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Data in Brief

Impact of aspirin on the transcriptome of *Streptococcus pneumoniae* D39

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ABSTRACT

Aspirin or acetylsalicylic acid (ASA) is a medicine used to treat pain, fever, and inflammation. Here, we for the very first time reported the genome-wide transcriptional profiling of aspirin-regulated genes in *Streptococcus pneumoniae* in the presence of 5 mM aspirin in chemically-defined medium (CDM) using microarray analysis. Our results showed that expression of several genes was differentially expressed in the presence of aspirin. These genes were further grouped into COG (Clusters of Orthologous Groups) functional categories based on the putative functions of the corresponding proteins. Most of affected genes belong to COG category E (Amino acid transport and metabolism), G (Carbohydrate transport and metabolism), J (Translation, ribosomal structure and biogenesis), and I (Lipid transport and metabolism). Transcriptional profiling data of aspirin-regulated genes was deposited to Gene Expression Omnibus (GEO) database under accession number GSE94514.

Specifications

Organism/cell line/tissue	*Streptococcus pneumoniae* D39
Sex	N/A
Sequencer or array type	Oligo-based DNA microarray
Data format	Raw and processed
Experimental factors	5 mM versus 0 mM Aspirin
Experimental features	Aspirin-dependent gene expression was explored by microarray comparison of *S. pneumoniae* D39 wild-type grown in CDM with 5 mM to 0 mM aspirin
Consent	N/A
Sample source location	Groningen, The Netherlands

1. Direct link to deposited data

The raw and processed DNA microarray dataset are accessible under the following link: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE94514.

2. Experimental design, materials and methods

2.1. Strains used and growth conditions for experiments

S. pneumoniae D39 wild-type strain was used for our experiments in this study. To analyze the effect of aspirin on the transcriptome of *S. pneumoniae*, the D39 wild-type strain was grown at 37 °C in replicates (50 ml each) in CDM with and without 5 mM aspirin and harvested at their respective mid-exponential growth phase.

2.2. RNA extraction, cDNA preparation and hybridization

RNA extraction and cDNA preparation was performed as described before [1]. The concentration of RNA was measured on NanoDrop Spectrophotometer (NanoDrop Technologies, Inc.). Agilent RNA analysis kit (Agilent technologies) was used to determine the quality of RNA. 10–15 μg of RNA was used for cDNA synthesis. DNA purification Kit (NucleoSpin, Gel and PCR clean-up kit) was used to purify the cDNA mixture according to the manufacturer's protocol. cDNA samples were labelled with DyLight-550 and DyLight-650 in dye-swap manner. Hybridization was performed with the labelled cDNA as described before [1]. After 16 h of hybridization at 45 °C, slides were washed with appropriate washing buffers.

2.3. Microarray data analysis

"GenePix Pro 6" software was used to pre-analyze scanned microarray slides as described previously [2]. Raw data files were deposited on GEO and can be accessed via GSE94514 (GSM2477254 and GSM2477255). In-house developed *Microprep* software package was used for further normalization and processing of the data [3]. Statistical analysis were performed as described previously [4]. CyberT implementation of a variant of *t*-test (http://bioinformatics.biol.rug.nl/cybert/index.shtml) was performed and false discovery rates (FDRs) were calculated as described [3]. Bayesian p-value of <0.001, FDR < 0.05 and a fold change cut-off 1.8 was applied to identify differentially expressed genes. Further computational analysis on the data for the regulatory

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networks prediction and data mining was done using different software packages [5,6].

