THE SYNTHESIS OF FIBRINOLYSIN BY FUNGI[1]

THANOMWANG AMATAYAKUL[2]

Department of Botany and Plant Pathology, The Ohio State University, Columbus, 10

The fungi have long been known to be capable of synthesizing a large number of enzymes, and there are many publications concerning the enzymic activity of fungi but to the best of my knowledge little or no work has been published on the fibrinolytic activity of fungi.

The fibrinolytic activity of hemolytic streptococci has been extensively studied by Tillet and Garner (1933), who reported that all 28 strains of hemolytic streptococci associated with human infections formed fibrinolytic substances which liquefied human blood clot while only 3 out of 18 hemolytic streptococci of animal origin were capable of causing fibrinolysis.

Van Deventer and Reich (1934) observed that fibrinolysin produced by hemolytic streptococci dissolves human but not animal plasma clots with the exception of rhesus plasma.

Madison (1934) studied fibrinolytic streptococci from lower animals and showed that an organism isolated from a particular animal species had a greater activity on the fibrin of that animal species than on the fibrin of another species.

Neter and Witebsky (1936), who studied fibrinolytic activity of hemolytic streptococci and other microorganisms observed that fibrinolysin production is not limited to hemolytic streptocci alone, if the sugar content of the culture medium is increased. They showed that other microorganisms such as *Streptococcus viridans*, *Enterococcus*, *B. coli*, *B. lactis*, etc. are capable of producing fibrinolysin when cultivated in 2 percent glucose broth but not in 0.5 percent glucose broth.

This present study is an attempt to find out whether fungi have the ability of synthesizing fibrinolysin. This work is a preliminary survey of species and strains of fungi in connection with their ability to synthesize fibrinolysin. The effect of certain environmental conditions on the production of the enzyme was also studied.

MATERIALS AND METHODS

The preliminary testing for ability to synthesize fibrinolysin was made by the technique described by Crabill and Reed (1915). The following medium has been used throughout the investigation.

$MgSO_4$	0.5 gm.
KCl	0.5 gm.
K^2HPO_4	1.0 gm.
Trace mineral salts	1 ml.
Ox blood fibrin	10 gm.
Agar	20 gm.
Distilled water	1000 ml.

Trace mineral salts dil. 1: 1000

$FeSO_4$	.04 g,./lit.
$ZnSO_4$	.025 gm./lit.
$MnSO_4$	.025 gm./lit.
$Na_2B_4O_7$	.025 gm./lit.
NH_4MoO_3	.025 gm./lit.
$CuSO_4$	.025 gm./lit.

The above culture medium was prepared by dissolving the salts in a portion of distilled water and then making to volume; blood fibrin was then added by

[1]Paper No. 578 from the Department of Botany and Plant Pathology, The Ohio State University.

[2]Present address: 370-K SriBumpen Rd., Toongmahamek, Thailand.

mixing in a Waring Blender in order to obtain uniform distribution through the medium. Agar was added and the medium was sterilized at 15 lbs. pressure for 20 min. Plates were poured directly from the flask with frequent shaking between pourings to prevent the settling of the finely-divided blood fibrin. Upon cooling, the medium was opaque due to the presence of the many fine particles of fibrin. Plates were inoculated by introducing spores at a single, central point on the plate. If a fungus is capable of synthesizing fibrinolysin, a clear area appears under the developing colony and sometimes with slow-growing fungi, a clear halo appears around the colony.

