CHAPTER 1
GENERAL INTRODUCTION

Bioproductivity in natural and artificial ecosystems is largely regulated by the availability of nutrients. Of these nitrogen is known to be chief limiting nutrient. The nutritional need of the world's growing population depends chiefly on the success of modern agriculture, which to a large extent is regulated by the availability of fixed nitrogen as chemical fertilizer. The supply of the chemical nitrogen fertilizer is dependent on petroleum based chemical factories and the cost of such chemical nitrogen fertilizer is galloping with a proportionate rise in the cost of fossil fuels. Thus, such artificial provisions of chemical nitrogen fertilizer are gradually becoming out of reach for poor countries like India whose nearly 80% population thrives primarily on agriculture produce. Further, extensive use of chemical nitrogen fertilizer in modern agriculture, over last three decades has caused pollution of aquatic and terrestrial ecosystems of rendering them eutrophic. These realisations have catalysed scientific research for developing alternative source of nitrogen supply in agriculture ecosystems. The most obvious candidates in this regard are biological nitrogen fixers called diazotrophs which are found as free living forms or symbiotic forms in various natural and artificial ecosystems.

The most prominent contributors of biological nitrogen input in terrestrial habitats are *Rhizobium-legume*. Symbiotic systems followed by *Frankia-woody* plant systems and cyanobacterial free living and symbiotic systems in given order. In fact diazotrophic cyanobacteria have been documented to play an indispensable role in nitrogen budget of rice agriculture (Singh, 1961). Since then research on understanding the molecular biology of cyanobacterial diazotrophy and NH₃ production have been intensified with some positive results (Stewart et al. 1987; Modi et al. 1991). A group of Indian workers led by Dr. G.S. Venkataraman has claimed to have developed techniques for using a mixture of free living diazotrophic cyanobacteria as biofertilizer in wet land rice agriculture (Venkataraman, 1975).
Cyanobacteria also exhibit tremendous potential for biotechnological applications in desert reclamation (Painter, 1993), waste water treatment (Wilde et al. 1991), bioremediation (Kuritz and Wolk, 1994) and reclamation of *usar* land (Singh, 1961). Many laboratories have also suggested the use cyanobacteria as biopesticide against mosquito larvae (Murphy and Stevens, 1992; Soltes-Rak et al. 1995) and as biocontrol measures against pathogenic fungi and bacteria (Kulik, 1995). The ability of cyanobacteria to grow at simple expense of light, water and air make them most ideal biological systems for such applications. However, the success of cyanobacteria in the given biotechnology would greatly depend on a clear and complete understanding of their molecular biology which is at the moment far from clear. The modern molecular biological techniques that have been developed during the last two decades for genetic analysis of cyanobacteria at molecular level include an introduction of foreign DNA into cyanobacteria by conjugation (Porter, 1987; Elhai and Wolk, 1988; Theil and Poo, 1989; Theil, 1994) and isolation of mutations tagged with transposons (Wolk et al. 1988) and to identify genes by the method of mutation complementation (Golden, 1988; Haselkorn, 1991). A technique has also been developed to make use of *lux* gene as a reporter gene to monitor the expression of *het* genes and regulatory genes during heterocyst differentiation and *nif* gene expression (Elhai and Wolk, 1990). The following description will narrate the history of major developments in molecular biology of cyanobacteria relevant to diazotrophy, biofertilizer technology, alkali metal nutrition and adaptation to salinity and osmotic stress.

