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Dinitrogen fixation by Cyanobacteria

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Abstract

Nitrogen is the most abundant element in the Earth's atmosphere in the form of di-nitrogen gas; it is not assimilable by plants or any other organism as such. Biological Nitrogen Fixation (BNF) is carried out by a specialized group of prokaryotes, like Cyanobacteria. These microorganisms fix the atmospheric nitrogen (N_2) to ammonia (NH_3) using a complex of enzymes called Nitrogenase. According to one estimate, total biologically fixed nitrogen approaches approx. 2×10^2 Mt annually, and the average nitrogen fixation is estimated to be approx. 90 kg N $ha^{-1}Y^{-1}$. Cyanobacteria occur in almost every conceivable habitat, including soil, on rocks, in freshwater, and saltwater, and these are cosmopolitan prokaryotes. Cyanobacteria have been divided into five 'subsections' or orders according to Bergey's Manual. Of these five subsections, three are for non-heterocystous types and two for heterocystous types. They have an unmatched range of symbiotic hosts (e.g., protists, fungi, sponges, and angiosperms) and are not restricted to roots only but may enter a symbiotic association with any plant part and need not be housed intracellularly within the host plants. All plant-associated cyanobionts can differentiate specialized nitrogen-fixing cells known as heterocysts. Heterocyst polar nodules may function as nitrogen storage products and share the same composition as cyanophycin granules, which are copolymers of aspartate and arginine. Heterocysts are distinguished from ordinary vegetative cells by their considerably larger and rounder form, decreased pigmentation, thicker cell envelopes, and typically noticeable cyanophycin granules at poles. Heterocysts' extra envelope layers shield the enzyme Nitrogenase from oxygen's harmful rays. There are about 20 genes organized as large contiguous genomic regions in a heterocyst, which are required to be expressed as upregulated in a coordinated fashion to synthesize heterocyst-specific envelopes. NtcA, as a transcription activator, has been identified to switch on the genes involved in heterocyst differentiation as well as in Nitrogenase synthesis. HetR, a serine-type protease also known as a specific master regulator, works in association with NtcA. Transcriptional regulation by NtcA is modulated per C:N balance of the cells, with 2-oxoglutarate serving as the actual effector molecule. The C/N ratio, thus, is crucial to setting early steps of heterocyst differentiation. The nif genes, which are activated by the NtcA protein, undergo programmed rearrangements during nitrogen fixation. Anabaena PCC 7120 has been shown to contain a stretch of 17 genes that encode the Nitrogenase complex. Two major clusters have been identified among these 17 genes: (a) nifB–fdxN–nifS–nifU gene cluster (of which the nifB, nifS, and nifU encode the complex FeMo cofactor assembly and fdxN encodes ferredoxin) and (b) nifH-nifD-nifK gene cluster, wherein nifH encodes Dinitrogenase reductase and nifD and nifK encode, respectively alpha and beta subunits of Dinitrogenase. The Nitrogenase enzyme complex is a multimeric complex of protein enzymes. Numerous filamentous and unicellular heterocystous and non-heterocystous cyanobacteria species, were able to fix molecular nitrogen both temporally and spatially when they synthesize the enzyme Nitrogenase complex. However, it protects the Nitrogenase enzyme complex from oxidative deactivation. There must be temporal shifts for oxygenic photosynthesis during the day and anoxic Nitrogenase activity during dark periods. The assimilatory form of N_2 for photosynthetic organisms is ammonium, which becomes incorporated into carbon skeletons metabolically. It is the most reduced inorganic form of nitrogen, which is available for assimilation. Fixed nitrogen as NH^{4+}

is energetically a favoured form. $NH^{4+,}$ either as direct uptake from the medium or as metabolically produced from alternative sources of nitrogen, is incorporated into carbon skeletons via the sequential activity of two enzymes, Glutamine Synthetase (GS) and Glutamate Synthase (Glutamine Oxoglutarate Aminotransferase-GOGAT), of a cycle commonly known as the GS-GOGAT pathway.

Keywords: *Nitrogen, Cyanobacteria (BGA), symbiosis, Heterocysts, nif genes, Nitrogenase enzyme, Glutamine synthetase and Glutamate synthase*

1. INTRODUCTION

Nitrogen is a major element of many critically important macromolecular components of cells, like proteins, chlorophylls, and nucleic acids, and many metabolically active simple molecules like ATP, GTP, etc., making it a critical limiting factor for plant growth and development. Although nitrogen is the most abundant element in the Earth's atmosphere in the form of di-nitrogen gas, it is not assimilable by plants or any other organism as such. Plants can intake nitrogen only as its reductively fixed derivatives. Plants acquire fixed nitrogen in four ways. (1) from ammonia and/or nitrate fertilizers or manures, (2) from biodegradation of dead and decaying matter (3) from naturally produced fixed nitrogen like lightning, etc., and (4) from biologically fixed nitrogen released in soil (Vance, 2001). Biological Nitrogen Fixation (BNF) is carried out by a specialized group of prokaryotes (Beijerinck, 1901). These microorganisms fix the atmospheric nitrogen (N_2) to ammonia (NH_3) using a complex of enzymes called Nitrogenase. Starting with NH_3, the fixed form of nitrogen, plants can manufacture all the molecules that are critical for plant life. These prokaryotic organisms include a variety of microbes like cyanobacteria, etc, which enter a symbiotic association with various groups of plants and lichen. (Postgate, 1982). Also known as blue-green algae, cyanobacteria are the most diverse photosynthetic bacteria. They are gram-negative bacteria endowed with chlorophyll a and photosystems I and II, allowing them to perform oxygenic photosynthesis. Cyanobacteria occur in almost every conceivable habitat, including soil, on rocks, in freshwater, and saltwater, and these are cosmopolitan prokaryotes (Hoffmann, 1989; Kulasooriya, 1998). They modify the physicochemical parameters of the environment in which they occur and increase the oxygen concentration (Mandal et al., 1998; Hu et al., 2003). Cyanobacteria secrete polysaccharides that bind soil particles and thereby promote soil stability, minimize soil erosion due to runoff, and ensure site availability for the germination of higher plants. Cyanobacteria thrive optimally in conditions of neutral to slightly alkaline pH of the soil and flourish in good diversity in nitrogen-deficient environments (Singh, 1961; Kaushik, 1991). However, acidic soils below pH 5 create growth stress for the cyanobacteria, although eukaryotic algae flourish well in such conditions. Thus, soil acidity had a selective effect on the succession and abundance of algal flora. Cyanobacterial species of Nostoc, Anabaena, Cylindrospermum, Scytonema, Westiellopsis, Tolypothrix, Aulosira, and several other genera are abundant in rice fields and are known to significantly contribute to their fertility (Kaushik,1991; Nayak et al., 2004). The formation of heterocysts is the most important event in cyanobacterial nitrogen fixation. The vegetative cells of the cyanobacterial filament are stimulated to differentiate into heterocysts when dissolved nitrogen in their habitat is poor in supply (Fig. 1).

Fig 1: Trichome (filamentous structure) with heterocysts of Anabaena Sp. (Tsukii., 2003)

A heterocyst is thick-walled and contains only photosystem I for cyclic photophosphorylation for the production of ATP as an adaptation for the requirement of an oxygen-free environment for nitrogen fixation by Nitrogenase enzyme complex, which becomes deactivated in the presence of oxygen. Diazotrophic cyanobacteria have acquired great ecological significance as "gatekeepers" of ecosystem productivity (Falkowski and Raven, 1997). N_2-fixing cyanobacteria have been recognized as potential agents of heavy metal bioremediation from the soil, biofertilizers, bioenergy production, and other high-value products (e.g., characteristic phycocyanin and phycoerythrin pigments as antioxidants, cosmetics derived from exopolysaccharides, UV sunscreens, etc.) (Heimann and Cires, 2015). In this review paper, we will discuss cyanobacterial characteristics, their classification, survival conditions, the structure of heterocysts, their differentiation, and their role in nitrogen fixation as an ecosystem function.

2. CLASSIFICATION OF CYANOBACTERIA

Cyanobacteria have been divided into five 'subsections' or orders according to Bergey's Manual. Of these five subsections, three are for non-heterocystous types and two for heterocystous types (Bergman et. al., 1997; Castenholz, 2001). These subdivisions of cyanobacteria are based on filament morphology and reproductive patterns.

(A) The non-heterocystous cyanobacteria:

Subsection (I): Chroococcales, include unicellular rods and cocci which reproduce by binary fission or budding. e.g., Gloeothece, Merismopedia, Microcystis, Aphanocapsa, Gloeocapsa

Subsection (II): Pleurocapsales, include rods and cocci, which are held together in aggregates, and daughter cells produced are smaller than the parent. e.g., Pleurocapsa, Chroococcidiopsis, Dermocarpella.

Subsection (III): Oscillatoriales, which include cyanobacteria that produce filaments of cells, are called trichomes. e.g., Lyngbya, Oscillatoria, Planktothrix, Microcoleus.

All three of the above subsections have N_2-fixing representatives also.

(B) The heterocystous cyanobacteria:

Subsection (IV): Nostocales, the vegetative cells in the trichome here and there are differentiated into heterocysts and akinetes. Both kinds of cells are thick-walled and serve as the sites of N_2 fixation and perennating structures, respectively. e.g., Nostoc, Calothrix, Anabaena

Subsection (V): Stigonematales, which include such representatives whose trichomes have heterogeneous cellular components along with heterocysts and akinetes. e.g., Stigonema, Mastigocladus.

N_2-fixing cyanobacteria are also classified based on differential behavioral activity regarding nitrogen fixation into categories.

A. Cyanobacteria that can fix N_2 aerobically

A1. Cyanobacteria that temporally separated N_2 fixation from oxygenic photosynthesis i.e., non-heterocystous genera, e.g., Gloeothece, Cyanothece, and Lyngbya.

A2. Cyanobacteria that spatially separated N_2 fixation from oxygenic photosynthesis i.e., heterocystous genera, e.g., Anabaena.

A3. Cyanobacteria that are temporally and spatially separated N_2 fixation from oxygenic photosynthesis. Include non-heterocystous genera, e.g., Trichodesmium and Katagnymene.

B. Cyanobacteria that can fix N_2 either anaerobically or microaerobically

B1. Many non-heterocystous cyanobacteria, e.g., Plectonemaboryanum

Strains of Cyanobacteria have also been classified into seven broader taxa based on morphological features as well as the ability to form mucilaginous colonies taken as major characters (Rippka et al., 1979; Grant et al.,1985). The following recognized taxa are

- **Nostoc group:** Heterocyclous strains with a dense outer layer, devoid of branching, and producing mucilaginous colonies with distinct morphology (Nostoc).
- **Anabaena group:** Heterocystous strains lacking trichome branching and without mucilaginous colonies of definite shape (Anabaena, Nodularia, Cylindrospermum, Anabaenopsis, etc.).
- **Fischerella group:** Heterocystous strains exhibiting true branching (Fischerella, Westiellopsis, Stigonema).
- **Scytoneina group:** Heterocystous strains having false branching, lacking polarity, and producing velvet-like patches on an agar medium (Scytonema).
- **Aulosira group:** Heterocystous strains having a thick sheath, usually without branching, and do not show diffuse colonies on agar medium (Aulosira).
- **Calothrix group:** Heterocystous strains having false branching, displaying polarity, and producing velvet-like patches on agar medium (Calothrix, Tolypothrix, Hassalia).
- **Gloeotrichia group:** Heterocystous strains, exhibiting polarity and generating mucilaginous colonies of distinct shape (Gloeotrichia, Rivularia).

3. THE ROLE OF ABIOTIC VARIABLES ON CYANOBACTERIA POPULATION AND SURVIVAL

The only prokaryotes capable of oxygenic photosynthesis are cyanobacteria. They can be found in a variety of environmental settings and are exposed to a range of natural stresses, including pollution, desiccation, pH, insolation, and nutrient distress (Herrero et al., 2001; Giardi et al., 1997; Stal, 2007). Increased levels of both organic and inorganic nutrients have a linear relationship with soil temperature, moisture content, and sunshine penetration, as does the algal population and its activities. Different algal taxa, however, respond differently to different soil pH values. For instance, green algae prefer more acidic soils ($pH \leq 5.5$), while cyanobacteria do better in alkaline soils ($pH \geq 7.0$). Pesticides, herbicides, fungicides, and soil fumigants are generally hazardous to cyanobacterial growth (Issa et al., 2013).

