Supporting information

Materials and Methods

Growth media and experimental conditions

All in vitro experiments were performed at 37˚C. O2 deprivation experiments were conducted under anaerobic conditions in a Concept 400 anaerobic incubator (Ruskinn, UK) . Overnight cultures were grown in Lysogeny Broth (LB) medium (the Substrate Department at the Panum Institute, Copenhagen, Denmark). All in vitro experiments were performed in ABT minimal medium (1) supplemented either by 0.5% CAS-amino acids (BD Bacto Casamino Acids, Becton, Dickinson and Company, USA) in growth experiments or 0,5% glucose (the Substrate Department at the Panum Institute, Copenhagen, Denmark) in oxygen deprivation experiments. An ABT minimal medium without any carbon/nitrate source was used in nutrient deprivation experiments.

Measurement of growth and sampling

Bacteria for Measurements of specific growth rate (ln2 hour-1) were cultured in 100 ml ABT minimal media supplemented with 0.5% CAS-amino acids in Erlenmeyer flask on shaker at 180 rpm. Optical densities (OD) at 450 nm of the culture were measured every half hour using a spectrophotometer. Five samples of 5 ml were aliquoted out at different time points within the first five hours after inoculation, and after 24 and 72 hours. 20 µl of each sample was fixated on Super Frost Plus slides (Thermo Scientific, USA) with GN Fixation Solution (AdvanDx, USA) at 65˚C for 20 min. 5 ml of RNAlater (Ambion, USA) was added to the remainder of the sample and stored at 4˚C.

Ribosome degradation under nutrient-limited conditions

Exponential growing cells were washed three times in 37˚C ABT minimal media without any carbon/nitrate sources. The cells were re-suspended in 50 ml 37˚C warm ABT minimal media without any carbon/nitrate. Samples were taken every half hour for the first 5 hours and additionally at 24 and 48 hours. Samples were fixated as described in previous section.

Ribosome degradation under oxygen limited conditions

Oxygen was removed from ABT minimal media by bubbling nitrogen (N2) though the media and subsequently keeping it in an anaerobic atmosphere. The final oxygen concentration in the media was 0.2% of atmospheric concentration. Exponential growing cells were washed three times in 37˚C warm anaerobic ABT minimal media supplemented with 0.5% glucose. The cells were re-suspended in the anaerobic media in airtight vials within an anaerobic chamber containing a gas mixture of 20% CO2 and 80% N2. The vials were incubated on a shaker at 37˚C. A sample vial were taken every half hour and fixated as described in the previous section.

Real Time – Polymerase Chain Reaction (RT-PCR)

To get a molecularly quantitative measurement of the ribosomal content per cell, RT-PCR was used. Primers targeting P. aeruginosa 16s rRNA was used to quantify the number of rRNA molecules, and P. aeruginosa 16s rDNA as a normalization factor. To determine the loss of DNA and RNA during the DNA/RNA purification process, nuclease treatment and cDNA generation, DNA and RNA were analyzed with spike-in internal controls as described by Alhede et al (2). The E. coli 16s rDNA was used as the DNA spike-in and produced by in vitro transcriptional PCR (IVT PCR). An overnight culture of wild type E. coli (MG1655) was lysed and DNA was purified with the Qiagen DNaesy Tissue Kit (Qiagen, Germany) following the manufacturer's instructions. IVT PCR was run on a mixture of 2 µl template DNA, 1 µl 25 mM MgCl2, 4 µl 2.5 mM dNTPs mix, 1 µl 20 µM forward primer (5'-GCT ACA ATG GCG CAT ACA AA- 3'), 1 µl 20 µM reverse primer (5'-TTC ATG GAG TCG AGT TGC AG-3') (3), 0.5 µl tag-polymerase, 5 µl PCR buffer, 35.5 µl MiliQ H2O to a final volume of 50 µl. The PCR program was as follows: 95°C for 2 min, 30 x (95°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 min), 72°C for 5 min, 4°C min. The PCR-product was purified with PCR Clean Kit QiAprep (Qiagen, Germany) according to the manufacturer's instructions. The purified product was run on a 1% agarose gel with 0.001% Etylium bromide to confirm that there was only one band of 101 bp following the PCR reaction. 1 mg ml-1 Promega Luciferase Control RNA (Promega, USA) was used as the RNA control spike-in as described by Johnson et al. (4). The control RNA consisted of 1807 bp, which was confirmed on a 1% agarose gel with 0.001% Etylium bromide.

