaminase activity, though weaker than C₄. Biological studies on the properties of these fungi showed that the former closely related to *Trichosporon cutaneum* and the latter to *Candida parapsilosis*, but differing in some respects they were postulated to be new species (2-5). Liao (6), on the other hand, found no thiaminase activity in the cultures of 100 strains belonging to *Candida*. Hayashi *et al.* (7,8) observed thiamine-decomposing activity of the cell suspensions of several strains belonging to *Candida*, and Ogata (9) detected the activity in the supernatant fluid of the destroyed cells of *Candida albicans*, *C. pseudotropicalis* and *C. crusei*.

The present authors have studied the thiaminase activity of the cell suspensions of 52 known stock strains of yeast-like fungi and found the majority to have thiaminase activity, though different in potency.

EXPERIMENTAL

Materials and Methods

1. Strains Tested.

The majority of the strains were obtained from the Institute for Fermentation, Osaka and a part from the Nagao Institute, Tokyo. C₄ strain of Yonezawa and Aoki was kindly supplied by Dr. Yonezawa.

2. Method of Cultivation.

Each strain was cultured on Sabouraud plates, and incubated at 25° for 48 hours.

3. Determination of Thiaminase Activity.

Cells grown on plates were harvested and a suspension containing 0.05 g wet cells per ml of water was prepared. 0.5 ml phosphate buffer (pH 6.5) and 1 ml thiamine solution (3 γ /ml) were added to 4 ml of the suspension, and the mixture was incubated at 45° for 2 hours. As a control, the cell suspension was heated at 80° for 20 minutes and treated further as described above. After incubation, the residual total thiamine was measured. The difference between

the total thiamine recovered from the control mixture and that of the main mixture was taken as the quantity of thiamine decomposed.

4. Optimum pH.

For testing the optimum pH for decomposing thiamine, 1.0 ml of thiamine solution (3 γ /ml) and 0.5 ml of buffer were added to 4 ml of the cell suspension. Acetate buffer was used for pH range 4.5–5.5, and phosphate buffer 5.5–8.0. After the reaction at 45° for 2 hours, the remaining thiamine was measured. The thiamine decomposed was calculated from the difference in the remaining vitamin between the control and main test.

5. Optimum Temperature.

To 4.0 ml of cell suspension were added 1.0 ml thiamine solution (3 γ /ml) and 0.5 ml phosphate buffer (pH 6.5). Each mixture was incubated for 2 hours at 10, 20, 30, 40, 50, 60, 70, and 80°, respectively and the remaining thiamine was determined in each case.

6. Measurement of Thiaminase Potency.

The cell suspension was assumed to be the enzyme solution and the thiaminase potency (Q') of representative strain was measured as follows according to Fujita (11). Three varying dilutions of the cell suspension were made and those showing a decomposition of 50 per cent or more of the added thiamine during one hour at their optimum pH and temperature were selected. From the per cent decomposition thus selected the thiaminase potency, Q' , *i.e.*, γ thiamine decomposed per g wet cells was calculated.

7. Determination of Thiamine.

On completion of the reaction, the pH of the reaction mixture was adjusted to 4.5 with $N/10$ HCl, and the mixture was heated for 20 minutes at 80° in order to stop the thiaminase activity and at the same time to extract thiamine from the cells. Then 1.0 ml of 6 per cent Takadiastase solution, pretreated with kieselguhr to remove the existing thiamine, was added. After hydrolysis at 45° for 1 hour and pH 4.5, the whole was centrifuged for 30 minutes at 3000 rpm and the supernatant was passed through a zeolite column to remove disturbing fluorescing substances. Thiamine was then measured by a cyanogen bromide method with a fluorophotometer.

8. Test for Thiamine Disulfurylase.

The mixture was heated for 15 minutes at 80° following completion of the reaction and then treated with cysteine according to the method of Tamaki *et al.* (13). The total thiamine after cysteine treatment was compared to that without cysteine treatment.

