Functional characterization of N metabolism whithin *Arthrospira* sp. PCC 8005, and its application for space flight

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SEM morphology observation of Arthrospira sp. PCC 8005 (Deschoenmaeker F.)

Introduction

For decades, humans have expressed a great interest for the exploration of the Universe, and planets close to the Earth like Mars. The main limiting factors come from the current technologies, which do not enable to sustain long-term manned space flight without regularly providing supplies (*e.g.,* oxygen & food). To overcome this constrain, the European Space Agency plans to develop an artificial closed system named Micro-Ecological Life Support System Alternative (hereafter, MELiSSA). This project has been conceived to support the life of crews by using microorganisms as well as higher plants differentially located in 5 compartments. Among the microorganisms used in MELiSSA project, *Arthrospira* sp. PCC 8005 (compartment IVa) is a cyanobacterium devoted to produce both edible biomass and oxygen from CO_2 and nitrogen sources generated by the other compartments.
Here, we analyzed the tolerance of *Arthrospira* sp. PCC 8005 to different nitrogen (N) sources and the degree of freedom in the cultivation of this cyanobacterium.

Results and Discussion

Arhtrospira sp. PCC 8005 was cultivated with 3 N sources, NO_3^-, NH_4^+ or urea at several concentrations (2, 6 & 12 mM of N) in batch mode (no pH control).
At 2 mM, no differences in growth was observed between the 3 N culture conditions (Fig. 1A). Sedimentation occurs at the same time (Fig. 1B) and was correlated to the exhaustion of N sources in the medium (Fig. 1C). At 6 and 12 mM, a lower growth was observed under NH_4^+ conditions (Fig. 1D & 1G). Morevore, OD_{750} values rapidly decreased after 264 hours at 12 mM NH_4^+, suggesting the death of bacteria (Fig. 1G).
No sedimentation was observed under 6 mM conditions (Fig. 1E), an increase in the sinking index (SI) was observed culture grown under 12 mM (Fig. 1H).

Is *Arthrospira sp. able to grow on high ammonium and urea concentrations ?*

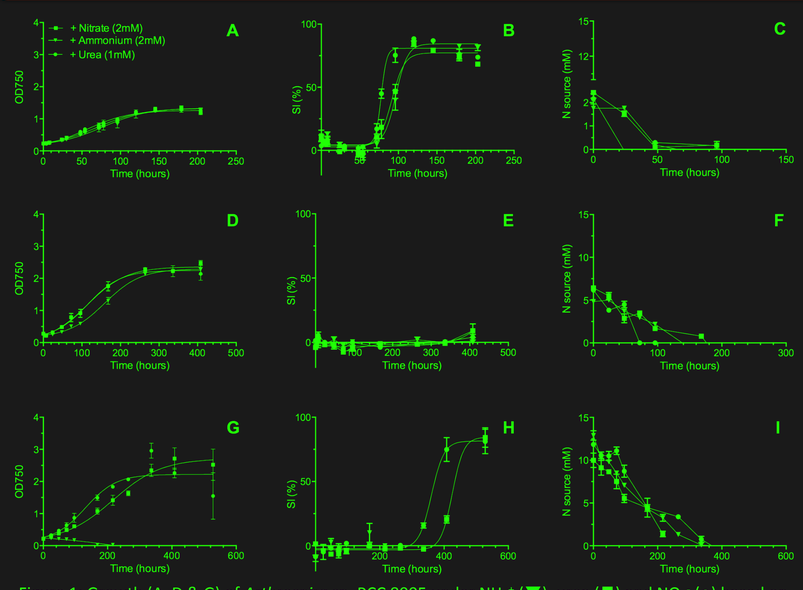

Figure 1: Growth (A, D & G) of *Arthrospira* sp. PCC 8005 under NH_4^+ (▼), urea (■) and NO_3^- (●) based on OD_{750} measures. The sinking (B, E & H) and the changes in nitrogen sources (C, F & I) were monitored.

How does *Arthrospira sp. assimilate N sources?*

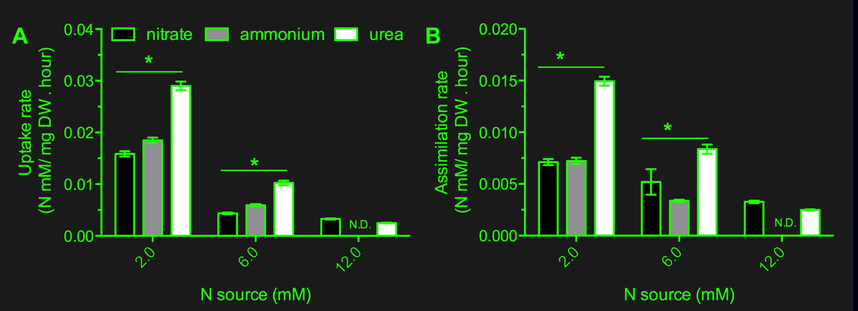

Figure 2: up-take (A) and assimilation (B) rates during growth of *Arthrospira* sp. PCC 8005 growing on NH_4^+ (▼), urea (■) and NO_3^- (●).