3. Discussion

Here, we investigate the impact of aspirin on the transcriptional profile of *S. pneumoniae* D39. To investigate the impact of aspirin on the transcriptome of *S. pneumoniae* D39, transcriptome of D39 wild-type grown in CDM with 5 mM aspirin was compared to the same strain grown in CDM without aspirin. The list of differentially expressed genes in the presence of aspirin is summarized in Table 1. Expression of many genes was altered in the presence of aspirin. After applying the criteria of, ≥1.8 fold difference as the threshold change and a p-value of <0.001, 51 genes were differentially expressed, of which 13 were upregulated and 38 were downregulated in the presence of aspirin. These genes have been further grouped into COG functional categories according to the putative function of respective proteins (Table 2). Pneumolysin, a key virulence factor produced by *S. pneumoniae*, is downregulated in the presence of aspirin. It is a focal point of the immune response to pneumococci [7] and its downregulation in the presence of aspirin indicates towards its importance as a potential target. A gene cluster putatively encoding chaperones and heat-shock proteins was upregulated in the presence of aspirin. Some genes involved in energy production and conversion were also among the ones upregulated in addition to genes having general function. Some amino acid transport and utilization genes were downregulated. Moreover, fatty acid biosynthesis genes (*fab* genes) were downregulated in the presence of aspirin. *S. pneumoniae* possesses a *fab* gene cluster within the genome coupled with an unusual system for unsaturated fatty acid biosynthesis [8] and enoyl-ACP reduction [8,9]. *fabT* is the second gene in the *fab* cluster and encodes a helix-turn-helix DNA-binding protein belonging to the MarR superfamily of transcriptional regulators that binds to a sequence-specific DNA palindrome present within the two promoters that control *fab* gene expression [10]. The detailed study of regulatory mechanisms and interactions of the *fab* genes in the presence of aspirin is warranted as they are pivotal in maintaining bacterial membrane lipid homeostasis and the potential to exploit these control systems for the development of novel antibacterial therapeutics. We could also observe several ribosomal proteins coding genes downregulated in the presence of aspirin. A couple of genes coding for alcohol dehydrogenases (*spd-1834* encoding an iron-containing dehydrogenase (AdhE) and *spd-1636* encoding a zinc-containing dehydrogenase) were upregulated in the presence of aspirin. *S. pneumoniae* D39 strain is ethanol tolerant and that alcohol upregulates AdhE [11]. Hemolytic activity, colonization, and virulence of *S. pneumoniae*, as well as host cell myeloperoxidase activity, proinflammatory cytokine secretion, and inflammation, were significantly attenuated in D39 Δ*adhE* compared to D39 wild-type [11]. Thus, AdhE appears to be a pneumococcal virulence factor [11]. These differentially expressed genes may provide us valuable targets for drugs and may be potential vaccine candidates.

Table 1
Summary of transcriptome comparison of *S. pneumoniae* D39 wild-type grown in CDM with and without 5 mM aspirin.

