DISCUSSION
4.1. DISCUSSION

Cyanobacteria are gram-negative bacteria, which, like plants and algae, are photosynthetic and have therefore been used as model systems to understand various physiological processes in plants, such as adaptation to salinity and low temperature acclimatization. Changes in the membrane lipid fatty acid composition of cyanobacteria following decrease in temperature has been studied in a number of cyanobacteria such as *Synechococcus* sp. PCC 7002 [Sakamoto et. al, 1997], *Anacystis nidulans* [Murata et. al, 1979], *Anabaena variabilis* strain M3 [Sato and Murata, 1980; 1981], *Synechocystis* sp. PCC 6803 [Wada and Murata, 1990], and *Thermosynechococcus elongatus*, a thermophilic bacterium [Kieseleva et. al, 2000] with a view to understand the molecular basis of cold adaptation. These studies indicated that common to all cyanobacteria appears to be the observed consistent increase in unsaturated fatty acids with decrease in temperature. In addition, a careful examination of the changes in the fatty acid composition also revealed a correlation between the change observed and the group to which the cyanobacteria belonged to [Murata et. al, 1992]. For instance, cyanobacteria in Group I cyanobacteria change both the extent of unsaturation and the chain length of fatty acids with alterations of growth temperature [Sato et. al, 1979]. But, in contrast to Group I cyanobacteria, the species belonging to the remaining three groups do not show any changes in the chain length of the fatty acids when exposed to low temperature. However, all these species consistently show an increase in the extent of unsaturation of the fatty acids [Sato et. al, 1979; Sato and Murata, 1980], the difference being that in species belonging to Group II, both the C16 and C18 fatty acids show increase in unsaturation, whereas in species in Groups III and IV only change in the extent of unsaturation of C18 fatty acids is observed [Wada and Murata, 1990; Murata et. al, 1992].

Increase in the proportion of unsaturated fatty acids with decrease in temperature is known to alter the fluidity of membranes, which is crucial for the
survival of the cyanobacteria [Wada and Murata, 1989; Wada et. al, 1990; Murata and Wada, 1995; Tasaka et. al, 1996; Macartney et. al, 1996; Nishida and Murata, 1996]. Therefore, it is indeed important to understand the process by which saturated fatty acids are converted to unsaturated fatty acids. This process is attributed to the enzymes called desaturases, which are known to act on membrane lipid-bound fatty acids [Murata and Wada, 1995]. In cyanobacteria there are four different types of desaturases and they have been classified as DesA (Δ12 desaturase), DesB (ω3 acyl-lipid desaturase), DesC (Δ9 desaturase) and DesD (Δ6 desaturase), based on their ability to introduce a double bond at a particular C atom of the fatty acid. As of now, the accepted model for cold adaptation in cyanobacteria is based on the two component signal transduction model, consisting of a membrane associated sensor molecule which, following reduction in membrane fluidity due to low temperature, gets phosphorylated and transfers its phosphate to a regulator molecule in the cytoplasm, which in turn brings about transcriptional activation of desaturase genes [Suzuki et. al, 2001], thus resulting in increase in mRNA, the corresponding desaturase and ultimately an increase in the proportion of unsaturated fatty acids.

Though considerable work has been done on these enzymes in mesophilic cyanobacteria, no work has been done so far on desaturases from psychrophilic organisms, i.e. organisms that are adapted to cold. These organisms, surviving in the cold for thousands of years are likely to have a different membrane lipid fatty acid composition, the mode of regulation of the desaturases may be different, and they may possess a different set of desaturases altogether, with an intrinsic change in their primary structure making them active at cold temperatures. Thus, with a view to get a more comprehensive insight into the molecular basis of cold adaptation the present study was undertaken using cyanobacteria from Antarctica, the untamed cold habitat, as a model system since these cyanobacteria would unequivocally form the best systems for such studies. The study was performed on two cyanobacteria from Antarctica, Nostoc sp. (strain 36) and Leptolyngbya sp.
(strain 9). (hereafter referred to as *Nostoc* sp. and *Leptolyngbya* sp.) with the following objectives:

vii) To monitor changes in the fatty acid composition of a psychrotolerant *Nostoc* sp. (strain 36) from Antarctica grown at different temperatures.

viii) To clone and characterise the desaturase genes.

ix) To understand the expression of the *des* genes.

