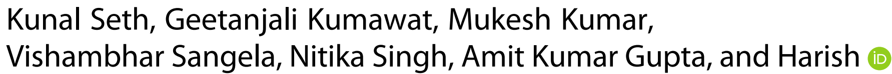

Nitrogen Metabolism in Cyanobacteria 12

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Abstract

Cyanobacteria are known to have unique capability of nitrogen fixation in their specialized cell known as heterocyst. However, differentiation of vegetative cell toward heterocyst reduces competitive ability of cyanobacteria because it led to a shift of energy allocation from carbon to nitrogen metabolism. Therefore, heterocyst formation is regulated to avoid the differentiation commitment due to short-term nitrogen fluctuation. Once nitrogen deficiency signal is sensed by the cyanobacteria, pattern of heterocyst formation is determined that ensures equidistance formation of heterocyst cells with about one heterocyst per ten vegetative cells. After differentiation, heterocyst provides anaerobic condition that is prerequisite for the nitrogenase complex to fix the atmospheric dinitrogen. Microoxic condition inside the heterocyst is attained by elimination of oxygen-producing photosystem II activity, increasing respiration rate, and by formation of thick heterocyst-specific exopolysaccharide and glycolipid layer. Nitrogen-fixing machinery is assembled and activated during heterocyst differentiation. The nitrogenase complex is encoded by *nif* gene family. Many of these genes are interrupted in the vegetative cells by interruption elements and these are excised during differentiation of heterocyst by a site-specific recombinase, leading to the activation of genes. In this chapter, we have outlined the molecular circuit of heterocyst differentiation and discussed the assembly of nitrogen-fixing machinery and role of key enzymes in the nitrogen metabolism in the cyanobacteria.

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Biofertilizer · Cyanobacteria · Nitrogen fixation · Heterocyst · Nitrogenase

12.1 Introduction

Nitrogen metabolism is the group of reactions, which includes the conversion of atmospheric dinitrogen (N_2) into glutamate at the expense of energy. This conversion is necessary because of two reasons; one is the storage of ammonia that is quite toxic to a cell and another is the amino acid glutamate that acts as a precursor for many metabolic pathways like 5-aminolevulinate, phycobilin, and chlorophyll biosynthesis (Flores and Herrero 1994). Nitrogen fixation, which is characteristic feature of some prokaryotes, is an important phenomenon to sustain nutrient cycling. It occurs in symbiotic bacteria like *Rhizobium* or in cyanobacteria like *Nostoc* and *Anabaena*.

Cyanobacteria are prokaryotic autotrophs which play an imperative role in food chain and have a diverse ecological niche. Cyanobacteria are not confined to oceanic condition yet their assorted variety in biochemistry has enabled these groups of species to occupy almost any terrestrial and aquatic living space on earth (Schirrmeister et al. 2013). Cyanobacteria normally live in marine or fresh water, some cyanobacteria live in a place with earthly biological system and some even thrive in outrageous conditions like desert, the polar area or warm water (Muro-Pastor and Hess 2020). Cyanobacteria are not in every case free-living yet many are fundamental for organizing complex microbial networks in endolithic form for example, in stromatolites, microbial mats, coastal and desert biological soil, and as symbionts of certain higher plants and fungi (Muro-Pastor and Hess 2020). They are also called as diazotrophs because they have capability to fix the atmospheric nitrogen (Lee 2018). Diazotrophic cyanobacteria species contribute considerable quantity of combined nitrogen into the biosphere by changing dinitrogen into ammonia, a procedure known as biological nitrogen fixation (Muro-Pastor and Hess 2020).

These are the oldest multicellular organism on earth (Herrero et al. 2016). According to paleobotanical study, these organisms were thought to evolve about 2.7 billion years ago and have characteristic blue green color due to the principal pigment c-phycocyanin and c-phycoerythrin. The cells of cyanobacteria are covered with mucilaginous sheath called capsule. Cyanobacterial cell wall shows similarity with gram-negative bacteria, a peptidoglycan layer is present at the outer side of the cell membrane. This layer is composed of NAM (N-acetyl muramic acid), NAG (N-acetyl glucosamine), and tetrapeptides, which are further linked by amide bond. They have porin protein on outer membrane which is permeable for all macro- and micromolecules. But the plasma membrane acts as a permeability barrier for these biomolecules.

