BIOCHEMICAL STUDIES ON T. QUINCKEANUM

By

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The field of medical mycology is becoming increasingly important. Studies on various aspects of medical mycology are currently being conducted but unfortunately few biochemical studies on pathogenic fungi particularly on the dermatophytes have been reported (Fujii 1955)[1]

The author, during his studies on " Hair penetration due to various fungi " (2–6) obtained some basic information on the proteolytic activity of some of perforating and non-perforating fungi. The results of the experiments done on T. quinckeanum are reported in this article.

EXPERIMENT I

To show the presence of proteolytic enzyme in the fungus mat of T. quinckeanum

100 ml. of Sabourauds broth was inoculated with *T. quinckeanum* and was grown for 30 days at 27°C. The enzyme activity of the mat was measured by the rate at which an aliquot hydrolysed a 1% solution of casein buffered at pH 7. This was prepared by mixing 40 ml. of 0.2 M NaH_2pO_4 solution and 60 ml. of 0.2 M Na_2HPO_4 solution. To this I g. of casein was added and the fluid was then immersed in boiling water bath for 15 minutes.

5 g. of fungus mat was washed in distilled water and then added to 50 ml. of 1% casein solution when cool.

I ml. of aliquot was removed immediately and added to 5 ml. of 5% trichloracetic acid and after periods of 1, 2, 3, 4 and 5 hours I ml. aliquots were removed subsequently and treated similarly.

Control tests were carried out by taking similar aliquots and treating these as above, (a) from the buffer mixture without added casein but with fungus added, and (b) from the buffer mixture with added casein but without the addition of fungus.

The solutions were allowed to stand for an hour and were then centrifuged at 3000 r. p. m. for 15 minutes. The optical density of the supernatant was measured in a Hilger spectrophotometer at 280 mu.

The results are shown in Table I.

TABLE I

Time	OPTICAL DENSITY 280 mu		
Approx. hours	Fungus+casein	Fungus+buffer	Buffer+casein
1	0.160	0.130	0.130
2	0.230	0.162	0.132
3	0.270	0.177	0.128
4	0.300	0.191	0.130
5	0.330	0.196	0.130

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CONCLUSIONS

1. The fungus mat of T. quinckeanum produces a proteolytic enzyme or enzymes splitting casein.
2. This enzyme or enzymes are produced even when the fungus is not in contact with the substrate.

EXPERIMENT 2

To determine the speed of extraction of the proteotytic fraction of the fungus mat of T. quinckeanum in physiological saline

0.05 g. of T. quinckeanum dried powdered mat was added to 2 ml. of physiological saline and left for 5 minutes after which time it was centrifuged at 1500 r. p. m. for 15 minutes. The supernatant was labelled A.

To the remaining precipitate, 2 ml. of saline was added, shaken and left for 30 minutes before centrifugation. The supernatant was labelled B.

The rest of the precipitate was suspended in 2 ml. of solution and similarly treated. The suspension was labelled C.

1 ml. aliquots were added to 10 ml. amounts of phosphate buffer casein solution at pH 7 and incubated at 37°C as in previous experiment.

Optical densities recorded are shown in Table 2.

TABLE 2

Time lapse (minutes)	OPTICAL DENSITY 280 mu		
	A	B	C
0	0.80	0.09	0.08
10	1.20	0.09	0.09
30	1.70	0.10	0.10
60	2.50	0.11	0.10

The remaining residue gave a violet colour with napthol and concentrated H_2SO_4, indicating the presence of carbohydrate.

CONCLUSIONS

1. Virtually all the proteolytic activity can be extracted from the dried fungal mat of T. quinckeanum with normal saline (25 mg/ml) in 5 minutes.
2. The saline-insoluble residue having little proteolytic activity is carbohydrate in nature.