The first conclusive evidence for heterocystous forms fixing N\(_2\) was provided by Drewes (1928), Fogg (1942), Fay et al. (1968) suggested heterocyst to be the site of nitrogen-fixation in heterocystous cyanobacteria, Stewart et al. (1969), Fleming and Haselkorn (1973) demonstrated occurrence of synthesis of nitrogenase with in heterocyst. These developments confirmed that heterocyst’s primary function in cyanobacteria is to provide site for nitrogen-fixation under aerobic conditions. This led to the belief that nitrogen-fixing ability in cyanobacteria belongs to heterocystous forms. However, this thinking soon underwent revision when Wyatt and Silvey (1969) discovered aerobic N\(_2\)-fixation in laboratory cultures of non-heterocystous unicellular cyanobacterium *Gleocapsa* sp. Stewart and Lex (1970) demonstrated microaerobic N\(_2\)-
fixation in the non heterocystous filamentous cyanobacterium *Plectonema boryanum*. These discoveries showed occurrence of diazotrophy in non-heterocystous unicellular and filamentous cyanobacteria as well. This led to the problem of heterocystous nitrogenase, vegetative cell nitrogenase and their protection from O₂ toxicity during oxygenic photosynthesis. A series of critical experiments established beyond doubt that heterocyst is the O₂-protection mechanism of cyanobacterial nitrogenase and it is also a requirement for expression of cyanobacterial *nif* genes (Fay, 1992; Gallon, 1992). Studies of Golden et al. (1985) led to the discovery of cyanobacterial *nif* gene rearrangement during heterocyst differentiation and thus explaining why *nif* genes are expressed for nitrogenase synthesis and activity only in heterocysts and not in vegetative cells.

The non-heterocyst cyanobacterial diazotrophs, unicellular or filamentous are known to carry out the antagonistic process of oxygenic photosynthesis and nitrogen-fixation with in the same cell. The question has been repeatedly asked about the mechanism of O₂-protection of their nitrogenase under such condition. This process has not been completely clarified (Fay, 1992; Gallon, 1992). At one extreme are forms like *Plectonema boryanum* which do not seem to process any effective O₂-protection mechanism because they keep fixing nitrogen only under anaerobic or microaerobic conditions. At the other extreme are forms like *Trichodesmium* which are capable of carrying N₂-fixation with in the same cell, under aerobic condition. At the intermediate level in forms like *Gloeocathecace* temporal separation of oxygenic photosynthesis and nitrogenase activity has been suggested as possible mechanism of oxygen protection (Gallon and Chapelin, 1988). In contrast *Gloeocathecace sp. PC 6909* had been shown to carry out N₂-fixation and oxygenic photosynthesis concurrently within the same cell (Ortego, Calvo and Stal, 1991).

Ammonium grown non-heterocystous filaments of heterocystous cyanobacteria on transfer to diazotrophic medium show differentiation of vegetative cells into heterocysts at regular intervals along their length. Nitrogen fixing mature heterocyst has been shown to lack PSII activity (Fay et al. 1968.). Ribulose 1-6 biphosphate carboxylase/oxygenase (Rubisco) activity (Cossar et al. 1985), nitrate reductase activity
(Kumar et al. 1985) and glutamate synthase (GOGAT) activity (Thomas et al. 1977) and to contain uptake hydrogenase activity (Peterson and Wolk, 1978) functional nitrogenase activity and PSI activity (Haselkorn, 1978). Biochemical basis of the role of heterocysts as O$_2$-protection mechanism of cyanobacterial nitrogenase activity has been analysed and shown to result from loss of PSII activity and presence of unique O$_2$-imperious glycolipid layer in the inner membrane of heterocyst envelope (Lambein and Wolk, 1973). Higher rate of aerobic respiration in heterocysts compared to that in vegetative cells coupled with operation of oxyhydrogen reaction exclusively localised in heterocysts suggest clearly the role of these two O$_2$-dependent processes as additional mechanism of oxygen protection of nitrogenase activity within heterocysts (Bothe, 1982). Analysis of the role of regulatory and structural genes of heterocyst differentiation and heterocyst pattern formation has demonstrated that het-R gene controls heterocyst differentiation by regulating the expression of het genes and pat-A gene controlling the pattern of heterocyst spacing by an unknown mechanism (Buikema and Haselkorn, 1991; Laing et al. 1992; Singh et al. 1994). A comparative study of the organisation of nif structural genes nif HDK in vegetative cells and in heterocysts has revealed a fundamental difference in that, while nif HDK in heterocysts are contiguous that in vegetative cells is interrupted by presence of a DNA element of 11 kb in nif-D gene. The loss of 11 kb DNA element from nif-D during differentiation of vegetative cells into heterocysts is believed to result in expression of nif HDK operon for nitrogenase synthesis (Golden et al. 1985). A second rearrangement involving deletion of a 55 kb long DNA segment located in fdx-N gene has also been shown to occur during vegetative cell differentiation into heterocyst (Golden, 1988). The exceptions to this characteristic nif gene rearrangement during heterocyst differentiation are the reports that nif structural genes are contiguous in both heterocysts and vegetative cells in Fischerella sp. AT 0027923 (Saville et al. 1987) and in Mastigocladus (Singh and Stevens, 1992; Elhai and Wolk, 1990) by the use of reporter gene lux fused to cyanobacterial nif. Promoter have shown clearly that expression of nif genes in the heterocyst is developmentally but not environmentally regulated. Recently experiments based on reporter gene expression for in situ localisation of nitrogenase gene activity has shown that in Anabaena variabilis there are two large clusters of nif genes. One of which functions under aerobic or anaerobic growth conditions exclusively in heterocysts. While the other functions only
under anaerobic growth conditions in vegetative cells and in heterocysts. While the \textit{nif} gene expression in heterocysts is developmentally regulated, that in vegetative cells is regulated by environmental factors (Theil, 1993).