4.1.1 *Nostoc* sp. (strain 36) and *Leptolyngbya* sp. (strain 9) are Group II cyanobacteria

Both *Nostoc* sp. and *Leptolyngbya* sp., which are psychrotolerant strains, are capable of growth at 10°C, but not at 35°C, unlike the mesophilic cyanobacteria, which are capable of growth between 20°C to 40°C. Psychrotolerant Antarctic *Nostoc* sp. and *Leptolyngbya* sp., which contain C16:0, C16:1(9), C18:1(9), C18:2(9,12) and C18:3(9,12,15) fatty acids (Table 3.2), could be classified as Group II cyanobacteria, according to the criteria of Kenyon [1972], Kenyon et. Al, [1972] and Murata et. al, [1992]. However, unlike some Group II cyanobacteria (e.g. *Anabaena* sp.), they lack C16:2(9,12) [Murata et. al, 1992; Stevens et. al, 1973].

The average number of double bonds per lipid molecule both in *Nostoc* sp. and *Leptolyngbya* sp. grown at 25°C is about 2.5, which is comparable to what is observed in other cyanobacteria grown at around this temperature. For example, the average number of double bonds per lipid molecule in *Nostoc muscorum* grown at 28°C is 2.7; for *Anabaena variabilis* grown at 22°C, it is 2.72; for *Synechococcus* (PCC 7002) grown at 22°C, it is 2.18; and for *Plectonema boryanum* grown at 28°C, it is 2.62 (all values calculated based on the data reported in Murata et. al, 1992). But, when *Nostoc* sp and *Leptolyngbya* sp. were grown at a lower temperature i.e., 10°C the average number of double bonds
increased to 3 and that of *Leptolyngbya* sp. to 2.8. This is a clear indication that the fatty acids were getting more and more unsaturated.

A prominent feature of these psychrotolerant species, grown either at 10°C or 25°C, was the presence of high mole % (84-90 %) of unsaturated fatty acids (C16:1\(_\text{cis-9}\), C18:1\(_\text{cis-9}\), C18:2\(_\text{cis-9,12}\) and C18:3\(_\text{cis-9,12,15}\)), compared to other mesophilic Group II cyanobacteria, such as *Anabaena variabilis* (68-70 %) and *Synechococcus* PCC 7002 (59-64 %) [Murata, 1989; Murata et al., 1992]. Both the psychrotolerant organisms also show a higher mole % of C16:1\(_\text{cis-9}\) compared to C16:0, unlike the mesophilic Group II cyanobacteria, in which the mole % of C16:0 is greater than C16:1\(_\text{cis-9}\) (Table 4.1), [Murata,1989; Murata et. al, 1992]. This would imply that in these psychrotolerant organisms the greater proportion of unsaturated fatty acids facilitates growth at low temperatures.

**Table 4.1.** Comparison of the mole % of C16 fatty acids in cyanobacteria belonging to Group II cyanobacteria*

<table>
<thead>
<tr>
<th>Cyanobacterium</th>
<th>Growth temperature (°C)</th>
<th>Fatty acid (mole %)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nostoc muscorum</em></td>
<td>28</td>
<td>C16:0</td>
<td>41</td>
</tr>
<tr>
<td><em>Plectonema boryanum</em></td>
<td>28</td>
<td>C16:1(_\text{cis-9})</td>
<td>14</td>
</tr>
<tr>
<td><em>Synechococcus</em> (PCC 7002)</td>
<td>28</td>
<td>C16:0</td>
<td>36</td>
</tr>
<tr>
<td><em>Anabena variabilis</em></td>
<td>28</td>
<td>C16:1(_\text{cis-9})</td>
<td>22</td>
</tr>
<tr>
<td><em>Nostoc</em> sp. (strain 36)</td>
<td>25</td>
<td>C16:0</td>
<td>12</td>
</tr>
<tr>
<td><em>Leptolyngbya</em> sp. (strain 9)</td>
<td>25</td>
<td>C16:1(_\text{cis-9})</td>
<td>30</td>
</tr>
</tbody>
</table>

* Data was compiled from Murata et.al [1992] except for the data of *Nostoc* sp. (strain 36) and *Leptolyngbya* sp., which was from the present study.

