Short Report

**Streptococcus pneumoniae** early response genes to human lung epithelial cells

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**Abstract**

**Background:** *Streptococcus pneumoniae* infection starts from colonization of the host respiratory tract where interaction with host respiratory tract epithelial cells occurs. To investigate pneumococcal genes that are involved in the early stage of interaction with host epithelial cells, transcriptional responses of an encapsulated pathogenic pneumococcal strain TIGR4 upon exposure to human lung epithelial cells A549 for 0.5 h and 1 h time periods were investigated by using TIGR (JCVI) microarray technology. Gene expression changes were validated by quantitative real-time PCR (qRT-PCR) analysis.

**Findings:** We observed different transcriptional profiles at two incubation time periods in which most gene expressions were down-regulated at 0.5 h but up-regulated at 1 h. Many genes associated with ribonucleotide biosynthesis were down-regulated at both time points, whereas the genes associated with cell envelope, energy metabolism, transport and protein synthesis were mostly up-regulated at 1 h. Furthermore, these profiles were compared to the transcriptomes of a TIGR4-derived strain in response to human macrophages for the same time periods. We found one set of genes that exhibited similar expression changes upon exposure to both types of host cells, including cell envelope-associated *bgaA* (SP0648) and *nanA* (SP1693), and uncharacterized gene clusters such as SP1677–SP1680 and SP1688–SP1690.

**Conclusion:** These data indicate that at the early stage of interaction with host epithelial cells, a complex gene regulation and expression change occur in bacteria. Some of them might play an essential role during pathogen-host interactions and for the establishment of infection.

**Findings**

**Background**

As a major bacterial pathogen, *Streptococcus pneumoniae* infection starts from colonization of the human upper respiratory tract, causing respiratory tract diseases such as pneumonia, bronchitis, otitis media and sinusitis. Under certain circumstances, bacteria invade host cells and evade host immunity, causing systemic infections such as bacteremia, sepsis and meningitis. Therefore, the interaction of *S. pneumoniae* with host respiratory tract epithelial cells is
an initial step for infection. Many factors that contribute to the colonization and/or invasion of host epithelial cells have been characterized in *S. pneumoniae* (recently reviewed by: [1-3]). However, it is becoming obvious that multiple factors are involved in this complex process [4].

Microarray-based transcriptome studies have been used in many pathogens, investigating their transcriptional responses to host cells [5]. However, they were rarely performed at an early stage of interaction time period, a stage that might be critical for microbes to establish an infection. This is likely due to the difficulty of obtaining sufficient bacterial RNA from a mixture of bacteria and host cells. In *S. pneumoniae*, transcriptome studies were initiated by Orihuela et al. [6] in which an unencapsulated derivative of TIGR4 was investigated following exposure to human pharyngeal epithelial cells (Detroit 562) for 3 h. By using self-spotted pneumococcal oligonucleotide (oligo) microarrays we have also examined gene expression changes of an encapsulated serotype 3 clinical isolate and one unencapsulated avirulent laboratory strain following incubation with human lung epithelial cells (A549) for 1 h and 3 h, respectively [7]. Nevertheless, a lack of information exists regarding pneumococcal gene expression at an early stage of interaction with host cells. The strain-specific gene regulation features of *S. pneumoniae* [8] also prompted our research interests on other serotype strains.

In this study, we have developed a system which can be used to isolate enough bacterial RNA for microarray analysis from encapsulated pathogenic strains following incubation with A549 cells for a short time period. By using TIGR microarrays, we performed transcriptome studies on an encapsulated wild-type strain TIGR4. This study highlighted the gene transcriptional profiles in *S. pneumoniae* and revealed the potential roles of some target genes during pathogen-host interactions.

