Bot. Bull. Academia Sinica 27: 53-62 (1986)

MORPHOLOGICAL DEVELOPMENT OF *NOSTOC* CY-1 UNDER LIGHT AND DARK CONDITIONS[1]

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(Received October 9, 1985; Accepted November 8, 1985)

Abstract

Nostoc CY-1 possessed apparent dimorphism which includes a straight motile hormogonium and a spiroid immotile heterocystous filament. The hormogonia had a gliding speed about 65 μm/min. They had phototropism and chemotaxis, but no N_2-fixing activity. The heterocystous filaments contained heterocyst and therefore had nitrogen-fixing activity. The morphological changes between these two forms happened regularly as the algae grown on the synthetic medium or on the soil surface without any special induction. Under the dark heterotrophic condition, the hormogonia could also convert to heterocystous form. However, the transformation from heterocystous filaments to hormogonia in the dark occurred only occasionally.

Key words: Dimorphism; cyanobacteria; *Nostoc* CY-1; hormogonium; heterocystous filament.

Introduction

Dimorphism, including a straight motile hormogonium and an immotile heterocystous form, exists commonly in *Nostoc*, *Calothrix*, and the branched type of cyanobacteria (Lazaroff, 1973; Rippka *et al.*, 1979). This phenomenon has been intensively studied only in a few strains. Based on the studies of *Nostoc muscorum* and *N. commune*, the morphogenesis could be induced by red light (Lazaroff and Vishniac, 1961; Robinson and Miller, 1970). Observation on cultures of *Nostoc* collected in this laboratory revealed that the ability of forming hormogonia varied among strains. Some isolates form hormogonia only under some special growth conditions. But for the others, transformation between hormogonium and heterocystous form is a sequential event in life cycle.

Strains with high potential of forming hormogonia could be used for the study of dimorphism mechanism. The presence of hormogonia in the culture was also

[1] Paper No. 301 of the Scientific Journal Series, Institute of Botany, Academia Sinica, Taipei, Taiwan, Republic of China.

ecologically and physiologically significant. The occurrence of hormogonia would affect the nitrogen-fixing activity of the culture, because the hormogonia do not have nitrogen-fixing activity. Nevertheless, the hormogonia could be important for the dispersal of *Nostoc* in nature due to their mobility. *Nostoc* CY-1 is one of the strains possessing apparent morphological changes under ordinary culture condition. In this communication, the general properties and the morphological changes of *Nostoc* CY-1 under illuminating condition and dark heterotrophic growth condition were reported.

Materials and Methods

Source of Cyanobacteria

Nostoc CY-1 was isolated from colloral roots of *Cycas*. The cyanobacterium was cultivated and maintained in the nitrate-free BG-11 medium (BG_o-11) (Stanier *et al.*, 1971). The isolate was checked routinely by transferring the culture to nutrient broth or examined microscopically to confirm there was no bacterial contamination.

Collection of Hormogonia

In order to obtain hormogonia, *Nostoc* CY-1 was incubated on a plate containing BG_o-11 for two weeks at 2,000 lux. About 50 mg algal material growing on such plate was put on a fresh plate and arranged in a line. The plate was then incubated in the dark overnight for motile hormogonia to glide away from the original site. The hormogonia after moving out of the line were collected by a loop for further study.

Observation of the Morphological Changes under Illuminating Condition

A simplified microculture technique was used to study the sequential events of the morphological changes of *Nostoc* CY-1. Three ml of the melted BG_o-11 agar were smeared on a sterilized slide. A small amount of hormogonia was spread on the agar surface after the medium had been hard. The slide was kept inside a petri dish which had been loaded with wet cotton for the maintenance of moisture. The culture was incubated at 28°C under continuous fluorescent light. The growth and the morphological development of *Nostoc* CY-1 were then microscopically examined at regular time intervals.

