Molecular Characterization of Osmoregulatory Genes in the cyanobacterium *Nostoc muscorum* Under Salt Stress

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Abstract

Salinity is one of the major environmental stresses affecting the growth, metabolism, and survival of cyanobacteria. *Nostoc muscorum*, a filamentous nitrogen-fixing cyanobacterium, exhibits remarkable adaptability to osmotic stress through various physiological and molecular mechanisms. The present study aimed to characterize osmoregulatory genes involved in salt stress tolerance in *Nostoc muscorum*. Cultures of *Nostoc muscorum* were exposed to different concentrations of sodium chloride (NaCl), and the expression patterns of selected osmoregulatory genes were analyzed. Growth rate, chlorophyll content, and compatible solute accumulation were evaluated to assess physiological responses. Results revealed significant upregulation of genes associated with compatible solute biosynthesis, ion transport, and stress signaling under elevated salinity conditions. Accumulation of sucrose and trehalose increased significantly in salt-treated cultures, indicating their role in osmo protection. The findings suggest that osmoregulatory genes contribute substantially to salt tolerance mechanisms in *Nostoc muscorum* and may serve as potential targets for improving stress tolerance in cyanobacteria used for agricultural and biotechnological applications.

Keywords: Nostoc muscorum, osmoregulation, salt stress, cyanobacteria, gene expression, compatible solutes, salinity tolerance.

1. Introduction

Cyanobacteria are among the earliest photosynthetic organisms and play a crucial role in global carbon and nitrogen cycles. Among them, *Nostoc muscorum* is a filamentous, nitrogen-fixing cyanobacterium widely distributed in fresh water and terrestrial habitats. Environmental stresses such as salinity, drought, and temperature fluctuations significantly influence cyanobacterial growth and productivity.

Salt stress induces osmotic imbalance and ionic toxicity, resulting in reduced photosynthetic efficiency, impaired metabolic functions, and oxidative damage. To survive under such conditions, cyanobacteria have evolved sophisticated osmoregulatory mechanisms involving the synthesis of compatible solutes, regulation of ion transport systems, and activation of stress-responsive genes.

Osmoregulation is a critical adaptive process that enables cells to maintain water balance and cellular integrity. Previous studies have demonstrated that cyanobacteria accumulate compatible solutes such as sucrose, trehalose, glucosylglycerol, and glycine betaine in response to osmotic stress. However, limited information is available regarding the molecular regulation of osmoregulatory genes in *Nostoc muscorum*.

Therefore, the present study was undertaken to characterize the expression of major osmoregulatory genes and evaluate their role in salt stress adaptation in *Nostoc muscorum*.

2. Review of Literature

Several studies have investigated salt stress responses in cyanobacteria. Hagemann (2011) reported that compatible solute accumulation is a universal strategy employed by cyanobacteria to counter osmotic stress. Singh et al. (2015) observed increased sucrose synthesis in *Nostoc* species under saline conditions. Mikkat et al. (2000) demonstrated that osmotic stress triggers alterations in protein expression profiles associated with stress adaptation.

Reed and Stewart (1985) identified sucrose and trehalose as primary osmoprotectants in nitrogen-fixing cyanobacteria. Marin et al. (2004) highlighted the importance of sodium/proton antiporters in maintaining ion homeostasis during salt stress. Recent transcriptomic studies have revealed differential expression of genes related to transport systems, stress signaling pathways, and compatible solute biosynthesis in cyanobacteria exposed to salinity.

These findings indicate that both physiological and molecular mechanisms contribute to salinity tolerance, although specific gene regulation in *Nostoc muscorum* remains insufficiently explored.

3. Objectives of the Study

1. To evaluate the effect of salt stress on the growth of *Nostoc muscorum*.
2. To analyze changes in chlorophyll content under different salinity levels.
3. To characterize the expression of osmoregulatory genes under salt stress.
4. To determine the accumulation of compatible solutes during osmotic stress.
5. To assess the relationship between gene expression and physiological adaptation.

4. Materials and Methods

Culture Maintenance: Pure cultures of *Nostoc muscorum* were maintained in Chu no. 10

medium under controlled laboratory conditions at 25-30 ± 1°C with a light-dark photoperiod and by white cool florescent tubes. Cultures were shaken by hand thrice daily.

Experimental Design: Cultures were divided into four treatment groups:

Treatment	NaCl Concentration
Control	0 mM
T1	50 mM
T2	100 mM
T3	150 mM

Each treatment was maintained in triplicate.

Growth Analysis: Growth was monitored by measuring optical density at 750 nm and determining dry biomass after 21 days of incubation.

