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COMPARATIVE GROWTH STUDTY OF *SCENEDESMUS BIJUGATUS* (TURP.) KUETZ. VAR. *GRAEVENITZII* (BERNARD) COMB. NOV. AND ITS MUTANT *SBM-I* WITH STREPTOMYCIN

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Cultures of *Scenedesmus bijugatus* and its mutant *Sbm-I* were treated with different doses of streptomycin ranging from 0 05 to 2.0 mg/100 ml. The impact of the antibiotic was recorded on growth,survival-pattern and morphology. Both parent as well as mutant were compared with each other for their resistance towards the drug. Cultures were successfully trained for higher concentrations of the antibiotic. Streptemycin supressed the growth as well as pigmet production. At higher concentrations, temporary bleaching took place. The durg did influence morphology and dimensions, but to a small extent.

Keywords : *Scenedemus bijugatus;* Mutant *Sbm-I; Growth;* Streptomycin.

Introduction

Antibiotic streptomycin is a carbohydrate molecule with glycosidic linkages and amino-orguanidine molecule (Umezawa, 1975). Bleaching action of streptomycin on chlorophyll pigments of *Euglena gracilis* has earlier been reported (Ebringer, 1962). This drug has extensively been employed for studying its effects on respiration, protein synthesis and membrane of bacterial system (Anand and Davis, 1960; Dubin *et al.*, 1963; Stern *et al.*, 1966).

There are a number of reports on the impacts of streptomycin on growth, survival-pattern and morphology of blue-green algal members (Kumar, 1964; Kumar and Kaushik, 1971; Reddy, 1977 a and b) and on green algae (Srivastava and Sarma, 1980; Sathaiah and Vidhyavati, 1983; Digambar and Vidyavati, 1984, Vidyavati and Sathaiah, 1985).

As little information is available on the effects of streptomycin on Chlorococcales (Srivastava and Nizam, 1974; Reddy, 1977 a and b), therefore the present study was aimed at investigating the effects of various doses of streptomycin on growth, survival and morphological changes of *Scenedesmus bijugatus* and its morphological mutant *Sbm-I.* Attempts were also made to raise resistant strains of these algal samples to higher concentrations by way of training the clonal cultures.

Materials and Methods

S. bijugatus and its morphological mu-

tant *Sbm-I* (Bajaj and Srivastava, 1985) formed the experimental materials of this paper. Cultures were grown photoautotropically at 26±2°C in Juller's solution (Mainx, 1931) irradiated with white fluorescent lamps for 8 hrs/day.

Streptomycin was added to the nutritive medium as sulphate (Ambistryn-S 1gm/vial, Sarabhai Chemicals). Stock solutions and desired dilutions were prepared in cold sterilized Juller's solution. Different concentrations tested were 0.05, 0.1, 0.5, 1.0 and 2.0 mg per 100 ml. Growth-pattern was followed through optical density as well as coenobial-counts at weekly interval for a period of six weeks. Drug-free medium was used as control Three ml of uniform freshly growing cultures were centrifuged and after discarding supernatant, the sediment was added to each test-tube containing various concentrations of the antibiotic.

For production of resistant strains, cultures from permissible dose were trained to resist to the higher concentration as has already been dond for other Chlorococcales (Srivastava and Nizam, 1969).

Results

S. bijugatus—Cultures of *S. bijugatus* treated with 0.05 mg/100 ml exhibited slightly enhanced growth in contrast to that of the untreated cultures in terms of optical density records (Table 1). While percentage of healrhy coenobia decreased for first two weeks and increased thereafter which reached a maximum of 86.95% by the end of the experiment (Fig. 1A). The percentage of healthy coenobia decrease for a period of fout weeks resulting in simultaneous increase of damaged and dead coenobia. But from fifth week it started increasing and was 51.75% on last observation. Higher doses such as 0.5, 1.0 and 2.0 mg./100 ml retarded the growth of the cultures which was quite evident from optical density as well as percentage of healthy coenobia records (Table 1; Fig. 1A).

In first two doses majority of coenobia were consisting of brilliant green, healthy cells with intact chloroplast and a distinct pyrenoid. Cells of different dimensions i. e., either slightly bulged or thin and long type were recorded. From third week, certain cells with slightly shrunken and granular chloroplast and indistinct pyrenoid were noted (Figs. $2B_5, C_4, C_5, D_2$ and D_3). Higher doses inflicted drastic effects on chloroplast as it had shrunken to a large extent in certain cells and was reduced to small bits with the advancement of time (Figs. $2D_5, E_{2-5}$). On final record, total bleaching and disintegration was observed.

