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Development of Gold-ISH for sensitive detection of microbial phylogeny with a NanoSIMS ion microprobe

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As yet most of prokaryotes in subseafloor are not culturable, linking prokaryotic phylogeny and metabolic activities at single cell resolution by culture-independent techniques is one of big concerns to understand biogeochemical nutrient cycles. Isotopic or radioisotopic labeling of microorganisms and subsequent phylogenetic identification by in situ hybridization of rRNA-targeted probes can directly link metabolic activity and phylogeny at single cell resolution. After the recent development of a NanoSIMS ion microprobe with high spatial resolution of ˜50 nm, isotope probing studies at single cell resolution are nowadays more popular to understand microbial metabolic activities related to carbon, nitrogen and sulfur metabolisms. Methods for simultaneous isotopic measurements and phylogenetic identification of single microbial cells were reported in 2008 from three different laboratories, and they all used halogen elements due to their high ionization yields and relatively low abundances in biomass. However, halogen-based these techniques still have drawbacks when apply to subseafloor samples, especially with halogen rich samples.

Gold is the one without exception for a SIMS analysis and it shows comparable ionization yield to halogen elements. In addition, gold is also relatively low natural abundance in biomass: it can make lower background signals. Furthermore, gold signals can be enhanced by gold enhancement to achieve high sensitivity. In this study, we focused on undecagold, which is consisted of 11 Au atoms with the diameter of only 0.8 nm. Here we present applicability of undecagold-labeled probes for the identification of single cells by an ion imaging analysis using the JAMSTEC NanoSIMS 50L.

For probe generation, mono-maleimide functionalized undecagold was successfully conjugated with thiol-linked oligonucleotide. After PAGE, more than two bands were observed. The band expected to be undecagolds labeled with single oligonucleotides was cut, purified, and used for in situ hybridization. Oligonucleotide probes were also labeled with Cy3, allowing verification of specific hybridization signals by epifluorescent microscopy before NanoSIMS analysis.

For the proof-of-concept, purely cultivated and 13C enriched E. coli cells and non-enriched M. maripaludis cells were mixed and used for FISH experiment with the EUB338 probe. Specific fluorescent signals were obtained only from E. coli cells and the undecagold-derived Au signals detected by nanoSIMS were identical to 13C signals, indicating only E. coli cells were successfully detected by the undecagold-labeled probe and the method has sufficient sensitivity for NanoSIMS analysis.

Further experiment was conducted using a granular sludge sample. The granular sludge was incubated with 13C-labeled lactate and sulfate to label lactate-utilizer under a sulfate-reducing environment. After hybridizing with the Desulfovibrionales-targeting SRB385 probe, specific FISH signals were obtained from rod-shaped cells. Undecagold-derived Au signals were identical to 13C signals by NanoSIMS analysis, indicating Desulfovibrionales is the main lactate-utilizer in the environment. The signals obtained from undecagod-labeled probes had high signal-to-noise ratio (approximately 10), enabling clear discrimination from background signals. These results indicated that undecagold is stable under the parameters used in this study and can be used for in situ hybridization study with NanoSIMS for ecological understanding in microbial ecology. Gold-ISH may open the door to decipher biogeochemical processes by linking uncultured microbial metabolisms with microbial phylogeny in complex microbial communities.

Keywords: Gold-ISH, NanoSIMS ion microprobe, Undecagold