URINIS Project: Optimization of Bacterial Cultivation and RNA Extraction for RNASeq

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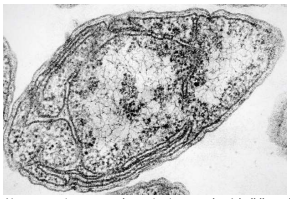
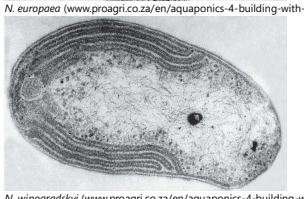
Introduction

The 'Urine Nitrification in Space' (URINIS) project aims to achieve full nitrification from urine during a flight experiment in the frame of ESA's 'Micro-Ecological Life Support System Alternative' (MELiSSA) program.

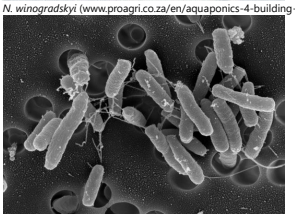
Urine contains a high amount of nitrogen in the form of urea. This nitrogen can be recovered as nitrate through the use of a synthetic bacterial community in a 3-step process: (i) ureolysis by *Comamonas testosteroni* produces ammonium. (ii) *Nitrosomonas europaea* (Ns) oxidizes this ammonium to nitrite, which is (iii) converted to nitrate by *Nitrobacter winogradskyi* (Nb). The URINIS project is a proof-of-concept to demonstrate that this community can perform full nitrification in space conditions. Optimal pre- and post-flight experiment storage conditions will be determined. Also, in a ground experiment, the effects of simulated microgravity and ionizing radiation will be assessed via phenotypical, transcriptomic, proteomic and metabolomic analyses. However, no protocol for microbial community maintenance has been established yet. Moreover, *N. europaea* and *N. winogradskyi* are characterized by a slow growth rate and low biomass production. This limits RNA availability for transcriptome analysis.



N. europaea (www.proagri.co.za/en/aquaponics-4-building-with-bacteria/)



N. winogradskyi (www.proagri.co.za/en/aquaponics-4-building-with-bacteria/)



C. testosteroni (Weiss *et al.*, 2013)

Objectives

(1) Define the optimal medium composition for growth and maintenance of the axenic strains, a co-culture of *N. europaea* and *N. winogradskyi,* and the synthetic bacterial community.

(2) Optimization of a protocol for RNA extraction for *N. europaea*. Once completed, the procedure has to be extended to *C. testosteroni, N. winogradskyi*, the nitrifying co-culture and the synthetic bacterial community.

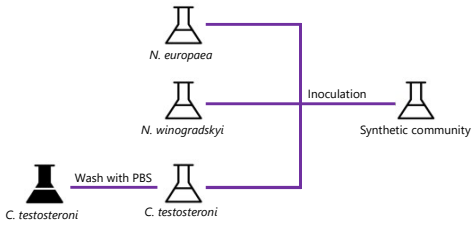
Methods

Bacterial Cultivation

The medium designed for the nitrifying co-culture (Ns and Nb) was used to grow a synthetic bacterial community. The nitrogen source in this medium, ammonium, was replaced with urea to enable ureolysis. Through urea conversion, ammonium will become available for the other members in the community. Also, different concentrations of EPPS buffer were tested to determine the optimal buffer capacity.

The medium was inoculated with equal volumes of axenic cultures of the 3 selected strains. *C. testosteroni* culture was washed with PBS to remove any organic carbon sources.

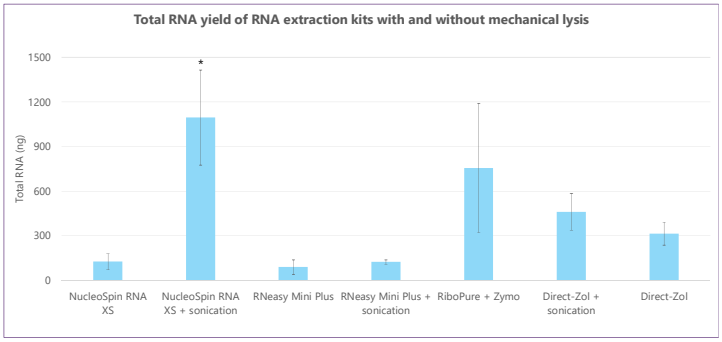
The OD600 value, pH value, conductivity, and ammonium, nitrite, and nitrate concentrations were measured.



RNA Extraction

RNA was extracted using the following commercial silica-column based kits: NucleoSpin® RNA XS (Machery-Nagel), RNeasy Mini Plus (Qiagen), RNeasy Micro Plus (Qiagen) and Direct-Zol™ RNA MiniPrep (Zymo Research).

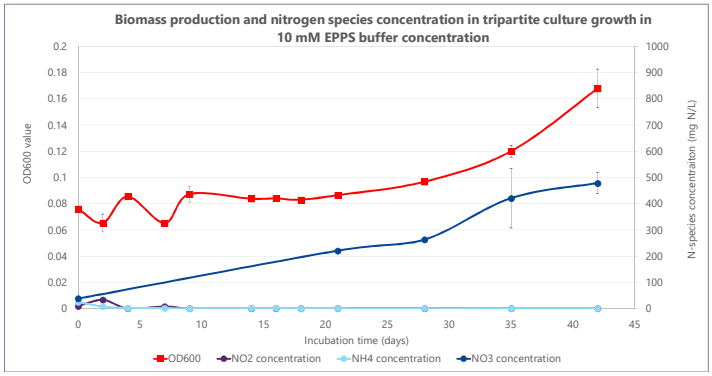
Moreover, an extra ultrasonication step was implemented during disruption and homogenization of the culture to assess the effect of mechanical lysis on total RNA yield. A protocol using bead-beating (Zymo+RiboPure) was also evaluated.



Results and Discussion

The observed conversion of the nitrogen species indicate that ureolysis and nitrification were occurring in the culture. pH of the medium also stabilized for all different buffer concentrations, albeit at varying pH values. In a medium with 10 mM EPPS, the pH stabilized at 7.9, close to the pH optimum of the nitrifiers (pH = 8).

The first indications of biomass production were observed after 5 weeks of incubation.



Total RNA yield gained using standard kit protocol did not significantly vary between most kits. Only Direct-Zol™ RNA MiniPrep showed a higher average RNA yield. However, sonication in combination with NucleoSpin® RNA XS significantly increased total RNA yield. The threshold amount for RNASeq (1 µg/L) was reached. RNA quality assessment showed a high level of RNA degradation. To minimize degradation and to make the samples suitable for RNASeq, further optimization is required. Shorter and/or less intense sonication periods did not prove effective. Total RNA yield decreased below the threshold while RNA quality did not increase adequately. Varying amounts of lysis buffer and chaotropic agents during the lysis step were also investigated, but did not provide any improvements.

Conclusion

We could successfully grow a gnobiotic synthetic community. This indicates that all 3 strains can grow together and feed each other. Observed biomass production is also encouraging. In future experiments, a synthetic urine salts solution (SUSS) will be used to grow the strains axenically, in nitrifying co-culture, and as a bacterial community. SUSS contains an organic carbon source for *C. testosteroni,* to ensure biomass production by this strain. Total RNA yield passes the threshold for RNASeq but RNA degradation is still an issue. In the near future, bead-beating instead of sonication will be investigated.

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