Mycological Fat Production in India. III. Effect of Temperatures on Fat Formation

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Temperature has manifold effects on cellular activities. It has been pointed out that those who are concerned with the industrial production of metabolites, should be aware of the fact that the temperature for production may not be the same as that for fungal growth (Deverall, 1965). Acracamone *et al.* (1961) found that *Claviceps purpurea* produced 30 times more lysergic acid derivative at 21° C than at 30° C, although there was no significant difference in growth at those two temperatures. Fries (1953) noticed that *Coprinus fimetarius* had an optimum growth at 40° C and grew vigorously at 44° C only if methionine synthesis continued. However, sometimes it is also desirable to grow fungus at one temperature to produce abundant mycelium and then change to the other to obtain a maximum yield of metabolite. Similar approach was emphasized by Owen and Johnson (1955) when they showed that *Penicillium chrysogenum* produced significantly more penicillin if started at 30° C while good growth was noticed at 20° C.

The optimal fat formation in microorganisms generally coincides with their optimal growth temperature (Kleinzeller, 1949; Fink, Haehn and Hoerburger, 1937; Heide, 1939; Pupowa and Putschkowa, 1967; Schmidt, 1938; Damm, 1943; Litvinova and Raevskaya, 1952; Gad and Walker, 1954; Gregory and Woodbine, 1953; and Zikes, 1919). The chemical composition of the fat formed may, however, vary with temperature (Bass and Hospodka, 1952; Pearson and Raper, 1927; Singh and Walker, 1956; and Terroine, Bonnet, Kopp and Vechot, 1927).

In the present investigation an attempt has been made to find out the effects of temperature on the growth, fat accumulation and sugar utilization by *Aspergillus allahabadii* Meh. and Agni., *A. indicus* Meh. and Agni., *Mucor circinelloides* van Tiegh. *M. peacockensis* Meh. and Nand *M. recurvus* Butler, *Penicillium oxalicum* Currey et Thom and *Phycomyces blakesleeanus* Burgeff.

Materials and Methods

Pure cultures of *Aspergillus* and *Penicillium* species were grown on a culture medium having the following composition: $NH_4NO/$,

3.0 g; KCl, 0.2 g; $MgCl_2$. $6H_2O$, 0.04 g; Na_2SO_4, 0.01 g; NaH_2PO_4. H_2O, 0,2 g; $ZnSO_4$. $7H_2O$, 4.5 mg.; $CuSO_4$. $5H_2O$, 0.08 mg.; sucrose, 25 g; distilled water, 1000 ml; and pH, 6.5. The species of *Mucor* and *Phycomyces* were grown on a medium of the following composition: KH_2PO_4, 0.5 g; $MgSO_4$. $7H_2O$, 0.25 g; asparagine, 2.0 g; thiamin chloride, 0.5 mg.; glucose, 25 g; distilled water, 1000 ml; and pH, 6.5. 30 ml. of the media were apportioned in 150 ml. Erlenmeyer flasks and were sterilized at 15 lbs pressure for 15 minutes. To remove the lag effect the flasks were then left undisturbed at least for three hours before incoculation at a temperature, at which the growth was to be observed. The different temperatures tried were 0° C, 10° C, 15° C, 20° C, 25° C, 30° C, 40° C and 48° C and 55° C. Three replicates were taken in each case.

The method of inoculation, incubation, harvesting, grinding, fat extraction and sugar estimation was the same as described in the first paper of this series (Mehrotra & Nand, 1970). Statistical analysis was done by the methods of Paterson (1939).

Results

The effects of differing temperatures on growth, fat synthesis and sugar utilization are given in Tables I—VII and graphically represented in Figs. 1—7. It is evident from the results that the growth, fat formation and sugar utilization were greatest for most of the species at 25° C. By means of "t" test, the most suitable temperature for growth and fat synthesis for all the species was found to be 25° C except for *Mucor recurvus* and *Phycomyces blakesleeanus* where highest growth and fat formation occurred at 20° C.

In general, the greater the relative amount of growth of an isolate at succeeding temperatures, the higher was the fat synthesis, sugar utilization, and economic and fat coefficients.

Discussion

Temperature affects the growth of different fungi by affecting a number of their metabolic processes as well as altering the physical nature of the substrate. But the temperature has not so profound effect on the total fat content (Foster, 1949; Kleinzeller, 1948), although striking differences in chemical nature of the fatty acids of the fat fraction were reported by Pearson and Raper, 1927; Terroine, Bonnet, Kopp and Vechot, 1927. Kleinzeller (1948) reported maximum fat formation at 25° C in *Torulopsis lipofera*. Zikes (1919) also found that a temperature range between 20°—30° C was the best for fat formation by *Mycoderma cerevisiae*, *Torula alba* and *Willia anomala*. Gregory and Woodbine (1953) working with *Aspergillus nidulans*, *Penicillium spinulosum* and

P. javanicum reported that 25°—30° C was found to be the most favourable for fat formation. Imai (1950) also reported that when *Penicillium chrysogenum* was grown at temperatures ranging from 4°—26° C, higher total fat content and phosphatide were obtained at higher temperatures. It is clear that for all isolates which were taken in this investigation, the optimum temperature for growth was most suitable for fat accumulation also. Temperatures which inhibited the growth resulted in lesser fat accumulation.