Morphological Changes of ***Nostoc*** *CY-1 Grown on the Soil*

A simple tensiometer was used to maintain the soil moisture for the cultivation of *Nostoc* CY-1 on the soil surface (Duniway, 1976). The soil taken from a rice field was put on a 9-cm diameter Büchner funnel with fritted glass plate. The height of the water column between the porous plate and the surface of a water reservoir

was 4 cm. The apparatus was kept inside a 28°C incubator with 2,000 lux fluorescent light. A loopful young culture (all in heterocystous form) of *Nostoc* CY-1 was cultivated on the surface of the soil. Samples were taken at regular time intervals for morphological examination.

Morphological Changes under Dark Heterotrophic Condition

The medium used for dark heterotrophic growth contained 1 g of glucose, 0.1 g of $NaHCO_3$ and 5 mg of proteose peptone in 100 ml of BG_o-11 medium (glucose-BG_o-11). Hormogonia were cultivated in a petri dish containing solid glucose-BG_o-11 medium. The cultures were then incubated at 28°C without illumination. Samples were taken from the plates at regular time intervals for the examination of morphology and assay of N_2-fixing activity.

Assay of N_2-fixing Activity

Nitrogen-fixing activity was measured by the acetylene reduction method established by Dilworth (1966). Acetylene and ethylene were quantitatively determined by a Shimadzu GC-3BF gas chromatograph equipped with FID detector. A stainless column of 2 m long and 3 mm i.d. packed with Porapak N was used for the seperation of the gases. The flow rate of nitrogen carrier gas was 80 ml/min. The column temperature was at 50°C, and the injection temperature was at 40°C.

Results

*Morphological Development of **Nostoc** CY-1*

Straight motile hormogonia and spiroid immotile heterocystous filaments usually coexisted in a non-synchronized culture of *Nostoc* CY-1 growing either on solid or in liquid medium (Fig. 1). The hormogonia as shown in Fig. 2 could be easily isolated from the plate by the method described above.

The sequential morphogenesis of *Nostoc* CY-1 was observed microscopically with a simplified microculture technique. As shown in Fig. 3-A and 3-B, all hormogonia spread on the solid medium were transformed into heterocystous filaments under continuous fluorescent light after one day of incubation. The transformed heterocystous filaments grew continuously and formed a dense cell cluster containing only heterocystous filaments (Fig. 3-C). After about one week of incubation, the spiroid heterocystous filaments started to differentiate into hormogonia. As shown in Fig. 3-D, the hormogonia developed from the heterocystous filaments of the cell cluster were gliding away, and many heterocyst were left at the original site. The gliding hormogonia would settle down at a new site, and then transformed again to form heterocystous filaments.

The same sequential events of morphological development also happened as the culture of *Nostoc* CY-1 was cultivated on wet soil surface (Fig. 4).

Properties of the Hormogonia and the Heterocystous Form of ***Nostoc*** *CY-1*

The hormogonium was straight and motile. It had an average cell size of about 3×5 μm. The heterocystous filament was immotile and arranged in a spiroid form. The vegetative cell of the heterocystous form was about 4.5×5 μm. The heterocyst contained in heterocystous trichome was about 5×9 μm. The hormogonia glided on agar plate at a speed about 65 μm/min. It had very sensitive phototropism (Fig. 2). A chemotaxis test indicated that the hormogonia had different responses against amino acids.

Both the hormogonia and the heterocystous filaments had similar compositions in chlorophyll, phycocyanin and phycoerythrin (data not shown). They also had similar CO_2-fixing activity (data not shown). Nevertheless, since the hormogonia did not contain heterocyst, both forms differed from each other in nitrogen-fixing activity. As shown in Fig. 5, when the hormogonia were transformed into heterocystous form, the nitrogen-fixing activity resumed rapidly. So the appearence of nitrogen-fixing activity could be used as an indicator for transformation of hormogonia into heterocystous form.

Dark Heterotrophic Growth and Morphological Changes

Nostoc CY-1 could use glucose as energy source for nitrogen-fixation under dark condition (Fig. 6). In the presence of glucose, it grew with a generation time of about 5 days under dark condition.

