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Polyamine biosynthesis and transport mechanisms are crucial for fitness and pathogenesis of *Streptococcus pneumoniae*

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Polyamines such as cadaverine, putrescine and spermidine are polycationic molecules that have pleiotropic effects on cells via their interaction with nucleic acids. *Streptococcus pneumoniae* (the pneumococcus) is a Gram-positive pathogen capable of causing pneumonia, septicaemia, otitis media and meningitis. Pneumococci have a polyamine transport operon (*potABCD*) responsible for the binding and transport of putrescine and spermidine, and can synthesize cadaverine and spermidine using their lysine decarboxylase (*cad*) and spermidine synthase (*speE*) enzymes. Previous studies from our laboratory have shown that an increase in PotD expression is seen following exposure to various stresses, while during infection, *potD* inactivation significantly attenuates pneumococcal virulence, and anti-PotD immune responses are protective in mice. In spite of their relative importance, not much is known about the global contribution of polyamine biosynthesis and transport pathways to pneumococcal disease. Mutants deficient in polyamine biosynthesis (Δ*speE* or Δ*cad*) or transport genes (Δ*potABCD*) were constructed and were found to be attenuated in murine models of pneumococcal colonization and pneumonia, either alone or in competition with the wild-type strain. The Δ*speE* mutant was also attenuated during invasive disease, while the *potABCD* and *cad* genes seemed to be dispensable. HPLC analyses showed reduced intracellular polyamine levels in all mutant strains compared with wild-type bacteria. High-throughput proteomic analyses indicated reduced expression of growth, replication and virulence factors in mutant strains. Thus, polyamine biosynthesis and transport mechanisms are intricately linked to the fitness, survival and pathogenesis of the pneumococcus in host microenvironments, and may represent important targets for prophylactic and therapeutic interventions.

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INTRODUCTION

Streptococcus pneumoniae is a Gram-positive pathogen that asymptomatically colonizes the nasopharynx of humans. However, in some cases it can cause pneumonia, septicaemia, otitis media and meningitis (Kadioglu *et al.*, 2008). Two capsular polysaccharide-based vaccines are currently licensed to prevent pneumococcal colonization and disease (Iyer *et al.*, 2005; Iyer & Camilli, 2007). However, these vaccines are ineffective in reducing disease incidence in children and the elderly (Fedson, 1999; Huang *et al.*, 2005). Pneumococci are one of the leading causes of community-acquired pneumonia and otitis media in the USA (Dagan, 2000; File, 2004). Worldwide, the situation is worse, as approximately one million children succumb to pneumococcal disease annually (O'Brien & Nohynek, 2003). The pneumococcus continues to be a serious public health concern, and there is a need for new and improved ways to combat and prevent infections.

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Abbreviations: ∑Xcorr, sum of cross correlations; 2D LC ESI MS/MS, 2D liquid chromatography-electrospray ionization tandem MS; ACN, acetonitrile, CI, competitive index; LR, lactated Ringer's solution; PCT, pressure cycling technology; SAM, *S*-adenosylmethionine; TP, trimethoprim.

Four supplementary tables, showing the results of tandem MS experiments, are available with the online version of this paper.

Significant advances have been made in the identification and characterization of classical bacterial virulence factors such as toxins, capsule, adhesins, secretory systems and immune evasion. However, the contributions of host and microbial metabolism to the establishment and progression of disease have often been underappreciated. Indeed, bacterial metabolic pathways such as the tricarboxylic acid cycle and gluconeogenesis play an important role in pathogenesis (Alteri *et al.*, 2009). Additionally, the availability of key nutrients in the host also modulates the expression of bacterial phenotypes that may affect disease outcome (Somerville & Proctor, 2009). A large proportion of the pneumococcal genome is devoted to basic metabolic functions (Tettelin *et al.*, 2001). This is of particular importance, as it spends most of its life cycle on nutritionally restricted mucosal surfaces, and the acquisition of scarce but essential nutrients represents a critical cellular function. Indeed, pneumococci possess multiple mechanisms for carbohydrate uptake and metabolism that are intricately linked to their pathogenesis (Iyer *et al.*, 2005; Iyer & Camilli, 2007). Transport and biosynthesis of amino acids, manganese and iron have been shown to be important for pneumococcal pathogenesis (Basavanna *et al.*, 2009; Gupta *et al.*, 2009; Nanduri *et al.*, 2008; Rosch *et al.*, 2009; Yesilkaya *et al.*, 2000). A thorough understanding of pneumococcal metabolism is required for designing effective therapeutic and prophylactic strategies.

Polyamines are small polycationic molecules with hydrocarbon backbones and are positively charged at physiological pH (Shah & Swiatlo, 2008). Intracellular polyamine pools are stringently regulated in all organisms, and polyamines are required for optimal cell growth and division (Shah & Swiatlo, 2008). Most bacteria have *de novo* biosynthesis pathways and membrane transporters to satisfy cellular polyamine requirements (Tabor & Tabor, 1985). Cadaverine, putrescine and spermidine are the most common and well-characterized bacterial polyamines (Tabor & Tabor, 1985). Recent reports also suggest that norspermidine (a derivative of spermidine with an extra carbon atom) plays important biological roles in *Vibrio* spp. (Lee *et al.*, 2009). Most research on polyamines in prokaryotes has focused on their effects on transcription and translation by virtue of their interactions with negatively charged nucleic acids. Little is known about the role of polyamines in the physiology and virulence of bacterial pathogens. However, over the last few years reports linking polyamines to cancer, biofilm formation, escape from phagolysosomes, bacteriocin production, toxin activity and stress responses have been published, providing insights about their other important but lesser known functions in bacteria (Shah & Swiatlo, 2008). Functional genomic analyses suggest that pneumococci have a membrane polyamine transporter encoded by *potABCD* (Sp_1386–1389) similar to the polyamine transport operon in *Escherichia coli* that binds and transports putrescine and spermidine (Igarashi *et al.*, 2001; Ware *et al.*, 2005). The pneumococcal chromosome also has annotated lysine decarboxylase (*cad*; Sp_0916) and spermidine synthase (*speE*; Sp_0918) genes, suggesting that it can synthesize cadaverine and spermidine from precursor amino acids. Signature-tagged mutagenesis screens have identified both the *pot* operon and lysine decarboxylase to be vital for pneumococcal disease formation in murine models (Hava & Camilli, 2002; Polissi *et al.*, 1998).

Previous studies from our laboratory have shown that inactivation of *potD* in a mouse-virulent capsular type 3 strain significantly attenuates the progression of disease in systemic and pulmonary murine models, supporting a role for polyamine uptake in pneumococcal pathogenesis (Ware *et al.*, 2006). We have also shown that polyamines may serve as functional analogues for choline molecules during *in vitro* growth, and a significant increase in PotD expression is seen when pneumococci are exposed to environmental stress and during murine septicaemia (Shah *et al.*, 2008; Ware *et al.*, 2005). Additionally, immunization with the surface-exposed PotD protects mice against colonization and lethal pneumococcal infections (Gupta *et al.*, 2009; Shah *et al.*, 2006; Shah & Swiatlo, 2006). Despite their relative importance, not much information is available about the global contribution of polyamines to pneumococcal disease. We hypothesized that genetic deficiencies in the ability to synthesize or transport polyamines would result in profound effects on pneumococcal colonization, invasive disease and stress responses. The data presented in this study show that polyamine biosynthesis and transport loci are conserved across multiple pneumococcal capsular serotypes. Mutant strains deficient in polyamine biosynthesis and transport genes were significantly attenuated in murine models of pneumococcal colonization, pneumonia and invasive infections. Measurement of intracellular polyamine pools and of survival during oxidative and pH stresses, and large-scale proteomic analyses were also performed with the mutant strains. Our data strongly suggest that polyamines are intricately linked to the *in vivo* fitness, pathogenesis and virulence factor expression of the pneumococcus, and may represent promising targets for novel prophylactic and therapeutic interventions against this pathogen.

MATERIAL AND METHODS

Bacterial strains and growth conditions. All experiments were performed with *S. pneumoniae* serotype 4 clinical isolate TIGR4 (Tettelin *et al.*, 2001). Pneumococci were routinely grown in Todd–Hewitt broth with 1 % yeast extract (THY) or on blood agar plates (BAP) at 37 °C in a 5% CO_2 atmosphere. Broth cultures were typically grown to a final OD_{600} of 0.4. All primers used in this study are listed in Table 1.

PCR analyses. PCR was used to examine the distribution of polyamine biosynthesis and transport genes among selected pneumococcal capsular serotypes. Primers were designed on the basis of 5′ and 3′ sequences of *cad* (*cad*F/*cad*R), *potD* (*potD*F/*potD*R) and *speE* (*speE*F/*speE*R) genes (Table 1). Bacterial cultures were statically grown in 2 ml THY medium at 37 °C in 5% CO_2, and harvested during the exponential phase of growth (OD_{600} 0.4) by centrifugation at

Table 1. Sequences of primers used in this study

Primer	Sequence* (5′–3′)
*cad*F	CACCTTGAAAGAGTTAGATCA
*cad*R	TTGACTTTTCTTATAGTTT
*potD*F	CACCATGTTAGATAGTAAAATCAAT
*potD*R	CTTCCGATACATTTTAAACTGTA
*speE*F	CACCATGGATTTATGGTTTTCT
*speE*R	TTTTTTTCCTTCCTCTTCTTCT
0916F1	AGCAAATATAAACCCGAGTAAAAA
0916R1	CAGGTACCGCTTGTGACCTGGAACATC
0916F2	CAGAGCTCGTTTCGGTTTGCGATTTT
0916R2	GATCTTCCGTCCCTTGGAG
1389-86F1	AGCCCCGATCGGTTAATCT
1389-86R1	CAGAGCTCAGAAAGTTTGCGGAT
1389-86F2	CAGGTACCACAGGGAAATATAGCGACC
1389-86R2	TATAAAGGTGCCTATCACCCAAT
0918FI	AAACTTTATATCCTTGTTCATGCAG
0918R1	CAGGTACCCTACTGCCAAAGCCCAA
0918F2	CAGAGCTCTTGCCCAAGTTGCTATTTT
0918R2	ACACCTGGGTCAAAACCAGA

*Underlined sequences are complementary to *S. pneumoniae* TIGR4 chromosomal DNA.

~17 000 ***g*** in an Eppendorf 5810 centrifuge, and pneumococcal chromosomal DNA was isolated with a MasterPure Gram-positive DNA purification kit (Epicenter) following the manufacturer's protocol. PCR was performed with GoTaq DNA polymerase (Promega Biotechnology), an equal concentration of chromosomal DNA was added to each mix and reactions were run for 30 cycles (94 °C, 1 min; 52 °C, 0.5 min; 72 °C, 1.5 min). Amplified products were separated on a 1% agarose gel and visualized following ethidium bromide staining.

Construction of Δ*potABCD*, Δ*cad* and Δ*speE* strains. The TIGR4 *potABCD* operon was replaced with a trimethoprim (TP)-resistance cassette, *tmp*^{r}. The *tmp*^{r} cassette was obtained from the *pkoT* plasmid by digestion with *Kpn*I and *Sac*I enzymes (Adrian *et al.*, 2000). Approximately 500 bp DNA fragments flanking the 5′ and 3′ ends of the *pot* operon were PCR-amplified from TIGR4 genomic DNA using primer pairs 1389-86F1/1389-86R1 and 1389-86F2/1389-86R2 (Table 1). One primer in each pair was designed to incorporate either a *Kpn*I (1389-86F2) or *Sac*I site (1389-86R1). The *tmp*^{r} cassette was ligated to the two PCR products flanking the *pot* operon, and the resulting construct was PCR-amplified using primer pair 1389-86F1 and 1389-86R2, and used for transforming *S. pneumoniae* TIGR4 as described by Lau *et al.* (2002). The double recombination event was selected by plating on BAP containing 50 μg TP ml^{-1}. Construction of *cad* and *speE* mutants was done in a similar manner by replacing the *cad* or *speE* genes with the *tmp*^{r} cassette. Briefly, approximately 500 bp fragments 5′ and 3′ of either *cad* (0916F1-0916R1 and 0916F2-0916R2) or *speE* genes (0918F1-0918R1 and 0918F2-0918R2) were PCR-amplified. One primer in each primer pair had either a *Kpn*I (0916R1 and 0918R1) or the *Sac*I site (0916F2 and 0918F2) (Table 1). The *tmp*^{r} cassette with *Kpn*I- and *Sac*I-generated ends was ligated with PCR fragments with complementary ends. PCR amplification was used for amplification of constructs of the correct size. All constructs were used for transformation of TIGR4 and transformants were selected on BAP with 50 μg TP ml^{-1} (Bricker & Camilli, 1999). PCR and DNA sequencing was performed to confirm the deletion of target genes.

Animal experiments. The institutional animal care and use committee approved all animal studies. All experiments were performed with 8- to 12-week-old CBA/N mice (Jackson Laboratory) (Briles *et al.*, 1982). Pneumococci were grown at 37 °C in 5% CO_2 in THY medium. Cells were harvested by centrifugation during exponential phase, and were resuspended in fresh medium containing 10% (v/v) glycerol and stored at −80 °C. One week prior to infection, stock cultures were thawed, serially diluted in lactated Ringer's solution (LR) and plated on BAP to determine c.f.u. Approximately 4×10^5 viable cells resuspended in 10 μl LR were used for nasopharyngeal colonization experiments (Briles *et al.*, 2003). Five days post-infection animals were killed and nasal cavities were washed with 1000 μl sterile LR, as previously described (Shah *et al.*, 2009). All collected samples were serially diluted and plated on BAP with 4 μg gentamicin ml^{-1} (for the wild-type strain) or 4 μg gentamicin ml^{-1} and 50 μg TP ml^{-1} (for mutant strains). For pneumonia experiments, approximately 4×10^5 cells were resuspended in 40 μl LR and administered intranasaly (i.n.) to anaesthetized mice. Forty-eight hours after infection, mice were euthanized, and lungs were aseptically harvested, homogenized and serially diluted in LR and plated on BAP with either 4 μg gentamicin ml^{-1} or 4 μg gentamicin ml^{-1} and 50 μg TP ml^{-1}. Retro-orbital puncture was used to collect blood from all animals in colonization and pneumonia experiments to assess bacteraemia. For comparison of *in vivo* growth and ability to cause septicaemia, mice were infected intravenously (i.v.) using approximately 1×10^4 cells. Blood was collected at regular intervals post-infection and was plated on BAP with and without TP to enumerate pneumococci. Infected animals were closely monitored and the survival time was recorded. *In vivo* competitive index (CI) experiments for colonization and pneumonia infections were essentially performed as described by Iyer & Camilli (2007). Pre-enumerated stock cultures were thawed, and mutant and wild-type cells were mixed to yield an input ratio of 1 : 1. Plating on media containing TP and regular BAP free of antibiotics was used to distinguish between competing strains. The CI [CI= ($mutant_{output}$/$competitor_{output}$)/($mutant_{input}$/$competitor_{input}$)] was calculated for each animal. A CI of 1 indicates that the mutant and competitor strains colonize to equal levels. A CI <1 indicates that the mutant is outcompeted and has a colonization/lung infection defect.