Summary

The influence of different temperatures (0° C, 10° C, 15° C, 20° C, 30° C, 40° C, 48° C and 55° C) on the growth, fat accumulation and sugar utilization by *Mucor circinelloides* van Tiegh., *M. recurvus* Butler, *M. peacockensis* Meh. and Nand. *Phycomyces blakesleeanus* Burgeff, *Aspergillus indicus* Meh. and Agni., *A. allahabadii* Meh. and Agni. and *Penicillium oxalicum* Currey et Thom, was studied under controlled conditions. Maximum fat content, growth, sugar utilization, and economic and fat coefficients were recorded at 20° C for *Mucor recurvus* Butler and *Phycomyces blakesleeanus* Burgeff and for the rest of the species at 25° C.

Acknowledgements

Grateful thanks are due to the University Grants Commission for the award of a Post-graduate Research Scholarship and to Prof. D. D. Pant, for providing the necessary laboratory facilities to the junior author.

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Table I. Effect of Temperature on Fat Synthesis, Felt Formation and Sugar Utilization by *Aspergillus allahabadii* Meh. and Agni

(Vol. of culture medium, 30 ml.; incubation period, 16 days, incubation temp. 25° C)

Temperature in centigrade		Dry wt of felt (mg/ per flask)	Sugar used (mg/per flask)	Fat (mg/ 100 mg dry felt)	*Economic coefficient	**Fat coefficient
0°		00.0	00.0	00.00	0.000	0.000
10°		22.8	310.0	12.00	7.354	0.882
15°		44.6	420.2	14.24	10.613	1.511
20°		180.2	450.6	16.26	37.528	6.096
25°		200.4	520.2	27.24	38.523	10.493
30°		210.8	544.2	22.00	38.735	8.521
40°		40.8	322.8	14.24	12.639	1.799
48°		3.2	111.2	14.00	2.877	0.402
55°		0.0	0.0	0.00	0.000	0.000
C. D.	5%	4.888	23.627	2.661		
	1%	7.536	37.535	4.102		

*) Mycelial felt formed Sugar used × 100
**) Fat formed Sugar used × 100

Table II. Effect of temperature on fat synthesis, felt formation and sugar utilization by *Aspergillus indicus* Meh. & Agni.

(Vol. of culture medium, 30 ml.; incubation period, 16 days; incubation Temp. 25° C)

Temperature in centigrade		Dry wt of felt (mg/per flask)	Sugar used (mg/per flask)	Fat (mg./ 100 mg dry felt)	*Deonomic coefficient	**Fat coefficient
0°		0.00	0.00	0.00	0.00	0.00
10°		30.2	430.0	10.20	7.023	0.716
15°		65.8	448.2	15.62	14.680	2.111
20°		112.4	450.6	20.24	25.166	5.081
25°		210.4	552.8	30.84	38.060	11.737
30°		208.4	540.1	20.46	36.732	7.913
40°		50.6	401.2	16.64	12.612	2.098
48°		4.2	11.2	15.42	3.750	0.578
55°		0.00	0.00	0.00	0.000	0.000
C. D.	5%	4.396	18.918	1.844		
	1%	6.669	29.165	2.844		

*) Mycelial felt formed Sugar used × 100
**) Fat formed Sugar used × 100

Table III. Effect of temperature on fat synthesis, felt formation and sugar utilization by *Mucor circinelloides* van Tiegh.
(Vol. of culture medium, 30 ml.; incubation period, 16 days; incubation temp. 25° C)

Temperature in centigrade		Dry wt of felt (mg/per flask)	Sugar used (mg/per flask)	Fat (mg/ 100 mg. dry felt)	*Economic coefficient	**Fat coefficient
0°		0.00	0.00	0.00	0.00	0.000
10°		30.6	114.2	34.26	2.679	0.927
15°		52.2	333.2	38.24	15.675	6.990
20°		82.6	398.2	40.66	20.718	8.434.
25°		130.4	522.1	45.24	24.976	11.299
30°		128.2	522.0	32.12	24.539	7.888
40°		32.4	324.2	30.42	9.993	3.020
48°		0.00	000.0	00.00	0.000	0.000
55°		0.00	000.0	00.00	0.000	0.000
C. D.	5%	2.548	16.700	1.392		
	1%	3.928	25.825	2.147		