Simultaneous purification of DNA and RNA from the same samples was conducted by the method described by Griffiths et al 2000 (5) with minor modifications. Cells were lysed by bead beating with a 0,5 mm Glass Bead Tube (Mobio, USA), in suspension of 0.5 ml 10% CTAB extraction buffer and 0.5 ml phenol-chloroform-isoamyl alcohol (25:24:1) (Fluka, Switzerland), beaded at 3000 rpm for 2 x 60 seconds separated by 60 seconds on ice. After lysation, spike-in DNA and RNA were added. Tubes were centrifuged at 16000 g for 5 min. The upper aqueous phase of the supernatant were transferred to a new 2 ml Eppendorf tube and gently mixed with 425 µl of chloroform-isoamyl alcohol (24:1) (Fluka, Switzerland) followed by centrifugation at 16000 g for 5 min. The upper phase were transferred to a new 2 ml Eppendorf tube and mixed with 800 µl 30% PEG with 1 µl glycogen (Applied Biosystem, USA) by pipetting. The mix was incubated for 2 hours on ice, subsequently centrifuged at 16000 g for 30 min. The pellet of nucleic acid appearing after the centrifugation was extracted by removing the supernatant and resuspending the pellet in 50 µl Nuclease-free water. The nucleic acid was further purified by using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The final dilution of nucleic acid from the membrane was done with 52 µl Nuclease-free water, resulting in a product of 50 µl

The resulting flow though was dividend in two parts, one for DNA and one for RNA. The part designated quantification of DNA were added 37.5 µl 1 N NaOH and incubated for 30 min at 65˚C, and subsequently neutralized with 37.5 µl 1 N HCl, bringing the final volume to 100 µl. The part designated quantification of RNA was treated with a mixture of 7.5 µl DNase I (Promega, USA), 5 µl 10x reaction buffer (Promega, USA) and 12.5 µl Nuclease-free water and incubate for 1 hour at

37˚C. The activity of the DNase I was inhibited by 7.5 µl DNase stop solution (Promega, USA). Last 42.5 µl RNase free water were added bringing the final volume to 100 µl.

cDNA for RT-PCR was produced from 1 µg RNA with High Capacity RNA-to-cDNA Master Mix (Applied Biosystems, USA). RT-PCR was conducted on a Step One Plus Thermal Cycler (Applied Biosystems, USA) with Power SYBR Green Master Mix (Applied Biosystems, USA). Ten times standard curves were used for direct quantification from forty cycles following the cycle, 95°C for 15 seconds, 55°C for 30 seconds, and 60°C for 45 seconds. The primers used for P. aeruginosa 16s rDNA and rRNA were: Forward 5'-CAA AAC TAC TGA GCT AGA GTA CG-3' and reverse 5'-TAA GAT CTC AAG GAT CCC AAC GGC T-3 (6). For spike-in lucI RNA: Forward 5'-GTG TTG GGC GCG TTA TTT ATC-3' and reverse 5'-ACT GTT GAG CAA TTC ACG TTC-3'. For spike-in E.coli 16s rDNA: Forward 5'-GCT ACA ATG GCG CAT ACA AA-3' and reverse 5'-TTC ATG GAG TCG AGT TGC AG-3' (3). The loss in spike in RNA and DNA were taking into account to get an exact quantification.

Reference List

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3. Lee C, Lee S, Shin SG, & Hwang S (2008) Real-time PCR determination of rRNA gene copy number: absolute and relative quantification assays with Escherichia coli. Applied microbiology and biotechnology 78(2):371-376.

4. Johnson DR, Lee PK, Holmes VF, & Alvarez-Cohen L (2005) An internal reference technique for accurately quantifying specific mRNAs by real-time PCR with application to the tceA reductive dehalogenase gene. Applied and environmental microbiology 71(7):3866-3871.

5. Griffiths RI, Whiteley AS, O'Donnell AG, & Bailey MJ (2000) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl.Environ.Microbiol. 66(12):5488-5491.

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Figure Legends

Figure S1. A. Change in fluorescence (FU) emitted by P. aeruginosa treated with PNA FISH probes targeting P. aeruginosa 16s rRNA over time when deprived of a carbon source. The data fits a model of Y = 20307 × exp(-0.5885 × X) + 8558 (R2 = 0.93). B. Change in fluorescence (FU) emitted by P. aeruginosa treated with PNA FISH probes targeting P. aeruginosa 16s rRNA over time when O2 is limited to less than 0.2% of the atmospheric concentration. The data fits a model of Y = 24582 × exp(-0.5141 × X) + 8267 (R2 = 0.91).

Figure S1