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

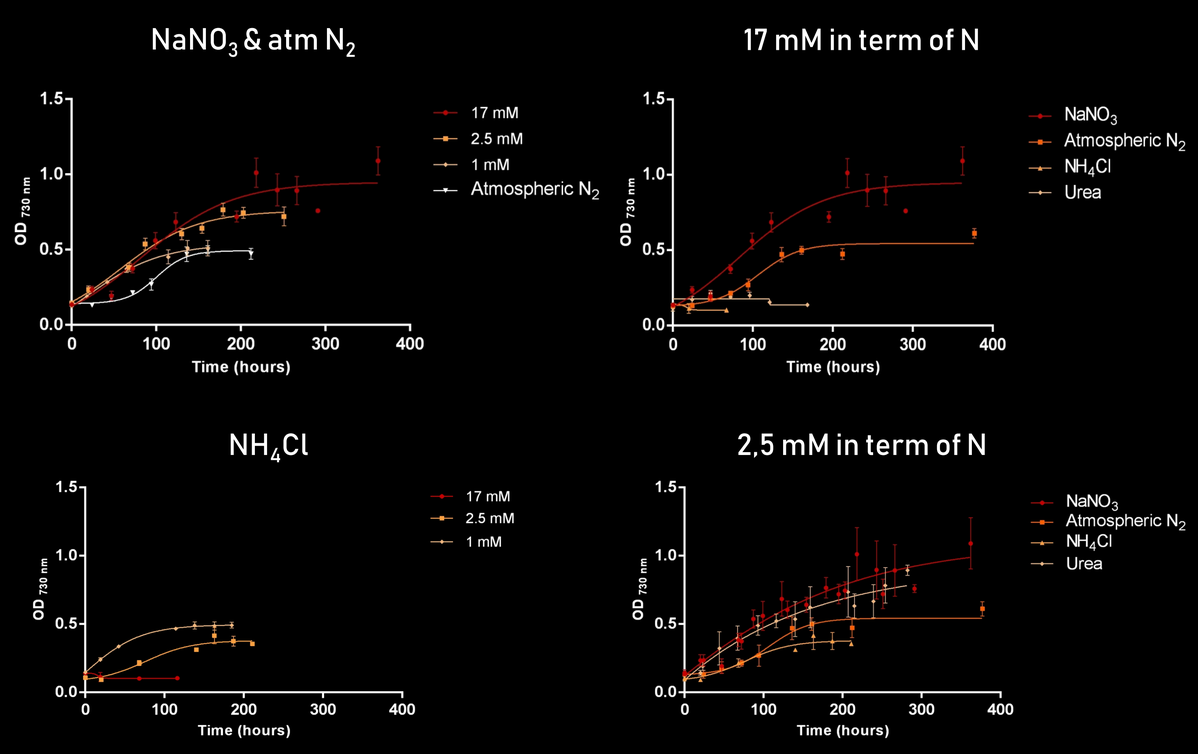

Fig. 1 : Growth curve of *Cyanothece sp*. PCC 7822 in BG 11 medium supplemented with different nitrogen sources at different concentration($NaNO_3$, NH_4Cl, urea at 1, 2.5 and 17 mM and atmospheric N_2). Concentrations are defined in term of N atom. The experiment was realised in 4 replicates.

As shown in Fig 1, the best growth is noticed in $NaNO_3$ condition independently of the concentration. Contrarily with literature, *Cyanothece* grows in presence of NH_4Cl at 1 and 2.5 mM. In urea condition, optimal growth is determined at 2.5 mM. Even if the strain is able to fix atmospheric N_2, growth is not as best as in $NaNO_3$ condition. It is interesting to highlight different growth profil of *Cyanothece* considering a potential relation with EPS production. As an example, environmental stress could induce an EPS production by bacteria. In presence of 1 mM of N sources, the C:N ratio increases and carbon excess could be drived in EPS production even if growth is reduced.

