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Transcriptional analysis of the functional tfdA gene in Ralstonia eutropha AEO106(pRO101) during MCPA herbicide degradation in natural soil and rhizosphere

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Existing protocols for direct transcription analysis in natural soil and rhizosphere microbial communities have so far been unsatisfactory. In this study, a simultaneous extraction of DNA and RNA from soil followed by hexamer-based construction of cDNA has been optimized successfully to yield a high-quality target for quantitative transcriptional analysis of functional genes and corresponding mRNA by real-time PCR. The quantification of both DNA and mRNA pools to create a normalized mRNA/DNA ratio was found necessary to eliminate the variable soil sample extraction efficiency. The optimized transcription analysis protocol was used to study tfdA expression in Ralstonia eutropha AEO106 (pRO101) in culture and inoculated in MCPA pesticide amended soil. tfdA supports the first step of MCPA degradation resembling that of 2,4-D (cleavage of ether band releasing the acid from the chloro-substituted phenol). The present culture study revealed that tfdA transcription activity had a maximum at MCPA levels of 2 ppm, and was inhibited at concentrations above 10 ppm. Natural soil microcosms were inoculated with 10^7 R.eutropha AEO106 (pRO101) cells g soil-1 and subsequently amended with 2 ppm MCPA to study tfdA transcription activity; mineralization rates for the herbicide was followed by 14CO2 emission from degradation of ring-labeled MCPA. The mRNA/DNA ratio for tfdA demonstrated a transient transcriptional activity from none-to-full expression within the first 2 h after MCPA addition, followed by a decline and complete loss of expression after about 20 h. During the same period, mineralization was highest after the early peak of transcription. Using the present microcosms, transcription activity could be detected using R. eutropha AEO106 (pRO101) inoculum as small as 10^5 cells g soil-1; constant mRNA/DNA values were thus independent of inoculum density over 2-3 logarithmic orders. The present study demonstrates that direct analysis of functional gene expression (transcription activity) can indeed be made in natural soil and rhizosphere, emphasizing the importance of optimized extractions to obtain high-quality nucleotides for PCR reactions and normalization towards the gene copy number (i.e. mRNA/DNA ratio).