

How the ammonia oxidizing archaeon *Nitrososphaera viennensis* responds to copper limitation

CAROLINA REYES^{1,2}, THOMAS PRIBASNIG^{2,3}, MELINA KEROU^{2,3}, BARBARA BAYER^{2,4}, CHRISTA SCHLEPER^{2,3}, STEPHAN M. KRAEMER^{1,2}

University of Vienna, ¹Dept. of Environmental Geosciences,

²Environmental Science Research Network

³Dept. of Ecogenomics and Systems Biology and

⁴Dept. of Limnology and Bio-Oceanography,

Althanstrasse 14, 1090, Vienna Austria

Copper (Cu) is central to many enzymes in ammonia oxidizing archaea (AOA) including plastocyanins and multicopper oxidases¹. Recently researchers have begun to explore the response of AOA to excess Cu and to Cu limitation^{2,3}. In this study, we explored the physiological response of the AOA soil isolate, *Nitrososphaera viennensis* (EN76^T)^{4,5} to Cu-limiting conditions. Additionally, the expression of genes under Cu-replete and Cu-limiting conditions was investigated. The chelator TETA as [1,4,8,11-tetraazacyclotetradecane] with selective affinity for Cu²⁺ was used to lower bioavailable Cu²⁺ in culture experiments as predicted by thermodynamic speciation calculations (PHREEQC). Ammonium (NH₄⁺) and nitrite (NO₂⁻) concentrations were measured in these cultures over time using colorimetric assays. Cell abundance was determined using flow cytometry. Gene expression was assessed by quantitative PCR (qPCR). Results show that at Cu concentrations of 6.18 x10⁻¹⁵ mol/L free Cu²⁺, the ammonia oxidation and cell growth abilities of *N.viennensis* are hindered. Genes potentially involved in Cu uptake were more highly expressed in Cu-limited cultures compared to Cu-replete cultures. The overall goal of the project is to contribute to the basic understanding of copper uptake by archaea.

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