When the hormogonia were cultivated on the solid glucose-BG_o-11 medium and incubated at 28°C in the dark, nitrogen-fixing activity appeared at the fourth day and then increased gradually (Fig. 7). This indicated that the hormogonia could transform into heterocystous form under dark heterotrophic growth condition. The heterocystous filaments also could transform into hormogonia occasionally, but it usually required more than one month of incubation under the same condition.

Discussion

The pattern of dimorphism existed in *Nostoc* CY-1 is similar to that occurred in *Nostoc muscorum* and other species (Robinson and Miller, 1970; Lazaroff, 1973; Isono and Fujita, 1981). However, in the case of *Nostoc* CY-1, the morphological changes occurred regularly without special induction. This phenomenon also existed when *Nostoc* CY-1 was cultivated on the soil, or even under dark heterotrophic growth condition. Therefore, the dimorphism of *Nostoc* CY-1 can be regarded as a regular phenomenon in its life cycle. The motile hormogonia show phototropism and chemotaxis, but no N_2-fixing capability. The heterocystous filaments can fix nitrogen, but are immotile. These implicate that the heterocystous form may be specific for vegetative growth, and the hormogonium for dispersal of the algal

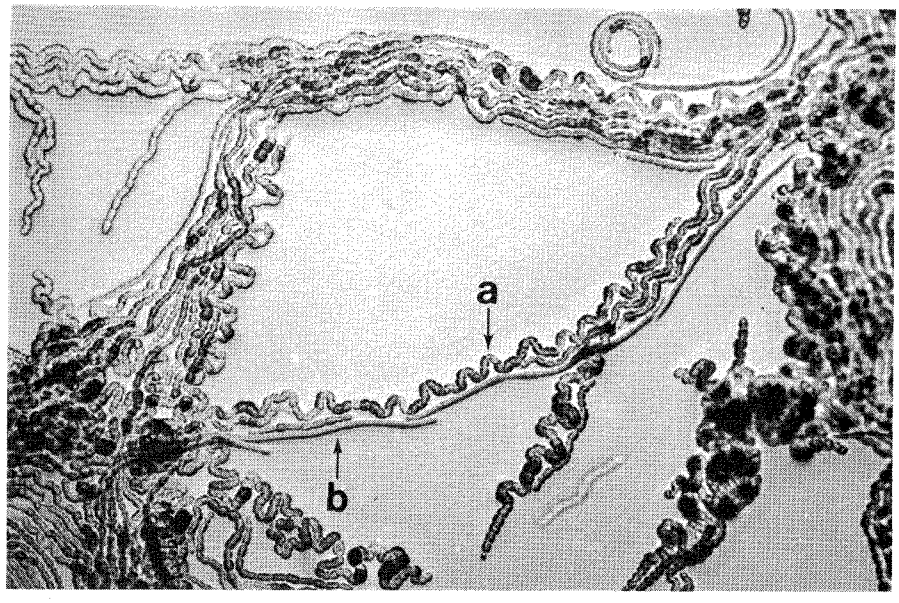

Fig. 1. Coexistence of the hormogonia and the heterocystous filaments of *Nostoc* CY-1 on a solid plate. ×250. Arrow a, heterocystous filament; arrow b, hormogonium.