Results

1. Decomposition of Thiamine by Fungi.

The decomposition of thiamine by the 52 strains examined is summarized in Table I, from which it can be seen that thiamine is destroyed in the majority of them. Since the total thiamine was measured, the action of "thiamine phosphorylase" (16) is excluded and since no rise in thiamine was found after adding cysteine in the strains showing the disappearance of more than 30 per cent of the thiamine added, this cannot be attributed to "thiamine disulfurylase" (12).

TABLE I
Thiamine Decomposition of 52 Strains Tested

Strains Tested		No. ^a	Thiamine decomposition per cent
<i>Torulopsis</i>	<i>miso</i>	0449	42
"	"	0445	52
"	"	0443	44
"	"	0442	45
"	"	0438	46
"	"	0440	49
"	"	0450	46
"	"	0446	65
"	"	0436	16
"	"	0444	45
"	"	0437	67
"	"	0447	27
"	<i>uvae</i>	0452	36
"	<i>colliculosa</i>	0381	43
"	<i>mandshurica</i>	0443	21
"	<i>lactica</i>	0432	33
"	<i>aeria</i>	0377	19
"	<i>xylinus</i>	0454	74
"	<i>sake</i>	0435	14
"	<i>candida</i>	0405	0
<i>Torulaspora</i>	<i>rosei</i>	0428	69
"	"	0430	83
"	"	0431	74
"	<i>delbrücki</i>	0426	52
"	"	0427	43
"	<i>fermentati</i>	0422	56
<i>Torula</i>	<i>dematia</i>	5371	91
"	<i>sacchari</i>	4440	75
"	<i>lactis</i>	0399	81
"	"	0400	89
"	"	0398	89
<i>Cryptococcus</i>	<i>albidus</i>	0378	80
"	"	0434	26
"	<i>luteolus</i>	0411	5
"	<i>laurentii</i>	0384	19
"	<i>neoformans</i>	0545	35
"	<i>glabrata</i>	0005	60
<i>Trichosporon</i>	<i>cutaneum</i>	0174	82
<i>Kloeckera</i>	<i>africana</i>	0177	0
"	<i>apiculata</i>	0175	0
"	"	0150	0
"	"	0151	0
<i>Rhodotorula</i>	<i>rubula</i>	0416	7
"	<i>minuta</i>	0387	83
"	<i>flava</i>	0407	5
"	<i>mucilaginoso</i>	0001	16
"	<i>logissima</i>	0386	17
"	<i>mucilaginoso</i>	0002	15
"	<i>glutinis</i>	0414	0
"	<i>gracilis</i>	0559	0
"	<i>species</i>	0415	40
<i>Kloeckera</i>	<i>species</i>	0153	16

^a All the figures are the strain numbers of the Institute for Fermentation, Osaka.

The reduction of thiamine determined is therefore assumed to be due to the decomposition of thiamine. Samples which decomposed more than 50 per cent

of 3 γ thiamine added included, among the fungi belonging to *Saccharomycetaceae*, 5 of the 6 strains of *Torulaspora*, and, among those belonging to *Pseudo-saccharomycetaceae*, 3 of the 20 strains of *Torulopsis*, 5 of the 5 strains of *Torula*, 2 of the 6 strains of *Cryptococcus*, 1 of the 9 strains of *Rhodotorula* and the one strain of *Trichosporon*. Aside from *Kloeckera* which showed on thiamine decomposition and *Trichosporon* of which the number of samples tested was small, a definite difference¹ in the activity of thiamine decomposition was found among four Genera, i.e., *Torulopsis*, *Torulaspora*, *Crptococcus* and *Rhodotosula*. The thiaminase potency was, if arranged in order of decreasing activity, *Torula*, *Torulaspora*, *Torulopsis*, *Cryptococcus* and *Rhodotorula*, the average percentage decomposition being 85, 62.8, 38.7, 37.5 and 20.3, respectively. For the following experiments, four species having relatively intensive thiaminase activity, i.e., *Trichosporon cutaneum* 0174, *Torula lactis* 0398, *Torulopsis xylinus* 454 and *Cryptococcus albidus* 0378, were used and their activities were compared with that of C₄ strain.

