

Copper uptake in the ammonia oxidizing archaeon *Nitrososphaera viennensis*

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Copper (Cu) is central to many enzymes in ammonia oxidizing archaea (AOA) including plastocyanins and multicopper oxidases [1]. Although it is known that Cu plays an important role in AOA physiology and metabolism, it is not known how AOA acquire and transport Cu into the cell. It has been hypothesized that AOA might secrete a chalkophore under Cu-limiting conditions to acquire Cu for ammonia oxidation [2]. In this study, we explored the physiological response of the AOA soil isolate, *Nitrososphaera viennensis* (EN76^T) [3,4] to Cu-limiting conditions. The chelators TETA as [1,4,8,11-tetraazacyclotetradecane 1,4,8,11-tetraacetic acid hydrochloride hydrate] or [3,6-diazoctane-1,8-diamine tetrahydrochloride], with selective affinities for Cu²⁺, were used to lower bioavailable Cu²⁺ in culture experiments as predicted by thermodynamic speciation calculations (PHREEQC). Ammonia (NH₄⁺) and nitrite (NO₂⁻) concentrations were measured in these cultures over time using colorimetric assays. Preliminary results show that *N. viennensis* is Cu-limited at concentrations as low as 6.18 x 10⁻¹⁵ mol/L free Cu²⁺ and below, as compared to standard conditions (3.26 x 10⁻¹² mol/L free Cu²⁺). This Cu²⁺ limiting threshold is higher than denitrifying bacteria (<10⁻¹⁶ mol/L) [5] and similar to other AOA [2], which also possess Cu containing enzymes. The overall goal of the project is to contribute to the basic understanding of copper uptake by archaea.

[1] C. Rensing, S.F. McDevitt, (2013), *Metallomics and the Cell* vol. 12, 417-450 [2] S.A. Amin *et al.*, (2013), *Limnol Oceanogr.* 58, 2037-2045 [3] M. Tourna *et al.*, (2011), *PNAS* 108:8423-8425 [4] M. Stiegelmeier *et al.*, (2014) *Int. J. Syst. Evol. Microbiol.* 64, 2738-2752. [5] J.W. Moffet *et al.*, (2012), *Limnol Oceanogr.* 57, 272-280.