THE RESPONSE OF A *CYLINDROSPERMUM* SPECIES TO DIFFERENT SOURCES OF NITROGEN

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***Cylindrospermum* sp. (IUCC 942) was grown for six weeks in a modified Bristol's solution that supplied N in different oxidation states and concentrations. The occurrence and position of heterocysts, the criteria used to identify this genus, were shown to be dependent on the source and concentration of N. Vegetative cell to heterocyst ratios increased as the N concentration increased. No heterocysts were formed in high concentrations of NH_4^+-N. Intercalary heterocysts were common in young cultures.**

C_2H_2 reduction was used to estimate the N_2-fixing capacity of the alga. Cells grown with NH_4^+-N at concentrations of 40 ppm and greater had no N_2-fixing activity, while cells with NO_3^- -N had activity even at 400 ppm.

The physiological and morphological responses to changes in N indicate the need for additional work on the taxonomy of the Nostocaceae.

The heterocyst, a specialized cell in the trichomes of the Nostocaceae, is distinguished from vegetative cells by its different size, shape, color, and function (1, 2). The position of the heterocyst in the trichome is of taxonomic importance at the generic level (3, 4, 5). The physiological function of the heterocyst has been debated extensively. Several major roles have been suggested. Among these are the regulation of cell division and growth of the trichome and N_2 fixation (1, 2, 6, 7).

The possibility for regulation of the activities of the trichome was supported by the demonstration of cytoplasmic connections between heterocysts and vegetative cells (8, 9). Wolk (10) reported a rather constant number of vegetative cells separating spores and suggested the presence of a gradient of some substance that somehow regulates the differentiation of vegetative cells.

The presence of nitrogenous compounds has been shown to affect the formation of heterocysts. *Anabaena cylindrica* had fewer heterocysts when grown on NO_3^--N or NH_4^+-N than when grown with only atmospheric N_2 (11, 12, 13). *Anabaena flos-aquae* A-37 responded in the same manner to NO_3^--N but in the presence of NH_4^+-N, heterocyst formation was completely inhibited (14). Likewise, *Cylindrospermum trichotosporum* grown on media with NH_4Cl or $(NH_4)_3PO_4$ at concentrations of 3mg/ml did not form heterocysts (15).

The isolation of mutant strains of *Cylindrospermum* that lack heterocysts has led to the identification of two categories of genetic regulation of heterocyst formation (16). The first category is an inducible-repressible system whereby combined N represses heterocyst formation. The other category is constitutive in that combined N does not affect the development of heterocysts, as in *C. majus* and *Anabaenopis raciborskii* (16).

Cylindrospermum sp. (IUCC 942) may be atypical for the genus in that it contains a high frequency of intercalary heterocysts in young cultures. In this paper we describe the pattern of formation of intercalary heterocysts and examine the effect of N supplied in different oxidation states and concentrations with respect to heterocyst formation and N_2 fixation.

MATERIALS AND METHODS

Cylindrospermum sp. (IUCC 942) was grown in soil-water medium and in Bristol's medium (17) modified to provide N in the following concentrations and oxidation states: atmospheric N_2; 40, 80, and 400 ppm NO_3^--N; 40, 80, and 400 ppm NO_3^--N; 40, 80, and 400 ppm NH_4^+-N; and 40, 80, and 400 ppm N as NH_4NO_3. The stock culture was examined for N_2-fixing contaminants.

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Aliquots of the stock culture were plated on N-free Bristol's, N-free Burk's minimal salts (18) and modified Line-Loutit (19) media and incubated in aerobic and anaerobic atmospheres. No growth, other than that of *Cylindrospermum* on Bristol's medium, was observed in any of the conditions.

One hundred ml of medium were placed in each of two 250-ml Erlenmeyer flasks per treatment. Each flask was inoculated with a 2-ml cell suspension from a stock culture that was previously conditioned to grow on N-free medium. The cultures were maintained on a 12-hour photoperiod at approximately 3000 lux illumination at 21 C. After 6 weeks a portion of the algal mass was removed from the medium with a wire transfer loop and placed on a slide for microscopic examination. Twenty-five trichomes on each slide which could be observed the entire length of the filament were selected for the counting of vegetative cells and heterocysts.

