

Investigating the redundancy of two Mn oxidases in *Pseudomonas putida* GB-1 in response to Mn(II) concentration

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Microbially-produced manganese oxides (MnOx) are potent solid-phase oxidants and scavengers of trace metal(loid)s, with the ability to significantly impact various geochemical cycles. Despite the importance of MnOx in the environment, the biological Mn oxidation and mineralization mechanisms remain largely unknown. Previous reports have shown that *Pseudomonas putida* GB-1, a known Mn-oxidizing bacterium, is capable of producing multiple Mn-oxidases. However, the functions and benefits of having multiple Mn oxidases have not been investigated. In this work, we examined the influence of aqueous Mn(II) concentrations on the activation of each promoter (P2447 and P2665), responsible for the transcription of the two primary pathways for Mn oxidation in *P. putida* GB-1 (*mnxG* and *mcoA*, respectively). We used fusion reporter technology and epifluorescence microscopy to monitor the activation of two promoters over time in response to a gradient of Mn(II) concentrations ranging from 0 to 500 μ M. Our findings show that both promoters are activated at early stationary phase, with sequential activation of P2447 followed by P2665. Although the average promoter expression remained constant, the number of cells reporting for promoter activity was positively influenced by the presence and increase of Mn(II) concentration. The presence of Mn(II) resulted in the activation of the promoter P2447 in the majority of the population (80% to 95%). However, higher Mn(II) concentrations (>250 μ M) led to an increase in the proportion of cells reporting for the second promoter (P2665) from 40% to 75%. Our results suggest a bistable expression of the Mn oxidizing encoding genes as a response to Mn(II), with an increasing proportion of the population expressing both promoters at high Mn(II) concentrations. The co-expression of both genes resulted in a significant enhancement of Mn(II) oxidation kinetics and subsequent MnOx precipitation. This indicates an additive effect of the two Mn oxidases, where both enzymes work together to increase the rate of MnOx precipitation in response to high Mn(II) concentrations. This study offers new insights into the time-dependent and Mn(II) concentration-dependent activation of MnxG and McoA in *Pseudomonas putida* GB-1.