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Alterations in Protein Synthesis in the Cyanobacterium Synechococcus sp. Strain PCC 6301 in Response to Calendula Micrantha Extract with the Molluscicidal Activity.

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خلاصة

تم في هذا البحث اختبار استجابة الطحلب الأخضر المزرق وحيد الخلية «سينيكوكوكس» للمعاملة بمستخلص النبات المصري كاليندولا ميكرانثا ذو الفاعلية ضد القواقع.

وأثبتت النتائج أن للتركيزات المنخفضة من مستخلص النبات تأثيرا طفيفا على النمو ومحتوى كلورفيل "a" في الخلية. بينما أوضح معدل تخليق البروتينات انخفاضاً تدريجياً عند زيادة تركيزات مستخلص النبات. وكان للمعالجة تأثيراً محفزاً للتخليق التفصيلي لعدد محدود من البروتينات (polypeptides). وعند حساب حركة هذه البروتينات بعملية الفصل الكهربائي (Gel electrophoresis)، أوضحت النتائج أن الوزن الجزيئي لهذه البروتينات 161، 96,7، 93,4، 85، 69,9، 59، 49، 45، 35، 32,4، 28، 24، 21,7، 18، 16 كيلو دالتون.

وعلى ذلك فإن النتائج تشير إلى أن مستخلص النبات له تأثير واضح في تحفيز التعديلات الناتجة في نمط تخليق البروتينات في السنيكوكوكس. وبالإضافة إلى ذلك فإن بعض هذه البروتينات المحفزة تشبه مثيلاتها التي تنتج عن التعرض لمؤثرات أخرى خاصة الإجهاد الحراري.

Abstract

The response to the extract of the Egyptian wild herb **Calendula micrantha,** with the molluscicidal activity, was examined in the unicellu-

lar nobacterium Synechococcus sp. strain PCC 6301. Growth and chlorophyll a of the cells were only slightly affected by low plant extract concentrations but were drastically reduced by high concentration. The rate of protein synthesis progressively decreased by increasing extract concentration. The cells preferntially induced the synthesis of a limited number of polypeptides in response to the treatment. Among the induced polypeptides were those with apparent molecular weights of 161K (161.000), 96.7K, 93.4K, 85K, 69.9K, 59K, 49K, 45K, 35K, 32.4K, 28K, 24K, 21.7K, 18K and 16K based on their mobilities in gel electrophoresis. These intial studies suggest that the plant extract exerted certain stress which stimulated alteration in the pattern of protein synthesis in synechococcus sp. Some of the induced polypeptides are similar to that known to occur in other stresses specially heat shock stress.

Introduction

The ecological impact of synthetic molluscicides have urged investigation on plant molluscicides. Several naturally occurring plants were known since ancient time as anthelmintics, however, only few examples of practical application exist (McCullough et al., 1980, WHO, 1981).

The water extract of a wild Egyptian herb Calendula micrantha officinalis was used as an anthelmintic against infective larvae and as molluscicide against **Biomphiaria alexandrina and Bulinus truncatus snails** (intermediate host of Schistosomiasis) (Lemma, 1970, Hassanain et al., 1991). They reported the importance of the dry powder of Calendula micrantha and recommended the use of this plant as molluscicide in large scale in Egypt. This called investigations on its impact on the surrounding environment and its mode of action.

Cyanobacteria are oxygen evolving photosynthesizing procaryotic organisms and are strongly influenced by their nutritional, chemical and physical environments. They adjust their growth to alterations in these conditions in their habitat (Harder & Dijkhuizen, 1983, Rozak & Colwell, 1987). Organisms respond to various stresses (heat shock, toxic chemicals), energy starvation and nutritional deprivation, by a decrease in their growth rate, and induction of stress proteins (Selye, 1956, Borbely et al., 1985, 1990, Suranyi et al., 1987). When cells are

subjected to deleterious environmental changes or stresses they exhibit changes in the pattern of protein synthesis.

Thus, the use of cyanobacterial system may be a valuable experimental approach for analysis of the potential differential effects of various tested Calendula mirantha concentrations, as environmental change of potential stress effect.

Synechcoccus sp. strain PCC 6301 is an obligate photoautotrophic cyanobacterium and would appear to have a good system for detailed studies of the possible physiological responses in these organisms to Calendula micrantlla as a potential natural molluscicide.