TABLE 1

Organisms used

STOCK NO.	
I-28	*Acrostalagmus cinnabarium* Corda
8	*Ascobolus striisporus* (Ellis & Dearn) Seaver
25	*Ascobolus striisporus* (Ellis & Dearn) Seaver
27	*Ascobolus striisporus* (Ellis & Dearn) Seaver
33	*Ascobolus striisporus* (Ellis & Dearn) Seaver
2	*Aspergillus fischeri* Wehmer
4	*Aspergillus fischeri* Wehmer
7	*Aspergillus (flavus oryzae)*
9	*Aspergillus fumigatus* Fresenius
10	*Aspergillus niger* Van Tieghem
14	*Aspergillus oryzae* (Ahlburg) Cohn.
15	*Aspergillus tamarii* Kita.
16	*Aspergillus terreus* Thom
17	*Aspergillus terreus* Thom
18	*Aspergillus terreus* Thom
19	*Aspergillus ustus* (Bainier) Thom, Church.
I-48	*Beauveria bassiana* (Bals.) Vuill.
I-41	*Bispora punctans* (Schw.) Hughes
23	*Coprinus sp.*
21	*Chaetomium globosum* Kze.
205	*Circinella* sp.
I-58	*Colletotrichum phomoides* (Sacc) Chester
I-42	*Coniothyrium fuckeli* Sacc.
I-45	*Cordana americana* Hughes
205	*Cunninghamella* sp.
I-4	*Curvulvaria* sp.
I-53	*Dendryphiopsis* atra
I- 5	*Fusarium lycopersici* Sacc.
I-31	*Gliocladium deliquescens* Sopp.
I-30	*Gliocladium roseum* Bain
116	*Hansenula ciferri* Lodder
I- 8	*Helminthosporium sativum* Pammel
I- 9	*Heterocephalum aurantiacum* Thaxt.
I-10	*Memnoniella echinata* (Rivolta) L.
I-37	*Melanconium fuligineum* Cav.
24	*Metarrhizium glutinosum* Pope
5	*Mucor* sp.
I-12	*Oidium lactis* Fres.
I-13	*Pestalozzia* sp.
I-14	*Phoma destructiva* Plowr.
69	*Phycomyces blakesleeanus* Burgeff
I-16	*Sclerotinia fructicola* (Wint) Rehm.
I-63	*Sclerotium rolfsii* Sacc.
37	*Sordaria humana* (Fekl)
I-19	*Spegazzinia* sp.
38	*Stachybotrys atra* Corda
I-21	*Stemphylium* sp.
I-23	*Trichoderma viride* A. et S.
I-25	*Verticillium puniceum* (Cook et Ellis) Grev.
206	*Zygorhynchus* sp.

For the initial screening experiments cultures were incubated at room temperature. Measurements of colony and clear zone diameters were made at 48 hr. and 72 hr. and each experiment was terminated at the end of this period. The average measurement was computed from two measurements at right angles to each other.

The organisms used were obtained from Dr. W. D. Gray, Department of Botany and Plant Pathology, The Ohio State University, and are listed in table 1 with their stock culture numbers.

The effect of sugar on the production of fibrinolysin was studied by using the above medium containing 2 percent dextrose.

The relation of temperature to fibrinolysin production was also determined. For this purpose the plates were inoculated and incubated at 15°C, 20°C, 21–25°C, 30°C, and 35°C.

The influence of pH of the medium on the production of fibrinolysin was also investigated. The initial pH of the medium was adjusted with N/1 HCl or N/1 NaOH before autoclaving and the final measurement was made with a Beckman Model H pH meter after autoclaving. Although all precautions were taken in making the medium, slight differences in pH were evident in each series. The six media termed a series were as follows: pH 3.2, 4.2 5.5, 6.2, 7.2, and 7.8. Each series was incubated at 25° and 30°C. All measurements reported represent the average of at least 9 replicated plates.

EXPERIMENTAL RESULTS

Screening experiments. The results of these experiments show that 23 out of the 50 fungi studied are capable of synthesizing considerable amounts of fibrinolysin. The organisms may be placed into three groups on the basis of the relative amounts of growth and fibrinolytic activity. The first group is characterized by rapid mycelial growth which extends well beyond the zone of fibrinolytic activity. The fungi falling into this group are (8, 25, 33, 23, 21, and 37) *Ascobolus striisporus, Ascobolus striisporus, Ascobolus striisporus,* Coprinus* sp., *Chaetomium globosum, Sordaria humana.* In the second group the clear zone free of blood fibrin extends beyond the edge of the colony, but the rate of growth is slow: (7, 14, 15, 16, 17, I–58, I–42, I–30, I–10, and 24) *Aspergillus* (*flavus oryzae*), *Aspergillus oryzae, Aspergillus tamarii, Aspergillus terreus, Aspergillus terreus, Colletotrichum phomoides, Coniothyrium fuckeli, Gliocladium roseum, Memnoniella echinate, Metarrhizium glutinosum,* are in this group. The third group is intermediate between the above two, the colony and clear zone being equal in size. The organisms of this group are (I–5, I–31, I–19, 38, I–21, and I–23) *Fusarium lycopersici, Gliocladium deliquescens, Spegazzinia* sp., *Stachybotrys atra, Stemphylium* sp., *Trichoderma viride.* The results are summarized in table 2.

