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Deciphering a Cu-dependent metabolism of the *Methylotuvimicrobium alcaliphilum* 20Z^{R} methanotroph by integration of transcriptomics data into a genome-scale metabolic model

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Motivation and Aim: Aerobic methane-oxidizing bacteria or methanotrophs have the unique ability to grow on methane as their sole source of carbon and energy. As a consequence, methanotrophs are ubiquitous in the environment and play a major role in the removal of the greenhouse gas methane from the biosphere [1, 2]. Despite the fact that the main metabolic steps of methane utilization by microorganisms have been identified and well studied to date, a detailed understanding of molecular genetic mechanisms that provide an adaptive response at the level of transcription regulation to various growth conditions, high and low pH, temperature, and salinity is still elusive.
Methods and Algorithms: *Transcriptomics data analysis*
To analyze original transcriptomic data on the growth of *M. alcaliphilum 20Z* ^{R} under various cultivation conditions, obtained as part of a collaboration with Prof. Marina Kalyuzhnaya (San Diego California State University, USA). The modified read sets were aligned to the *M. alcaliphilum* 20Z^{R} reference genome available in the NCBI database (ASM96853v1) using the Bowtie2 algorithm [3]. To quantify gene expression based on reads aligned to the reference genome, the featureCounts package was used [4]. At this stage, we used a genomic annotation built within the MicroScope project (MAGE_000000532.1)[5]. The normalization of the obtained data and the subsequent search for differentially expressed genes were performed using the edgeR package [6].
Development of the genome-scale metabolic model
We also integrated published transcriptome data into the previously developed iIA407 metabolic flow model [7]: gene expression data (FPKM values) under 4 culture conditions: 1) growth on methane in the presence of calcium and copper; 2) growth at low methane concentration in the presence of calcium and copper; 3) growth on methanol in the presence of calcium without copper; 4) growth on methanol in the presence of La [8] were integrated into a mathematical model using the E-Flux2 algorithm [9]. To reproduce the cultivation conditions for 20Z^{R} growth in the absence of Cu, the mathematical model was modified using the CobraPy software [10]. After integrating the transcriptome data into the model, analysis of the flow balance with biomass maximization was performed using the OptFlux program [11] for each of the cultivation conditions.
Results: At the stage of analysis of transcriptomic data sets for 20Z^{R}, it was possible to identify biologically significant changes in expression patterns for the ectoine operon

upon varying the osmolarity of the cultivation medium and for mxa-methanol dehydrogenase as a result of the inclusion or absence of copper in the nutrient medium. Integration of transcriptome data into the previously developed model made it possible not only to more accurately predict growth rates under the cultivation conditions used, but also to reveal quantitative differences in the distribution of fluxes depending on the substrate and the presence of key metals for bacterial growth.

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