Afterwards, the up-take rate (i.e., calculated on the period of time for N exhaustion) and assimilation (i.e., calculated on the entire growth curves) were calculated. As shown in Figure 2, urea was up-taken quicker than NH_4^+ and NO_3^- (Fig. 2A) at 2 and 6 mM N. Moreover, the assimilation of urea was higher in the same conditions. However, NO_3^- and urea were similarly assimilated at 12 mM N (Fig. 2B), suggesting that maximal activity in up-take and assimilation was reach at 12 mM N. Since a lysis was observed in 12 mM NH_4^+, no rate was determined (Fig. 2A-B).

How does *Arthrospira* sp. adapt to changes in N sources?

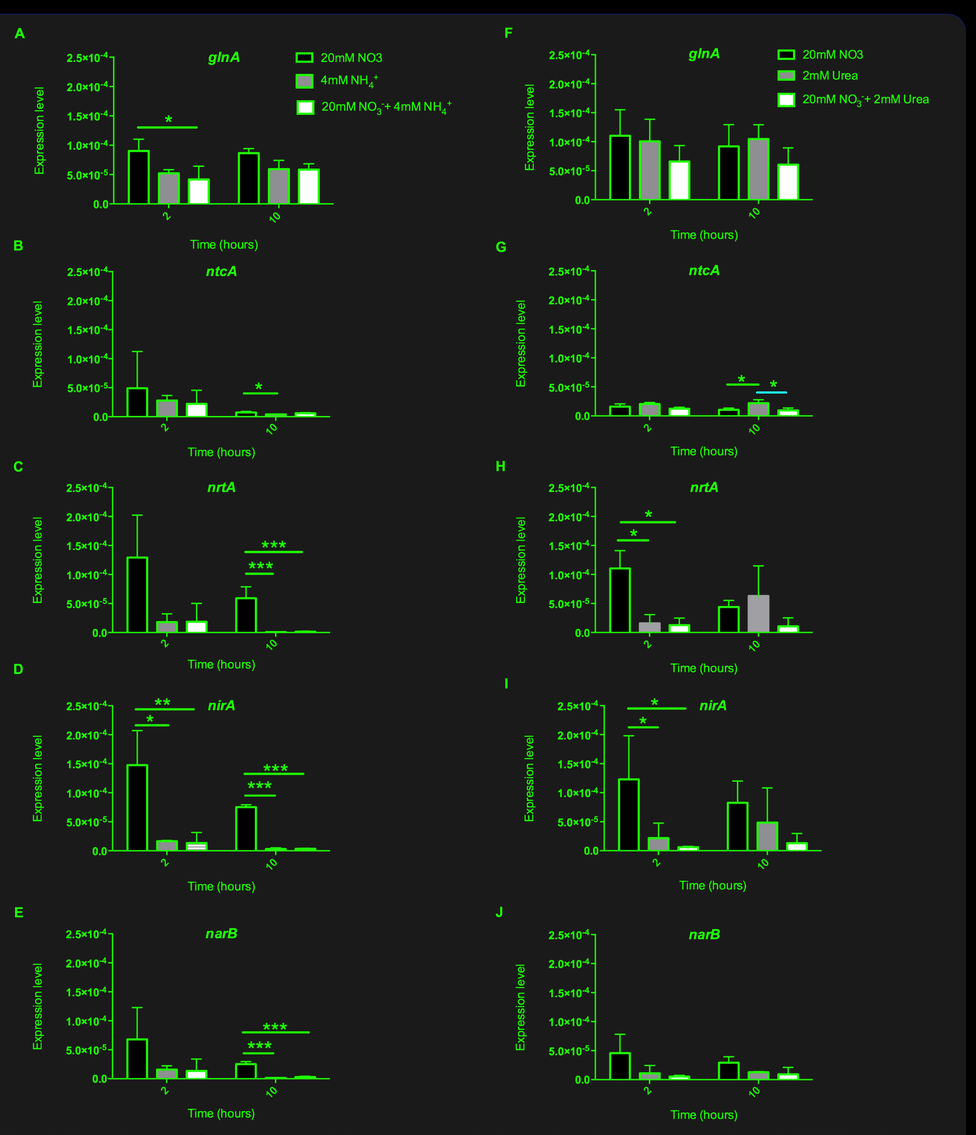

Figure 3: Expression level of the nitrogen-assimilatory genes *nrtA, nirA, narB, glnA, ntcA* in *Arthrospira* sp. PCC 8005 cultivated with NO_3^- and/or a second nitrogen source: NH_4^+ (A-E) or urea(F-I). The expression level was evaluated by RTqPCR.