A significant quantity of EPS are spotted in $NaNO_3$ conditions (Fig 2A, 2B). The absence of growth with NH_4Cl and urea 17 mM correlated with microscopy observation suggests an EPS formation as a solution to prevent the stress caused by N sources (Fig 2C, 2E). EPS configuration in these 2 conditions is also contrasting with $NaNO_3$ conditions. In atm. N_2 condition, EPS are dispersed in the medium, characterised by a visquous culture (Fig 2G). EPS seems more abundant in urea 1 mM condition that in urea 17 mM (Fig 2E, 2F) whereas the opposite observation is done in NH_4Cl condition (Fig 2C, 2D).

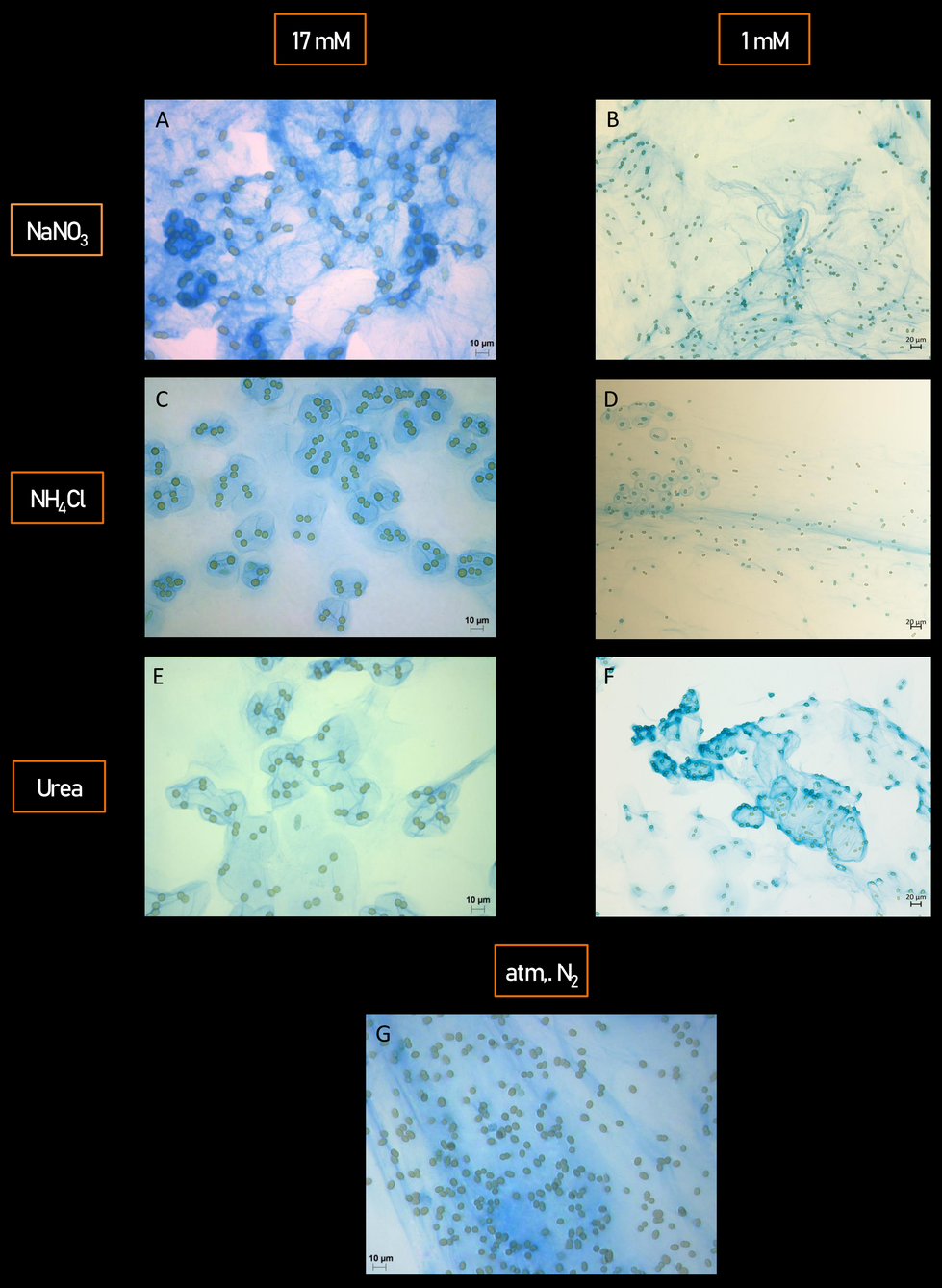

Fig. 2 : Observation of *Cyanothece sp.* PCC 7822 exopolysaccharides by using a positive alcian blue staining at pH 2.5 in presence of 4 different nitrogen sources at different concentrations. Experiments were performed in 4 replicates and over the growth.

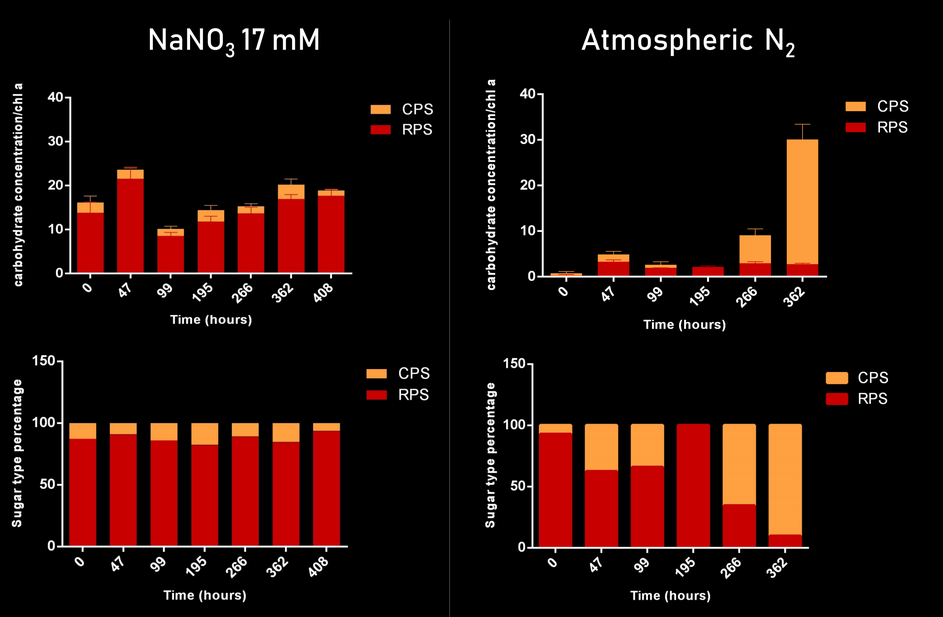

Fig. 3 : Total carbohydrate quantification of exopolysaccharides. RPS represent the released polysaccharides and CPS represent the capsular polysaccharides (attach to cell surface), D-glucose is used for standard curve and values are standardised by chlorophyll *a* concentration

Fig. 3 reveals that RPS are dominant in $NaNO_3$ condition representing 80% of total EPS quantified (RPS + CPS). Despite the concentration fluctuation during the growth, the ratio between RPS and CPS remains stable. Globally, less EPS are formed in atm. N_2 condition and the ratio is also different with a stable RPS concentration and an increase of CPS produced over the growth. Surprisingly, The maximum EPS obtained is 33 µg/mL at the end of the growth in atm. N_2 condition.
Future acid uronic and sulphated sugar quantification will help us to select various conditions for a monosaccharide composition investigation.

Conclusion

Variation of N sources and concentrations are relevant because of their influence on C:N ratio and carbon metabolism. Interestingly, modification of this parameter has an impact on *Cyanothece sp.* PCC 7822 growth but also on EPS production and configuration. Projected experiments will go further to better understand the effect on EPS composition and structure. A mass spectrometry analysis of the protein profil is also planed to improve knowledge of cyanobacterial EPS biosynthesis pathways.

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