The evolutionary significance of 'obligate' photoautotrophy of cyanobacteria

Cyanobacteria are a major group of oxygen-evolving photosynthetic microbes. Their evolutionary antiquity and the ability to fix CO_2 using water as an electron donor provided them with a decisive edge over other microbes to dominate the biosphere in the past[1–3] and inhabit extreme environmental niches[4–6]. In diverse aquatic ecosystems that are presently inhabited by cyanobacteria along with other phototrophs, mixotrophy is a common phenomenon within the microbial food web[7–10]. Tittel *et al.*[7] have suggested that mixotrophy is expected because natural selection which combined the two specialist modes – photoautotrophic growth by photosynthetic CO_2 fixation under conditions when dissolved organic carbon is depleted due to competition with heterotrophs, and switch over to heterotrophy when food is abundant – could confer an advantage within the same cell over that of obligate photoautrophy. Such a selection should be advantageous, irrespective of the niches photosynthetic microbes occupy. Paradoxically, however, laboratory culturing suggests the contrary. Majority of the extant species of cyanobacteria are obligate photoautotrophs, though a small fraction is mixotroph (or photoheterotroph)[11]. The present correspondence attempts to explain the origin of this paradox.

Mixotrophy in ecological studies generally refers to population characteristics, and not that of individual cells. It is attributed to a complex food web due to the presence of chemoheterotrophs and non-obligate eukaryotic microbes such as algae and other protists[7–10,12]. Obligate photoautotrophy, which characterizes strict dependence for growth on organic carbon synthesized within the cell by photosynthetic CO_2 fixation – one of the specialist modes referred to by Tittel *et al.*[7] – is more complex than simply loss of transport of organic carbon into the cell. For instance, an attempt to supplement with glucose transport gene in an obligate photoautotroph to facilitate photoheterotrophic growth with exogenously added glucose did not sustain growth for long, even under normal conditions[13].

We propose the hypothesis that photoheterotrophy in cyanobacteria has been selected against in the wild because of simultaneous inheritance of vital negative trait – loss of resistance to UV stress from solar radiation. If this is true, it could explain the abundance of 'obligate' photoautotrophs in their native habitats. The hypothesis cannot be tested by simply comparing the UV sensitivities of different strains of obligate photoautotrophs and photoheterotrophs from the wild. This is because arguments based on genetic heterogeneity among any number of independent isolates of the two types could invalidate the conclusions, even if the correlation proposed in the hypothesis exists. The criticism would hold even if it was possible to grow all the isolates in the same medium under identical growth conditions. On the other hand, uncertainty due to genetic variability can be eliminated by comparing the UV sensitivities of a mixotrophic strain between mixotrophic and photoautotrophic modes of growth. Here we report the result of such an experiment with *Synechocystis* strain PCC6803. This strain was identified as a photoheterotroph, capable of growth on glucose in the medium, by Ripka *et al.*[11], in an exhaustive survey characterizing cyanobacterial strain collections for their ability to grow on organic carbon sources, besides other phenotypes.

Figure 1 shows the comparison of UV sensitivities of *Synechocystis* PCC 6803 under photoautotrophic and mixotrophic modes of growth. Clearly, UV sensitivity of cells from mixotrophic mode is higher than that from autotrophic mode, irrespective of whether the culture was in light or dark prior to UV irradiation. In these experiments UV irradiated cells and corresponding controls were incubated in dark at ambient temperature for 24 h to block photoreactivation, before transferring to light for colony development. Significantly, cells from the photoautotrophic culture incubated in dark prior to UV irradiation were more resistant than actively growing cells in light. Thus a light–dark UV sensitivity switch operates under photoautotrophic growth of the mixotrophic *Synechocystis* strain. The switch is not disabled even in homozygous *recA* insertion mutant derivative, though its UV sensitivity is high compared to wild type[14]. We had reported[15] the existence of such a switch in *Anacystis nidulans* strain BD-1, which could grow only photoautotrophically. This strain was isolated in a suburb of Mumbai and verified as *Synechococcus* species by ribosomal 16s RNA sequencing (reported in the cyanobacteria database). It cannot utilize any of the organic carbon compounds such as glucose, fructose, succinate, acetate, maltose, glycerol and several other common sugars that were tested (unpublished obs.). The differential UV sensitivity between cells from light and dark phases was observable on blocking