The N_2-fixing capacity of this *Cylindrospermum* species was examined also. Bristol's medium having NH_4^+-N at 0, 5, 10, 20, 40, 80, and 400 ppm and NO_3^--N at the same concentrations were used. Two 250-ml Erlenmeyer flasks containing 100 ml of medium received a 2-ml cell suspension as described earlier. After 1 week of growth the cells from each concentration were collected on a filter pad and returned to 5 ml of the medium for determination of N_2ase activity. The C_2H_2-reduction assay of Hardy *et al.* (20) as modified by Stutz and Bliss (21) was used to estimate the N_2-fixing capacity of the cells. The cells were incubated for 24 hr in 0.1 atmosphere C_2H_2. Sampling at various times indicated a linear response through 24 hours. C_2H_4 content was measured with a Becker 409 gas chromatograph equipped with a 3 mm × 3 m column containing Porapak N.

General observations on the morphology of our cultures in soil-water medium have been made over the past four years.

RESULTS AND DISCUSSION

The following features of the life cycle of *Cylindrospermum* sp. (IUCC 942) were observed in soil-water cultures. Perhaps unusual for *Cylindrospermum* is our observation that intercalary heterocysts are a regular feature of young cultures. The greatest development of intercalary heterocysts occurs in cultures which are about two weeks old and continues until the cultures are a month old. At that time akinete formation begins next to the terminal heterocyst and the formation of intercalary heterocysts slows down. After 2 months, no intercalary heterocysts can be found, although akinete development continues. We do not know when akinete germination first occurs but we have had excellent germination of akinetes in cultures up to 1½ years old. The germling is a single elongated cell which divides, eventually giving rise to a filament with a single terminal heterocyst. A second terminal heterocyst develops on the opposite end of some filaments but such old cultures appear never to have intercalary heterocysts.

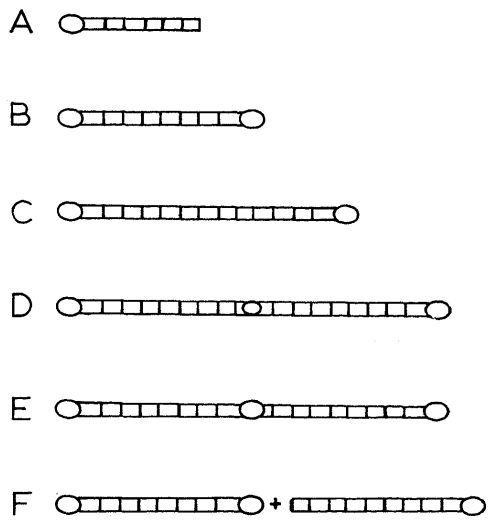

FIGURE 1. The sequence of formation of intercalary and terminal heterocysts in young cultures of *Cylindrospermum* sp. (IUCC 942). A—filament with a single terminal heterocyst gives rise to B; B—filament with two terminal heterocysts gives rise to C; C—filament elongating; D—filament elongating and an intercalary heterocyst forming; E—intercalary heterocyst fully formed; F—filament fragments at the juncture between the heterocyst and vegetative cell.