Cyanobacteria are for the most part described by their high protein content. In these, nitrogen metabolism is directed by a saved calibrating framework, which

detects the cell balance among carbon and nitrogen level (Forchhammer and Lüddecke 2016). In *Synechocystis* PCC 6803, for example, photosynthetic rates are found to be positively correlated with amino acid and protein levels, but not with growth rates (Esteves-Ferreira et al. 2017). The growth of any cyanobacteria requires two interdependent cell types, viz., vegetative cells for oxygenic photosynthesis and heterocyst for dinitrogen fixation. The fix ratio of two macronutrients carbon and nitrogen (5:1) plays an important role in metabolic homeostasis. Vegetative cells supply reduced carbon to heterocyst, similarly heterocyst supply fixed nitrogen to vegetative cells and maintains the carbon-nitrogen pool. The balanced metabolism of C and N is essential for optimal growth. Heterocysts are connected with vegetative cells through microplasmodesmata or septosome for minerals and substrates, so it manifests the best example of cell-cell communication in cyanobacteria. Heterocyst itself is a modified vegetative cell, thick walled, pale yellow in color due the principal pigment carotenoid, lacks oxygen evolving PSII activity, and creates a microoxic environment for the key enzyme nitrogenase (Harish 2020).

Heterocysts develop from vegetative cells by decomposition of granular inclusions (carboxysomes and glycogen granules), disintegration of photosynthetic thylakoids, and formation of new membrane structures. They neither fix carbon dioxide nor produce oxygen, but have a high oxygen consumption rate via respiration, surrounded by thick layered laminated cell wall. A special system "Honey comb" is present close to heterocyst poles and has a role in respiration and photosynthesis. The differentiation of heterocyst is completed in two steps—first step is reversible in which the vegetative cell senses the nitrogen-deprived condition and converts it into proheterocyst and the next step is irreversible in which conversion of proheterocyst to heterocyst occurs and *nif* gene is activated. Proteins like NtcA, HetR, HetC, PatA, PatS, and PatB participate in heterocyst differentiation and pattern formation (Harish 2020). Nitrogen-deprived condition induces the vegetative filament for heterocyst differentiation, accumulation of 2-oxoglutarate (an intermediate of tricarboxylic acid (TCA) cycle), which acts as a signaling molecule for heterocyst differentiation and pattern formation (Esteves-Ferreira et al. 2018). The 2-OG provides a carbon framework for ammonia assimilation through GS-GOGAT cycle (Zhang et al. 2018; Forchhammer and Selim 2020). Heterocyst differentiation is the suitable example of remodeling and cell differentiation.

The key enzyme of biological nitrogen fixation is nitrogenase. There is a temporal and spatial separation in cyanobacteria to prevent denaturation of nitrogenase. Nitrogenase has two components, first is dinitrogenase reductase (a type of iron protein) and second is dinitrogenase (a type of molybdenum-iron protein) (Flores and Herrero 2005). Ammonia is the most preferable nitrogen source because it diffuses passively through the membrane. The ammonia is further converted into glutamate through GS-GOGAT pathway by succeeding reaction held by glutamine synthetase (GS) and glutamate synthase (GOGAT). The reaction catalyzed by GS is ATP-dependent and GOGAT is an amino-transferase which transfers amide group of glutamine to 2-OG resulting in formation of glutamate amino acid. Cyanobacteria can assimilate many organic and inorganic nitrogen-containing compounds other

than atmospheric nitrogen, these may be nitrate, nitrite, ammonium, urea, cyanate, and amino acids such as arginine, glutamine, and glutamate, but ammonia is the favored nitrogen source (Esteves-Ferreira et al. 2017). The concentration of ammonia in a medium acts as a regulator (inducer or repressor) for the signal transduction pathway; this pathway is closely correlated with plants because the evolution of plastid is phylogenetically correlated with cyanobacteria by endosymbiosis theory. Nitrogen fixation is a metabolically expensive process because it involves 16 ATP for fixing each molecule of nitrogen. Like GS-GOGAT cycle, there are some amino acids like arginine and aspartate that combinedly form a nitrogen storage reservoir called cyanophycin. It is a nonribosomal synthesized protein like polymer which is arranged in a poly-aspartate form (Lee 2018).