**Methods**

**Incubation of bacteria and host cells**

Culturing and incubation of pneumococcal strain TIGR4 (provided by Dr. Caroline A. Obert, St. Jude Children’s Research Hospital) and human lung epithelial cells A549 were performed as previously described [7] with minor modifications. Briefly, bacteria grown to early logarithmic-phase at OD$_{600}$ 0.3 were collected by centrifugation, re-suspended in antibiotic-free MEM complete medium supplemented with 1% fetal bovine serum (FBS), and incubated with host cells in T75 flasks at a multiplicity of infection 120:1. After incubation, non-adherent bacteria were removed by washing 3 times with 5 ml of antibiotic-free cell culture medium. Host cells were removed by incubation with a host cell lysis buffer containing guanidine thiocyanate (Sigma), β-mercaptoethanol, phenol and ethanol at room temperature for 10 min. Bacterial samples were collected by centrifugation for RNA isolation. Bacteria incubated with cell culture medium for different time points, treated with RNALater (Ambion), were collected as medium control samples.

**Preparation of bacterial RNA**

Isolation of bacterial RNA was performed with RiboPure™-Bacteria Kit (Ambion) or a modified method using RNeasy MiniKit (Qiagen) as previously described [7]. From each flask of cell infection, about 2–4 μg bacterial total RNA with less than 10% of eukaryotic RNA contamination could be generated. Medium control RNA samples at each incubation time point were generated by pooling RNAs isolated from 3 separate assays. Genomic DNA contamination was removed by the treatment with RNase-free of DNase I (Ambion).

**Microarray experiment and analysis**

TIGR (J. Craig Venter Institute) *S. pneumoniae* 70-mer oligo microarray (version 6), provided by the Pathogen Functional Genomics Resource Center (PFGRC), was used in this study. The cDNA synthesis, Cy-dye labelling, and microarray hybridization were carried out according to TIGR’s standard operating procedures (SOPs) [9]. This includes flagging of marker spots, background correction, printTip Loess normalization with Limma, and statistical analysis with Limma’s eBayes moderated t-test [10]. Gene expressions of fold change ≥ 2.0 (bacteria incubated with host cells vs. bacteria incubated with media) with statistical significance (p ≤ 0.05) were classified as being significantly changed. In this study, eight independent hybridizations, including four labelled in dye flips, using RNA samples isolated from eight separate assays were performed for each incubation time point.

**Quantitative real-time PCR (qRT-PCR) analysis**

The oligo primers used for qRT-PCR analysis (Table 1) were designed from *S. pneumoniae* TIGR4 genome sequences by using Clone Manager Suite 7 (Scientific & Educational Software) and synthesized by Invitrogen. The qRT-PCR reaction and analysis were performed as previously described [7]. For each gene, duplicate reactions were performed on the RNA samples isolated from at least two separate assays for each incubation time point.

**Results and discussion**

**Transcriptional responses of S. pneumoniae to host epithelial cells**

Microarray analysis revealed many gene expression changes following exposure to A549 cells (Table 2). At 0.5
h, most gene expressions were down-regulated (35 vs. 16) and a smaller number of genes changed (Fig. 1). At 1 h, more genes were changed and most of them were up-regulated (50 vs. 25) (Fig. 2). Furthermore, most of those changed genes were only defined at a certain incubation time period (Fig. 3). These data indicate divergent transcriptional profiles between 0.5 h and 1 h incubation time periods. Repressed transcriptional profiles at 0.5 h (Fig. 1) suggest that the interaction with human respiratory tract epithelial cells, a natural reservoir for \textit{S. pneumoniae}, might be a favourable situation for pneumococci. This is in contrast to the \textit{S. pneumoniae} transcriptomes to macrophages, where most genes that showed transcriptional changes at the early stage of interactions were up-regulated (Song XM, Connor W, Hokamp K, Babiuk LA, Potter AA: Transcriptome studies on \textit{Streptococcus pneumoniae}, illustration of early response genes to THP-1 human macrophages, submitted). When incubated for 1 h, bacterial survival, growth and virulence mechanisms appear to be activated, apparent from an induced expression of genes in cell envelope, energy metabolism, transport, protein synthesis, and hypothetical proteins (Fig. 2).