The higher proportion of unsaturated C16 fatty acids at *sn*-2 (since C16 fatty acids are bound to the *sn*-2 position in Group II cyanobacteria) observed in these psychrotolerant organisms is probably an adaptive response to the survival in the cold. Introduction of a double bond into a fatty acyl chain results in a kink
Discussion

(Fig. 4.1), which causes steric hindrance for the packing of the lipid molecules. For a lipid molecule with unsaturated fatty acids at sn-1, steric hindrance produced will be less compared to that of a molecule in which both sn-1 and sn-2 positions are bound by unsaturated fatty acids (Fig. 4.1). The proportion of such kind of lipids, with unsaturated fatty acids at both the positions, is higher in these Antarctic cyanobacteria and probably is a general feature of all cyanobacteria from cold habitats. Since the general rule for the lipids of cyanobacteria belonging to Group II is that the C18 fatty acids are bound to sn-1 and C16 fatty acids are bound to sn-2 and the percentage of C18:0 is negligible, it may be assumed that the sn-1 position is almost always occupied by unsaturated fatty acids. Thus the increase in C16:1(9) (assuming that all of it is bound to the sn-2 position) is a direct indication of the increased proportion of lipids with unsaturated fatty acids at both the positions. With the above assumptions, the approximate percentages of such lipids in different Group II cyanobacteria could be inferred from table 4.1 as about 13-14% in *Nostoc muscorum*, 19-22% in *Plectonema boryanum*, 17-19% in *Synechococcus* (PCC 7002), 22% in *Anabena variabilis* and 30% in both *Nostoc* sp. and *Leptolyngbya* sp. It may be worthwhile to mention that the difference in the mole % of saturated and unsaturated fatty acids in mesophilic and psychrotolerant cyanobacteria is essentially because of the difference in the proportions of the C16:1(9) which is 10-15% more in psychrotolerant cyanobacteria.

Earlier studies have indicated that, in cyanobacteria, temperature shift-down brings about an increase in desaturation of fatty acids [Sato and Murata, 1980; 1981; Murata et. al, 1979; Wada and Murata, 1990]. But, the fatty acid that is desaturated varies, depending on the fatty acid composition of the cyanobacterium. For instance, in Group I (e.g. *Anacystis nidulans*), conversion of C16:0 to C16:1(9) occurs; in Group II (e.g. *Anabaena variabilis*), transient conversion of C16:0 to C16:1(9) and then to C16:2(9,12) occurs along with C18:0 sequentially getting converted to C18:1(9), C18:2(9,12) and then finally to C18:3(9,12,15); in Group III, desaturation of C18 at sn-1 occurs [Wada and Murata,
1988]; and in Group IV (e.g. *Synechocystis* PCC 6803), C18:3\((6,9,12)\) and C18:4\((6,9,12,15)\) are synthesised after shift-down in temperature [Sato and Murata, 1980; 1981; Murata et al, 1979; Wada and Murata, 1990]. Both the psychrotolerant organisms in the present study, though to different extents, show an increase in the unsaturated fatty acid content, similar to that typically observed in *Anabaena variabilis* [Sato and Murata, 1980; 1981].

Fig. 4.1. Schematic representation of membrane lipids (MGDG and DGDG) of Group II cyanobacteria. Introduction of double bonds in the lipid fatty acids at both sn-1 and sn-2 positions has a higher ‘fluidising’ effect as compared to having unsaturated fatty acids only at the sn-1 position because of lesser steric hindrance in the later case.

