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EFFECT OF FEW AMINO ACID ANALOGUES IN *NOSTOC SPONGIAEFORME*

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The effect of amino acid analogues (AAA) L-canavanine and L-methionine—DL-sulfoximine (MSO) on the growth and development of *Nostoc spongiaeforme* was studied. L-canavanine suppressed the growth at 4 μg/ml while MSO inhibited the growth at 1 μg/ml. AAA at lower concentrations stimulated the growth of *Nostoc spongiaeforme* concomit with the higher quantities of biochemical metabolities. Progressive reduction in growth of alga by AAA resulted in lowering the quantities of the cellular metabolities. The heterocyst frequency and per cent sporulation correspondingly decreased with enhanced concentrations of the AAA, except the lower concentration of MSO.

Keywords : L-methionine-DL-Sulfoximine; AAA—Amino Acid Analogues; *Nostoc spongiaeforme.*

Introduction

Amino acid analogues are amino acid antagonists which severely affect the various micro-organisms by inhibiting the growth and metabolism in various ways (Richmond, 1962). L-canavanine, a naturally occuring analogue of L-arginine, has been shown to inhibit the growth and heterocyst differentiation of blue-green algae (Ladha and Kumar, 1978; Kumar and Kumar, 1980, 1981). MSO, an analogue of methionine and alanine, strongly inhibits the growth of *Nostoc linckia* but produced an unusual number of heterocysts with alteration in spacing in both nitrogen free and combined nitrogen media (Ladha and Kumar, 1978)and two fold increase in heterocyst frequency in *Anabaena doliolum* and *Anabaena sp.* To date there is no systematic study of the effect of aminoacids analogues on spore formation, hence the present study.

Material and Methods

Clonal and axenic culture of *Nostoc spongiaeforme* an isolate from cultures collection of IARI, New Delhi was raised from single spore and grown

in modified Chu No. 10 medium (Gerloff *et al.*, 1950). The experimental and stock cultures were incubated in fluorescent light (600 lux) and maintained at 28±2°C. The growth was measured in terms of optical density of 80 per cent acetone extracted chlorophyll-a pigment. The techniques for quantitative estimation of cellular metabolities are reported earlier (Sarada, 1988). Heterocyst frequency was expressed in terms of average number of heterocysts present in 100 vegetative cells in twenty five actively growing vegetative filaments. Four days lag phase, 20 days exponential phase and more than seven days declining phase were observed on the growth of *Nostoc spongiaeforme*. The percentage of sporulation was calculated as the number of spores per hundred vegetative cells in a filament. The percentage of sporulation is based on average of ten sporulating filaments.

Results and discussion

The growth of *Nostoc spongiaeforme* was inhibited at 4 μg/ml canavanine. The canavanine supplemented cultures (0.1 and 1 μg/ml) showed less growth upto 14th day, more growth rate from 16th day to the end of declining phase as compared to control. The growth of *Nostoc spongiaeforme* was retarded at 1.0 μg/ml of MSO indicating its inhibitory action. The lower concentration (0.1 μg/ml) of MSO promoted the growth as well as enhanced biochemical metabolites and heterocyst frequency. It is evident from the data given in tables 1 and 3 that the heterocyst frequency reduced considerably with the increasing levels of canavanine and MSO except the lower concentration (0.1 μg/ml) where the frequency of heterocyst was slightly high.

Apart from the inhibitory growth (4.0 μg/ml) and promotory effect of 0.1 and 1.0 μg/ml of canavanine, the cellular metabolites were reduced (Table 2). In addition to the inhibition of growth and heterocyst development, MSO caused reduction of cell metabolites such as chlorophyll, carotenoids, proteins, carbohydrates and nucleic acids except at 0.1 and 0 25 μg/ml (Table 3). The spore formation in *Nostoc spongiaeforme* was inhibited at 4.0 μg/ml canavanine and 1.0 μg/ml MSO and there was no change in the spore initiation day both in control and experimental cultures (Figs. 1, 2). The percent sporulation decreased with the increase in concentrations of both amino acid analogues tested.

From the foregoing results, it is obvious that canavanine and MSO act as growth inhibitors in *Nostoc spongiaeforme* at higher concentrations. These findings are in agreement with the results obtained in *Anabaena doliolum*, *Anabaena sp.* (Kumar and Kumar, 1980; 1981), and