12.2 Heterocyst Differentiation

Heterocyst differentiation is a quite complex mechanism and many proteins are involved in its regulation. Multiple layers of regulation ensure that cyanobacteria do not commit heterocyst formation due to short-term fluctuations in the soil nitrogen source content. When cyanobacterial filament receives a lasting signal for nitrogen depletion condition, it led to synthesis of 2-oxoglutarate. This is considered as first sensing signal for induction of heterocyst formation to overcome the nitrogen starvation condition. 2-oxoglutarate or α-ketoglutarate of the Krebs cycle and this metabolite connect C and N metabolism (Huergo and Dixon 2015). Increase in concentration of 2-oxoglutarate triggers the synthesis of NtcA protein also known as global nitrogen regulator, due to its role in overall regulation of nitrogen metabolism in the cyanobacterial filament. Further, in its downstream cascade signal perpetuation, synthesis of HetR protein occurs. This protein is known as specific master regulator, due to its specific role in heterocyst differentiation. HetR itself is regulated by Pkn22 Kinase, cyABrB1, some other genes like *asl1930, alr3234,* and *alr2902*. Multiple layers of regulation ensure fine tuning of development mechanism for heterocyst differentiation. Further, HetR itself has autocatalytic activity and phosphorylation of serine residue present at 130 positions in HetR protein is essential for its activity. HetR protein then interacts with HetP and HetZ proteins and developmental signal is passed to these proteins. HetP protein led to irreversible commitment toward heterocyst formation.

Number of heterocyst cells with respect to vegetative cells are regulated by pattern determination. Because too many heterocyst cells will incur huge energy cost in terms of entire filament and will reduce the competitive ability of the filament. Heterocyst differentiation is energy intensive phenomenon for the cyanobacteria. Therefore, equal distribution of heterocyst cells throughout the filament ensures the equal distribution of fixed nitrogen compound to neighboring vegetative cells, and also conserves the energy for carbon metabolism in the filament. Pattern distribution is therefore equally important aspect when considering the heterocyst differentiation. Proteins of *Pat* gene family regulate this aspect in the cyanobacteria. HetR protein is involved in synthesis of PatS, which is an inhibitor of the HetR protein itself, thereby

controlling the number of heterocyst cells in the filament. PatS is processed to short peptide that acts as concentration-dependent manner to inhibit the heterocyst formation. HetC protein is known for its role in transport of short peptides of PatS. The concentration ratio of PatS and HetR determines the development of heterocyst and position of the heterocyst cell in the filament. PatX is another protein that inhibits heterocyst formation and there is functional overlap between PatS and PatX. PatC protein ultimately selects the cell for differentiation into heterocyst and thereby governs the spatial pattern determination of heterocyst in cyanobacterial filament. Other proteins are also identified that play role in regulation of heterocyst frequency like PatD, PatN, and PknH.

Cell wall of the heterocyst is thick to keep the oxygen concentration minimum in the interior of the cell. Therefore, entire remodeling of the cell wall is done during differentiation of vegetative cell to the heterocyst cell. Cyanobacteria are gram-negative as far as their cell wall organization is concerned, but the thickness of peptidoglycan layer is intermediate (15–35 nm) between gram-positive bacteria and gram-negative bacteria and cross-linking is also higher in cyanobacterial cell wall in comparison to gram-negative bacteria. During heterocyst differentiation, two additional layers are developed. The external polysaccharide layer is known as hep layer and internal glycolipid layer is known as hgl layer. HepA protein is involved in synthesis of hep layer and gene of this protein is regulated by HetR and HepK proteins. Some more genes involved in synthesis of hep layer are *hepA, hepK, hepN, hepS, henR, murB, murC, hcwA, amiC1, amiC2, pbp6, sepJ (fraG), fraC, fraD, and sjcF1*. Additionally, hgl layer prevents the entry of oxygen inside the cell and therefore ensures low oxygen concentration for functioning of nitrogenase complex. Some genes, which regulated the formation of hgl layer, have been identified like *hgdB, hgdC, devBCA* operon, *devH,* and *hglE* (Table 12.1).