Chlorophyll Estimation: Total chlorophyll content was estimated spectrophotometrically using methanol extraction.

RNA Isolation and Gene Expression Analysis: Total RNA was extracted from control and salt-treated cultures. Complementary DNA (cDNA) was synthesized using reverse transcriptase. Quantitative real-time PCR (qRT-PCR) was employed to analyze expression levels of selected osmoregulatory genes including:

- **spsA** (Sucrose phosphate synthase)
- **treS** (Trehalose synthase)
- **nhaS** (Na+/H+ antiporter)
- **otsA** (Trehalose-6-phosphate synthase)
- **hspA** (Heat shock protein)

Gene expression was normalized against 16S rRNA.

Compatible Solute Analysis: Sucrose and trehalose concentrations were quantified using high-performance liquid chromatography (HPLC).

5. Results

Effect of Salt Stress on Growth: Increasing salinity significantly reduced growth and biomass accumulation. The highest reduction was observed at 150 mMNaCl.

Treatment	Biomass (g/L)
Control	1.85 ± 0.08
50 mM	1.62 ± 0.06
100 mM	1.29 ± 0.05
150 mM	0.94 ± 0.04

Chlorophyll Content: Chlorophyll content decreased progressively with increasing salt concentration, indicating stress-induced impairment of photosynthesis.

Gene Expression Analysis: qRT-PCR analysis revealed significant up regulation of osmoregulatory genes under salt stress.

Gene	Fold Change at 150 mMNaCl
spsA	4.8
treS	5.2
nhaS	6.1
otsA	4.5
hspA	3.9

The highest expression was observed for the sodium/proton antiporter gene (**nhaS**).

Compatible Solute Accumulation: Salt-treated cultures accumulated significantly higher concentrations of sucrose and trehalose compared with control cultures.

Treatment	Sucrose (mg/g DW)	Trehalose (mg/g DW)
Control	8.5	4.2
50 mM	12.4	7.6
100 mM	18.7	11.5
150 mM	25.9	16.8

6. Discussion

The present investigation demonstrates that *Nostoc muscorum* responds to salinity stress through coordinated physiological and molecular adaptations. Reduced growth and chlorophyll content under high salinity are consistent with previous studies reporting osmotic and ionic stress effects on cyanobacteria.

The significant up regulation of **spsA**, **treS**, and **otsA** genes suggests enhanced biosynthesis of compatible solutes, which protect cellular macromolecules and maintain osmotic balance. Increased expression of **nhaS** indicates activation of ion transport mechanisms responsible for sodium exclusion and intracellular ion homeostasis.

Accumulation of sucrose and trehalose further supports their critical role as osmoprotectants. These compounds stabilize proteins, membranes, and photosynthetic complexes under stressful conditions. The observed molecular responses collectively contribute to improved stress tolerance and survival.

7. Conclusion

The study highlights the importance of osmoregulatory genes in the adaptation of *Nostoc muscorum* to salt stress. Enhanced expression of genes associated with compatible solute synthesis and ion transport was closely linked to physiological adjustments under saline conditions. Accumulation of sucrose and trehalose served as a key mechanism for maintaining cellular osmotic balance. These findings improve our understanding of cyanobacterial stress biology and provide a foundation for future genetic engineering approaches aimed at developing salt-tolerant strains for agricultural and biotechnological applications.

References

1. Hagemann, M. (2011). Molecular biology of cyanobacterial salt acclimation. *FEMS Microbiology Reviews*, 35(1), 87–123.
2. Reed, R.H., & Stewart, W.D.P. (1985). Osmotic adjustment and organic solute accumulation in cyanobacteria. *Marine Biology*, 88, 1–9.

3. Marin, K., et al. (2004). Salt-dependent expression of Na+/H+ antiporters in cyanobacteria. *Journal of Bacteriology*, 186(24), 8471–8478.
4. Singh, S.P., et al. (2015). Salinity-induced physiological and biochemical responses in cyanobacteria. *World Journal of Microbiology and Biotechnology*, 31, 121–132.
5. Mikkat, S., et al. (2000). Proteome analysis of salt stress responses in cyanobacteria. *Proteomics*, 1, 560–568.
6. Hagemann, M., & Erdmann, N. (1997). Environmental stress adaptation in cyanobacteria. *Plant Physiology*, 113, 107–114.
7. Klähn, S., &Hagemann, M. (2011). Compatible solute biosynthesis in cyanobacteria. *Environmental Microbiology*, 13(3), 551–562.
8. Allakhverdiev, S.I., et al. (2000). Salt stress effects on photosynthetic machinery. *Plant Physiology*, 123, 1047–1056.