Figure 1 presents the developmental sequence for heterocyst formation in the young cultures used in this study. The filament initially has a single terminal heterocyst. As the filament elongates beyond some critical length, which varies with the different sources and amounts of nitrogen, a second heterocyst develops on the end opposite the initial heterocyst. Further elongation of the filament increases the

TABLE 1. *Average ratio of vegetative cells to heterocysts after 6-week growth in different concentrations and sources of N.*

Source	N_2	NO_3^--N			NH_4^+-N			NO_3^--N + NH_4^+-N		
N conc, ppm	Atmos.	40	80	400	40	80	400	40	80	400
Avg. ratio	27[a]	57[bc]	66	84	100[d]	100[a]	∞[e]	200[d]	200	∞[e]

a. Difference between N_2 and NO_3^--N 40, 80, and 400 ppm significant at the 0.01 level or better.
b. Difference between NO_3^--N 40 ppm and NO_3^--N 80 ppm significant at 0.05 level or better.
c. Difference between NO_3^--N 40 ppm and NO_3^--N 400 ppm significant at 0.01 level or better.
d. Estimate not subject to statistical test.
e. No heterocysts formed.

distance between the heterocysts and the vegetative cells in the middle of the filament, resulting in the formation of an intercalary heterocyst. The intercalary heterocyst easily separates from the adjacent cells, resulting in two filaments, one with a single terminal heterocyst and one with two terminal heterocysts. Reddy and Talpasayi (15) described a similar sequence for *Cylindrospermum trichotosporum* except that no intercalary heterocysts were formed in their cultures.

The response to the various sources of N (Table 1) was quite dramatic. The vegetative cell: heterocyst ratio increased as the amount of combined N in the medium increased. It was not possible to make accurate determinations of cell numbers for the NH_4^+-N media because the growth habit was greatly affected. Filaments grown in the presence of NH_4^+-N were coiled and twisted making it impossible to trace the entire length of the filament. Filament length was estimated at about 100 vegetative cells /heterocyst in the 40 and 80 ppm NH_4^+-N media and about 200 vegetative cells/heterocyst for the 40 and 80 ppm NH_4NO_3 -N.

The most striking morphological response was in the 400 ppm NH_4^+-N and 400 ppm NH_4NO_3-N media. In addition to the coiling nature there was an obvious absence of heterocysts. The few heterocysts that were found were probably introduced in the initial transfer. No heterocysts were found in the areas of new growth.

C_2H_2 reduction by the cells grown on NH_4^+-N (Figure 2) indicates N_2ase activity is completely suppressed at concentrations of 40 ppm NH_4^+-N and above. Nitrate (Figure 2), however, did not cause this suppression. Even at concentrations of 400 ppm NO_3^--N there was considerable C_2H_2 reduction. These data indicate that *Cylindrospermum* sp. 942, unlike *C. majus*, has the inducible-repressible genetic control of heterocyst formation and N_2 fixation described by Singh (16).

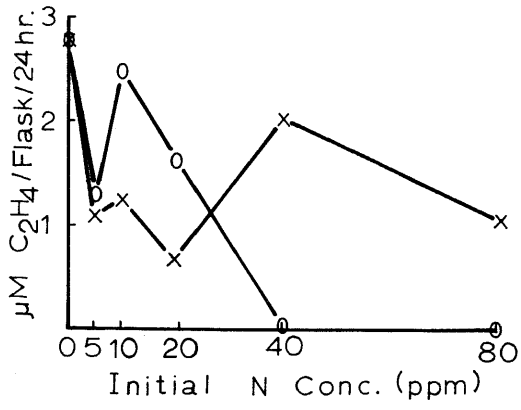

FIGURE 2. C_2H_2 reduction by *Cylindrospermum* sp. in Bristol's medium with various concentration of NH_4^+-N (O) and NO_3^--N (X).

Finally the phenotypic plasticity of heterocystic blue-green algae with respect to the presence of the heterocyst and even the position in the trichome exhibits a wide latitude of responses. *Cylindrospermum* sp. (IUCC 942), as reported here, forms heterocysts in response to N in the medium, with NH_4^+-N having the greatest effect. The genus *Cylindrospermum* is variable, having species without heterocysts (15), with only terminal heterocysts (4, 5), and with intercalary heterocysts. This variability along with the responses to N for *Cylindrospermum* sp. (IUCC 942) and for *Anabaena flos-aquae* A-37 (13) indicates the need for a critical examination of Nostocaceae in culture to provide more reliable taxonomic characters.

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