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The Effect of *In-vitro* Temperature on Actinomycetes and their Antibiotic Activity

R.B. Srivastava[1], P.K. Upadhyay[2] and S.K. Sinha[3]

[1]Department of Botany, Amardeep (PG) College, Firozabad - 283 203, India.
[2]Department of Genetics & Plant Breeding, R.B.S. (PG) College, Agra, India.
[3]Department of Botany, Agra College, Agra, India.

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The effect of different in-vitro temperature were investigated in relation to the growth and production of antibiotic substance by *Streptomyces carcinomycicus* in order to find out the maximum production of antibiotic substance occurred at 25°C, 30°C and 35°C *in vitro* temperature. The results of experiment are inconformity with the earlier observations that actinomycetes generally appears to be mesophilic according to the temperature relations.

Key words: Temperature durations, Actinomycetes, Antibiotic activity, Beef extract medium.

The study of in-vitro temperature is an important physical factor effecting the growth and metabolic activity of actinomycetes. Waksman *et.al.* (1944) obtained maximum *Streptomycin* production at 30°C temperature. The antibiotic yield was found to decrease sharply when incubated at temperature above 30°C although there was no decline in mycelium growth upto 37°C, optimum temperature for the production of *Neomycin* was found to be 30°C to 32°C (Mangallum *et.al.* 1974) and maximum antiobiotic production by *Streptomyces* species was found at 28°C (De & Chandra 1978).

* To whom all correspondence should be addressed.
E-mail: dr.rbsrivastava@gmail.com

MATERIAL AND METHODS

In the present investigation, 8 different incubation temperature were tested for the growth and production of antibiotic substance by *S. carcinomycicus*. Different incubation temperature listed in table-1 were incorporated individually, 30 ml of Beef extract medium (Glucose 10.0 gm, Peptone 0.4 gm, Beef extract 0.5 gm, Sodium Chloride 0.5 gm, Distilled water 1.0 liter) was taken in 250 ml Erlenmeyer flasks in triplicate and sterilized at 10 lbs pressure for half an hour. Each flask was inoculated with spore suspension of *S. carcinomycicus* and incubated for 15 days at 28^0C($+2^0$C). The antibiotic activity of the culture filtrate was assayed in terms of percentage inhibition of spore germination of *Alternaria solani* after 5, 10 and 15 days incubation adopting the "Hanging drop method" of Brain and his co-workers (1960). The spore suspension of *A.solani*

was prepared in sterilized distilled water from a 10 days old culture grown on oat-meal agar medium. The dilution of spores in the suspension was adjusted to 10-15 spores per microscopic field in low power. 0.1 ml of spore suspension was added to 5 ml of the cultural filtrate to be assayed to ensure uniform distribution of spore and throughout the filtrate. Drops of the filtrate containing spores of *A. solani* were placed on slides which were inverted and kept in moist chamber on 'V' tubes aseptically and incubated for 8 hours at $30^0C(+2^0C)$. The drops were then examined under the microscope and number of ungerminated spores were counted. The percentage inhibition of spore germination was calculated. After 15 days of growth of *S. carcinomycicus* in different inoculation termperature, mycelial mat was harvested by filtration through previously weighed whatman no. 1 filter papers. The mycelial mat was thoroughly washed with distilled water and the dry weight was recorded for each treatment.

RESULTS AND DISCUSSION

The results presented in table-1 indicate that the Actinomycetes are effected at different incubation temperature. The table shows further that *Streptomyces carcinomycius* is able to utilize various incubation temperature for growth and production of antibiotic substance but to a varying degree considering the critical difference (C.D. = 21.78) at 5% probability (table-1) The production of maximum antibiotic substance at 25°, 30°, 35°C as their respective percentage inhibition were 89.32%, 96.10% and 80.86% respectively. Mycelial dry weight recorded in these three cases being 1.8512 gm, 1.7512 gm, and 1.4121 gm respectively. There was however, a sharp fall in antibiotic activity from 35°C to 40°C where as it was gradual as temperature decreases from 25°C to 15°C, 5°C to 10°C the micro-organism was unable to grow and the assay values achieved were consequently low. Maximum growth of mycelium was obtained at 30°C where as the maximum antibiotic production occurred at 30°C.

Table 1. The effect of incubation temperature on the production of antibiotic by the strain of *Streptomyces carcinomycicus* and its growth

S. No.	Temperature durations (°C)	Percentage inhibition of spore germination *Alternaria solani*			Mycelial dry wt. in gm 15 days of growth	Final pH
		5 days	10 days	15 days		
1.	5	3.90	4.25	5.02	0.1421	6.2
2.	10	4.41	5.10	6.18	0.2132	5.7
3.	15	8.60	28.54	41.42	0.6728	6.0
4.	20	12.20	34.34	76.73	0.9028	6.6
5.	25	32.14	58.98	89.32	1.8512	6.8
6.	30	38.54	76.10	96.10	1.7512	7.0
7.	35	36.56	74.18	80.86	1.4121	6.2
8.	40	39.30	51.30	48.16	0.9901	6.4

Table 1A: Analysis of Variance

S. No.	Source of variance	Degree of Freedom DF	Sum of Squares SS	Mean Squares MSS	Variance Ratio (F) Calculated Value	Table Value 1%	Table Value 5%
1.	Duration	2	4538.242	2269.121	14.663	6.51	3.74
2.	Treatment	7	13793.091	1970.442	12.732	4.30	2.77
3.	Residual	14	2166.550	154.7532			
Total		23	20497.883				

In present investigation, optimum temperature for growth and the production of antibiotic substance was found to be 30°C which is general confirms the previous reports (Waksman and Schatz 1945, Waksman et.al. 1944). Temperature at 5°C interval between 5°C to 40°C, growth was excellent between 25°C to 35°C, maximum antibitoic production occurred at 30°C and maximum growth t 35°C. Thus, the actinomycetes appears to be mesophilic according to the temperature relations. Thornberry and Anderson (1948) also reported that above 30°C, there is a sharp cut in antibiotic production although good growth was obtained at temperature as high as 37°C.

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