EXPLORATION OF EXOPOLYSACCHARIDE PRODUCTION BY *CYANOTHECE SP.* PCC 7822

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Introduction

The Algotech project was established to progress in the field of high-added value compounds production by Microalgae/Cyanobacteria completing by a circular economy consideration. In this way, we investigate the exopolysaccharide (EPS) production of *Cyanothece sp.* PCC 7822, well-known for its diazotrophic metabolism. Impact of C:N ratio and N source on EPS configuration and composition will be explored.

Modification of N sources & C:N ratio

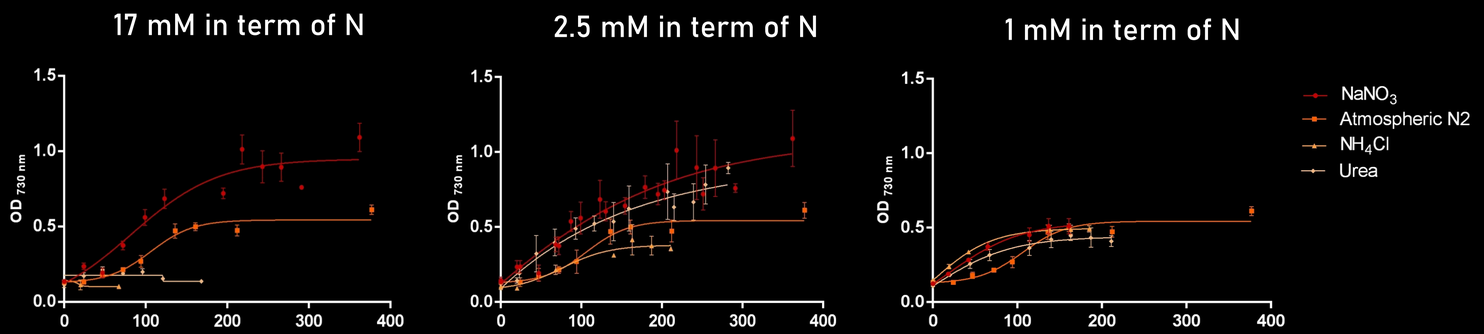

Fig. 1 : Growth curve of *Cyanothece sp.* PCC 7822 in BG 11 medium supplemented with different nitrogen sources at different concentrations ($NaNO_3$, NH_4Cl, urea at 1, 2.5 and 17 mM and atmospheric N_2). Concentrations are defined in term of N atom and cultures are performed at 25°C and continuous light (10 µmol photons.m-².s-¹).