Morphology of cells and coenobia was not much influenced by this drug. Streptomicin resulted in decreased dimensions of cells and coenobia and it was directly proportionate to

the dose of the drug.

Mutant Sbm-I—Lower concentrations such as 0.05 and 0.1 mg/100 ml supported the growth of the cultures of *Sbm-I*, which almost paralleled the growth of the alga in drug–free medium (Table 2). However, a slight decline in the percentage of healthy cells was recorded upto a period of three weeks which increased afterwards (Fig. 1B). Under the impact of 0.5 mg/100ml percentage of healthy cells was gradually reduced to zero by fifth week, with onest of the sixth week, cultures revived with 1.85% of healthy cells (Fig. 1B). Concentrations 1 and 2 mg/100 ml did not support the growth of the cultures.

In concentrations 0.05 and 0.1 mg/100 ml, cells were bright green, healthy, normal with intact chloroplast and a distinct pyrenoid for first two weeks. But from third week onwards, cells with granular and slightly shrunken chloroplast were also recorded. Two types of cells i. e, globose and thin and long cells were noted (Figs. $2F_1$ & F_4). Certain bilobed (Fig. $2G_5$) and narrow ending cells were observed (Figs. $2G_2$ & G_3). In higher doses i. e., 0.5, 1.0 and 2.0 mg per 100 ml, chloroplasr was reduced to small dots-like structures (Fig.$2H_5$). Very few bulged cells were recorded Fig. $2H_3$). Average length and width decreased under the impact of various doses of the drug.

Cultures at sublethal concentrations were trained for higher doses of the drug by successive subculturings. Finally, *S. bijugatus* and *Sbm-I* could resist to a conceutration as high as 2 and 4 mg/100 ml respectively, which is double times more than the initial tolerance.

Discussion

Cultures of *S. bijugatus* and mutant *Sbm-I* looked apparently dead at 0.5 and 1.0 mg/100 ml cocentrations of streptomycin respectively. But they resumed their green coloration and normal growth phenomenon after

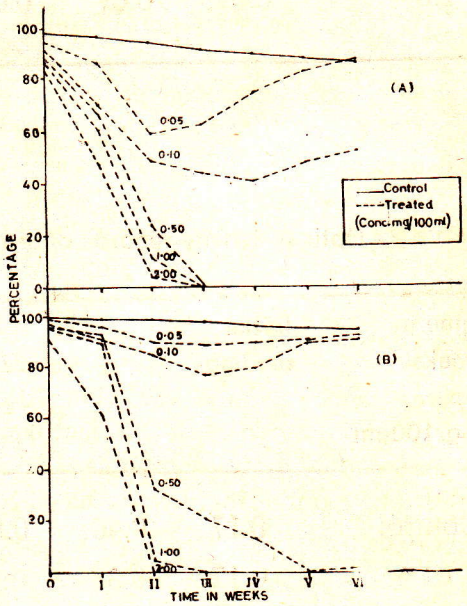

Fig. 1. [A] Percentage of healthy coenobia of *S. bijugatus* after streptomycin treatment. [B] Percentage of healthy cells of *Sbm-I* after streptomycin treatment.

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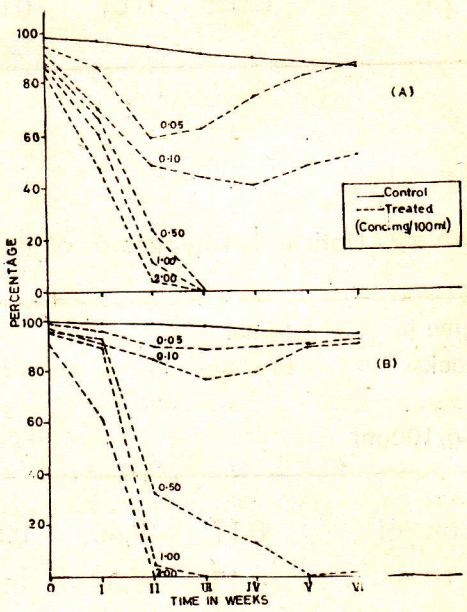

Fig. 1. [A] Percentage of healthy coenobia of *S. bijugatus* after streptomycin treatment. [B] Percentage of healthy cells of *Sbm-I* after streptomycin treatment.