The availability of ammonia in a medium, acts as a regulator (inducer or repressor) for the signal transduction pathway. Where global nitrogen regulator gene *ntcA* and signal transducer P-II (which is encoded by *glnB*) control the activity of many genes like *henA*, *hetR*, *hetC*, *patA*, *patB,* and *patC* which are responsible for the heterocyst differentiation and pattern formation. NtcA is a bacterial transcription factor which is a member of catabolic repressor protein. NtcA can inactivate GS-activity by coding inhibitory polypeptides (IF-7 and IF-17) by protein – protein interaction (Muro-Pastor and Florencio 2003). By this, cyanobacteria maintain the metabolic homeostasis. In nitrogen-starved condition, storage level of 2-OG is very high and NtcA self-regulates the expression of *hetR* for heterocyst differentiation (Muro-Pastor et al. 2001). HetR is a kind of serine type protease and also a DNA-binding protein. In in vivo condition, HetR performs as a homodimer and this homodimer is essential for DNA-binding activity and heterocyst differentiation (Huang et al. 2004). Another gene *patS* inhibits this DNA-binding affinity (Huang et al. 2004). The nitrogen regulatory protein PII (PII) interacts with 2-OG and brings conformational changes of PII leading to the release of the PII interacting protein X (PipX). PipX interacts with the nitrogen control factor (NtcA) of cyanobacteria.

Table 12.1 Role of different protein identified during heterocyst differentiation and nitrogen metabolism in cyanobacteria

Protein/gene	Function
NtcA	An autocatalytic protein acts as a transcriptional regulator of many genes of nitrogen metabolism
HetR	A homodimer DNA-binding protein (Ser type protease) regulates heterocyst differentiation
Pkn22	A hanks-type kinase which phosphorylates HetR protein at 130th position of Ser residue
cyABrB1	Negatively controls heterocyst formation
asl1930	Negatively controls HetR protein by holding up the commitment of heterocyst differentiation
alr2902	Negatively regulatory proteins which obstruct the development of heterocyst
alr3234	Delays heterocyst differentiation by inhibiting HetR
HetP	Interacts with HetR and down-regulates heterocyst differentiation
HetZ	Interconnects with HetZ and alr2902 and acts as a regulatory protein of pattern formation phase of cellular differentiation
PatS	Negatively controls heterocyst differentiation by post translational degradation of HetR
PatD	Controls heterocyst frequency
PatN	Plays an important role in pattern formation
PatA	Helps in pattern formation
PatC	Plays a key role in pattern formation by selecting cells for differentiation
PatX	Negatively controls heterocyst differentiation as PatS
PatB (CnfR)	Activates *Nif-B* gene
HetN	Helps in fate determination
PknH	Maintaining the heterocyst system by connecting the vegetative cell and heterocyst.
HepA	Plays a key role in the formation of hep layers
HepK	Controls the expression of HepA and functions as a bacterial two component regulatory system
HepN, HepS	Subsidiary genes which are essential for Hep layer formation
HenR	Subsidiary gene which is essential for Hep layer formation
MurB	Transforms UDP-N-acetylglucosamine enolpyruvate to N-acetyl muramic acid
MurC	Attaches pentapeptide chains
HcwA	Involved in rearrangement of the peptidoglycan layer
AmiC1	Amidase encoding gene, forms nanopore at septal junction and is also involved in rearrangement of peptidoglycan layer
AmiC2	Amidase encoding gene, forms nanopore at septal junction and is also involved in rearrangement of peptidoglycan layer
Pbp6	A penicillin-binding protein functions as origination of the peptidoglycan layer and maturing it
Yfr1 (sRNA)	Reduces heterocyst differentiation along with ten different m-RNA
SepJ (FraG)	Maintains the number of nanopores
FraC	Maintains the number of nanopores
FraD	Maintains the number of nanopores

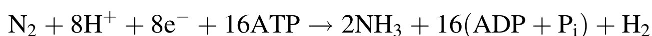
(continued)

Table 12.1 (continued)

Protein/gene	Function
SjcF1	Regulates the size of nanopore diameter
HgdB	A kind of membrane fusion protein which helps in to find out the correct configuration and ratio of glycolipids in heterocyst
HgdC	A type of permease which helps in to find out the correct configuration and ratio of glycolipids in heterocyst
DevBCA	Exports glycolipid along with ABC exporter
DevH	Positively regulates the gene expression of HglE
HglE	Helps in synthesis of hgl
NifH1	Main nitrogen fixation gene
NifD	Plays a key role in coding the alpha subunit of nitrogenase
HupL	Helps in nitrogen fixation
Primase P4	Helps in nitrogen fixation
NifB	Essential for the formation of Fe-Mo cofactor
NifS	Essential for the formation of Fe-Mo cofactor
NifU	Essential for the formation of Fe-Mo cofactor
FdxN	Encodes ferredoxin
NifH	Encodes dinitrogenase complex
NifK	Plays a key role in coding the beta subunit of nitrogenase
NifB	Activated by Cnfr
Cox2	A respiratory oxidase which helps in enhancing the respiration rate in heterocyst
Cox3	A respiratory oxidase which helps in enhancing the respiration rate in heterocyst
Flv1B	Decreases oxygen concentration to form water solely in heterocyst
Flv3B	Decreases oxygen concentration to form water solely in heterocyst
PetH	Encodes ferredoxin-NADP oxidoreductase
GlnA	Encodes glutamine synthetase
CphA1	Encodes cyanophycin synthetase which synthesizes cyanophycin granules
CphB1	Encodes cyanophycinase which forms cyanophycin granules