We also observed a common change between two incubation time points, that more than 10 purine and pyrimidine ribonucleotide biosynthesis genes, including purine and pyrimidine regulatory genes purR and pyrR, were consistently down-regulated (Table 2; Figs. 1, 2). The roles of ribonucleotide biosynthesis and their gene regulation mechanism in \textit{S. pneumoniae} are largely unknown. However, down-regulation of these genes in pneumococci appears to occur only at an early stage of interaction with host epithelial cells, but not at 3 h [6,7]. It also might be specific to the pneumococcal strains and the types of host cells because most of those ribonucleotide biosynthesis genes were unchanged in a serotype 3 strain [7] or when the TIGR4-derived strain was exposed to the host macrophages (Song XM, Connor W, Hokamp K, Babiuk LA, Potter AA: Transcriptome studies on \textit{Streptococcus pneumoniae}, illustration of early response genes to THP-1 human macrophages, submitted). Perhaps this is the shift of bacteria to parasitism enabling the uptake of substrates from the host cells [11], or the indication of metabolic changes in different pneumococcal strains in different host environment.

| Table 1: Oligonucleotide primers used for qRT-PCR analysis |
|------------------------------------------------------------|
| **Gene name**  | **TIGR4 genome acc. No.** | **Oligonucleotide primers 5’ to 3’** | **Amplified product (bp)** |
|----------------|--------------------------|--------------------------------------|--------------------------|
| purH           | SP0050                   | Sense: TCAAGCAACCAATGCTTTACGTGAG     | 110                      |
|                |                          | Anti-sense: TCTGCGGATCAGGCTTTAGGA    |                          |
| strH           | SP0057                   | Sense: GTGCTACCGAAGCATGCTTTAGGA      | 128                      |
|                |                          | Anti-sense: GCTGCGGATCAGGCTTTAGGA    |                          |
| cbpI           | SP0069                   | Sense: GCATGCGGATCAGGCTTTAGGA        | 133                      |
|                |                          | Anti-sense: GCTGCGGATCAGGCTTTAGGA    |                          |
| nrrD           | SP0202                   | Sense: TCGAGAAGCATGCTTTAGGA          | 99                       |
|                |                          | Anti-sense: TCGAGAAGCATGCTTTAGGA     |                          |
| caps4A         | SP0287                   | Sense: TCGAGAAGCATGCTTTAGGA          | 103                      |
|                |                          | Anti-sense: TCGAGAAGCATGCTTTAGGA     |                          |
| purR           | SP0346                   | Sense: GTCAGAAGCATGCTTTAGGA          | 159                      |
|                |                          | Anti-sense: CAGAGAAGCATGCTTTAGGA     |                          |
| strH           | SP0648                   | Sense: GCATGCGGATCAGGCTTTAGGA        | 128                      |
|                |                          | Anti-sense: CAGAGAAGCATGCTTTAGGA     |                          |
| gyrA           | SP1219                   | Sense: GCATGCGGATCAGGCTTTAGGA        | 142                      |
|                |                          | Anti-sense: GCATGCGGATCAGGCTTTAGGA   |                          |
| pyrR           | SP1278                   | Sense: GCATGCGGATCAGGCTTTAGGA        | 115                      |
|                |                          | Anti-sense: CAGAGAAGCATGCTTTAGGA     |                          |
| strH           | SP1679                   | Sense: GCATGCGGATCAGGCTTTAGGA        | 149                      |
|                |                          | Anti-sense: CAGAGAAGCATGCTTTAGGA     |                          |
| strH           | SP1680                   | Sense: GCATGCGGATCAGGCTTTAGGA        | 127                      |
|                |                          | Anti-sense: CAGAGAAGCATGCTTTAGGA     |                          |
| strH           | SP1688                   | Sense: GCATGCGGATCAGGCTTTAGGA        | 136                      |
|                |                          | Anti-sense: CAGAGAAGCATGCTTTAGGA     |                          |
| nanA           | SP1693                   | Sense: GCATGCGGATCAGGCTTTAGGA        | 117                      |
|                |                          | Anti-sense: CAGAGAAGCATGCTTTAGGA     |                          |
| purR           | SP1979                   | Sense: GCATGCGGATCAGGCTTTAGGA        | 120                      |
|                |                          | Anti-sense: CAGAGAAGCATGCTTTAGGA     |                          |

