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Rhythmic nitrogenase activity of *Synechococcus* sp. RF-1 established under various light-dark cycles

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Abstract. *Synechococcus* sp. RF-1 established rhythmic nitrogen-fixing activity under different diurnal LD cycles varying from 20 L:4 D to 6 L:18 D. The rhythmic nitrogen-fixing pattern could also be attained under LD cycles with a period length different from 24 h, such as 18:18 or 6:6. The results of these experiments indicate that under diurnal LD cycle, a dark period of 4 h is sufficient for *Synechococcus* sp. RF-1 to establish the rhythmic nitrogenase activity within one LD cycle when this culture is incubated at 3000 lux, 28°C. Once the nitrogenase activity has started, it will run for a certain minimum period before it stops. With very short dark periods, it continues fixing nitrogen into the subsequent light period. On the other hand, with very long light periods, the nitrogenase activity commences before the onset of darkness. The results suggest that, in addition to darkness, the concentration of photosynthetic products has a strong influences on the initiation of nitrogenase activity.

Key words: Blue-green algae; Cyanobacterium; Light-dark cycles; Nitrogen fixation; Rhythmic nitrogenase activity; *Synechococcus*.

Introduction

Because cyanobacteria produce O_2 during photosynthesis and because nitrogenase is O_2 labile, the simultaneous occurrence of photosynthesis and nitrogen fixation in a single cyanobacterial cell would appear to be highly unlikely. However, a few unicellular cyanobacteria are able to fix N_2 aerobically under continuous light or light-dark condition. Wyatt and Silvey (1969) first reported that an ensheathed unicellular cyanobacterium, assigned to *Gloeocapsa*, can fix nitrogen aerobically. This strain was subsequently re-identified as a member of the genus *Gloeothece* and several additional nitrogen-fixing isolates of the same genus were purified and characterized (Stanier *et al*., 1971; Rippka *et al*., 1979; Huang & Chow, 1988). Recently some sheathless unicellular strains, identified as *Synechococcus*, were isolated (Huang and Chow, 1986; León *et al*., 1986) which exhibit high aerobic nitrogen-fixing activities.

These aerobic nitrogen-fixing unicellular cyanobacteria exhibit unique rhythmic nitrogen-fixing activities when grown in a diurnal LD cycle. The pattern of the rhythmic nitrogen-fixing activities established under a 12 L:12 D cycle or in LD cycles close to 12:12 have been extensively examined (Mullineaux *et al*., 1981; Grobbelaar *et al*., 1986; León *et al*., 1986). Since light is the energy source for *Synechococcus* sp. RF-1, the period length or the light intensity of light phase may have important effects on the establishment of the rhythmic nitrogenase activity. In this communication, the nitrogen-fixing patterns of *Synechococcus*

sp. RF-1 established at various LD cycles are examined.

Materials and Methods

Organism and Cultivation

Synechococcus sp. RF-1 was isolated from a rice paddy field in the Ping-Tong district, southern Taiwan. It was purified as an axenic culture in this laboratory. The culture was grown in nitrate-free BG-11 medium ($BG\text{-}11_0$) (Stanier *et al.*, 1971), supplemented with 0.01 M EPPS buffer, pH 8.0. Unless stated otherwise, cultures in flasks were incubated without aeration or shaking at 28°C under about 3000 lux (35 μmol photon $\cdot m^{-2} \cdot s^{-1}$) of light from white fluorescent tubes (Toshiba FL20D/18, Taiwan Fluorescent Lamp Co.). Cell concentration was estimated from measurements of optical density made with a Klett-Summerson photoelectric colorimenter fitted with a No. 42 blue filter. The total protein content in 10^8 cells was 0.21 mg as determined by the Lowry method (Lowry *et al.*, 1951) after the cells were hydrolysed with 1 M NaOH in a waterbath at 100°C for 10 min.

Nitrogenase Activity

Nitrogenase activity was assayed by the acetylene reduction method (Dilworth, 1966). Samples (1 ml) were removed from the cultures and placed into 14 ml test tubes. The test tubes were sealed with rubber stoppers and 1.4 ml commercial acetylene was added with a gas-tight syringe. Gas samples (0.5 ml) were analysed for their ethylene content at the beginning of the incubation period and again one hour later by a Shimadzu GC-3BF gas chromatograph equipped with a flame ionization detector. The samples were incubated under the same conditions of light and temperature as the parent cultures.

