Functional characterisation of N metabolism whithin *Arthrospira* sp. PCC 8005, and its application for the MELiSSA loop

UMONS Université de Mons

biosciences
UMONS RESEARCH INSTITUTE FOR BIOSCIENCES

[1]Bayon-Vicente G.,[1]*Deschoenmaeker* F., [1]Sachdeva N., [1]Leroy B., [2]Raffestin S., [2]Paille C., [2]Lasseur C. & [1]Wattiez R.

[1]Department of Proteomics and Microbiology, Research Institute for Biosciences, University of Mons, Place du Parc 20, 7000 Mons, Belgium
[2]ESA-ESTEC Noordwijk, Netherlands

Introduction

One of the biggest dream for humans has always been to travel in space. After years and years of research they have succeeded in space travel and landing on the moon. However, some planets remain inaccessible due to the distance separating them from the Earth and our lack of technology (*i. e.* food, fuel problem, oxygen, ...). In this context, the European Space Agency (ESA) established a closed artificial ecosystem, named the MELiSSA loop, enables to produce enough food, oxygen and water to sustain crew needs during space travels to Mars. *Arthrospira* sp. PCC 8005 has been selected to settle in the fourth compartment (compartment IVa) of this loop. Its role is devoted to the production of biomass and oxygen for the crew through the photosynthesis and the uptake of nitrogen sources and carbon dioxyde.

In the frame of this loop, a characterisation of the nitrogen metabolism in presence of different nitrogen sources constitute a fundamental axe of research.

Growth and transcriptomic analyses of *Arthrospira sp.* PCC 8005 on different nitrogen sources

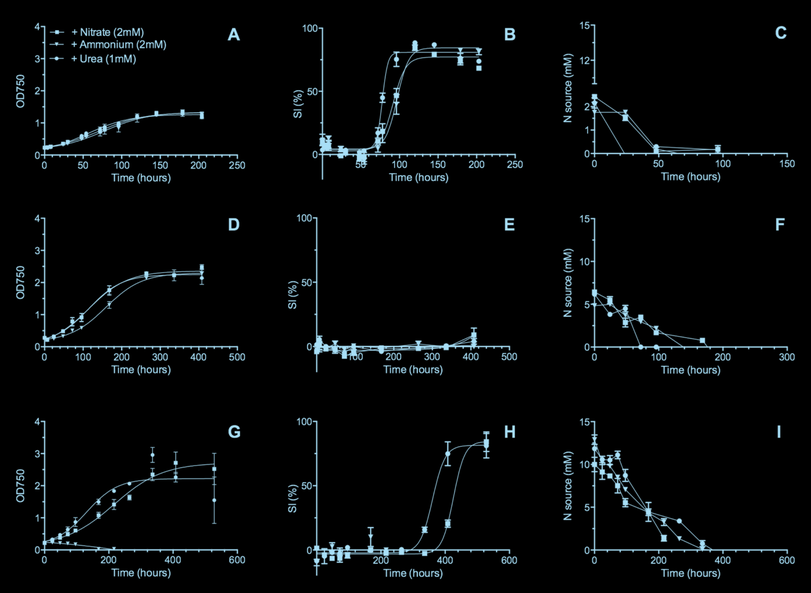

Figure 1: Growth (A, D & G) of *Arthrospira* sp. PCC 8005 under NH_4^+ (▼), urea (■) and NO_3^- (●) based on OD_{750} measures. The sinking (B, E & H) and the changes in nitrogen sources (C, F & I) were monitored.

Arthrospira 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 were 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). Moreover, 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 in culture grown under 12 mM (Fig. 1H).