Rearrangements of \textit{nif}-genes and their regulation has been studied in great detail in \textit{Anabaena} PCC 7120. During the differentiation of heterocysts the \textit{nif} cluster is organised in such a way that 11 kb DNA element of \textit{nif}-\textit{D} gene and 55 kb element of \textit{fdx}-\textit{N} gene are precisely excised and in the mature heterocyst the entire \textit{nif} cluster becomes continuous as in \textit{Klebsiella pneumoniae} (Golden et al. 1985). The results suggest operation of two site-specific recombination events to take place simultaneously in the elimination of the two given DNA element during heterocyst differentiation. It has been shown that \textit{Xis}-\textit{A} gene encoding the enzyme excises functions in deletion of 11 kb element from \textit{nif}-\textit{D} gene (Lammers et al. 1986). The genetics of the mechanism involved in addition of 55 kb DNA element from \textit{fdx}-\textit{N} gene is not yet clarified. The gene \textit{Xis}-\textit{A} is essential for N\textsubscript{2}-fixation since its inactivation by site directed mutagenesis blocks the 11 kb rearrangement and also inhibits N\textsubscript{2}-fixation (Golden and Wiest, 1988).

Recently molecular genetic approaches has revealed three genetically distinct nitrogenases in \textit{Azatobacter vinelandii}. They are molybdenum containing nitrogenase called conventional nitrogenase (nitrogenase-1) vanadium containing nitrogenase called V-nitrogenase (nitrogenase-2) and Mo or V free iron containing nitrogenase (nitrogenase-3) (Bishop et al. 1986). The three nitrogenase are regulated differently by molybdenum or vanadium. Evidence has been presented for the possible occurrence of V-nitrogenase in addition to Mo-nitrogenase in the cyanobacterium \textit{Nostoc muscorum} (Singh et al. 1993 a&b) and in \textit{Anabaena variabilis} (Kentemich et al. 1988). Lack of appropriate molecular techniques at the moment is the limiting factor for examining the role of Mo and V in the regulation of cyanobacterial Mo-nitrogenase and V-nitrogenase. Cyanobacterial nitrogenase like nitrogenases of other prokaryotes is an enzyme reducing molecular nitrogen to NH\textsubscript{3} and protein to molecular N\textsubscript{2} simultaneously. Occurrence of uptake hydrogenase activity within nitrogen fixing heterocyst provides as effective mechanism for utilisation of nitrogenase generated molecular hydrogen for production of reductants, ATP and protection of nitrogenase activity from O\textsubscript{2}-toxicity (Bothe, 1982).
Glutamine synthetase (GS) and Glutamate synthase (GOGAT) are the primary enzymes of assimilation of NH3 resulting from fixation of N2 reduction of NO3− or supply of exogenous NH3. Nitrogen regulation of GS enzyme has been studied in some cyanobacteria and glutA gene coding for this enzyme has been demonstrated to be regulated from two promoters, one relatively more efficient functioning under diazotrophic condition and the other relatively less efficient functioning under NH3 assimilating condition (Tumer et al. 1983). Heterocyst differentiation, nitrogenase synthesis and activity, NH4+ transport activity all exhibit nitrogen control in the sense that they show NH4+ repression/derepression by a molecular mechanism which is still unknown (Haselkorn 1978; Stewart 1980; Rai et al. 1984; Singh et al. 1985; Singh et al. 1989). In enterobacterial systems well defined ntr genes have been shown to operate in control of nitrogen assimilation (Merrick, 1988). In contrast, molecular basis of nitrogen control in cyanobacterial nitrogen nutrition has not been clear until now. Recently cyanobacterial regulatory gene containing nitrogen nutrition has been discovered and given the name ntc-A whose product is a global nitrogen regulation for the activation of NH4+ repressible gene in diverse cyanobacteria (Luque et al. 1994) ntc-A gene has also been shown to be essential for heterocyst differentiation in Anabaena PCC 7120 (Wel et al. 1994). Preliminary evidence for the operation of ntr like regulatory gene in positive control of heterocyst formation, nitrogenase activity and NH4+ transport system has been demonstrated in Nostoc muscorum (Singh et al. 1989).