Environmental physicochemical imbalances can have a negative impact on a specific species, but they can also encourage the growth and abundance of other species, allowing many species to successively arise over time (Muthukumar et al., 2007). High temperatures promote increased productivity in blue-green algae and phytoplankton (Roger and Reynaud, 1979). In rice fields with abundant soil and water organic matter, cyanobacteria thrive quickly (Hasan, 2020). pH is the most essential soil property that influences cyanobacteria growth, establishment, and diversity. It has been observed that cyanobacteria prefer neutral to slightly alkaline pH for optimal growth (Chandra and Rajashekhar, 2016), while certain species, such as *Aulosira fertilissima* and *Calothrix brevissima*, are widespread at lower pH in Kerala rice fields (pH 3.5 to 6.5). Subhashini and Kaushik (1981) also documented a decrease in soil pH in rice fields when treated with cyanobacteria. On the contrary, Prasanna and Nayak (2007) found that cyanobacteria were more abundant in rice fields with high pH. Cyanobacteria have also been shown to increase soil physicochemical qualities by supplementing them with carbon, nitrogen, and phosphorus (Abinandan et al., 2019).

Cyanobacteria are spectacular organisms because they not only fix CO_2 through photosynthesis, but they can also fix atmospheric molecular nitrogen to NH_3. Both of these processes play significant roles in humification. Further, cyanobacteria produce considerable amounts of polysaccharides, which help aggregate soil colloids, improving soil structure and infiltration and percolation of water. Subsurface soil cyanobacteria have also been linked to encouraging root growth by producing hormones and stimulating the activity of other beneficial root-associated microbes (Singh et al., 2011; Gonçalves, 2021).

4. SYMBIOSIS AND ONTOGENY OF CYANOBACTERIA

Symbiotically competent cyanobacteria have a prized distinction of being included among N_2-fixing symbioses with crops of commercial interest, like cereals. Unlike rhizobia, most symbiotic cyanobacteria have mechanisms to protect Nitrogenase from oxidative inactivation (e.g., heterocysts). They have an unmatched range of symbiotic hosts (e.g., protists, fungi, sponges, and angiosperms) and are not restricted to roots only but may enter a symbiotic association with any plant part and need not be housed intracellularly within the host plants (Warshan, 2017; Barman et al., 2019). Cyanobionts, as they are referred to vis-à-vis the other symbiotic partner, generally supply fixed nitrogen to their hosts, although they can supply fixed carbon too in case the symbiotic partner is heterotrophic. The major symbiotic plant hosts are the water-fern Azolla, bryophytes, cycads, the angiosperm, Gunnera, and fungi (to form obligate symbiotic associations called lichens). Despite being photoautotrophic, cyanobacteria can also exhibit facultative heterotrophy and hence are not limited to photolit parts of the plant body, but can be found in roots, stems, leaves, and thalli (Meeks, 2003). All plant-associated cyanobionts share two major characteristics: (i) the ability to differentiate specialized nitrogen- fixing cells known as heterocysts (Rascio et al., 2008), and (ii) the ability to produce short, motile filaments known as hormogonia, which provide a means of dispersal and propagation for otherwise immotile cyanobacteria (Meeks, 1998).

Heterocysts are typically found singly at semi-regular intervals within vegetative cell filaments (Golden and Yoon, 2003; Zhang et al., 2006). Hormogonia is the primary mode of propagation in most plant symbioses, and some, if not all, plants emit chemical signals that cause the development of these propagules, while chemoattractants direct them into plant tissue. The most frequent plant cyanobionts are from the Nostoc genus and are free-living (Rai et al., 2002). Meeks and Elhai (2002) discovered that the hormogonia acts as both a method of dissemination and plant infection in the case of Nostoc sp. Hormogonia develops in response to a range of environmental conditions, including plant-derived chemical signals. Rapid and synchronous divisions in heterocystous cyanobacterial thallus cells reduce cell size, causing breakdown at heterocyst-vegetative cell junctions and hormogonia (Meeks and Elhai, 2002). Hormogonia lacks heterocysts and represents a transient stage in the Nostoc life cycle, returning to vegetative growth and continuing the generation of hormogonia in a typical manner. Hormogonia locates the symbiotic tissue of a plant host by connecting to tissue surfaces via extracellular polysaccharides and pili (fimbriae) (Adams, 2000; Bhaya, 2004; Duggan et al., 2007). Hormogonia growth in cyanobionts is triggered by symbiotic hosts synthesizing hormogonia-inducing factors (HIFs), which have been discovered in the hornwort Anthoceros punctatus (Meeks, 2003), cycads, and the angiosperm Gunnera (Rasmussen et al., 1994; Ow et al., 1999). In Anthoceros punctatus, HIF is a tiny, heat-labile substance produced when the organism is depleted of combined nitrogen (Meeks, 2003). According to Taylor and Krings (2005) and Villarreal and Renzaglia (2006), fossil specimens of the plant provide evidence that cyanobacteria first colonized the substomatal chambers before spreading over the outer cortical tissue. Once inside the host plant, the cyanobacterium undergoes some morphological, developmental, and physiological changes, such as suppression of hormogonia development, stimulation of heterocyst development, reduction in cell division to prevent cyanobacteria from outgrowing the host, elevation of the nitrogen fixation rate, decrease in CO_2 fixation rate, etc. The most prevalent symbiotic relationship is that which exists between the cyanobacteria *Anabaena azollae* and the pteridophytes Azolla.

5. ROLE OF CYANOBACTERIA AS A BIOFERTILIZER

Inorganic nitrogen fertilizers require high inputs in the form of fossil fuels and hence incur huge production costs. A powerful alternative to this, which is environmentally sustainable and largely cost-effective, is biologically sourced nitrogen (Vance, 2001; Mahanty et al., 2017). Biological systems with the ability to fix atmospheric nitrogen have been identified and standardized (Vaishampayan et al., 2001). According to one estimate, total biologically fixed nitrogen approaches approx. 2×10^2 Mt annually (Guerrero et al., 1981). Metting (1988) estimates the average nitrogen fixation to be approximately 90 kg N $ha^{-1}Y^{-1}$. Many free-living blue-green algae that fix atmospheric nitrogen neither compete with crop plants nor with heterotrophic soil microflora for either

energy or carbon because of being autotrophic. Nitrogen-fixing ability is not the sole preserve of only filamentous heterocystous cyanobacteria, even many non-heterocystous and unicellular cyanobacteria can also fix nitrogen. Cyanobacteria can add up to 20-40 kg N ha^{-1} $season^{-1}$ along with organic matter to the soil, which is quite significant for the economically weaker farmers who find it hard to bear heavy input costs of inorganic fertilizers. Trichodesmium, a non-heterocystous genus, contributes approximately 100 Tg N y^{-1}. Tropical conditions favour blooms of free-living cyanobacteria, and traditionally, Asian countries have been using their inocula in paddy fields as biofertilizers to replenish nitrogen requirements. Besides paddy, crop plants like vegetables, wheat, sorghum, maize, cotton, sugarcane, etc., also respond to cyanobacterial biofertilizers (Abd-Alla et al., 1994). In subtropical regions, Azolla-Anabaena symbiosis is traditionally used as a biofertilizer (Kimura, 2000; Kirk, 2004). In addition to providing nitrogen to crops, cyanobacteria also help plants grow by generating gibberellins, auxins (such as Indole-3-Acetic Acid, Indole-3-Propionic Acid, etc.), vitamin B12, free amino acids (such as serine, arginine, glycine, aspartic acid, threonine, glutamic acid, etc.), and extracellular and intracellular polysaccharides (such as xylose, galactose, fructose, etc.). According to El-Enany and Issa (2000), these compounds have several advantages, including enhancing soil structure, promoting the growth of beneficial microbes and crop plants, and chelating heavy metals. Numerous research investigations have examined the prevalence and significance of cyanobacteria in the biological nitrogen fixation process that sustains soil fertility in paddy fields (Fogg and Stewart, 1968). Rice fields are often algalized using mixed cultures of free-living cyanobacteria (Chittora et al., 2020). Cyanobacteria *Anabaena azollae* harbored in fronds of water fern Azolla releases ammonium into the water when paddy fields are inoculated with foam-immobilized *A. azollae* strains (Kannaiyan et al., 1997). Azolla can absorb ammonium/nitrates from the water, but it, instead, absorbs ammonia secreted by Anabaena within its leaf cavities and thus Azolla-Anabaena symbiosis plays a very important role in rice production. China and other Asian countries have been traditionally using Azolla and its symbiotic partner Anabaena for centuries as green manures to fertilize rice paddies to increase production. According to some estimates, in the absence of crop rotation, Azolla can increase rice yields by up to 158% each year without lowering output year after year. *Anabaena doliolum* and *A. fertilissima* inoculation, either with or without urea, can result in increased biomass and nutritional value as well as a notable improvement in grain yield in paddy fields (Dubey and Rai, 1995). Numerous cyanobacterial species have been linked to increased rice field productivity in Chile, including *Anabaena iyengarii* var. tenuis, *A. fertilissima, Nostoc commune, N. ellipsosporum, N. linckia, and Gloeotrichia natans* (Pereira et al., 2009). An estimate of 12.5 kg ha^{-1} of cyanobacterial biofertilizer is needed to boost rice production sufficiently both quantitatively and qualitatively (Rai and Sharma, 2006; Rastogi and Sinha, 2009; Toribio et al., 2020).

6. HETEROCYST: ULTRASTRUCTURE, LIFECYCLE, AND ROLE IN CYANOBACTERIA

6.1. Ultrastructure

The plasma membrane and an outer membrane with a layer of peptidoglycan sandwiched in between are the two separate membranes that make up the Gram-negative cell wall of cyanobacteria. Compared to other gram-negative bacteria, they have a thicker peptidoglycan coating (Hoiczyk and Hansel, 2000). The glycocalyx, which is rich in carbohydrates and may have varying proportions of three distinct layers—a defined capsule, a closely related sheath, and loosely associated slime is located outside the cell envelope. It shields the cells from desiccation and, hopefully, from phages and predators. In the cytoplasm of their cells, cyanobacteria have extensive thylakoid membranes that act as locations for photosynthesis (Fig. 2). Heterocysts are distinguished from ordinary vegetative cells by their considerably larger and rounder form, decreased pigmentation, thicker cell envelopes, and typically noticeable cyanophycin granules at poles (Fig. 2). Heterocysts' extra envelope layers shield the enzyme Nitrogenase from oxygen's harmful rays. Mature heterocysts spatially separate oxygenically photosynthetic vegetative cells to ensure the microoxic environment needed for nitrogen fixation. Heterocyst-forming cells go through a variety of morphological and metabolic alterations (Golden and Yoon, 1998). Two extra envelope layers are deposited around the heterocyst as part of the morphological changes: an exterior layer of polysaccharide (HEP) and an inner layer of glycolipids peculiar to heterocysts (HGL) (Cardemil and Wolk,

1981; Nicolaisen et al., 2009). Pro-heterocyst thylakoid membranes reorganize into mature heterocysts' so-called honeycomb membranes (Fig. 2a, b). Because heterocysts have a thicker envelope and necked ends, the septa between them are narrower than those between vegetative cells, resulting in a smaller area of exchange (Walsby, 2007). Respiratory enzymes at honeycomb membranes close to neighboring cell junctions reduce the quantity of oxygen that enters the heterocyst. Furthermore, the heterocysts have adaptively stopped O_2 evolving photosystem-II in order to maintain photosystem-I for cyclic photophosphorylation and the RUBISCO system for regular CO_2 fixation to carbohydrates (Maldener et al., 2010; Zhao and Wolk., 2008). This has allowed them to maintain a minimal O_2 titer. The genes responsible for Nitrogenase subunits and cofactor production are activated to initiate N_2 fixation and resume active filament development once the heterocyst reaches a microaerobic state. Terminally developed cells that don't divide are known as mature heterocysts (Wolk et al., 1994; Xu et al., 2008).