2. Optimum pH.

As shown in Fig. 1, the optimum pH for thiamine decomposition of *Trichosporon cutaneum* 0174, *Torula lactis* 0398, *Cryptococcus albidus* 0378 and the C₄ strain was found to be 6.0. At pH 4.5, the activity failed to be detected in

FIG. 1 Effect of pH on Thiamine Decomposition by Cell Suspensions

Reaction system consisting of 1 ml of thiamine solution containing 3.0 γ , 0.5 ml buffer solution and 4.0 ml cell suspension, was incubated at 45° for 2 hr.

FIG. 2 Effect of Temperature on Thiamine Decomposition

Reaction system consisting of 1.0 ml thiamine solution containing 3.0 γ , 0.5 ml buffer solution (pH 6.5) and 4.0 ml cell suspension, was incubated at 45° for 2 hr.

¹ Ratio of unbiased estimates of variance, $0.82 > F_{41}^4$ (0.01)

any of the strains. But at pH 8.0, a moderate activity was noted with the exception of *Trichosporon* and *Cryptococcus*. On the other hand, *Torulopsis xylinus* 0454 was somewhat different, the optimum pH being 6.5.

TABLE II.
Thiaminase Potency of Several Strains Tested

Strain tested	Thiaminase potency (Q')
C ₄	146.0
<i>Cryptococcus albidus</i> 0378	44.5
<i>Trichosporon cutaneum</i> 0174	30.5
<i>Torula lactis</i> 0398	22.8
<i>Torulopsis xylinus</i> 0454	14.5
<i>Candida crusei</i> 7492 ^a	14.5

^a Received from the Nagao Institute, Tokyo

whereas in the case of *Torulopsis xylinus* 0454, the optimum was 60°, definitely higher than the others.

4. Thiaminase Potency.

The results given in Table II show that the activity of C₄ strain is markedly higher than the others.

5. Thiamine disulfurylase.

Strains showing thiamine decomposition of more than 30 per cent were examined but thiamine disulfurylase was not detected.

DISCUSSION

It has been shown that the thiamine-decomposing action of C₄ strain isolated by Yonezawa *et al.* is not due to thiamine disulfurylase, the decomposition products being hydroxymethyl-pyrimidine and thiazole moiety. It has also been found that neither base exchange reaction nor thiamine phosphorylation takes place, showing that the enzyme belongs to thiaminase II (17). Yonezawa *et al.* (15), however, failed to demonstrate thiaminase activity in 62 strains of *Candida albicans* which they isolated, though some strains of *Candida* have been reported to decompose thiamine (9, 14).

The author have tested 52 known stock strains of fungi and found that the added thiamine was more or less decomposed in the majority of cases. The action was demonstrated neither to be due to thiamine phosphorylase nor thiamine disulfurylase. It is thermolabile and has an optimum pH and temperature. These findings lead to the conclusion that the thiamine was decomposed by thiaminase.

SUMMARY

52 known stock strains of yeast-like fungi were tested for thiaminase activity and the following results were obtained.

1. Of the 51 strains tested, 16 (30 per cent) decomposed more than 50 per cent, 9 (18 per cent) 30–50 per cent, while 27 (52 per cent) less than 30 per cent of the thiamine added at pH 6.5 and 45° in 2 hours in the presence of 3 γ thiamine.

2. The thiaminase activity of representative strains, *Trichosporon cutaneum* 0174, *Torula lactis* 0398, *Torulopsis xylinus* 0454, *Cryptococcus albidus* 0378 and C₄ strain of Yonezawa and Aoki was studied and found that the optimum pH was 6.0 and the optimum temperature 40°. *Torulopsis xylinus*, however, differed in that the optimum pH was 6.5 and the optimum temperature 60°.

3. Determination of the thiaminase potency according to Fujita showed that C₄ strain had the highest value, 146. *Cryptococcus albidus* 0378 had the value, 44.5 and *Trichosporon cutaneum* 0174, 30.5, only one-fourth that of C₄.

4. It is concluded that thiaminase is widely distributed in yeast-like fungi.

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