4.1.2. Increased activity of DesA and DesB in Antarctic *Nostoc* sp. (strain 36) and *Leptolyngbya* sp. (strain 9)

*Nostoc* sp. and *Leptolyngbya* sp. grown isothermally at 10°C or when grown at 25°C and then transferred to 10°C, show an increase in mole % of
C18:3(9,12,15) at the expense of C18:1(9) and (or) C18:2(9,12), implying an increase in the activity of DesA and DesB. Increase in polyunsaturated fatty acids, like C18:3[(6,9,12) or (9,12,15)] and C18:4(6,9,12,15), at the expense of the corresponding mono-, di- or tri-unsaturated fatty acids, is a phenomenon commonly observed in Group II, Group III and Group IV cyanobacteria, when they are either grown at lower growth temperature or are transferred from higher growth temperature to lower growth temperature [Murata et. al, 1979; 1992; Sato and Murata, 1980; Wada and Murata, 1990; Sakomoto et. al, 1997]. However, in Group I cyanobacteria, which do not possess polyunsaturated fatty acids [Murata et. al, 1992], conversion of C16:0 to C16:1(9) fatty acids occurs after a temperature shift-down; subsequently, the proportion of shorter chain-length fatty acids also increases [Murata et al., 1979]. Increase in C16:2(9,12) was observed in Anabaena variabilis, which belongs to Group II, with decrease in growth temperature [Murata et al., 1992; Sato and Murata, 1980; Sato et al., 1979]. But, neither of the psychrotolerant species synthesize C16:2(9,12) at either of the temperatures.

In both Nostoc sp. and Leptolyngbya sp., following downshift in temperature from 25°C to 10°C, increase in mole % of C18:3(9,12,15) in the individual lipid fractions; namely MGDG (400 % and 400 %), DGDG (150 and 200 %), PG (500 % and 300 %) and SQDG (250 % and 300 %); Further under similar conditions C16:1(9) increased by 100 % only in PG and SQDG of Leptolyngbya sp. following down-shift in temperature. These results are in contrast to the changes observed in Anabaena variabilis [Sato and Murata, 1980], where many-fold increase in the levels of C18:3(9,12,15) was observed in all the four individual lipids, C16:1(9) in SQDG, and C16:2(9,12) in MGDG and DGDG. It is also of interest to note that these changes in Anabaena variabilis occurred 10 h after down-shift in temperature, whereas in Nostoc sp., it was observed after 20 h. Furthermore, in both the strains, desaturation of C18:1(9) and C18:2(9,12) to C18:3(9,12,15) appeared to be the most rapid response in MGDG. This differential response observed between the psychrotolerant strains and Anabaena variabilis [Sato and Murata, 1980] may indicate that the modes of acclimation in a
mesophilic and a psychrotolerant cyanobacterium are not identical, despite the fact that the two cyanobacteria under comparison belong to Group II cyanobacteria. In fact, the rapid decrease in C16:0 and the increase of C16:1(9) in DGDG prior to the increase in C18:3(9,12,15), observed in *Anabaena variabilis*, is considered as an emergency response to increase the membrane fluidity to abrupt temperature changes [Sato and Murata, 1980]. The emergency response lasts for 10 h, after which C18:3(9,12,15) increases and maintains the membrane fluidity. In these psychrotolerant strains, the emergency response to down-shift in temperature is not observed and both C16:1(9) and C18:3(9,12,15) increase at the same time. Fujii and Fulco [1977] had demonstrated a transient increase in Δ5 desaturase in *Bacillus megaterium* when shifted from 35°C to 22°C.