Results and Discussion

Synechococcus sp. RF-1 fixed nitrogen continuously but fluctuated irregularly when grown in continuous light (LL) (Fig. 1). When the continuous illuminating cultures were transfered to a diurnal light-dark (LD) cycle of 22:2, 20:4, 16:8 or 12:12 respectively, the rhythmic nitrogenase activity was phased up within one LD cycle for those cultures exposed to 20:4, 16:8 or 12:12 LD cycle (Fig. 1). However, exposure to a 22:2 LD regimen did not establish a nitrogenase activity rhythm as was the case with the other LD regimens, although a nitrogen-fixing peak was induced during the dark phase. As shown in Fig. 1, when *Synechococcus* sp. RF-1 was grown in a diurnal 12L:12D regimen, the period of nitrogenase activity lasted for about 12 h, with the activity exclusively within the dark phase. When the dark period was very short or the light period was very long, such as in 20L:4D, the period of nitrogenase activity also lasted for about 12 h, with the nitrogenase activity commenced before the onset of the dark phase and continuing into the subsequent light period. Fig. 1 also showed that the rhythmic nitrogenase activity of cultures growing at 20:4 and 16:8 both started at about 14 h after the onset of illumination, despiting that there were 4 h difference between the two LD cycles.

The results of Fig. 1 suggest that darkness is required for the establishment of rhythmic nitrogenase activity, but it is not the only factor to rhythmically initiate the nitrogenase activity, because the nitrogenase activity may appear before the onset of darkness. The nitrogenase activities all initiated about 14 h after the onset of light phase when the cultures were exposed to 16L:8D or 20L:4D. The results suggest that the accumulation of photosynthetic products might have a strong influence on the initiation of nitrogenase activity. With very long light periods, the accumulation of photosynthates may induce the synthesis of nitrogenase which acts as a sink and will consume photosynthetic products for both the actural fixation and assimilation of nitrogen.

When *Synechococcus* sp. RF-1 was grown in diurnal LD cycles with the light phase shorter than the dark phase, the rhythmic nitrogenase activity could also be fully established. As shown in Fig. 2, although the LD cycles varied from 6L:18D to 10L:14D, the rhythmic nitrogenase activity all started within dark period with a timing about 12 h after the onset of light period. The results suggest that when the light phase was not very long, the initiation of nitrogenase activity might be mainly affected by darkness.

The nitrogen-fixing patterns of *Synechococcus* sp. RF-1 exposed to LD cycles different from 24 h were also studied. As shown in Fig. 3, the rhythmic nitrogen-fixing pattern of *Synechococcus* sp. RF-1 could be induced by LD cycles ranging from 6L:6D to 18L:18D. When the culture was exposed to 10:10, 8:8 or 6:6 LD