12.3 Nitrogenase and Alternate Nitrogenase

In order to enter the biogeochemical cycle, atmospheric N_2 must be first reduced to a form that can be readily assimilated by organisms in a process known as nitrogen fixation. In cyanobacteria and other N_2-fixing prokaryotes, molecular dinitrogen (N_2) is reduced in multiple electron transfer reactions requiring 16 ATPs per N_2 fixed, resulting in the synthesis of ammonia and the release of hydrogen as a by-product. H_2 generated during the N_2 fixation process may be oxidized by a hydrogenase in a subsequent step (Esteves-Ferreira et al. 2018).



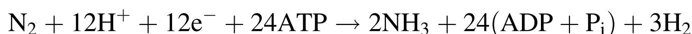
$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + 16(ADP + P_i) + H_2$$

The reduction of molecular nitrogen to ammonium is catalyzed in all nitrogen-fixing organisms via the nitrogenase enzyme complex. Nitrogenase can also reduce many other substances, such as acetylene, hydrogen azide, hydrogen cyanide, or nitrous oxide. Of these, acetylene reduction to ethylene can be monitored because both acetylene and ethylene can be detected easily by gas chromatography (Fay 1992). Based on the type of metal center, there are three well-known types of nitrogenases: iron and molybdenum (Fe/Mo) nitrogenase, iron and vanadium (Fe/V) nitrogenase, and iron only (Fe) nitrogenase. The Fe/Mo-type is the most commonly found in cyanobacteria and rhizobia. The Fe-only and V-nitrogenases are referred as alternative nitrogenases and are considered as "backup" enzymes when Mo is limiting (McRose et al. 2017). The Fe/Mo-nitrogenase is encoded by nitrogen fixation genes (*nifHDK*), the V-nitrogenase by vanadium-dependent nitrogen fixation genes (*vnfHDK*), and the Fe-nitrogenase by alternative nitrogen fixation genes (*anfHDK*) (McRose et al. 2017).

In nonheterocystous cyanobacteria, nitrogenase enzyme is present in all vegetative cells, while in heterocystous form it is localized only in heterocysts. The enzyme nitrogenase that is expressed in heterocyst is Mo-dependent nitrogenase (Nif-1), which has two components—a Mo-Fe protein (molybdoferredoxin or dinitrogenase) and Fe protein (azoferredoxin or dinitrogenase reductase). The dinitrogenase (Mo-Fe protein) is an $\alpha_2\beta_2$ tetramer and its subunits are encoded by *nifD* and *nifK* genes, respectively. The other component, dinitrogenase reductase (Fe protein) is a dimer of two identical subunits (γ) encoded by *nifH* gene. Fifteen nitrogen fixation-related genes are found clustered together in six transcriptional units: *nifB-fdxN-nifS-nifU*, *nifHDK*, *nifEN*, *nifX-orf2*, *nifW-hesA-hesB*, and *fdxH*. A gene-designated *glbN* is found positioned between *nifU* and *nifH,* which encodes monomeric hemoglobin called cyanoglobin. A second functional Mo-dependent nitrogenase Nif2 has been reported in *Anabaena variabilis* ATCC 29413 which is synthesized in the vegetative cells solely under anoxic conditions after the cells have been starved of nitrogen and long before heterocysts form (Schrautemeier et al. 1995; Thiel et al. 1997). Nif2 has also been observed in vegetative cells of nonheterocystous species (Berman-Frank et al. 2003).