Table 1. Optical density records of streptomycin treated *S. bijugatus*

Time in weeks Dose mg/100 ml	Initial reading	*I*	*II*	*III*	*IV*	*V*	*VI*
Control	0.06	0.18	0.34	0.45	0.56	0.66	0.76
0.05	0.06	0.19	0.37	0.48	0.58	0.68	0.78
0.1	0.06	0.18	0.33	0.43	0.52	0.60	0.68
0.5	0.06	0.14	0.13	0.10	0.07	0.06	0.05
1.0	0.005	0.11	0.10	0.08	0.065	0.05	0.02
2.0	0.055	0.07	0.07	0.06	0.045	0.03	0.015

Table 2. Optical density records of streptomycin treated *Sbm-I*

Time in weeks Dose mg/100 ml	Initial reading	*I*	*II*	*III*	*IV*	*V*	*VI*
Control	0.17	0.40	0.68	0.88	1.2	1.6	1.7
0.05	0.17	0.38	0.64	0.80	1.0	1.5	1.6
0.1	0.17	0.38	0.64	0.80	1.0	1.5	1.6
0.5	0.17	0.38	0.58	0.66	0.66	0.64	0.74
1.0	0.17	0.38	0.48	0.49	0.45	0.37	0.23
2.0	0.17	0.22	0.22	0.22	0.14	0.05	0.05

Fig. 2. Streptomycin treated *S. bijugatus* and mutant *Sbm-I* showing damage to chloroplast and morphological variations. A *S. bijugatus* untreated coenobium; A_1 — E_5 Treated coenobia of *S. bijugatus;* F Mutant *Sbm-I* untreated cell; F_1 — J_5 Treated cells of *Sbm-I*

being transferred to fresh drug-free nutritive medium indicating that the cultures suffered temporary bleaching and these concentrations were not lethal. As cultures of *S. bijugatus* and mutant *Sbm-I* could not revive back from the deleterious effects of 1.0 and 2.0 mg/100 ml concentrations of streptomycin respectively, these doses proved lethal for them. Even the lag-phase of *Sbm-I* at sublethal concentration was also less than that of *S. bijugatus*.

The antibiotic had an inhibitory effect on growth of both the algal samples. This is said to be as a result of interference of the drug with the biological reactions which are essential for their growth (Newton, 1965; Zahner and Mass, 1970). While the lethal effects of the drug may be due to its damaging effects on RNA and polyamine metabolism (Stern *et al.*, 1966; Freda and Cohen, 1966).

Streptomycin brought about damage both to the plastids and pigments as chloroplast turned faint yellow and was contracted from the cell wall. At higher concentrations cells were partly or totally bleached. But this chlorosis was temporary and reversible in lower doses and this behaviour was different from that of *Euglena* (Provasoli *et al.*, 1948; Rosen and Gowalik, 1961; Nora *et al.*, 1965). The loss of pigment colour may be due to conversion of some precursor of either the pigment or plastid by streptomycin (Rosen and Gowalik, 1961). In eukaryotes, organelles like chloroplast and mitochondria areknown to possess 70s ribosomes which behave like bacterial ribosomes in respect to sedimentation velocity and sensitivity to antibiotics (Boschetti *et al.*, 1974; Behn and Arnold, 1974; Speiss, 1977). The structure of this antibiotic has been related to its bleaching action (Ebringer, 1962).

Streptomycin did not influence the morphology of cells or coenobia of *S. bijugatus*, but *Sbm-I* exhibited a few abnormal forms. The drug had a decreasing effect on dimensions of cells and coenobia of *S. bijugatus* and *Sbm-I* both and decrease was directly proportionate to the increase in concentration of antibiotic. However, lethal doses had greater decreasing impact than the sublethal concentrations of the drug.

After training, cultures of *S. bijugatus* and *Sbm-I* could tolerate a high concentration of 2 and 4 mg/100 ml respectively. The resistance acquired by mutant *Sbm-I* was double the resistance acquired by its parent *S. bijugatus*, but adaptation was very slow. Growth of algal samples in higher doses of streptomycin leads to the fact that physiological adaptation played an important role.

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