CARBON FIXATION IN SYMBIOTIC *NOSTOC* FROM CYCAS

CORALLOID roots of Cycas contain symbiotic blue-green alga *Nostoc*, in the midcortical zone. It is shown that the alga fixes nitrogen which is partly utilised by the host[1]. However, little is known about the photosynthetic activities of the symbiont. We report the presence of two important carboxylating enzymes, RuBPCase and PEPCase involved in carbon fixation by the alga.

The alga was isolated from healthy tips of the coralloid roots freshly collected from Cycas plants growing in Botanical Gardens of the University. The tips, about 10 mm in length, were homogenised in a blender with water and filtered through nylon mesh to remove the tissue debris. The suspension was first centrifuged at 1000 rpm for 5 min and the algal cells present mostly in the supernatant were sedimented at 5000 rpm for 20 min. The algal mass washed thrice by centrifugation with distilled water was suspended in tris-HCl-buffer, 10 μM, pH 8·2 and sonicated with Vibronics Ultrasonic disintegrator for 15 min at 200 mA for 15 min, in an ice bath. The homogenate was centrifuged at 5000 rpm for 20 min and the supernatant was used for the enzyme assays.

Nostoc from the coralloid roots isolated aseptically from surface-sterilised root tips was cultured in Allen and Arnon's medium[2] at 26° C under fluorescent light. Cell-free extract of the cultured alga was also prepared as above.

Assay of PEPCase was according to Hatch and Slack[3] and RuBPCase by the method of Morris and Farrel[4]. ^{14}C-bicarbonate solution (25 μCi) was added to the reaction mixture and incubated in a water-bath maintained at 30° or 40° C as required. The reaction was stopped by adding 0·1 ml of 4% HCl. For the determination of photosynthetic carbon fixation by intact cells, the alga isolated as above from coralloid roots, was suspended in Allen and Arnon's medium and exposed to light (28,000 lux) for 15 min at 30° C, in the presence of ^{14}C-bicarbonate. At the end of incubation 0·1 ml of dilute HCl was added and acid-stable radioactivity was determined.

Radioactivity of all samples was determined using Bray's solution as scintillant, with Beckman LC100 counter. Chlorophyll was estimated by the method of Talling and Driver[5]. Protein determinations were according to Lowry *et al.*[6].

Photosynthetic Carbon Fixation

The alga, freshly isolated from coralloid roots showed conversion of ^{14}C-bicarbonate into acid-stable products in the presence of light as well as in the dark. However, the light-fixation rate was nearly 12 times the dark rate at 30° C (Fig. 1 A).