***In vitro* growth assays.** *S. pneumoniae* mutant and wild-type strains were grown at 37 °C in THY broth following inoculation with approximately 10^6 cells. Exponentially growing cells were diluted in fresh medium to achieve an OD_{600} of approximately 0.04 and OD_{600} readings were taken every 60 min. For enumeration of c.f.u. during growth, equal numbers of pre-enumerated mutant or wild-type cells were inoculated in THY broth at 37 °C, aliquots were taken at periodic intervals, and serial dilutions and plate counts were performed.

Determination of polyamine concentrations. Bacterial cells in exponential phase growth were harvested by centrifugation and washed three times in a glucose citrate buffer [100 mM sodium citrate (pH 5.5), 2% glucose] followed by sterile PBS. Cells were resuspended in 5% $HClO_4$, vigorously vortexed, and centrifuged. The bacterial cell extract solution was adjusted with 8.0 M NaOH to pH 3–4 to precipitate proteins, and the supernatant was removed after centrifugation at 10 000 ***g*** for 15 min at 4 °C. Precipitates were washed two times with 0.1 M $HClO_4$. Polyamines were derivatized with fluorescein-5-isothiocyanate and quantified by the capillary electrophoresis method (Du *et al.*, 2004).

Exposure to oxidative and acid stress. To determine the sensitivity of mutant and wild-type strains to superoxide, exponentially growing cultures in THY broth were treated with 50 mM paraquat, a generator of intracellular superoxide (Hassett *et al.*, 1987). Untreated cultures were used as negative controls. At defined time points, samples were removed from treated and untreated cultures, serially diluted in sterile LR, and plated on BAP to obtain c.f.u. The assay was performed in triplicate. For acid-stress experiments, mutant and wild-type bacteria were inoculated

in THY broth (pH 7.15) and incubated at 37 °C until all cultures reached OD_{600} ~0.4. Cells were then collected by centrifugation, washed in LR and resuspended in sterile THY broth (pH 5.5). Cells were incubated for 3 h at 37 °C. For each strain, c.f.u. were determined pre- and post-exposure.

Proteomic analyses

Cell lysis using pressure cycling technology (PCT). Bacterial cells were processed using a ProteoSolve-SB kit (Pressure Biosciences) followed by lysis by PCT using a Barocycler NEP2017 pressure cycling instrument (Pressure Biosciences). In PCT, samples are subjected to alternating cycles of ambient and high pressure, up to 35 000 p.s.i. (241 500 kPa), resulting in cell lysis (Smejkal *et al.*, 2006).

Trypsin digestion and tandem MS analysis. Proteomic analyses were carried out with proteins isolated from independent triplicate cultures of wild-type and Δ*potABCD* and *speE* mutant strains. Proteins were trypsin-digested and desalted as previously described (Nanduri *et al.*, 2008). Briefly, approximately 20 µg protein was reduced with 5 mM dithiothreitol at 65 °C for 5 min and alkylated with 10 mM iodoacetamide at 30 °C for 30 min. Trypsin digestion was carried out using molecular biology grade porcine trypsin (2 µg; 37 °C, 16 h; 50 : 1 ratio of protein : trypsin; Promega). Tryptic peptides were desalted using a peptide macrotrap (Michrom BioResources), eluted in 0.1% triflouroacetic acid, 95% acetonitrile (ACN) solution, vacuum-dried, and resuspended in 20 µl 0.1% formic acid for 2D liquid chromatography-electrospray ionization tandem MS (2D LC ESI MS/MS). Liquid chromatography (LC) analysis was accomplished by reverse-phase LC coupled directly in-line with an ESI ion trap mass spectrometer (LCQ Deca XP Plus, ThermoElectron). Tryptic peptides were loaded onto a BioBasic C18 reversed-phase column (Thermo 72105-100266) that was equilibrated for 20 min with 5% ACN, 0.1% formic acid. The HPLC flow rate was set at 500 nl min^{-1} and all solvents contained 0.1% formic acid. Peptide separation was achieved with an ACN gradient: 5–25% ACN in 450 min, followed by 25–50% in 130 min, followed by a 20 min wash with 95% ACN and equilibration with 5% ACN for 55 min. Data were collected for 655 min over the duration of the HPLC run using repetitive MS scans immediately followed by three MS/MS scans of the three most intense MS peaks. Dynamic exclusion was enabled with a duration of 2 min with a repeat count of two.

Protein identification. Mass spectra and tandem mass spectra were searched against an *in silico* trypsin-digested protein database for *S. pneumoniae* TIGR4 downloaded from the National Center for Biotechnology Information (NCBI). All searches were done using TurboSEQUEST (Bioworks Browser 3.2, ThermoElectron) (Eng *et al.*, 1994). Cysteine carboxyamidomethylation and methionine single and double oxidation were included in the search criteria. Decoy searches from a reversed version of the *S. pneumoniae* TIGR4 protein database were derived using the reverse database function in Bioworks 3.2. The reversed database was *in silico* trypsin-digested and used for searches with tandem mass spectra as described above. The probability for peptide identification was estimated using a method described for Sequest data analysis and was set at $P \leqslant 0.05$ (Qian *et al.*, 2005). Probabilities of protein identifications being incorrect were calculated using published methods (López-Ferrer *et al.*, 2004; MacCoss *et al.*, 2002). Differential protein expression analysis based on ∑Xcorr was carried out using ProtQuant (Bridges *et al.*, 2007). When comparing two datasets, ProtQuant utilizes tandem mass spectra present at Xcorr values below the user-defined threshold for peptide identification to fill in the missing Xcorr values in a dataset, thus improving the specificity (i.e. decreasing type I errors), provided at least three peptides are identified in the corresponding dataset at a user-defined identification threshold. ProtQuant generated an ANOVA-based *P* value for significant changes in protein expression. The *P* values were corrected for multiple testing using the Benjamini–Hochberg method (Benjamini & Hochberg, 1995), and proteins with an adjusted *P* value of $\leqslant 0.05$ were considered to be significantly differentially expressed. We calculated fold-changes based on ∑Xcorr for proteins that had a significant change in expression using a published method (Old *et al.*, 2005). For protein identifications where ∑Xcorr=0 in a dataset, this method accounts for the discontinuity in identification by including a correction factor. We used a correction factor of 0.5 (Nanduri *et al.*, 2008) for reporting the $\log_2$ ratio of protein abundance.

Statistical analyses. All pneumococcal c.f.u. are reported as $\log_{10}$ values where indicated. Statistical analysis was performed using the GraphPad software program (GraphPad Software). Bacterial counts obtained from the nasopharynx, lungs and blood were compared using Mann–Whitney two-sample rank tests. Data on survival of mice were analysed by using the Kaplan–Meier graph and log rank test. *P* values less than 0.05 were considered to represent significant differences between groups.

RESULTS

Distribution of pneumococcal polyamine biosynthesis and transport genes

Sequence alignment and analyses showed that the pneumococcal Cad protein is similar to other bacterial pyridoxal-dependent decarboxylases. It has a conserved lysine residue that can bind pyridoxal phosphate and decarboxylates lysine to cadaverine (Fig. 1). The amino acid sequence of pneumococcal spermidine synthase has the characteristic glycine-rich aminopropyltransferase motif that catalyses the production of spermidine from putrescine and decarboxylated *S*-adenosylmethionine (SAM) (Fig. 1). Similarly, *S. pneumoniae* PotD has a bacterial spermidine/putrescine-binding motif and is similar to other polyamine ABC transporters (Fig. 1). PCR analyses showed that *cad*, *speE* and *potD* genes were present in 12 different *S. pneumoniae* clinical isolates (Table 2). Additionally, BLAST searches using *cad*, *potABCD* and *speE* sequences as templates showed that these genes were conserved, with more than 99% identity in the genomes of all sequenced pneumococcal isolates (http://strepneumo-sybil.igs.umaryland.edu/).

The polyamine biosynthesis genes *cad* and *speE*, and *potABCD* contribute to pneumococcal nasopharyngeal colonization

In *E. coli*, the lysine decarboxylase gene is co-transcribed with a membrane transporter *cadB* involved in lysine–cadaverine exchange and pH regulation (Soksawatmaekhin *et al.*, 2004). The pneumococcal lysine decarboxylase locus annotated as *cad* is transcribed independently, and this is suggested by the presence of a 5′ promoter and 3′ stem–loop transcriptional terminator element and by transcriptional analyses of wild-type TIGR4 cells grown under laboratory conditions. MEGABLAST searches using the *cadB* sequence from *E. coli* confirmed the absence of a homologous membrane transporter in all sequenced pneumococcal genomes (results not shown). Inter-

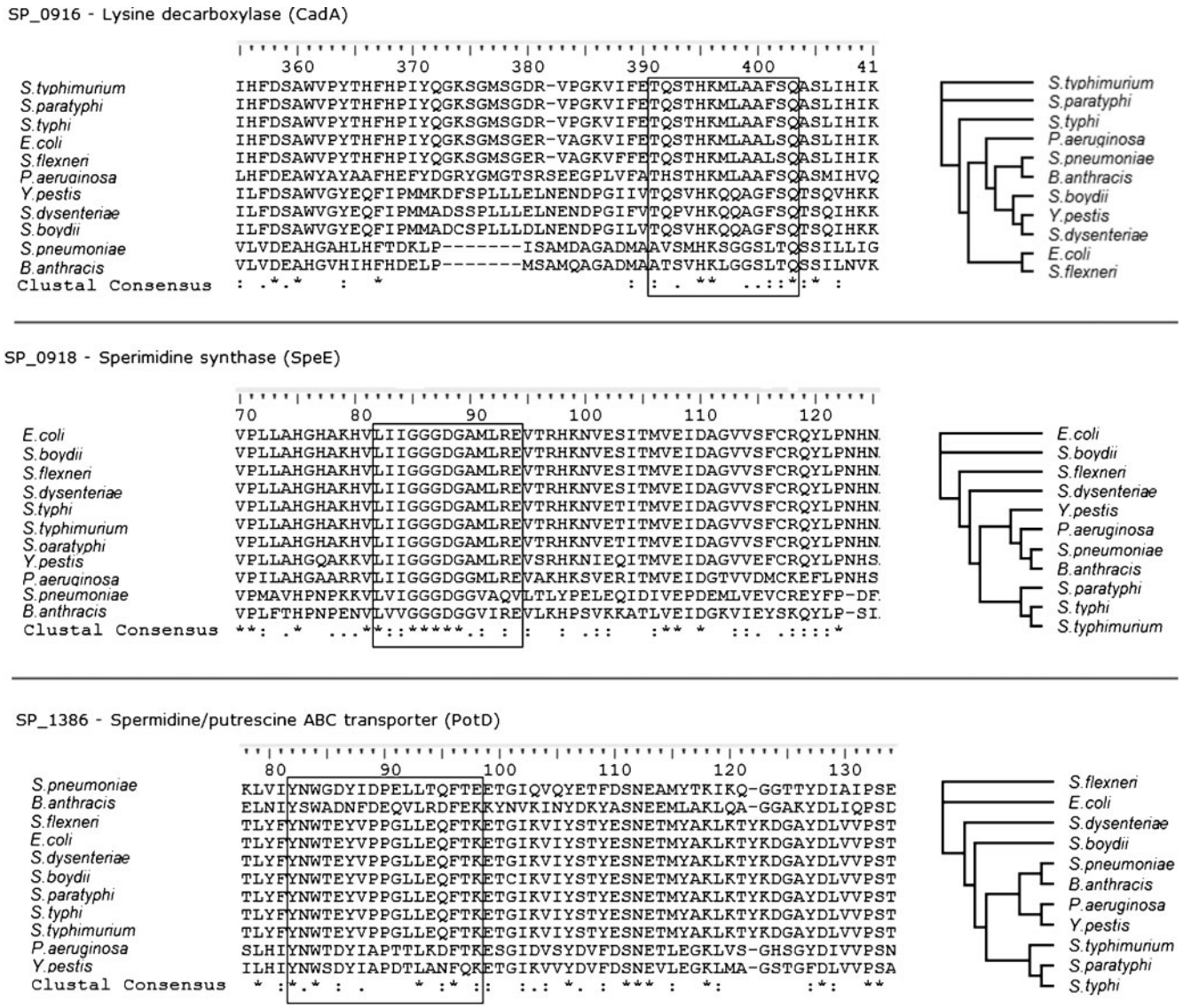

Fig. 1. Sequence alignments of a representative set of polyamine biosynthesis and transport domains. Alignment of the conserved lysine decarboxylase, spermidine synthase and the spermidine-binding domains of PotD from *S. pneumoniae* TIGR4 and other human bacterial pathogens. The boxed sequences in the alignment represent signature motifs in each domain representing either polyamine biosynthesis or transport functions. Asterisk, identical amino acid residues in all sequences; colon, highly conserved amino acids; period, similar amino acids; blank, dissimilar amino acids or gaps in sequences.

estingly, sequence analyses also showed that a *cadB* homologous sequence is absent in other Gram-positive pathogens. These findings suggest that Gram-positive bacteria that do not have lysine–cadaverine antiporters may use cadaverine for novel intracellular functions in addition to pH regulation. The pneumococcal *cad* mutant was significantly attenuated in nasopharyngeal colonization compared with the wild-type strain (Fig. 2a). It was also outcompeted by the isogenic parent during nasopharyngeal colonization in CI experiments (Fig. 2b).