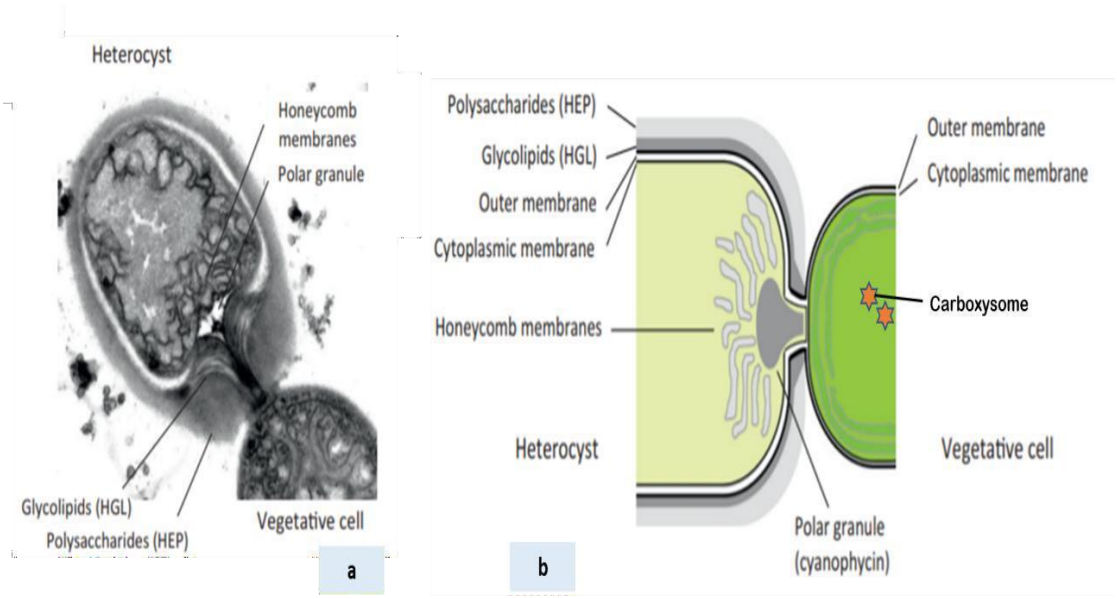

Fig.2. The Heterocyst and heterocyst-vegetative system. (a). A transmission electron micrograph of a terminal heterocyst and its neighbouring vegetative cell from the filament of Anabaena sp. PCC 7120 (or Nostoc sp. PCC7120). The cyanophycin granule (CPG) is often destroyed during sample preparation for electron microscopy, producing a white void in the micrograph. (b). The diagrammatic section shows the neighbouring vegetative cell and the heterocyst. Along the filament, there is a continuous periplasm that is bounded by the cytoplasmic and outer membranes (CM and OM) and contains the peptidoglycan (HGL & HEP) (Courtesy of Iris Maldener, Universität Tubingen, Germany).

6.2. Lifecycle of Heterocysts formation and their role:

Heterocyst differentiation occurs in response to a deficiency of combined nitrogen in the environment (Garcia-Pichel, 2009). When coupled nitrogen is restored in a filament environment, heterocysts retro differentiate into intervening vegetative cells and begin dividing (Flores E, Herrero, 2010). In other heterocyst-forming strains, some vegetative cells differentiate into akinetes as overwintering structures in response to energy-limited conditions, and when favorable conditions return, they germinate into filaments with or without heterocysts, depending on the availability of combined nitrogen. *Nostoc punctiforme* PCC 73102, like many other filamentous cyanobacteria, can produce hormogonia, which can act as infection units to build symbiotic relationships with plants. Hormogonia has been shown to differentiate when stimulated by certain environmental conditions, such as nitrogen deficiency, as well as by a hormogonium-inducing substance produced by a symbiotic co-host (Meeks and Elhai, 2002). Hormogonia form filaments that may or may not contain heterocysts, depending on the availability of combined nitrogen (Fig. 3).

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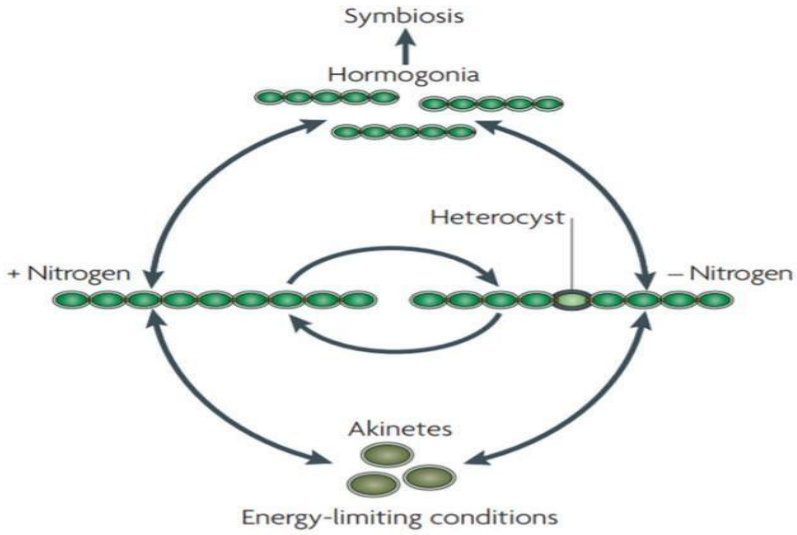

Fig.3. Life cycle of Heterocysts formation under deprived soil Nitrogen (Adapted and modified)

Heterocysts are highly specialized structures for performing fixation of dinitrogen under aerobic conditions. They represent a melange of adaptations aimed at preventing oxic deactivation of Nitrogenase complex in an environment of oxygenic photosynthesis. Heterocysts are the only cyanobacterial cells in which nif (nitrogen fixation) genes are expressed, and Nitrogenase is synthesized. The reductant for nitrogen fixation is funneled to heterocysts by the adjacent vegetative cells in exchange for fixed nitrogen from the heterocyst in the form of amino acids (mostly glutamine). Thick enveloping layers, absence of photosystem-II, and increased rates of cellular respiration to mop up diffusive oxygen help heterocysts keep the partial pressure of oxygen significantly low to prevent oxidative inactivation of Nitrogenase. Heterocysts provide fixed nitrogen in exchange for fixed carbon and reductant from symbiotically associated vegetative cells (Wolk et al., 994) (Fig.4).

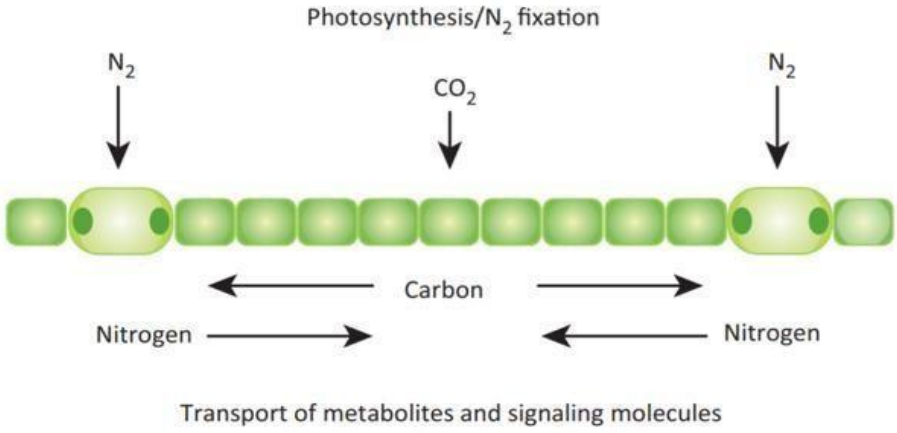

Fig.4: Role of Vegetative and Heterocyst cells

Vegetative cell

Photolysis of water
$2H_2O \rightarrow 4H^+ + 4e^- + O_2$
Carbon dioxide fixation
$CO_2 + 4H^+ + 4e^- \rightarrow [CH_2O] + H_2O$

Heterocyst

Nitrogen fixation
$N_2 + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2$
Hydrogen uptake
$2H_2 + O_2 \rightarrow 2H_2O$

Both cell types

Carbohydrate oxidation
$[CH_2O] + H_2O \rightarrow CO_2 + 4H^+ + 4e^-$
Respiration
$4H^+ + 4e^- + O_2 \rightarrow 2H_2O$

7. HETEROCYSTS DIFFERENTIATION AND MATURATION

Heterocysts are specialized nitrogen-fixing cells that differentiate in the trichome at semi-regular intervals (after 10-20 vegetative cells) during nitrogen deprivation (Wei et al., 1994; Muro-Pastor and Hess, 2012). Nitrogenase activity and NifH biosynthesis occur only in heterocysts (Plominsky et al., 2013). The growth of heterocysts requires around 25% of the genome (roughly 1000 genes), as well as communication between outside signals and vegetative filaments (Flores and Herrero, 2010). Enzymes such as RUBISCO, Nitrate reductase, and Glutamate synthase (GOGAT) are inactive in mature heterocysts (Rai and Bergman, 1986; Kumar et al., 2010). When the supply of coupled nitrogen is reduced, heterocysts become anatomically distinct from vegetative cells after around 8 to 9 hours in a typical laboratory environment. They thicken and narrow their necks, and septal growth at vegetative cell ends results in decreased exchange regions (Fig.2). Under nitrogen deficiency conditions, vegetative cells intended to develop into heterocysts degrade the phycobilisome (PBS) and first differentiate into proheterocysts (presumptive heterocysts), an intermediate stage of incomplete differentiation of a mature heterocyst (Fig. 5). The pro-heterocystous stage persists as long as the microoxic environment is not reached for functional Nitrogenase activity. Proheterocysts also lack the nifHDK gene rearrangement necessary for the formation of functioning Nitrogenase enzyme complexes (Haselkorn et al., 1998).

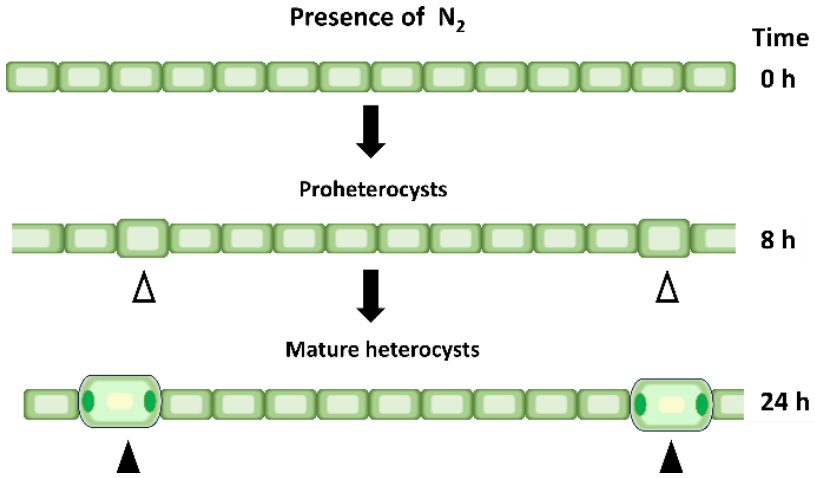

Fig.5. Pattern differentiation of Heterocysts in the absence of combined nitrogen

Proheterocysts pass through seven developmental stages to turn into heterocysts. In the I and II stages, there develop fibrous layers that delimit the proheterocyst from the vegetative cells. In the III and IV stages, photosynthetic lamellae undergo disorganization with a concomitant increase in intercellular spaces and laying inner laminated layer being laid down. In stage V, heterocysts acquire their distinct appearance, and in the last two stages (stages VI and VII), polar nodules become organized and the heterocyst becomes fully mature. Teramoto et al. (2018) observed temporal changes in the distribution of the cellular C/N ratio in a soft X-ray imaging experiment on nitrogen-starved cells of the filamentous cyanobacterium Anabaena sp. PCC 7120. The experiment proved that cells with high C/N ratios were destined to differentiate into heterocysts.