*) Mycelial felt formed Sugar used × 100
**) Fat formed Sugar used × 100

Table IV. Effect of temperature on fat synthesis, felt formation and sugar utilization by *Mucor peacockensis* Meh. & Nand.
(Vol. of culture medium, 30 ml.; incubation period, 16 days; incubation temp. 25° C)

Temperature in centigrade		Dry wt of felt (mg/per flask)	Sugar used mg/per flask)	Fat (mg/ 100 mg. dry felt)	*Economic coefficient	**Fat coefficient
0°		0.00	0.00	0.00	0.000	0.000
10°		20.2	182.0	30.28	11.098	3.360
15°		46.8	300.0	36.42	15.600	4.938
20°		72.6	320.0	38.24	22.687	8.675
25°		86.4	400.0	40.62	21.600	8.773
30°		60.0	400.0	36.00	15.000	5.400
40°		00.0	000.0	00.00	00.000	0.000
48°		00.0	000.0	00.00	00.000	0.000
55°		00.0	000.0	00.00	00.000	0.000
C. D.	5%	3.276	16.695	1.711		
	1%	5.051	25.738	2.637		

*) Mycelial felt formed Sugar used × 100
**) Fat formed Sugar used × 100

Table V. Effect of temperature on fat synthesis, felt formation and sugar utilization by *Mucor recurvus* Butler

(Vol. of culture medium, 30 ml.; incubation period, 16 days; incubation temp. 25° C)

Temperature in centigrade		Dry wt of felt (mg/per flask)	Sugar used (mg/per flask)	Fat (mg./ 100 mg dry felt)	*Economic coefficient	**Fat coefficient
0°		00.00	000.0	00.00	00.000	0.000
10°		20.0	160.8	32.42	12.437	4.032
15°		55.2	340.0	26.82	16.235	6.301
20°		68.0	360.0	38.42	18.888	7.256
25°		60.0	350.0	37.24	17.142	6.385
30°		40.8	288.0	36.42	14.166	5.163
40°		00.0	000.0	00.00	00.000	0.000
48°		00.0	000.0	00.00	00.000	0.000
55°		00.0	000.0	00.00	00.000	0.000
C. D.	5%	4.847	14.301	2.103		
	1%	7.472	22.048	3.242		

*) Mycelial felt formed Sugar used × 100
**) Fat formed Sugar used × 100

Table VI. Effect of temperature on fat synthesis, felt formation and sugar utilization by *Penicillium oxalicum* Currey et Them

(Vol. of culture medium, 30 ml; incubation period, 16 days; incubation Temp. 25° C)

Temperature in centigrade		Dry wt of felt (mg/per flask)	Sugar used (mg/per flask)	Fat (mg./ 100 mg dry felt)	*Economic coefficient	**Fat coefficient
0°		00.0	000.0	00.00	00.000	0.000
10°		20.2	313.0	20.24	6.453	1.306
15°		50.6	441.2	22.42	11.468	2.571
20°		160.2	562.2	25.62	28.495	7.300
25°		180.4	582.2	28.82	39.859	8.930
30°		162.2	552.2	28.24	29.351	8.295
40°		10.0	110.2	26.24	9.074	2.381
48°		2.2	100.2	25.20	2.195	0.553
55°		00.0	000.0	00.00	00.000	0.000
C. D.	5%	2.582	18.964	2.333		
	1%	3.981	29.236	3.597		

*) Mycelial felt formed Sugar used × 100
**) Fat formed Sugar used × 100

Table VII. Effect of temperature on fat synthesis, felt formation and sugar utilization by *Phycomyces blakesleeanus* Burgeff

(Vol. of culture medium, 30 ml; incubation period, 16 days; incubation Temp. 25° C)

Temperature in centigrade		Dry wt of felt (mg/per flask)	Sugar used (mg/per flask)	Fat (mg./ 100 mg dry felt)	*Economic coefficient	**Fat coefficient
0°		0.00	0.00	0.00	0.00	0.00
10°		30.2	430.0	10.20	7.023	0.716
15°		65.8	448.2	15.62	14.680	2.111
0°		00.0	000.0	00.00	00.000	0.000
10°		59.4	460.0	32.61	12.913	4.212
15°		65.8	490.0	34.46	13.428	4.627
20°		98.2	508.4	38.24	19.315	7.386
25°		86.4	494.0	36.20	17.489	6.533
30°		44.4	400.0	34.00	11.100	3.779
40°		00.0	000.0	00.00	00.000	0.000
40°		00.0	000.0	00.00	00.000	0.000
55°		00.0	000.0	00.00	00.000	0.000
C. D.	5%	2.538	14.121	1.166		
	1%	4.914	19.770	1.798		

*) Mycelial felt formed Sugar used × 100

**) Fat formed Sugar used × 100