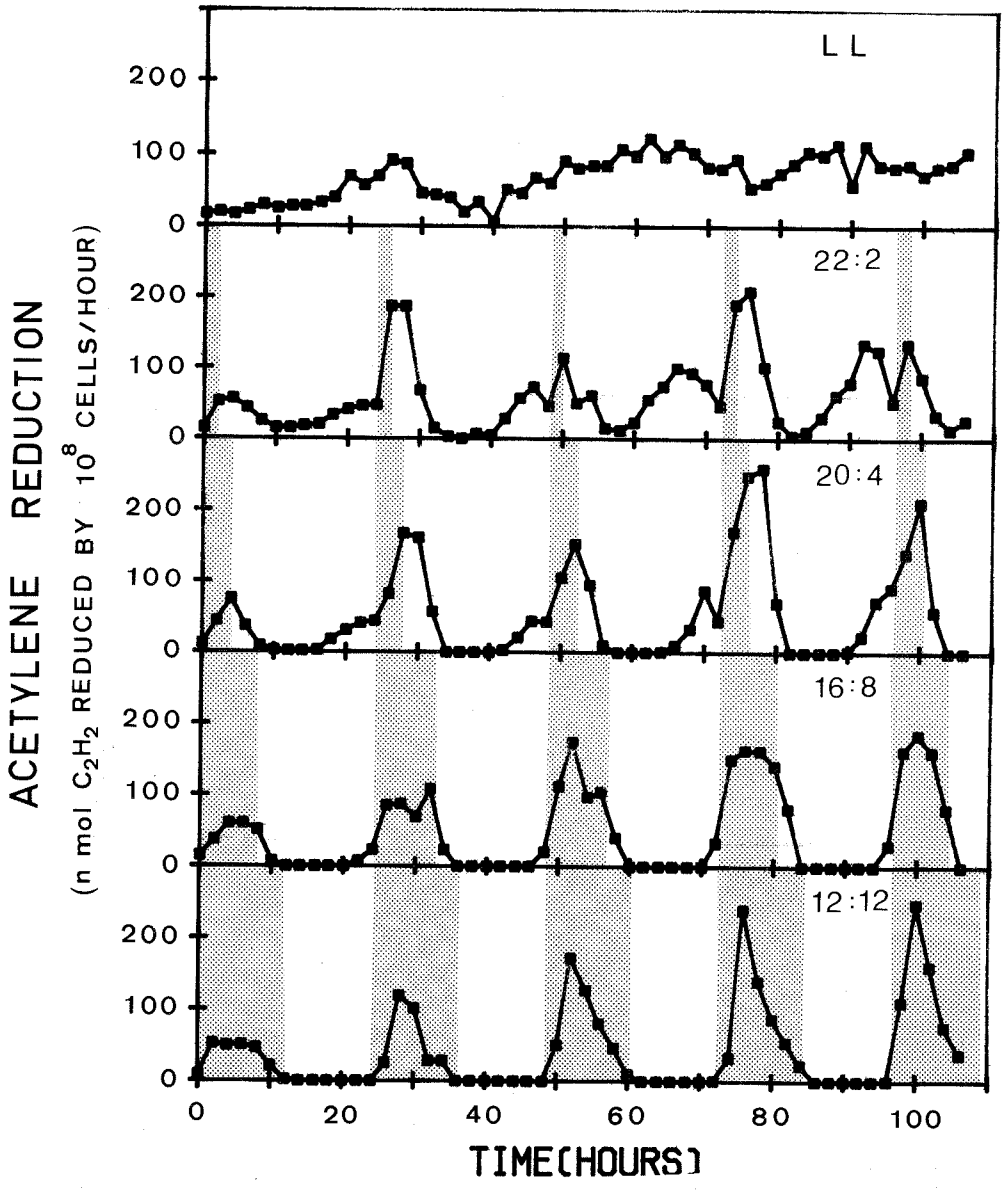

Fig. 1. Nitrogen-fixing pattern of *Synechococcus* sp. RF-1 established at LL and different diurnal LD regimens. Cultures adapted to LL condition were transferred to 22:2, 20:4, 16:8 and 12:12 LD regimens respectively. The nitrogenase activity of the culture maintained under LL and those newly transferred to different LD conditions were assayed at two hours intervals for a period of 5 days.

cycles, the nitrogenase activity commenced after the onset of darkness and continued into the subsequent light period. When the culture was exposed to 14:14, 16:16 or 18:18 LD cycles, the nitrogenase activity commenced at about 13 h after the onset of illumination, although the culture was still in the light phase. The results support our deductions based on Fig. 1 that, in addition to darkness, the accumulation of photosynthetic products probably has a strong influence on the initiation of nitrogenase activity, especially when the culture is grown in the LD regimen with very long light phase.