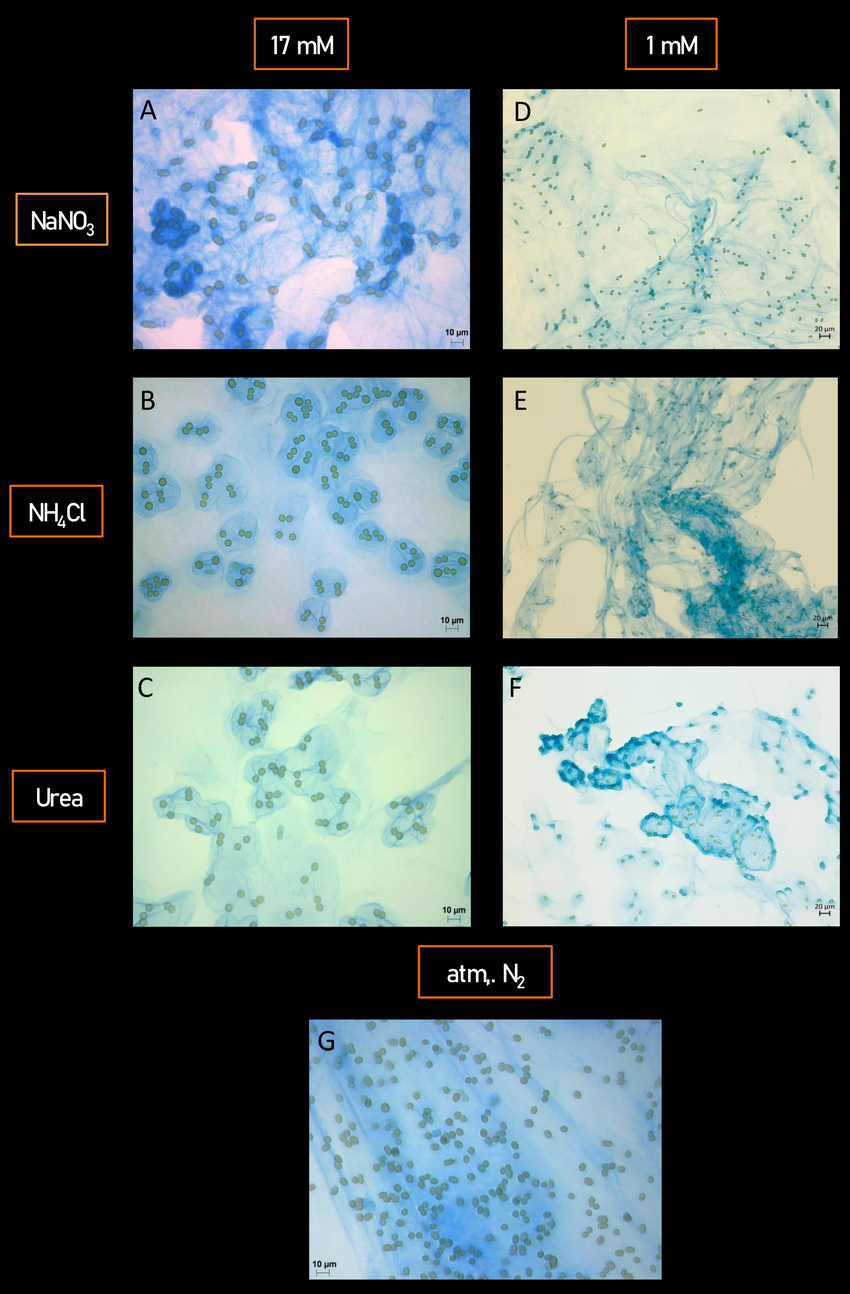

Fig. 2 : Observation of *Cyanothece sp.* PCC 7822 exopolysaccharides by a alcian blue staining in presence of 4 different nitrogen sources at different concentrations.

Metabolic impact of C:N ratio modulation

- Total uptake of N sources at the mid-log phase in all 1 mM conditions associated with a reduction of cyanophycin and phycocyanin content

→N-limiting conditions related with the use of intracellular nitrogen stock

- $NaNO_3$ 17 mM is a N-excess condition associated with stable phycocyanin and cyanophycin contents

→Nitrogen stored as cyanophycin granules

- Atm. N_2 condition reveals a profile similar as a N-limiting condition with a complete degradation of cyanophycin granules after 100 hours of culture

→Consumption of nitrogen stock due to a low nitrogenase efficiency

Growth analysis

- Maximal growth in $NaNO_3$ conditions independently of the concentration
- Growth in presence of NH_4Cl 1 mM and 2.5 mM (not correlated with literature : MIC 1 mM)
- In urea condition, best growth at 2.5 mM
- In presence of atm. N_2, growth is similar to N-limiting conditions (1mM)

→ Relation growth – EPS production ?

1. Environmental stress
2. C:N ratio & carbon excess

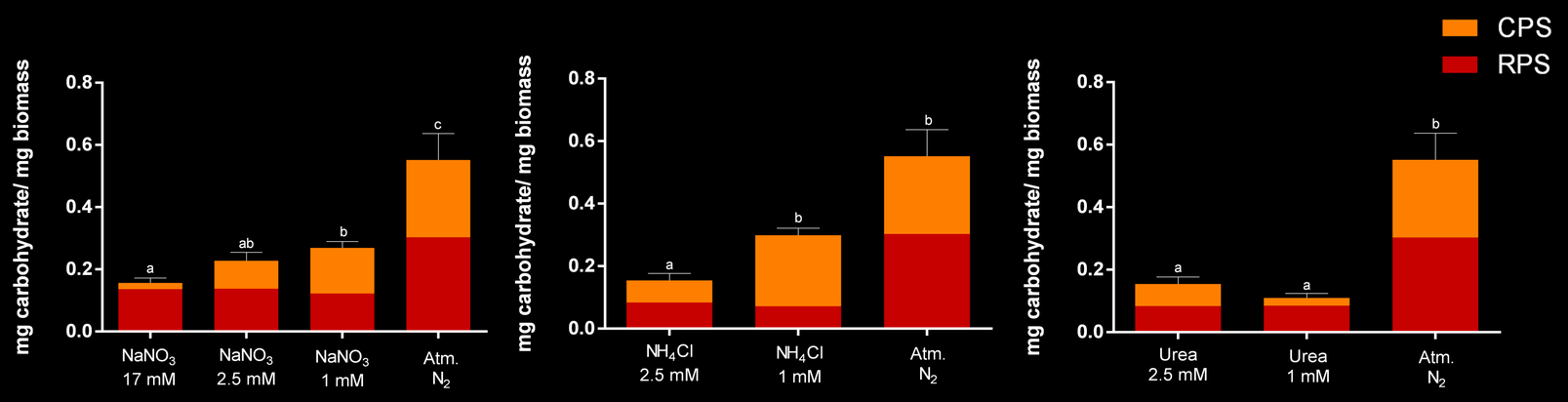

Fig. 3 : Quantification of exoplysaccharides by the method of Dubois et al., 1956. EPS extraction is based on the protocol of Di Pippo et al., 2013. Value obtained by the colorimetric method are standardised by dry biomass and the experiment is performed in 4 replicates.

