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Bioavailability of dissolved copper species to *Pseudomonas fluorescens*

Peter E. Holm[1], Yusuf M. Ibrahim[1,2], Andreas Tom-Petersen[2], Kristian K. Brandt[2], Ole Nybroe[2]

[1]Department of Natural Sciences (peho@kvl.dk); [2]Department of Ecology (oln@kvl.dk), The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK.

INTRODUCTION

Total concentrations of soluble Cu often correlate poorly with toxicity and bioavailability of the metal to microorganisms pointing to a need for corresponding chemical and biological analysis. Bioavailable Cu to specific microorganisms can be determined by the use of Cu-specific reporter strains. However, knowledge on the specific Cu-species that are able to elicit a response from reporter strains is so far limited. The objective of the present work was to determine the bioavailability of different dissolved Cu species to a Cu-specific bioluminescent reporter strain of the common soil bacterium *Pseudomonas fluorescens*.

METHODS

Strains and bio-sensors

Pseudomonas fluorescens strain DF57-Cu15 is a copper-specific bioluminescent reporter strain, which carries a chromosomal insertion of a promoter-less Tn*5::luxAB* cassette controlled by a copper-induced promoter (Tom-Petersen *et al.*, 2001). Another strain, *P. fluorescens* DF57-40E7, shows a constitutive expression of bioluminescence from the Tn*5::luxAB* cassette and is used to measure sample toxicity through inhibition of bioluminescence. This strain has a copper tolerance comparable to that of strain DF57-Cu15.

P. fluorescens DF57-Cu15 and DF57-40E7 reporter assays

Standard reporter assays were conducted as follows: Reporter cells harvested in the exponential growth phase were mixed with sample or copper standard and incubated for 90 min before bacterial bioluminescence (relative light units; RLU) was measured.

Cupric ion selective electrode measurements and geochemical modelling

The cupric ion activity ($pCu = -\log[Cu^{2+}]$) in the test solutions, without the biosensor was measured on a cupric ion selective electrode coupled with a double-junction reference electrode. The test solutions contained varying concentrations of EDTA, citrate or DOC. The total copper concentration (Cu_{tot}) of the test solutions was 0.04 µM.

A calibration curve was established according to Sauvé et al. (1997). The electrode potential (EP) determined by Cu-ISE was then plotted against Cu^{2+} activity calculated by the computer equilibrium model MINTEQA2 assuming that the solutions were in thermodynamic equilibrium.

RESULTS AND DISCUSSION

When the test medium that initially contained 0.04 µM Cu and no chelating agents was amended with increasing concentrations of citrate, the response from the reporter strain was high and relativity stable. Cu-ISE measurements showed a marked decrease in free ion activity and speciation analysis showed that all Cu was present as citrate complexes at the highest citrate concentrations. For media containing a high citrate concentration, increasing

concentrations of EDTA decreased the bioluminescence response from the reporter. In this experiment free ion activity was too low to measure, and geochemical equilibrium calculations showed a transition from Cu-citrate to Cu-EDTA complexes.

The effect on DOC on Cu bioavailability and speciation is illustrated in Figure 1.

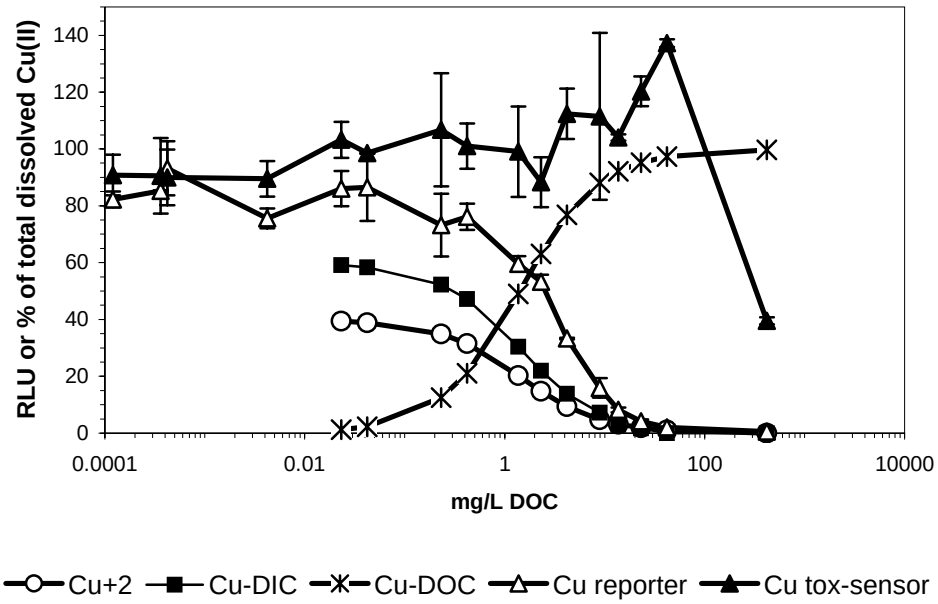

Figure 1. Cu speciation and bioluminescence expressed as relative luminescence (RLU) as function of varying DOC concentrations.

The results with DOC from a forest soil showed that Cu-DOC complexes were not bioavailable to *P. fluorescens*, as the Cu reporter signal remained relatively constant with increasing DOC up to 0.4 mg/L but declined for higher DOC concentrations where Cu-DOC became the dominant dissolved Cu species. Amendment with increasing citrate concentrations was not able to increase bioavailability. Again changes in bioavailability were not solely correlated to changes in free ion activity of Cu.

CONCLUSIONS

- The *P. fluorescens* Cu-reporter provides a sensitive and quantitative measurement of bio-available Cu in solution.
- Bio-available Cu does not merely correspond to free Cu^{2+} or total dissolved Cu.
- Free Cu ions and Cu-citrate complexes appear to be bio-available to the Cu-reporter whereas EDTA and DOC from forest soil form non-available complexes with Cu.
- Parallel use of bionsensors and chemical speciation analysis will improve our understanding of factors influencing “bio-availability”, and hence toxicity of Cu.

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