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Enhancement of *Spirulina* Biomass Productivity by a Protein Hydrolysate

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ABSTRACT

The effect of a hydrolyzed protein preparation on the growth and biomass yield of the blue-gree alga *Spirulina platensis* was studied. At the optimum dosage, the hydrolysate enhanced *Spirulina* productivity by almost 40% when compared to untreated cultures. This increased productivity correlated with an increase in the level of nitrogen assimilating enzymes, nitrate reductase and glutamine synthetase.

Index Entries: *Spirulina;* biomass; nitrate reductase; glutamine synthetase.

INTRODUCTION

The cyanobacterium *Spirulina* is a rich source of proteins, water soluble vitamins, β-carotene, and polyunsaturated fatty acids, and hence is identified by the United Nations Organization as a "wonderful food" (*1,2*). To realize its full potential as a nutritional supplement, efforts are under progress to mass produce *Spirulina* by intensive growth in artificial ponds (*3,4*). Mass production of *Spirulina* attempts to maximize conversion of solar radiant energy and inorganic chemicals into biomass. The huge gap between the theoretical possibility for conversion of the resources into

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biomass (*5*) and the practical realities achieved thus far in both experimental and commercial ponds, indicate the lacuna in our understanding the bottlenecks of this conversion process. As a result, the cost of *Spirulina* is high and its nutritional potential remains underutilized (*6*).

With the high demand placed on agricultural productivity, and the concomitant increase in our understanding of plant physiology, several performance chemicals to modulate plant productivity have evolved (*7*). Since there are a lot of biochemical similarities between cyanobacteria and higher plants, we explored the possibility of stimulating the growth of *Spirulina* with a cereal protein hydrolysate that was reported to enhance the productivity of several crops (*8*).

MATERIALS AND METHODS

Materials

Spirulina platensis, Sp6, was obtained from Ballarpur Industries Limited (New Delhi). Reagent chemicals used for analyses were from SD's Chemicals Limited (Bombay).

Protein Hydrolysate

Cereal protein was isolated by wet milling (*9*) and hydrolyzed. The composition of the hydrolysate was 50% total solids, 3.8% alpha-amino nitrogen, 5.3% total nitrogen, 24.0% minerals, pH 4.9 (*8*).

Spirulina Growth Conditions

Sp6 was cultivated in glass trays (26 × 16 × 5 cm) in an optimized growth medium (*10*). The culture was adjusted to 0.7 OD at 560 nm, incubated in a temperature (30°C) controlled green house, illuminated by sunlight and agitated periodically. The biomass was diluted every fourth day by adding fresh medium (containing the appropriate amount of protein hydrolyzate) to maintain *Spirulina* culture density at 0.7 OD.

Analytical Procedures

Daily growth was monitored by measuring the optical density (OD) of the cultures. Consumption of inorganic nutrients (nitrate and phosphate) in the culture filtrates were assayed according to APHA methods (*11*).

Biomass was harvested by filtration through polyester-cotton (80:20) cloth and thoroughly washed with demineralized water to remove inorganic nutrients. Chlorophyll, nitrate reductase, and glutamine synthetase assays of the washed biomass were performed according to procedures described by McKinney (*12*), Herrero et al. (*13*), and Shapiro and Stadman (*14*), respectively.

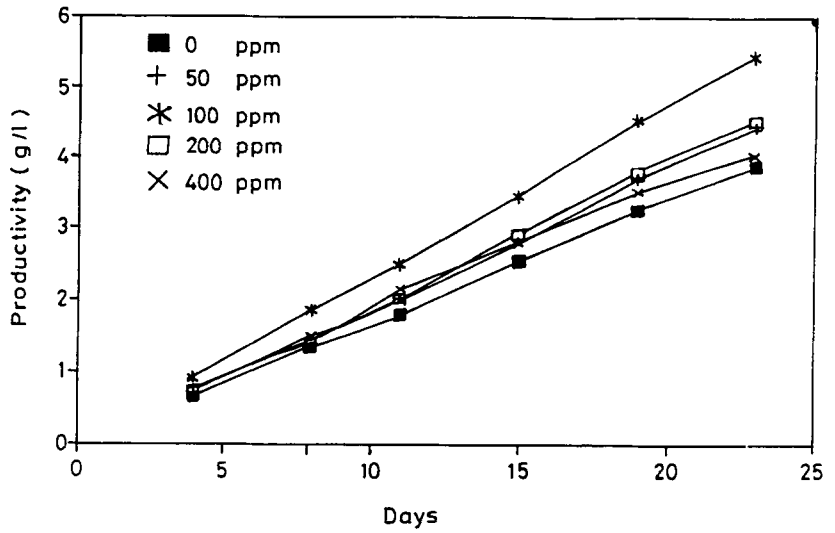

Fig. 1. *Spirulina* biomass productivity in protein hydrolysate treated cultures. *Spirulina* cultures were supplemented with the indicated amount of the protein hydrolyzate (v/v). For recovery of biomass, the culture ODs were adjusted to 0.7 with fresh medium and the biomass harvested from the additional volume. Total biomass harvested from the different cultures are plotted against days in culture.