* Obtained from [7].
Table 2: Microarray identified genes in pneumococcal strain TIGR4 upon exposure to A549 cells for 0.5 h and 1 h time periods

| Function/gene name | Protein | TIGR4 genome acc. No. | Incubation time |
|--------------------|---------|-----------------------|-----------------|
|                    |         | 0.5 h | 1 h |
| **Cell envelope**  |         |       |     |
| cbpI    | choline binding protein I | SP0069 | 2.8 |
| cps4A   | capsular polysaccharide biosynthesis protein Cps4A | SP0346 | 2.9 |
| cps4B   | capsular polysaccharide biosynthesis protein Cps4B | SP0347 | 2.0 |
| cps4C   | capsular polysaccharide biosynthesis protein Cps4C | SP0348 | 3.3 |
| cps4E   | capsular polysaccharide biosynthesis protein Cps4E | SP0350 | 2.9 |
| bgaA    | l-galactosidase | SP0648 | 17.0 |
| nanA    | neuraminidase A, authentic frameshift | SP1693 | 16.5 |
| **Energy metabolism** |         |       |     |
| agoS    | sugar isomerase domain protein AgaS | SP0065 | 5.6 |
| pyk     | pyruvate kinase | SP0897 | -2.7 |
| glgA    | glycogen synthase | SP1124 | 3.8 |
| zwf     | glucose-6-phosphate 1-dehydrogenase | SP1243 | -2.7 |
| scrB    | sucrose-6-phosphate hydrodrolase | SP1724 | 3.0 |
| galT    | galactose-1-phosphate uridylyltransferase | SP1852 | 2.7 |
| galK    | galactokinase | SP1853 | 2.3 |
| recP    | transketolase | SP2030 | -3.6 |
| arcA    | arginine deiminase | SP2148 | 4.6 |
| gplK    | glycerol kinase | SP2186 | 3.0 |
| **Hypothetical proteins** |         |       |     |
| conserved hypothetical protein | SP0024 | -2.6 |
| hypothetical protein | SP0026 | -2.3 |
| hypothetical protein | SP0052 | -3.5 |
| hypothetical protein | SP0067 | 2.4 |
| conserv hypothetical protein | SP0095 | -2.4 |
| hypothetical protein | SP0159 | -2.3 |
| hypothetical protein | SP0190 | 2.3 |
| conserv hypothetical protein | SP0203 | -2.5 |
| conserv hypothetical protein | SP0207 | -2.1 |
| conserv hypothetical protein | SP0288 | -4.2 |
| conserv hypothetical protein | SP0742 | -2.9 |
| conserv hypothetical protein | SP0951 | 2.4 |
| conserv hypothetical protein | SP1003 | 2.1 |
| hypothetical protein | SP1049 | 2.0 |
| hypothetical protein | SP1059 | 4.4 |
| conserv hypothetical protein | SP1174 | 2.4 |
| hypothetical protein | SP1198 | 2.7 |
| hypothetical protein | SP1199 | 2.9 |
| conserv hypothetical protein | SP1601 | 2.4 |
| hypothetical protein | SP1677 | 10.3 |
| hypothetical protein | SP1678 | 2.9 |
| hypothetical protein | SP1679 | 4.6 |
| conserv hypothetical protein | SP1680 | 5.3 |
| hypothetical protein | SP2183 | 2.7 |
| **Others** |         |       |     |
| bacteriocin, putative | SP0109 | 2.3 |
| lacC    | lactose phosphotransferase system repressor, degenerate | SP0169 | 2.2 |
| dihydropterone synthase | SP0289 | -2.2 |
| acpP    | acyl carrier protein | SP0418 | -2.0 |
| fabF    | 3-oxoacyl-(acyl-carrier-protein) synthase II | SP0422 | -2.4 |
| accD    | acetyl-CoA carboxylase, carboxyl transferase subunit beta | SP0426 | -2.4 |
| accA    | acetyl-CoA carboxylase, carboxyl transferase subunit alpha | SP0427 | -3.4 |
### Table 2: Microarray identified genes in pneumococcal strain TIGR4 upon exposure to A549 cells for 0.5 h and 1 h time periods