7.1 Regulation of heterocyst differentiation and pattern formation

There are about 20 genes organized as large contiguous genomic regions in a heterocyst, which are required to be expressed as upregulated in a coordinated fashion to synthesize heterocyst-specific envelopes (Fa et al., 2005; Huan et al., 2005). Nitrogen deprivation leads to dramatic changes in the pattern of gene expression. The NtcA protein is a transcriptional regulator belonging to the cAMP receptor protein (CRP) family. It has been observed to be involved in global nitrogen regulation in cyanobacteria (Herrer et al., 2001). NtcA, as a transcription activator, has been identified to switch on the genes involved in heterocyst differentiation as well as in Nitrogenase synthesis (Peter, 1997).

HetR, a serine-type protease also known as a specific master regulator, working in association with NtcA, has been implicated in the regulation of heterocyst differentiation and pattern formation in cyanobacteria

(Valladares et al., 2016; Kaushik et al., 2017) (Fig. 6). However, HetR is not exclusive to heterocyst-forming cyanobacteria. It has been reported in non-heterocystous cyanobacteria also (Zhang et al., 2009). Transcriptional regulation by NtcA is modulated in accordance with C:N balance of the cells with 2-oxoglutarate serving as the actual effector molecule (Zhao et al., 2010). C/N ratio, thus, is crucial to set early steps of heterocyst differentiation (Frıas, J.E. et al. 1994; Wei, T-F. et al. 1994). In cells differentiating into heterocysts, NrrA (a response regulator) is linked with NtcA and HetR (Ehira and Ohmori, 2006a). However, under N_2-fixing conditions, nrrA has also been found to be regulated by NtcA (Ehira and Ohmori, 2006b; Muro-Pastor et al., 2006). Activation/overexpression of nrrA gene significantly influences the expression of the hetR gene and, thereby, the differentiation of heterocysts (Ehira and Ohmori, 2006a, b). Similarly, HetF, a protease, also influences development of heterocysts by inducing expression of hetR gene in cells differentiating into heterocysts (Wong and Meeks, 2001) (Fig. 6). Additionally, PatA, a response regulator, influences heterocyst development and pattern formation by nullifying PatS and HetN induced inhibitory signals (Munoz-Garcia and Ares, 2016; Orozco et al., 2006). HetC is an ATP-binding cassette-type exporter. It has been implicated in the early steps of heterocyst differentiation by regulating the morphogenesis of its envelope. If the hetC gene becomes impaired, it triggers a developmental checkpoint that would prevent further differentiation of the heterocyst (Adams, 2000). Specific structural, as well as physiological changes, have been observed during different stages of heterocyst differentiation, which initiates the morphogenesis of its envelope. The outer polysaccharide and inner glycolipid layers of the envelope minimize the entry of oxygen into the heterocyst (Fay, 1992; Halimatul et al., 2014). The delimitation of the exterior polysaccharide layer is one of the initial morphological alterations that characterize heterocyst development. The production of the polysaccharide layer involves the proteins DevR and HepK, which form a two-component regulatory mechanism (Zhou and Wolk, 2003). The hep genes (hepA,B,C) have been identified as necessary for polysaccharide layer deposition (Huang et al. 2005; Wang et al., 2007). The glycolipid layer of the heterocyst is formed beneath the polysaccharide layer via the glycosylation of fatty alcohols. The hgl gene (helB,C,D,hglE) and the DevH genes regulate glycolipid synthesis (Campbell et al., 1997; Fan et al. 2005). DevH is a trans-acting regulatory protein that either directly influences gene expression or indirectly through other gene products (Fig. 6; Ramirez et al. 2005). The hglK gene has been linked to glycolipid location and may also play a role in deposition (Black et al., 1995). The gene hglT is anticipated to encode a glycosyl transferase that is necessary for glycolipid layer production (Awai and Wolk, 2007; Halimatul et al. 2014). DevBCA and HgdD proteins, which are assumed to represent an outer membrane efflux tunnel and a glycolipid exporter, respectively, are additionally necessary for the differentiation and maturation of heterocysts (Fiedler et al., 1998; Moslavac et al., 2007a). Heterocyst maturation is subject to cell wall remodelling, which is affected by an autolysin, viz. HcwA (Zhu et al., 2001). The larger and rounder shape that heterocysts acquire could be the result of the interplay of autolysin activity and turgor pressure. Many more regulatory genes like hepK, hepN, henR, and hepS have been implicated in the normal maturation of heterocysts (Lechno-Yossef et al., 2006).

The nif genes, which are activated by the NtcA protein, undergo programmed rearrangements during nitrogen fixation. Anabaena PCC 7120 has been shown to contain a stretch of 17 genes which encode the Nitrogenase complex. Two major clusters have been identified among these 17 genes: (a) nifB–fdxN–nifS–nifU gene cluster (of which the nifB, nifS, and nifU encode the complex FeMo cofactor assembly and fdxN encodes ferredoxin) and (b) nifH-nifD-nifK gene cluster (wherein nifH encodes Dinitrogenase reductase and nifD and nifK encode respectively alpha and beta subunits of Dinitrogenase) (Rubio and Ludden, 2008).

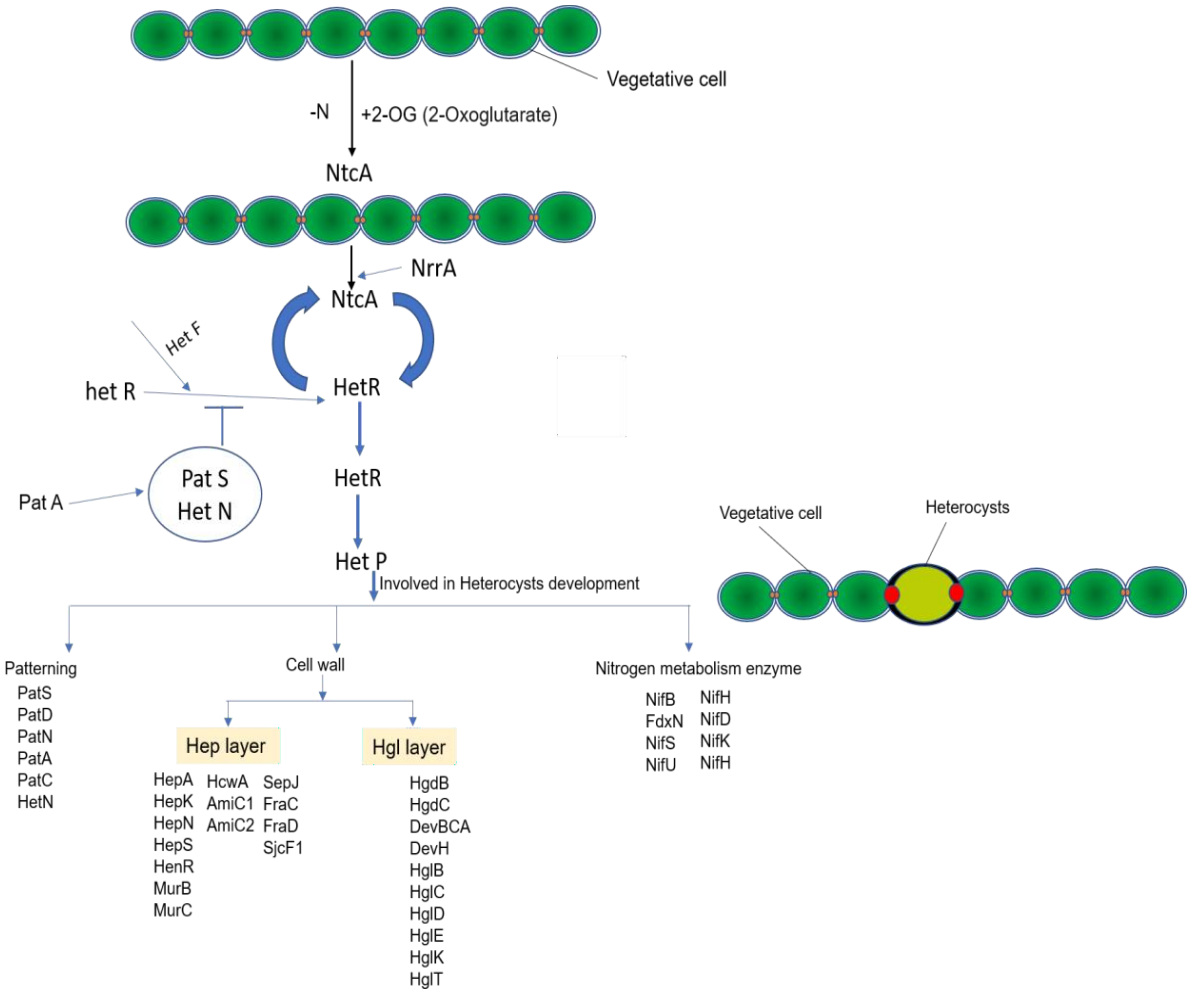

Fig.6. Schematic diagram showing various genes and proteins regulate heterocyst differentiation, maturation, and nitrogen fixation under Nitrogen deprivation.

8. BIOCHEMISTRY OF HETEROCYSTS AND THEIR ROLE IN NITROGEN FIXATION

8.1. The structure of the Nitrogenase Enzyme Complex

A gene family known as nif genes encodes the Nitrogenase complex involved in N_2-fixation. N_2 fixation is catalyzed by this enzyme complex, and its functions are encoded by three structural genes: nifD, nifK, and nifH. The first two codes for the enzyme Nitrogenase, which is responsible for holding the Fe-S cluster (also referred to as the P cluster) and the Fe-Mo cofactor, which is necessary for the nitrogen fixation reaction. Nitrogenase reductase, which carries a Fe-S cluster, binds Mg^{2+}-ATP and transfers electrons to Nitrogenase, is encoded by nifH, the final of the three structural genes (Rubio and Ludden, 2008). Labile electron carriers like ferredoxin and flavodoxin are sources of electrons. Notably, Fe-V or Fe-Fe cofactors are used by alternative Nitrogenases (Mus et al., 2018). Like other N_2-fixing bacteria, the nifHDK genes in Anabaena are grouped as a single operon with genes encoding electron donors and Nitrogenase maturation proteins (Flores et al., 2015).

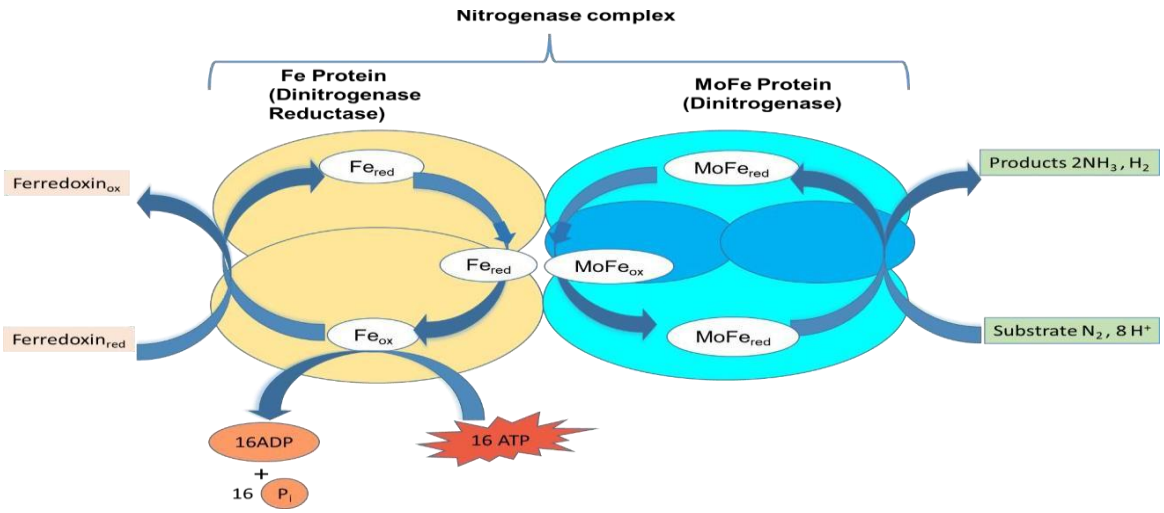

Fig.7: Schematic structure of Nitrogenase enzyme complex (Taiz and Zeiger, 2016)