RESULTS AND DISCUSSION

Effect of Protein Hydrolyzate on *Spirulina* Growth

The cereal protein hydrolyzate was added to the growth medium at varying concentrations, and its effect on the growth and biomass productivity of *Spirulina* was monitored. Results from one representative experiment are shown in Fig. 1. Under the conditions of experimentation, the growth of *Spirulina* was maximum in medium supplemented with 100 ppm protein hydrolysate. This resulted in an increase in biomass productivity by about 40%. The increasing difference in biomass productivity between treated and untreated culture indicated that the growth stimulating effect of the hydrolysate was consistent during the period of study and did not show signs of saturation or refractoriness during the growth phase studied. However, at 50 ppm level there was an initial lag period before a marginal increase in the biomass productivity became apparent. Similarily, at 200 ppm, there was only a marginal increase in the biomass yield, whereas in 400 ppm of the hydrolysate, the biomass yield was no better than the untreated culture, suggesting an adverse effect at this high concentration on the growth of *Spirulina*. Thus, the protein hydrolysate stimulated *Spirulina* growth only within a narrow range of concentration.

Table 1
Nitrogen Assimilating Enzyme Levels in Treated *Spirulina*[a]

Enzyme	Protein hydrolysate, ppm				
	0	50	100	200	400
Nitrate reductase	711 ± 11	750 ± 27	797 ± 20	745 ± 16	720 ± 16
Glutamine synthetase	34.4 ± 0.6	37.5 ± 0.8	39.2 ± 0.3	37.0 ± 0.6	35.3 ± 0.5

[a]Samples were collected every fourth day, for 24 d. Enzyme activities are presented as an average of six samples ± standard deviation, nitrate reductase in *n* mol/mg biomass/min, glutamine synthetase in μmol/mg biomass/min.

The cultures used in these experiments were not axenic, and hence the possibility of contaminants owing to the addition of protein hydrolysate overwhelming *Spirulina* growth was watched during the course of the study. It was observed that, at the concentrations of the hydrolysate used and the duration of the experiments (3–6 wk), the cultures did not indicate any sign of deterioration of *Spirulina* or overgrowth of any other organism.

Nitrate Reductase and Glutamine Synthetase Levels

To gain a better understanding of the principles involved in the increase of *Spirulina* growth and biomass productivity, levels of two enzymes involved in nitrogen metabolism were analyzed (Table 1). Both nitrate reductase and glutamine synthetase activities, measured over a period of 4 wk, were higher in *Spirulina* cells grown in the presence of protein hydrolysate at 50, 100, or 200 ppm. The samples of biomass were obtained from cultures over a period of 24 d, thus representing cells at different stages of growth. The increased enzyme levels in these samples suggest that the increase in enzyme levels was inherent in the samples and was not owing to cells representing different phases of growth. However, *Spirulina* grown in the presence of 400 ppm hydrolysate expressed these enzymes at the same level as cells from untreated culture. Thus, there was a good correlation between the increased level of these enzymes and the biomass increase, suggesting that stimulation of the nitrogen assimilation pathway alleviated some restrictions in the conversion of inorganic nutrients to biomass. These observations further suggest that the amino acids, although are not directly involved in the reaction catalyzed by nitrate reductase, might have some hormone-like activity in inducing cellular metabolic pathways.

Table 2
Reduction in Nutrient Levels[a]

	Protein hydrolysate, ppm				
Nutrient	0	50	100	200	400
Nitrate	62 ± 21	102 ± 33	163 ± 46	102 ± 28	62 ± 20
Phosphate	20 ± 0	45 ± 14	52 ± 12	32 ± 7	27 ± 5

[a]Reduction in nitrate and phosphate concentrations in culture filtrates are expressed as mg/L ± standard deviation.

Nutrient Utilization

To evaluate the efficiency of conversion of nutrients into biomass, consumption of nitrate and phosphate were monitored in culture filtrates (Table 2). In the presence of the protein hydrolysate at 50, 100, and 200 ppm, the nitrate levels were substantially reduced compared to untreated or 400 ppm hydrolysate treated culture filtrates. This is perhaps reflective of the increased level of nitrogen assimilating enzymes in these treated cells. The phosphate levels in the culture filtrates showed a similar pattern as nitrate levels. This suggested an increased utilization of these nutrients. However, this higher consumption rate was not commensurate with the observed increase in biomass.

Cereal protein hydrolysate has been reported to increase the yield of wheat and moong-bean (green gram) by 20–30% (*7*). its mechanism of action on these plants has not been clearly elucidated, although some increase in chlorophyll content was reported. However, addition of the hydrolysate did not affect the chlorophyll content of *Spirulina* (data not shown). In general, cyanobacteria are capable of utilizing inorganic nitrogen and may not be expected to respond to complex nitrogen. However, the data presented in this study support the idea that amino acids in some form may stimulate the growth of not only higher plants but also the growth of the cyanobacterium *Spirulina*. This suggests that the hydrolysate may modulate some intrinsic process common to both these groups of organisms.

Some amino acids are known to be plant morphogens, indicating that they stimulate some specific differentiation pathways in plants. In the context of cyanobacteria, although the concept of morphogen is invalid, the amino acids may function as inducers of certain other metabolic pathways that might lead to altered growth characteristics. The present studies suggest the possibility of using *Spirulina* for the evaluation of plant growth stimulatory/regulatory substances. In conclusion, the protein

hydrolysate may find application in improving the growth of the nutritive cyanobacterium *Spirulina*, and it would be worthwhile to explore the feasibility of its application in improving *Spirulina* production in outdoor ponds.

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