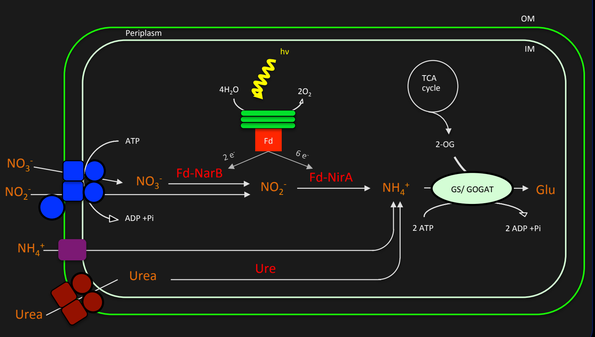

Figure 4: Diagram sketch of the N assimilatory pathway (modified from Flores *et al*. 2005): (Fd) ferredoxin, (GS/GOGAT) glutamine synthetase/ glutamate cycle, (IM) inner membrane (NarB) nitrate reductase, (NirA) nitrite reductase, (OM) outer membrane, (TCA) tricarboxylic cycle, (Ure) urease.

To understand the mechanisms underlying the acclimation to changes in the presence of N sources, mRNA of target genes (*ntcA, nirA, narB, glnA* & *nrtA*) were evaluated in *Arthrospira* sp. cultivated with nitrate and/or another N source (NH_4^+ or urea). The results clearly showed a decrease in mRNA abundance of *glnA* and *nirA* after 2 hours of incubation in presence of NH_4^+ (Fig. 3A & 3D). After 10 hours of incubation, a significant decrease in mRNA abundance was observed for the genes *ntcA, nrtA, nirA* and *narB* (Fig. 3B-E). Because the degradation of urea releases NH_4^+ ions (Fig. 4), the mRNA levels were also investigated after 2 and 10 hours of incubation with urea (with and without NO_3^-). Significant decreases in *nrtA* (coding for the subunit A of the nitrate transporter) and *nirA* (coding for the nitrite reductase) abundances were observed after 2 hours of incubation (Fig. 3H-I), whereas *ntcA* (N metabolism regulator) was up-regulated after 10 hours of incubation in presence of urea in the medium (Fig. 3G). These results showed a down-regulation of N-assimilatory genes when *Arthrospira* sp. was cultivated in presence of NH_4^+. In contrast, urea induced the expression of these genes after 10 hours of incubation, which could explain the higher assimilation rate of urea.
Proteomic analyses were also performed in the same conditions of incubation. The preliminary results clearly indicated that an oxidative stress occurred in presence of NH_4^+ ions. Indeed, the presence of ammonium in the medium induced an increase in the abundance of the ferredoxin thioredoxin (fold change: 3.48; peptides: 2) after 2 hours of incubation. After 10 hours of NH_4^+ presence in the medium, the superoxide dismutase was in higher abundance (fold change: 1.29; peptides: 5). Additionally, NH_4^+ ions induced a decrease in the nitrate reductase NirA after 2 hours (fold change: 0.55; peptides: 6) and 10 hours (fold change: 0.20; peptides: 5) of incubation. Whereas NirA was down-regulated under urea condition after 10 hours (fold change: 0.20 peptides: 5), the glutamine synthetase GlnA showed a higher abundance after 2 hours of incubation in presence of urea (fold change: 1.22; peptides: 5).
Taken together, the transcriptomic and proteomic analyses indicated a NH_4^+-promoted repression inducing a down-regulation of the NO_3^- uptake and assimilation (Fig. 4). Whereas urea also down-regulated proteins involved in NO_3^- assimilation, this N source up-regulated GlnA. This latter enzyme could use NH_4^+ released during urea catabolism.

Conclusion

The strain PCC 8005 of the genus *Arthrospira* showed abilities to grow either with nitrate or urea despite their concentration. Moreover, urea seemed to be assimilated more efficiently respective to the up-regulation in genes and proteins. In contrast, ammonium induced repression at both gene and protein levels when its concentration pass over 4 mM. Consequently, the impact in co-existence of NH_4^+ and other N source in the medium of *Arthrospira* sp. PCC 8005 must be deeply studied.

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