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Scientific Paper:

Frontiers in Microbiology (2018) Vol. 9, Article 2696

Transcriptome-Stable Isotope Probing Provides Targeted Functional and Taxonomic Insights Into Microaerobic Pollutant-Degrading Aquifer Microbiota

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Abstract:

While most studies using RNA-stable isotope probing (SIP) to date have focused on ribosomal RNA, the detection of ^{13}C-labeled mRNA has rarely been demonstrated. This approach could alleviate some of the major caveats of current non-target environmental "omics". Here, we demonstrate the feasibility of total RNA-SIP in an experiment where hydrocarbon-degrading microbes from a BTEX-contaminated aquifer were studied in microcosms with ^{13}C-labeled toluene under microoxic conditions. From the total sequencing reads (~ 30 mio. reads per density-resolved RNA fraction), an average of 1.2 % of reads per sample were identified as non-rRNA, including mRNA. Members of the *Rhodocyclaceae* (including those related to *Quatrionicoccus* spp.) were most abundant and enriched in ^{13}C-rRNA, while well-known aerobic degraders such as *Pseudomonas* spp. remained unlabeled. Transcripts related to cell motility, secondary metabolite formation and xenobiotics degradation were highly labeled with ^{13}C. mRNA of phenol hydroxylase genes were highly labeled and abundant, while other transcripts of toluene-activation were not detected. Clear labeling of catechol 2,3-dioxygenase transcripts supported previous findings that some of these extradiol dioxygenases were adapted to low oxygen concentrations. We introduce a novel combination of total RNA-SIP with calculation of transcript-specific enrichment factors (EFs) in ^{13}C-RNA, enabling a targeted approach to process-relevant gene expression in complex microbiomes.

Keywords: RNA-seq, RNA-SIP, metatranscriptomics, hydrocarbon degradation, groundwater, dioxygenases