The exopolysaccharide production by *Cyanothece sp*. PCC 7822 through an adapted metabolism

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Introduction

The Algotech project was established to progress on the field of high-added value compounds production by Microalgae/Cyanobacteria. In this way, we investigate the exopolysaccharide (EPS) production of *Cyanothece sp*. PCC 7822 well-known for its diazotrophic metabolism in light/dark cycle condition. The influence of several parameters on EPS production and composition will be examined throughout this study.

Impact of nitrogen sources

Nitrate, ammonium and N_2 conditions are characterised by comparable lag and exponential phases. The main difference consists in the highest reached $OD_{730\ nm}$. In opposition, the strain can't grow in presence of 8.5 mM urea.
At 300h of culture in NO_3 condition, a second exponential rise in the OD follows the stationary phase. It could be related to a complete use of NO_3 by the bacterium and a switch to a diazotrophic metabolism (Fig. 1). However, NO_3 quantification exhibits 8 mM of NO_3 when the OD increases for the second time (results not shown).

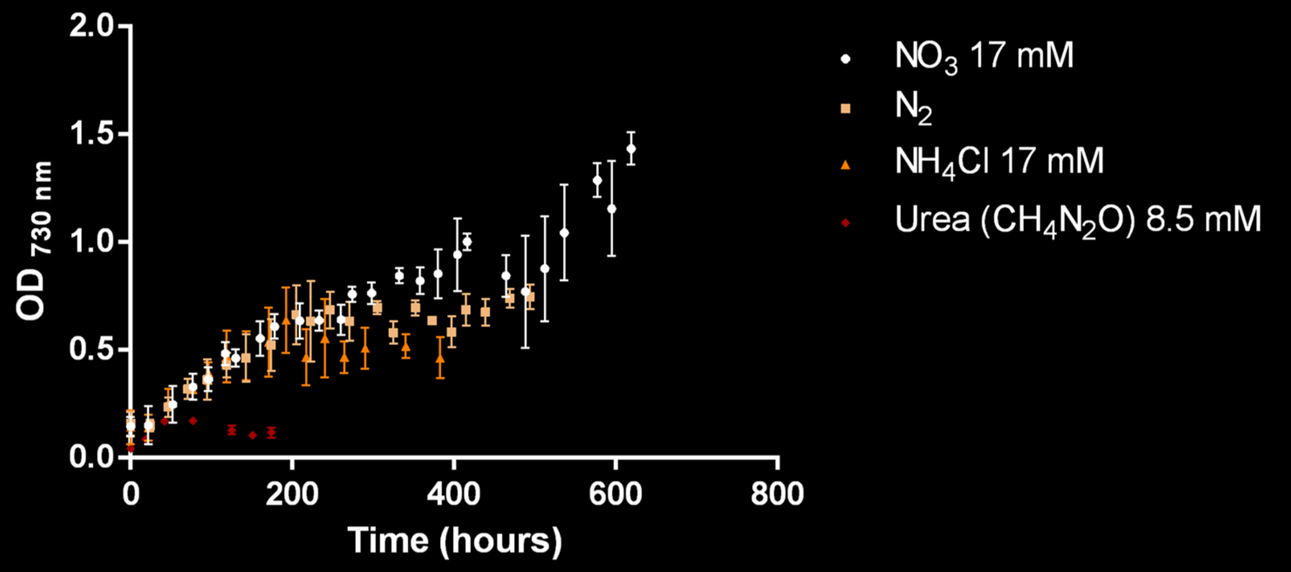

Fig. 1 : Growth curve of *Cyanothece sp.* PCC 7822 in BG 11 medium supplemented with different nitrogen sources : NO_3 17mM (●), NH_4Cl 17 mM (▲), urea 8.5 mM (◆) and N free (■) (atmospheric N_2). The experiment was realised in 4 replicates.