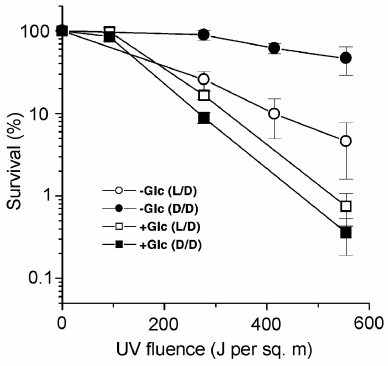

Figure 1. UV dark-survival of *Synechocystis* sp. PCC 6803 in photoautotrophic (–Glc) and photoheterotrophic (+Glc) mode of growth under different pre-UV light–dark regime. L/D, Pre-UV light/post-UV in dark for 24 h; D/D, Pre-UV dark for 24 h/post-UV in dark for 24 h. Bars represent SE values from three or more independent experiments. *Synechocystis* PCC6803 was used as the wild-type strain[26] (kindly provided by Takakazu Kaneko). All cultures were grown in BG-11 medium[27] with light intensity of ~800 lux, at 33°C. A constant mixed illumination of white light and tungsten lamp was used for growth in liquid as well as on plates as described[14]. Air from an aquarium pump was filtered and used for bubbling the liquid cultures. Glucose (5 mM) was added to both liquid and solid media for photoheterotrophic growth. For UV exposures the procedure described by Minda *et al.*[14] was used. Aliquots (100 µl) of various serial dilutions of log-phase cultures were spread on BG-11 plates. With the lids removed, the plates were immediately irradiated with UV light (254 nm, fluence rate: 9.2 J per sq. m per s) for different lengths of time. All the plates were then kept in dark overnight at room temperature (about 22°C) to prevent photo-reactivation. After dark incubation, the plates were transferred to light growth chamber till growth was visible to the naked eye as colony (~10–14 days).

photoreactivation by a prolonged dark incubation following UV irradiation before transfer to light for colony development, as in the experiments reported here. Therefore, survival following UV exposure was presumably due to dark-repair activity.

We explain the enhancement of UV sensitivity in mixotrophic mode of growth at the phenomenological level as follows (Figure 1). In these experiments, prolonged (24 h) post-UV incubation in dark at ambient temperature was maintained. Since the dark-repair systems continue to be active during the dark period following UV irradiation, inhibition or poor rate of growth of cells would have an advantage over the potential ability to grow efficiently, like chemoheterotrophs, even in the presence of organic carbon sources. Such an adaptation would allow the damage accumulated in the DNA by UV and high intensities of photosynthetically active radiation in the day, with minimum risk of lethal collapse at the replication fork, as demonstrated in heterotrophs during housekeeping activity[16,17]. Such an experimental protocol mimicked light–dark transition of the natural habitat in the day–night cycle, though the fraction of time in light and dark phases and temperatures in natural habitats would be variable due to dependence on the latitude where the habitat was located. Prolonged dark incubation at night would be expected to ensure saturation of, or maximal DNA repair in a given habitat, even in the presence of organic carbon source, by arresting the growth in obligate photoautotrophs until the next phase of light exposure. Such a phenomenon of enhanced survival on transferring cells immediately following irradiation to the buffer medium without carbon source, known as LHR (liquid holding recovery), had been observed earlier in heterotrophs[18,19]. The enhanced survival was due to extended DNA dark-repair prior to challenge for growth[19]. Thus by adaptation to obligate dependence on photosynthetically produced organic carbon source for growth that occurs within the cell only in the light phase, the selection of high UV resistance could be ensured in cyanobacteria, without the dependence on the availability of light for repair of DNA damage by photoreactivation. This loss of capacity to grow in the presence of organic carbon in the medium could be a simple spontaneous event that occurred as a consequence of error-prone repair of DNA damage in groups of cyanobacteria, independently. The trait could also spread through horizontal gene transfer, ensuring higher resistance than the heterotrophic prokaryotes that are more efficient in utilizing organic carbon sources in the media. Such a model predicts that loss of the ability to utilize multiple organic carbon sources in the dark would ensure higher survival value, thereby explaining why most photoheterotrophic cyanobacteria cannot utilize multiple carbon sources[11].

The adaptation to such a lifestyle ensuring higher dark-survival resulted in abundance of 'obligate' photoautotrophs among cyanobacteria in all their ecological niches. The emergence of mixotrophs or facultative photoheterotrophs, as they are sometimes referred to, may have been a later event in the evolution, when UV stress from solar radiation reaching the earth's surface diminished because of the build-up of an ozone screen following accumulation of gaseous oxygen. In the Precambrian era, 'obligate' photoautotrophs may have been more abundant than are represented in extant communities, which breathed out molecular oxygen that was eventually responsible for the build-up of ozone layer. The trait, perhaps, was generated simply by loss of the ability to utilize organic carbon from an external medium. Consequently, the growth phase was tightly regulated by the light–dark cycle. Thus UV resistance could be enhanced without inventing any novel DNA-repair system that was not already present in their non-photosynthetic progenitors. Instead, an additional mechanism of screening UV radiation had evolved, in which UV-absorbing compounds were metabolized following induction to protect the cyanobacterial cells due to stress from the UV component of solar radiation[20].