Although the pneumococcal polyamine transporter has been implicated in both pneumonia and murine septicaemia, its role in nasopharyngeal colonization was unknown (Polissi *et al.*, 1998; Ware *et al.*, 2006). Similar to the *cad* deletion strain, a *potABCD* mutant was significantly attenuated in a murine respiratory tract colonization model (Fig. 2a). CI experiments with the mutant strain also showed that the Δ*potABCD* mutant was outcompeted by wild-type TIGR4 during colonization of respiratory mucosal surfaces (Fig. 2b).

Spermidine biosynthesis in Gram-negative bacteria is usually carried out by the *spe* operon, consisting of the *speD* and *speE* genes (Xie *et al.*, 1989). The *speD* locus seems to be absent in all sequenced pneumococcal isolates.

Table 2. Conservation of *cad*, *potD* and *speE* genes among different *S. pneumoniae* capsular serotypes implicated in disease

Strain	Serotype	Patient sex	Age (years)	Sample
AW234	3	F	78	Sputum
TIGR4	4	M	30	Blood
AW61	6A/B	M	53	Sputum
AW130	7F	F	57	Sputum
AW230	9V	M	83	Sputum
SP11-BS70	11	Child	11	NP*
AW315	14	F	41	Sputum
AW207	18	M	58	Sputum
G54	19F	NA†	NA†	Sputum
AW66	23F	M	78	Sputum
AW212	33F	F	69	Sputum
AW205	35B	F	60	Sputum

*Nasopharynx.
†Not available.

Additionally, similar to *cadB*, the *speD* locus is also absent in a large subset of Gram-positive pathogenic bacteria (data not shown). Thus, lysine–cadaverine antiporters and SAM decarboxylases may be non-essential for some Gram-positive bacteria, and other compensatory or novel pathways may account for polyamine biosynthesis. To elucidate the role of spermidine in pneumococcal colonization, an in-frame *speE* deletion mutant was constructed. Similar to *cad* and *potABCD* mutants, the *speE* deletion strain was less fit to colonize the nasophraynx, either alone or in competition with the isogenic parent strain (Fig. 2a, b).

Polyamine biosynthesis and transport loci play an essential role in pneumococcal pneumonia

Deletion of *cad* or the polyamine transporter resulted in severe attenuation of the mutants in a pneumonia model, either alone or in competition with the wild-type strain (Fig. 3). It was also shown for the first time, to our knowledge, that the biosynthesis of spermidine is also a key determinant in pneumococcal pneumonia, as the *speE* deletion mutant was significantly outcompeted by the wild-type parent (Fig. 3).

Role of pneumococcal polyamine biosynthesis and transport during invasive infection

To elucidate the *in vivo* growth kinetics of mutant strains, an i.v. model of murine bacteraemia was used. Fewer mutant strains were recovered from infected mice, suggesting that they could be cleared more rapidly compared with wild-type TIGR4 (Fig. 4a). The mean time to death of mice infected with the *potABCD* and *speE* mutants ($P<0.05$) was longer compared with the wild-type parent (Fig. 4b). The *cad* locus, on the other hand, did not confer a significant survival or growth advantage (Fig. 4a, b).

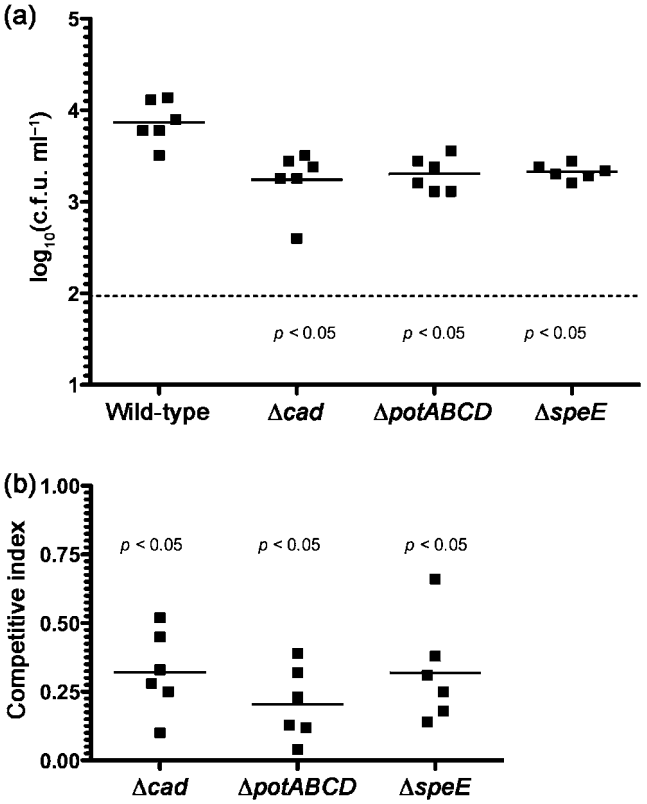

Fig. 2. Lysine decarboxylase, *speE* and the *potABCD* operon contribute to pneumococcal nasopharyngeal colonization. Inactivation of *cad*, *potABCD* or *speE* results in significant attenuation of the ability of the pneumococcus to colonize the murine respiratory tract following infection either alone (a) or in competition with the wild-type parent (b). Data represent $\log_{10}$(c.f.u.) of either the mutant or the wild-type strain (a), or the CI ratios (mutant : wild-type) recovered from the nasopharynx (b) of infected mice ($n=6$ in each group) 5 days post-infection. The dashed line in (a) represents the lower limit of bacterial detection for the colonization experiment.

In vitro growth

Both the *cad* and the *speE* mutant grew similarly to the wild-type TIGR4 *in vitro* (Fig. 5a). The *potABCD* mutant initially grew more slowly but eventually attained OD_{600} values similar to those of the wild-type parent (Fig. 5a). Provision of excess polyamines (0.5 μM) in the growth medium did not affect the growth of the *potABCD* strain (data not shown). Colony counts of all mutant and wild-type cells during growth in THY also showed that all strains had identical growth kinetics *in vitro* either alone (Fig. 5b) or in competition with the wild-type TIGR4 (data not shown).

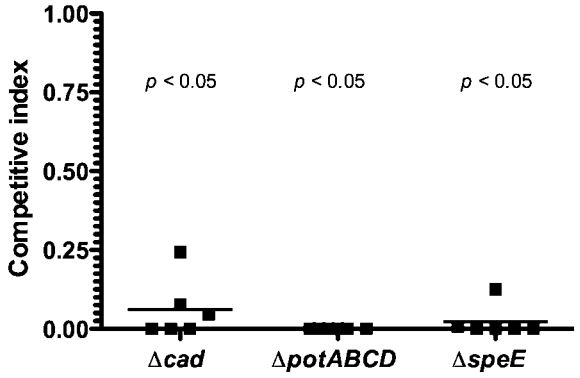

Fig. 3. Polyamine biosynthesis and transport genes are essential for pneumococcal pneumonia formation. Deletion of *cad*, *speE* or the *potABCD* genes results in severe attenuation in the pneumonia model of infection in competition with the wild-type strain. Data represent CI ratios (mutant : wild-type) of the $\log_{10}$(c.f.u.) recovered from lung homogenates of mice (n=6 in each group) 48 h post-infection.

Polyamine measurement

Intracellular polyamine pools of mutant strains were measured and compared with that of the wild-type TIGR4. Similar depletion in cadaverine, putrescine and spermidine levels was seen in all mutant strains (Table 3). Spermidine was the most abundant polyamine in the pneumococcus, followed by cadaverine and putrescine. Indeed, genes responsible for putrescine biosynthesis (*speABC*) are absent in the TIGR4 chromosome, suggesting that most of its intracellular putrescine is acquired from the environment via the polyamine transporter or synthesized by as yet unknown pathways.

Susceptibility to oxidative and pH stress

Polyamines have been implicated in oxidative stress responses in many bacterial species (Chattopadhyay *et al.*, 2003; Ha *et al.*, 1998; Jung & Kim, 2003a, b; Khan *et al.*, 1992). It has previously been shown that environmental polyamine acquisition might play a role in oxidative stress responses, as expression of PotD significantly increases when pneumococci are exposed to sublethal H_2O_2 levels and temperature stress (Shah *et al.*, 2008). Mutants and wild-type cells were exposed to an oxidizing stress-inducing agent (paraquat) and survival was enumerated by plate counts. No significant differences in survival rate were observed between mutant and wild-type cells following paraquat exposure at all time points (Fig. 6b). We further tested our mutant strains by using different concentrations of paraquat and taking earlier time points but saw no difference in survival rates (data not shown). Similarly, when rapidly dividing mutant or wild-type cells were transiently exposed to a low-pH environment, no significant differences in survival were observed (Fig. 6a).

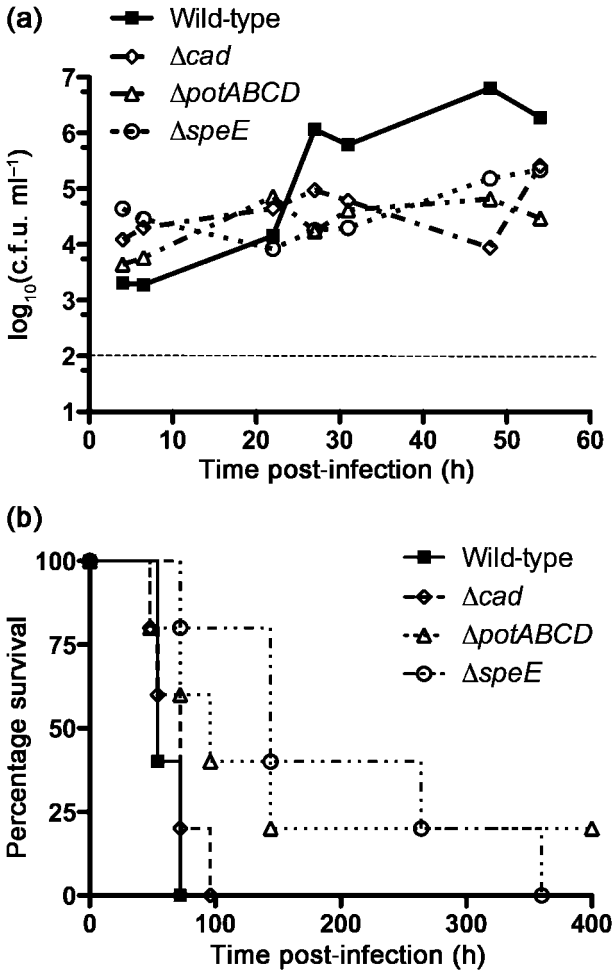

Fig. 4. *S. pneumoniae* polyamine biosynthesis and transport genes affect pneumococcal survival and virulence during septicaemia. (a) Deletions in *cad*, *speE* or the *potABCD* genes affect pneumococcal septicaemia in mice following intravenous challenge with mutant strains. Each data point represents $\log_{10}$(c.f.u.) of either the mutant or the wild-type strain recovered from blood of infected mice at various time points. (b) The median survival of mice intravenously infected with the Δ*speE* (P<0.05) and Δ*potABCD* strains is longer than that of mice infected with wild-type TIGR4. The dashed line in (a) represents the lower limit of bacterial detection for the experiment.

Proteomic analyses

2D LC ESI MS/MS analyses were used to identify proteins that are uniquely or differentially expressed in the Δ*potABCD* and Δ*speE* mutant strains compared with the wild-type TIGR4, as these mutants showed severe attenuation during colonization, pneumonia and invasive infection. Proteomic analyses with the Δ*potABCD* strain and wild-type TIGR4 identified 92 proteins that were detected only in wild-type, while 79 proteins were exclusive to the Δ*potABCD* mutant strain and 359 proteins were common to both datasets (Supplementary Table S1).

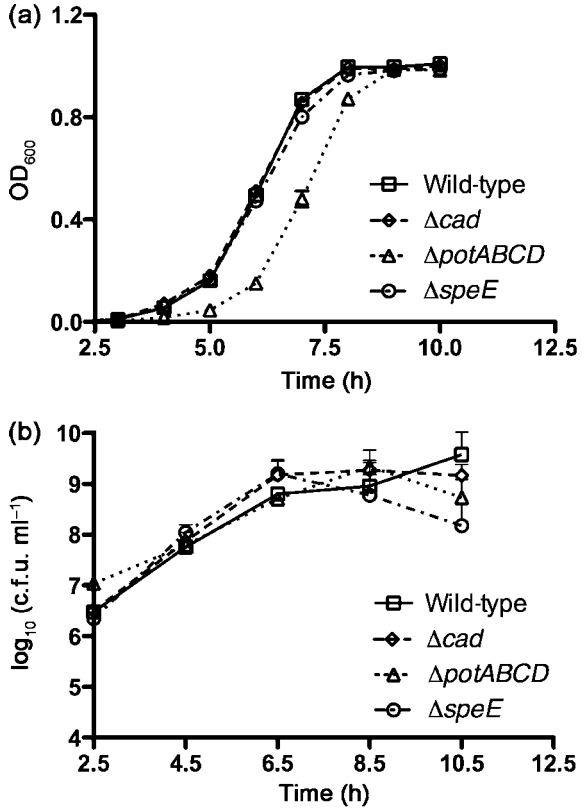

Fig. 5. Growth of wild-type TIGR4 and *cad*, *potABCD* and *speE* deletion strains. (a) OD_{600} measurements and growth curves of wild-type TIGR4 and the *cad*, *potABCD* and *speE* deletion strains in THY medium. (b) Growth rate and c.f.u. of the *cad*, *potABCD* and *speE* deletion strains and wild-type TIGR4 in THY medium. All assays were performed in triplicate and data are presented as mean ± SEM.

Differential expression analysis based on ∑Xcorr showed a significant increase in the expression of approximately 35 proteins, while 76 proteins were downregulated in the Δ*potABCD* mutant strain compared with wild-type TIGR4 (Supplementary Table S2). While 16 proteins showed a significant increase in expression, 23 proteins were downregulated in the Δ*speE* mutant strain compared with wild-type TIGR4 (Supplementary Table S3). Tandem MS analysis with the Δ*speE* mutant strain and wild-type TIGR4 also showed that 124 proteins were unique to the wild-type strain, 96 were detected in the Δ*speE* strain alone, and 535 were common to both datasets (Supplementary Table S4). Differentially expressed proteins in mutant strains included known virulence factors, such as capsular polysaccharide, pneumolysin, zinc metalloprotease, amino acid transporters, and several growth and replication factors, signifying the involvement of polyamines in regulating pneumococcal physiology and pathogenesis. We are currently characterizing pneumococcal transcriptional responses to various polyamines to corroborate the reduced expression of key virulence factors.