The Nitrogenase enzyme complex is a multimeric complex of protein enzymes. It is made up of two different components: Iron-protein (Fe-protein), a homodimer with each subunit between 24-36 kD which acts as Dinitrogenase reductase and molybdenum-iron protein (Mo-Fe protein) protein, a tetramer or more specifically a heterodimer of homodimers with a total molecular weight of about 220 kD which acts as Dinitrogenase (Fig.7). The reactions take place when N_2 becomes bound to the Nitrogenase complex. The reductive circuitry proceeds with the Fe protein first getting reduced by electrons from ferredoxin. The reduced Fe-protein then binds ATP and further reduces the molybdenum-iron (Mo-Fe) protein, which in turn, finally transfers electrons to N_2, which becomes reduced to HN=NH. The subsequent two reduction cycles, as the first (each using electrons furnished by ferredoxin), reduce HN=NH to $H_2N\text{-}NH_2$ and, finally, $H_2N\text{-}NH_2$ to $2NH_3$ (Bothe et al., 2010). Reduction of one mole of N_2 gas to one mole of NH_3 by Nitrogenase complex consumes 16 moles of ATP, electrons, and protons (H^+). Schindelin et al. (1997) concluded that binding ATP to Fe--protein brings about substantial conformational change in the latter, causing efficient feeding of electrons to the Fe-Mo redox site. Against each electron fed to the Fe-Mo protein active site, two molecules of ATP molecules are hydrolyzed. The overall reaction stoichiometry is,

$N2 + 8\ H^+ + 8\ e^- + 16\ ATP \rightarrow 2NH3 + H2 + 16\ ADP + 16\ Pi.$

8.2. Nitrogenase enzyme activity in filamentous heterocystous cyanobacteria

In filamentous heterocystous cyanobacteria, an anaerobic environment is favoured by a strong gas diffusion barrier through a multilayer envelope, increased respiratory activity, and adaptive abortion of the oxygenic photosystem II (Scherer et al., 1982). Vegetative cells transport reductants such as ferredoxin for Nitrogenase to the heterocysts, where ATP is produced via cyclic photophosphorylation involving photosystem I (Stewart and Rowell, 1986). Most heterocystous strains identified thus far preferentially fix nitrogen in the light during a light-dark cycle (Khamees et al., 1987; Horne, 1979). A heterocyst with adjacent vegetative cells is depicted schematically in the fig. 8. The heterocyst envelope is made up of two layers: polysaccharides on the outside and glycolipids inside. Heterocysts get carbohydrates in return for glutamine from vegetative cells (Heli, 2014). A cell-free system derived from heterocysts demonstrated that the following substrates supported Nitrogenase activity: sucrose (less active), glycogen, glucose, maltose, and fructose; Glucose-6-Phosphate (G6P), Di Hydroxy Acetone Phosphate (DHAP), Glyceraldehyde 3-Phosphate (GAP), and other intermediates of the Oxidative Pentose-Phosphate Cycle (OPPC) were particularly active. Acetylene reduction by heterocyst extract did not occur because glycolytic substrates such as Phospho Enol Pyruvate (PEP) and Pyruvate (Pyr) were either inactive or inhibitive. The action of the OPPC and, presumably by, Isocitrate Dehydrogenase enhance reductive nitrogen fixation during dark times. As depicted, electrons are directed to a heterocyst-specific ferredoxin (FdxH) via

Ferredoxin-NADP-Reductase (FNR) and then to the two components of the Nitrogenase complex (Fe-protein and FeMo-protein). NAD(P)H and hydrogen both give electrons to Respiratory Electron Transfer (RET), which generates the ATP required for Nitrogenase action. During the day, when there is enough sunlight, cyclic photophosphorylation mediated by photosystem I generates a large portion of the ATP required for the activity. PS-I may also photoreduce ferredoxin using electrons from hydrogen and NAD(P)H (Fig. 8).

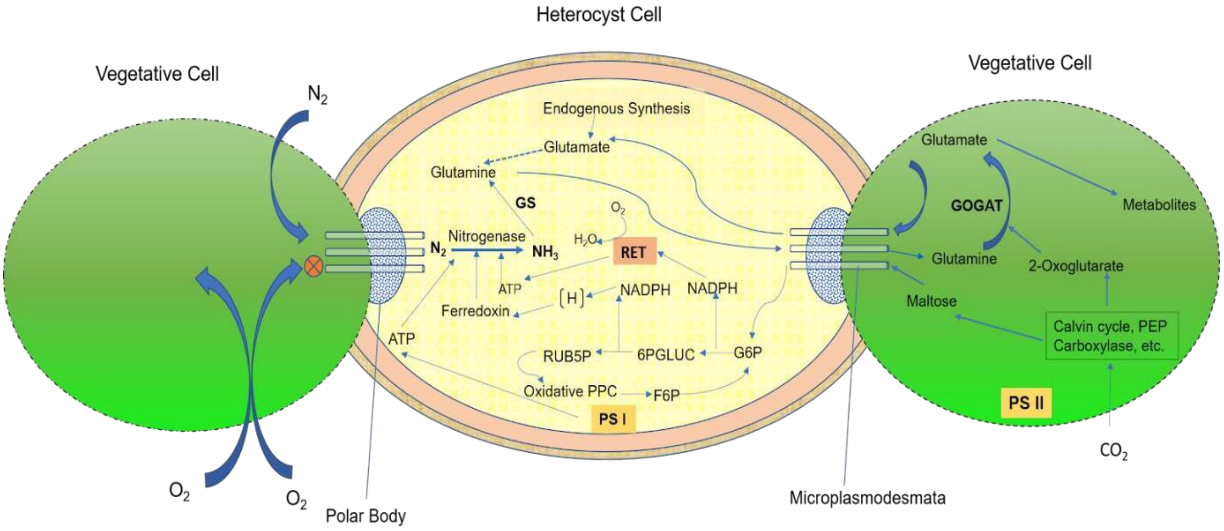

Figure 8. Shows a diagrammatic representation of the exchange of nitrogen and carbon between the vegetative cells and the heterocyst. Photosystem I (PS I) and Photosystem II (PS II) GS, Glutamine synthetase; GOGAT, Glutamate synthase; RIB5P, Ribulose-5-phosphate; 6PGLUC, 6-Phosphogluconic acid; G6P, Glucose-6-phosphate (Adapted and modified).

8.3. Nitrogenase activity in filamentous and unicellular non-heterocystous cyanobacteria

Non-heterocystous cyanobacteria are also capable of fixing nitrogen, a fact initially reported by Wyatt and Silvey in 1969. According to Stanier and Cohen-Bazire (1977), cyanobacteria are oxygenic photoautotrophs, which means that their natural habitats are aerobic and oxygen-supersaturated. Numerous filamentous and unicellular non-heterocystous cyanobacteria species, including Trichodesmium, Cyanothece, and Gloeothece spp., are able to fix molecular nitrogen both temporally and spatially when they synthesize the enzyme Nitrogenase complex (Stewart, 1980). However, to protect the Nitrogenase enzyme complex from oxidative deactivation, there must be temporal shifts for oxygenic photosynthesis during the day and anoxic Nitrogenase activity during dark periods (Stal and Krumbein, 1985a; Sherman et al., 1998). Nitrogenase synthesis in Gloeothece initiates two hours before it enters the dark period. Stored glucan reserves that had accumulated during the light period source the needed intermediates like ATP, reductants, and other carbon skeletons for synthesis and activity of Nitrogenase after the onset of darkness (Gallon & Chaplin, 1988; Gallon, 1992). A burst of respiratory activities at the start of the dark period not only depletes much of the cellular O_2 to protect Nitrogenase from oxidative inactivation but also provides some of the energy required for nitrogen fixation. Photosynthetic propensity is also at a minimum during the time they enter the dark period (Sherman et al., 1998), which further keeps O_2 titer low to protect Nitrogenase from oxygen damage. Cyanophycin granules are the stored forms of fixed nitrogen that become fully depleted before they enter the next cycle of the light period. During the daytime, the products of photosynthesis are stored as glycogen granules, which are rapidly consumed as respiratory substrates early in the dark period (Sherman et al., 1998).

In the spatial separation of the antagonistic pathways, the central non-photosynthetic cells carry out nitrogen fixation, and the outer green cells carry out the oxygenic photosynthesis. Such adaptive separation allows the non-heterocystous unicellular cyanobacteria to protect Nitrogenase from the damaging effects of atmospheric and photosynthetically evolved oxygen. However, organisms have been observed to grow in continuous light too, at the expense of molecular nitrogen, allowing Nitrogenase activity under such conditions (Grobbelaar et al., 1986). Mitsui et al. (1986) observed that Nitrogenase activity and oxygen evolution followed a reciprocal pattern in an experiment involving synchronized cultures of Synechococcus sp. They also observed that oxygen evolution virtually declined to zero when Nitrogenase activity was maximum and, in one case, it even became negative, i.e., respiration exceeded the rate of oxygen production. There is further evidence that photosynthetic activity is regulated through cyclic degradation and resynthesis of phycobiliproteins. When the cellular content of phycobiliproteins was low, Nitrogenase activity was induced with concomitant cessation of oxygen evolution (Weare and Benemann,1974; Giani and Krumbein,1986). The phycobiliprotein content of actively nitrogen-fixing cells in Oscillatoria is also lower than that of cultures maintained on nitrate (Stal and Krumbein, 1985b). Because of this adaptive reciprocity, the photosynthetic apparatus, which is necessary for Nitrogenase activity, is not as active. However, total protein and phycobiliprotein did not change in N_2-rich cultures (Scherer and Böger, 1982).

9. NITROGEN ASSIMILATION IN HETEROCYSTOUS AND NON-HETEROCYSTOUS CYANOBACTERIA

Heterocystous cyanobacteria are filamentous organisms. They can perform aerobic N_2-fixation due to heterocysts, which are differentiated as specialized non-photosynthetic cells. As an adaptation to ensure anoxic conditions inside heterocysts possess only photosystem-I for ATP generation needed for N_2 fixation, but have aborted water-splitting and oxygen-producing second light-harvesting complexes, i.e. photosystem-II. In addition to this physiological peculiarity, heterocysts have acquired structural novelties as well in the form of the bilayered envelope which further minimizes the mechanical influx of O_2. The outer layer of the envelope is of polysaccharides and serves as a mechanical support, whereas the inner layer is a laminated structure of unusual glycolipids and specifically limits the diffusion of oxygen inside (Wörmer et al., 2012). Lack of Ribulose-1,5-Bishosphate Carboxylase (RuBisCO) is another peculiarity of heterocysts due to which they are incapable of fixing CO_2 and rely on adjacent photosynthetic vegetative cells to meet carbon needs (Figure 10). Organic carbon is delivered by adjacent photosynthetic vegetative cells to the heterocysts as disaccharides (e.g., maltose), which are metabolized via the oxidative pentose phosphate pathway enzymes (glucose-6-phosphate and 6-Phosphogluconate dehydrogenases) for generating reducing power (NADPH) required for reductive fixation of N_2. The heterocystous glutamate pool is either endogenously synthesized or transported from the vegetative cells, which, in return, get glutamine export from heterocysts. Vegetative cells then synthesize glutamates from glutamine via Glutamate synthase (GOGAT), which are incorporated as vegetative cell metabolites. Glutamate returns to the heterocyst to assimilate ammonia further (Hager et al., 1983) (Fig. 10).