Materials and methods

Plant water extract

The wild Egyptian herb Calendula micrantha officinalis, Family Compositae was shade dried, and finely powdered. A stock water extract solution was prepared by soacking the plant dry powder overnight at room temperature and sieving. The plant water extract was used at the concentrations in the range from 0.1 to 1.0 % by adding to the logarithmically growing cultures of Synechococus sp.

Cyanobacterial strain and its growth

Synechococcus sp. strain PCC 6301 (Anacystis nidulans, ATCC 27144) was grown in the liquid medium of Allen (Allen, 1968). Cultures were kept in glass vessels thermostatically maintained at 39°C and illuminated with warm white fluorescent light 3.6 x 10^4 mWm^{-2}). Aeration was achieved by bubbling with sterile air containing 2 - 3 % CO_2 (V/V). Growth of the cultures was monitored spectrophotometrically at 800 nm and by measurement of their chlorophyll a content in 90 % acetone extracts in ,µg per milliliter (Kallas & Castenholz, 1982).

Isotope labelling conditions

Logarithmically growing Synechococcus cultures were treated with plant water extract concentration from 0 to 1.0 % and 1ml aliquots of cell suspension were removed and pulse labelled at each concentration with 2.4 MBq of L - [^{35}S] methionine per ml for 1h.

Polyacrylamide gel electrophoresis of proteins

Pulse labelled cyanobacterial cells were chilled on ice, collected by centrifugation, and washed with water. The pellets were dissolved in the sample buffer described by Laemmli (1970). 5µl aliquots were removed to determine the rates of total protein synthesis during the treatment as described by Borbely *et al* (1985). Electrophoresis was performed on 10 to 18% linear sodium dodecyl sulfate (SDS) polyacrylamide gradient gels as described previously (Laemmli, 1970).

After electrophresis, the gels were processed for staining and fluorography, dried (gel slab drier model 224; Bio - Rad Laboratories, Richmond, Calif.), and exposed to X- ray films. The low-molecular-weight calibration kit of Pharmacia Fine Chemicals AB was used as molecular weight markers.

Results

Effect of Calendula micrantha extract on growth of the cyanobacterium Synechococcus sp. strain PCC 63Ol.

Log phase cultures of *Synechococcus sp.* were treated for 8 h . with *Calendula micrantha* plant water extract (PWE) at 0.1, 0.3, 0.5 and 1.0%. The rate of growth, as determined by optical density measurements was slightly affected by low concentrations (Fig.1 A). Treatment with higher concentration 1.0 % drastically inhibited growth specially by incubation for 8hr. A similar tendency was obtained by measuring the chlorophyll a (Chl a) content (Fig.1 B). When growth was monitored by measuring Chl a content there was a more obvious decrease in the growth rate after treatment with 1.0% extract. Growth was detected in cultures treated with 1.0% throughout the experiments although at a highly reduced rate.

Effect of PWE on protein synthesis

To study the effect of Calendula micrantha PWE on protein synthesis in treated cells, cells were pulse labelled with L- [^{35}S] methionine. The rate of protein synthesis in Synechococcus cells immediately decreased after treatment (Fig.2), and gradually reduced in response to increased PWE concentration. At concentrations of 0.7% and higher the rate of protein synthesis declined to a steady - state level (Fig. 2) reaching minimum value.