The first and third groups do not produce a halo on blood fibrin-agar but grew well and dissolved blood fibrin in the agar which was in contact with the mycelium. These are considered by Crabill and Reed as weak enzyme producers, and the presence of a halo around the edge of a fungus colony is indicative of stronger ensymic activity.

The effect of sugar content of the medium on the production of fibrinolysin. For studies on the effect of sugar on fibrinolysin production, 18 different fungi were used, and it was found that dextrose inhibits the formation of fibrinolysin by most of the fungi studied. The degree of inhibition varies with the organism. The results of this experiment as shown in table 3 indicate that Cultures 21, I–42, I–5, I–31, I–10, I–19, 38, I–21, and I–23 produce no fibrinolysin in the medium containing 2 percent dextrose, and Cultures 8, 25, 27, 33, 15, 16, 23, and 37 exhibit only traces of fibrinolysin. Culture I–30 produces more fibrinolysin in the presence

*Three different monospore strains were used.

of dextrose than in dextrose-free medium. The sugar content of the medium apparently has no effect on the production of fibrinolysin by Culture 24. In all cases growth is greater when cultivated in 2 percent dextrose medium.

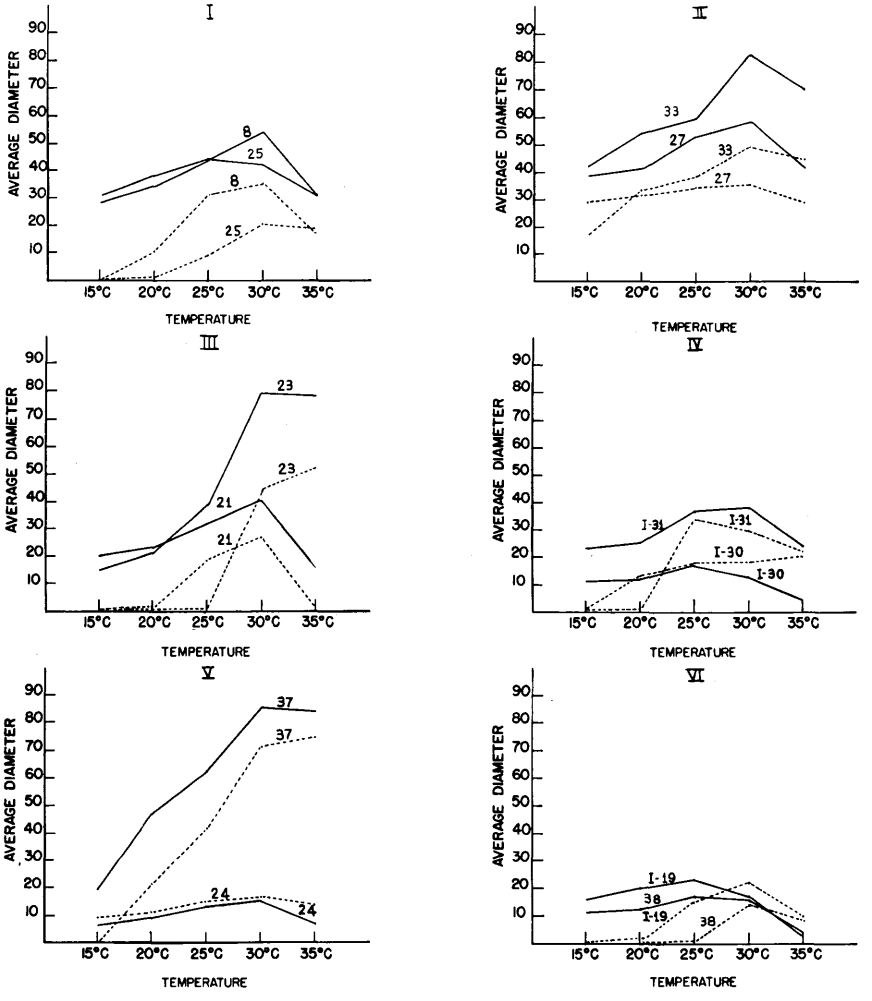
TABLE 2

Average diameter in millimeters of colony and zone of fibrinolytic activity of all fungi studied

STOCK NO. OF ORGANISM	48 HRS.		72 HRS.	
	COLONY	CLEAR ZONE	COLONY	CLEAR ZONE
I-28	–	–	+	–
8	35.31	26.61	48.84	41.96
25	24.96	14.5	31.82	18.9
27	48.46	36.59	65.27	51.86
33	45.03	31.75	64.52	49.0
2	17.67	+	28.57	+
4	12.28	–	19.97	–
7	14.63	21.69	35.2	42.55
9	18.35	+	27.87	+
10	10.0	–	13.45	–
14	12.28	17.43	20.78	26.87
15	13.5	16.06	21.08	25.44
16	11.56	16.06	15.42	19.09
17	13.25	+	18.71	+
18	11.53	15.09	15.8	22.06
19	15.59	–	22.62	–
I-41	–	–	+	–
I-48	–	–	+	–
23	37.06	20.03	61.25	34.37