The assimilatory form of N_2 for photosynthetic organisms is ammonium, which becomes incorporated into carbon skeletons metabolically. It is the most reduced inorganic form of nitrogen, which is available for assimilation. However, the nitrogen forms found in nature are generally in their oxidized forms like nitrate (NO_3^-), nitrite (NO_2^-), and dinitrogen (N_2). Dinitrogen is reductively fixed to ammonium by the Nitrogenase complex (Herrero et al., 2001; Valladares et al., 2002). Fixed nitrogen as NH_4^+ is energetically a favored form vis a vis other sources of fixed nitrogen for uptake from the reservoir (Flores and Herrero, 2005). Ammonium, either as direct uptake from the medium or as metabolically produced from alternative sources of nitrogen, is incorporated into carbon skeletons via the sequential activity of two enzymes, Glutamine synthetase (GS) and Glutamate synthase (Glutamine Oxoglutarate Aminotransferase -GOGAT), of a cycle commonly known as the GS-GOGAT pathway (Fig. 9) GS mediated reaction involves the ATP-dependent amidation of glutamate to

produce glutamine (Purich et al., 1998) which is the substrate for Glutamate synthase (GOGAT) catalyzed reductive transfer of the amide group to 2-Oxoglutarate yielding two molecules of glutamate (Vanoni and Curti, 1999). 2-oxoglutarate, thus, is the carbon skeleton that is required for ammonium assimilation and is synthesized by Isocitrate Dehydrogenase (IDH), an enzyme involved in the tricarboxylic acid cycle. In some cyanobacteria, 2-oxoglutarate undergoes direct amination by Glutamate Dehydrogenase (GDH) (Heeswijk et al., 2013).
Thus, depending on the cyanobacterial species, there are two paths for glutamate production, as depicted in Fig. 9. The GDH pathway is depicted in Fig. 9(a), and the cyclic GS-GOGAT pathway is shown in Fig. 9(b). Glutamate dehydrogenase is the only enzyme in the GDH pathway. The reductive amination of 2-oxoglutarate yields one net glutamate; ATP is not required, and GDH has a low affinity for ammonium. In contrast, the two enzymes that make up the cyclic GS-GOGAT pathway, Glutamine synthetase (GS) and Glutamate synthase (GOGAT), function in a cyclic fashion to also yield one net glutamate through the reductive amination of 2-oxoglutarate. Nevertheless, GS has a comparatively high affinity for ammonium, and one ATP is used for each amino group that is absorbed. Due to its pivotal role in metabolism, the GS-GOGAT pathway is influenced by various environmental factors, particularly the accessibility of carbon and nitrogen. NtcA is a transcriptional factor that controls GS activity and, as a result, is essential for controlling nitrogen absorption. Moreover, NtcA has been linked to the inhibition of the synthesis of two inhibitory polypeptides (IF7 and IF17) that interplay with other proteins to render the GS inactive (Herrero et al., 2001).

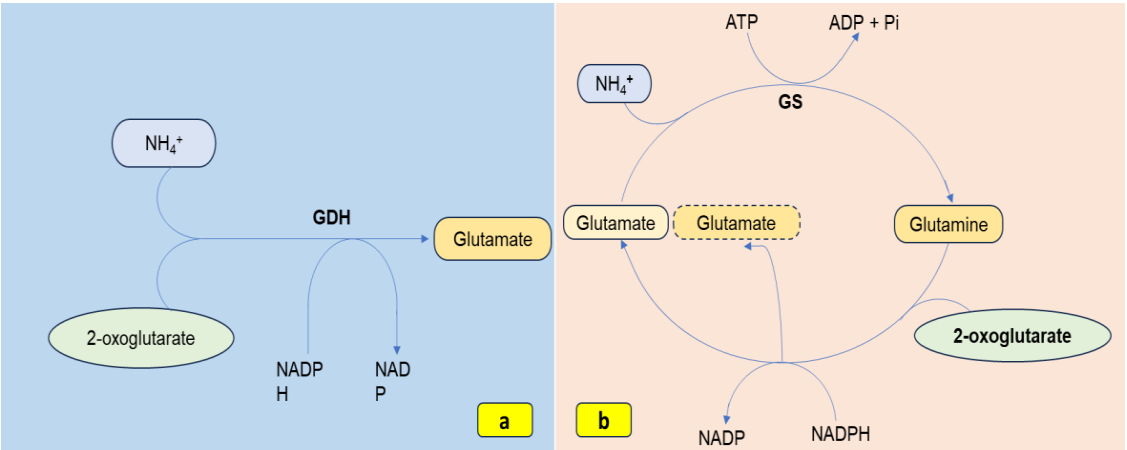

Fig 9: The two pathways for glutamate synthesis. (a) scheme of the GDH pathway and (b) scheme of the cyclic GS-GOGAT pathway.

9.1.a. In heterocysts of free-living cyanobacteria

Free-living cyanobacteria produce both Nitrogenase and Glutamine synthetase (GS) at the same time. Glutamine, which is the end product of primary ammonium absorption, is exported to the vegetative cells, where GOGAT transfers the amino groups to 2-oxoglutarate (Fig. 8).

9.2.b. In heterocysts of symbiotic Cyanobacteria:

The heterocysts of symbiotic cyanobacteria within the host plants differ from those of free-living heterocysts. Unlike the free-living cyanobacteria, the heterocysts of symbiotic members like Nostoc have no GS activity, and the fixed ammonium is directly exported into the host plant cells. Inside host cells, ammonium is assimilated via the usual GS-GOGAT pathway involving 2-oxoglutarate. Heterocysts receive fixed carbon from host cells as symbiotic reciprocation **(Fig.10).**

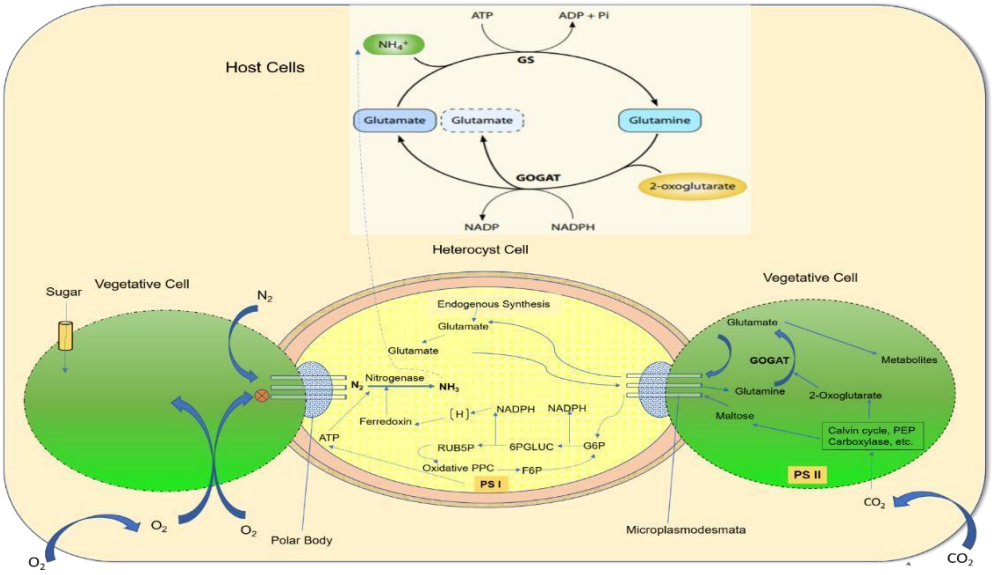

Fig.10. Schematic view of N_2-fixation in heterocysts of symbiotic diazotrophic filamentous heterocystous cyanobacteria and Carbon-Nitrogen exchanges with vegetative cells, Heterocysts, and Host cells ((Adapted and modified).

9.3.c. In non-heterocystous unicellular Cyanobacteria:

The same process used by Glutamine synthetase/Glutamate synthase (GS/GOGAT) in individual cells also facilitates the assimilation of nitrogen in these species. It has been proposed that GS is necessary for NH_4^+ assimilation independent of the primary form of N being used, based on high rates of GS transferase activity relative to rates of total N uptake in both natural and cultured populations of Trichodesmium spp. (Mulholland and Capone, 1999). Rates of GS biosynthesis and Transferase in Trichodesmium spp. are both enhanced in the afternoon when N_2 fixation is at its peak. On the other hand, during the peak N_2 fixation phase, the ratio of GS transferase to biosynthetic activity drops, suggesting that during the day, the proportion of the biosynthetically active GS pool grows. The biosynthetic ability of GS is so strong that over the course of a day, colonies of Trichodesmium spp. can triple their cellular nitrogen content, suggesting that N-assimilation occurs and that there is no upper limit to the rate at which cells can utilize N, even when N_2 fixation peaks in the midday (Kramer et al., 1996; Mulholland et al., 1999).

10. CONCLUSION

Nitrogen is a limiting nutrient despite being the most abundant element in Earth's atmosphere (nearly 80%) because of being biologically unavailable as such. Diazotrophs are a group of bacteria and archaea that can grow on molecular nitrogen (N_2) without being fed on biologically or chemically fixed nitrogen. No eukaryote is known to fix diatomic molecular nitrogen to an assimilable form, due to which animals, plants, and other eukaryotic organisms depend on bacteria and archaea to meet their nutritional needs for nitrogen. Nitrogen is an essential macronutrient for plant growth, most plants obtain it through uptake from the soil as fixed forms and only a few plants acquire it through symbiotic association with microorganisms. Nitrogen-containing fertilizers are widely applied agronomically for enhanced plant growth and yield. However, such artificial fertilizers make agricultural practices quite expensive and have serious negative environmental effects like degrading soil quality and runoff leading to hazardous contamination of water resources. Blue-green algae (Cyanobacteria) are versatile organisms that can utilize nitrogen in inorganic or organic forms. Gram-negative prokaryotes that carry out oxygen-evolving photosynthesis are known as cyanobacteria. A variety of nitrogen sources, including urea, dinitrogen, nitrate, and ammonium, can be utilized by cyanobacteria. Nitrate is favored

over ammonium, and they are favored over dinitrogen. Most N-fixing cyanobacteria are filamentous, consisting of a chain of vegetative cells and some giant specialized cells known as heterocysts, which serve as the organism's N-fixing mechanism. These cells are specialized for nitrogen fixation and differentiate from vegetative cells at a regular spacing of 10-15 cells when combined nitrogen is limited in the media. Oxygen permanently inactivates Nitrogenase, a nitrogen-fixing enzyme. Heterocysts create a microoxic environment for Nitrogenase by thickening their envelopes, changing photosynthetic activity, and boosting respiration. They create symbiotic relationships with both lower vascular plants and angiosperms. The development of heterocysts is activated by a lack of combined nitrogen, which triggers the initiation of heterocyst development, as demonstrated by elegant experiments involving growing colonies of Nostoc or Anabaena in N- free medium, where 5-10% of cells in the filaments differentiate into heterocysts. Heterocysts are relatively big, with strong walls that prevent oxygen transport within the cells. As a further adaptation to ensure a microaerobic environment inside heterocysts in order to prevent oxidative inactivation of Nitrogenase, photosystem-II has been entirely detached, and only cyclic photophosphorylation to create ATP and $NADPH_2$ occurs without the formation of O_2. Cyanobacteria spread rapidly within these glands and infiltrate neighboring plant cells by dissolving walls at points of contact. Once inside the cytoplasm, the cyanobacteria are encased by symbiosome membranes, and up to 80% of the cyanobacterial cells are eventually transformed into N_2-fixing heterocysts. In response to the environmental cue of nitrogen deficit, some cells at semi-regular intervals in the filament develop into N_2-fixing heterocysts, a process that requires a specialized gene expression program. Two proteins required for differentiation include the cyanobacterial nitrogen control transcription factor NtcA and the heterocyst differentiation control protein (HetR). Inhibitors such as the tiny, diffusible heterocyst inhibition-signalling peptide (PatS) or a PatS-related molecule produced by differentiating heterocysts limit excessive vegetative cell differentiation into heterocysts. The evolved Anabaena filament, which contains two types of interdependent cells, CO_2-fixing vegetative cells and N_2-fixing heterocysts, is a fascinating example of bacterial multicellularity. The various stages of heterocyst development have demonstrated unique structural and physiological changes that trigger the morphogenesis of the heterocyst envelope. The heterocyst is made up of two layers: an outside polysaccharide layer and an interior glycolipid layer, which limit the amount of oxygen that can enter. A two-component regulatory system (involving DevR and HepK proteins) and hep genes (hepABC) are responsible for polysaccharide layer deposition, whereas hgl genes (hglBCDEK) and DevH (a transacting regulatory protein) are responsible for aglycone synthesis, localization, and deposition of the inner glycolipid layer. The hglT gene (which encodes a heterocyst glycoside synthase) is responsible for the final phase of Hgl biosynthesis: the conversion of glucose to fatty alcohol. Furthermore, DevBCA and HgdD are implicated in glycolipid export, which is important for heterocyst differentiation and maturation. Many other regulatory genes, including hepK, hepN, henR, and hepS, have been linked to normal heterocyst maturation. During nitrogen fixation, the nif genes activated by the NtcA protein are programmed to rearrange themselves. It has a stretch of 17 genes that encode the Nitrogenase complex. The Nitrogenase enzyme is extremely sensitive to molecular oxygen. As a result, nitrogen-fixing cyanobacteria have developed ways to protect Nitrogenase from oxygen-induced deactivation. The enzyme complex Nitrogenase (E.C.1.18.6.1) is made up of a dimeric Fe-protein (the Dinitrogenase reductase) that serves as an electron carrier for the tetrameric MoFe-protein (the Dinitrogenase), which converts molecular nitrogen to ammonia. Carbon molecules are transferred from vegetative cells to nitrogen-fixing heterocysts via the oxidative pentose pathway, where they produce the necessary NADPH. Nitrogen fixation produces ammonia, which is digested by Glutamine synthetase (GS), and the bulk of glutamine is transferred to the vegetative cell and transformed into glutamate by Glutamate synthase (GOGAT).

