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.

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**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 Microprop 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]. fabD 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 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 ΔadhE 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 taga | Functionb | Ratioc |
|----------|-----------|--------|
| spd_0775 | Hypothetical protein | 2.7 |
| spd_1932 | Maltoolxidr 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, GroE | 2.2 |
| spd_0460 | Chaperone protein, DnaK | 2.1 |
| spd_1636 | Alcohol dehydrogenase, zinc-containing | 2.1 |
| spd_2002 | Undecaprenol-phosphate-poly (glycerophosphate subunit) | 1.9 |
| spd_0868 | D-alanine transfer protein, DltD | 1.9 |
| spd_0420 | Formate acetyltransferase, PFIb | 1.8 |
| spd_1006 | Glucose-1-phosphate adenyltransferase, GltC | 1.8 |
| spd_1007 | Glucose-1-phosphate adenyltransferase, GltD | 1.8 |
| spd_0927 | Neopullulanase, NplT | 1.8 |
| spd_0979 | 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-Oxooxoc-(acyl-carrier-protein) synthase III, FabH | 1.8 |
| spd_0409 | Threonine dehydratase, FabZ | 1.8 |
| spd_1158 | NADP-specific glutamate dehydrogenase, GdhA | 1.9 |
| spd_0646 | Hypothetical protein | 1.9 |
| spd_0382 | 2-fatty-ACP reductase II, FabK | 1.9 |
| spd_1727 | Hypothetical protein | 1.9 |
| spd_0674 | Ribosomal protein S16 | 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 |

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 | 0 |
| I: Lipid transport and metabolism | 07 0 07 | 0 |
| J: Translation, ribosomal structure and biogenesis | 10 10 | 0 |
| K: Transcription | 03 0 03 | 0 |
| L: Replication, recombination and repair | 0 0 0 | 0 |
| M: Cell wall/membrane/envelope biogenesis | 01 0 01 | 0 |
| O: Posttranslational modification, protein turnover, chaperones | 03 0 03 | 0 |
| P: Inorganic ion transport and metabolism | 01 0 01 | 0 |
| Q: Secondary metabolites biosynthesis, transport and catabolism | 01 0 01 | 0 |
| R: General function prediction only | 01 0 01 | 0 |
| S: Function unknown | 01 0 01 | 0 |
| T: Signal transduction mechanisms | 0 0 0 | 0 |
| U: Intracellular trafficking, secretion, and vesicular transport | 0 0 0 | 0 |
| V: Defense mechanisms | 0 0 0 | 0 |
| Others | 06 0 05 | 0 |

Number of genes 51 13 38

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*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.
Conflict of interest

The authors have no conflicts of interest.

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