21	32.21	17.18	49.7	38.25
205	32.79	–	37.0	–
I-58	6.58	11.5	16.08	19.41
I-42	8.75	13.08	13.08	19.16
I-45	10.0	–	16.66	–
204	60.7	–	70.0	–
I- 4	19.33	–	29.83	–
I-53	–	–	+	–
I -5	23.16	23.16	37.33	37.33
I-31	29.65	29.65	43.28	43.28
I-30	11.93	14.53	18.65	21.46
116	–	–	+	–
I- 8	15.83	–	29.0	–
I- 9	9.3	+	14.27	+
I-10	8.37	11.25	13.25	17.75
I-37	9.0	–	16.66	–
24	9.87	14.75	14.82	20.88
5	34.55	+	54.85	+
I-12	17.33	–	24.0	–
I-13	22.25	–	34.33	–
I-14	22.85	–	33.25	–
69	12.1	+	17.0	+
I-16	–	–	–	–
I-63	38.33	–	57.66	–
37	64.0	48.07	90.0	90.0
I-19	14.23	14.23	20.55	20.55
38	10.45	10.45	15.35	15.35
I-21	20.0	20.0	27.83	27.83
I-23	37.15	37.15	47.25	47.25
I-25	3.91	+	8.75	+
206	43.4	–	69.25	–

+ Denotes slight growth and slight fibrinolytic activity.
– Denotes no growth and no fibrinolytic activity.

The influence of temperature. Twelve fungi were used in the temperature studies and all grew throughout the temperature range used. The optimum for growth is 25°–30°C. Essentially no fibrinolysin was formed by most of the fungi at 15°C with the exception of Cultures 27, 33, 24, and 37 which are capable of synthesizing fibrinolysin at this temperature. In most cases, the optimum temperature for the production of fibrinolysin is at 30°C.



FIGURES 1 to 6. Relation of temperature to growth and fibrinolysin production by fungi. All measurements made after 72 hr. incubation except those for culture 37 (48 hr.) Unbroken line indicates colony diameter, broken line represents diameter of clear area.

The behavior of I–19 and I–30 differs from the others in that the optimum temperature for growth is at 25°C ,while the optimum for formation of fibrinolysin lies at 35° and 30°C. The production of fibrinolysin increases with increase of temperature from 15° to 35°C, while growth decreases with increase of temperature from 25° to 35°C. The results are summarized in table 4 and expressed graphically in fig. 1 to 6.