Table 3. Polyamine concentrations of wild-type TIGR4 and the Δ*cad*, Δ*potABCD* and Δ*speE* strains grown in THY

Values represent mean results from three independent experiments ± SEM.

Strain	Polyamine	Concentration (pM)
Wild-type	Cadaverine	9.17 ± 1.20
	Putrescine	4.14 ± 2.03
	Spermidine	14.22 ± 1.21
Δ*cad*	Cadaverine	5.59 ± 0.19
	Putrescine	1.64 ± 0.40
	Spermidine	9.60 ± 0.29
Δ*potABCD*	Cadaverine	3.29 ± 1.77
	Putrescine	1.99 ± 1.04
	Spermidine	11.29 ± 0.30
Δ*speE*	Cadaverine	5.95 ± 0.92
	Putrescine	1.74 ± 0.91
	Spermidine	9.12 ± 1.15

DISCUSSION

Bacterial pathogens face diverse environmental stresses during growth in a host and have consequently made multiple adaptations to survive and multiply under difficult conditions. Recently, a growing body of literature has alluded to the role of basic metabolites and carbon metabolic pathways in the overall growth, fitness and virulence of bacterial pathogens (Barelle *et al.*, 2006; Muñoz-Elías & McKinney, 2005; Naderer *et al.*, 2006; Shelburne *et al.*, 2008; Tchawa Yimga *et al.*, 2006). In this study a similar degree of attenuation was seen for both transport and biosynthesis mutants during nasopharyngeal colonization, suggesting that genetic deficiencies affecting the availability of certain polyamines are equally detrimental to successful mucosal colonization. These data are the first demonstration, to our knowledge, that polyamines may be important for the growth and fitness of the pneumococcus on nutritionally restricted mucosal surfaces.

Pneumococcal pneumonia requires prolonged bacterial persistence in the lungs of infected individuals, and most patients succumb to the subsequent massive influx of polymorphonuclear neutrophils and lobar pneumonia in the absence of bacteraemia (McCullers & Tuomanen, 2001). The metabolic requirements governing *S. pneumoniae* persistence during pulmonary infection are crucial for understanding pneumococcal disease. Severe attenuation in a murine model of pneumococcal pneumonia was observed for *potABCD* and *cad* mutant strains in competition with wild-type TIGR4. Additionally, spermidine biosynthesis seems to be equally important during pneumococcal pneumonia. These data strongly suggest that the biosynthesis and acquisition of polyamines are crucial for pneumococcal fitness and disease during lower respiratory tract infections, and represent novel targets for prophylactic or therapeutic interventions.

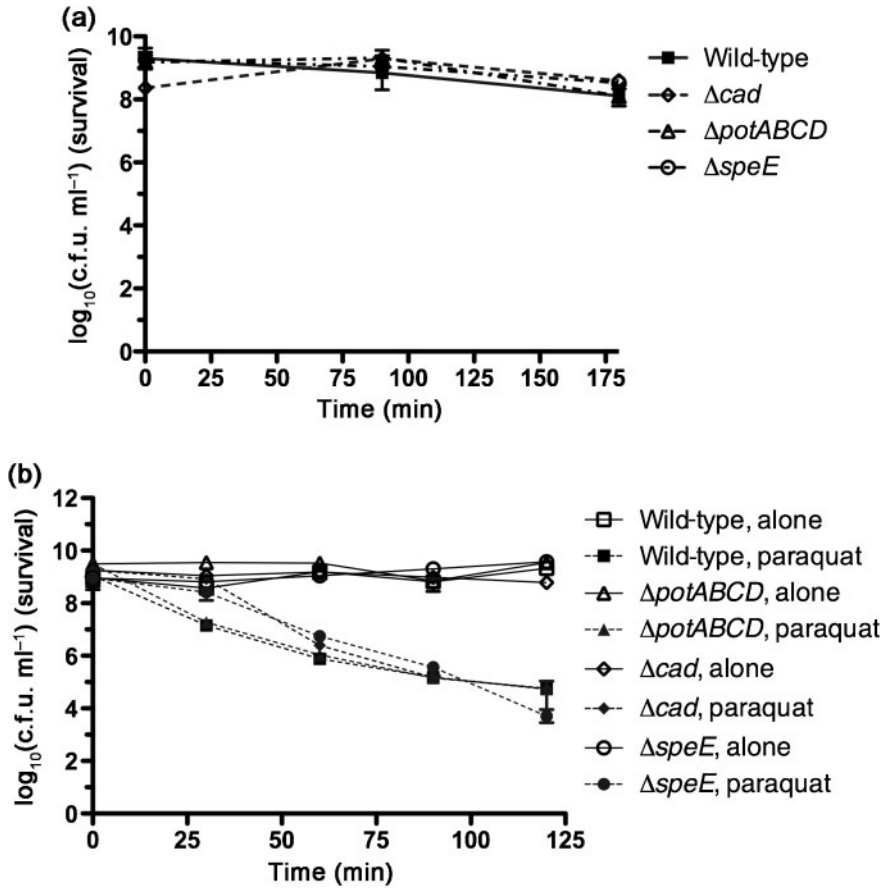

Fig. 6. Stress responses. (a) Wild-type TIGR4 and the *cad*, *potABCD* and *speE* mutant strains were exposed to a low-pH (5.0) environment and post-exposure c.f.u. enumerated. (b) Wild-type TIGR4 and the *cad*, *potABCD* and *speE* mutant strains were incubated with or without 50 mM paraquat, and survival was determined at various time points. All assays were performed in triplicate and data are presented as mean ± SEM.

In septicaemia experiments the time to death of mice infected with either the *potABCD* or the *cad* mutant strain was almost identical to that for wild-type TIGR4, suggesting that these loci are dispensable for the pneumococcus during invasive infections. However, mice infected with the Δ*speE* strain had a median time to death of 144 h compared with 54 h for animals infected with the wild-type parent. These data suggest that the biosynthesis and intracellular availability of spermidine play a role in pneumococcal pathogenesis during invasive infections. The functions of the *cad* and *potABCD* loci may be compensated for by genetic redundancy or other unknown compensatory mechanisms, and hence do not affect disease outcome. It is also possible that spermidine is the key polyamine required by pneumococci during murine septicaemia, and that *cad* (deficient in cadaverine) and *potABCD* (deficient in putrescine) mutants synthesize excess spermidine to compensate for the deficiency of other polyamines. However, the infection of mice with *potABCD* or *cad* mutant strains grown in media with excess spermidine did not result in increased virulence (data not shown), suggesting as yet unknown functions of spermidine during infection. These data suggest that deficiencies in cadaverine biosynthesis and putrescine or spermidine transport have marginal or no effects on pneumococcal growth and virulence during invasive infections. Spermidine biosynthesis, however, may confer certain survival and fitness advantages upon pneumococci and play an adjunctive role during septicaemia.

Overall, the *in vivo* data show that deletion of genes involved in polyamine biosynthesis or transport results in severe attenuation during pneumonia formation, followed by upper respiratory tract colonization, and little or no effect during murine septicaemia. Interestingly, the availability of free polyamines is highest in the blood, followed by the nasal mucosa, and is severely limited in pulmonary tissues (Shah & Swiatlo, 2008). At least two different, although not necessarily mutually exclusive, mechanisms may be responsible for polyamine-mediated pneumococcal disease outcomes. (i) Polyamines may be important nutrients that are indispensable for pneumococcal growth,

replication and persistence during colonization and infection. For example putrescine and spermidine are eventually shuttled into the tricarboxylic acid cycle as carbon and nitrogen sources (Chou *et al.*, 2008). (ii) Deficiencies in polyamines may modulate the expression of key pneumococcal growth and virulence factors that are indispensable for *in vivo* fitness and infection, and result in an attenuated phenotype. Indeed, polyamines regulate the translation of numerous proteins, many of which are key regulators of growth, replication and virulence (Shah & Swiatlo, 2008; Tabor & Tabor, 1985).

We tested both these hypotheses by measuring intracellular polyamine pools of mutant and wild-type bacteria and *in vitro* growth kinetics, and by performing large-scale proteomics with the Δ*potABCD* and Δ*speE* strains, as they showed significant attenuation in all disease models. Capillary electrophoresis analyses showed a reduction in intracellular pools of cadaverine, putrescine and spermidine in all mutant strains compared with the wild-type TIGR4. Depletion of intracellular polyamines, however, does not affect the *in vitro* growth kinetics of mutant strains, suggesting that polyamines are dispensable for growth under routine laboratory conditions in an enriched medium (THY). There are no well-defined minimal media for *S. pneumoniae*, partly due to its complex growth requirements. We tested the growth kinetics of mutant strains in a completely defined medium that provides all essential nutrients in measured amounts and again saw no difference in growth rates (data not shown). These results are similar to the observations made by Chattopadhyay and Tabor, which showed the non-essentiality of polyamines for aerobic growth of *E. coli* (Chattopadhyay *et al.*, 2009). Large-scale proteomic analyses with the *potABCD* mutant revealed a significant decrease in the expression of oligopeptide and amino acid ABC transporters involved in pathogenesis, as well as several well-characterized virulence factors such as capsular polysaccharide biosynthesis proteins, pneumolysin and pneumococcal surface protein A (Kadioglu *et al.*, 2008). Decreased expression of several proteins involved in growth and replication was also seen in the polyamine transport-deficient strain. Tandem MS analysis with the *speE* mutant strain also showed a reduction in the expression of important virulence factors such as oligopeptide and amino acid ABC transporters, zinc metalloprotease ZmpB and choline-binding protein PcpA. Similar to the Δ*potABCD* strain, a decrease in the expression of several growth and cell division proteins, such as DivIVA, MreC and FtsX, was also seen in the spermidine synthase mutant strain. Interestingly, the *speE* mutant strain showed increased lysine decarboxylase expression. It is possible that increased cadaverine production compensates for spermidine deficiency during infection and may be a counteracting mechanism. These results suggest that polyamines regulate the expression of proteins required for pneumococcal replication as well as virulence. The attenuated phenotype of the mutant strains could be a collective outcome of reduced expression of proteins responsible for regulating pneumococcal growth, replication and/or virulence, with the exact contribution of each group of proteins probably dependent on the host micro-environment and the level of polyamines in it.

Polyamines have often been implicated in the transcriptional and translational control of genes and transcripts involved in stress responses. However, all mutants had survival rates similar to that of the wild-type strain following paraquat and low-pH exposure. Also, the pneumococcus might employ polyamine-mediated defence mechanisms to overcome nitrosative stress during infection, as described for *E. coli* via the *merR* transcriptional regulator (Bower *et al.*, 2009; Potter *et al.*, 2010). Thus, subtle perturbations in intracellular polyamine levels may not significantly affect pneumococcal stress responses *in vitro*. However, further evaluation by constructing mutants with additional deletions in polyamine biosynthesis and transport pathways is required to fully comprehend the role of polyamines in *S. pneumoniae* survival during stress.

Our data strongly suggest that polyamines are one of the key nutrients utilized by the pneumococcus during *in vivo* growth, and that they regulate the expression of certain virulence factors during infection. These results also establish, for the first time to our knowledge, a link between *S. pneumoniae* polyamine biosynthesis and transport and colonization and disease outcomes. Similar observations have been made with respect to the roles of sucrose, sialic acid metabolism and manganese transport in pneumococcal pathogenesis (Manco *et al.*, 2006; Rosch *et al.*, 2009; Yesilkaya *et al.*, 2008). It is becoming clear that understanding the metabolic requirements of a pathogen may promote a deeper understanding of the underlying disease aetiology and result in novel prophylactic and/or therapeutic interventions.

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Polyamine biosynthesis and transport mechanisms are crucial for fitness and pathogenesis of *Streptococcus pneumoniae*

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Supplementary Table S4. Tandem MS analysis of *S. pneumoniae* TIGR4 and TIGR4 Δ*speE*

Protein ID	Protein name	Number of peptides	
		S. pneumoniae* TIGR4	***S. pneumoniae* TIGR4 Δ*speE****
NP_346439.1	Glyceraldehyde-3-phosphate dehydrogenase	301	351
NP_344811.1	Elongation factor G	190	222
NP_345941.1	Elongation factor Tu	180	199
NP_345598.1	Phosphopyruvate hydratase	178	204
NP_345812.1	50S ribosomal protein L7/L12	170	182
NP_345017.1	Phosphoglycerate kinase	138	140
NP_345231.1	Pyruvate oxidase	134	136
NP_345035.1	Molecular chaperone DnaK	129	123
NP_346623.1	30S ribosomal protein S2	111	127
NP_346455.1	Transketolase	111	93
NP_346387.1	DNA-directed RNA polymerase subunit beta	110	130
NP_346451.1	Bifunctional acetaldehyde-CoA/alcohol dehydrogenase	105	96
NP_345350.1	30S ribosomal protein S1	104	105
NP_345117.1	Fructose-bisphosphate aldolase	102	91
NP_346336.1	Chaperonin GroEL	100	74
NP_346527.1	Maltose/Maltodextrin ABC transporter, maltose/maltodextrin-binding pro	95	96
NP_345686.1	L-lactate dehydrogenase	91	86
NP_344765.1	50S ribosomal protein L6	88	78
NP_345151.1	Serine protease	87	90
NP_346622.1	Elongation factor Ts	84	83
NP_345336.1	Lipoprotein	83	85
NP_346019.1	Non-heme iron-containing ferritin	81	80
NP_345769.1	Glutamate dehydrogenase	80	77
NP_346105.1	Cell division protein FtsZ	80	76
NP_345364.1	PTS system, fructose specific IIABC components	78	64
NP_345462.1	Foldase protein PrsA	77	109
NP_344663.1	Pneumococcal surface protein A	77	71
NP_344944.1	3-ketoacyl-(acyl-carrier-protein) reductase	77	56
NP_345384.1	Pyruvate kinase	76	109
NP_346636.1	Inositol-5-monophosphate dehydrogenase	76	61
NP_344902.1	6-phosphogluconate dehydrogenase	74	79
NP_344804.1	D-fructose-6-phosphate amidotransferase	74	56
NP_345020.1	Glutamine synthetase, type I	73	84
NP_344810.1	30S ribosomal protein S7	70	73
NP_344923.1	Trigger factor	69	88
NP_345294.1	Aminopeptidase N	69	74
NP_345590.1	Glyceraldehyde-3-phosphate dehydrogenase, NADP-dependent	66	75
NP_345949.1	Phosphoglucomutase	66	70
NP_345285.1	Methionyl-tRNA synthetase	65	72
NP_344761.1	50S ribosomal protein L5	65	54
NP_346388.1	DNA-directed RNA polymerase subunit beta	63	77
NP_345645.1	Phosphoenolpyruvate-protein phosphotransferase	63	70
NP_346397.1	Asparagine synthetase AsnA	61	59
NP_345923.1	NADH oxidase	61	56
NP_346499.1	Arginyl-tRNA synthetase	60	78
NP_344633.1	30S ribosomal protein S4	60	75
NP_345142.1	50S ribosomal protein L1	60	68
NP_346525.1	Glycogen phosphorylase family protein	58	77
NP_346087.1	Endopeptidase O	57	63
NP_345648.1	Ribonucleotide-diphosphate reductase subunit alpha	56	53
NP_346094.1	Phosphoglyceromutase	55	52
NP_345748.1	Hypothetical protein SP_1284	55	44
NP_344979.1	Formate acetyltransferase	53	73
NP_346493.1	Glucose-6-phosphate isomerase	53	53
NP_344751.1	50S ribosomal protein L23	53	38
NP_345989.1	30S ribosomal protein S6	52	46
NP_344822.1	PTS system, mannose-specific IIAB components	52	44
NP_345959.1	F0F1 ATP synthase subunit beta	51	56

