## Supplementary Information

## Regulation of a nickel/cobalt efflux system and nickel homeostasis in a soil actinobacterium *Streptomyces coelicolor*

Hae Mi Kim, Bo-Eun Ahn<sup>‡</sup>, Ju-Hyung Lee, Jung-Hye Roe\*

Laboratory of Molecular Microbiology, School of Biological Sciences, and Institute of Microbiology, Seoul National University, Seoul 151-742, Korea

\* To whom correspondence should be addressed. Tel: 82-2-880-6706; Fax:82-2-882-6706; Email: jhroe@snu.ac.kr

**Fig S1. NmtR binding to the** *nmtA* **promoter region.** Extracts (40  $\mu$ g) from the wild type (M145) and  $\Delta nmtR$  mutant cells were incubated with or without 50  $\mu$ M NiSO<sub>4</sub>, followed by incubation with the SCO6460 promoter DNA fragment to perform electrophoretic mobility shift assay (EMSA) as described in M&M.

Fig. S1



**Fig S2. Concentration-dependent binding of purified NmtR to the** *nmtA* **promoter probe DNA.** (A) Increasing amounts (0.25 ~ 250 nM final concentration) of purified NmtR were incubated with the nmtA promoter DNA fragment for EMSA as described in experimental procedure. (B) Autoradiographic signals were plotted and Kd was estimated by using SigmaPlot 10.0 (SPSS Inc).

Fig. S2

В





Fig S3. Effect of cobalt on the growth and induction of *nmtA* transcription in *S. coelicolor*. (A) The wild type *S. coelicolor* cells were grown in liquid YEME media containing 0, 50, 100, 250, 500, and 1000  $\mu$ M CoCl<sub>2</sub>. Cell growth was monitored by OD<sub>600</sub> after inoculation of spores (4x10<sup>7</sup>) to 200 ml media and incubation at 30°C. (B) S1 mapping analysis of nmtA transcripts. RNA samples were prepared from cells grown to OD<sub>600</sub> of 0.7-0.8 as demonstrated in panel A. Ribosomal RNAs in each sample were shown as a control.







Fig S4. Effect of cobalt on the induction of nickel-regulated genes in *S. coelicolor*. The wild type cells were growth in YEME to  $OD_{600}$  of 0.9, and then treated with  $CoCl_2$  for 40 min at 10 nM to 1 mM. Transcripts from *nmtA*, *sodF* and *nikA* genes were analysed by S1 mapping. Ribosomal RNAs in each sample were shown as a control to indicate the quality of RNA samples.

Fig. S4



**Fig S5. Induction of** *nmt* **genes in** *\Deltanur* **and** *\DeltasodN* **mutants.** The wild type and mutant cells were growth in YEME to OD<sub>600</sub> of 1.0, followed by treatment with 50  $\mu$ M to 1 mM NiSO<sub>4</sub> for 40 min. Transcripts from *nmtA* and *nmtR* genes were analysed by S1 mapping.

Fig. S5