EXPERIMENT 3

To dertermine whether the proteolytic active fraction of trichophyton quinckeanum can be extracted with alumina

15 g. of dried T. quinckeanum mycelium was ground with an equal amount of alumina (Hopkin and Williams, M. F. C. 100-200 mesh) in 200 ml. of phosphate buffer solution at pH 7. The homogenate was centrifuged at 1,500 r. p. m. for 20

minutes and 175 ml. of supernatant fluid was obtained. The proteolytic activity of this was assayed by adding 1 ml. of this solution to 10 ml. of casein phosphate buffer solution (pH 6.8) and incubating at 37°C. Aliquots were taken at specified intervals and tested for optical dentisty at 280 mu. The results are shown in table 3.

TABLE 3

Time lapse (minutes)	Optical density at 280 mu
0	0.50
5	0.58
15	0.64
30	0.82
60	1.13
90	1.30

CONCLUSION

By grinding with alumina the proteolytic active fraction of T. quinckeanum mycelium is extractable.

EXPERIMENT 4

To determine whether the alumina extract of trichophyton quinckeanum mycelium is altered in activity when dialysed and freeze-dried

175 ml. of the extract from previous experiment was dialysed four times on each occasion against 1 litre of distilled water for two days. The final solution was freeze-dried and made into a powder. 0.5 g. of this powder was dissolved in 1 ml, of a buffer of pH 7 and 0.2 ml. of the solution then added to 10 ml. of 1% casein-phosphate buffer solution of pH 6.2 and incubated at 37°C. Aliquots were taken at specified intervals for estimation of optical density (see Table 4).

TABLE 4

Time lapse (minutes)	Optical density at 280 mu
0	0.31
5	0.36
15	0.44
30	0.59
60	0.86
90	0.96

CONCLUSION

The processes of dialysing and freeze-drying do not affect the enzyme activity of the alumina extract of T. quinckeanum mycelium.

EXPERIMENT 5

To determine whether the extract of T. quinckeanum retains its proteolytic activity after dialysis

A strain of T. quinckeanum was grown in several bottles of Sabouraud's broth for four weeks. The mats were washed and dried for three weeks in a vacuum desiccator at 4°C and then pulverized. 10 grams of dry powder was extracted with 50 ml. phosphate buffer (pH 6.0) at room temperature for 30 minutes and the solution then filtered. To the residue a further 50 ml. of buffer solution was added and left for 30 minutes before filtration. The filtrates were combined and in total volume measured 70 ml.

0.5 ml. of this extract was added to 10 ml. of buffered 1% casein solution (pH 6.0) and incubated at 37°C and 1 ml. aliquots taken at time intervals (shown in Table 5) were tested for optical density (O. D.) at 280 mu.

TABLE 5

Time lapse (minutes)	O. D. Reading	Time lapse (minutes)	O. D. Reading
0	0.80	20	.86
5	0.82	40	1.02
10	0.84	60	1.24

The remaining filtrate was dialysed for three days at 4°C against distilled water. 0.5 ml. of this dialysed filtrate, which measured 102 ml. was mixed with 10 ml. of buffered 1% casein solution, incubated at 37°C and aliquots taken at similar time intervals for assessment of optical density. The results are shown in Table 6.

TABLE 6

Time lapse (minutes)	Optical Density 280 mu
0	0.16
5	0.20
10	0.24
20	0.31
40	0.46
60	0.50

CONCLUSIONS

The filtrate retains the proteolytic activity after undergoing dialysis against distilled water.

The remainder of the dialysed T. quinckeanum extract was freeze-dried and powdered ready for use in subsequent experiments.

EXPERIMENT 6

To determine whether the proteolytic fraction of T. quinckeanum is precipitated by ammonium sulphate solution

1 ml. of fungus extract was added to 6 ml. of saturated $(NH_4)_2SO_4$ solution. It was left at 4°C overnight and centrifuged at 1500 r. p. m. for 15 minutes. 1 ml. of supernatant was added to 1 ml. of casein–buffer solution (pH 6.2). After the measured time interval, the solution was neutralised with 5% trichloracetic acid and its optical density determined–(Reading A). The optical density of supernatant added to buffer without casein formed the control (Reading B). These results are shown in Table 7.