Protein ID	Protein name	Number of peptides	
		S. pneumoniae TIGR4*	*S. pneumoniae* TIGR4 *ΔspeE**
NP_345813.1	50S ribosomal protein L10	51	48
NP_344891.1	Capsular polysaccharide biosynthesis protein Cps4J	50	53
NP_344945.1	3-oxoacyl-(acyl carrier protein) synthase II	49	48
NP_344566.1	Cell division protein FtsH	48	64
NP_344959.1	Aspartyl/glutamyl-tRNA amidotransferase subunit A	48	52
NP_344750.1	50S ribosomal protein L4	47	60
NP_344774.1	30S ribosomal protein S13	47	59
NP_346020.1	Triosephosphate isomerase	47	35
NP_346519.1	Tyrosyl-tRNA synthetase	46	60
NP_345899.1	Bifunctional GMP synthase/glutamine amidotransferase protein	46	45
NP_344964.1	Hypothetical protein SP_0443	46	42
NP_345841.1	Alanyl-tRNA synthetase	43	43
NP_346140.1	Preprotein translocase subunit SecA	43	41
NP_344802.1	Prolyl-tRNA synthetase	43	34
NP_345141.1	50S ribosomal protein L11	43	29
NP_344571.1	Adenylosuccinate synthetase	43	27
NP_345383.1	6-phosphofructokinase	41	46
NP_345499.1	Serine hydroxymethyltransferase	40	47
NP_345072.1	Translation initiation factor IF-2	40	44
NP_345990.1	Asparaginyl-tRNA synthetase	40	43
NP_346638.1	ABC transporter, ATP-binding protein	40	40
NP_344934.1	Seryl-tRNA synthetase	39	46
NP_345706.1	Amino acid ABC transporter, amino acid-binding protein/permease protei	39	34
NP_344820.1	PTS system, mannose-specific IID component	39	26
NP_346089.1	Manganese ABC transporter, manganese-binding adhesion liprotein	38	45
NP_346106.1	Cell division protein FtsA	37	46
NP_346492.1	Glutamyl-tRNA synthetase	37	41
NP_346479.1	Alcohol dehydrogenase, zinc-containing	37	37
NP_344894.1	Oligopeptide ABC transporter, oligopeptide-binding protein AliA	37	37
NP_345273.1	30S ribosomal protein S16	37	32
NP_346570.1	SPFH domain-containing protein/band 7 family protein	37	30
NP_346322.1	Oligopeptide ABC transporter, oligopeptide-binding protein AmiA	37	29
NP_344764.1	30S ribosomal protein S8	37	23
NP_345928.1	Glycyl-tRNA synthetase subunit beta	36	35
NP_346237.1	General stress protein 24, putative	35	41
NP_344941.1	Acyl carrier protein	35	33
NP_344776.1	DNA-directed RNA polymerase subunit alpha	35	31
NP_344767.1	30S ribosomal protein S5	35	30
NP_344565.1	Hypoxanthine-guanine phosphoribosyltransferase	35	29
NP_345968.1	Hypothetical protein SP_1518	34	56
NP_344892.1	Capsular polysaccharide biosynthesis protein Cps4K	34	39
NP_344832.1	50S ribosomal protein L13	34	34
NP_344585.1	Aromatic amino acid aminotransferase	34	30
NP_344755.1	30S ribosomal protein S3	33	48
NP_345646.1	Phosphocarrier protein HPr	33	44
NP_346421.1	Aminotransferase AlaT	33	31
NP_346066.1	30S ribosomal protein S15	33	28
NP_344756.1	50S ribosomal protein L16	33	23
NP_344754.1	50S ribosomal protein L22	32	37
NP_345757.1	50S ribosomal protein L19	32	34
NP_346469.1	Acetate kinase	32	28
NP_346426.1	Catabolite control protein A	32	26
NP_344823.1	Alcohol dehydrogenase	32	23
NP_346026.1	Sugar ABC transporter, ATP-binding protein	32	23
NP_345878.1	Nicotinate phosphoribosyltransferase	31	33
NP_344777.1	50S ribosomal protein L17	31	29
NP_345982.1	Putative manganese-dependent inorganic pyrophosphatase	31	25
NP_344557.1	Translation-associated GTPase	31	17
NP_345320.1	Phosphopentomutase	30	19
NP_344760.1	50S ribosomal protein L24	29	30
NP_344943.1	Acyl-carrier-protein S-malonyltransferase	29	24
NP_344890.1	UDP-N-acetylglucosamine-2-epimerase	29	24
NP_345186.1	Elongation factor Tu family protein	28	33
NP_345961.1	F0F1 ATP synthase subunit alpha	28	29
NP_345557.1	Methionine aminopeptidase	28	27
NP_345819.1	Homoserine dehydrogenase	28	20
NP_346351.1	Pneumolysin	27	31
NP_344555.1	DNA polymerase III subunit beta	27	26
NP_346319.1	Oligopeptide ABC transporter, ATP-binding protein AmiE	27	22

Protein ID	Protein name	Number of peptides	
		S. pneumoniae TIGR4*	*S. pneumoniae* TIGR4 *ΔspeE**
NP_345244.1	Uracil phosphoribosyltransferase	27	18
NP_346098.1	Isoleucyl-tRNA synthetase	26	36
NP_344752.1	50S ribosomal protein L2	26	29
NP_345578.1	50S ribosomal protein L27	26	22
NP_346316.1	Trehalose PTS system, IIABC components	26	19
NP_344946.1	Acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	26	16
NP_344690.1	ABC transporter, substrate-binding protein	25	33
NP_344748.1	30S ribosomal protein S10	25	31
NP_344957.1	Elongation factor P	25	25
NP_345649.1	Ribonucleotide-diphosphate reductase subunit beta	25	24
NP_344948.1	Acetyl-CoA carboxylase biotin carboxylase subunit	25	23
NP_346297.1	Glutamyl-aminopeptidase	25	23
NP_346615.1	50S ribosomal protein L9	25	21
NP_346511.1	UTP-glucose-1-phosphate uridylyltransferase	25	21
NP_345348.1	Pyrrolidone-carboxylate peptidase	25	14
NP_346051.1	UDP-glucose 4-epimerase	24	25
NP_345084.1	Valyl-tRNA synthetase	24	25
NP_344893.1	UDP-N-acetylglucosamine 2-epimerase	24	24
NP_345943.1	Glycerol uptake facilitator protein, putative	24	20
NP_345069.1	Transcription elongation factor NusA	24	20
NP_344742.1	Anaerobic ribonucleoside triphosphate reductase	24	14
NP_344793.1	Leucyl-tRNA synthetase	23	35
NP_344888.1	Hypothetical protein SP_0355	23	22
NP_346315.1	Dextran glucosidase DexS, putative	23	21
NP_344938.1	Enoyl-CoA hydratase	23	20
NP_345362.1	Lactose phosphotransferase system repressor	23	20
NP_344759.1	50S ribosomal protein L14	23	19
NP_346339.1	Hypothetical protein SP_1910	23	14
NP_346321.1	Oligopeptide ABC transporter, permease protein AmiC	23	13
NP_344833.1	30S ribosomal protein S9	22	28
NP_345214.2	Lysyl-tRNA synthetase	22	27
NP_345101.1	Polynucleotide phosphorylase/polyadenylase	22	25
NP_346617.1	Ribosomal subunit interface protein	22	23
NP_345567.1	Ribose-phosphate pyrophosphokinase	22	22
NP_346071.1	Threonyl-tRNA synthetase	22	21
NP_344936.1	Aspartate kinase	22	18
NP_344958.1	Aspartyl/glutamyl-tRNA amidotransferase subunit B	22	17
NP_345541.1	Phosphoenolpyruvate carboxylase	22	17
NP_346434.1	Transcription antitermination protein NusG	22	13
NP_346533.1	Aspartyl-tRNA synthetase	21	33
NP_345967.1	Transcription elongation factor GreA	21	23
NP_344775.1	30S ribosomal protein S11	21	21
NP_345304.1	Septation ring formation regulator EzrA	21	20
NP_345708.1	Glucose-6-phosphate 1-dehydrogenase	21	19
NP_345269.1	Peptidyl-prolyl cis-trans isomerase, cyclophilin-type	21	16
NP_345248.1	Branched-chain amino acid ABC transporter, amino acid-binding protein	21	14
NP_345572.1	Phosphotransacetylase	21	14
NP_345584.1	DNA-binding protein HU	21	12
NP_345442.1	50S ribosomal protein L20	20	33
NP_344578.1	Ribose-phosphate pyrophosphokinase	20	24
NP_346138.1	Phospho-2-dehydro-3-deoxyheptonate aldolase	20	19
NP_345877.1	NAD synthetase	20	18
NP_344628.1	Trk family potassium uptake protein	20	18
NP_345830.1	Hypothetical protein SP_1372	20	15
NP_345262.1	Dihydroorotate dehydrogenase 1A	20	14
NP_345932.1	Aldo/Keto reductase family oxidoreductase	20	11
NP_345763.1	50S ribosomal protein L31 type B	19	21
NP_346646.1	Serine protease	19	20
NP_346122.1	Sugar ABC transporter, sugar-binding protein	19	17
NP_344896.1	Penicillin-binding protein 1A	18	36
NP_344771.1	Adenylate kinase	18	23
NP_346209.1	Thioredoxin	18	22
NP_344968.1	Ketol-acid reductoisomerase	18	21
NP_345095.1	Phenylalanyl-tRNA synthetase subunit beta	18	21
NP_344819.1	Aminopeptidase C	18	18
NP_345428.1	Ribosome recycling factor	18	15
NP_345467.1	UDP-N-acetylglucosamine pyrophosphorylase	18	15
NP_345991.1	Aspartate aminotransferase	18	12
NP_345135.1	Dipeptidase PepV	18	7

Protein ID	Protein name	Number of peptides	
		S. pneumoniae TIGR4*	*S. pneumoniae* TIGR4 *ΔspeE**
NP_345546.1	RNA polymerase sigma factor RpoD	17	19
NP_345216.1	Lactate oxidase	17	17
NP_346605.1	ATP-dependent Clp protease, ATP-binding subunit	17	15
NP_345573.1	Hypothetical protein SP_1102	17	15
NP_346540.1	Histidyl-tRNA synthetase	17	12
NP_344779.1	Hypothetical protein SP_0239	17	12
NP_345140.1	Hypothetical protein SP_0629	17	12
NP_346318.1	Oligopeptide ABC transporter, ATP-binding protein AmiF	17	12
NP_345707.1	Amino acid ABC transporter, ATP-binding protein	17	11
NP_344816.1	Aminopeptidase PepS	17	11
NP_346100.1	Cell division protein DivIVA	17	6
NP_344876.1	Glucan 1,6-alpha-glucosidase	16	27
NP_346482.1	Queuine tRNA-ribosyltransferase	16	25
NP_346006.1	Phosphoglucomutase/phosphomannomutase family protein	16	21
NP_346407.1	cmp-binding-factor 1	16	19
NP_345910.1	Peptide deformylase	16	19
NP_346037.1	Proline dipeptidase	16	19
NP_346270.1	Capsular polysaccharide biosynthesis protein, putative	16	16
NP_345034.1	Heat shock protein GrpE	16	14
NP_346489.1	Threonine synthase	16	10
NP_344961.1	Peptide chain release factor 3	16	8
NP_345256.1	PTS system, IIABC components	16	8
NP_344749.1	50S ribosomal protein L3	15	17
NP_345173.2	Glucokinase	15	17
NP_346526.1	4-alpha-glucanotransferase	15	16
NP_346005.1	Hypothetical protein SP_1558	15	15
NP_344768.1	50S ribosomal protein L30	15	14
NP_345345.1	Branched-chain amino acid aminotransferase	15	14
NP_344883.1	Capsular polysaccharide biosynthesis protein Cps4E	15	13
NP_346032.1	ATP-dependent RNA helicase, putative	15	12
NP_345853.1	Phosphate transport system regulatory protein PhoU, putative	15	12
NP_346113.1	Phosphosugar-binding transcriptional regulator, putative	15	11
NP_345854.1	Phosphate ABC transporter, ATP-binding protein, putative	15	9
NP_345751.1	Signal recognition particle protein	14	21
NP_346238.1	Hypothetical protein SP_1805	14	20
NP_345484.1	Peptidase T	14	19
NP_345714.1	Guanosine 5 -monophosphate oxidoreductase	14	17
NP_345125.1	Metallo-beta-lactamase superfamily protein	14	16
NP_345667.1	GTP-binding protein LepA	14	15
NP_345821.1	Hypothetical protein SP_1363	14	15
NP_345817.1	Bifunctional methionine sulfoxide reductase A/B protein	14	14
NP_346406.1	Purine operon repressor	14	14
NP_344882.1	Capsular polysaccharide biosynthesis protein Cps4D	14	13
NP_346147.1	GTP-binding protein EngA	14	10
NP_345507.1	Iron-compound ABC transporter, iron compound-binding protein	14	10
NP_345755.1	Cof family protein	14	5
NP_346337.1	Co-chaperonin GroES	14	3
NP_344664.1	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase	14	3
NP_345260.1	S-adenosylmethionine synthetase	13	15
NP_344773.1	50S ribosomal protein L36	13	14
NP_344667.1	Metallo-beta-lactamase superfamily protein	13	13
NP_344972.1	Hypothetical protein SP_0451	13	12
NP_345012.1	CTP synthetase	13	11
NP_346030.1	Transcriptional repressor CodY	13	11
NP_345354.1	ABC transporter, ATP-binding protein	13	10
NP_345741.1	Aspartate carbamoyltransferase catalytic subunit	13	7
NP_346516.1	2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase, putati	13	6
NP_346554.1	Choline binding protein PcpA	12	21
NP_345138.1	Hypothetical protein SP_0627	12	18
NP_345835.1	3-dehydroquinate dehydratase	12	15
NP_346153.1	Hypothetical protein SP_1715	12	14
NP_345988.1	Single-strand DNA-binding protein	12	14
NP_345363.1	1-phosphofructokinase, putative	12	13
NP_344881.1	Capsular polysaccharide biosynthesis protein Cps4C	12	11
NP_345987.1	30S ribosomal protein S18	12	10
NP_345912.1	Thioredoxin reductase	12	10
NP_346386.1	Nucleoside diphosphate kinase	12	9
NP_345950.1	Bacterocin transport accessory protein	12	8
NP_346023.1	Adenine phosphoribosyltransferase	12	6

