Biodetoxification of 2, 4-Dichlorophenoxyacetic acid
by a Cyanobacterium Synechococcus aeruginosus Nageli

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Abstract

The O2 evolving photosynthetic Cyanobacteria are vastly distributed in the tropical rice-fields of various countries and are important to maintain the soil fertility through elemental nitrogen fixation. Various herbicides which are being applied in modern agriculture practices to eradicate the target organisms (i.e.) weeds, but also simultaneously affect the non-target beneficial Cyanobacteria. 2, 4-D is a strong selective herbicide and generally used to control many broad leaf weeds in rice-fields. Since, the unicellular blue-green alga Synechococcus aeruginosus grow abundantly in rice-fields of Andhra Pradesh in India were chosen to observe the biodetoxification efficiency in vitro of herbicide 2,4-D in this study. The results suggest that the Synechococcus aeruginosus showed a remarkable tolerance to the herbicide up to lethal doses (1400 µg/ml) and showed growth and survival. However, growth was retarded at super lethal dose (2000 µg/ml). It can be inferred that the herbicide 2, 4-D could be detoxified by Synechococcus aeruginosus even at lethal doses.

Keywords: biodetoxification; Cyanobacteria; 2, 4-Dichlorophenoxyacetic acid

Introduction

Several pesticides have been employed by the farmers in order to control the pests in rice-fields in the modern practices of agriculture. Excessive application of the pesticides and herbicides not only cause pollution but also affect the metabolism of the microorganisms and in addition the pesticides and herbicides are also detoxified by the microorganisms such as blue-green algae and may serve as co-metabolite (Giuseppe Forlani et al., 2008; Sanaa A Ibrahim et al., 2009). Reports on the mechanism of biodetoxification of pesticides by algae are seldom, though the pesticide effects on growth and nitrogen fixation have been recorded (Das and Singh, 1977b; Valentine, 1973). Different experimental studies were conducted on the tolerance and growth of blue-green algae to pesticides and herbicides in the laboratory (Hale and Aslim, 2013). Valentine (1973) reported the detoxification of 2, 4-D by the algae and hexachlorocyclohexane by blue-green algae Anabaena raciborskii and Anabaena aphanizomenoides (Das and Singh, 1977a) carbofuran and hexachlorocyclohexane (BHC) by Nostoc muscorum and Wollea bharadwajae (Kar and Singh, 1979). The role of nitrogen fixing blue-green algae in the rice-fields has been well documented in augmenting the soil fertility and crop productivity.
Recently farmers have been introducing the blue-green algae as a biofertilizer in the rice-fields of India and other tropical countries. Among the pesticides, 2, 4-D is a strong selective herbicide and generally used to control many broad leaf weeds in agricultural fields (Brian, 1964).

Since the pesticides are often vastly applied by farmers indiscriminately in agriculture, an extensive study on pesticide biodetoxification by algae in general is essential from ecological and physiological point of view. A study was initiated to observe the growth response of *Synechococcus aeruginosus* to 2, 4-D to evaluate the ability of algae to remove 2, 4-Dichlorophenoxyacetic acid from rice-fields under a variety of environmental conditions and to determine the stability of 2, 4-D in an algae-laden environment. Hence, the present study deals with the study of biodetoxification of 2, 4-dichlorophenoxyacetic acid by unicellular cyanobacterium, *Synechococcus aeruginosus*. This study is also expected to give an account of ecophysiological aspects of algae and suggests to employ specific algae to combat the pesticide pollution.

**Materials and Methods**

The blue-green algae *Synechococcus aeruginosus* chosen for the present study was isolated from rice-fields of Andhra Pradesh. Isolation and purification were performed by the usual protocols of dilution and plating. The blue-green alga, *Synechococcus aeruginosus* was grown in Chu. No. 10 medium as modified by Gerloff et al. (1950). In this study, the detoxifying capacity of the blue-green algae against 2, 4-D toxicity even at sub-lethal, lethal and super lethal doses were observed and the act of detoxification was expressed in enhancement of growth in terms of chlorophyll-a and proteins and cell number. The various doses of 2, 4-D were chosen that arbitrarily in this study based on the growth rate experiments.

The cooled, sterilized growth media containing different doses of 2, 4-D (900, 1200, 1400, 2000 µg/ml) were inoculated with equal number of vegetative cells (50x10^4) of *Synechococcus aeruginosus*. Growth was observed up to 30 days of life cycle period. After every 5 days, the algae were removed by centrifugation and fresh inoculum of the same strength and age algal cells were inoculated aseptically in the sterile spent medium which was incubated under growth conditions and growth was recorded in terms of chlorophyll-a as per the formulae of Mac Lachlan and Zalik (1963) and proteins by the method of Lowry et al. (1951). Growth in terms of cell number was estimated with Neubauer’s Haemocytometer.

**Results**

The detoxification of lethal and super lethal doses of 2, 4-D was observed by inoculating the equal number of same age algal cells of *Synechococcus aeruginosus* in liquid basal media. The growth measured on every 5th day up to 30 days was varied with algal culture incubation period and the concentrations of 2, 4-D (i.e.) 900, 1200, 1400 and 2000 µg/ml). Table 1 shows the detoxifying capacity of *Synechococcus aeruginosus* against the different concentrations of 2, 4-D. The blue-green algae *Synechococcus aeruginosus* showed better growth at 900 µg/ml of 2, 4-D concentration on every subsequent inoculation of algae and as a consequence, the growth rate in terms of chlorophyll-a, proteins and cell number enhanced. At lethal dose (1200 µg/ml) and super lethal doses (1400 and 2000 µg/ml), the growth of the algae was completely suppressed during the first
inoculation period (5 days) without enhancement of chlorophyll-a pigment and proteins (5 days period). The concentration 900 µg/ml 2, 4-D proved as algistatic since there was a growth of algae whereas the other concentrations (1200, 1400, 2000 µg/ml) completely inhibited the growth of algae and were proved as algicidal. After second subsequent algal inoculation, the growth of the algae was observed on the next 5 days period (10th day) in terms of chlorophyll-a, protein and cell number in the medium containing lethal dose of 2, 4-D (1200 µg/ml), whereas at super lethal concentration (1400 µg/ml) of 2, 4-D, the growth of the Synechococcus aeruginosus appeared only after the third subsequent algal inoculation i.e. after 15 days duration. There was no growth of the algae even at 2000 µg 2, 4-D per ml containing basal medium after every subsequent inoculation observed up to 30 days and proved as algicidal.

