

## UPREGULATION OF THE *nifHDKEN* OPERON IN *LEPTOSPIRILLUM* SPECIES SUBJECTED TO SOLUBLE NITROGEN STARVATION

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**Background:** Members of the genus *Leptospirillum* routinely dominate bioleaching systems. Understanding their metabolic complexity is necessary for the improvement of bioleaching technologies. Nitrogen is an essential element required for the cellular growth of all microorganisms. In this study *Leptospirillum* species were assessed for their nitrogen fixation capabilities under aerobic and anaerobic conditions, in the absence of soluble ammonia.

**Methods:** *L. ferrooxidans*, *L. ferriphilum* and *L. ferrodiazotrophum* were grown in liquid suspension without soluble nitrogen and maintained under aerobic and anaerobic settings. Culture growth was monitored by cell counting and iron oxidation. Genes selected to study nitrogen fixation were the *nifHDKEN* operon required for nitrogenase production and the *nifSU-hesB* operon for protein assembly. Primers were designed from sequences obtained from the NCBI database and gene expression levels were analysed by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR).

**Results:** All three *Leptospirillum* strains demonstrated an initial decrease in cellular proliferation after soluble nitrogen starvation. Increases in RNA expression levels of the *nifHDKEN* and *nifSU-hesB* operons were detected in *L. ferrooxidans* and *L. ferrodiazotrophum* under both anaerobic and aerobic conditions. *L. ferriphilum* lacked expression of both selected genes.

**Conclusions:** As nitrogen fixation requires a large amount of energy, a decrease in cell growth was to be expected. The increased expression of nitrogen fixing genes after soluble nitrogen starvation indicates that *L. ferrooxidans* and *L. ferrodiazotrophum* can fix atmospheric nitrogen and may provide the first source of fixed nitrogen for other bioleaching micro-organisms.