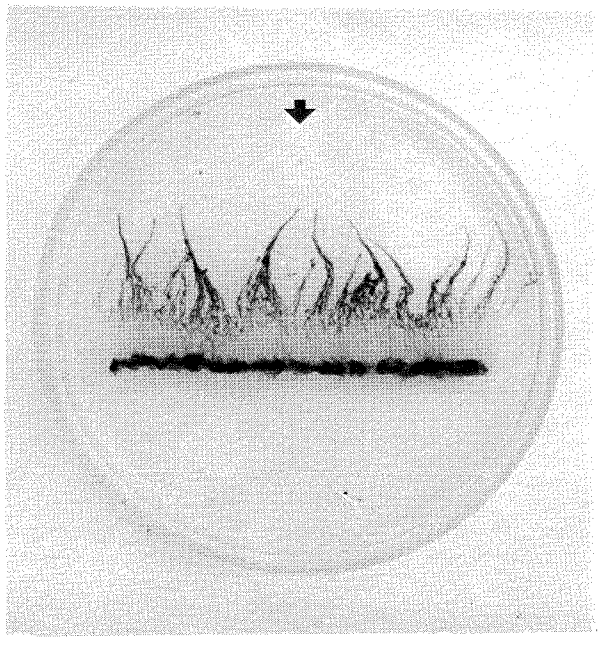

Fig. 2. Seperation of the hormogonia and the heterocystous filaments on plate. The hormogonia glided toward the source of illumination (indicated by arrow).