The effect of pH. The data represented in table 5 indicates that the range of pH that will permit growth and formation of fibrinolysin depends on the individual organism, the initial reaction of the medium and the temperature. In the majority of cases growth and production of fibrinolysin occur at temperatures of 25° and 30°C within the range of pH 4.2 to 7.8. At a temperature of 30°C there is a complete inhibition of growth and formation of fibrinolysin in all cases at pH 3.2 with the exception of Culture 37 which exhibited growth and fibrinolytic activity over a wide pH range (3.2 to 7.8).

Cultures 24 and 33 showed growth and fibrinolytic activity within the range of pH 3.2 to 7.8 at a temperature of 25°C and pH 6.2 is the optimum at both temperatures incubated.

TABLE 3

The effect of sugar content of the medium on growth and production of fibrinolysin by fungi. Average diameter in millimeters of the colony and clear zone in 72 hours.

Stock No of Organisms	Sugar-free medium		Medium containing 2% dextrose	
	Colony	Clear Zone	Colony	Clear Zone
8	51.73	40.30	90.0	+
25	48.75	26.0	63.5	+
27	77.58	54.54	90.0	+
33	63.05	47.0	85.0	+
15	15.0	20.0	31.0	+
16	16.5	18.25	18.25	+
23	59.25	24.33	72.25	+
21	46.29	32.12	46.66	−
I–42	13.08	19.16	18.58	−
I– 5	37.33	37.33	42.83	−
I–31	43.62	43.62	90.0	−
I–30	19.41	21.04	17.07	22.5
I–10	13.25	17.75	13.75	−
24	14.8	19.07	18.03	19.45
37	90.0	90.0	90.0	+
I–19	20.45	20.45	26.3	−
38	15.16	15.16	14.65	−
I–21	27.5	27.5	31.91	−
I–23	48.20	48.20	90.0	−

+ Denotes slight growth and slight fibrinolytic activity.
− Denotes no growth and no fibrinolytic activity.

The optimum pH for growth and production of fibrinolysin by Cultures 8 and 27 is shifted to a less acid reaction at higher temperatures.

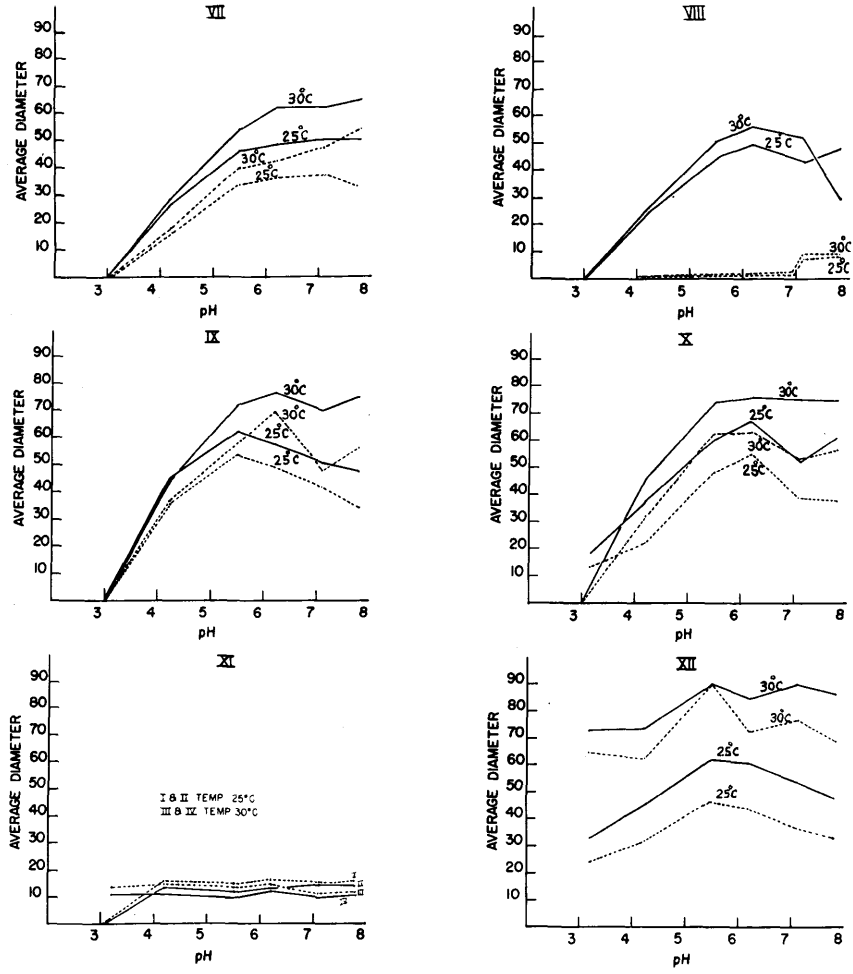
In all cases growth and fibrinolytic activity are greater at 30°C than at 25°C within the same range of pH.