Discussion

Indiscriminate application of pesticides in rice-fields resulted in disturbance of ecosystem where some of the pesticides persist and finally bioaccumulated in microorganisms such as blue-green algae (Alexander, 1972) and the blue-green algae are prone to become resistant to herbicides at higher concentrations (Hale and Belma, 2013). The herbicide resistant strains of algae inhabiting in natural ecosystem and rice fields may play an important role in detoxifying the pesticides pollution. Tiwari and Pandey (1981) reported the 2, 4-D resistant mutant strains and abnormal cells of Anacystis nidulans which were induced by higher concentration (2 µg/ml) of 2, 4-D. Surendra Singh et al. (2012) isolated the multiple herbicide resistant strain [Av(MHR)]½ of Anabaena variabilis against four rice field herbicides viz., Arozin, Alachlor, Butachlor and 2, 4-D.

The present results showed that Synechococcus aeruginosus proved to be effective detoxifier of 2, 4-D and was capable of removing the herbicide from the medium as evidenced from the enhancement of algal growth and growth rate (Table 1). Therefore the present results suggest that the increase in growth of Synechococcus aeruginosus even in the lethal doses of 2, 4-D (1200 and 1400 µg/ml) with repeated inoculation and removal of these algae ensure gradual detoxification of 2,4-D. While growth in terms of chlorophyll-a, protein and cell number were not recorded in 2000 µg/ ml cultures up to 25 days. However, the exact site and mechanism of degradation and detoxification of 2, 4-D is not understandable with the present results. Probably, the uptake of 2, 4-D in Synechococcus aeruginosus would have been taken up through adsorption, absorption and translocated through cell permeable membrane to the membranes of thylakoids where the herbicide must have been accumulated in these algae as suggested in Chlorella pyrenoidosa (Bertagnolli and Nadakavukaren, 1974) and thereby inhibited the photosynthetic pigment synthesis as evidenced in the reduction of chlorophyll-a quantity and affect the other primary metabolic products such as proteins as evidenced in table 1, as a consequence of cause and effect, finally the growth of the algae was inhibited as evidenced in super lethal doses of 2, 4-D(2000 µg per ml) in these algae. During detoxification the metabolites were increased in these algae, which suggests that the absorbed 2, 4-D might have been steiochemically changed and utilized as nutrients for synthesis of cellular metabolites (Table 1).
Table 1. Detoxification of 2, 4-D by Synechococcus aeruginosus

<table>
<thead>
<tr>
<th>Concentration of 2, 4-D (µg/ml)</th>
<th>5th Day</th>
<th>10th Day</th>
<th>15th Day</th>
<th>20th Day</th>
<th>25th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth of the algae</td>
<td>Chl-a mg/g</td>
<td>No. of cells ml/10^4</td>
<td>Prot mg/100 mg.f.w.</td>
<td>Chl-a mg/g</td>
</tr>
<tr>
<td>Control</td>
<td>Growth of the algae</td>
<td>12.5</td>
<td>0.218</td>
<td>16.8</td>
<td>14.3</td>
</tr>
<tr>
<td>900</td>
<td>-</td>
<td>-</td>
<td>6.2</td>
<td>0.058</td>
<td>6.3</td>
</tr>
<tr>
<td>1200</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.1</td>
</tr>
<tr>
<td>1400</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Alternatively, the adsorbed and absorbed herbicide 2, 4-D might be conjugated with amino acids of extracellular products of these algae at the permeable membrane as suggested by Feung et al. (1974) and subsequently the complex structure utilized as carbon and nitrogen sources as suggested by Tiwari and Pandey (1981) in growth of the algae or the extracellular substances in the medium antagonize the toxicity of 2, 4-D by forming complex, and as a result the permeability of cell wall may be changed (Rivere and Pennera, 1979) and the availability of quantity of 2, 4-D would be reduced as a result the cometabant 2, 4-D quantity was recorded in the medium which leads to enhancement of the growth and other primary metabolites of these algae that might be occurring either in rice-fields or ponds containing high quantity of pesticides. Similarly if the process takes place in natural ecosystem the algae create better environment for their better survival in the pesticide free environment. These findings suggest that these microorganisms progressively detoxify the herbicides in the medium by adsorption, absorption and accumulation inside the organisms.

**Conclusion**

Blue-green algae have a certain ability to bioaccumulate, to immobilize, to sequester and to biotransform different herbicides and related organic compounds, thus contributing to a substantial remediation of the aquatic habitats polluted with xenobiotics (Chong et al., 2000). The present study suggests that, blue-green algal biomass could be used to reduce the pesticide pollutants in waters, either in natural ponds or rice fields since these algae have the capacity to uptake the 2, 4-D by adsorption and absorption and also utilized as co-metabolites for promoting the growth of algae which is an indication of herbicide detoxification in the environment.

**References**


Singh RN (1961). The role of blue-green algae in nitrogen economy of Indian Agriculture. Indian Council of Agricultural Research, New Delhi, India. pp 1-175.