Vanadium-containing nitrogenase was first reported in *Anabaena variabilis*, which significantly reduced acetylene (C_2H_2) to ethane (C_2H_6) under Mo deficiency and in the presence of vanadium (V). It was further identified that the V-nitrogenase is encoded by *vnf* genes cluster (*vnfDGKEN*) in *A. variabilis* (Thiel 1996). The V-dinitrogenase is actually encoded by *vnfDGK* gene cluster, while, *vnfEN* gene cluster located downstream of *vnfDGK* is found to be essential for V-nitrogenase activity. In addition to *vnfDGKEN* gene cluster, four other *vnfH* genes are located 23 kb downstream of *vnfN and* are responsible for encoding dinitrogenase reductase of V-nitrogenase.

The V-nitrogenase is a heterooctomer consisting of two α-subunit (VnfD), two β-subunit (VnfK), four δ-subunits (VnfG), and two Fe-V cofactors (Thiel and Pratte 2014). In comparison to Mo-nitrogenase, the V-nitrogenases have lower substrate-binding efficiency; therefore, it reduces less dinitrogen and produces three times more hydrogen than the Mo-nitrogenase (Thiel and Pratte 2014).



$$N_2 + 12H^+ + 12e^- + 24ATP \rightarrow 2NH_3 + 24(ADP + P_i) + 3H_2$$

Nitrogenase is extremely oxygen sensitive. The oxygen is kept far away from nitrogenase by biochemical pathways like the Mehler-reaction or by special oxygen scavenging molecules such as cyanoglobin that binds oxygen reversibly, with high affinity and noncooperatively (Thorsteinsson et al. 1996). In addition, cyanobacteria are diverse group of gram-negative bacteria which coordinate two mutually exclusive process; O_2-evolving photosynthesis and O_2-sensitive nitrogenase-dependent nitrogen fixation. Cyanobacteria have an efficient way to protect O_2-sensitive nitrogenase from O_2_evolved during photosynthesis. In cyanobacteria, these processes are either separated temporally (as in nonheterocystous form/unicellular cyanobacteria, where alternate cycles of nitrogen fixation and photosynthesis take place) or spatially (as in heterocystous forms). Interestingly, heterocyst lacks photosystem II activity; therefore, they do not evolve oxygen that inhibits nitrogen fixation.

Numerous nonheterocystous cyanobacterial strains can fix and reduce atmospheric N_2 to ammonium when confronting nitrogen hardship, for example, *Synechocystis* and *Arthrospira* (*Spirulina*) *maxima* (Esteves-Ferreira et al. 2017). N-fixation is a costly metabolic reaction catalyzed by nitrogenase, which is restrained by O_2 (Esteves-Ferreira et al. 2018). To shield nitrogenase from O_2, photosynthesis and N-fixation are transiently isolated. High nitrogenase activity peaks 12 h after the peak of photosynthesis, at the same time with higher respiratory rates. An alternate N-fixation methodology is solely seen in strains of the genera *Trichodesmium* (Bergman et al. 2013). In these genera, nitrogenase is situated in roughly 20% cells of the filament, and inquisitively these cells display high N_fixation rates at midday (Rodriguez and Ho 2014).

12.4 Uptake of Nitrogen Sources

The most commonly used nitrogen sources by cyanobacteria are nitrate, ammonium, urea, and dinitrogen. Ammonium is the most reduced inorganic form of nitrogen and preferred source of nitrogen for cyanobacteria. When present in the environment, a decrease in the abundance of nitrogen assimilatory enzymes and a reduced expression of nitrogen transport systems leads to a process referred as global nitrogen control (Esteves-Ferreira et al. 2018). Ammonium indirectly represses the expression of *nif* genes by blocking the transcription of NtcA. In natural environments, ammonium is generally present at low concentrations; therefore, specific permeases namely Amt1, Amt2, and Amt3 are required for efficient cellular uptake of ammonium (Esteves-Ferreira et al. 2018). It has been identified that Amt1 is the main permease for ammonium uptake in *Synechocystis* (Montesinos et al. 1998).

