Partial characterization of nitrate signaling pathway of *Arthrospira platensis* PCC 7345

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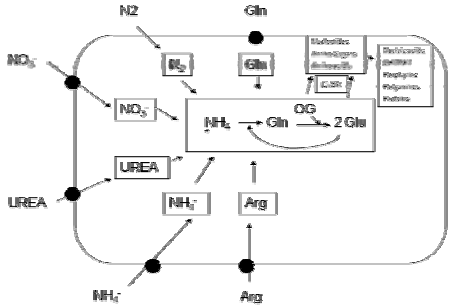
Introduction

- *A platensis* is a photosynthetic, filamentous, spiral-shaped, multicellular and green-blue microalga.
- Cannot fix atmospheric nitrogen
- Its chemical composition includes proteins (55%-70%), carbohydrates (15%-25%), essential fatty acids (18%) vitamins, minerals and pigments like carotenes, chlorophyll *a* and phycocyanin.
- Excellent food, lacking toxicity and having corrective properties against viral attacks, anemia, tumor growth and malnutrition.



- The genes involved in nitrate assimilation are not constitutively expressed but are carefully regulated by factors like light, metabolite and cell type
- Nitrate as a primary signal molecule triggering the activation of transcription of nitrate assimilation and related genes.

Objectives

- To examine whether nitrate assimilatory pathway is induced by nitrate/light signaling in cyanobacteria
- To study the *expression* of genes involved in Nitrogen metabolism as influenced by *nitrate/light* simulation
- To identify the signaling steps/molecules involved in the transduction of the above signals, leading to the regulation of primary carbon and nitrogen metabolism.

Methods

Biochemistry	Semi-quantitative RT PCR	RAPD-Differential Display
Nitrate Free Treatment	Nitrate Free Treatment	Nitrate Free Treatment
↓	↓	↓
+Nitrate (Control)	-Nitrate (Control)	-Nitrate (Control)
+Inhibitors + Nitrate	+Nitrate	+Nitrate
↓	↓	↓
Nitrate uptake	Total RNA Isolation (Qiagen RNeasy Kit)	Total RNA Isolation (Qiagen RNeasy Kit)
NR	↓	↓
Nir	Qiagen One Step RT PCR- with Gene Specific Primers	Qiagen One Step RT PCR- with Random Primer sets
GS		

Modeling and Simulation

- Modeling: Modeller9v7
- Energy Minimization: Amber9
- Docking: Molegro Virtual Docker 4.0.2

Results

Effects of Phosphodiesterase inhibitors on Nitare uptake, NR, NiR and GS

Figure 1 Nitrate uptake

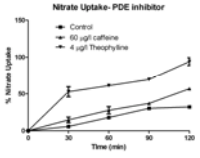
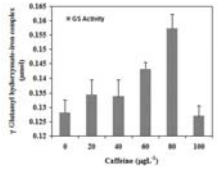


Figure 2 GS



Nitrate uptake and GS increases significantly

Figure 3 NiR

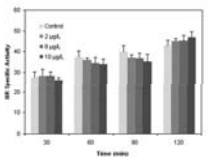
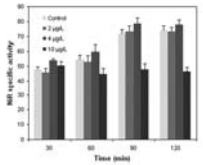


Figure 4 NiR



No significant change in NR and NIR

Semi-quantitative RT PCR

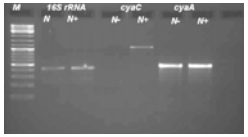


Figure 5 :Expression of Adenylate cyclase *cyaC* with nitrate

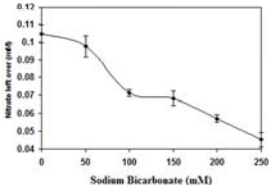


Figure 6 Nitrate uptake with bicarbonate

Nitrate uptake increases with HCO_3^- and was absent in the absence of bicarbonate

Note: Unlike other non-nitrogen fixing cyanobacteria, *A platensis* has a Adenylate cyclase mediated regulation of nitrate assimilation

A. platensis NtcA Modeling

(NtcA-a global nitrogen-control regulator)

Figure 7 Sequence Analysis

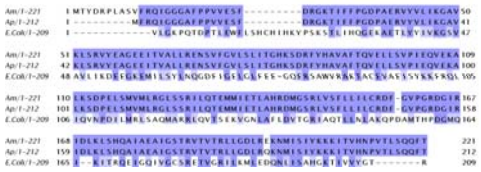
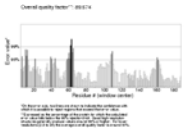


Table 1 Changes in cAMP binding site

Amino Acid Position	E. Coli	A. Platensis	A. Maxima
72	Glu	Val	Val
82	Arg	Arg	Arg
83	Ser	Phe	Phe
123	Arg	Arg	Arg

Homology Modeling (Template: 3D0S) Figure 8



Docking Studies

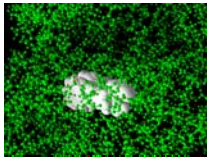
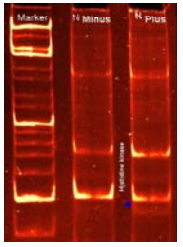


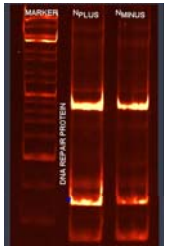
Figure 9 NtcA docked with cAMP

- cAMP binding affinity to be compared between template and modeled structure
- Binding affinity of ntcA evaluated compared after molecular simulation

Differential Display [RAPD-DDRT-PCR]

Figure 10





- Two genes (Multi sensor histidine kinase and a DNA repair protein) involved in regulating nitrate assimilatory pathway were identified and sequenced (GenBank accession number : GO428516 , GO451842)

Conclusions

- In *A. platensis,* nitrate induces its uptake, NR, NiR, GS
- A signaling cascade viz., adenylate cyclase / cAMP is involved in nitrate assimilatory pathway of *A. platensis*
- *A. platensis* NtcA is likely to have binding affinity to cAMP
- Multi sensor histidine kinase and a DNA repair protein are involved in the process

Acknowledgement

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