

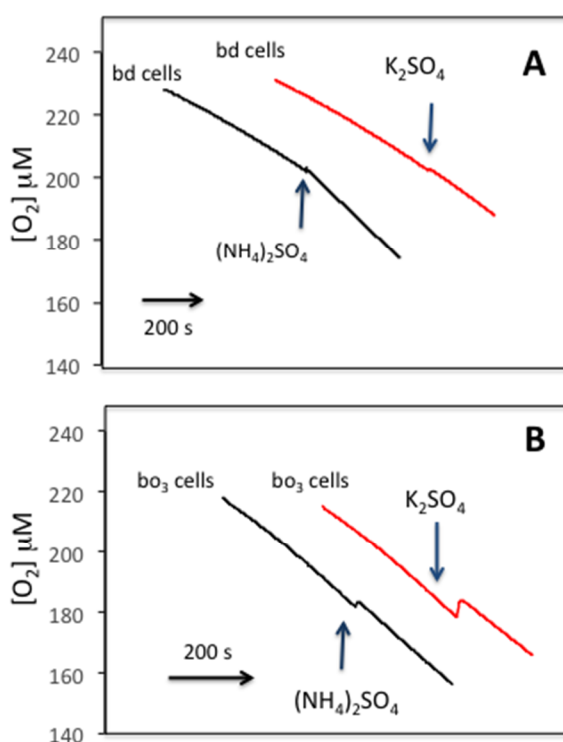
Supplementary Materials for

Article

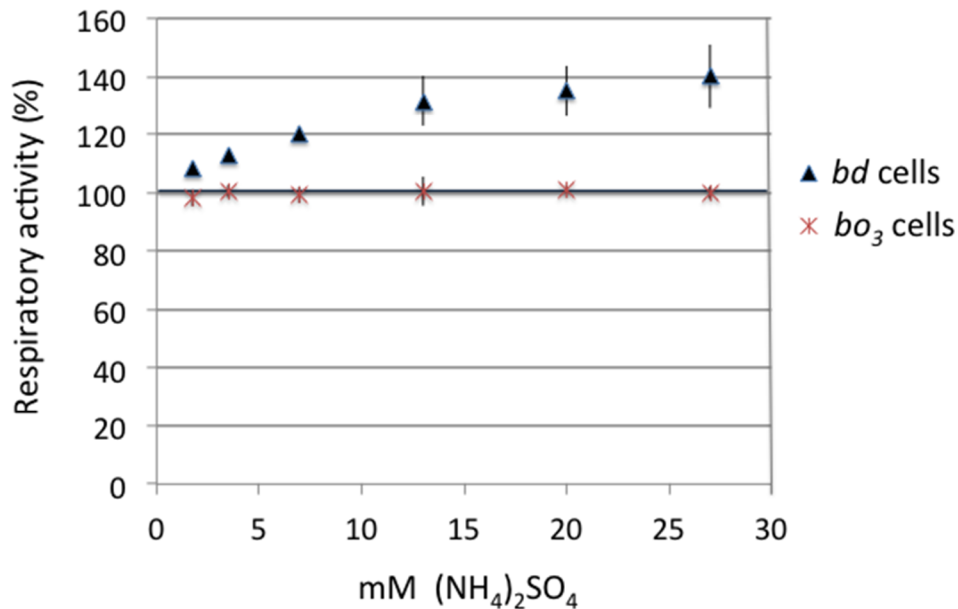
In *Escherichia coli* Ammonia Inhibits Cytochrome *bo*₃ but Activates Cytochrome *bd*-I

Elena Forte, Sergey A. Siletsky and Vitaliy B. Borisov

Supplementary Figures



Supplementary Figure S1. O₂ consumption traces showing the effect of (NH₄)₂SO₄ on the respiration of *E. coli* in respiratory mutants at pH 8.3. **A)** The addition of 13 mM (NH₄)₂SO₄, but not 13 mM K₂SO₄, increases O₂ consumption by cell suspensions of the mutant strain expressing cytochrome *bd*-I as the only terminal oxidase (400 μl cells with OD₆₀₀ = 2.3). **B)** Neither 13 mM (NH₄)₂SO₄ nor 13 mM K₂SO₄ affects respiration in the mutant strain expressing cytochrome *bo*₃ as the sole oxidase (300 μl cells with OD₆₀₀ = 2.15).



Supplementary Figure S2. Effect of (NH₄)₂SO₄ on the respiration of *E. coli* in respiratory mutants. The respiratory activity measured increased after the addition to mutant *E. coli* cells of (NH₄)₂SO₄ at increasing the ligand concentrations ranging from 1.75 mM to 27 mM. Data (mean ± standard deviation, *n* = 3) refer to the control activity measured before the addition of (NH₄)₂SO₄ (taken as 100%). Addition of K₂SO₄ in the same range of concentrations does not affect the respiratory activity of both mutants.

Supplementary Methods

Bacterial strains and growth conditions

The *E. coli* respiratory mutant strains used, derived from the K-12 derivative MG1655 (RKP5416), were TBE025 (MG1655 ΔcydB nuoB appB::kan) and TBE037 (MG1655 ΔappB nuoB cyoB::kan), respectively expressing cytochrome *bo*₃, and *bd*-I as the only terminal oxidase (mutants kindly given by Alex Ter Beek and Joost Teixeira de Mattos, University of Amsterdam).

E. coli cells were grown in 50 mL-Falcon tubes in 5 mL Luria Bertani (LB) medium supplemented with 30 μg/ mL kanamycin at 37 °C and 200 rpm.

Assay conditions

The respirometric assays were performed at 25 °C in 100 mM Tris-phosphate (pH 8.3).