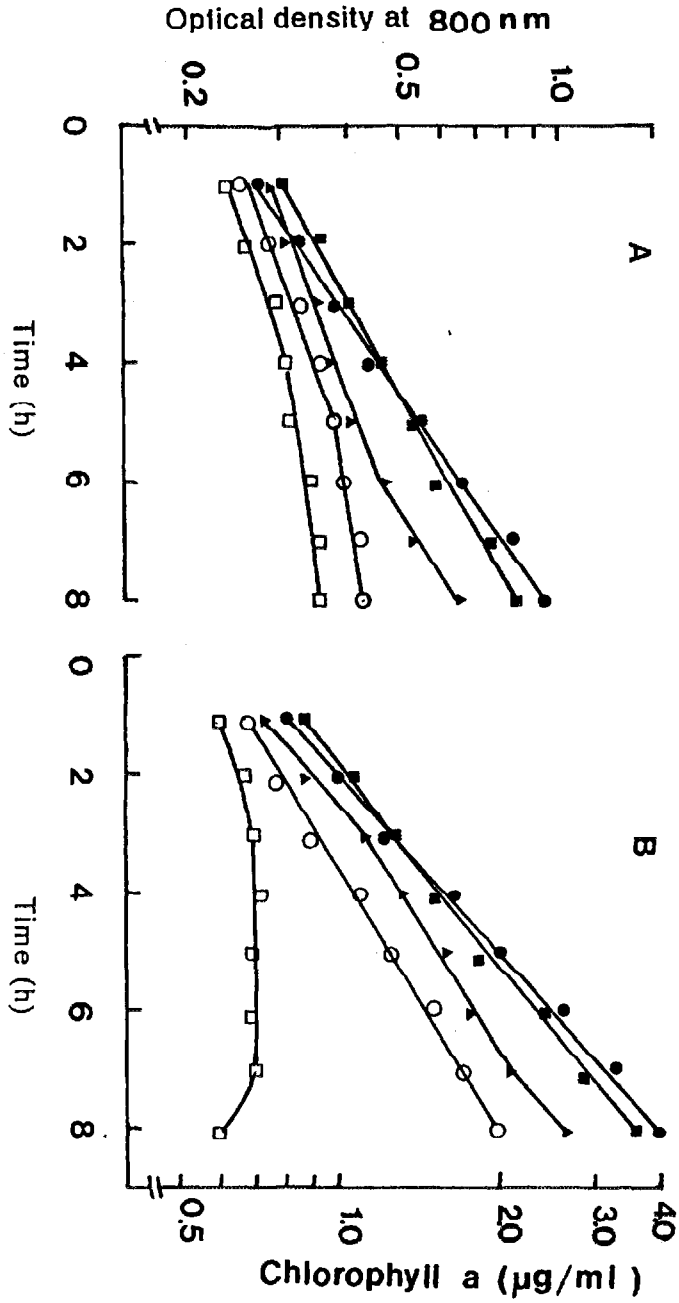

Fig. 1. Growth of Synechococcus sp. strain PCC 6301 in response to treatment with Calendula micrantha extract. Growth was monitored by measuring optical density at 800 nm (A) and chlorophyll a content ($\mu g.ml^{-1}$) (B), at o (●), 0.1 (■), 0.3 (▲), 0.5 (O) and 1.0% (❑) plant extract.