**4.1.3. Desaturase genes of *Nostoc* sp. (strain 36)**

Wada et. al, [1990] were the first to clone an acyl lipid desaturase from *Synechocystis* (PCC 6803). Ever since, various acyl lipid desaturases have been cloned from a variety of cyanobacteria, such as from *Synechocystis* PCC6714 [Sakamoto et. al, 1994a; Murata et. al, 1996], *Synechococcus* PCC7002 [Sakamoto et. al, 1994a-c; 1997; Murata et. al, 1996], *Synechococcus vulcanus* [Kiseleva et. al, 2000], *Spirulina platensis* PCC6714 [Sakamoto et. al, 1994a; Murata et. al, 1996] and *Anabaena variabilis* [Reddy et. al, 1993; Sakamoto et. al, 1994a; Murata et. al, 1996]. This is the first report on the desaturase genes namely desA (AJ621245), desB (AJ621246), desC (AJ621244) and desC2 (AJ621247) from a psychrotolerant cyanobacterium, *Nostoc* sp. All the four genes exhibit high homology with the previously reported homologs from cyanobacteria, thus indicating that these genes are highly conserved. It was also observed that the des genes exhibited highest homology with *Anabaena* sp., and *Nostoc punctiforme* which belong to *Nostocales*, compared to other cyanobacteria. The deduced amino acid sequence of all the desaturases exhibited the three histidine clusters [Murata and Wada, 1995; Los and Murata, 1998] (Table 3.9), characteristic of desaturases and clusters of hydrophobic regions which are the putative membrane
spanning domains (Table 3.9, Fig. 3.12) [Murata and Wada, 1995]. Based on the high homology of the Antarctic *Nostoc* sp. des genes with homologues in cyanobacteria three of the four genes of *Nostoc* sp. have been identified as desA for Δ12 desaturase, desB for ω3 acyl-lipid desaturase and desC for Δ9 desaturase. Further, desC2, an isoform of desC, functional at the Δ9 position of fatty acids bound at sn-2 position of the lipid has also been identified. But as yet, very little is known about this gene, which exhibits high homology with other known Δ9 desaturases. Within the genus the homology at protein level between DesC1 and DesC2 is 63 %; between DesC2 and DesA it is 36 %; between DesC2 and DesB it is 37 %; and between DesA and DesB it is 44 %. There is no significant homology of DesC1 with either DesA or DesB.

Sequence analysis at the BDGP Neural Network Promoter Prediction site (http://www.fruitfly.org/seq_tools/promoter.html) has revealed putative promoter sequences for desC, desA, desB (Fig. 3.8) and desC2 (Fig. 3.11). −10 and −35 sequences, normally found upstream of the transcription initiation sites are also identified for the four genes. GTG seems to be the start codon for desB in this organism, as opposed to the commonly used ATG. Three out of the four desaturase genes, namely desC, desA and desB, are in tandem. The length of intergenic region between desC and desA is 213 bp and that between desA and desB is 218 bp. All the four genes show diad symmetries, which indicate putative rho dependent termination sites (Figs. 3.8 and 3.11).

### 4.1.4. Positional specificity of DesC2

DesC or the Δ9 desaturase of Group II cyanobacteria, with specificity at sn-1 position, is known to catalyse the conversion of C18:0 to C18:1(9) and does not recognise C16:0 [Sakamoto et. al, 1994b]. But the existence of a single Δ9 desaturase with specificity restricted only to sn-1 position cannot explain the high mole % of C16:1(9) which is positionally assigned to sn-2 position of lipids. This indicated the probable existence of a Δ9 desaturase specific to fatty acids bound to
sn-2 position of the lipid. In fact, based on the fatty acid composition of Group II cyanobacteria which have higher proportion of C16:1(9) at sn-2 position [Murata et al, 1992; Wada and Murata, 1998], it is probable that the presence of two Δ9 desaturases is a more common feature of Group II cyanobacteria than described. This indeed may be so, as evident from the complete genome sequences of *Anabena* PCC 7120, *Thermosynechoccus elongates* BP-1 and *Gloeobacter violaceus* PCC 7421 which show two or more than two putative Δ9 acyl-lipid desaturases (http://www.kazusa.or.jp/cyano/).