High UV resistance during photoautotrophic growth compared to photoheterotrophic growth in both light and dark phases, suggests at least one regulatory mechanism of protection/repair that operates only in the photoautotrophic mode. Unfortunately, not much is known about DNA repair and chromosome replication in cyanobacteria[21]. Therefore, several alternative mechanisms have to be considered at this stage. Two possibilities are as follows. (1) A component or subassembly of photosynthetic electron transport system that is expressed only in photoautotrophic mode may repair or protect against UV-induced injury. For instance, a study[22] using microarray technique shows that switching from photoautotrophy to heterotrophy in PCC6803 inhibits the transcription of photosynthetic electron-transport component genes, but enhances the expression of SOS regulator gene *lexA*. (2) The enhanced UV resistance can also be due to an epigenetic process of repair through induced homologous recombination that may be RecA-dependent. A knockout homozygous *recA* mutant of *Synechocystis* PCC 6803 shows high sensitivity to UVC both in light and dark phases, though the switching is not eliminated[14]. Yet the promotor of the *recA* gene in this strain does not contain the LexA-binding site required for the induction of *recA*–*lexA*-dependent network of DNA repair system[23]. Though the absence of LexA-binding site does not necessarily rule out a modified SOS response in this strain, the RecA protein might be involved in a yet unknown function for repair of lesions on DNA that accumulate from oxidative damage during autotrophic growth in light. Some components of homologous recombination in cyanobacteria may be induced by injury to DNA that ensures repair for several generations, as has been seen in higher plants but not reported for chemoheterotrophs[24].

The light–dark cycle is perhaps the most significant and persistent selection agent that has shaped the physiology of photoautotrophs all through their origin and evolution. This study clearly points to a dominant role of diurnal rhythm on regulation of the mode of carbon utilization in cyanobacteria. A strict obligatory dependence of growth during light exposure would force a prolonged period of DNA dark-repair activity synchronous with the dark phase in the diurnal rhythm, to ensure healthy beginning of growth processes under the morning sun. Restriction of growth and intense housekeeping activity without cell division respectively, to light and dark phases, may be entrained by the light–dark cycle in the natural habitats of cyanobacteria. Such entrainment, in turn, may be forced by metabolic cycles because of dependence of growth primarily on photosynthases and redox state of the cells. Tu and McKnight[25] suggest that diverse biological oscillations driven by metabolic cycles have a common evolutionary origin across all living organisms, because of economy of nutrient utilization and survival value.

Though they did not consider microbes in formulating their general conclusion, the oscillation of UV sensitivity of obligate photoautotrophic cyanobacteria in their natural habitats may be the most striking example of survival strategy among microbes, substantiating the proposal.

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RENU MINDA[1]
VASHUDHA P. JOSHI[2]
SWAPAN K. BHATTACHARJEE[3,*]

[1]*Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Colaba, Mumbai 400 005, India*
[2]*Molecular Biology Division, Bhabha Atomic Research Centre, Mumbai 400 085, India*
[3]*School of Life Sciences, Devi Ahilya Vishwavidyalaya, Vigyan Bhawan, Khandwa Road, Indore 452 001, India*
**For correspondence.*
e-mail: swapan1943@yahoo.co.in

Assessment of air pollution in Aizawl city

A study of air pollution was carried out in Aizawl city during 2006–07. The study sites selected and their station types are given in Table 1.

The objectives of study were to estimate suspended particulate matter (SPM), respirable suspended particulate matter (RSPM), nitrogen dioxide (NO_2) and sulphur dioxide (SO_2).

The ambient air quality at four different stations was monitored from 28 September to 24 November 2006, and from 2 March to 29 June 2007. Analysis was done once every week and the sample monitored for 8 h. The absorbing reagents and filter paper were kept a day before analysis. After monitoring for 8 h, the sample was then taken back to the laboratory for analysis.

SPM in the atmosphere was determined using high volume method. RSPM in the ambient air was determined using the cyclonic flow technique. A respirable dust sampler was used for the estimation of RSPM and SPM.

NO_2 in the atmosphere was determined using Jacob and Hochheiser modified (sodium arsenite) method[1]. SO_2 in the air was determined using the modified West and Gaeke method[2].

The present study shows that the concentration of SPM, RSPM, NO_2 and SO_2 varies greatly from one station to another. The study was done based on the National Ambient Air Quality Standards given by the Central Pollution Control Board[3]. The average concentration of the estimated particulate matter and gases is given in Table 2.

The average concentration of SPM in Bawngkawn was the highest at 131.85 $\mu g/m^3$ and was lowest at the MZU Campus (38 $\mu g/m^3$). Khatla with an average of 83.95 $\mu g/m^3$ is in the medium range as well as Laipuitlang (63.56 $\mu g/m^3$). Also, from the data obtained, SPM analysed in 2007 was comparatively higher compared to 2006, which could be due to the slash and burn method of agriculture practised widely in the Northeast and commonly in Mizoram. Various activities like power generation, demolition, spraying, grinding, agriculture and stone quarrying generate SPM. Automobile exhaust has been found to contain 40–50 $\mu g/l$ SPM; thus some areas with high vehicle density like Bawngkawn have the highest SPM.

RSPM is injurious to health. Particulate in the size range up to 10 mm can be considered as RSPM. The RSPM concentration at all the stations was more or less similar, ranging from 38.06 $\mu g/m^3$