



“Gheorghe Asachi” Technical University of Iasi, Romania



EVOLUTIONARY INSIGHTS INTO NICKEL TOLERANCE IN BACTERIA. WHERE DO *NRE* GENES COME FROM?

F. Pini^{1*}, M. Migliore², A. Florio², A. Benedetti², M. Galardini¹, G. Spini¹,
M. Bazzicalupo¹, A. Mengoni¹

¹Department of Evolutionary Biology, University of Florence, via Romana 17, I-50125 Firenze, Italy; ²C.R.A.—Centro di Ricerca per lo Studio delle Relazioni tra Pianta e Suolo, Via della Navicella 2-4, 00184 Rome, Italy; *present address: Institut de Recherche Interdisciplinaire-IRI CNRS USR3078 Parc de la Haute Borne 50 avenue de Halley F-59658 Villeneuve d'Ascq Cedex France

Abstract

Heavy-metal tolerance in bacteria is a widespread phenotype, particularly occurring in strains isolated from heavy-metal contaminated sites, which often are of recent origins. Due to its rapid appearance, it is likely a few genes may confer metal tolerance via a limited number of mutations, and that these genetic determinants conferring heavy-metal resistance could have orthologs present in most of nonresistant strains, which may contributed to some other cellular functions. Concerning nickel tolerance several genetic determinants have been characterized and the *nre* system, which employs a Ni/H⁺ antiporter (NreB), has been found as one of the most diffused, being present in both strains isolated from polluted sites and in soils naturally rich in Ni (serpentine soils). Interestingly, an ortholog of *nreB* gene is also present in the nonresistant legume symbiont strain *Sinorhizobium meliloti* Rm1021 and recent genome sequencing efforts have shown this gene to be present in other *S. meliloti* strains, all of them nonresistant to Ni.

Here, we report a comprehensive phylogenetic analysis of *nreB* genes in all completely sequenced bacterial genomes, showing that this gene was subjected to strong horizontal gene transfer events, possibly mediated via mobile genetic elements, often present in the proximity of its genomic locations. Moreover, a deletion mutant for *nreB* was obtained in *S. meliloti* Rm1021 and shown to be more sensitive to Ni than the parental strain, this deletion mutant was also characterized by Phenotype Microarray analysis and symbiosis with legumes. A strain overexpressing *nreB* under a *lac* inducible promoter was also constructed and compared with a strain overexpressing all the operon *nreAB*. Moreover two *Escherichia coli* strains overexpressing *nreB* of *S. meliloti* and the ortholog from the model heavy-metal resistant strains *Cupriavidus metallidurans* CH34 were also compared, in order to elucidate differences between these two orthologs.

In conclusion *nreB* is probably involved also in other cellular functions than Ni tolerance. Ni-tolerant strains may consequently arise via a facilitated variation evolutionary pathway, explaining their independent appearance in many different heavy-metal rich environments.