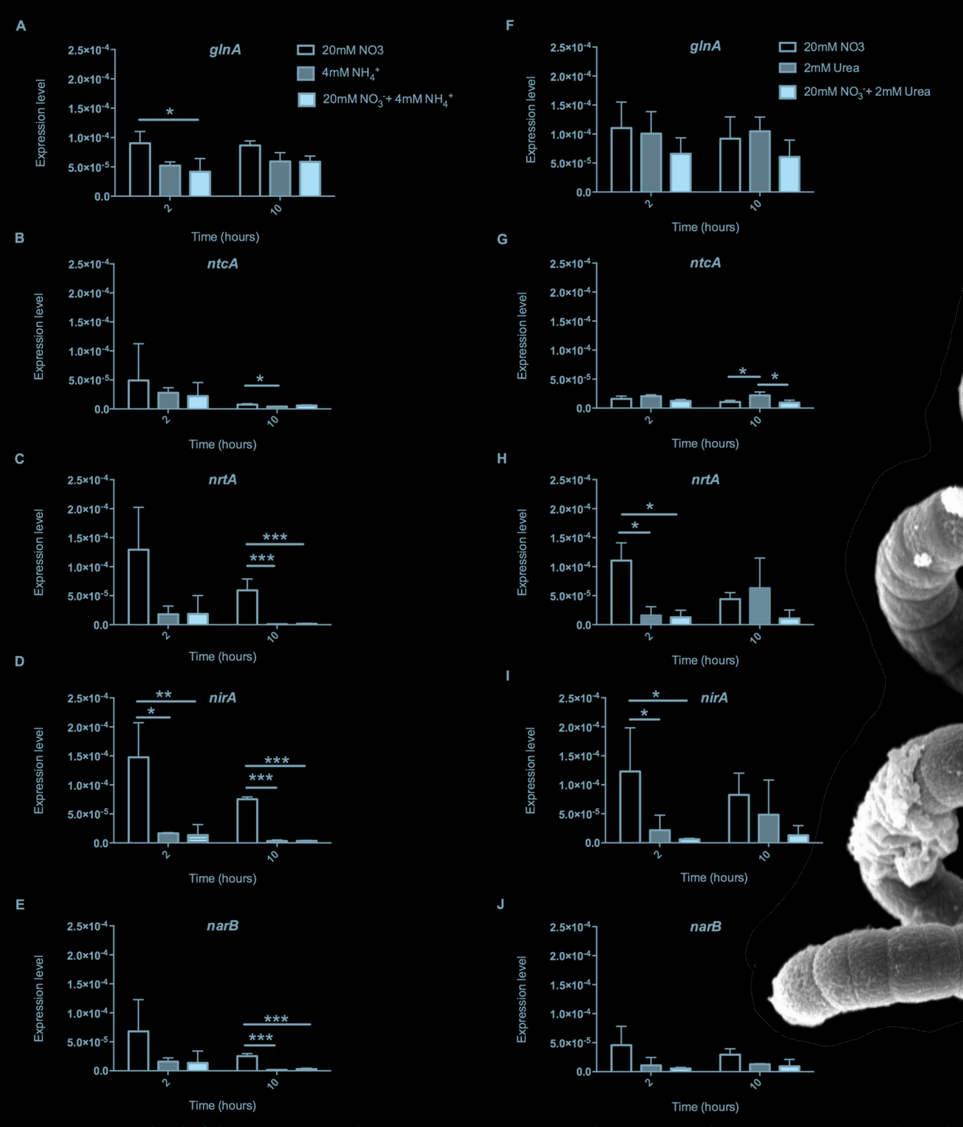

Figure 2: Expression level of the nitrogen-assimilatory genes *nrtA, nirA, narB, glnA, ntcA* in *Arthrospira* sp. PCC 8005 cultivated with NO_3^- and/or a second nitrogen source: NH_4^+ (A-E) or urea(F-I). The expression level was evaluated by RTqPCR.

