Probing metabolically active cells in soils using BONCAT

ESTELLE COURADEAU[1], JOELLE SCHLAPFER[1], DANIELLE GOUDEAU[1], REX MALMSTROM[1], TRENT NORTHEN[1]

[1]Joint Genome Institute, DOE, Walnut Creek, CA, USA
ecouradeau@lbl.gov

Soils are arguably the most biologically diverse ecosystems on Earth. Microorganims inhabiting soils play a critical role in large scale biogeochemical cycles such as C and N, however our understanding of the function of soil microorganisms is limited by their overwhelming diversity and interconnected metabolic pathways. Here we implemented a recently developped method called BONCAT (biorthogonal non-canonical amino-acid tagging) that enables us to identify the metabolically ative microorganims in soil and reduce the long census of soil microorganisms into a meaningfull functional list. BONCAT is a two step process that fluorescenty labels protein making cells as a proxy for cellular activity. BONCAT was developed for oceanic microbial communities and we report here its first impementation in terrestrial systems. Our sample is a silt dominated soil from Oak Ridge (TE) that was collected at 30 cm depth and preserved unfrozen until processing. A subsample was incubated in triplicate in close to natural conditions with the non-canonical amino acid homopropargylglycine (HPG) that cells incorporated *in vivo*. Single cells were detached from the soil and HPG was further coupled to a fluorescent dye using a copper-catalyzed azide-alkyne cycloaddition. Fluorescently labelled cells were sorted based on their double BONCAT and DNA counter-stain fluorescent labels using fluorescence-activated cell sorting. BONCAT positive cells will be identified using 16S rDNA library Illumina sequencing and compared to the whole soil community. Single BONCAT positive cells will also be sorted alive into media with the prospect of producing new relevant soil microbial isolates. BONCAT could also be coupled to stable isotope probing and mass spectrometry to further assess how active microbes incorporate relevant substrates. This pioneer study will likely open new prospects to re-assess key metabolic traits of the active sub-set of soil microbiome.