Protein ID	Protein name	Number of peptides	
		S. pneumoniae TIGR4*	*S. pneumoniae* TIGR4 *ΔspeE**
NP_346510.1	NAD(P)H-dependent glycerol-3-phosphate dehydrogenase	12	5
NP_344644.1	Hypothetical protein SP_0097	11	45
NP_344650.1	Capsular polysaccharide biosynthesis protein, putative	11	20
NP_345929.1	Glycyl-tRNA synthetase subunit alpha	11	17
NP_345576.1	50S ribosomal protein L21	11	14
NP_345986.1	Cof family protein/peptidyl-prolyl cis-trans isomerase, cyclophilin ty	11	14
NP_344942.1	Enoyl-(acyl-carrier-protein) reductase	11	13
NP_346465.1	jag protein, putative	11	13
NP_345960.1	F0F1 ATP synthase subunit gamma	11	9
NP_345268.1	ABC transporter, ATP-binding protein	11	8
NP_346164.1	Hydroxymethylglutaryl-CoA synthase	11	7
NP_345871.1	HPr kinase/phosphorylase	11	6
NP_345403.1	Hypothetical protein SP_0919	11	0
NP_345381.1	x-prolyl-dipeptidyl aminopeptidase	10	16
NP_346544.1	Dihydroxy-acid dehydratase	10	15
NP_344757.1	50S ribosomal protein L29	10	13
NP_345460.1	Oligoendopeptidase F	10	12
NP_346637.1	Tryptophanyl-tRNA synthetase II	10	12
NP_345500.1	Hypothetical protein SP_1025	10	10
NP_344949.1	Acetyl-CoA carboxylase subunit beta	10	9
NP_345951.1	Amino acid ABC transporter, amino acid-binding protein	10	9
NP_345295.1	DNA-binding response regulator CiaR	10	9
NP_345761.1	Flavodoxin	10	9
NP_345134.1	Nitroreductase family protein	10	8
NP_346139.1	Phospho-2-dehydro-3-deoxyheptonate aldolase	10	8
NP_344960.1	Aspartyl/Glutamyl-tRNA amidotransferase subunit C	10	6
NP_344728.1	Peptidase M24 family protein	10	5
NP_346278.1	Exodeoxyribonuclease	10	1
NP_345329.1	30S ribosomal protein S20	9	16
NP_344766.1	50S ribosomal protein L18	9	16
NP_345264.1	Superoxide dismutase, manganese-dependent	9	12
NP_345254.1	Cell division ABC transporter, ATP-binding protein FtsE	9	11
NP_345235.1	Mannose-6-phosphate isomerase	9	10
NP_346394.1	Hypothetical protein SP_1967	9	9
NP_345731.1	licC protein	9	9
NP_346173.1	DNA-directed RNA polymerase subunit omega	9	8
NP_346014.1	Endoribonuclease L-PSP	9	8
NP_346088.1	Manganese ABC transporter, ATP-binding protein	9	8
NP_346405.1	Diaminopimelate decarboxylase	9	7
NP_346412.1	Dimethyladenosine transferase	9	6
NP_346158.1	Fructokinase	9	6
NP_346115.1	N-acetylneuraminate lyase, putative	9	6
NP_346601.1	Choline binding protein A	9	5
NP_345583.1	degV family protein	9	3
NP_344947.1	(3R)-hydroxymyristoyl-ACP dehydratase	9	2
NP_345936.1	Gfo/Idh/MocA family oxidoreductase	9	1
NP_345450.1	GTP-binding protein Era	8	15
NP_345193.1	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	8	15
NP_346402.1	OxaA-like protein precursor	8	13
NP_346480.1	N-acetylglucosamine-6-phosphate deacetylase	8	10
NP_345037.1	dnaJ protein	8	9
NP_344753.1	30S ribosomal protein S19	8	8
NP_344733.1	Hypothetical protein SP_0192	8	8
NP_345322.1	Purine nucleoside phosphorylase	8	8
NP_345659.1	Tagatose 1,6-diphosphate aldolase	8	7
NP_346002.1	Dihydrodipicolinate reductase	8	6
NP_346599.1	Hsp33-like chaperonin	8	6
YP_873931.1	Lysozyme	8	6
NP_344693.1	ABC transporter, ATP-binding protein	8	5
YP_873928.1	ATP cone domain-containing protein	8	5
NP_345832.1	Chorismate synthase	8	5
NP_346285.1	Galactokinase	8	5
NP_345066.1	Hypothetical protein SP_0549	8	5
NP_344780.1	Phosphoglycerate mutase family protein	8	5
NP_344829.1	GTP cyclohydrolase I	8	4
NP_344875.1	Hypothetical protein SP_0341	8	4
NP_346312.2	Fused deoxyribonucleotide triphosphate pyrophosphatase/unknown domain	8	3
NP_344874.1	S-ribosylhomocysteinase	8	3
NP_345276.1	16S rRNA-processing protein	8	2

Protein ID	Protein name	Number of peptides	
		S. pneumoniae TIGR4*	*S. pneumoniae* TIGR4 *ΔspeE**
NP_345426.1	tRNA (uracil-5-)-methyltransferase Gid	8	2
NP_346016.1	ATP-dependent protease ATP-binding subunit	7	14
NP_344769.1	50S ribosomal protein L15	7	12
NP_345552.1	GTPase ObgE	7	10
NP_345865.1	HAD superfamily hydrolase	7	10
NP_345739.1	Carbamoyl phosphate synthase large subunit	7	9
NP_344827.1	Dihydropteroate synthase	7	9
NP_345104.1	Cysteinyl-tRNA synthetase	7	7
NP_345495.1	Peptide chain release factor 1	7	7
NP_344884.1	Capsular polysaccharide biosynthesis protein Cps4F	7	6
NP_345814.2	Chlorohydrolase	7	6
NP_345183.1	Hypothetical protein SP_0678	7	6
NP_345416.1	Gamma-glutamyl phosphate reductase	7	5
NP_345858.1	Phosphate ABC transporter, phosphate-binding protein, putative	7	5
NP_346103.1	ylmF protein	7	5
NP_345740.1	Carbamoyl phosphate synthase small subunit	7	4
NP_346226.1	Hypothetical protein SP_1793	7	4
NP_345027.1	Type I restriction-modification system, M subunit	7	4
NP_346608.1	ABC transporter, substrate-binding protein, putative	7	3
NP_344720.1	Holliday junction DNA helicase motor protein	7	3
NP_346370.1	Transcriptional regulator, putative	7	3
NP_344792.1	Glycerol dehydrogenase	7	2
NP_346306.1	Ribosomal large subunit pseudouridine synthase B	7	2
NP_346410.1	Ribulose-phosphate 3-epimerase	7	2
NP_345554.1	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	7	2
NP_345358.1	Intein-containing protein	7	0
NP_346552.1	50S ribosomal protein L32	6	13
NP_345957.1	Hypothetical protein SP_1506	6	12
NP_346213.1	Oligoendopeptidase F, putative	6	11
NP_345337.1	Sugar ABC transporter, ATP-binding protein	6	11
NP_346350.1	Hypothetical protein SP_1922	6	9
NP_346174.1	Guanylate kinase	6	7
NP_346035.1	Hypothetical protein SP_1589	6	7
NP_344607.1	GntR family transcriptional regulator	6	6
NP_346365.1	Autolysin	6	5
NP_346311.1	Hypothetical protein SP_1879	6	5
NP_344700.1	Hypothetical protein SP_0158	6	4
NP_346013.1	Hypothetical protein SP_1566	6	4
NP_345993.1	Hypothetical protein SP_1546	6	3
NP_345255.1	Cell division ABC transporter, permease protein FtsX	6	1
NP_346454.1	Preprotein translocase subunit YajC	6	1
NP_345685.1	DNA gyrase subunit A	5	14
NP_346175.1	Hypothetical protein SP_1739	5	12
NP_344587.1	Putative glycerol-3-phosphate acyltransferase PlsX	5	10
NP_345407.1	Cof family protein	5	8
NP_344666.1	tRNA uridine 5-carboxymethylaminomethyl modification enzyme GidA	5	7
NP_345241.1	Hypothetical protein SP_0742	5	6
NP_345565.1	Hypothetical protein SP_1093	5	6
NP_345971.1	UDP-N-acetylmuramate--L-alanine ligase	5	6
NP_345489.1	Aspartate-semialdehyde dehydrogenase	5	5
NP_345038.1	Hypothetical protein SP_0520	5	5
NP_345636.1	Dihydroorotase	5	4
NP_346171.1	Methionyl-tRNA formyltransferase	5	4
NP_345647.1	NrdH-redoxin	5	4
NP_346046.1	phnA protein	5	4
NP_344588.1	Acyl carrier protein, putative	5	3
NP_345011.2	DNA-directed RNA polymerase subunit delta	5	3
NP_345039.1	HIT family protein	5	3
NP_346048.1	Hypothetical protein SP_1604	5	3
NP_345326.1	Purine nucleoside phosphorylase	5	3
NP_345491.1	tRNA modification GTPase TrmE	5	3
NP_344950.1	Acetyl-CoA carboxylase subunit alpha	5	2
NP_345447.1	Adherence and virulence protein A	5	2
NP_344801.1	eep protein	5	2
NP_345349.1	Hypothetical protein SP_0861	5	2
NP_346353.1	Hypothetical protein SP_1925	5	2
NP_345274.1	KH domain-containing protein	5	2
NP_345855.1	Phosphate ABC transporter, ATP-binding protein, putative	5	2
NP_344772.1	Translation initiation factor IF-1	5	2

Protein ID	Protein name	Number of peptides	
		S. pneumoniae TIGR4*	*S. pneumoniae* TIGR4 *ΔspeE**
NP_345833.1	3-dehydroquinate synthase	5	1
NP_346414.1	ABC transporter, ATP-binding protein	5	1
NP_345916.1	Hypothetical protein SP_1462	5	1
NP_345742.1	Pyrimidine regulatory protein PyrR	5	1
NP_346352.1	Hypothetical protein SP_1924	5	0
NP_345019.1	MerR family transcriptional regulator	5	0
NP_346110.1	D-alanyl-alanine synthetase A	4	9
NP_346600.1	NifR3 family TIM-barrel protein	4	7
NP_345734.1	Alcohol dehydrogenase, zinc-containing	4	6
NP_345999.1	Cation efflux family protein	4	6
NP_345738.1	licD2 protein	4	6
NP_345735.1	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	4	4
NP_346047.1	Cytidylate kinase	4	4
NP_346185.1	GTP-binding protein YqeH	4	4
NP_344899.1	Hypothetical protein SP_0372	4	4
NP_346029.1	Isochorismatase family protein	4	4
NP_345443.1	Lactoylglutathione lyase	4	4
NP_345784.1	Neuraminidase, putative	4	4
NP_345589.1	Pullulanase, putative	4	4
NP_346010.1	Pyridine nucleotide-disulphide oxidoreductase family protein	4	4
NP_346368.1	Recombinase A	4	4
NP_345440.1	Translation initiation factor IF-3	4	4
NP_345848.1	UDP-N-acetylenolpyruvoylglucosamine reductase	4	4
NP_344977.1	Undecaprenyl pyrophosphate phosphatase	4	4
NP_344873.1	ATP-dependent Clp protease, ATP-binding subunit, putative	4	3
NP_345998.1	Cation transporter E1-E2 family ATPase	4	3
NP_345640.1	HAD superfamily hydrolase	4	3
NP_345995.1	Hypothetical protein SP_1548	4	3
NP_346007.1	Hypothetical protein SP_1560	4	3
NP_345709.1	Signal recognition particle-docking protein FtsY	4	3
NP_344974.1	Amino acid ABC transporter, amino acid-binding protein/permease protei	4	2
NP_345958.1	F0F1 ATP synthase subunit epsilon	4	2
NP_344778.1	Hypothetical protein SP_0238	4	2
NP_344963.1	Hypothetical protein SP_0442	4	2
NP_345471.1	Hypothetical protein SP_0992	4	2
NP_345547.1	Hypothetical protein SP_1074	4	2
NP_345926.1	Oxidoreductase, putative	4	2
NP_345301.1	4-methyl-5(b-hydroxyethyl)-thiazole monophosphate biosynthesis protein	4	1
NP_345710.1	Cof family protein	4	1
NP_344642.1	Hypothetical protein SP_0095	4	1
NP_345385.1	Hypothetical protein SP_0899	4	1
NP_346550.1	Hypothetical protein SP_2132	4	1
NP_346504.1	Phosphate ABC transporter, phosphate-binding protein	4	1
NP_345319.1	Ribose-5-phosphate isomerase A	4	1
NP_345427.1	Uridylate kinase	4	1
NP_345169.1	Zinc metalloprotease ZmpB, putative	4	0
NP_346215.1	Ribosomal protein L11 methyltransferase	3	9
NP_345884.1	U32 family peptidase	3	9
NP_345886.1	U32 family peptidase	3	9
NP_345559.1	ATP-dependent DNA helicase PcrA	3	5
NP_344575.1	Hypothetical protein SP_0024	3	5
NP_344645.1	Hypothetical protein SP_0098	3	5
NP_346631.1	Hypothetical protein SP_2223	3	5
NP_345174.1	Thymidylate synthase	3	5
NP_345733.1	Choline kinase	3	4
NP_345415.1	Gamma-glutamyl kinase	3	4
NP_345298.1	Hypothetical protein SP_0801	3	4
NP_345980.1	Hypothetical protein SP_1531	3	4
NP_346403.1	Pyruvate formate-lyase-activating enzyme	3	4
NP_346393.1	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	3	4
NP_345334.1	Deoxyribose-phosphate aldolase	3	3
NP_344668.1	Hypothetical protein SP_0122	3	3
NP_346314.1	Hypothetical protein SP_1882	3	3
NP_346613.1	Hypothetical protein SP_2202	3	3
NP_345468.1	MutT/nudix family protein	3	3
NP_345492.1	4-oxalocrotonate tautomerase	3	2
NP_346000.1	ABC transporter, ATP-binding protein	3	2
NP_344605.1	Adenylosuccinate lyase	3	2
NP_345852.1	Amino acid ABC transporter, amino acid-binding protein	3	2

