

Regulation of growth and biomolecule of *Aphanothece* sp. by phenoxy herbicide 2, 4-D

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ABSTRACT

Toxicity of the herbicide 2, 4-Dichlorophenoxy acetic acid to unicellular cyanobacterium *Aphanothece* Sp. has been studied. Stimulation in cyanobacterial growth was observed at 100 $\mu\text{g ml}^{-1}$ while higher concentrations had inhibitory effect with 1600 $\mu\text{g ml}^{-1}$ being completely lethal. The sublethal concentration (1200 $\mu\text{g ml}^{-1}$) allowed few cell (frequency approx 10^{-7}) of cyanobacterium population into filamentous aseptate structure and regained the normal course of the unicellular pattern of differentiation in absence of the herbicide. 2, 4-D resulted in decline of chlorophyll *a* and no effect on DNA content even at the growth stimulatory concentration (100 $\mu\text{g ml}^{-1}$). However, cell protein, RNA and phycobillin contents were increased up to 400 $\mu\text{g ml}^{-1}$, while higher concentrations have inhibitory effects. Cellular abnormalities by higher concentration and possible mode of action of 2, 4-D at the level of cell division are discussed.

Key words: Growth Biomolecules, *Aphanothece* sp. Phenoxy herbicide, 2, 4-D.

INTRODUCTION

Cyanobacteria (Blue-green algae) are now recognized as a major group in the prokaryotic kingdom as judged by variety and number of species. They represent the most ancient and diverse group of organism and like higher plants perform oxygenic photosynthesis involving two photosystem. Because of their O_2 evolving photosynthetic features, identical to those of higher plant chloroplast, cyanobacteria occupy an unique position in the continuum of life.

The phenoxy acetic acid herbicide, 2, 4-D, is a synthetic auxin and generally used to control weeds in agriculture and ponds. Cyanobacteria are reported to tolerate higher concentrations of 2, 4-D although lower concentrations (100 $\mu\text{g ml}^{-1}$) are stimulatory to their growth and cell multiplication (Venkataraman and Rajyalakshmi, 1972; Tiwari *et al.* 1981) The literature concerned with

effect of herbicides on nucleic acid metabolism and protein synthesis has been reviewed by Cherry (1976) and Moreland (1980). Many herbicide block nucleic acid and protein synthesis by reducing ATP production. The present paper summarizes the possible mode of action of 2, 4-D for regulation of growth and cellular constituent of *Aphanothece* sp.

MATERIAL AND METHODS

Aphanothece sp. a unicellular cyanobacterium was isolated from local rice fields. The cyanobacterium was raised to axenic population employing standard microbial techniques. The clonal and axenic cultures were routinely grown in modified Chu-10 medium (Safferman and Morris, 1964) supplemented with calcium nitrate (1 mM), as the source of nitrogen, at $25 \pm 1^\circ\text{C}$ in a culture from. The cultures were illuminated with cool white fluorescent light (approx 2200 lux) for 14 h photoperiod.

The mid-log cultures were invariably used for each experiment.

Growth of the cyanobacterium was estimated by measuring the changes in the absorbance in Bausch and Lomb spectronic 20 spectrophotometer at 665 nm or by monitoring changes in the total dry mass, after 12 days. The cyanobacterial samples were centrifuged, washed and pellets were transferred to pre-weighed stainless steel planchet for dry weight determination. The samples were heated at 80°C for dryness until a constant weight.

Centrifuged and washed pellets of the cyanobacterial samples were used to determine the various biomolecules. Chlorophyll *a* was extracted in acetone (80%v/v) at 4°C for 14 h in dark and estimated by measuring the optical density at 665nm. It was quantified by using the extinction coefficient 82.04 (Myers and Kratz, 1955). The phycobillin content was determined by using an extinction coefficients of 7.5 (Brody and Brody, 1961) at 625 nm of water soluble pigment extracted by repeated freezing and thawing. Total protein was estimated by using Folin-phenol reagent (Herbert *et al.*, 1971) RNA was determined by orcinol reagent method and DNA by diphenylamine method (Herbert *et al.*, 1971)

The herbicide 2, 4-D was dissolved in the sterile medium. The required concentrations (100 to 1600 µg ml⁻¹) were prepared by diluting the filter-sterilized (Millipore filter size 0.22µm) stock solution and added to the culture medium after inoculation with the cyanobacterium.

RESULTS AND DISCUSSION

Growth of *Aphanothece* sp. in the basal medium (C+NO₃) with or without 2, 4-D, was compared. Cultures exhibited essentially similar patterns of growth upto 400 µg ml⁻¹ with enhanced growth yield and morphologically healthy cells at 100 µg ml⁻¹. There was a 2 to 4 days lag in the initiation of exponential growth with 2, 4-D except 100 µg ml⁻¹ of 2, 4-D. Subsequent increase of the herbicide concentrations inhibited growth and 1600 µg ml⁻¹ proved to be lethal as evident by the loss of turbidity and the absence of the cells in the

microscopic observations. The reliability of the toxic effect was also confirmed by plating the flask contents, after centrifugation and washing, on herbicide free nutrient agar. The cells could not revive even after incubation of 20 days. The final growth of the cyanobacterium (dry mass mg ml⁻¹) after 12 days, followed similar pattern of the growth as in liquid medium. The sublethal concentration (1200 µg ml⁻¹) of 2, 4-D, allowed multiplication of a fraction (frequency approx. 10⁻⁷) of the algal population with filamentous aseptate structure. The abnormalities became observable after 20 to 24 days of the growth of the cyanobacterium in the 2, 4-D medium. Such abnormalities could not be inherited when these abnormally differentiated cultures were grown in the medium free of 2, 4-D where as in about 5-6 days, the cells regained the normal course of unicellular pattern of differentiation.