Transcriptomic analyses have been performed in order to evaluate the influence of ammonium ions on N metabolism. Those analyses revealed, as we could guess, that all genes encoding for nitrate assimilation (*Fig.* 2 C,D,E)(*i.e. nirA, narB and nrtA*) were down-regulated. Likewise, the nitrate reductase NirA shows a lower relative abundance in presence of 4 mM NH_4^+ after 2 (fold change: 0.55, peptide: 6) and 10 (fold change: 0.30, peptide: 6) hours of culture. The presence of ammonium ions in the medium seems to induce a slight, but significant, decrease of the *glnA (Fig.* 2A) transcript. However, no significant differences have been observed for the global nitrogen control transcription factor, NtcA at the transcriptomic and proteomic level. Despite the release of two ammonium ions, urea seems to be suitable for the growth of *Arthrospira* sp. PCC 8005 (*Fig* 1 A, D, G). Thus, we decided to investigate the influence of this compound at the transcriptomic and proteomic level. Our data demonstrate a similar action than ammonium ions to the genes encoding for the nitrate assimilation (*Fig.* 2 H,I and J). Effectively, after 2 hours of culture, a significant decrease in those transcripts has been observed. At the proteomic level, we monitored a lower relative abundance of the nitrate reductase after 10 hours of culture in presence of urea (fold change: 0.20, peptide: 6). Concerning *glnA*, no significant differences have been detected at the trascriptomic level *(Fig. 2 F)*, but we observed a higher relative abundance of the corresponding protein after 2 hours of culture (fold change: 1.22 , peptides: 5).

Growth of *Arthrospira sp.* PCC 8005 in an urine-like medium

Table 1: Synthetic urine composition based on NASA reports of 1979 and Chang et al., protocole in 2013.

Compounds	Concentration (mM)
Urea	223.11
Creatinine	13.26
Urate	3.08
Hippurate	6.98
Citrate	3.38
EDTA	0.53
NaCl	136.89
$MgSO_4.7H_2O$	3.18
K_2SO_4	15.09
$CaCl_2$	3.42
$K_3PO_4.3H_2O$	0.88
$KHCO_3$	6.60

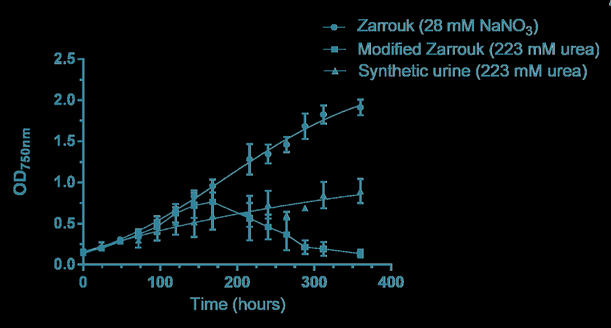

Figure 3: Growth of *Arthrospira* sp. PCC 8005 under classical Zarrouk (●), modified Zarrouk (■) or in urine-like medium (▲) based on OD_{750} measures.