Sakamoto *et al.* [1997] were the first to describe the presence of two Δ9 acyl lipid desaturases in *Synechococcus* PCC7002. But, the functional specificity of the genes was not specified. In the present study, the specificity of *desC2* from *Nostoc* sp. (strain 36) was ascertained by expressing *desC2* in *Synechocystis* sp. PCC 6803 and by evaluating the fatty acid distribution in the transformant. Fatty acid analysis of the major lipid fraction, MGDG, of *Synechocystis* PCC6803 transformed with *desC2* showed higher levels of C16:1(9) compared to the one transformed with the vector (Table 3.11). More interestingly, a major fraction of C16:1(9) was bound to sn-2 position of MGDG, indicating the specificity of the enzyme to sn-2 position. *desC2* seems to show some head group specificity since high contents of C16:1(9) was associated with MGDG and DGDG of *Nostoc* sp. (strain 36) and not in either PG or SQDG (Table 3.3). In other Group II cyanobacteria like *Leptolyngbya* sp. (strain 9) (Table 3.4), *Nostoc muscorum*, *Plectonema boryanum* and *Synechococcus* PCC7002, also the levels of C16:1(9) fatty acid were significantly higher only in MGDG and DGDG [Murata et. al, 1992]. This would indicate a probable specificity of *desC2* to lipids with glucose/galactose in their head group. This increase in levels of C16:1(9) is not observed in Group III and Group IV cyanobacteria [Murata et. al, 1992]. Earlier studies had indicated that the Δ6 desaturase is specific to MGDG, but the Δ9 and Δ12 desaturases do not discriminate between the polar head groups [Sakamoto et. al, 1994b; Wada et. al, 1993]
4.1.5. Expression of the desaturase genes of *Nostoc* sp. (strain 36)

From the above discussion, it is evident that shifting the cultures from higher to lower temperatures or growing the cultures at lower temperature results in an increase in the unsaturated fatty acid content of the membrane. Increase in the unsaturated fatty acid content could be because of transcriptional activation of the desaturase genes or activation of pre-existing enzymes. Studies on cyanobacteria till date have shown that essentially, a reduction in growth temperature activates the transcription of desaturase genes, resulting in an increase in the amount of enzyme [Los et. al, 1993; 1997; Sakamoto et. al, 1997; Deshnium et al., 2000]. This is followed by increase in unsaturation of fatty acids. To test whether the same mechanism of regulation exists in Antarctic cyanobacteria, RT-PCR analysis was performed on RNA from *Nostoc* sp.

RTR-PCR results indicated no significant changes in the transcript levels of the four desaturase genes (*desA*, *desB*, *desC* and *desC2*) of the Antarctic *Nostoc* sp. when cells grown at 25°C were shifted to 10°C for up to 150 min. These results clearly indicate that the desaturase genes in the Antarctic *Nostoc* sp., which is cold tolerant and adapted to growth at low temperatures, are constitutively expressed, unlike the mesophilic cyanobacteria where some of the desaturase genes are transcriptionally activated at lower temperatures of growth. In *Synechococcus* sp. PCC 7002, a mesophilic Group II cyanobacterium, *desB* and *desC* genes are up-regulated within 15 min after shift-down to 22°C from 34°C [Sakamoto et. al, 1997]. In *Spirulina platensis* C1, *desD* is up-regulated transcriptionally in response to low temperature incubation [Deshnium et. al, 2000], whereas in *Synechocystis* sp. PCC 6803, the expression of *desC* was not induced but *desA*, *desB* and *desD* are induced, following cold shock from 34°C to 22°C [Los et. al, 1993, 1997]. In *Synechocystis* sp. PCC 6803, the enhanced levels of *desA*, *desB* and *desD* mRNAs results from both induction of gene expression and enhancement of mRNA stability [Sakamoto et. al, 1997, Los et. al, 1997]. Although the mRNA levels for the desaturase genes in these systems increased very rapidly, the fatty acid composition of membrane lipids changed only very
slowly during a 10–12 h period following the temperature shift-down [Sakamoto et. al, 1997]. This probably demonstrates that the rate-limiting step for increasing the desaturation of membrane lipids at low temperature is post-transcriptional and this may be the case in Antarctic Nostoc sp. Activation of pre-existing desaturase in *Synechococcus vulcanus*, a thermophilic cyanobacterium in response to cold has been proposed [Kieseleva et. al, 2000].