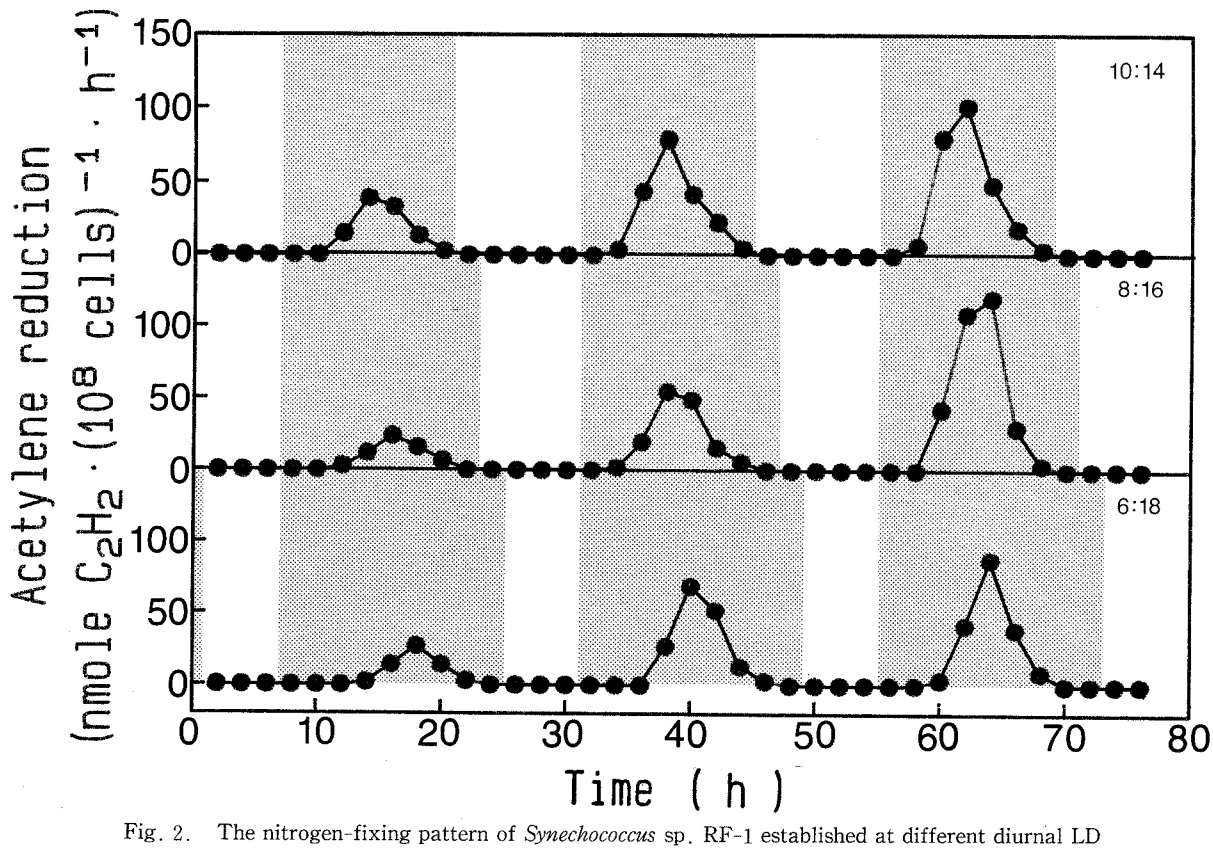

Fig. 2. The nitrogen-fixing pattern of *Synechococcus* sp. RF-1 established at different diurnal LD regimens with the light phases shorter than the dark phases. The nitrogenase activity was assayed at two hours intervals for a period of three days after the cultures had been adapted to the stated LD cycles for one week.