| Gene  | Description                                      | Symbol/Number | Log2 Ratio |
|-------|--------------------------------------------------|---------------|------------|
| ilvB  | Acetolactate synthase, large subunit, biosynthetic type | SP0445        | -2.8       |
| zmpB  | Zinc metalloprotease ZmpB                         | SP0664        | -2.1       |
| ilvE  | Branched-chain amino acid aminotransferase        | SP0856        | -2.0       |
| preprotein translocase, SecG subunit, putative | SP0974        | 2.5        |
| asd   | Aspartate-semialdehyde dehydrogenase             | SP1013        | -2.0       |
| bta   | Bacterocin transport accessory protein            | SP1499        | -2.7       |
|       | Transcriptional regulator, MerR family            | SP1856        | 2.0        |
| groEL | Chaperonin, 60 kDa                               | SP1906        | -2.4       |

#### Protein synthesis

| Gene  | Description                                      | Symbol/Number | Log2 Ratio |
|-------|--------------------------------------------------|---------------|------------|
| rpsD  | Ribosomal protein S4                             | SP0085        | 2.7        |
| rpsJ  | Ribosomal protein S10                            | SP0208        | 4.1        |
| rplW  | Ribosomal protein L23                            | SP0211        | 2.9        |
| rpsC  | Ribosomal protein S3                             | SP0215        | 2.0        |
| infA  | Translation initiation factor IF-1               | SP0232        | 2.4        |
| valS  | Valyl-tRNA synthetase                            | SP0568        | -2.1       |
| rplK  | Ribosomal protein L1 I                           | SP0630        | 2.5        |
| infC  | Translation initiation factor IF-3               | SP0959        | 2.5        |
| rpsR  | Ribosomal protein S18                            | SP1539        | 2.8        |
| rpsF  | Ribosomal protein S6                             | SP1541        | 2.9        |
| rpmN  | Ribosomal protein L34                            | SP1993        | 2.4        |
| rpmG  | Ribosomal protein L33                            | SP2135        | 2.1        |
| yfIA  | Ribosomal subunit interface protein              | SP2206        | -3.9       |

#### Purine and pyrimidine ribonucleotide biosynthesis

| Gene  | Description                                      | Symbol/Number | Log2 Ratio |
|-------|--------------------------------------------------|---------------|------------|
| purA  | Adenylosuccinate synthetase                       | SP0019        | -2.5       |
| purC  | Phosphoribosylaminomimidazole-succinocarboxamide synthase | SP0044 | -5.1       |
| purH  | Phosphoribosylaminomimidazolecarboxamide formyltransferase-IMP cyclohydrolase | SP0050 | -15.3      |
| purE  | Phosphoribosylaminomimidazole carboxylase, catalytic subunit | SP0053 | -6.4       |
| purK  | Phosphoribosylaminomimidazole carboxylase, ATPase subunit | SP0054 | -2.4       |
| nrdD  | Anaerobic ribonucleoside-triphosphate reductase   | SP0202        | -4.4       |
| nrdG  | Anaerobic ribonucleoside-triphosphate reductase activating protein | SP0205 | -3.4       |
| thyA  | Thymidylate synthase                             | SP0669        | -2.2       |
| pyrK  | Dihydroorotate dehydrogenase, electron transfer subunit | SP0963 | -3.6       |
| nrdH  | NrdH-redoxin                                     | SP1178        | -2.1       |
| carB  | Carbamoyl-phosphate synthase, large subunit       | SP1275        | -4.2       |
| pyrR  | Pyrimidine operon regulatory protein              | SP1278        | -2.1       |
| guaA  | GMP synthase                                     | SP1445        | -2.4       |
| purR  | Pur operon repressor                             | SP1979        | -2.7       |

