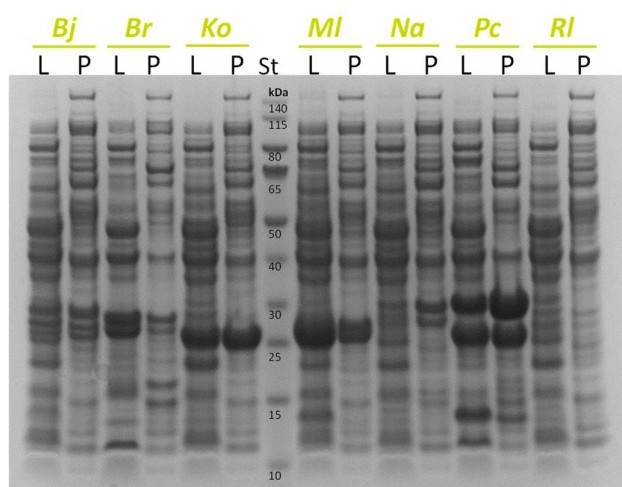


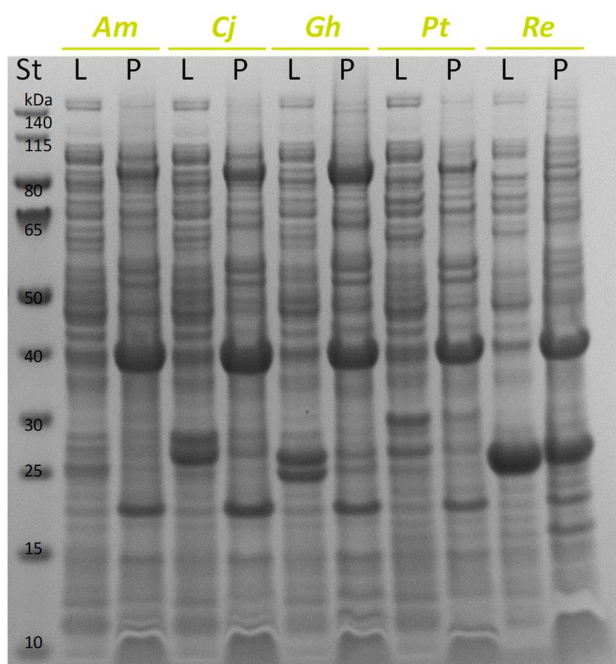
# Functional expression and characterization of a panel of cobalt and iron dependent nitrile hydratases

Birgit Grill, Maximilian Glänzer, Helmut Schwab, Kerstin Steiner, Daniel Pienaar, Dean Brady, Kai Donsbach, and Margit Winkler