Nitrate and nitrite are the most frequent sources of nitrogen for cyanobacteria. In order to be assimilated by cyanobacteria, nitrate is reduced to ammonium via two sequential reactions catalyzed by enzymes nitrate reductase and nitrite reductase. The reductions of nitrate to nitrite and nitrite to ammonium are Fd-dependent and

energetically costly (Flores and Herrero 2005). Nitrate uptake and nitrate reductase system are not found in heterocyst. Nitrate and nitrite are actively transported by the ABC-type NrtABCD transporter in freshwater cyanobacterial strains (Maeda et al. 2015). However, it has been reported that nitrate utilization by cyanobacteria in saline environments may be mediated by NapA (NrtP) rather than NrtABCD transporters. The genes for NrtABCD transporter (*nrtA, nrtB, nrtC, and nrtD*) are commonly present in the *nirA* operon (i.e., *nirA-nrtABCD-narB*). The *nirA* and *narB* genes encode the enzymes Fd-nitrite reductase (NirA) and Fd-nitrate reductase (NarB), respectively, which lead to the formation of ammonium. In *Synechocystis*, *nirA* has been found to be separated from *nrtABCD-narB* (Ohashi et al. 2011). Certain marine and saline water cyanobacterial strains have nitrite transporter of the formate/nitrite transporter (FNT) family, and the cyanate ABC-type transporter which transport nitrite with a much lower affinity than for cyanate (Maeda and Omata 2009; Maeda et al. 2015). A transporter, encoded by the gene *nrtP*, additionally displays high affinity for nitrate and nitrite and was distinguished in the genome of cyanobacterial strains from freshwater and marine conditions (Sakamoto et al. 1999; Bird and Wyman 2003; Maeda et al. 2015).

Many cyanobacteria have shown to import urea at concentrations as low as 0.1–0.6 mM (Mitamura et al. 2000). But before assimilation, urea needs to be hydrolyzed to ammonium and CO_2 catalyzed by a Ni^{2+}-dependent urease. The urease is typically a constitutive enzyme which is not regulated by nitrogen-containing compounds (Ludwig and Bryant 2012). However, a low urease action has been noted in some cyanobacterial strains in presence of ammonium (Singh 1992). These cyanobacteria, such as *Synechocystis*, have high-affinity urea ABC-type transporter responsible for urea uptake at concentrations lower than 1 mM (Esteves-Ferreira et al. 2018). The urea ABC-type transporter is encoded by *urt* genes *urtA, urtB, urtC, urtD,* and *urtE*. These genes are normally organized in an operon, although in *Synechocystis* they have been found to spread along the chromosome (Valladares et al. 2002).

12.5 Ammonium Incorporation into Carbon Skeletons

In cyanobacteria, ammonium is incorporated into carbon skeletons mainly through the glutamine synthetase/glutamine oxoglutarate aminotransferase (GS/GOGAT) cycle. GS catalyzes the ATP-dependent incorporation of ammonium into glutamate to form glutamine. In the following reaction, GOGAT (glutamate synthase) catalyzes the transfer of amide group from glutamine to 2-oxoglutarate (2-OG) to form two molecules of glutamate. Subsequently, aminotransferases can transfer the amino group from glutamate to other carbon skeletons to form other amino acids (Esteves-Ferreira et al. 2018).

In cyanobacteria, there is only one GS (GSI) which is encoded by the gene *glnA*. GSI activity is negatively regulated in presence of ammonium by protein-protein interaction of two inactivating factors (i.e., IF7 and IF17). In contrast, under nitrogen deficiency or in the presence of nitrogen sources other than ammonium, *glnA*

expression is up-regulated (Esteves-Ferreira et al. 2018). Interestingly, in some cyanobacterial strains such as *Synechocystis*, *Synechococcus,* and *Gloeocapsa* sp. PCC 7428, a second type of GS encoded by *glnN*, referred as GS type III (GSIII) has also been observed (Reyes and Florencio 1994). It has been observed that *glnN* transcription is more sensitive to ammonium and nitrate than *glnA*, and maximal GSIII activity can reach at most 24% of GSI activity in cells under nitrogen starvation (Reyes and Florencio 1994). In *Pseudanabaena* sp. strain PCC 6903, only the GSIII has been found to be responsible for all ammonium assimilation. This indicates that the presence of an additional glutamine synthetase (GSIII) in some cyanobacterial strains indicates its possible role in increasing the efficiency of ammonium assimilation under nitrogen deficiency.