C:N ratio is a good measure of nitrogen status of prokaryotic cell. In enterobacteria the ratio of alfa-keto glutarate to glutamine is used to estimate the nitrogen status of the cell. If the ratio is high the cells generate nitrogen starvation signal and if the ratio is low the cells accordingly generate nitrogen replete signals.

Such nitrogen signal transduction mechanisms have been well characterised at genetic and molecular level in enterobacteria. The sensor molecule of this ratio is a biofunctional enzyme called Uridylyl Transferase (UT)/Uridylyl Removing (UR) enzyme, which uridylylates PII protein under nitrogen limitation and deuridylylates, uridylylated PII protein under nitrogen sufficiency. The uridylylated PII protein is an activator of Ntr-
B encoded protein kinase activity leading to phosphorylation of Ntr-C protein (a product of ntr-C gene). The resulting phosphoprotein is an activator of genes under the control of nitrogen regulated promoters including nif promoters. Thus nitrogen starvation signal is sensed through uridylylating activity of the sensor molecule and translated into the production of phosphorylated Ntr-C protein, the form required for initiation of the nif genes leading to fixation of molecular N₂. The nitrogen replete signal is transduced through uridylylating activity of the sensor molecule for dephosphorylation of Ntr-C protein, the form inactive in activation of nif genes and other similarly nitrogen regulated operons. Recently the gene for PII protein has been discovered in a wide range of cyanobacteria. Studies on regulation of its activity has shown it to undergo phosphorylation in the absence of NH₄⁺ (nitrogen source) and/or under PSII light, i.e., functioning of PSII is favoured over PSI under the conditions of phosphorylated PII protein. This finding has directly implicated a role of PII protein in co-ordination of oxygenic photosynthesis and inorganic nitrogen metabolism (Tsinoremas et al. 1991; Allen, 1992). More studies along this line in diazotrophic cyanobacteria are expected to define the fundamentals of coupling mechanism within photosynthesis and nitrogen nutrition.