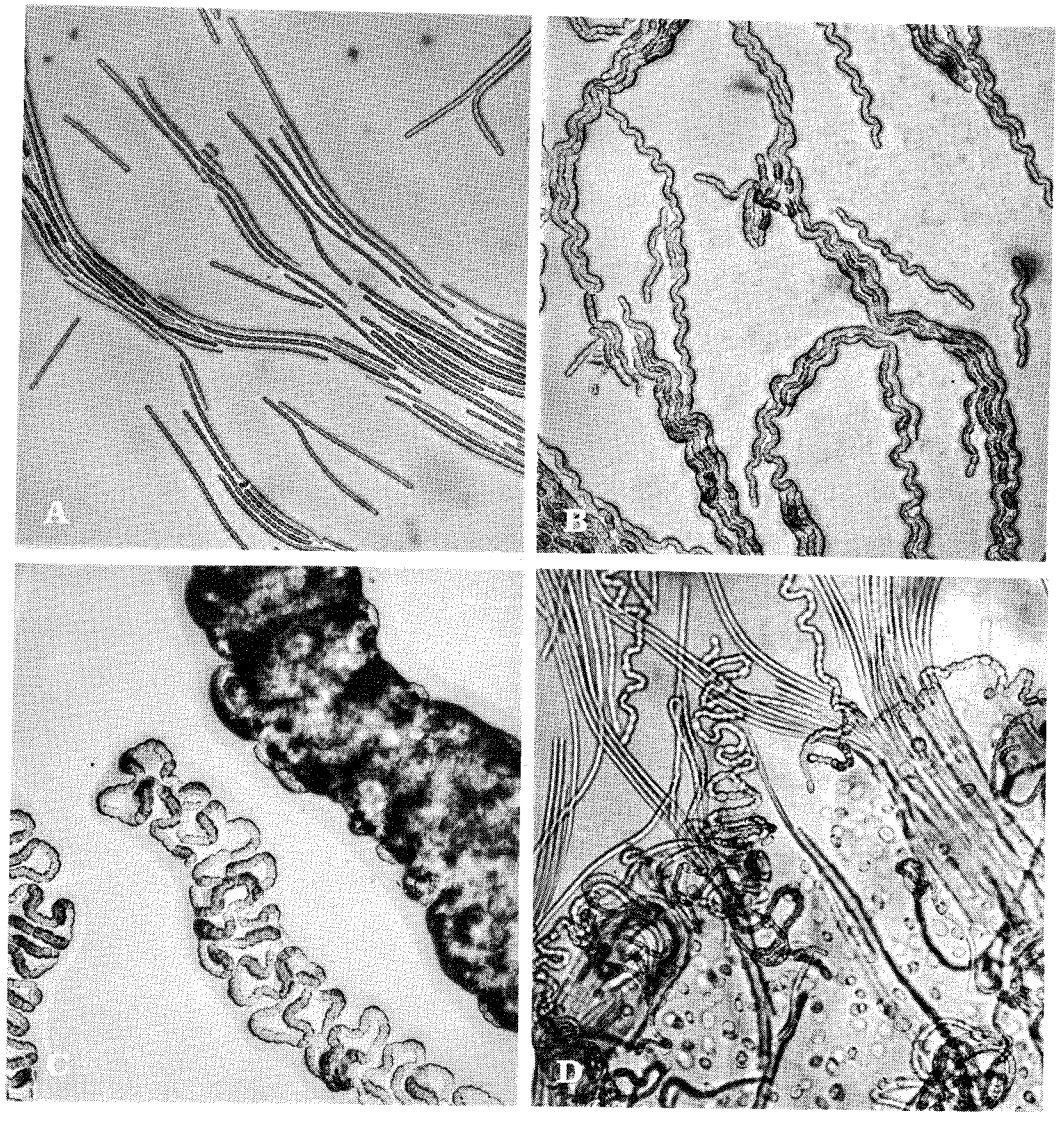

Fig. 3. Developmental cycle of *Nostoc* CY-1. ×250. A. Hormogonia gliding away from the original inoculated site, B. Differentiation from hormogonia into the immotile heterocystous filaments, C. Continuous growth of heterocystous filaments to form a huge cell cluster, D. Differentiation from heterocystous filaments into hormogonia when the culture getting old.