Protein ID	Protein name	Number of peptides	
		S. pneumoniae TIGR4*	*S. pneumoniae* TIGR4 *ΔspeE**
NP_345490.1	Dihydrodipicolinate synthase	3	2
NP_346152.1	GntR family transcriptional regulator	3	2
NP_344900.1	Hypothetical protein SP_0373	3	2
NP_345175.1	Hypothetical protein SP_0670	3	2
NP_344821.1	PTS system, mannose-specific IIC component	3	2
NP_346586.1	adc operon repressor AdcR	3	1
NP_345312.1	ATP-dependent Clp protease, ATP-binding subunit ClpE	3	1
NP_345252.1	Branched-chain amino acid ABC transporter, ATP-binding protein	3	1
NP_346292.1	Choline transporter	3	1
NP_345429.1	Hypothetical protein SP_0946	3	1
NP_345680.1	Hypothetical protein SP_1213	3	1
NP_345868.1	Hypothetical protein SP_1410	3	1
NP_345869.1	Hypothetical protein SP_1411	3	1
NP_346197.1	Hypothetical protein SP_1762	3	1
NP_345697.1	Phosphopantothenoylcysteine decarboxylase	3	1
NP_345455.1	Preprotein translocase subunit SecG	3	1
NP_345973.1	Snf2 family protein	3	1
YP_873932.1	Transketolase	3	1
NP_345914.1	Amino acid ABC transporter, ATP-binding protein	3	0
NP_345695.1	Formate--tetrahydrofolate ligase	3	0
NP_344673.1	Hypothetical protein SP_0127	3	0
NP_345469.1	Hypothetical protein SP_0990	3	0
NP_346304.1	Iron-compound ABC transporter, iron-compound-binding protein	3	0
NP_345629.1	Lipoate-protein ligase, putative	3	0
NP_344652.1	L-serine dehydratase, iron-sulfur-dependent, alpha subunit	3	0
NP_345093.1	Phenylalanyl-tRNA synthetase subunit alpha	3	0
NP_346626.1	Rod shape-determining protein MreC	3	0
NP_345180.1	Short chain dehydrogenase/reductase family oxidoreductase	3	0
NP_345829.1	3-phosphoshikimate 1-carboxyvinyltransferase	2	12
NP_345259.1	DEAD-box ATP dependent DNA helicase	2	7
NP_345393.1	Transcriptional regulator, putative	2	6
NP_344691.1	Lipoprotein	2	5
NP_345952.1	Amino acid ABC transporter, ATP-binding protein	2	4
NP_344973.1	Amino acid ABC transporter, ATP-binding protein	2	3
NP_345623.1	Immunoglobulin A1 protease	2	3
NP_345470.1	5 -methylthioadenosine/S-adenosylhomocysteine nucleosidase	2	2
NP_346590.1	D-alanine--D-alanyl carrier protein ligase	2	2
NP_346004.1	degV family protein	2	2
NP_345085.1	Hypothetical protein SP_0570	2	2
NP_345314.1	Hypothetical protein SP_0822	2	2
NP_345679.1	tRNA pseudouridine synthase B	2	2
NP_345915.1	Amino acid ABC transporter, permease protein	2	1
NP_345953.1	Amino acid ABC transporter, permease protein	2	1
NP_346248.1	Anthranilate phosphoribosyltransferase	2	1
NP_346148.1	Nitroreductase family protein	2	1
NP_346518.1	Penicillin-binding protein 1B	2	1
YP_873927.1	Peptide chain release factor 2	2	1
NP_345073.1	Ribosome-binding factor A	2	1
NP_345368.1	Thiamine biosynthesis protein ThiI	2	1
NP_345630.1	Acetoin dehydrogenase complex, E3 component, dihydrolipoamide dehydrog	2	0
NP_345820.1	Adaptor protein	2	0
NP_346329.1	Alpha-galactosidase	2	0
NP_344554.1	Chromosomal replication initiation protein	2	0
NP_345937.1	DEAD-box ATP dependent DNA helicase	2	0
NP_345704.1	Excinuclease ABC subunit B	2	0
NP_345963.1	F0F1 ATP synthase subunit B	2	0
NP_344849.1	Glycosy hydrolase family protein	2	0
NP_345108.1	Hypothetical protein SP_0595	2	0
NP_345355.1	Hypothetical protein SP_0868	2	0
NP_346528.1	Maltodextrin ABC transporter, permease protein	2	0
NP_346223.1	Recombination factor protein RarA	2	0
NP_346340.1	Thioredoxin, putative	2	0
NP_345600.1	Transcriptional regulator	2	0
NP_345126.1	Tributyrin esterase	2	0
NP_344799.1	Undecaprenyl diphosphate synthase	2	0
NP_345502.1	Hypothetical protein SP_1027	1	8
NP_346168.1	Serine/threonine protein kinase	1	8
NP_345585.1	ABC transporter, ATP-binding protein	1	3
NP_344932.1	Hypothetical protein SP_0409	1	3

Protein ID	Protein name	Number of peptides	
		S. pneumoniae TIGR4*	*S. pneumoniae* TIGR4 *ΔspeE**
NP_346063.1	Cation transporter E1-E2 family ATPase	1	2
NP_344573.1	Deoxyuridine 5 -triphosphate nucleotidohydrolase	1	2
NP_345548.1	Glycosyl transferase CpoA	1	2
NP_345309.1	Hypothetical protein SP_0816	1	2
NP_345556.1	Hypothetical protein SP_1083	1	2
NP_346372.1	Hypothetical protein SP_1944	1	2
NP_344939.1	MarR family transcriptional regulator	1	2
NP_346497.1	ABC transporter, ATP-binding/permease protein	1	1
NP_344591.1	Competence factor transporting ATP-binding/permease protein ComA	1	1
NP_345724.1	Copper homeostasis protein CutC	1	1
NP_345421.1	DNA replication intiation control protein YabA	1	1
NP_345727.1	DNA topoisomerase I	1	1
NP_344649.1	Glycosyl transferase	1	1
NP_344797.1	Holliday junction DNA helicase B	1	1
NP_345542.1	Hypothetical protein SP_1069	1	1
NP_346008.1	Hypothetical protein SP_1561	1	1
NP_346218.1	Hypothetical protein SP_1785	1	1
NP_346521.1	Hypothetical protein SP_2102	1	1
NP_346539.1	Hypothetical protein SP_2120	1	1
NP_344620.1	Immunoglobulin A1 protease	1	1
NP_345696.1	Phosphopantothenate--cysteine ligase	1	1
NP_345660.1	Tagatose-6-phosphate kinase	1	1
NP_346245.1	Tryptophan synthase subunit beta	1	1
NP_345283.1	ABC transporter, ATP-binding protein	1	0
NP_345839.1	ABC transporter, ATP-binding protein	1	0
NP_346128.1	ABC transporter, permease protein	1	0
NP_345632.1	Acetoin dehydrogenase, E1 component, beta subunit, putative	1	0
NP_345494.1	Acetyltransferase	1	0
NP_345498.1	Acetyltransferase	1	0
NP_346287.1	Alcohol dehydrogenase, zinc-containing	1	0
NP_345288.1	Aldo/keto reductase family oxidoreductase	1	0
NP_344806.1	Alkaline amylopullulanase, putative	1	0
NP_345850.1	Alpha-acetolactate decarboxylase	1	0
NP_345121.1	Amino acid ABC transporter, amino acid-binding protein	1	0
NP_344606.1	Beta-N-acetylhexosaminidase	1	0
NP_345251.1	Branched-chain amino acid ABC transporter, ATP-binding protein	1	0
NP_344886.1	Capsular polysaccharide biosynthesis protein Cps4H	1	0
NP_346520.1	Cation transporter E1-E2 family ATPase	1	0
NP_346310.1	CBS domain-containing protein	1	0
NP_344870.1	Cell division protein FtsL	1	0
YP_873926.1	Cell wall surface anchor family protein	1	0
NP_344905.1	Choline binding protein J	1	0
NP_345335.1	Cytidine deaminase	1	0
NP_344714.1	DNA mismatch repair protein	1	0
NP_344582.1	DNA polymerase I	1	0
NP_345545.1	DNA primase	1	0
NP_344903.1	DNA-binding response regulator	1	0
NP_346281.1	DpnD protein	1	0
NP_345800.1	Drug efflux ABC transporter, ATP-binding/permease protein	1	0
NP_345446.1	Endo-beta-N-acetylglucosaminidase	1	0
NP_345130.1	Excinuclease ABC subunit C	1	0
NP_345621.1	Exonuclease RexA	1	0
NP_345620.1	Exonuclease RexB	1	0
NP_344791.1	Fructose-6-phosphate aldolase	1	0
NP_345747.1	Heat shock protein HtpX	1	0
NP_346022.1	Homoserine O-succinyltransferase	1	0
NP_344699.1	Hypothetical protein SP_0157	1	0
NP_344746.1	Hypothetical protein SP_0206	1	0
NP_344813.1	Hypothetical protein SP_0275	1	0
NP_344935.1	Hypothetical protein SP_0412	1	0
NP_344956.1	Hypothetical protein SP_0434	1	0
NP_345068.1	Hypothetical protein SP_0552	1	0
NP_345075.1	Hypothetical protein SP_0559	1	0
NP_345079.1	Hypothetical protein SP_0563	1	0
NP_345080.1	Hypothetical protein SP_0564	1	0
NP_345149.1	Hypothetical protein SP_0639	1	0
NP_345184.1	Hypothetical protein SP_0679	1	0
NP_345247.1	Hypothetical protein SP_0748	1	0
NP_345287.1	Hypothetical protein SP_0790	1	0

Protein ID	Protein name	Number of peptides	
		S. pneumoniae TIGR4*	*S. pneumoniae* TIGR4 *ΔspeE**
NP_345293.1	Hypothetical protein SP_0796	1	0
NP_345332.1	Hypothetical protein SP_0841	1	0
NP_345622.1	Hypothetical protein SP_1153	1	0
NP_345712.1	Hypothetical protein SP_1247	1	0
NP_345760.1	Hypothetical protein SP_1296	1	0
NP_345862.1	Hypothetical protein SP_1404	1	0
NP_345946.1	Hypothetical protein SP_1494	1	0
NP_346235.1	Hypothetical protein SP_1802	1	0
NP_346354.1	Hypothetical protein SP_1926	1	0
NP_346392.1	Hypothetical protein SP_1965	1	0
NP_346483.1	Hypothetical protein SP_2059	1	0
NP_346543.1	Hypothetical protein SP_2125	1	0
NP_346596.1	Hypothetical protein SP_2185	1	0
NP_346415.1	Immunity protein, putative	1	0
NP_346180.1	Iojap-related protein	1	0
NP_346232.1	LacI family sugar-binding transcriptional regulator	1	0
NP_345651.1	Lactose phosphotransferase system repressor	1	0
NP_346531.1	Maltose operon transcriptional repressor	1	0
NP_346086.1	Metallo-beta-lactamase superfamily protein	1	0
NP_345588.1	NAD-dependent DNA ligase LigA	1	0
NP_344675.1	O-sialoglycoprotein endopeptidase	1	0
NP_346263.1	Phosphate transport system regulatory protein PhoU, putative	1	0
NP_345463.1	Phosphoglycerate mutase family protein	1	0
NP_345789.1	Phosphosugar-binding transcriptional regulator, putative	1	0
NP_346453.1	Phosphotyrosine protein phosphatase	1	0
NP_345172.1	Pneumococcal surface protein, putative	1	0
NP_344997.1	PTS system, lactose-specific IIBC components	1	0
NP_345997.1	Putative glutathione S-transferase YghU	1	0
NP_346459.1	Putative L-xylulose 5-phosphate 3-epimerase	1	0
NP_345922.1	Pyridoxine biosynthesis protein	1	0
NP_346111.1	Recombination protein RecR	1	0
NP_346502.1	Response regulator	1	0
NP_344716.1	Riboflavin synthase, beta subunit	1	0
NP_345413.1	Ribosomal large subunit pseudouridine synthase D	1	0
NP_346411.1	Ribosome-associated GTPase	1	0
NP_345874.1	S-adenosylmethionine:tRNA ribosyltransferase-isomerase	1	0
NP_346338.1	Short chain dehydrogenase/reductase family oxidoreductase	1	0
NP_344925.1	Signal peptidase I	1	0
NP_345123.2	Single-stranded-DNA-specific exonuclease RecJ	1	0
NP_345402.1	Spermidine synthase	1	0
NP_345497.1	Sua5/YciO/YrdC family protein	1	0
NP_345338.1	Sugar ABC transporter, permease protein, putative	1	0
NP_346242.1	Transcriptional regulator	1	0
NP_344843.1	Transcriptional regulator, putative	1	0
NP_345670.1	Transcriptional repressor, putative	1	0
NP_345373.1	Type I restriction-modification system, M subunit, putative	1	0
NP_345028.1	Type I restriction-modification system, R subunit	1	0
NP_345794.1	Type II DNA modification methyltransferase Spn5252IP	1	0
NP_346583.1	Zinc ABC transporter, zinc-binding lipoprotein	1	0
NP_344758.1	30S ribosomal protein S17	0	9
NP_345562.1	Redox-sensing transcriptional repressor Rex	0	5
NP_344940.1	3-oxoacyl-(acyl carrier protein) synthase III	0	4
NP_345400.1	Lysine decarboxylase	0	4
NP_345067.1	tRNA (guanine-N(7))-methyltransferase	0	4
NP_345406.1	Carbon-nitrogen hydrolase family protein	0	3
NP_346208.1	Hypothetical protein SP_1775	0	3
NP_346320.1	Oligopeptide ABC transporter, permease protein AmiD	0	3
NP_346169.1	Phosphatase, putative	0	3
NP_344770.1	Preprotein translocase subunit SecY	0	3
NP_346273.1	ABC transporter, ATP-binding/permease protein	0	2
NP_345702.1	Acetyltransferase	0	2
NP_345840.1	Cytoplasmic alpha-amylase	0	2
NP_345359.1	D-alanyl-D-alanine carboxypeptidase	0	2
NP_345263.1	DNA polymerase III subunit delta	0	2
NP_346418.1	Hydrolase, putative	0	2
NP_344975.1	Hypothetical protein SP_0454	0	2
NP_345754.1	Hypothetical protein SP_1290	0	2
NP_346134.1	Hypothetical protein SP_1696	0	2
NP_345431.1	PhoH family protein	0	2