#### Transport

| Gene  | Description                                      | Symbol/Number | Log2 Ratio |
|-------|--------------------------------------------------|---------------|------------|
| PTS system, II A component | | SP0064 | 2.2 |
| PTS system, mannose-specific IID component | | SP0282 | -3.7 |
| xanthine-uracil permease family protein | | SP0287 | -8.4 |
| O-antigen transporter RibX, putative | | SP0356 | 2.5 |
| PTS system, IIC component, putative | | SP0647 | 4.3 |
| Sugar ABC transporter, ATP-binding protein | | SP0846 | -2.1 |
| ABC transporter, permease protein | | SP1688 | 5.3 |
| ABC transporter, permease protein | | SP1689 | 2.8 |
| ABC transporter, substrate-binding protein | | SP1690 | 2.1 |
| msmE | Sugar ABC transporter, sugar-binding protein | SP1897 | 2.1 |
| molD | maltodextrin ABC transporter, permease protein | SP2110 | 2.6 |

#### Unknown function

| Gene  | Description                                      | Symbol/Number | Log2 Ratio |
|-------|--------------------------------------------------|---------------|------------|
| vanZ  | Protein, putative                                | SP0049        | -2.9       |
| ACT   | Domain protein                                   | SP0238        | -2.1       |
| HIT   | Family protein                                   | SP0521        | -2.4       |
| gid   | Gid protein                                      | SP0943        | -2.2       |
| flavoprotein | | SP1231 | -2.0 |
| usp4S | Secreted 45 kd protein                           | SP2216        | 2.1        |

*a genes that are also involved in pathogenesis according to TIGR genome annotation*
Microarray data have been deposited in the ArrayExpress microarray database http://www.ebi.ac.uk/arrayexpress under accession No. E-FPMI-15.

Microarray data validation
To confirm gene expression changes identified in microarray analysis, we performed qRT-PCR analysis on 16 selected genes at different incubation time point, most of them associated with cell envelope, ribonucleotide biosynthesis, SP1677-SP1680 and SP1688-SP1690 gene clusters. Except for the unchanged SP1680 at 0.5 h, all the other gene expressions changed in accordance to the microarray data, but at a greater average fold change in the qRT-PCR analysis (Figs. 4, 5). Expression change of SP0057 at 1 h was only obtained from qRT-PCR assay because the strain-specific oligo probes were absent on the microarrays (Fig. 5).

Common response genes to host cells
In a separate transcriptome study, we have investigated gene expression changes of a TIGR4-derived unencapsulated strain following incubation with human THP-1 derived macrophages for different time points (0.5 h, 1 h and 3 h) (Song XM, Connor W, Hokamp K, Babiuk LA, Potter AA: Transcriptome studies on Streptococcus pneumoniae, illustration of early response genes to THP-1 human...
The exoglycosidase family genes

In *S. pneumoniae*, the *bgaA*-encoded β-galactosidase (BgaA) and the *nanA*-encoded neuraminidase (NanA) belong to a family of exoglycosidases exposed on the bacterial surface. Studies have demonstrated that both enzymes, especially NanA, are involved in adherence to host respiratory tract epithelial cells, possibly by clearing host cell surface structures and secreted components to enhance pathogen-host interactions [12-15]. These reports demonstrated the importance of *S. pneumoniae* to deglycosylate human targets during colonization and/or pathogenesis.