In *Synechocystis* sp. (PCC 6803), expression of the ω3 desaturase desB was shown to be under the control of a membrane bound sensor, Hik33 [Suzuki et. al, 2001]. Hik33 knock out directly affected the loss of desB promoter activity as evident from a knock out study done by Suzuki and his co-workers [2001]. The first direct evidence for the two-component signal transduction mechanism involved in sensing cold has come from recent studies on *Bacillus subtilis* (Fig. 1.6) which consisted of the DesK / DesR system, that recognizes and tranduces low temperature signals, thus activating the transcription of Δ5 desaturase [Mansilla et. al, 2003]. Though the knock out study of Hik33 and the demonstration by footprinting assay that DesR binds upstream to the Δ5 desaturase indicates its direct role in upregulation of the gene, this mechanism of the two-component system involved in temperature sensing and activation of the desaturase gene does not seem to be universal. In *Nostoc* sp. des gene activation does not seem to be responsible for the restoration of the membrane fluidity in response to a rigidification brought about by cold shock. It is worthwhile to mention that a similar phenomenon of activation of pre-existing enzyme, rather than induction of the gene has been observed in *Synechococcus vulcanus*, a thermophilic cyanobacterium. This cyanobacterium when exposed to cold showed increase in unsaturation with no apparent difference in the transcript levels [Kieseleva et. al, 2000]. Thus, it may be a common feature of these extremophilic organisms to have a post-transcriptional mechanism of regulation, like activating a pre-existing enzyme, rather than activating the production *de novo*.

Many organisms, as an acclimative response to lower temperatures, adopt post-transcriptional and post-translational mechanisms. For example, β-tubulin
from an Antarctic microbe, *Euplote focardii*, is heavily phosphorylated [Pucciarelli et al, 1997]; ω3 fatty acid desaturase in wheat roots is temperature dependent and is translationally regulated [Horiguchi et al, 2000]; maize plants exposed to lower temperatures respond by phosphorylating a minor chlorophyll a/b protein [Bergantino et al, 1995]; and in rats, 5 h exposure to acute cold resulted in a 6-fold increase in lipoprotein lipase activity, but only a two-fold increase in mRNA level [Giralt et al, 1990]. Exposure of poikilothermic animals, such as carp, when temperature is shifted from 30°C to 23°C, resulted in a significant increase in the amount of desaturase proteins [Tiku et al, 1996], modest increases in enzyme products, but no apparent changes in transcription of desaturase gene. Desaturases in rat liver stearoyl-Co-A desaturase has also been reported not to be regulated at the transcript level [Mziaut et al, 2000; Heinemann and Ozols, 1998].

In case of the desaturases from Antarctic *Nostoc sp.*, the regulation of the enzyme is also probably brought about by post-translational modifications. Understanding these mechanisms could provide insights into the mechanisms underlying the molecular basis of cold adaptation. Preliminary *in silico* studies on the deduced desaturase protein sequences of *Nostoc* sp. have shown putative phosphorylation sites. Characterisation of these sites with respect to the function of the enzyme using a site directed mutagenesis approach may shed light on the post transcriptional regulation of the activity of the enzyme.

4.1.6. Increased activity of DesC2 in Antarctic cyanobacteria

The higher proportion of unsaturated fatty acid bound to sn-2 position of the lipid in *Nostoc* sp. is suggestive of higher activity of the enzyme DesC2. Such an increased activity of this enzyme is probably an essential feature for the survival of this organism in the cold since increase in the activity of DesC2 would increase the proportion of unsaturated fatty acids in the membrane thus facilitating an increase in membrane fluidity, which is crucial for survival. Further, it is probably not the mere presence of the enzyme, but its regulation in
these organisms that make their membrane composition different from other cyanobacteria. Thus both the presence and regulation of DesC2 activity could be the differentiating factor by which mesophilic and psychrotolerant cyanobacteria adapt to low temperature.