Diazotrophic cyanobacteria are known to occur in a wide range of ecological habitats ranging from hot springs brakish water, usar soils to desert habitats (Fay and Van Baalen, 1987). In tropical countries a majority of diazotrophic cyanobacteria grow and multiply luxuriantly in wet land rice ecosystem during rainy season. They prefer alkaline pH, high humidity and high temperature to grow in water logged paddy fields of tropical countries (Singh, 1961). They have been suggested to function as biological nitrogen source for nitrogen supply in rice ecosystems. (Singh, 1961). Indian group led by (G.S. Venkataraman, 1975) has advocated a technology for production and use of diazotrophic cyanobacteria as biofertilizers in rice agriculture. The biofertilizer potential of diazotrophic cyanobacteria in recent years has attracted interest of cyanobacteriologists all over the world to understand the molecular basis of biological NH₃ production, NH₃ assimilation, NH₄⁺ transport and amino acid metabolism. The concept of using diazotrophic cyanobacteria as potential source of NH₃ arose from the fact that Anabaena azollee in symbiotic association with its host provides N₂-derived
NH₃ to its host through a mechanism in which GS-GOGAT pathway of NH₃ assimilation remains repressed (Stewart et al. 1987; Rai et al. 1984). Cyanobacterial NH₄⁺ transport systems has been found to be characteristically biphasic and NH₄⁺ repressible (Rai et al. 1984; Singh et al. 1985, 87, 89 & 90). Experimentally generated strains of free living diazotrophic cyanobacteria defective in the GS activity for NH₃ assimilation have been found to liberate extracellularly N₂ derived NH₃ (Stewart et al. 1987; Singh et al. 1983, 1992). Further experiments with such NH₃-excreting cyanobacterial strain have shown then to supply N₂-derived NH₃ to rice and wheat plants in laboratory conditions (Lattore et al. 1986; Spiller et al. 1993). The molecular mechanism of linkage between NH₄⁺ transport systems, NH₃ excretion and NH₃ assimilation still remains to be understood clearly. However, such knowledge is a pre-requisite for construction of useful cyanobacterial bio-fertilizer strains. Ideally a cyanobacterial biofertilizer strain needs to have the following attributes.

1. Derepression of diazostrophy.
2. NH₃ excreting during N₂-fixation
3. Resistance to herbicides and pesticides
4. Resistance to salinity or osmotic stress
5. Ability to compete successfully against native strains.

Although derepressed N₂-fixing strains of cyanobacteria are known (Kerby and Stewart 1988; Kumar et al. 1988) but as yet there is no clear understanding of the repression/derepression control mechanism in any diazotrophic cyanobacteria. Some rice field herbicides have been shown to be extremely toxic and mutagenic to cyanobacteria (Singh and Vaishampayan, 1978).

Thus cyanobacterial biofertilizer strains need to have rice field herbicide resistant phenotypes. Multiple herbicide resistant, NH₃ excreting strains of Nostoc muscorum with biofertilizer potential have been constructed successfully by Modi et al. (1991) using the techniques of genetic transformation and mutation.
Studies on cyanobacterial adaptation to pH stress, salinity stress and osmotic stress have started recently, available evidences suggest them to be having a very effective mechanism for such adaptation. Diazotrophic cyanobacteria are known to exhibit alkalophily in their ecology. The mechanism of cyanobacterial alkalophily has not been worked out. In comparison, this aspect has been investigated in much detail in bacteria. The pH of natural habitats is known to vary from 1 to 11 and the organisms growing there are expected to have evolved mechanism of pH homeostasis to overcome the adversity of external pH on their growth and survival. The primary function of the pH homeostatic mechanisms is to maintain the cytoplasmic pH close to neutrality by regulating the production and consumption of H⁺ with in the cell or the exchange of H⁺ across the cellular membranes (Raven, 1985). The electrogenic system of K⁺ uptake associated with activation of H⁺ extrusion by the H⁺-pumping importing antiporter in the pH range at or above 9 are the two known basic mechanism of pH homeostasis in bacterial systems (Karpel et al. 1991; Koyama et al. 1986; Krulwich and Gulfanti, 1989; Mclaggan et al. 1991).

Cyanobacteria are oxygenic photosynthetic prokaryotes growing and multiplying in aquatic habitats of alkaline nature (Singh, 1961). A majority of them are diazotrophs contributing greatly to the nitrogen budget of their respective habitats. Knowledge of the cyanobacterial pH homeostasis mechanism would be extremely useful in understanding the critical regulatory role of pH in their growth, development and ecology. Few previous studies on the pH related growth characteristics have shown Synechococcus leopoliensis (Miller et al. 1984), Spirulina plantensis (Belkin and Baussiba, 1991) and Halosiphon welwitschii (Dwivedi et al. 1992) to be alkalophiles showing preferential growth in medium of alkaline pH (Krulwich et al. 1990). Virtually nothing is known about the mechanism of pH homeostasis in any cyanobacterium.