In this study, expression of *bgaA* (SP0648) and *nanA* (SP1693) was highly induced when incubated with A549 cells for 1 h in both microarray and qRT-PCR analyses (Table 2; Fig. 4). Further qRT-PCR assay revealed an unchanged expression of *strH* (SP0057) (Fig. 5), correlated to the previous observation that StrH was not involved in the adherence [15]. The enhanced expression of *bgaA* and *nanA* was also observed in a TIGR4-derived strain when exposed to human macrophages for 0.5 h and 1 h time periods (Table 3). It suggests that both *bgaA* and *nanA* belong to a family of conserved early response genes. Clearing host cell surface components and accessing to the host cells are a priority for bacteria at the early stage of pathogen-host interactions.

Other genes

The *cbpi* (SP0069), encoding choline binding protein I, was also up-regulated in expression (Table 2; Fig. 4). The choline binding proteins (CBPs) are a family of surface proteins, many of them are involved in colonization of nasopharynx [16]. However, *cbpi* was the only CBP gene that was identified in this study. The function of CbpI is still unclear but its crystal structure has been solved [17]. Whether it is important in colonization, most CBPs might not be required at the early stage of interaction with host epithelial cells.

Because of strain-specific gene regulations in *S. pneumoniae* [7,8], different microarray technologies and experimental conditions, some potential gene targets might be missed in our transcriptome studies. For example, the *pspC* (SP1417) gene was reported to be up-regulated in a serotype 2 strain D39 within 1 h post-infection in mice [18]. However, expression change of *pspC* was not identified in our assays, despite of a degenerated PspC carried by the TIGR4 genome (TIGR). Another unchanged gene cluster was the *rlrA* pathogenicity islet genes (SP0461-SP0468) encoding pneumococcal pili [19,20]. All of these TIGR4-specific oligo probes were carried by the TIGR microarrays, and they were clearly identified in our studies of the regulation mechanisms for the pilus locus genes (Song XM, Connor W, Hokamp K, Babiuk LA, Potter AA: The growth phase-dependent regulation of the pilus locus genes by two-component system TCS08 in *Streptococcus pneumoniae*, submitted). We could therefore exclude the technical concern for these genes in our microarray analysis. Earlier studies suggested that pneumococcal pili were mainly involved in the host cell adhesion [21]. Recently, Rosch, et al. defined the restricted functions of pili in invasion of host lung epithelial cells [22], suggesting its roles at a late stage of pathogen-host interactions. If this is the...
### Table 3: Common response genes to both A549 cells and THP-1 derived macrophages at 0.5 h and 1 h incubation time periods