GOGAT (glutamine 2-oxoglutarate aminotransferase) is a constitutive expressed enzyme. Two different forms of GOGAT have been described in photosynthetic organisms, one is a Fd-GOGAT encoded by *glsF* (*gltS*), and, second is a complex of NADH-GOGAT encoded by *gltB* and *gltD gltB* (large subunit) and *gltD* (small subunit) (Muro-Pastor and Florencio 2003). All cyanobacteria encompass Fd-GOGAT (Muro-Pastor et al. 2005) though NADH-GOGAT has been also identified in *Synechocystis* and *Plectonema boryanum* (Wang et al. 2004). Although, both the forms of this enzyme are simultaneously active in these strains, but Fd-GOGAT is found to be more active and has a more prominent role in ammonium assimilation and growth.

Alternatively, the glutamate dehydrogenase (GDH) can catalyze the formation of glutamate directly from 2-oxoglutarate and ammonium in an NADPH-dependent reaction. However, since GDH has higher K_m (and consequently low affinity) for ammonium than GSI, hence, this enzyme is not likely to be intricated in main ammonium assimilation in cyanobacteria. It has been suggested that this enzyme is involved in the removal of excess cellular ammonium, so as to guard the proton gradient within the thylakoid and periplasmic spaces (Chávez et al. 1999).

12.6 Cyanobacteria as Biofertilizer

Due to ability of cyanobacteria to fix the atmospheric nitrogen, they are conventionally used as biofertilizers in agriculture field. Their importance as biofertilizer has increased mainly to avoid usage of synthetic fertilizers. Cyanobacteria not only increase the nitrogen content in the soil, but also improve the soil structure via released polysaccharides, increase the soil aeration by their filamentous structure, improve soil health by releasing growth hormones, decrease the soil salinity, and improve water holding capacity of the soil (Kuraganti et al. 2020). Because of their ecofriendly organic nature and economic feasibility, cyanobacteria are commonly used as biofertilizer mainly in the paddy field. Generally, *Anabaena*, *Nostoc*, *Aulosira,* and *Tolypothrix* are used as biofertilizer. However, use of *Azolla-Anabaena* symbiotic N_2-fixing complex has also been reported and it is found to have this symbiotic complex as more advantageous than free-living cyanobacteria from agronomic point of view.

12.7 Conclusions

Nitrogen-fixing capability of cyanobacteria has made this group of organisms very important for the research purpose. Cyanobacteria require two interdependent cell types for growth, i.e., vegetative cells for oxygenic photosynthesis and heterocyst for nitrogen fixation. Heterocyst differentiation process involves many proteins like NtcA, HetR, HetP, HetZ, PatS, PatX, PatC, PatD, PatN, PknH, HepA, HepK, etc. The first sensing signal for induction of heterocyst formation is synthesis of 2-oxoglutarate which is a part of Kreb cycle as alpha ketoglutarate. Increase in concentration of 2-oxoglutarate triggers the synthesis of NtcA protein. NtcA further activates the other genes involved in heterocyst differentiation. Development of heterocyst and their spatial distribution both are equally important. Proteins of *het* gene family are involved in differentiation and proteins of *Pat* gene family regulate distribution pattern.

In this chapter, it is discussed that most commonly used nitrogen sources by cyanobacteria are nitrate, ammonium, urea, and dinitrogen, but ammonium is most preferred nitrogen source. In nitrogen fixation, dinitrogen is reduced to ammonia in multiple electron transfer reactions requiring 16 ATPs per N_2 fixed. In nitrogen assimilation by cyanobacteria, nitrate is reduced to ammonium via two sequential reactions catalyzed by enzymes nitrate reductase and nitrite reductase. Nitrate and nitrite are actively transported by the ABC-type NrtABCD transporter in freshwater cyanobacterial strains, and in saline environments may be mediated by NapA (NrtP). Urea is hydrolyzed to ammonium and CO_2 by a Ni^{2+}-dependent urease. The urea ABC-type transporter is encoded by *urt* genes.

In cyanobacteria, ammonium is combined into carbon skeletons mostly through the GS/GOGAT cycle. GS is encoded by the gene *glnA*. Two diverse forms of GOGAT have been described in photosynthetic organisms, a Fd-dependent GOGAT (*glsF*) and NADH-dependent GOGAT (*gltB* and *gltD*). Alternatively, GDH catalyzes the synthesis of glutamate from 2-oxoglutarate and ammonium in an NADPH-dependent reaction, albeit with low affinity.

Studies involving nitrogen metabolism led to understanding of molecular mechanism of nitrogen fixation on exposure to various nitrogen sources. Better understanding of the mechanisms involved in nitrogen metabolism would increase the biotechnological potential of these organisms (Zehr and Capone 2020).

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