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Conflicts of Interest:
The authors declare that they have no conflicts of interest.

Authors' Contribution

Vandana and Kuldeep wrote the manuscript with support and expert guidance from Gyan.
Vinod, Vishal, Alka, and Piyush contributed to the completion of the figure and table.
Devendra, Sunil, and Pratibha contributed to the final version of the manuscript by providing critical feedback.
All authors reviewed the paper and approved the final version of the manuscript.

11. REFERENCES

1. Abd-Alla, M.H., Mahmoud A.L. E. & A.A. Issa. (1994). Cyanobacterial biofertilizer improved the growth of wheat. Phyton 34, 11-18.
2. Abinandan, S., Subashchandrabose, S. R., Venkateswarlu, K., & Megharaj, M. (2019). Soil microalgae and cyanobacteria: the biotechnological potential in the maintenance of soil fertility and health. Critical reviews in biotechnology, 39(8), 981-998.
3. Adams, D. G. (2000). Heterocyst formation in cyanobacteria. Curr. Opin. Microbiol. 3, 618– 624.
4. Adams, D. G., & Duggan, P. S. (2011). Signalling in cyanobacteria–plant symbioses. In Signaling and communication in plant symbiosis (pp. 93-121). Berlin, Heidelberg: Springer Berlin Heidelberg.
5. Awai K, Wolk CP. (2007). Identification of the glycosyl transferase required for synthesis of the principal glycolipid characteristic of heterocysts of Anabaena sp. strain PCC 7120. FEMS Microbiol Lett 266:98–102.
6. Barman, S., Das, S., & Bhattacharya, S. S. (2019). The prospects of bio-fertilizer technology for productive and sustainable agricultural growth. In New and future developments in microbial biotechnology and bioengineering (pp. 233-253).
7. Benson D., Kerry K., Malin G. (2014). Algal biofuels: impact significance and implications for EU multi-level governance. J. Clean. Prod. 72 4–13. 10.1016/j.jclepro.2014.02.060
8. Beijerinck, M. W. (1901). Uber oligonitrophile mikroben. Zentralbl. Bakterol. Parasitenkd. Infektionskr. Hyg. Abt. II., 7, 561-582.
9. Bergman, B., Gallon, J. R., Rai, A. N., & Stal, L. J. (1997). N2 fixation by non-heterocystous cyanobacteria. FEMS Microbiology reviews, 19(3), 139-185.
10. Bhaya, D. (2004). Light matters: phototaxis and signal transduction in unicellular cyanobacteria. Molecular microbiology, 53(3), 745-754.
11. Bothe, H., Schmitz, O., Yates, M.G. and Newton, W.E. (2010). Nitrogen fixation and hydrogen metabolism in cyanobacteria. Microbiology and molecular biology reviews, 74(4), pp.529- 551.
12. Burnat, M., Herrero, A., & Flores, E. (2014). Compartmentalized cyanophycin metabolism in the diazotrophic filaments of a heterocyst-forming cyanobacterium. Proceedings of the National Academy of Sciences, 111(10), 3823-3828.
13. Cameron, H. J., and Julian, G. R. (1988). Utilisation of hydroxyapatite by cyanobacteria as their sole source of phosphate and calcium. Plant Soil 109, 123–124. doi: 10.1007/BF02197589.
14. Campbell EL, Cohen MF, Meeks JC. (1997). A polyketide-synthase-like gene is involved in the synthesis of heterocyst glycolipids in Nostoc punctiforme strain ATCC 29133. Arch Microbiol 167:251- 258.
15. Cardemil L, Wolk CP. (1981). Isolated heterocysts of Anabaena variabilis synthesize envelope polysaccharides. Biochim Biophys Acta 674: 265 –276.
16. Castenholz, R. W. (2001). Phylum B. X. Cyanobacteria. Oxygenic photosynthetic bacteria. In R. Boone and R. W. Castenholz (Eds.), Bergey's manual of systematic bacteriology (Vol. 1, pp. 473-599). New York: Springer.
17. Chandra, S. K., & Rajashekhar, M. (2016). Effect of pH on Freshwater Cyanobacteria Isolated from Different Habitats of Southern Karnataka. International Journal of Life Sciences and Technology, 9(7), 56.
18. Chittora, D., Meena, M., Barupal, T., Swapnil, P., & Sharma, K. (2020). Cyanobacteria as a source of biofertilizers for sustainable agriculture. Biochemistry and biophysics reports, 22, 100737.
19. Dodds, W. K., Gudder, D. A., & Mollenhauer, D. (1995). The ecology of Nostoc. Journal of Phycology, 31(1), 2-18.
20. Dubey, A. K., and Rai, A. K. (1995). Application of algal biofertilizers (Aulosira fertilissimatenuis and Anabaena doliolum Bhardwaja) for sustained paddy cultivation in Northern India. Isr. J. Plant. Sci. 43, 41–51.
21. Duggan, P.S, Gotardello P, & Adams D.G. (2007). Molecular analysis of genes in Nostoc punctiforme involved in pilus biogenesis and plant infection. J Bacteriol 189, 4547–4551.
22. Ehira S, Ohmori M. (2006a). NrrA directly regulates the expression of hetR during heterocyst differentiation in the cyanobacterium Anabaena sp. strain PCC 7120. J Bacteriol 188: 8520– 8525.
23. Ehira S, Ohmori M. (2006b). NrrA, a nitrogen-responsive response regulator facilitates heterocyst development in the cyanobacterium Anabaena sp. strain PCC 7120. Mol Microbiol 59: 1692–1703.

24. El-Enany, A.E. & Issa, A.A. (2000). Cyanobacteria as a biosorbent of heavy metals in sewage water. Envir Toxicol Pharmacol 8, 95-101.
25. Falkowski, P. G., & Raven, J. A. (1997). Aquatic photosynthesis (Vol. 256). Malden, MA: Blackwell Science.
26. Fan Q, Huang G, Lechno-Yossef S, Wolk CP, Kaneko T, Tabata S. (2005). Clustered genes required for synthesis and deposition of envelope glycolipids in Anabaena sp. strain PCC 7120. Mol Microbiol 58: 227–243.
27. Fay P. (1992). Oxygen relations of nitrogen fixation in cyanobacteria. Microbiol Rev 56: 340– 373.
28. Fiedler G, Arnold M, Hannus S, Maldener I (1998). The DevBCA exporter is essential for envelope formation in heterocysts of the cyanobacterium Anabaena sp. strain PCC 7120. Mol Microbiol 27:1193–1202.
29. Flores E, Herrero A (2005) Nitrogen assimilation and nitrogen control in cyanobacteria. Biochem Soc Trans 33(Pt 1):164–167.
30. Flores E, Herrero A. (2010). Compartmentalized function through cell differentiation in filamentous cyanobacteria. Nat Rev Microbiol. Jan;8(1):39-50. doi: 10.1038/nrmicro2242. PMID: 19966815.
31. Flores, E., López-Lozano, A., and Herrero, A. (2015) Nitrogen fixation in the oxygenic phototrophic prokaryotes (cyanobacteria): the fight against oxygen. In Biological Nitrogen Fixation, Vol. 2, de Bruijn, F. J. (ed), Wiley, pp. 879–889.
32. Fogg, G. E., and Stewart, W. D. P. (1968). In situ determinations of biological nitrogen fixation in Antarctica. Br. Antarct. Surv. Bull. 15, 39–46.
33. Frıas, J.E. et al. (1994) Requirement of the regulatory protein NtcA for the expression of nitrogen assimilation and heterocyst development genes in the cyanobacterium Anabaena sp. PCC 7120. Mol. Microbiol. 14, 823–832 17.
34. Gallon, J. R. (1992). Tansley Review No. 44. Reconciling the incompatible: N2 fixation and O2. New Phytol 122, 571-609.
35. Garcia-Pichel, F. (2009). Cyanobacteria. In Encyclopedia of microbiology, third edition (pp. 107-124). Elsevier.
36. Giani, D., & Krumbein, W. E. (1986). Growth characteristics of non-heterocystous cyanobacterium Plectonema boryanum with N2 as nitrogen source. Archives Microbiol., 145, 259-265.
37. Golden JW, Yoon HS. (1998). Heterocyst formation in Anabaena. Curr Opin Microbiol 1: 623– 629.
38. Golden, J.W., &Yoon, H.S. (2003). Heterocyst development in Anabaena. Cur Opin Microbiol 6, 557–563.
39. Gonçalves, A. L. (2021). The use of microalgae and cyanobacteria in the improvement of agricultural practices: a review on their biofertilising, biostimulating and biopesticide roles. Applied Sciences, 11(2), 871.
40. Grant, IF., Roger, PA., Watanabe, I. (1985) Effect of grazer regulation and algal inoculation on photodependent nitrogen fixation in a wetland rice field. Bio1 Fertil Soils 1:61-72.
41. Grobbelaar, N., Huang, T. C., Lin, H. Y., & Chow, T. J. (1986). Dinitrogen-fixing endogenous rhythm in Synechococcus RF-1. FEMS Microbiol. Lett., 37, 173-177.
42. Guerrero, M. G., Vega, J. M., and Losada, M. (1981). The assimilatory nitrate-reducing system and its regulation. Annu. Rev. Plant Physiol. 32, 168–204. doi: 10.1146/annurev.pp.32.060181.001125.
43. Halimatul, H.S.M., Ehira, S., Awai, K., (2014). Fatty alcohols can complement functions of heterocyst specific glycolipids in Anabaena sp. PCC 7120. Biochem. Biophys. Res. Commun. 450, 178–183.
44. Hasan, M. A. (2020). Cyanobacteria from Rice Field and Comparative Study of Their Performances as Biofertilizer on Rice Plants. J. Glob. Biosci, 9, 8078-8087.
45. Haselkorn, R., Schlictman, D., Jones, K., & Buikema, W. J. (1998). Heterocyst differentiation and nitrogen fixation in cyanobacteria. In Biological Nitrogen Fixation for the 21st Century: Proceedings of the 11th International Congress on Nitrogen Fixation, Institut Pasteur, Paris, France, July 20–25, 1997 (pp. 93-96). Springer Netherlands
46. Haselkorn, R. (2007). Heterocyst differentiation and nitrogen fixation in cyanobacteria. Associative and endophytic nitrogen-fixing bacteria and cyanobacterial associations, 5, 233- 255.
47. Heeswijk, W.C.V., Westerhoff, H.V., Boogerd, F.C., (2013). Nitrogen assimilation in Escherichia coli: putting molecular data into a systems perspective. Microbiol Mol Biol Rev. 2013 Dec;77(4):628-95. doi: 10.1128/MMBR.00025-13. PMID: 24296575; PMCID: PMC3973380.
48. Heli, S. H. M. (2014). Physiological analysis of heterocyst specific glycolipid and production of its aglycone, fatty alcohol in Anabaena sp. PCC 7120.
49. Heimann, K., & Cirés, S. (2015). N2-fixing cyanobacteria: ecology and biotechnological applications. In Handbook of marine microalgae (pp. 501-515). Academic Press.
50. Herrero. A, Muro-Pastor, A.M., Flores, E., (2001). Nitrogen control in cyanobacteria, J. Bacteriol. 183. 411–425.
51. Hoffmann, L. (1989). Algae of terrestrial habitats. The botanical review, 55, 77-105.
52. Hoiczyk E, Hansel A (2000) Cyanobacterial cell walls: news from an unusual prokaryotic envelope. J Bacteriol 182:1191–1199.