Lack of efficient homeostatic mechanism is known to cause metabolically toxic fluctuations in the cytoplasmic pH of organisms upon their exposure to changes in the external pH. Intracellular production or consumption of H⁺ or exchange of H⁺ or H⁻ equivalents across the boundary membranes in response to variations in external pH are the known cellular mechanisms of pH homeostasis in plants and microbes (Raven, 1985).
Facultative alkophilic bacteria are known to maintain their cytoplasmic pH homeostasis in the neutral to near neutral pH range by alkalization of their cytoplasm through the activity of plasma membrane located tightly coupled systems of electrogenic K⁺ uptake by K⁺ transport system and H⁺ extrusion by H⁺-pumping respiratory system and in the alkaline range by acidification of their cytoplasm through the activity of the alkaline pH-regulated Na⁺/H⁺ antiporter system (Koyama et al. 1985). The activity of the Na⁺/H⁺ antiporter is suggested to be the known mechanism of cytoplasmic pH homeostasis in the cyanobacterium S. leopoliensis growing in the growth medium of alkaline pH (Miller et al. 1984). Na⁺ is known to regulate alkaliophilic metabolic processes in cyanobacteria. The Na⁺/H⁺ antiporter in bacterial systems is the known mechanism of cytoplasmic pH homeostasis and of salt adaptation in the alkaline pH range (Krutwich and Gufanti, 1989). Much information is available on the molecular biology of tolerance to temperature, desiccation, salinity or osmotic stresses in bacteria. In cyanobacterial systems tolerance to desiccation is known to result from desiccation induced production of proteins called dehydrins (Bartels et al. 1993; Potts, 1994). Genetic evidence suggest that acclimation induced cold tolerance, like desiccation tolerance, is a quantitative characteristic controlled by number of additive genes (Guy, 1990). Recent biochemical and genetic engineering experiments have demonstrated a definite role of fatty acid desaturases in acquisition of cold tolerance in higher plants (Murata et al. 1992) and in cyanobacteria (Wada et al. 1992). These new developments suggest genetic engineering for generating plants and cyanobacteria tolerant to desiccation and cold. Problem of tolerance to salinity/osmolarity has been investigated at a very preliminary level in certain cyanobacteria mainly with a view to identify nature of compatible solute that occur in cyanobacterial forms naturally adapted to grow in hypersaline/hyperosmotic habitats. The general conclusion is that cyanobacteria like other bacteria and higher plants accumulate compatible solutes like glycine betain, sucrose, trehlosene, glucosyl glycerol as principle osmolytes conferring tolerance to them against salinity/osmotic stress (Mackay et al. 1984; Warr et al. 1988). Analysis of the mechanism of adaptation of enterobacterial forms to salinity/osmotic stress has identified a fundamental role of kdp system of potassium transport as a primary signal leading to their adaptation to salinity/osmotic stress (Lucht and Bremer, 1994). Recently Apte and Haselkorn (1990) have found involvement of about 100 genes in conferring salt tolerance in Anabaena torulosa.
Further studies on the role of proline in conferring salinity/osmotolerance in *Nostoc muscorum* have found osmoinducible over production of proline as a biochemical mechanism of adaptation to such stresses.