D39 tag[a]	Function[b]	Ratio[c]
spd_0775	Hypothetical protein	2.7
spd_1932	Maltodextrin phosphorylase, MalP	2.6
spd_1933	4-alpha-Glucanotransferase, MalQ	2.3
spd_1834	Alcohol dehydrogenase, iron-containing	2.3
spd_0459	Heat shock protein, GrpE	2.2
spd_0460	Chaperone protein, DnaK	2.1
spd_1636	Alcohol dehydrogenase, zinc-containing	2.1
spd_2002	Undecaprenol-phosphate-poly (glycerophosphate subunit) D-alanine transfer protein, DltD	1.9
spd_0868	Protease maturation protein, putative	1.9
spd_0420	Formate acetyltransferase, PflB	1.8
spd_1006	Glucose-1-phosphate adenylyltransferase, GlgC	1.8
spd_1007	Glucose-1-phosphate adenylyltransferase, GlgD	1.8
spd_0927	Neopullulanase, NplT	1.8
spd_0379	Transcriptional regulator, marr family protein	−1.8
spd_0401	Ribosomal protein L28	−1.8
spd_0652	Branched-chain amino acid ABC transporter, amino acid-binding protein, LivJ	−1.8
spd_1726	Pneumolysin, Ply	−1.8
spd_0192	Ribosomal protein S10	−1.8
spd_0380	3-Oxoacyl-(acyl-carrier-protein) synthase III, FabH	−1.8
spd_0409	Threonine dehydratase, IlvA	−1.8
spd_1158	NADP-specific glutamate dehydrogenase, GdhA	−1.9
spd_0646	Hypothetical protein	−1.9
spd_0382	*trans*-2-Enoyl-ACP reductase II, FabK	−1.9
spd_1727	Hypothetical protein	−1.9
spd_0674	Ribosomal protein S16	−1.9
spd_0385	3-Oxoacyl-[acyl-carrier-protein] synthase II, FabF	−1.9
spd_0161	Hypothetical protein	−1.9
spd_0448	Glutamine synthetase, type I, GlnA	−1.9
spd_1370	Ribosomal protein S6	−2
spd_1525	ABC transporter, ATP-binding protein	−2
spd_0383	Malonyl coa-acyl carrier protein transacylase, FabD	−2
spd_0219	Ribosomal protein L17	−2.1
spd_1964	Ribosomal protein L33	−2.2
spd_0334	Oligopeptide ABC transporter, oligopeptide-binding protein, AliA	−2.3
spd_0447	Transcriptional regulator, MerR family protein	−2.3
spd_0251	Ribosomal protein S12	−2.3
spd_0387	Beta-hydroxyacyl-(acyl-carrier-protein) dehydratase, FabZ	−2.3
spd_0388	Acetyl-coa carboxylase, biotin carboxylase, AccC	−2.3
spd_0404	Acetolactate synthase, large subunit, biosynthetic type, IlvB	−2.3
spd_0390	Acetyl-coa carboxylase, carboxyl transferase, alpha subunit, AccA	−2.3
spd_0405	Acetolactate synthase, small subunit, IlvN	−2.4
spd_0408	Hypothetical protein	−2.4
spd_0407	Hypothetical protein	−2.4
spd_1963	Ribosomal protein L32	−2.4
spd_0389	Acetyl-coa carboxylase, carboxyl transferase, beta subunit, AccD	−2.4
spd_0274	Ribosomal protein L13	−2.5
spd_1524	Transcriptional regulator, GntR family protein	−2.6
spd_0386	Acetyl-coa carboxylase, biotin carboxyl carrier protein, AccB	−2.8
spd_0406	Ketol-acid reductoisomerase, IlvC	−2.8
spd_1526	Hypothetical protein	−2.9
spd_0275	Ribosomal protein S9	−3

[a] Gene numbers refer to D39 locus tags.
[b] D39 annotation [12].
[c] Ratio (>1.8 or <−1.8) represents the fold increase/decrease in the expression of genes in the presence of aspirin in CDM.

Table 2
Number of genes significantly affected in D39 wild-type grown in CDM with 5 mM aspirin compared to that grown in CDM without aspirin. Genes affected at least 1.8 fold in the presence of aspirin are shown in COG functional categories.

Functional categories	Total	Up	Down
C: Energy production and conversion	02	02	0
E: Amino acid transport and metabolism	08	01	07
F: Nucleotide transport and metabolism	0	0	0
G: Carbohydrate transport and metabolism	05	05	0
H: Coenzyme transport and metabolism	01	0	01
I: Lipid transport and metabolism	07	0	07
J: Translation, ribosomal structure and biogenesis	10	0	10
K: Transcription	03	0	03
L: Replication, recombination and repair	0	0	0
M: Cell wall/membrane/envelope biogenesis	01	01	0
O: Posttranslational modification, protein turnover, chaperones	03	03	0
P: Inorganic ion transport and metabolism	01	0	01
Q: Secondary metabolites biosynthesis, transport and catabolism	01	0	01
R: General function prediction only	01	0	01
S: Function unknown	01	0	01
T: Signal transduction mechanisms	0	0	0
U: Intracellular trafficking, secretion, and vesicular transport	0	0	0
V: Defense mechanisms	01	0	01
Others	06	01	05
Total number of genes	51	13	38

Conflict of interest

The authors have no conflicts of interest.

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