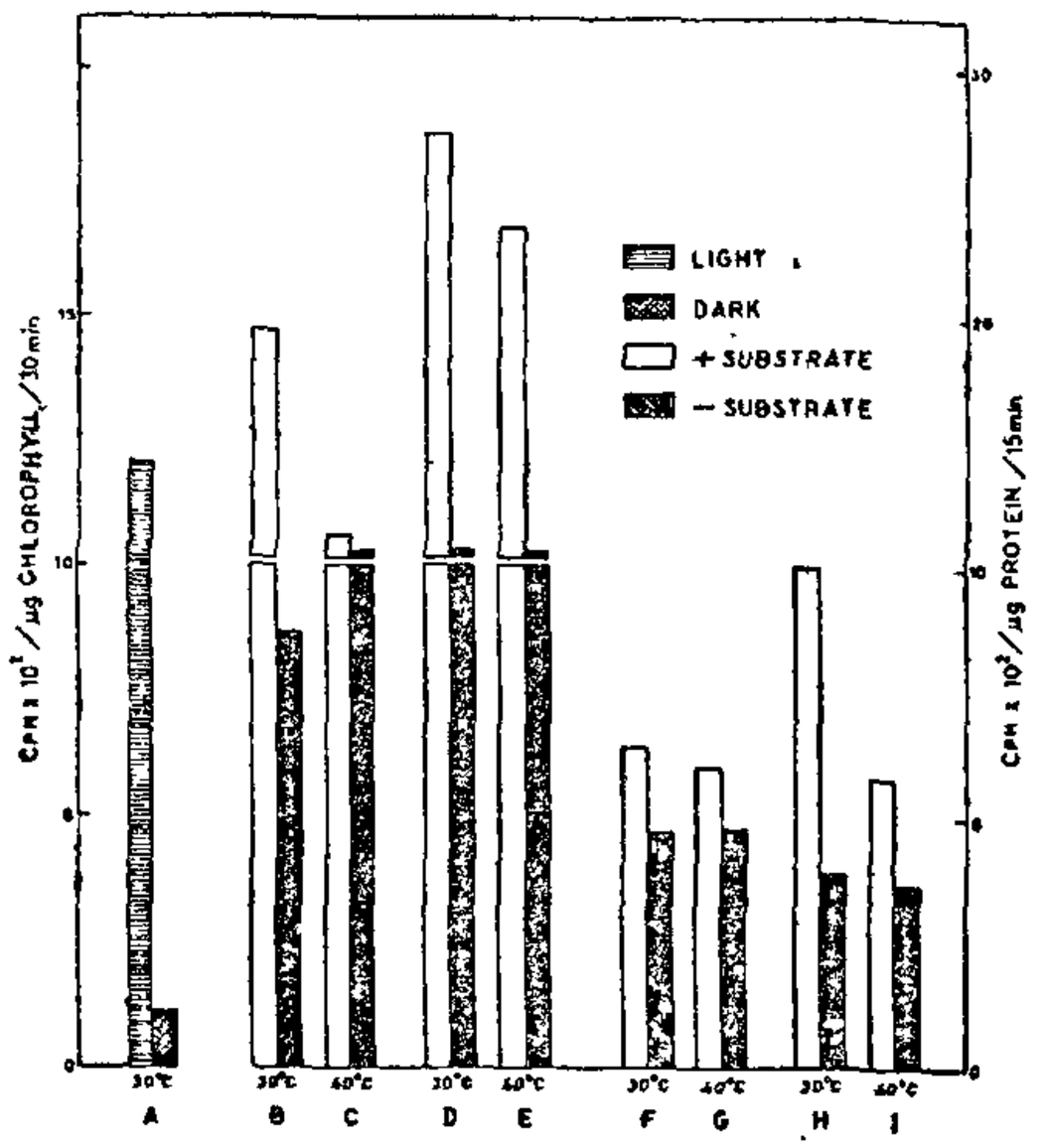

FIG. 1. A—Carbon fixation by freshly isolated symbiotic *Nostoc*. B, C—PEPCase and D, E—RuBPCase of the cultured alga; F. G—PEPCase and H, I—RuBPCase of freshly isolated alga.

Carboxylases

The activities of both the carboxylases, RuBPCase and PEPCase, were found in the cell-free extracts of freshly isolated alga and also in the cultured alga (Fig. 1, B–I), the activity being higher in the latter. Further, both the enzymes showed lesser activity at 40° C than at 30° C, however, it was seen that PEPCase was much less affected than RuBPCase. There was also considerable fixation of ^{14}C-bicarbonate even without the addition of RuBP or PEP. This may be due to the presence of endogenous substrates in the crude algal extracts.

The carbon fixation pathway in blue-green algae is generally considered to be of the Calvin cycle type[7]. The key enzyme of the cycle, RuBPCase is shown to occur in blue-green algal cells in the form of polyhedral bodies (carboxysomes)[8]. A number of workers have also reported the presence of PEPCase activity in blue-green algae[9]. The endosymbiont *Nostoc*, while it fixes molecular nitrogen also fixes carbon dioxide. Like the products of nitrogen fixation, the products of carbon fixation may also be used at least in part by the host. The host may in turn control the biochemical activities of the alga. In the light of our findings, it is necessary to determine the relative importance of the two carboxylating enzymes in the photosynthetic fixation of carbon and their control in the symbiont.

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MASS MIGRATION AND MORTALITY OF *AMYNTHAS* (= *PHERETIMA*) *ALEXANDRI* (BEDDARD) (MEGASCOLECIDAE: OLIGOCHAETA)

MASS migration is an occasional phenomenon seen in earthworms[1]. This phenomenon was recorded in *Perionyx* sp. in Burma[2,3], and in *Eisenia foetida* in Holland[4]. However, only one such report exhisted till today in India, in which little was mentioned regarding the location, and species of the earthworm[5]. During 1979, the mass migration leading to mortality, of a geophagous earthworm, *Amynthus* (= *Pheretima*) *alexandri* (Beddard) was recorded at Medziphema (lat. 25° 45′ N ; long. 93° 53′ E ; and ca altitudinal range 420–450 m MSL), in Kohima District, Nagaland, which experienced a tropical climate with heavy rainfall. The uncultivated fields of the place were covered with thick undergrowth consisting of grasses, *Imperata cylendrica*, and *Thysanolaena maxima* ; and herbs, *Mikania scadens*, and *Eupatorium odoratum*. This report is a brief account of the observations of the above mentioned phenomenon.

The mass migration was observed during September–October, 1979, the post monsoon and early winter season of the place, most probably when the soil was hardened. During this period the worms were recorded crawling on the surface aimlessly in the early mornings (0500–0700 hrs) in huge numbers, almost covering the earthern roads, tracks, paths and other open places. The direction of their migration was downward the hill slopes. About 5,000 migratory worms were collected and preserved in 5% formalin. It was estimated that abont 99·99% of them were matured. Gate[2,3] also reported similar types of migratory movements, and assumed that these worms were moving downwards in search of water and food. Madge[5] stated that the mass migration was in response to the raiding activity of the predatory ants. In contrast, the mass migration of earthworms in India as reported by Gates[3] was during the early part of the monsoon, and worms were moving upward. This upward movement was probably in search of suitable habitat.

During the day time, in the forenoon when the temperature of the atmosphere, and soil was increased gradually, the locomotion of the worms were stoped. Then, they dried up and died secreting a whitish yellow body fluid and rolling in the surrounding soil. Small predatory ants, black in colour were observed attacking these worms and they were quickly killed and caten[5]. During the afternoon, these worms were not found except a few fresh carcases in certain places. In some other places the worm carcases were seen in hundreds lying near each other on the surface. Similar type of mass mortality which was described by Gates[2,3] as "Mortal wandering", has been recorded in species belonging to the genus *Eutyphoeus*, *Desmogaster* or *Pheretima* in western hills of Burma.

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