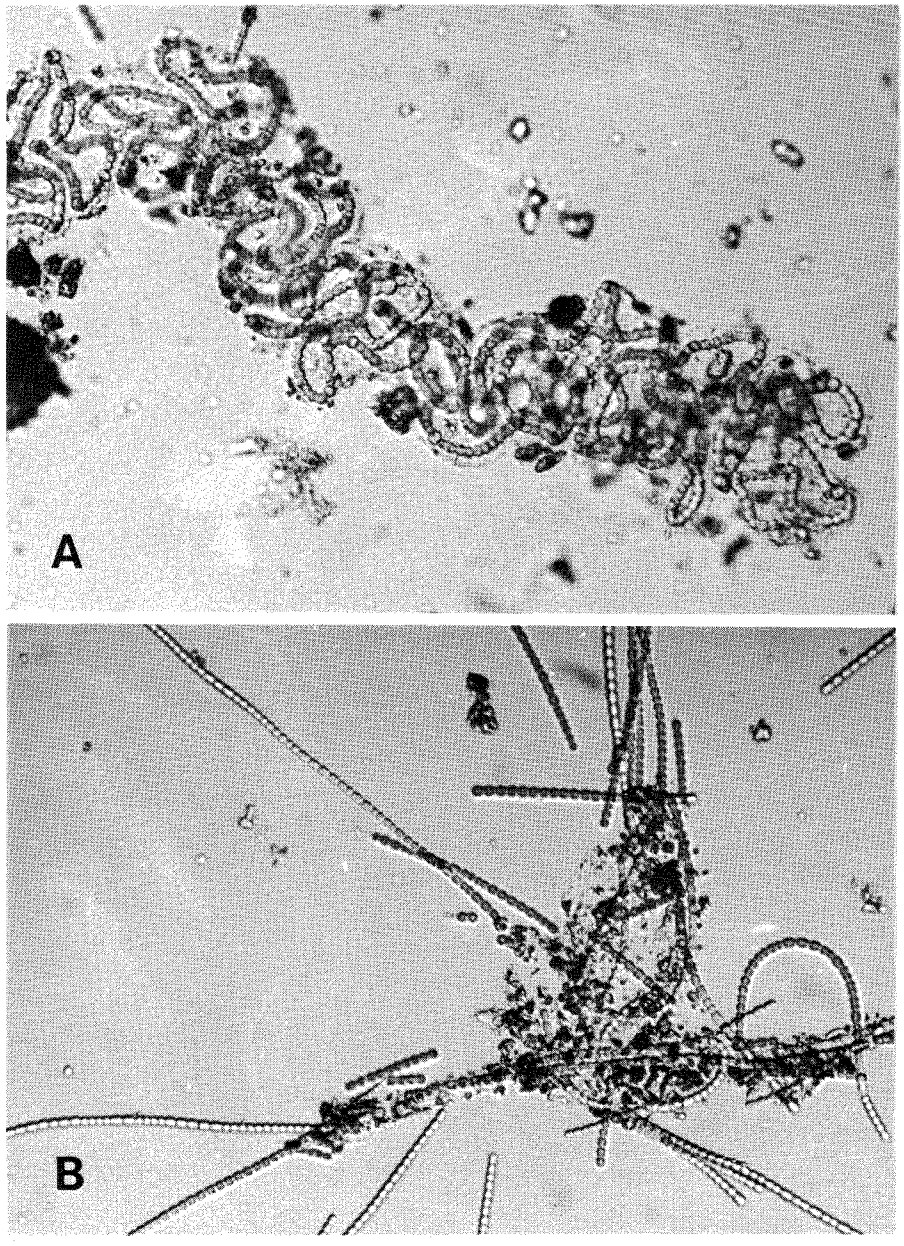

Fig. 4. Morphology of ***Nostoc*** CY-1 cultivated on the wet soil surface. ×400. A. Heterocystous filaments, B. Hormogonia.

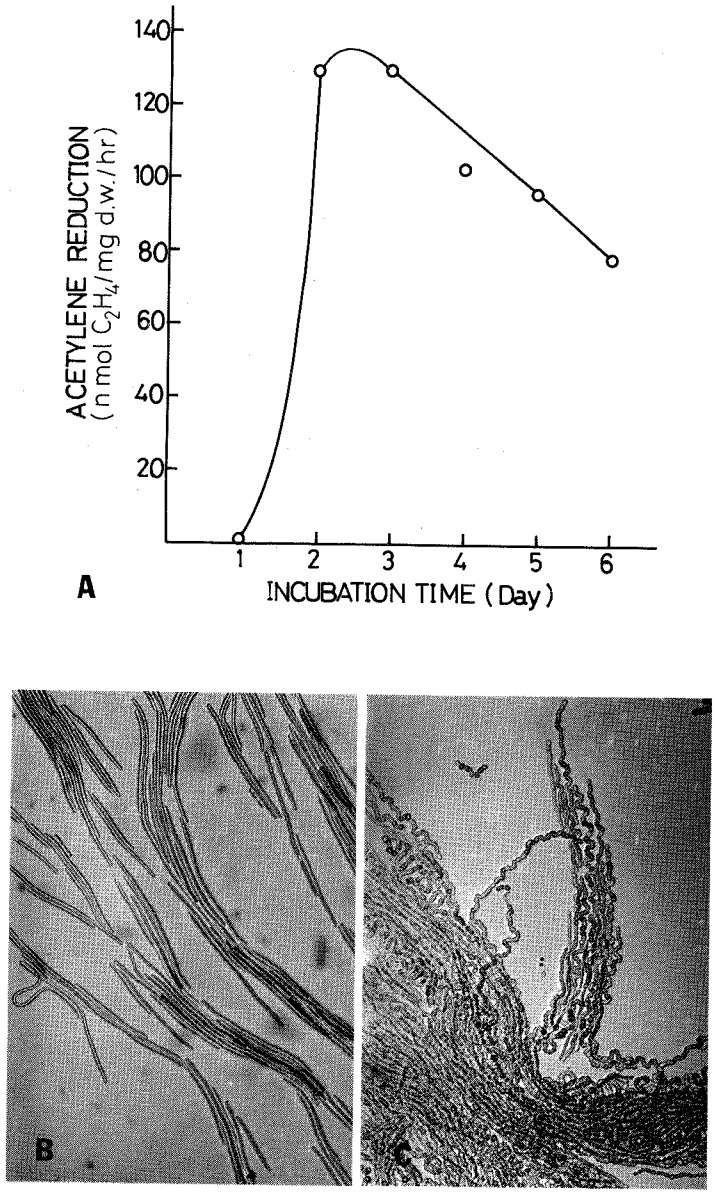

Fig. 5. Variations of the nitrogen-fixing activity along with the morphogenesis of *Nostoc* CY-1. A. Change of nitrogen-fixing activity of *Nostoc* CY-1 during differentiation from hormogonia into heterocystous filaments. The activity was assayed at 25°C and 4,000 lux, B. The cell morphology of the sample taken at day 1, C. The cell morphology of the sample taken at day 2.

