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Effects of Caffeine on the Sporulation and Spore Germination in the Green Alga *Stigeoclonium pascheri*

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ABSTRACT. The treatment of vegetative cells of *Stigeoclonium pascheri* with caffeine delayed the initiation of sporulation and decreased the percentage sporulation. However, the treatment of spores with 500 and 1000 ppm caffeine for short durations accelerated the initiation of spore germination and increased the percentage spore germination.

The methylated oxypurine caffeine has been extensively used on cells of higher plants, animals, as well as some procaryotic organisms to study cytological and genetic effects of the drug (Kihlman and Levan 1949; Gimenez-Martin *et al.* 1969; Zuk and Swietlinska 1973; Lehmann and Kirkbell 1974; Kihlman 1977; Singh and Kashyap 1977; Timson 1977; Solberg *et al.* 1978) but so far no data are available on the effects of caffeine on green algal sporulation and spore germination. The present investigation deals with the effects of pretreatment with caffeine on the sporulation and spore germination in *Stigeoclonium pascheri* (VISCHER) COX and BOLD, a green alga belonging to *Chaetophorales*.

MATERIAL AND METHODS

The alga was collected from a fresh-water pond situated at Sarnath near Varanasi. Clonal cultures were raised from single germinating akinetes and maintained under cultural conditions in Bold's basal medium (BBM) (Cox and Bold 1966) at 22 ± 1 °C and illuminated at *c.* 2 klx light intensity from daylight fluorescent tubes for 16 h/d. The mode of reproduction of this alga was observed to be only through the formation of akinetes, hereafter called spores. Initiation of sporulation, observed in 30-d-old cultures growing on agar plates, is characterized by (1) change in colour of the filaments from deep-green to light-yellowish orange, (2) constriction of cells at partition wall and consequent barrel-like shape, and (3) increase in the width of cells, their individual lengths remaining more or less constant. Mature spores obtained 30 d after initiation of sporulation, can germinate directly into

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TABLE I. Effects of pretreatment with caffeine on the time taken for initiation of sporulation and percentage sporulation in *S. pascheri*

Concentration ppm	Time of treatment h	Initiation of sporulation d	Sporulation %
500	0	30	65.0
	3	30	65.0
	6	30	61.3
	12	30	60.0
	24	30	52.0
	48	30	41.7
1000	0	30	65.0
	3	30	62.5
	6	30	53.6
	12	33	47.7
	24	35	40.2
	48	38	32.8
2000	0	30	65.0
	3	30	57.6
	6	32	46.1
	12	36	39.8
	24	39	30.6
	48	40	21.5

new filaments, following their incubation in a fresh medium. Aged spores remaining in the medium in which they had been formed usually did not germinate. The first indications of spore germination observed was the change in colour from reddish-yellow to light-green and subsequent emergence of a small protuberance breaking through the wall of the spore, which subsequently develops to a germling. The emergence of the protuberance was taken as a criterion of germination.

In order to observe the effects of caffeine on sporulation and spore germination, the 7-d-old, actively growing, vegetative filaments and the mature spores were pretreated for different time periods with an aqueous solution of caffeine, ranging from 500 to 2000 ppm. The treated vegetative filaments and spores were then carefully washed with distilled water several times to remove the caffeine that may have been carried with the material and then were inoculated into Petri dishes containing the basal media. They were then placed in a growth chamber under standard experimental conditions. Untreated material plated simultaneously for each set served as control. Observations were made to determine the time taken for initiation of sporulation, spore germination, percentage sporulation and spore germination under different conditions.

RESULTS

In *S. pascheri* sporulation generally begins in 30-d-old cultures under controlled cultural conditions. There was a delay of 3, 5 and 8 d in the time taken for initiation of sporulation in cells treated with 1000 ppm caffeine

TABLE II. Effects of pretreatment with caffeine on the time taken for initiation of spore germination and percentage spore germination of *S. pascheri*

Concentration ppm	Time of treatment h	Initiation of spore germination d	Germination %
500	0	2	12.5
	1	2	23.0
	2	1	40.3
	3	2	31.5
	4	2	26.0
	5	2	20.3
	6	2	13.6
1000	0	2	12.5
	1	1	41.8
	2	1	53.4
	3	1	47.2
	4	1	39.4
	5	1	27.8
	6	1	21.5
2000	0	2	12.5
	1	2	5.9
	2	2	4.5
	3	3	3.2
	4	4	2.6
	5	5	1.5
	6	5	1.0

for 12, 24 and 48 h, respectively. Initiation of sporulation was delayed by 2, 6, 9 and 10 d in cells treated with 2000 ppm caffeine for 6, 12, 24 and 48 h, respectively. No change was observed in the time taken for initiation of sporulation in any of the remaining treatments. Percentage sporulation was recorded in 60-d-old cultures, which decreased with increasing concentration of caffeine and duration of treatment (Table I).

Pretreatment of spores with caffeine was found to influence spore germination. In control material, spores begin to germinate 2 d after inoculation. Treatment of spores with 500 ppm caffeine has no substantial effect, that with 1000 ppm accelerates the initiation of spore germination. On the other hand, prolonged treatment of spores with 2000 ppm caffeine delays the initiation of germination (Table II).

The percentage germination of spores was recorded 4 d after inoculation. Spores pretreated with 500 ppm or with 1000 ppm caffeine showed the highest increase in the percentage germination in the 2-h variant. However, continuous decrease in percentage germination of spores was observed following their treatment with 2000 ppm of caffeine for 1—6 h (Table II)

DISCUSSION

Caffeine increased the sporulating ability of *Saccharomyces cerevisiae* at concentrations of 100—1000 ppm (Tsuboi and Yanagishima 1975). Cells

grown in the presence of caffeine have a higher content of RNA and protein. In the present study, the vegetative cells of *S. pascheri* subjected to treatment of caffeine showed a decrease and delay in sporulation. The sporulation gradually declined with increase in concentration of caffeine and the time of treatment. Although caffeine was found to inhibit the sporulation of vegetative cells, nothing is known about its exact mode of action.

Caffeine is known to inhibit the mitosis and to cause failure of cell division in some algae and higher plants. In *Gymnodinium inversum*, Sarma and Shyam (1975) observed that treatment of cells with lower doses of caffeine prevented cell division resulting in the production of bi- and tetranucleate cells. Kihlman and Levan (1949) found that treatment with 0.4 % caffeine for 24 h in *Allium* sp. resulted in total suppression of mitosis. However, the concentration of 0.02 % (200 ppm) or even less resulted in mitosis at a normal or higher than normal rate. In human lymphocytes lower concentrations of caffeine increase the frequency of mitosis (Timson 1970).

The treatment of *S. pascheri* spores with 500 and 1000 ppm of caffeine for short durations increased their percentage germination and decreased the time taken for initiation of germination. However, irrespective of the concentration employed, caffeine decreased the percentage sporulation. Thus spores behave differently from vegetative cells at concentrations of caffeine up to 1000 ppm.

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