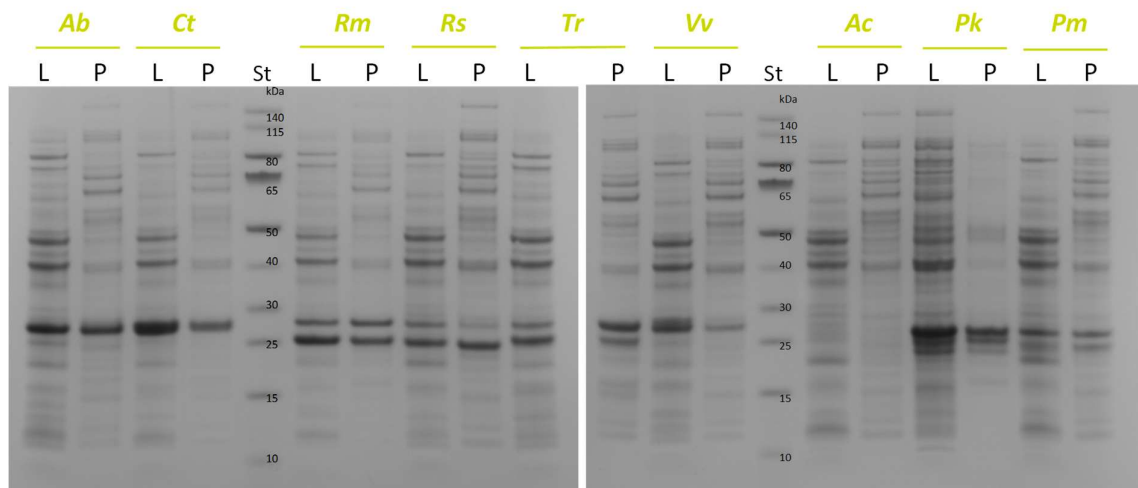
## Supplementary Information



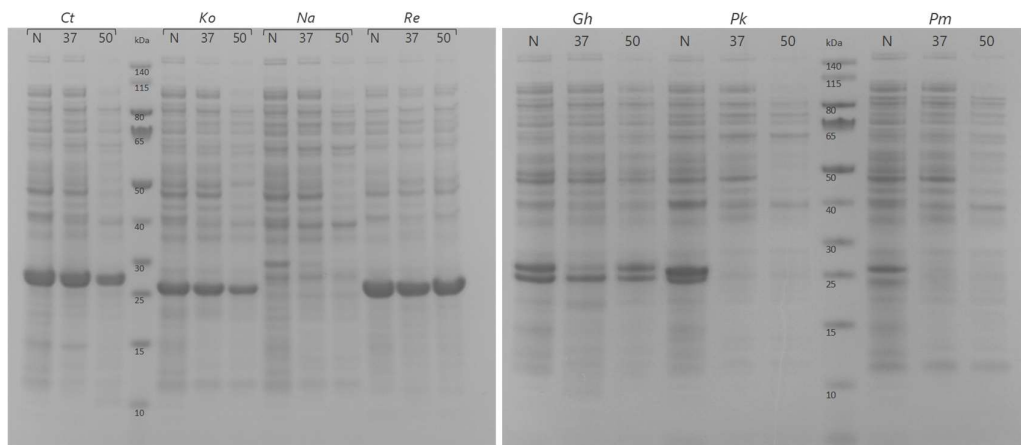
**Figure S1.** SDS-PAGE gel of expression of Co-type NHases. Cell-free lysate (L); insoluble pellet fraction (P). Expected sizes of NHase subunits (in kDa): *Bj*  $\alpha$  26,  $\beta$  25; *Br*  $\alpha$  25,  $\beta$  26.5; *Ko*  $\alpha$  22.5,  $\beta$  24.5; *MI*  $\alpha$  23,  $\beta$  24; *Na*  $\alpha$  24,  $\beta$  26; *Pc*  $\alpha$  25,  $\beta$  27; *RI*  $\alpha$  29,  $\beta$  24. St: PageRuler Prestained Protein Ladder.



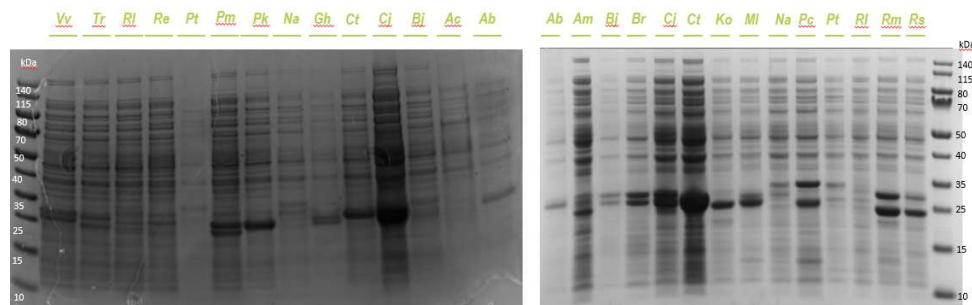
**Figure S2.** SDS-PAGE gel of expression of NHases. Cell-free lysate (L); insoluble pellet fraction (P). Expected sizes of NHases subunits (in kDa): *Am*  $\alpha$  +  $\beta$  24; *Cj*  $\alpha$  +  $\beta$  24; *Gh*  $\alpha$  23,  $\beta$  24; *Pt*  $\alpha$  23,  $\beta$  26.5; *Re*  $\alpha$  23,  $\beta$  23.5. St: PageRuler Prestained Protein Ladder.



**Figure S3.** SDS-PA gel of expression of NHases. Cell-free lysate (L); insoluble pellet fraction (P) Expected sizes of NHase subunits (in kDa): *Ab*  $\alpha$  25,  $\beta$  24; *Ct*  $\alpha$  23,  $\beta$  24; *Rm*  $\alpha$  23,  $\beta$  25; *Rs*  $\alpha$  23,  $\beta$  24; *Tr*  $\alpha$  23,  $\beta$  27; *Vv*  $\alpha$  24,  $\beta$  24; *Ac*  $\alpha$  22,  $\beta$  24; *Pk*  $\alpha$  22,  $\beta$  24; *Pm*  $\alpha$  22,  $\beta$  24; St: PageRuler Prestained Protein Ladder.



**Figure S4.** SDS-PA gel of NHase-CFE incubated at pH 7.2 at 37°C or 50°C overnight compared to non-incubated CFE (N). Expected sizes of NHase subunits (in kDa): *Ct*  $\alpha$  23,  $\beta$  24; *Ko*  $\alpha$  22.5,  $\beta$  24.5; *Na*  $\alpha$  24,  $\beta$  26; *Re*  $\alpha$  23,  $\beta$  23.5; *Gh*  $\alpha$  23,  $\beta$  24; *Pk*  $\alpha$  22,  $\beta$  24; *Pm*  $\alpha$  22,  $\beta$  24; St: PageRuler Prestained Protein Ladder.



**Figure S5.** SDS-PA gel of NHase-CFE cultivated in the presence of 1 mM  $\text{CoCl}_2$  or 2.5 mM  $\text{FeSO}_4$ , respectively. Expected sizes of NHase subunits see Figures above. St: PageRuler Prestained Protein Ladder.