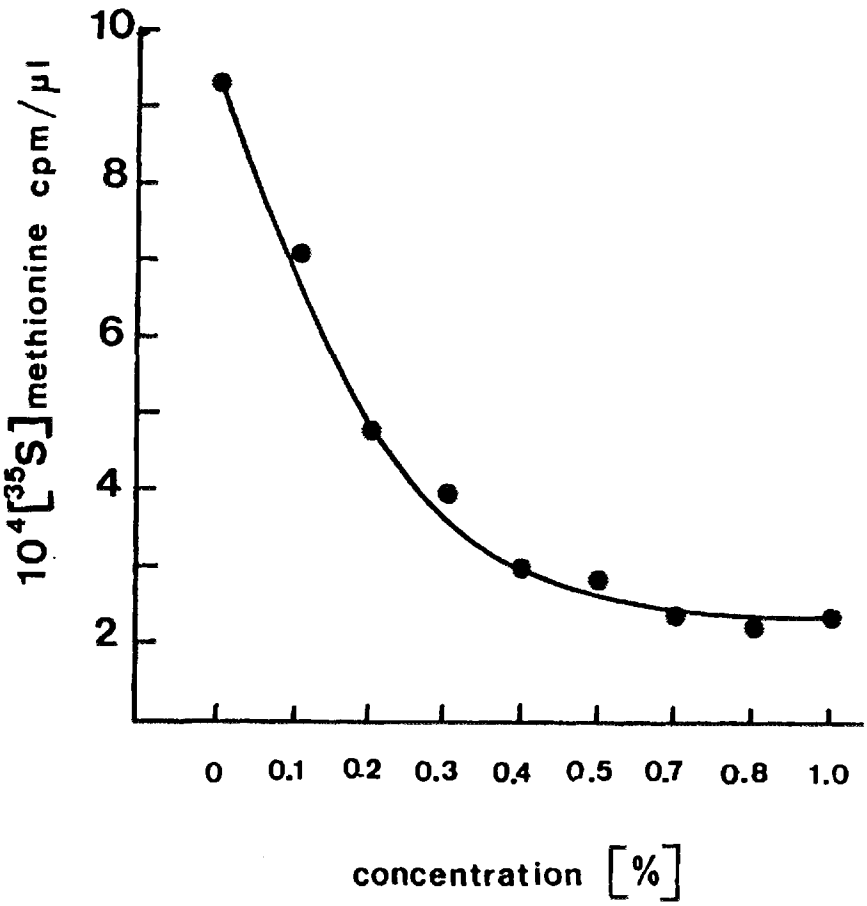

Fig. 2. Effect of Calendula micrantha extract on the rate of protein synthesis in Synechococcus sp. strain PCC 6301 cultures. Cells were grown in Allen medium containing different concentrations of the plant extract and labelled with L-(^{35}S) methionine. The rate of protein synthesis was determined as described in the text.

Effec of PWE on the pattern of protein synthesis.

Aliquots (1 ml) of Synechococcus were labelled for 1h with L-[^{35}S] methiomine at different PWE concentrations [0 - 1.0%]. Cell extracts were prepared from the labelled cells and analyzed by SDS -

polyacrylamide gel electrophoresis (Fig.3).Generally the synthesis of protein normally present in cells was reduced (Fig.3). A number of newly synthesized polypeptide bands may be seen in the extracts obtained from PWE treated cells when compared with the proteins synthesized in the untreated cultures. Some of these bands, can already be detected after exposure of cells to 0.1% extract, whereas their preferential synthesis progressively increased by increasing extract concentration.

The apparent molecular weights of the polypeptides synthesized under treatment are indicated in (Fig. 3). Three classes of proteins can be observed in the cyanobacterium;(1) the bulk of cellular proteins synthesized under normal conditions; (2) polypeptides synthesized under both control and treated cultures but at different rate;(3) proteins induced specifically under treatment. The molecular weights of these inducible polypeptide chains have been -estimated on the basis of their mobilities in SDS - polyacrylamide gels to be 161K (161.000), 96.7K, 93.4K, 85K, 69.9K,59K, 49K, 45K, 35K, 32.4K, 28K, 24K, 21.7K, 18K and 16K. Proteins synthesized under normal conditions (control) and progressively or completely disappeared specially by increasing treatment concentration; 103K, 80K, 54K, 52.5 - 50 K, 45.9K, 42.8K, 40K, 38K, 33.8K, 31.5K, 20.8K and 16.9 K.

Discussion

The cyanobacterium synechococcus sp. strain PCC 6301 is a single - celled photosynthetic procaryote which responds to a variety of physiological stesses by altering the pattern of growth and protein synthesis (Ashburner,1982; Borbely *et al.*, 1985; Webb *et al.*, 1990).

The application of total dry powder of Calendula micrantha proves an important economic value (Hassanain *et al.*, 1991). The plant water extract demonstrated anthelmintic activity with different efficiency with concentrations of 1 .0% and less (El-Emam et al., 1986)./ Generally, the above results indicate that the growth rate of Synechococcus sp. cells treated with PWE decreases. The increase in concentration from 0. 1 to 1.0% in logarithmically growing cultures is rapidly followed by changes in protein synthesis. At 1.0 % extract, protein synthesis is considerably reduced compared with untreated cultures. Furthermore, Synechococcus sp. respond to increased concentration of PWE by a transient induction of a specific set of polypeptides and raising their cellular level

markedly. Analysis of the detailed results indicates that treatment with concentrations higher than 0.1% increases the incorporation of label into these inducible proteins. Their apparent molecular weights estimated on the basis of their mobilities in SDS - polyacrylamide gels (97, 93.4, 85, 59, 49, 45, 32.4, 24, 18, 16K) falls into the range which is known to be charactaristic for heat shock (HS) stress (Borbely et al., 1985). They indicated the synthesis of 4 size classes (91,79 to 61,49 to 45 and 24 to 11 K) in Synechococcus sp. strain PCC 6301 under HS at 47°. A 65 K protein was synthesized both in light and in dark, where as the 11 K protein was only made when HS - cells were incubated in light.