| Function/gene name | Protein | TIGR4 genome acc. No. | A549<sup>a</sup> | THP-1<sup>b</sup> |
|--------------------|---------|-----------------------|-----------------|-----------------|
| **Cell envelope**  |         |                       | 0.5 h | 1 h | 0.5 h | 1 h |
| cbpI<sup>c</sup>   |         |                       | 2.8   | 8.4 |       |     |
| bgA<sup>c</sup>    |         |                       | 17.0  | 26.9 |       |     |
| nanA<sup>c</sup>   |         |                       | 16.5  | 3.9 | 47.1  |     |
| **Energy metabolism** |         |                       |       |     |       |     |
| agoS<sup>c</sup>   |         |                       | 5.6   | 10.3|       |     |
| gga<sup>a</sup>    |         |                       | 3.8   | 5.4 |       |     |
| scrb<sup>b</sup>   |         |                       | 2.7   | 4.9 | 6.0   |     |
| gatT                |         |                       | 2.7   | 2.6 | 4.4   |     |
| goIK                |         |                       | 2.3   | 2.8 |       |     |
| recP                |         |                       | 3.0   | 4.1 |       |     |
| **Hypothetical proteins** |       |                       |       |     |       |     |
| hypothetical protein |         |                       | -3.5  | -5.6 | -2.6 | -3.5 |
| hypothetical protein |         |                       | 2.4   | 2.1 |       |     |
| conserved hypothetical protein |       |                       | -2.3  | -2.0|       |     |
| conserved hypothetical protein |       |                       | -2.9  | -6.5 | -3.2 |     |
| conserved hypothetical protein |       |                       | 2.1   | 2.1 | 3.4   |     |
| hypothetical protein |         |                       | 4.4   | 56.3| 16.0  |     |
| conserved hypothetical protein |       |                       | 2.4   | 2.7 |       |     |
| hypothetical protein |         |                       | 2.7   | 2.6 | 2.8   |     |
| hypothetical protein |         |                       | 2.9   | 2.0 | 2.2   |     |
| hypothetical protein |         |                       | 2.9   | 2.0 | 2.2   |     |
| hypothetical protein |         |                       | 10.3  | 14.6|       |     |
| hypothetical protein |         |                       | 2.9   | 6.1 | 6.9   |     |
| hypothetical protein |         |                       | 4.6   | 9.6 | 6.6   |     |
| conserved hypothetical protein |   |                       | 5.3   | 11.5| 2.0   | 14.6|
| **Others**          |         |                       |       |     |       |     |
| lactose phosphotransferase system repressor, degenerate | | SP0169 | 2.2 | 15.4 | 6.0 |
| acpP                |         |                       | -2.0  | -2.3|       |     |
| fabF<sup>b</sup>    |         |                       | -2.4  | -5.1|       |     |
| bta<sup>c</sup>     |         |                       |       |     |       |     |
| **Protein synthesis** |       |                       |       |     |       |     |
| rpsD<sup>c</sup>    |         |                       | 2.7   | 2.4 |       |     |
| rpsJ<sup>c</sup>    |         |                       | 4.1   | 2.9 |       |     |
| rpsC<sup>c</sup>    |         |                       | 2.0   | 2.2 |       |     |
| infC<sup>c</sup>    |         |                       | 2.5   | 2.2 | 2.3   |     |
| rpmI<sup>c</sup>    |         |                       | 3.9   | 2.2 |       |     |
| rpsF<sup>c</sup>    |         |                       | 2.9   | 3.0 | 2.2   |     |
| yflA<sup>c</sup>    |         |                       | -3.9  | -2.6| -2.5  |     |
| **Purine and pyrimidine ribonucleotide biosynthesis** |       |                       |       |     |       |     |
| purC<sup>c</sup>    |         |                       | -5.1  | -4.7 | -2.4 | -7.8 |
| purH<sup>c</sup>    |         |                       | -15.3 | -4.1 | -4.3 | -5.5 |
| purE<sup>c</sup>    |         |                       | -6.4  | -8.5 | -4.2 |     |
| corB<sup>c</sup>    |         |                       | -4.2  | -4.2 |       |     |
| pyrR<sup>c</sup>    |         |                       | -2.1  | -7.8 | -2.3 | -4.4 |
| **Transport**       |         |                       |       |     |       |     |
| PTS system, IIA component |       |                       | 2.2   | 3.4 | 6.5   |     |
| PTS system, mannose-specific IID component | | SP0282 | -3.7 | -2.4|      |     |
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Table 3: Common response genes to both A549 cells and THP-1 derived macrophages at 0.5 h and 1 h incubation time periods

| Gene Name                        | Log2 Ratio | Time 0.5 h | Time 1 h |
|----------------------------------|------------|------------|----------|
| xanthine-uracil permease family  | SP0287     | -8.4       | -5.3     | -2.3     |
| PTS system, IIIC component, putative | SP0647   | 4.3        | 3.5      | 2.2      |
| ABC transporter, permease protein | SP1688    | 5.3        | 2.4      | 1.3      |
| ABC transporter, permease protein | SP1689    | 2.8        | 3.7      | 18.9     |
| ABC transporter, substrate-binding protein | SP1690 | 2.1        | 1.9      | 21.6     |
| msmE                             | SP1897     | 2.1        | 4.1      |
| molD                             | SP2110     | 2.6        | 2.1      | 6.4      |

Unknown function

| Gene Name                        | Log2 Ratio |
|----------------------------------|------------|
| vanZ protein, putative           | SP0049     |
| ACT domain protein               | SP0238     |
| HIT family protein               | SP0521     |
| flavoprotein                     | SP1231     |

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