The data are represented graphically in figures 7 to 12.

DISCUSSION

From the preceding results it is obvious that some fungi are capable of synthesizing an extracellular enzyme which digests blood fibrin; this enzyme is known as fibrinolysin. The extent of fibrinolytic activity depends upon the type of organism. In general, the slow-growing ones appear to have strong fibrinolytic activity while those which are rapid growers have weak fibrinolytic activity. The accumulation of fibrinolysin by the fast growing ones appears to be greater than the slow-growing organisms in a definite period of time.

Different species and strains of fungi behave differently on medium containing sugar. Waksman (1918) reported that the sugar content of the medium has no decided influence upon the production of proteolytic enzyme by *Aspergillus niger*.



FIGURES 7 to 12. The relation of pH to growth and fibrinolysin activity of fungi at 25° and 30° C. All measurements made after 72 hr. except those for culture 37 (48 hr.) Unbroken line indicates colony diameter, broken line represents diameter of clear area.

The work of Jones (1916) showed that *B. proteus* will not form gelatinase on gelatin when glucose is present, but as soon as the glucose has been used up the gelatinase appears. He concluded that this enzyme is elaborated in response to need.

Neter and Witebsky (1936) pointed out that *Streptococcus virdians* and Enterococcus did not show any fibrinolytic activity when cultured in 0.05 percent glucose-broth but produced the fibrinolytic substance in 2 percent glucose-broth.

From the result of this work it appears that the effect of dextrose on the production of fibrinolysin varies with species and strains of fungi studied.

TABLE 4

Average diameters in millimeters of colonies and clear zones at different temperatures

Stock No. of Organism	Incubation Period	15° C Colony	15° C Clear-zone	20° C Colony	20° C Clear-zone	25° C Colony	25° C Clear-zone	30° C Colony	30° C Clear-zone	35° C Colony	35° C Clear-zone
8	48 hrs.	18.38	+	20.88	8.33	28.38	15.0	36.25	22.33	22.88	11.22
	72 "	31.33	+	38.91	10.0	44.66	31.58	54.91	35.12	31.75	17.16
25	48 "	19.42	−	21.05	+	28.0	7.83	27.83	8.61	19.77	9.77
	72 "	28.33	−	34.95	+	44.27	9.58	42.89	20.55	31.75	19.91
27	48 "	31.11	18.0	34.42	19.72	35.05	20.77	37.94	22.33	28.56	20.12
	72 "	39.0	29.16	41.25	31.16	53.96	34.5	58.16	35.51	41.70	29.58
33	48 "	28.38	11.75	37.66	19.33	44.0	28.5	55.5	43.0	37.94	25.16
	72 "	42.0	17.5	54.5	33.91	59.62	38.75	82.08	49.0	70.58	44.16
21	48 "	13.66	+	14.5	+	17.61	10.66	22.5	11.71	12.41	+
	72 "	20.97	+	23.86	+	32.0	19.77	40.61	27.16	16.93	+
23	48 "	8.22	−	9.88	−	25.05	+	49.83	19.61	55.55	28.88
	72 "	15.0	−	21.0	−	39.38	+	79.94	44.0	78.38	52.66
I–31	48 "	15.61	+	18.29	+	25.88	20.08	30.2	27.0	17.58	15.61
	72 "	23.38	+	25.17	+	37.94	34.25	38.88	30.16	24.31	22.5
I–30	48 "	7.88	+	8.81	9.20	11.61	13.26	10.30	15.0	3.05	16.33
	72 "	11.66	+	12.83	13.33	17.25	18.46	13.88	18.5	4.22	20.08
24	48 "	4.8	7.61	6.27	9.08	8.05	10.61	9.13	11.33	6.02	11.27
	72 "	6.62	9.08	9.83	11.16	13.08	15.41	15.73	17.58	7.54	14.75
37	48 "	19.41	+	47.1	26.6	62.68	41.25	85.66	71.88	83.12	74.0
	72 "	40.5	16.54	68.66	52.75	90.0	90.0	90.0	90.0	90.0	90.0
I–19	48 "	11.16	−	15.61	+	18.0	9.72	9.69	10.83	3.44	8.77
	72 "	16.61	−	20.97	+	23.22	15.5	17.72	22.66	3.5	10.22
38	48 "	5.41	−	6.66	−	10.25	+	9.08	8.88	3.91	5.87
	72 "	11.16	−	12.22	−	17.05	+	16.27	14.05	4.09	8.12