EPS staining

- Significant quantity of stained EPS in $NaNO_3$ conditions (Fig 2A, 2D)
- EPS observation in NH_4Cl and urea 17 mM conditions and the absence of growth could be explained by an EPS production necessary to deal with environmental stress (Fig 2E, 2F)
- In atm. N_2 condition, EPS uniformly dispersed and in high quantity (Fig 2G)
- Visual abundance of EPS higher with 1 mM of urea than 17 mM (Fig 2C, 2F). Opposite observation in NH_4Cl condition (Fig 2B, 2E)

→**Variable EPS configuration between the different conditions could reveal differences in monosaccharide composition**

EPS quantification

- EPS quantity increases when N concentration is reduced in $NaNO_3$ and NH_4Cl conditions

→ **Influence the C:N ratio & carbon excess drives EPS production**

- No impact of urea concentration on EPS accumulation
- Highest value obtained in **atm. N_2** condition with **0.55 mg/mg DW**

→ **Surprising result comparing with literature**

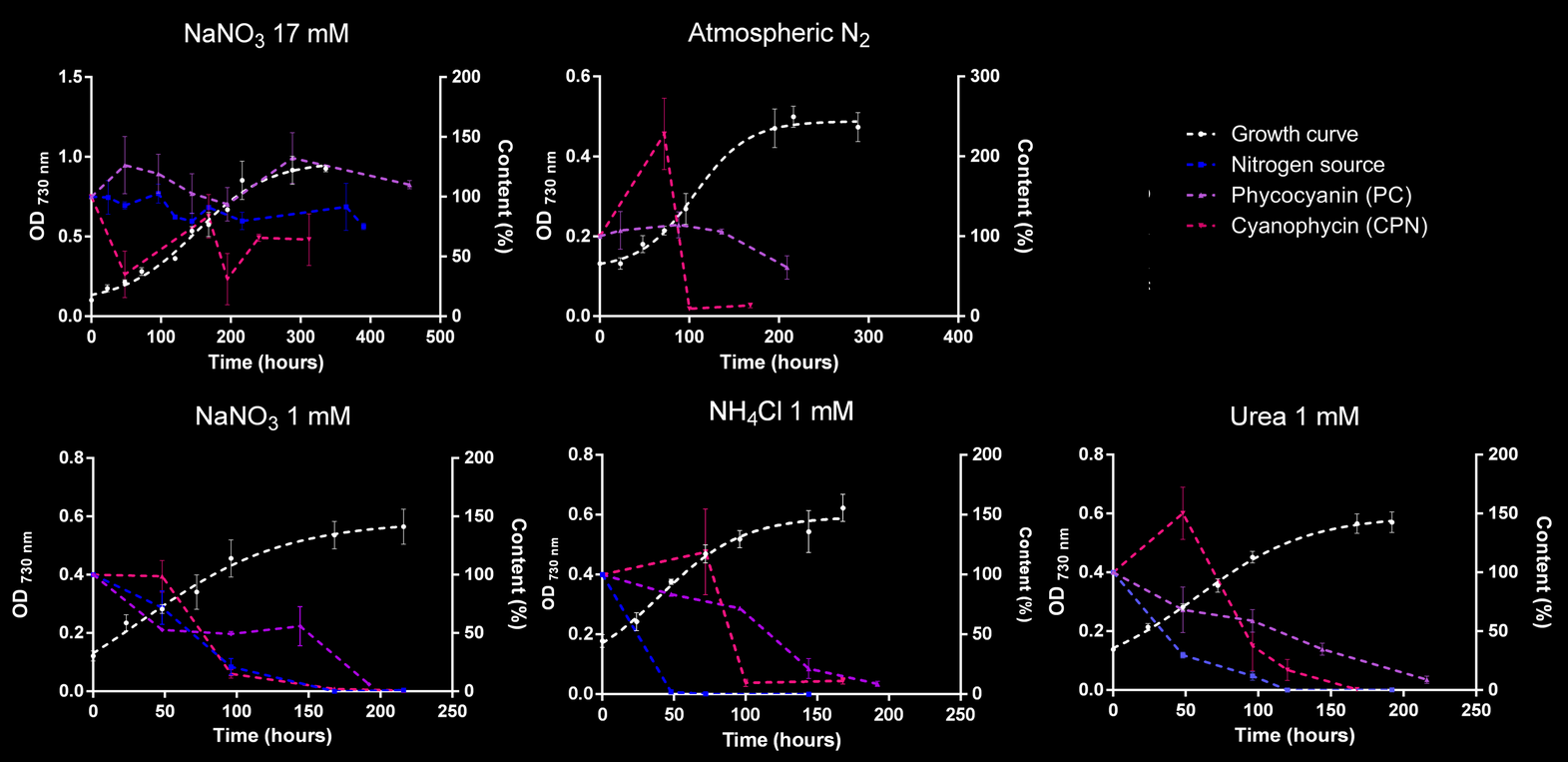

Fig. 4 : Firsts insights on *Cyanothece sp.* PCC 7822 metabolism by nitrogen sources, phycocyanin and cyanophycin quantification when the bacterium is cultivated in presence different nitrogen sources and concentrations.

Conclusion

Modulation of N source is relevant because of its impact on C:N ratio and carbon metabolism. Interestingly, modification of this parameter has an opposite effect on bacterial growth and EPS accumulation. Atmospheric N_2 is the best condition to produce EPS which is interesting in an industrial point of view. Deeper analyses of polymer composition and EPS metabolism will permit to hypothesise on its future field of application.

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