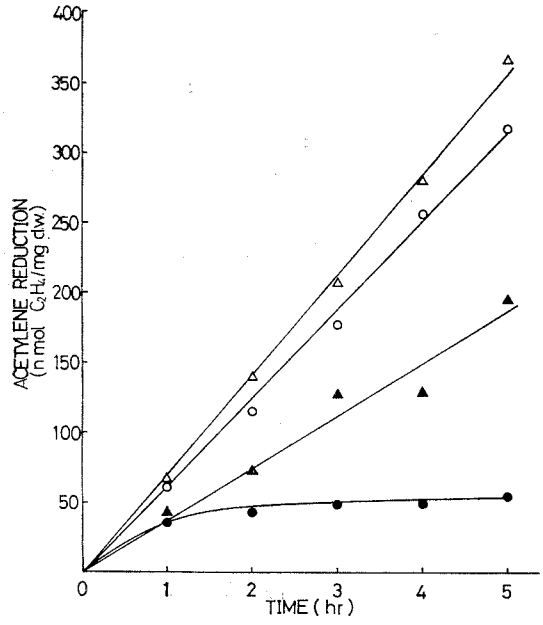

Fig. 6. Nitrogen-fixing activities of *Nostoc* CY-1 under light and dark conditions. △—△, with illumination and in the presence of glucose; ○—○, in BG_0-11, with illumination but no glucose; ▲—▲, in the presence of glucose and in darkness; ●—●, in the absence of glucose and in darkness.

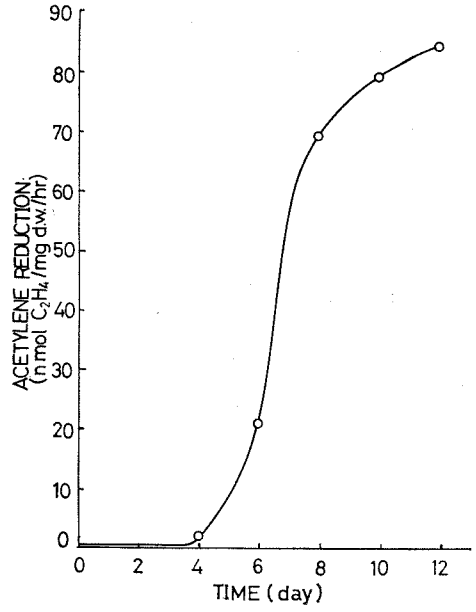

Fig. 7. The occurrence of nitrogen-fixing activity during the development of heterocystous filaments from the hormogonia of *Nostoc* CY-1 under the dark heterotrophic condition.

population.

The hormogonia always glide away from the original site and form a new colony at a fresh place. Based on our observation, aging or the increase of population density of the culture usually enhances the appearance of hormogonium. Therefore, it is possible that the morphogenesis of *Nostoc* CY-1 may be regulated by metabolic products accumulated in the colony, or by the change of microenvironment around the colony.

Acknowledgement

This study was supported by a National Science Council grant NSC 73-0201-B001-13. We thank Dr. Lin-Yuan Su for critically reading of the manuscript.

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Nostoc CY-1 在光照與黑暗下之形態變化

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Nostoc CY-1 具有雙型性，此雙型性包括一種直線形能滑動之同型體，及一種螺旋狀不能滑動之異型體。同型體以每分鐘約 65 μm 的速度滑動，具向光性與向化性，但不能固氮。異型體具異形孢子，故有固氮活性。將此藻培養於合成培養基或土壤表面時，同型體和異型體之間呈規律性之相互轉化。在黑暗異營條件下，同型體亦可轉化爲異型體。