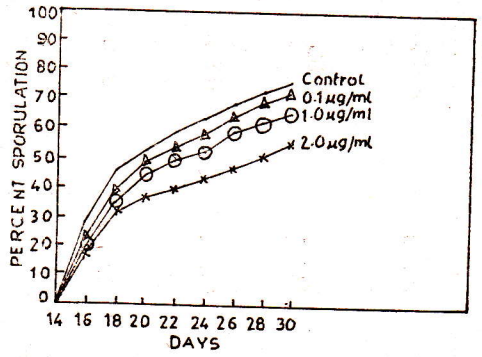

Fig. 1. Effect of L-canavanine on sporulation in *Nostoc spongiaeforme.*

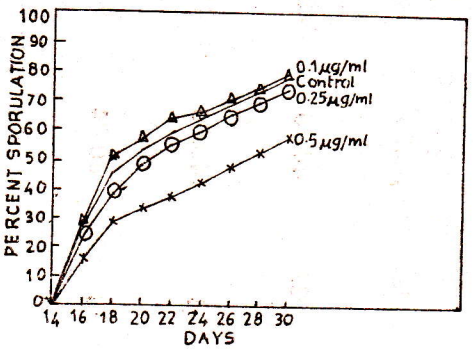

Fig. 2. Effect of MSO on sporulation in *Nostoc spongiaeforme.*

Table 1. Effect of canavanine on heterocyst frequency in *N. spongiaeforme.*

	% Heterocyst frequency						
	DAYS						
	2	4	6	8	10	12	14
Basal medium (control)	—	—	3.45	2.91	2.70	3.00	3.20
Basal medium +0.1 µg/ml canavanine	—	—	2.81	2.25	1.97	1.87	2.29
Basal medium + 1.0 µg/ml canavanine	—	—	—	2.14	1.89	1.86	2.17
Basal medium + 2.0 µg/ml canavanine	—	—	—	1.87	1.79	1.77	1.81
Basal medium + 4.0 µg/ml canavanine	—	—	—	—	—	—	—

Table 2. Effect of canavanine on biochemical substances in *N. Spongiaeforme*.

Concentrations	Chloro-phyll-a (mg/g)	Carote-noids (mg/ml)	Proteins (μg/100) mg fresh weight)	Carbo-hydrates (μg/100 mg fresh weight)	R N A (μg/100 mg fresh weight)	D N A (μg phos-phate/100 mg fresh weight)
Basal Medium (Control)	0.1156	0.0029	39.28	361.25	44.05	6.08
Basal Medium +0.1 μg/ml canavanine	0.1936	0.0039	44.35	491.58	72.72	6.68
Basal medium +1 0 μg/ml canavanine	0.1507	0.34	40.00	414.67	58.33	5.54
Basal medium +2.0 μg/ml canavanine	0.1029	0.28	30.24	250.00	27.38	0.86
Basal medium +4.0 μg/ml canavanine	—	—	—	—	—	—

Table 3. Effect of MSO on heterocyst frequency in *N. spongiaeforme*.

	% Heterocyst frequency						
	DAYS						
	2	4	6	8	10	12	14
Basal medium (control)	—	—	3.45	2.91	2.70	3.00	3.28
Basal medium + 0.1 μg/ml MSO	—	—	4.44	3.17	2.91	3.21	3.75
Basal medium + 0.25 μg/ml MSO	—	—	—	2.74	2.41	2.75	3.11
Basal medium + 0.5 μg/ml MSO	—	—	—	—	—	2.40	2.22
Basal medium + 1.0 μg/ml MSO	—	—	—	—	—	—	—

in *Nostoc linckia* (Ladha and Kumar, 1978). Besides the growth inhibitory effects of the amino acid analogues on *Nostoc spongiaeforme* the quantities of various metabolites reduced with increase in concentrations of the chemicals except lower concentrations where the cellular metabolites were enhanced. Based on the inhibitory range of metabolites, amino acid analogues significantly affected the chlorophyll synthesis, and proteins (63%) followed by nucleic acids (55–60%). The inhibition of growth of *Nostoc spongiaeforme* by canavanine might be due to the formation of canavanyl proteins (i.e., canavanine substituted proteins) which are located in cell wall membrane as mentioned by Rosenthal (1977). MSO inhibited the growth by inactivating the glutamine synthesis and nitrogenase enzyme as suggested by several workers (Stewart and

Table 4. Effect of MSO on metabolic products in *N. spongiaeforme*.

Concentrations	Chloro-phyll–a (mg/g)	Carote-noids (mg/ml)	Proteins (μg/100 mg fresh weight)	Carbo-hydrates (μg/100 mg fresh weight)	R N A (μg/100 mg fresh weight)	D N A (μg phos-phate/100 mg fresh weight)
Basal medium (control)	0.1156	0.0029	39.28	361.25	44.05	6.08
Basal medium + 0.1 μg/ml MSO	0.1486	0.0048	45.71	320.00	71.67	2.00
Basal medium + 0.25 μg/ml MSO	0.1295	0.0038	41.74	225.00	68.00	0 95
Basal medium + 0.5 μg/ml MSO	0.0791	0.0013	16.00	100.00	24.21	—
Basal medium + 1.0 μg/ml MSO	—	—	—	—	—	—

Rowell, 1975; Thomas *et al.*, 1977; Haselkorn, 1978; Ladha and Kumar, 1978; Meeks, *et al.*, 1978).

Canavanine and MSO inhibited the heterocyst formation in *Nostoc spongiaeforme* (Table 1 and 3). It has been established that the heterocyst differentiation is dependent upon the synthesis of nascent proteins and also on the product of photosynthetic process (Singh and Kumar, 1971; Tyagi, 1975; Haselkorn, 1978). Heterocyst formation might be affected by the defective canavanyl proteins (Kumar and Kumar, 1981) or inactivation of glutamine synthetase (Stewart and Rowell, 1975; Haselkorn, 1978; Ladha and Kumar, 1978; Kumar and Kumar, 1980, 1981).

Both canavanine and MSO reduced the spore formation and per cent sporulation as compared to control (Figs. 1 and 2) without delay in spore initiation time. Kumar and Kumar (1981) supported the results of Simon (1971) on the basis of absence of cynophycean granules in canavanine treated cultures. Inactivation of glutamine synthesis by MSO may affect the alteration of metabolites in the cell leading to the reduction of spore formation. It is thus obvious that this subject needs further work before suitable conclusions are drawn.

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