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Data in Brief

Differential gene expression of *Moraxella catarrhalis* upon exposure to human serum

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ABSTRACT

The complement system is an important part of the innate defense against invading pathogens (Blom et al., 2009; [1]). The ability to resist complement-mediated killing is considered to be an important virulence trait for the human-restricted respiratory tract pathogen *Moraxella catarrhalis*, as most disease-associated *M. catarrhalis* isolates are complement-resistant (Wirth et al., 2007; [2]). Here we provide a detailed overview of the experimental methods that we have used to study the molecular basis of *M. catarrhalis* complement-resistance by transcriptome profiling of the bacterium upon exposure to 10% normal human serum (NHS), associated with the study of de Vries et al. published in Molecular Microbiology in 2014 [3].

Specifications	
Organism/cell line/tissue	*Moraxella catarrhalis* BBH18
Sex	Not applicable
Sequencer or array type	NimbleGen 4 × 72 K custom designed array (GPL17283)
Data format	Analyzed
Experimental factors	Normal human serum (NHS) versus serum dilution buffer
Experimental features	After 1 h exposure of *M. catarrhalis* BBH18 to 10% NHS or the NHS dilution buffer alone, total RNA was isolated and labeled cDNA was generated according to standard Nimblegen gene expression array protocols and hybridized to 4 × 72 K NimbleGen *M. catarrhalis* expression arrays for read-out.
Consent	Not applicable
Sample source location	*M. catarrhalis* strain BBH18 was originally isolated from sputum of a patient with COPD during an exacerbation [4].

Direct link to deposited data

Microarray data is deposited at the NCBI Gene Expression Omnibus (GEO) database under GEO Series accession number GSE52552 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52552).

Experimental design, materials and methods

Exposure of M. catarrhalis BBH18 to normal human serum

To gain insight in the mechanisms of complement resistance in *M. catarrhalis*, the transcriptional response of *M. catarrhalis* strain BBH18, a complement resistant isolate [4,5], upon exposure to 10% pooled normal human serum (NHS) was analyzed by microarray expression profiling. This concentration was chosen because test experiments demonstrated that a *M. catarrhalis* BBH18 gene deletion mutant lacking the key complement resistance factor UspA2H [6] was rapidly killed in 10% NHS (data not shown). To reduce the effect of day to day variation, two fully independent microarray expression profiling experiments were performed, indicated hereafter as experiments A and B (Table 1). *M. catarrhalis* BBH18 was pre-cultured overnight on brain heart infusion (BHI) agar plates at 37 °C in an atmosphere containing 5% CO_2. Bacteria were harvested from plates and resuspended in PBS supplemented with 0.15% gelatin (PBS-G). Next, 5 ml BHI medium was inoculated to an $OD_{600\ nm}$ of ~0.075 and cultured until mid-log phase ($OD_{600\ nm}$ of ~1.0), obtaining cultures with a density of 6×10^7 CFU/ml. Of this culture, 4 ml was harvested by centrifugation, washed in 16 ml PBS-G, and resuspended in 20 ml PBS-G with 0.2 mM $MgCl_2$ and 1 mM $CaCl_2$ (Ca^{2+} and Mg^{2+}). 5.5 ml of this suspension was mixed with 5.5 ml of either PBS-G (Ca^{2+} and Mg^{2+}) (control; experiment A; n = 3, experiment B; n = 2) or with 20% pooled NHS (GTI Diagnostics) in PBS-G (Ca^{2+} and Mg^{2+}) (experiment A; n = 3, experiment B; n = 3) and incubated at 37 °C with agitation (200 rpm). After 0, 30 (experiment A only), and 60 min, a 3.5 ml aliquot was taken, harvested by centrifugation, and immediately treated with 1 ml of RNAprotect Bacteria reagent (Qiagen).

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Table 1
Details of samples used in microarray and RT-qPCR analysis.