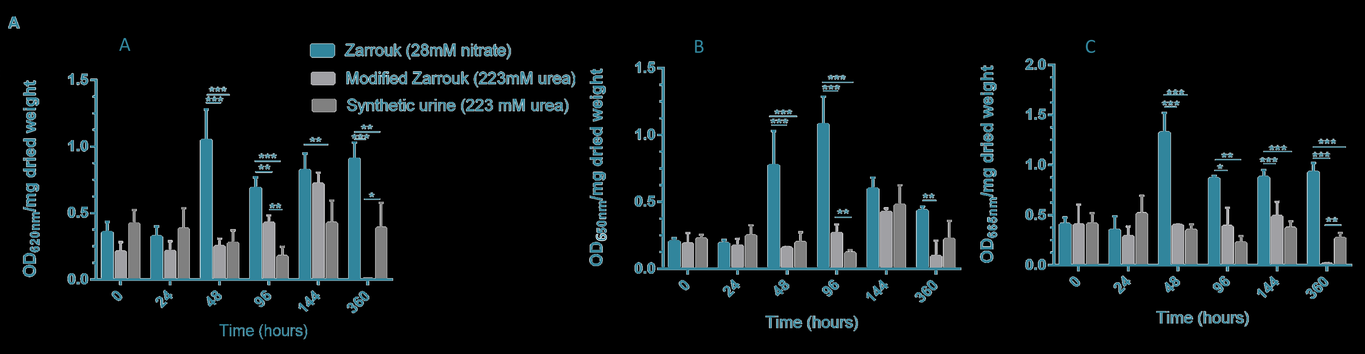

Figure 4: Monitoring of phycocyanin (A), allophycocyanin (B) and chlorophyll *a* (C) content when *Arthrospira* sp. PCC 8005 was cultivated in presence of classical Zarrouk (▬), modified Zarrouk (▬) or in urine-like medium (▬). Means are based on 5 independent replicates.

In a way to permit the direct steering of the urine from the crew compartment to the fourth compartment, the influence of such a liquide on the bacterial growth has to be tested. Therefore, we have observed the growth of *Arthrospira* sp. PCC 8005 (*Fig.* 3) in a medium comprising important characteristics of the human urine (organic compounds, high concentration of urea and salt) (*Table* 1)and compared it to growth in a classical Zarrouk medium and a modified Zarrouk medium in which we have replaced the NO_3^- by 224 mM of urea (concentration found in urine). Until 160 hours no differences have been observed *(Fig.* 3). However, afterwards, a cellular death is noticed for bacteria cultivated in modified Zarrouk medium (comprising 224 mM of urea). On the contrary, bacteria cultivated in synthetic urine succeed in growing, indicating that some components of the urine-like medium permit the growth of *Arthrospira* sp. PCC 8005 despite the high concentration of urea *(Fig. 3)*. To go further in the questionning, an analysis of the pigment content has been performed. The collected data depict a decrease in the phycocyanin (*Fig. 4A*), allophycocyanin (*Fig. 4B)* and chlorophyll *a* (*Fig. 4C)* content after 48 hours of culture. Further analyses are required to investigate if this drop is accompanied by a decline of oxygen production, protein content or biomass accumulation.

Conclusion

Despite the high concentration of nitrates and urea, *Arthrospira* sp. PCC 8005 demonstrates the ability to grow. However, high concentration of ammonium ions constrain its growth. Presence of ammonium ions clearly induces a down-regulation of nitrate assimilation at the transcriptomic and the proteomic level as it is mentioned in literature. Furthermore, ammonium ions released by the hydrolysis of urea seem to induce a similar phenomenon. Nevertheless, in order to be as close as possible to the MELiSSA, further researches have to focus on a 3-nitrogen sources based medium. In this context, it will be possible to observe transcriptomic and proteomic adaptation of this strain to a mixture of nitrogen sources as it could be the case in the MELiSSA loop. Pigment analyses of *Arthrospira* sp. PCC 8005 provide important indications on its adaptation to urine-like medium and reveal a worrying impact on pigment content. But, as a promising observation, no cellular death has been observed. Further experiments are required to better understand which component of the synthetic urine induce the decline of the pigment content and if it is possible to reduce this effect.

Acknowledgements

This research was kindly supported by the European Space Agency (GSTP5 melgen-3', Biorat-2 ELIPS4, PRODEX).

Presenting author: Guillaume.BAYON-VICENTE@student.umons.ac.be
Corresponding author: Ruddy.Wattiez@umons.ac.be

References

Chang, Y., Wu, Z., Bian, L., Feng, D., & Leung, D. Y. C. (2013). Cultivation of Spirulina platensis for biomass production and nutrient removal from synthetic human urine. *Applied Energy*, *102*, 427–431. doi:10.1016/j.apenergy.2012.07.024

Putman, D. F. (1971). Composition and Concentrative Properties of Human Urine. *NASA Contractor Report*, (July), 109. Retrieved from http://hdl.handle.net/2060/19710023044