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Transcriptomic analysis of chloride tolerance in *Leptospirillum ferriphilum* DSM 14647 adapted to NaCl

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Abstract:

Chloride ions are toxic for most acidophilic microorganisms. In this study, the chloride tolerance mechanisms in the acidophilic iron-oxidizing bacterium *Leptospirillum ferriphilum* DSM 14647 adapted to 180 mM NaCl were investigated by a transcriptomic approach. Results showed that 99 genes were differentially expressed in the adapted versus the non-adapted cultures, of which 69 and 30 were significantly up-regulated or down-regulated, respectively. Genes that were up-regulated include carbonic anhydrase, cytochrome *c* oxidase (*ccoN*) and sulfide:quinone reductase (*sqr*), likely involved in intracellular pH regulation. Towards the same end, the cation/proton antiporter CzcA (*czcA*) was down-regulated. Adapted cells showed a higher oxygen consumption rate (2.2×10^{-9} ppm O_2 $s^{-1}cell^{-1}$) than non-adapted cells (1.2×10^{-9} ppm O_2 $s^{-1}cell^{-1}$). Genes coding for the antioxidants flavohemoprotein and cytochrome *c* peroxidase were also up-regulated. Measurements of the intracellular reactive oxygen species (ROS) level revealed that adapted cells had a lower level than non-adapted cells, suggesting that detoxification of ROS could be an important strategy to withstand NaCl. In addition, data analysis revealed the up-regulation of genes for Fe-S cluster biosynthesis (*iscR*), metal reduction (*merA*) and activation of a cellular response mediated by diffusible signal factors (DSFs) and the second messenger c-di-GMP. Several genes related to the synthesis of lipopolysaccharide and peptidoglycan were consistently down-regulated. Unexpectedly, the genes *ectB*, *ectC* and *ectD* involved in the biosynthesis of the compatible solutes (hydroxy)ectoine were also down-regulated. In line with these findings, although hydroxyectoine reached 20 nmol mg^{-1} of wet biomass in non-adapted cells, it was not detected in *L. ferriphilum* adapted to NaCl, suggesting that this canonical osmotic stress response was dispensable for salt adaptation. Differentially expressed transcripts and experimental validations suggest that adaptation to chloride in acidophilic microorganisms involves a multifactorial response that is different from the response in other bacteria studied.

Keywords: chloride ions, *Leptospirillum ferriphilum*, transcriptomic analysis, intracellular pH regulation, oxygen consumption, NaCl adaptation