+ Denotes slight growth and slight fibrinolytic activity.
− Denotes no growth and no fibrinolytic activity.

TABLE 5

The relation of pH in relation to growth and production of fibrinolysin by 6 fungi. Based on the average diameter in millimeters of colony and clear zone in 72 hr.

Stock No. of Organisms	Temp.	pH 3.2		pH 4.2		pH 5.5		pH 6.2		pH 7.1		pH 7.8	
		Colony	Clear-zone	Colony	Clear-zone	Colony	Clear-zone	Colony	Clear-zone	Colony	Clear-zone	Colony	Clear-zone
8	25° C.	—	—	26.79	17.54	47.42	34.28	49.33	37.0	51.04	38.33	49.88	34.99
	30° C.	—	—	30.92	17.78	55.5	40.33	63.11	43.5	63.29	48.7	66.27	55.0
25	25° C.	—	—	25.94	+	44.33	+	49.88	+	43.64	8.0	48.16	8.33
	30° C.	—	—	26.41	+	50.92	+	56.93	+	52.56	9.9	29.73	9.07
27	25° C.	+	—	45.16	36.55	62.61	53.13	58.64	49.88	51.11	42.16	48.11	37.33
	30° C.	—	—	44.91	37.5	72.38	59.44	77.5	69.83	70.05	48.05	75.5	57.27
33	25° C.	19.5	13.87	38.5	22.44	60.55	48.22	67.16	55.41	52.77	39.66	61.27	38.81
	30° C.	—	—	46.81	32.37	74.40	62.5	76.58	63.75	75.11	53.47	75.35	57.0
24	25° C.	11.72	14.16	11.66	15.11	10.8	14.94	12.44	15.22	10.05	12.88	11.38	12.5
	30° C.	—	—	10.15	14.5	12.33	15.94	14.77	17.44	15.05	16.94	15.61	16.22
37	25° C.	75.83	61.5	79.5	71.5	82.87	82.0	81.37	72.62	77.87	67.28	73.5	55.28
	30° C.	81.3	75.5	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0

\+ Denotes slight growth and slight fibrinolytic activity.
— Denotes no growth and no fibrinolytic activity.

Dextrose inhibits the formation of fibrinolysin by most of the fungi, but some produce more fibrinolysin in the presence of dextrose, and others do not appear to be affected by the presence of dextrose. The results of this work do not agree with those of Waksman which concerned only *Aspergillus niger*.

It is quite probable that the presence of a substance which is more easily decomposed than blood fibrin hinders fibrinolysin formation. The degree of inhibition depends upon the type of organism. The presence of substances which diminish the utilization of a particular nutrient in the substrate brings about a diminution in the production of the corresponding enzyme.