The cell constituents nucleic acids (DNA, RNA), protein, Chl.*a* and Phycocyanin of the cyanobacterium during active phase with or without 2, 4-D after 72h of treatment, are presented in Table 1. Total protein and RNA, declined from 193.3±4.93 to 157.9±4.47 µg mg⁻¹ dry mass and 4.33±0.72 to 2.50±0.58 µg mg⁻¹ dry mass, respectively at 1200 µg ml⁻¹ of 2, 4-d while stimulated upto 400 µg ml⁻¹ (194.6±1.41 and 5.35±1.03 µg ml⁻¹ dry mass respectively) although 100 µg ml⁻¹ supported more than control (Table 1). No effect on DNA content was observed at growth stimulatory concentration (100 µg ml⁻¹) while reduction in DNA was observed from 0.625 0.04 µg mg⁻¹ dry mass to 0.262±0.02 µg mg⁻¹ dry mass of the cyanobacterium at 1200 µg ml⁻¹. Control cells exhibited normal pigment composition while 2, 4-D treated cultures showed more inhibition of Chlorophyll *a* than phycocyanin. Chlorophyll *a* showed a marked decrease from 4.66±0.35 to 1.97±0.02 ug mg⁻¹ drymass. On the other hand phycocyanin content was increased from 10.55±0.27 to 11.00±.20 µg mg⁻¹ dry mass at 100 µg ml⁻¹ and finally decreased to 6.97±0.08 µg mg⁻¹ dry mass at 1200 µg ml⁻¹. The ratio of phycocyanin/ Chlorophyll *a* was increased with increasing concentrations of 2, 4-D and the values ranged from 2.25±0.65 to 3.53±0.65 also suggesting the inhibition of chlorophyll *a* synthesis in 2, 4-D medium. The altered pigment composition may interfere in the energy generation and CO₂ fixation which could affect the synthesis of other cellular constituents.

2, 4-D is commonly used as herbicide in agriculture to control the weed and the effect is more on aerobic micro organisms (Kligman, 1961). The results demonstrate that the test cyanobacterium tolerates much higher concentrations of 2, 4-D, many times higher than the recommended dose (1 to 40 ppm by weight basis) for field application (Klingman, 1961). Lower concentrations (100 mg/ml⁻¹) stimulated growth and cell multiplication of the cyanobacterium suggesting for growth stimulatory action of 2, 4-D. The chlorinated phenoxy-acetic acid(2, 4-D) acts in plant as analogs of auxin, indolyl acetic acid, a natural growth promoting substance. Therefore, 2, 4-D can not be regraded as growth inhibitor. The filamentous growth of the alga seems to be physiologically induced by higher concentrations of 2, 4-D and provides evidence to support the hypothesis that higher dosages of such growth regulatory herbicides causes abnormal cell division and growth in higher plants (Handon and Slife, 1969). Reduction in protein content (Table 1) and coenocytic filament formation is also suggesting it possible involvement with protein synthesis at higher concentrations as reported by Wolk (1973) that the chemicals known to affect protein synthesis, causes filament formation in unicellular algae. A positive correlation was found between RNA and protein content upto 400 µg ml⁻¹ while DNA was reduced with increasing concentrations of 2, 4-D Table 1.

The reduction in DNA content might be due to depression of certain DNA parts by combination of 2, 4-D as observed in higher plants (Seiler, 1978). The lowering in protein content at higher concentrations has already been attributed to possible utilization of phycocyanin by cyanobacterial cells in presence of 2, 4-D (Table 1). A positive correlation between phycocyanin, Chlorophyll a ratio is suggesting that the reduction in photoautotrophic growth of the cyanobacterium is due to inhibition of CO₂ fixation, not because of nitrogen, since phycocyanin serves as readymade source of fixed nitrogen (Allen and Arnold, 1969). The increasing ration of phycocyanin and Chlorophyll a is also suggesting that Chlorophyll a is target of 2, 4-D action as reported earlier in other cyanobacteria and green algae (Pandey and Srivastava, 1984, Bertagnolli and Nada Kavukaran, 1974).

Table 1: Effect of 2, 4-D* on the cell constituents of *Aphanathece* sp. The values are an averages of six experiments with their respective confident limits (at P=.99)**

2, 4-D(µg ml ⁻¹)	DNA	RNA	Protein	Chl.a(665)	Phycocyanin	Phyco./Chl.a
Control	0.625±0.04	4.33±0.72	193.3±4.93	4.66±0.35	10.55±0.27	2.25±0.52
100	0.625±0.12	6.14±0.77	196.3±0.39	4.00±0.20	11.00±0.20	2.75±0.82
400	0.429±0.10	5.35±1.03	194.6±1.41	3.83±0.07	11.96±0.08	3.12±0.06
800	0.410±0.07	3.45±0.07	189.4±3.83	2.42±0.14	9.24±0.23	3.81±0.45
1200	0.262±0.02	2.50±0.58	157.9±4.74	1.97±0.02	6.97±0.08	3.53±1.65
1600***						

* After 72h treatment.

**Cell constituents presented in ug. mg-1 drymass.

***No growth (lethal concentration)

The inhibition of photosynthetic pigment (Chl.a) may limit the carbon demands of the cyanobacterium for respiratory energy, ATP and reducing power NADPH which ultimately may lead to phytotoxicity as reported in other photosynthetic systems (Moreland, 1980). Interference with production of energy

required to drive the biosynthetic reaction would inhibit the protein and nucleic acids which ultimately limit the algal growth. Therefore, inhibition in the algal growth may ascribed their primary effect on photosynthesis which will cause several secondary effects as proposed by Moreland, 1980.

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