Experiment	Treatment	Exposure time (min)	Replicate	Read-out
A	10% NHS	30	1	Microarray and RT-qPCR
A	10% NHS	30	2	Microarray and RT-qPCR
A	10% NHS	30	3	Microarray and RT-qPCR
A	10% NHS	60	1	Microarray
A	10% NHS	60	2	Microarray
A	10% NHS	60	3	Microarray
B	10% NHS	60	1	Microarray and RT-qPCR
B	10% NHS	60	2	Microarray and RT-qPCR
B	10% NHS	60	3	Microarray and RT-qPCR
A	PBS-G	30	1	Microarray and RT-qPCR
A	PBS-G	30	2	Microarray and RT-qPCR
A	PBS-G	30	3	Microarray and RT-qPCR
A	PBS-G	60	1	Microarray
A	PBS-G	60	2	Microarray
A	PBS-G	60	3	Microarray
B	PBS-G	60	1	Microarray and RT-qPCR
B	PBS-G	60	2	Microarray and RT-qPCR

Microarray gene expression analysis

Total RNA was isolated using the RNeasy mini kit (Qiagen) according to manufacturer's instructions. Co-purified genomic DNA was removed by a double DNase treatment using the DNA-free kit (Ambion). Five micrograms of total RNA obtained from samples that were exposed for 60 min to NHS or control conditions was used for microarray expression profiling. The RNA was reverse transcribed and labeled for microarray hybridization according to standard NimbleGen gene expression array protocols. Two micrograms of Cy3-labeled cDNA probes was hybridized overnight at 42 °C to custom-designed NimbleGen *M. catarrhalis* BBH18 expression arrays (4 plex arrays; 72 K) and subsequently washed; all procedures were performed according to manufacturer's instructions. The microarrays harbor 8 probes per predicted coding sequence (CDS). Array images were acquired with a NimbleGen MS200 scanner, and images were first processed with NimbleScan software. Raw expression data (.pair files) were processed using Arraystar (DNASTAR) and normalized using the Quantile RMA method yielding an average signal intensity per CDS. To identify genes differentially expressed in 10% NHS compared to the control condition, statistical analysis was executed using CyberT unpaired two-condition data analysis (http://cybert.ics.uci.edu/). Genes were considered to be differentially expressed when expression was increased or decreased by > 2.5-fold and at a Bonferroni corrected $P < 0.05$. To eliminate genes with no or very low expression from our analysis, we generated frequency plots of $\log_2$ average probe signal intensity per gene; two distributions could be observed (data not shown). Based on these frequency plots we eliminated genes with an average normalized probe signal intensity of $\log_2 < 6$ in all tested conditions from further analysis. Using these criteria we identified 84 genes with a >2.5 fold increased expression and 134 genes that showed a >2.5 fold reduced expression in 10% NHS compared to incubation in PBS-G (NHS dilution buffer). To determine if exposure to 10% NHS resulted in the increased expression of genes grouped in the same functional classes, potential enrichment was analyzed with the Fishers exact (one-tail) test and corrected for multiple testing according to Storey and Tibshirani [7].

Validation of microarray data by RT-qPCR analysis

To validate microarray expression data, real-time quantitative PCR (RT-qPCR) analysis was performed for a selection of 18 genes on samples obtained after 60 min exposure to NHS or PBS-G (experiment B). A sub-selection of 11 genes was tested on samples obtained after 30 min (experiment A) (Table 1). DNA-free total RNA (500 ng) was reverse transcribed into cDNA using the iScript cDNA synthesis kit (Bio-Rad). RT-qPCR was performed in duplicate for each sample using SsoAdvanced SYBR Green Supermix (Bio-Rad) and analyzed on a CFX96 real-time PCR machine (Bio-Rad). Expression fold-changes (NHS versus PBS-G) between the different groupings were calculated using the ΔΔCt method and the *gyrA* gene was used for normalization [8]. Correlation between microarray and RT-qPCR data was assessed with a two-tailed Pearson correlation test using GraphPad Prism software.

Acknowledgments

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