Temperature influences the enzymic processes in general parallel with the rate of growth. It is evident from this experiment that growth and formation of fibrinolysin increases with increase in temperature up to 30°C and declines at 35°C while the optimum lies at 25° to 30°C. The decrease of fibrinolytic activity with the decrease of temperature may be due to the fact that the time required for the accumulation of fibrinolysin in sufficient quantity to start the liquefaction of blood fibrin is longer at low temperature than at high temperature. Inhibition of the production of fibrinolysin occurs at 15°C by most of the fungi studied with the exception of Nos. 27, 33, 24, and 37 (*Ascobolus striisporus*, *Ascobolus striisporus*, *Metarrhizium glutinosum*, and *Sordaria humana*) which exhibit fibrinolytic activity at a temperature as low as 15°C.

Waksman (1918) studied the proteolytic enzymes of soil fungi and actinomycetes, and observed that a temperature of 29°C to 34°C is optimum for enzyme activity of *Aspergillus niger* and that the activity dropped at 39°C. This observation by Waksman coincides with that made in the present studies.

The pH limit for growth and production of fibrinolysin is influenced by temperature as shown in this investigation since pH 3.2 inhibits growth and formation of fibrinolysin at 30°C. The pH range is widest at 25°C. In some cases, at the higher temperature the optimum pH for growth and fibrinolytic activity is higher than at lower temperatures. All fungi showed greater growth and fibrinolytic activity at 30°C than at 25°C within the same range of pH.

Wolpert (1924) working with the growth of wood-destroying fungi in relation to hydrogen-ion concentration stated that the range of pH in which those fungi will grow and the amount of mycelial growth depend upon the individual organism, the composition of nutrient solution, the initial active acidity and the temperature. The major portion of the growth curves of all of these fungi is on the acid side of neutrality and in the majority of cases wholly on the acid side.

Karrer (1921) working with the effect of hydrogen-ion concentration upon the accumulation and activation of amylase produced by certain fungi concluded that a relation, which varies with the organism seemed to exist between the hydrogen-ion concentration of the medium and the accumulation of extra- and intra-cellular amylase. An optimum zone of activity between pH 3.0 and pH 6.0 existed for *Penicillium italicum*, while in the other fungi the optimum was more sharply defined at 6.0. Inactivation occurred on the alkaline side.

Brancato and Golding (1933) in their studies of the influence of pH on the diameter attained by colonies of *Aspergillus niger*, *A. flavus*, *Penicillium notatum*, *P. expansum*, *P. roqueforti*, and *Geotrichum candidum* reported that pH 5.5 is optimum or very near optimum for all of these organisms.

Webb (1921) in his studies of the germination of spores of certain fungi in relation to hydrogen-ion concentration observed that inhibition of growth in all cases occurred at pH 3.0.

The relation of temperature to the effect of hydrogen-ion concentration (Wolpert, 1924) showed that the pH range of *Armillaria mellea* is 2.5 to 7.5 at 15°C, 2.0 to 7.8 at 25°C and 2.5 to 7.4 at 35°C when cultivated on a sucrose-peptone medium.

The finding of the present studies is similar to that of Wolpert in that the

pH range in which the fungi will grow depends on the individual fungus, the initial reaction of the medium and the temperature.

SUMMARY

1. Fifty fungi have been surveyed for their ability to synthesize fibrinolysin, using an *invivo* method described by Crabill and Reed. Of all fungi investigated only 23 species and strains are capable of synthesizing a considerable amount of fibrinolysin.

2. The sugar content of the medium show a marked inhibitory effect on the production of fibrinolysin by most of the fungi.

3. The optimum temperature for the production of fibrinolysin lies between 25° and 30°C and inhibition occurs at 15°C in most cases. Growth takes place over a wide range of temperature (15° to 35°C).

4. Temperature influences the pH limits for growth and formation of fibrinolysin. At 30°C inhibition occurs at pH 3.2. Under favorable temperatures (about 25°C) pH range is widest for growth and fibrinolytic activity. The optimum pH for the majority of fungi is between pH 5.5 and 7.1.

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