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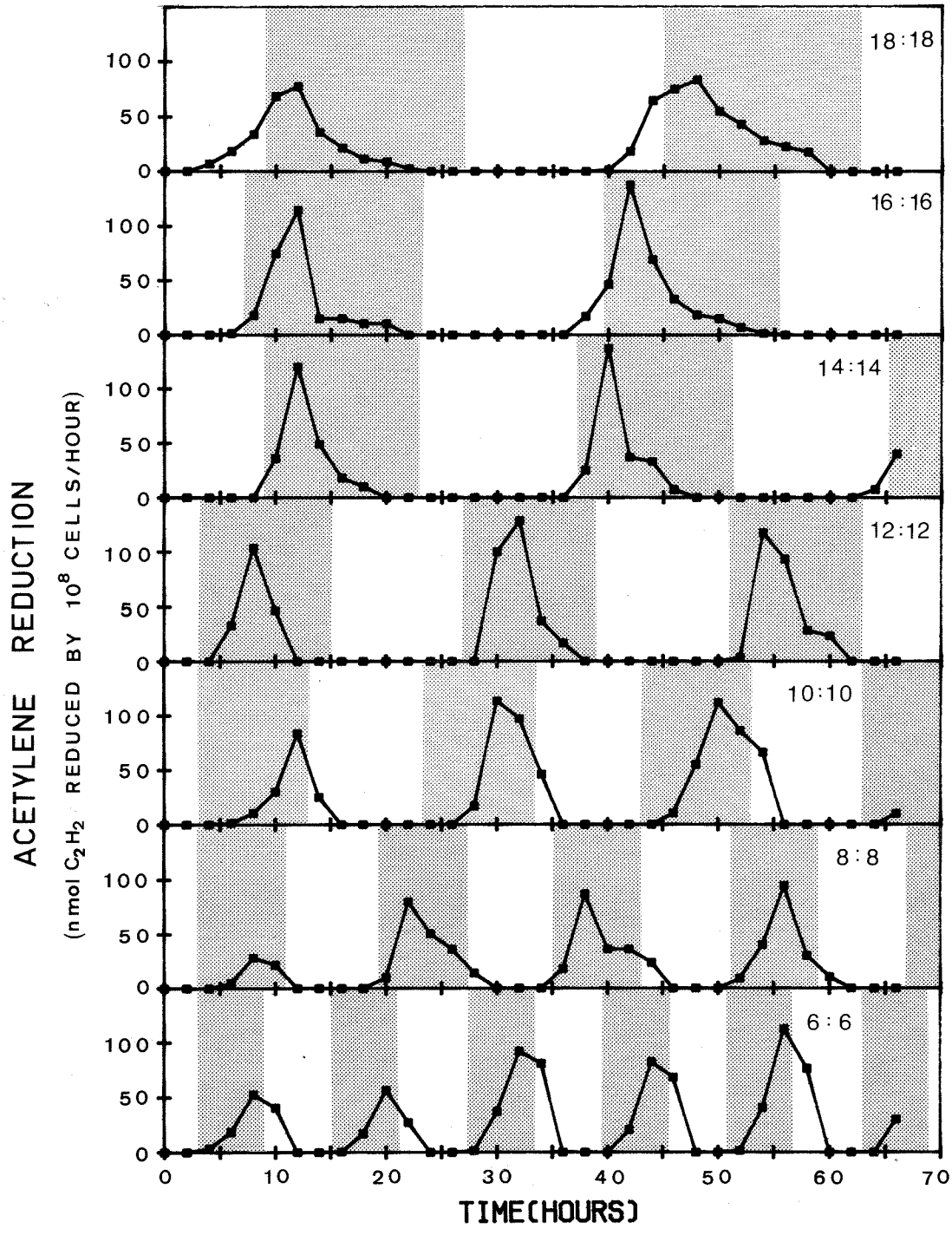

Fig. 3. Comparison of the nitrogen-fixing patterns of *Synechococcus* sp. RF-1 established in 18:18, 16:16, 14:14, 12:12, 10:10, 8:8, and 6:6 LD regimens. The nitrogenase activity was assayed at two hours intervals for a period of three days after the cultures had been adapted to the stated LD cycles for one week.

聚球藻 RF-1 品系在不同光-暗週期所建立之韻律性固氮活性

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聚球藻 RF-1 品系在光-暗爲 20:4 或 6:18 的週期下均可建立韻律性固氮活性，當光-暗週期長或短於 24 小時，如 18:18 或 6:6，亦可建立。將此藻培養於 3000 lux，28°C 時，雖然暗期只有 4 小時(即 20L:4D)，其韻律性固氮活性在經過一次光-暗週期處理後即可建立。當韻律性固氮活性建立後通常會維持一段時間，故當暗週期很短時，固氮活性會持續進行到光期內，而當光期很長時，固氮活性會在進入暗期前出現。此實驗結果顯示除了需要暗條件外，光合作用產物之累積對於固氮活性之出現時間亦可能有重要的影響。