TABLE 7

Time lapse (minutes)	OPTICAL DENSITY 280 mu	
	Readings A	Readings B
0	0.60	0.61
30	0.62	0.60
60	0.62	0.61
90	0.61	0.62

The precipitate remaining was dissolved in 6 ml. of buffer and the proteolytic activity of 1 ml. of this solution was assayed at the measured time intervals (Series C) and these compared with results for a control solution without dissolved precipitate (Series D). The results are shown in Table 8.

TABLE 8

Time lapse (minutes)	OPTICAL DENSITY 280 mu	
	Readings C	Readings D
0	0.63	0.64
30	1.29	0.64
60	1.85	0.65
90	2.25	0.65

CONCLUSION

The proteolytic fraction of T. quinckeanum is precipitated by ammonium sulphate solution, while the supernatant has negligible proteolytic properties.

EXPERIMENT 7

To determine the optimum pH for the proteolytic activity of the dialysed extract of T. quinckeanum

1 g. of casein was dissolved in 40 ml. of Ellis buffer solution (pH 7) containing the following solutions:

0.2 M. Sodium carbonate

TABLE 9

Time (Minutes)	pH 4	4.5	5	5.5	6	6.5	7	7.6	8	8.2	8.6	9.0	10.0	10.5	11	11.5	12	Blank
0	0.27	0.27	0.27	0.27	0.27	0.27	0.28	0.27	0.28	0.23	0.23	0.23	0.23	0.22	0.22	0.22	0.21	0.24
15	0.37	0.40	0.50	0.48	0.44	0.44	0.45	0.47	0.51	0.45	0.44	0.41	0.38	0.36	0.35	0.25	0.24	0.23
30	0.40	0.80	1.30	1.10	0.44	0.47	1.00	1.20	1.50	1.10	1.00	0.90	0.85	0.70	0.70	0.25	0.26	0.23
60	0.60	1.10	2.40	2.30	0.80	0.90	1.80	2.00	2.50	2.00	1.90	1.75	0.90	0.80	0.70	0.60	0.28	0.24
90	0.80	2.0	3.20	3.00	1.80	1.90	2.70	3.00	3.50	2.80	2.40	2.20	1.50	1.3	1.00	0.90	0.34	0.25

CONCLUSION

T. quinckeanum extract has maximal proteolytic attivity in the pH range 5–8.

0.2 M. Ammediol (2–Amino–2–methylin 1 : 3 propaidiol)
Eastern Kodak reagent grade.

0.2 M. Sodium dihydrogen orthophosphate.

0.2 M. Citric acid.

Solutions of pH range 4–12 were prepared by the addition of acid or alkali to this basal solution.

20 ml. of casein substrate of each pH value was added to 1 ml. of dialysed fungus extract and incubated at 37°C. After measured time intervals 1 ml. aliquots were taken and added to 3 ml. of 5% trichloracetic acid and the optical density measured at 280 mu.

A control solution was prepared by adding 1 ml. of dialysed fungus extract to 20 ml. of distilled water and incubating at 37°C. As in the tests above aliquots were taken at the same time intervals.

The results are shown in Table 9.

SUMMARY

1. The fungus mat of T. quinckeanum produces proteolytic enzyme/enzymes which splits casein. This enzyme is produced even when the fungus is not in contact with the substrate.
2. The proteolytic activity can be extracted with normal saline (25 mgm/ml) in 5 minutes. The saline insoluble residue is carbohydrate in nature, which shows little proteolytic activity.
3. The proteolytic fraction can be extracted by grinding with alumina. Dialysis and freeze drying do not affect the enzyme activity of the alumina extract.
4. The filtrate of T. quinckeanum retains the proteolytic activity even after dialysis against water.
5. Almost the entire proteolytic fraction is precipitated by ammonium sulfate solution.
6. The maximal proteolytic activity of T. quinckeanum is in the pH range of 5–8.

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