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Data in Brief

The *Porphyromonas gingivalis* ferric uptake regulator orthologue does not regulate iron homeostasis

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ABSTRACT

Porphyromonas gingivalis is a Gram-negative anaerobic bacterium that has an absolute requirement for iron which it transports from the host as heme and/or Fe^{2+}. Iron transport must be regulated to prevent toxic effects from excess metal in the cell. *P. gingivalis* has one ferric uptake regulator (Fur) orthologue encoded in its genome called Har, which would be expected to regulate the transport and usage of iron within this bacterium. As a gene regulator, inactivation of Har should result in changes in gene expression of several genes compared to the wild-type. This dataset (GEO accession number GSE37099) provides information on expression levels of genes in *P. gingivalis* in the absence of Har. Surprisingly, these genes do not relate to iron homeostasis.

Specifications	
Organism/cell line/tissue	*Porphyromonas gingivalis* ATCC 33277
Sex	
Sequencer or array type	Microarray platform GPL1438
Data format	Raw data: .tar, Analyzed data: SOFT, MINiML, Series Matrix (.txt) files
Experimental factors	Gene expression in bacteria
Experimental features	Gene expression profiles of wild-type and *har* deleted *P. gingivalis* were obtained from RNA extracted from chemostat-grown cells
Consent	
Sample source location	Melbourne Dental School, The University of Melbourne, Melbourne, Australia

1. Direct link to deposited data

Deposited data can be found here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE37099.

2. Experimental design, materials and methods

2.1. Bacterial samples

Porphyromonas gingivalis 33277 wild-type and Har mutant ECR455 [1] were grown in continuous culture under conditions of heme-excess (BHI with 5 μg/mL hemin and 5 μg/mL Vitamin K). Six biological replicate samples for each strain were stabilized with 0.2 volumes of 5% phenol in absolute ethanol, then pelleted by centrifugation and frozen in liquid nitrogen.

2.2. RNA preparation

Total RNA was extracted using TRIzol (Invitrogen) according to the manufacturer's instructions but enhanced with mechanical lysis (Precellys 24 homogenizer — Bertin Technologies, Lysing Matrix B Glass Beads — MP Biomedicals). RNA was further purified using the Illustra RNAspin Mini RNA Isolation kit (GE) according to the manufacturer's instructions including on-column DNase treatment. cDNA was synthesized from 5 μg total RNA using the SuperScript Plus Indirect cDNA labeling system (Invitrogen) primed with 5 μg random hexamers. cDNA was labeled using either the Cy5 or Cy3 post-labeling reactive dye pack (GE) and purified using the purification module of the Invitrogen labeling system.

2.3. Microarray hybridization

P. gingivalis W83 microarray slides version 1 were obtained from the Pathogen Functional Genomics Resource Centre of the J. Craig Venter

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Institute. Paired samples were compared on the same microarray using a two-color system. A total of 6 paired microarray hybridizations were performed representing 6 biological replicates, where a balanced dye design was used, with the overall analyses including three microarrays where *P. gingivalis* 33277 samples were labeled with Cy3 and the paired Har mutant ECR455 samples were labeled with Cy5 and three other microarrays where samples were labeled with the opposite combination of fluorophores. Prior to hybridization, microarray slides were immersed for 1 h in blocking solution (35% formamide, 1% BSA, 0.1% SDS, 5× SSPE [1× SSPE is 150 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA]) at 42 °C. After blocking, slides were briefly washed in H_2O followed by 99% ethanol and then dried by centrifugation. Labeled cDNAs were resuspended in 55 μL of hybridization buffer (35% formamide, 5× SSPE, 0.1% SDS, 0.1 mg/mL Salmon Sperm DNA) and denatured at 95 °C for 5 min, and then applied to slides and covered with LifterSlips (Erie Scientific). Hybridization was performed at 42 °C for 16 h. Following hybridization, slides were successively washed in 0.1% SDS plus 2× SSC [1× SSC is 150 mM NaCl, 15 mM sodium citrate] (5 min at 42 °C, all further washes performed at room temperature), 0.1% SDS plus 0.1× SSC (10 min), 0.1× SSC (4 washes, 1 min each), and then quickly immersed in 0.01× SSC, then 99% ethanol followed by centrifugation to dry the slides. Microarray slides were scanned with a GenePix 4000B microarray scanner (Molecular Devices) at 532 nm (Cy3) and 635 nm (Cy5) with a 10 μm resolution and laser power at 10%. PMT setting adjusted to obtain a 1:1 ratio of Cy3:Cy5. Pictures of both channels were saved as 16-bit tiff files.

2.4. Data normalization and analysis

Image analysis was performed using the GenePix Pro 6.0 software (Molecular Devices), and "morph" background values were used as the background estimates in further analysis. The LIMMA software package [2–4] was used to normalize the within-array data by subtracting the morph background and using Print Tip Loess. Between-array normalization was also carried out for all arrays in the series using the VSN method [5]. To identify differentially expressed genes, the LIMMA software package was used with a cutoff of P values <0.05. Within-array normalization was performed by fitting a global loess curve through the microarray sample pool control spots and applying the curve to all other spots. The Benjamini–Hochberg method was used to control the false discovery rate to correct for multiple testing [6].

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