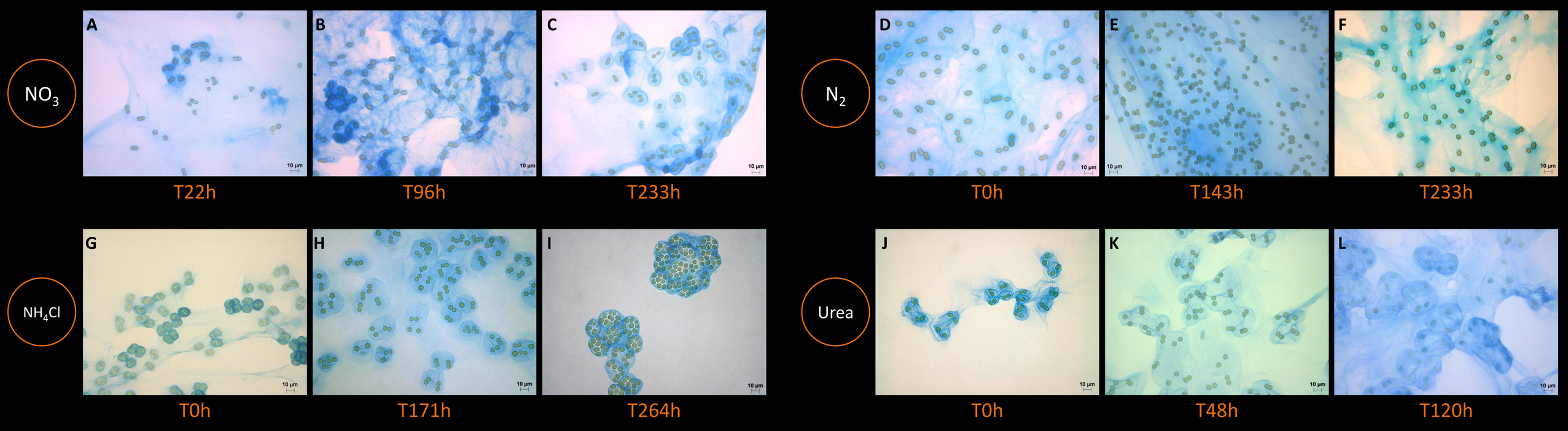

Fig. 2 : Observation of *Cyanothece sp.* PCC 7822 exopolysaccharides by using a positive alcian blue staining at pH 2.5 in presence of 4 different nitrogen sources (17 mM NO_3, N_2, 17 mM NH_4Cl and 8.5 mM urea). Exopolysaccharides are observed at different moment of the growth curve and experiment are performed in 4 replicates.

Fig. 2 depicts that *Cyanothece sp.* PCC 7822 synthesises EPS in the four conditions but EPS configuration differs. In NO_3 condition, EPS are uniformly spreaded in the medium (Fig. 2A, 2B) then formation of a halo around the cell is visible (Fig. 2C). In N_2 condition, no morphological changes are distinguished during the growth. Effectively, they are homogeneously dispersed into the medium (Fig. 2D, 2E and 2F). The presence of ammonium leads to a contrasting distribution of EPS. Spheres of EPS surround bacteria in division inducing formation of aggregates (Fig. 2I). The same observation is done in urea condition (Fig. 2J and 2K) and cell death is noticed after 5 days of culture as seen in Fig. 2L. A connection between the growth and EPS observation could indicate that in more stressful conditions, EPS structure and composition change.

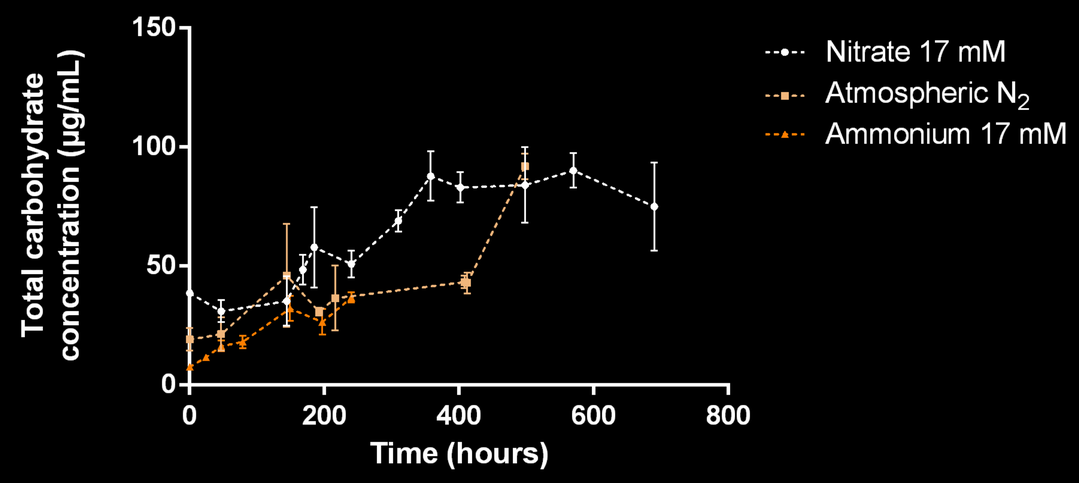

Fig. 3 : Total carbohydrate quantification of released polysaccharides by the phenol/sulfuric acid method when *Cyanothece sp.* PCC 7822 is cultivated in BG 11 supplemented by 3 different nitrogen sources: NO_3 17mM (●), NH_4Cl 17 mM (▲) and N free (■) (atmospheric N_2). D-glucose is used for standard curve.

The presence of nitrate enhances exopolysaccharide production in the form of RPS (Released PolySaccharide) (Fig. 3). Usually, the presence of combined nitrogen sources increases EPS formation because of the lower energy required for their assimilation unlike N_2 fixation. In addition, a quantification of CPS (Capsular PolySaccharide) is necessary to obtain a global view of EPS produced by the strain. The rise of RPS measured at the end of the curve in N_2 condition could be related to bacteria degradation and intracellular polysaccharide release.

Conclusion

We chose to modify the N source because of its influence on C:N ratio and so on carbon metabolism. Interestingly, modification of this parameter has an impact on *Cyanothece sp.* PCC7822 growth but also on EPS production and appearance. Future experiments will go further into the effect on EPS composition and structure but also on EPS biosynthesis pathways. The N source is not the only parameter regulating EPS production. Effectively carbon source, light intensity or salt concentration could also affect EPS. Therefore, some of these will be attractive to analyse in addition to nitrogen supply variation.

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