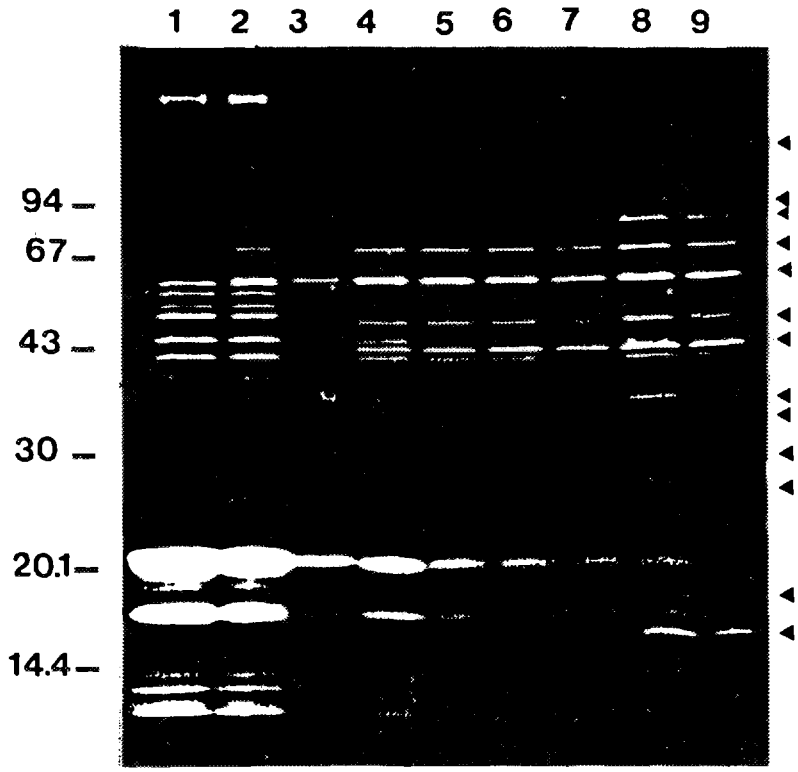

Fig.3. Effect of different concentrations of Calendula micrantha extract on polypeptide synthesis in Synechococcus sp. strain PCC 6301 cells. Log - phase cultures were labelled with L-(^{35}S) methionine for 1h, separated on a 10 to 18% linear gradient SDS - polyacrylamide gel and processed for fluorography as described in the text. The position of molecular weight markers (right) as well as of polypeptides induced by the treatment ( ) are indicated in kilodalton (K). Lane 1 (control) and lanes 2-9 are 0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 0.8 and 1.0% treatment, respectively.

In the filamentous cyanobacterium **Anabaena sp.** strain L - 31, HS induced the synthesis of proteins of 92,75, 65 and 32 K (Bhagwat & Apte, 1989). Polypeptide synthesis may, therefore, represent a part of the cellular adoptation mechanism for survival in cyanobacteria. They respond to various stresses by a decrease in their growth rate, an induction of stress proteins and a concomitant accumulation of guanosine tetraphosphate (Doolittle, 1979). It has been shown that heavy metal in stress and HS induce the synthesis of adenylated nucleotides, compounds which are suspected of being metabolic signals of various environmental stress conditions (Suranyi *et al.*, 1987, Palfi *et al* ., 1991).

El - Emam *et al.* (1980) reported that the active principle of Calendula micrantha is saponin triterpene which is easily soluble in water . They revealed the importance of the plant as a potential natural molluscicide in Egypt (Hassanain *et al.*, 1991). In view of the above results, different PWS concentrations had preferential effect on the obligate photoautotrophic cyanobacterium Syechococcus sp. strain 6301. Low concentrations slightly influenced growth and Chl a content whereas, high concentrations were inhibitory. Protein pattern was greatly altered with a progressive inhibitory effect upon increasing plant extract concentration. Several polypeptides were induced in response to the stress exerted by high concentration. The polypeplides found to be inducible may be the products of a cyanobacterial stress regulon (Borbely et al., 1990). Finally, the total constituents of Calendula micrantha extract and the separation of the active principle remains to be investigated.

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