53. Horne, A. J. (1979). Nitrogen fixation in Clear Lake, California. 4. Diel studies on Aphanizomenon and Anabaena blooms. Limnol. Oceanogr. 24, 329-341.
54. Hu, C., Zhang, D. Huang, Z., Liu, Y. (2003): The vertical microdistribution of cyanobacteria and green algae within desert crusts and the development of the algal crusts – Plant Soil 257: 97-111.
55. Huang G, Fan Q, Lechno-Yossef S, Wojciuch E, Wolk CP, Kaneko T, Tabata S (2005). Clustered genes required for the synthesis of heterocyst envelope polysaccharide in Anabaena sp. strain PCC 7120. J Bacteriol 187:1114-1123.
56. Issa, A.A., Adam, M.S. & Fawzy, M.A. (2013). Alternations in some metabolic activities of scenedesmus quadriquada and Merismopedia glauca in response to glyophsate herbicide. J. Biol Earth Sci. 3,17-23.
57. Kannaiyan S. (1990). Biotechnology of Biofertilizers for Rice Crop, p. 225. Tamil Nadu Agricultural University, Coimbatore (India).
58. Kannaiyan, S., Aruna, S. J., Kumari, S. M. P., and Hall, D. O. (1997). Immobilized cyanobacteria as a biofertilizer for rice crops. J. Appl. Phycol. 9, 167–174.
59. Karthikeyan N, Prasanna R, Sood A, Jaiswal P, Nayak S, Kaushik, BD. (2009). Physiological characterization and electron microscopic investigations of cyanobacteria associated with wheat rhizosphere. Folia Microbiol 54:43–51.
60. Kaushik, B.D. (1991): Cyanobacterial response of crops in saline irrigated with saline groundwater. – In Shastree, N.K (ed.) Current Trends in Limnology, Vol. 1. Narendra Publishing House, New Delhi.
61. Khamees. H. S. K. Gallon, J. R., & Chaplin. A. E. (1987). The pattern of acetylene reduction by cyanobacteria grown under alternating light and darkness. Br. Phycol. J. 22: 55-60.
62. Kramer, J. G., Wyman, M., Zehr, J. P., & Capone, D. G. (1996). Diel variability in the transcription of the structural gene for glutamine synthetase (glnA) in natural populations of the marine diazotrophic cyanobacterium Trichodesmium thiebautii. FEMS microbiology ecology, 21(3), 187-196.
63. Kulasooriya, S.A. (1998). Cyanobacteria and Azolla as biofertilizer for rice. – In: Subramanian, G.S., Kaushik, B.D. and. Venkataraman, G.S (eds) Cyanobacterial Biotechnology, New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd.
64. Kumar, K., Mella-Herrera, R.A., Golden, J.W., (2010). Cyanobacterial heterocysts. Cold Spring Harb. Perspect. Biol. 2 (4), a000315. Kutz. J. Gen. Microbiol. 128, 2203–2205.
65. Lechno-Yossef S, Fan Q, Ehira S, Sato N, Wolk CP. (2006). Mutations in four regulatory genes have interrelated effects on heterocyst maturation in Anabaena sp. strain PCC 7120. J Bacteriol 188:7387–7395.
66. Mahanty, T., Bhattacharjee, S., Goswami, M., Bhattacharyya, P., Das, B., Ghosh, A., et al. (2017). Biofertilizers: a potential approach for sustainable agriculture development. Environ. Sci. Pollut. Res. 24, 3315–3335.
67. Maldener, I. and Muro-Pastor, A. (2010) Cyanobacterial heterocysts, In Encyclopedia of Life Sciences (ELS), John Wiley & Sons.
68. Mandal, B., Vlek, P.L.G., Mandal. L.N. (1998): Beneficial effect of blue green algae and Azolla excluding supplying nitrogen, on wetland rice fields: a review. – Biology and Fertility of Soils 27: 329-342.
69. Meeks, J.C. (1998). Symbiosis between nitrogen-fixing cyanobacteria and plants. BioScience 48, 266–276.
70. Meeks, J.C. (2003). Symbiotic interactions between Nostoc punctiforme, a multicellular cyanobacterium, and the hornwort Anthoceros punctatus. Symbiosis 35, 55–71.
71. Meeks JC, Elhai J (2002) Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant-associated symbiotic growth states. Microbiol Mol Biol Rev 66:94–121.
72. Metting, B. (1988). "Microalgae in agriculture," in Micro-Algal Biotechnology, eds M. A. Borowitzka and L. J. Borowitzka (Cambridge: Cambridge University Press), 288–304.
73. Mitsui, A., Kumazawa, S., Takahashi, A., Ikemoto, H. & Arai, T. (1986). Strategy by which nitrogen-fixing unicellular cyanobacteria grow photoautotrophically. Nature 323, 720–732.
74. Moslavac S, Nicolaisen K, Mirus O, Al Dehni F, Pernil R, Flores E, Maldener I, Schleiff E.(2007a). A TolC-like protein is required for heterocyst development in Anabaena sp. strain PCC 7120. J Bacteriol 189:7887–7895.
75. Mulholland, M.R. and Capone, D.G., (1999). Nitrogen fixation, uptake and metabolism in natural and cultured populations of Trichodesmium spp. Marine Ecology Progress Series, 188, pp.33-49.
76. Munoz-Garcia, J., & Ares, S. (2016). Formation and maintenance of nitrogen-fixing cell patterns in filamentous cyanobacteria. Proceedings of the National Academy of Sciences, 113(22), 6218-6223.
77. Mus, F., Alleman, A. B., Pence, N., Seefeldt, L. C., and Peters, J. W. (2018) Exploring the alternatives of biological nitrogen fixation. Metallomics 10: 523–538.
78. Mus, F., Colman, D. R., Peters, J. W., & Boyd, E. S. (2019). Geobiological feedbacks, oxygen, and the evolution of Nitrogenase. Free Radical Biology and Medicine, 140, 250-259.

79. Muthukumar, C., Muralitharan, G., Vijayakumar, R., Panneersevam, A. and Thajuddin, N., (2007). Cyanobacterial biodiversity from different freshwater ponds of Thanjavur, Tamilnadu (India). Acta botanica malacitana, 32, pp.17-25.
80. Nayak, S., Prasanna, R., Dominic, T.K., Singh, P.K. (2004): Effect of BGA- Azolla biofertilizers on nitrogen fixation and chlorophyll accumulation at different depths in soil cores. – Biology and Fertility of Soils 40: 67-72.
81. Nicolaisen K, Hahn A, Schleiff E. (2009). The cell wall in heterocyst formation by Anabaena sp. PCC 7120. J Basic Microbiol 49: 5 –24.
82. Obreht Z., Kerby N. W., Gantar M., Rowell P. (1993). Effects of root-associated N2-fixing cyanobacteria on the growth and nitrogen content of wheat (Triticum vulgare L.) seedlings. Biol. Fertil. Soils 15 68–72. 10.1007/BF00336292.
83. Pereira, I., Rodrigo, O., Barrientos, L., Moya, M., Reyes, G., and Kramm, V. (2009). Development of a biofertilizer based on filamentous nitrogen-fixing cyanobacteria for rice crops in Chile. J. Appl. Phycol. 21, 135–144.
84. Peter J. Lea, Primary Nitrogen Metabolism, Editor(s): P.M. Dey, J.B. Harborne, Plant Biochemistry, Academic Press, 1997, Pages 273-313, ISBN 9780122146749,
85. Plominsky, A.M., Larsson, J., Bergman, B., Delherbe, N., Osses, I., Vasquez, M., 2013. Dinitrogen fixation is restricted to the terminal heterocysts in the invasive cyanobacterium Cylindrospermopsis raciborskii CS-505. PLoS ONE 8 (2), e51682.
86. Postgate, J. R. (1982). The fundamentals of nitrogen fixation (p. 252pp).
87. Prasanna, R., Sharma, E., Sharma, P., Kumar, A., Kumar, R., Gupta, V., et al. (2013). Soil fertility and establishment potential of inoculated cyanobacteria in rice crop grown under nonflooded conditions. Paddy Water Environ. 11, 175–183.
88. Prasanna, S. Nayak. R. (2007). Soil pH and its role in cyanobacterial abundance and diversity in rice field soils. Appl. Ecol. Environ. Res, 5(2), 103-113.
89. Purich, D.L., (1998). Advances in the enzymology of glutamine synthesis, in: D.L. Purich (Ed.), Amino Acid Metabolism, vol. 72, John Wiley & Sons Inc, New York, 1998, pp. 9–42.
90. Rai, A. K., and Sharma, N. K. (2006). Phosphate metabolism in the cyanobacterium Anabaena doliolum under salt stress. Curr. Microbiol. 52, 6–12.
91. Rai, A.N., Bergman, B., & Rasmussen, U. 2002. Cyanobacteria in symbiosis. Dordrecht: Kluwer Academic Publishers.
92. Rai, A.N., Bergman, B., 1986. Modification of NO3 ─ metabolism in heterocysts of the N2-fixing cyanobacterium Anabaena 7120 (ATCC 27893). FEMS Microbiol. Lett. 36, 133–137.
93. Ramirez ME, Hebbar PB, Zhou R, Wolk CP, Curtis SE 2005. Anabaena sp. strain PCC 7120 gene devH is required for synthesis of the heterocyst glycolipid layer. J Bacteriol 187:2326-2331.
94. Ramona Aldea, M., Kumar, K., & Golden, J. W. (2008). Heterocyst development and pattern formation. Chemical communication among bacteria, 75-90.
95. Rascio, N., La Rocca, N., Sven Erik, J., & Brian, F. (2008). Biological nitrogen fixation. Encyclopedia of Ecology.
96. Rasmussen, U., Johansson, C. & Bergman B. 1994. Early communication in the Gunnera–Nostoc symbiosis – plant-induced cell differentiation and protein synthesis in the cyanobacterium. Mol Plant Microb Inter 7, 696–702.
97. Rastogi, R. P., and Sinha, R. P. (2009). Biotechnological and industrial significance of cyanobacterial secondary metabolites. Biotechnol. Adv. 27, 521–539.
98. Rippka R., J. Deruelles, J. B. Waterbury, M. Herdman and R. Y. Stanier, 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111: 1 – 61.
99. Roger, P.A. and Reynaud, P.A. 1979. Ecology of blue-green algae in paddy fields. In: Nitrogen and rice, pp. 289–309. International Rice Research Institute, Los Banos.
100. Rubio, L. M.; Ludden, P. W. 2008. Biosynthesis of the Iron-Molybdenum Cofactor of Nitrogenase. Annu. Rev. Microbiol., 62, 93–111, DOI:10.1146/annurev.micro.62.081307. 162737.
101. Scherer, S., & Böger, P. 1982. Respiration of blue-green algae in the light. Arch Microbiol
102. Schindelin, H., Kisker, C., Schlessman, J.L., Howard, J.B. and Rees, D.C., 1997. Structure of ADP· AIF4–-stabilized Nitrogenase complex and its implications for signal transduction. Nature, 387(6631), pp.370-376.
103. Singh, R.N. (1961): Role of blue-green algae in nitrogen economy of Indian agriculture. – Indian Council of Agricultural Research, New Delhi.
104. Singh, J. S., Kumar, A., Rai, A. N., & Singh, D. P. (2016). Cyanobacteria: a precious bio- resource in agriculture, ecosystem, and environmental sustainability. Frontiers in microbiology, 7, 529.