Table 4.2. Putative phosphorylation sites of desaturases of Nostoc sp. (strain 36) predicted by in silico analysis. Numbers in the bracket indicate the amino acid number.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Serine phosphorylation sites</th>
<th>Threonine phosphorylation sites</th>
<th>Tyrosine phosphorylation sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>DesA</td>
<td>SIINSKQLS (8), KTRQSAFEL (155), KDQSSIKLS (191), LAYSIIKEN (304)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DesB</td>
<td>LFKPSEKWD(197), KGAISIDR(272)</td>
<td>-</td>
<td>TESQYKDMP(152)</td>
</tr>
<tr>
<td>DesC</td>
<td>TIATSTKQP(6), HHLHSDTDA(104)</td>
<td>HRLVTHRSF(64), RSFQTPKWL(70)</td>
<td>-</td>
</tr>
<tr>
<td>DesC2</td>
<td>HRLSHKSF(69)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Low temperature per se may not be the factor that determines the activity of DesC2 and thereby the survival of the cyanobacterium at low temperature. Growth of Antarctic cyanobacteria at 25°C also shows higher proportion of the unsaturated fatty acid, a temperature where the mesophilic cyanobacteria fail to show this phenomenon (Table 4.1). One of the probable reasons for these organisms not to survive at 35°C could be cell lysis, a result of higher proportion of unsaturated fatty acid.

Increased activity of DesC2 in Nostoc sp. could be either because of existence of an additional trans-acting factor or is probably a result of structural change in the protein. It appears more likely that the increased activity is because of a structural change in the protein. This is evident from the fact that
**Discussion**

*Synechocystis* sp. PCC 6803 transformed with desC2 shows a similar phenotype to that of the Antarctic *Nostoc* sp. i.e higher levels of C16:1(9) compared to C16:0 (Table 3.11). If the higher proportion of unsaturated fatty acids at the sn-2 position of the membrane lipid is because of existence of an additional regulatory mechanism enhancing the activity of DesC2 in *Nostoc* sp., (say, a trans-acting factor) the increase in the unsaturated fatty acids of the desC2 transformed *Synechocystis* sp. should be comparable to the levels of unsaturation observed in mesophilic cyanobacteria belonging to Group II (Table 4.1). Low temperature tolerance and survival are probably critically defined by this factor.

**4.2. CONCLUSIONS**

Taken together, the results of the present study indicate that psychrotolerant cyanobacteria differ from mesophilic cyanobacteria in the mode of regulation of membrane fluidity. To the best of our knowledge, this is the first study of fatty acid composition and its regulation in a psychrotolerant cyanobacterium. The salient findings of the present study are as follows:

- The psychrotolerant Antarctic cyanobacteria, *Nostoc* sp. and *Leptolyngbya* sp. belong to Group II cyanobacteria.
- Both the cyanobacteria show a higher proportion of unsaturated fatty acids when compared to the fatty acid composition of other mesophilic cyanobacteria grown at around the same temperature.
- The proportion of polyunsaturated fatty acids increases in both the organisms either when the cultures are grown at a lower temperature, or when shifted to a lower temperature.
- Desaturases from *Nostoc* sp. (*desA, desB, desC* and *desC2*) show high homology to the other reported desaturases of mesophilic organisms.
- Desaturases of *Nostoc* sp. are constitutively expressed and the expression levels are not modulated by low temperature. But, since the natural
habitat of Nostoc sp. experiences temperature lower than the temperatures studied in the laboratory, it may be logical to assume that the des genes are constitutively expressed.

- Nostoc sp. has two isoforms of Δ9 desaturases viz. desC and desC2.
- Heterologus expression of desC2 in Synechocystis sp. PCC 6803 confirms that the enzyme DesC2 is functional on fatty acids bound to sn-2 position of a membrane lipid.
- The enzyme DesC2 also seems to show head group specificity, since the unsaturation of sn-2 bound fatty acids is specifically found only in galactolipid of the membrane (MGDG and DGDG).
- DesC2 appears to be a more common feature of species belonging to Group II than described, as evident from the fatty acid comparison of the organisms under study with the ones already characterised.
- Higher proportion of unsaturated fatty acids at the sn-2 position of MGDG and DGDG in these Antarctic cyanobacteria than what is normally observed in mesophilic cultures indicates that the enzyme is more active in the Antarctic cyanobacteria, and probably in all cold adapted cyanobacteria. This is probably an adaptive response of cyanobacteria surviving in the cold, since the higher proportion of unsaturated fatty acids in these organisms is because of the action of this enzyme.

Regulation of DesC2 activity is still an unsolved mystery. Understanding this phenomenon would explain the differences in the strategies used by mesophilic and psychrotolerant organisms to adapt to temperature, at least at the membrane level.