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IDENTIFICATION AND ANALYSIS OF A NICKEL-INDUCIBLE CATION DIFFUSION FACILITATOR-NICOT COMBINED SYSTEM IN *RHIZOBIUM LEGUMINOSARUM* BV. VICIAE

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Nickel, like other transition metals, can be toxic to cells even at moderate concentration (low microM range) by displacing essential metals from their native binding sites or by generating reactive oxygen species that cause oxidative DNA damage. For this reason, cells have evolved mechanisms to deal with excess nickel. Efflux systems include members of the Resistance-Nodulation-cell Division (RND) protein family, P-type ATPases, cation diffusion facilitators (CDF) and other resistance factors. Nickel-specific exporters have been characterized in *Cupravidus metallidurans*, *Helicobacter pylori*, *Achromobacter xylosoxidans*, *Serratia marcenses* and *Escherichia coli*.

Analysis of *Rhizobium leguminosarum* bv. viciae strain 3841 genome sequence revealed a genetic system (RL1175 through RL1178), potentially involved in nickel homeostasis. The predicted RL1175 gene product shows sequence similarity to cation diffusion facilitator proteins, whereas RL1178 encodes a high-affinity nickel uptake transporter of the NiCoT family (nickel-cobalt transport) and the RL1176 gene product is an NreA-like nickel-responsive regulator. The confluence of uptake and efflux transport proteins potentially linked in the same genetic system resembles the organization of the *ncrABC* and *nreBAC* operons from *Serratia marcenses* and *Legionella pneumophila*, involved in nickel and cobalt resistance. Gene expression analysis using promoter fusions to *lacZ* and qRT-PCR assays revealed that the identified *R. leguminosarum* genes are organized as a single operon whose transcription is strongly induced by nickel, but not cobalt, ions in free-living cultures, as well as in pea bacteroid cells. Generation of mutants affected in this system is in progress and will hopefully help to define the role of the interplay between metal uptake and metal efflux systems in nickel resistance.