Researches on physiology of alkali cations have remained confined mainly to sodium and potassium in eucaryotes and prokaryotes. Study on the ecology of cyanobacteria growing luxuriant in alkaline and saline habitats including *usar* soils implicated a definite role of sodium in their physiology, metabolism and growth (Singh, 1961). During the last 5 decades several reports have been made suggesting the need for sodium especially in nitrate grown cultures of many unicellular non-diazotrophic cyanobacteria such as *Chroococcus sp.*, *Microcystis aeruginosa* (Mclachlan and Gorhem, 1961), *Anacystis nidulans* (Kratz and Myers, 1955). Such Sodium requirement observed in no.1 diazotrophic cyanobacterial forms was found to operate in filamentous heterocystous diazotrophic cyanobacteria as well *Anabaena variabilis*, *Nostoc muscorum* (Kratz and Myers, 1955). Allen and Arnon (1955) found that *Anabaena cylindrica* specifically required sodium during diazotrophic mode of nutrition. Subsequently Apte and Thomas (1980, 1984) found on absolute growth requirement of sodium for N₂-fixing cultures of brackish water cyanobacterial form *Anabaena torulosa* and fresh water cyanobacterial form *Anabaena L-31* form. The sodium requirement was found to be highly specific as it could not be replaced by K⁺, Rb⁺, Cs⁺, Li⁺, Mg²⁺ and Ca²⁺ (Allen and Arnon 1955; Apte and Thomas, 1984).

Molecular biology of salt tolerance has not been studied in much detail although cyanobacterial forms are known some of which grow in brine waters and some in fresh water habitats. In view of cyanobacterial requirement of sodium for growth and in view of sodium being the chemical component involved in induction of salt stress. It becomes necessary to study the mechanism of Na⁺ transport in relation to the mechanism of NaCl induced salt tolerance.

Following types of systems have been implicated in general in the physiology of cyanobacterial salt tolerance:-
1. Exclusion of sodium and maintenance of low intracellular sodium concentration appears to be one mechanism of salt tolerance in *Anabaena torulosa*. In comparison fresh water cyanobacterial forms like *Anabaena L-31* lack an efficient process of sodium efflux as found in *Anabaena torulosa* (Apte and Thomas, 1983). According to this view relative efficiency of cyanobacteria Na\(^+\) influx and efflux is the primary determinant of cyanobacterial salt tolerance.

2. The other mechanism of salt tolerance in cyanobacteria involves salt stress induced production of organic osmotica in terms of sugars, polyols, amino acids and quaternary amines (Reed and Stewart, 1988).

Cyanobacterial systems have not been studied as much in detail for K\(^+\) as they have been studied in for Na\(^+\). In contrast much of the physiology of K\(^+\) is known from studies on bacterial cells and higher plant systems (Walderhaug et al. 1987). K\(^+\) is known to activate a number of cell enzymes from animal cells, plant cells and bacteria. Ions of similar size, such as Rb\(^+\) are known to replace K\(^+\) physiologically in large number of microbial systems (Walderhaug et al. 1985). The other important function of K\(^+\) in cellular physiology is their role as primary inorganic osmoticum catalysing the adaptation of cells to a salinity or osmotic stress (Epstein and Schultz, 1965). Biology of caesium in some cyanobacteria has been studied in some detail and results suggest their Cs\(^+\) toxicity results from intracellular replacement of K\(^+\) by non-functional Cs\(^+\) (Avery et al. 1991). It has also been shown that K\(^+\), Na\(^+\) or NH\(_4\)^+ mitigates or eliminates Cs\(^+\) toxicity by preventing the entry and accumulation of Cs\(^+\) (Avery et al. 1992 a and b; Avery et al. 1993; Singh et al. 1994). There is also report suggesting that Rb\(^+\) can replace K\(^+\) for growth of *Anabaena nidulans* (Kumar and Purohit, 1972). There are also studies on the physiology of K\(^-\) and Rb\(^-\) transport systems in cyanobacteria. According to which there is one common transport system for Rb\(^-\) and K\(^-\) transport (Reed, Rowell and Stewart, 1981). Virtually nothing is known about the physiology of Li\(^+\) in any cyanobacteria. Although in this connection it is important to mention here that a role for K\(^-\) has been assigned in salt/osmotic adaptation of certain cyanobacteria (Miller et al. 1976; Yopp et al. 1978). The following chapters incorporate in brief the main novel findings on the
physiology of alkali cations in the nutrition and adaptation of *Nostoc muscorum* to salinity and osmotic stress.