Protein ID	Protein name	Number of peptides	
		S. pneumoniae **TIGR4***	***S. pneumoniae*** **TIGR4 *ΔspeE****
NP_345736.1	Polysaccharide biosynthesis protein, putative	0	2
NP_345831.1	Prephenate dehydrogenase	0	2
NP_346435.1	Preprotein translocase subunit SecE	0	2
NP_345448.1	Putative metalloprotease	0	2
NP_346614.1	Replicative DNA helicase	0	2
NP_345044.1	Response regulator BlpR	0	2
NP_345844.1	Spermidine/putrescine ABC transporter, spermidine/putrescine-binding p	0	2
NP_345023.1	Type I restriction-modification system, S subunit, putative	0	2
NP_346163.1	3-hydroxy-3-methylglutaryl-CoA reductase	0	1
NP_345694.1	A/G-specific adenine glycosylase	0	1
NP_345040.1	ABC transporter, ATP-binding protein	0	1
NP_345146.1	ABC transporter, ATP-binding protein	0	1
NP_346607.1	ABC transporter, ATP-binding protein	0	1
NP_344966.1	Acetolactate synthase catalytic subunit	0	1
NP_344595.1	Amidophosphoribosyltransferase	0	1
NP_345211.1	Amino acid ABC transporter, ATP-binding protein	0	1
NP_345316.1	Amino acid ABC transporter, ATP-binding protein	0	1
NP_345367.1	Aminotransferase, class-V	0	1
NP_345903.1	C3-degrading proteinase	0	1
NP_344739.1	Cardiolipin synthetase	0	1
NP_346390.1	CBS domain-containing protein	0	1
NP_345195.1	Cell division protein DivIB	0	1
NP_346266.1	Cell wall surface anchor family protein	0	1
NP_346293.1	Choline transporter	0	1
NP_346629.1	Cobalt transporter ATP-binding subunit	0	1
NP_345711.1	Cof family protein	0	1
NP_346424.1	Cof family protein	0	1
NP_344909.1	Diphosphomevalonate decarboxylase	0	1
NP_345303.1	DNA gyrase subunit B	0	1
NP_344586.1	DNA repair protein RecO	0	1
NP_345716.1	Endonuclease, putative	0	1
NP_345673.1	Exodeoxyribonuclease VII small subunit	0	1
NP_346582.1	Fucose operon repressor, putative	0	1
NP_346495.1	Glutamine amidotransferase, class-I	0	1
NP_346202.1	Glycosyl transferase family protein	0	1
NP_346015.1	GTPase EngB	0	1
NP_344563.1	Hypothetical protein SP_0010	0	1
NP_344646.1	Hypothetical protein SP_0099	0	1
NP_344725.1	Hypothetical protein SP_0184	0	1
NP_344901.1	Hypothetical protein SP_0374	0	1
NP_345129.1	Hypothetical protein SP_0617	0	1
NP_345147.1	Hypothetical protein SP_0637	0	1
NP_345178.1	Hypothetical protein SP_0673	0	1
NP_345387.2	Hypothetical protein SP_0902	0	1
NP_345481.1	Hypothetical protein SP_1004	0	1
NP_345575.1	Hypothetical protein SP_1104	0	1
NP_345577.1	Hypothetical protein SP_1106	0	1
NP_345882.1	Hypothetical protein SP_1425	0	1
NP_346054.1	Hypothetical protein SP_1610	0	1
NP_346408.1	Hypothetical protein SP_1981	0	1
NP_346452.1	Hypothetical protein SP_2027	0	1
NP_346633.1	Hypothetical protein SP_2225	0	1
NP_345570.1	Inorganic polyphosphate/ATP-NAD kinase	0	1
NP_346515.1	M20/M25/M40 family peptidase	0	1
NP_344723.1	MccC family protein	0	1
NP_344929.1	MutS2 family protein	0	1
NP_345194.1	N-acetylglucosaminyl transferase	0	1
NP_346112.1	Penicillin-binding protein 2B	0	1
NP_344871.1	Penicillin-binding protein 2X	0	1
NP_345535.1	Protein kinase, putative	0	1
NP_345654.1	PTS system, lactose-specific IIBC components	0	1
NP_346635.1	Recombination protein F	0	1
NP_345179.1	Ribonuclease Z	0	1
NP_345872.1	Ribosomal protein S21	0	1
NP_345504.1	RNA methyltransferase	0	1
NP_346603.1	Sensor histidine kinase	0	1
NP_345116.1	Sensor histidine kinase VncS	0	1
NP_345847.1	Spermidine/putrescine ABC transporter, ATP-binding protein	0	1
NP_345422.1	Tetrapyrrole methylase family protein	0	1

Protein ID	Protein name	Number of peptides	
		S. pneumoniae* TIGR4	***S. pneumoniae* TIGR4 *ΔspeE****
NP_345164.1	Thioredoxin family protein	0	1
NP_345419.1	Thymidylate kinase	0	1
NP_345090.1	Transcription antiterminator Lict	0	1
NP_344786.1	Transcriptional regulator	0	1
NP_344627.1	Trk family potassium uptake protein	0	1
NP_345379.1	Type I restriction-modification system, R subunit, putative	0	1
NP_346109.1	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate–D-alanyl-D-	0	1

*Number of peptides identified at a peptide probability of $P \leq 0.05$.

Polyamine biosynthesis and transport mechanisms are crucial for fitness and pathogenesis of *Streptococcus pneumoniae*

By: Pratik Shah, Bindu Nanduri, Edwin Swiatlo, Yinfa Ma and Ken Pendarvis

Supplementary Table S3. Tandem MS analysis and differential protein expression in the Δ*speE* strain compared with wild-type TIGR4

Protein ID	Protein name	∑Xcorr *S. pneumoniae* TIGR4*	∑Xcorr *S. pneumoniae* TIGR4 Δ*speE**	$\log_2$ ratio of abundance
NP_344947.1	(3R)-hydroxymyristoyl-ACP dehydratase	20.8	3.7	–2.3
NP_345276.1	16S rRNA-processing protein	23.6	5.9	–1.9
NP_346516.1	2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase, putati	41.5	16.5	–1.3
NP_344774.1	30S ribosomal protein S13	122.7	160.9	0.4
NP_344758.1	30S ribosomal protein S17	0	17.3	5.2
NP_345329.1	30S ribosomal protein S20	29.2	52.7	0.9
NP_344755.1	30S ribosomal protein S3	110.2	151.6	0.5
NP_344633.1	30S ribosomal protein S4	179.3	223.6	0.4
NP_344764.1	30S ribosomal protein S8	134.6	80.2	–0.7
NP_345833.1	3-dehydroquinate synthase	13.8	2.3	–2.3
NP_344944.1	3-ketoacyl-(acyl-carrier-protein) reductase	233.4	158.1	–0.5
NP_344940.1	3-oxoacyl-(acyl carrier protein) synthase III	0	10.7	4.5
NP_345829.1	3-phosphoshikimate 1-carboxyvinyltransferase	7.0	33.0	2.2
NP_345141.1	50S ribosomal protein L11	139.8	96.8	–0.5
NP_344756.1	50S ribosomal protein L16	101.5	65.1	–0.6
NP_345442.1	50S ribosomal protein L20	68.3	108.4	0.7
NP_344751.1	50S ribosomal protein L23	150.3	111.4	–0.4
NP_346552.1	50S ribosomal protein L32	17.7	42.5	1.3
NP_344750.1	50S ribosomal protein L4	154.8	197.4	0.4
NP_346414.1	ABC transporter, ATP-binding protein	12.5	2.4	–2.2
NP_344946.1	Acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	73.5	45.4	–0.7

Protein ID	Protein name	∑Xcorr *S. pneumoniae* TIGR4*	∑Xcorr *S. pneumoniae* TIGR4 Δ*speE**	$\log_2$ ratio of abundance
NP_346023.1	Adenine phosphoribosyltransferase	41.3	21.1	–0.9
NP_344571.1	Adenylosuccinate synthetase	132.4	82.1	–0.7
NP_344823.1	Alcohol dehydrogenase	99.1	76.2	–0.3
NP_345932.1	Aldo/keto reductase family oxidoreductase	61.2	35.7	–0.7
NP_345914.1	Amino acid ABC transporter, ATP-binding protein	10.1	0	–4.4
NP_345707.1	Amino acid ABC transporter, ATP-binding protein	63.9	38.1	–0.7
NP_344742.1	Anaerobic ribonucleoside triphosphate reductase	71.4	41.3	–0.8
NP_346499.1	Arginyl-tRNA synthetase	191.2	242.4	0.4
NP_345741.1	Aspartate carbamoyltransferase catalytic subunit	43.2	20.8	–1.0
NP_346533.1	Aspartyl-tRNA synthetase	65.0	99.9	0.7
NP_346016.1	ATP-dependent protease ATP-binding subunit	24.3	40.4	0.8
NP_345248.1	Branched-chain amino acid ABC transporter, amino acid-binding protein	79.6	47.8	–0.7
NP_344650.1	Capsular polysaccharide biosynthesis protein, putative	32.2	57.9	0.9
NP_345406.1	Carbon-nitrogen hydrolase family protein	0	7.2	4.0
NP_345255.1	Cell division ABC transporter, permease protein FtsX	16.9	2.6	–2.4
NP_346100.1	Cell division protein DivIVA	59.5	23.7	–1.3
NP_344566.1	Cell division protein FtsH	153.1	212.8	0.5
NP_346336.1	Chaperonin GroEL	352.9	247.9	–0.5

*Number of peptides identified at a peptide probability of $P \leq 0.05$.

Polyamine biosynthesis and transport mechanisms are crucial for fitness and pathogenesis of *Streptococcus pneumoniae*

By: Pratik Shah, Bindu Nanduri, Edwin Swiatlo, Yinfa Ma and Ken Pendarvis

Supplementary Table S2. Tandem MS analysis and differential protein expression in the Δ*potABCD* strain compared with wild-type TIGR4

Protein ID	Protein name	∑Xcorr *S. pneumoniae* TIGR4*	∑Xcorr *S. pneumoniae* TIGR4 *Δ potABCD**	$\log_2$ ratio of abundance
NP_345363.1	1-phosphofructokinase, putative	54.2	25.8	–0.8
NP_345350.1	30S ribosomal protein S1	195.0	132.1	–0.3
NP_345273.1	30S ribosomal protein S16	89.6	118.6	0.7
NP_346623.1	30S ribosomal protein S2	126.1	63.0	–0.7
NP_344767.1	30S ribosomal protein S5	188.3	124.3	–0.3
NP_344764.1	30S ribosomal protein S8	111.4	79.2	–0.2
NP_344833.1	30S ribosomal protein S9	31.0	49.8	1.0
NP_344945.1	3-oxoacyl-(acyl carrier protein) synthase II	153.6	98.0	–0.3
NP_345142.1	50S ribosomal protein L1	92.2	58.2	–0.4
NP_345141.1	50S ribosomal protein L11	110.9	58.8	–0.6
NP_344759.1	50S ribosomal protein L14	66.7	46.9	–0.2
NP_344769.1	50S ribosomal protein L15	0	11.4	4.9
NP_344756.1	50S ribosomal protein L16	0	25.9	6.0
NP_344752.1	50S ribosomal protein L2	0	51.0	7.0
NP_345578.1	50S ribosomal protein L27	7.7	21.9	1.8
NP_344757.1	50S ribosomal protein L29	13.0	0	–4.4
NP_344749.1	50S ribosomal protein L3	0	9.6	4.6
NP_346552.1	50S ribosomal protein L32	0	13.2	5.1
NP_344761.1	50S ribosomal protein L5	247.6	170.4	–0.2
NP_345812.1	50S ribosomal protein L7/L12	305.3	246.4	0.0
NP_345383.1	6-phosphofructokinase	106.3	64.0	–0.4
NP_344902.1	6-phosphogluconate dehydrogenase	186.9	124.7	–0.3

Protein ID	Protein name	∑Xcorr *S. pneumoniae* TIGR4*	∑Xcorr *S. pneumoniae* TIGR4 *Δ potABCD**	log_2 ratio of abundance
NP_345354.1	ABC transporter, ATP-binding protein	20.5	50.6	1.6
NP_344946.1	Acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	0	12.9	5.0
NP_344823.1	Alcohol dehydrogenase	402.9	298.0	–0.1
NP_346479.1	Alcohol dehydrogenase, zinc-containing	197.5	134.8	–0.2
NP_345294.1	Aminopeptidase N	90.1	50.7	–0.5
NP_344936.1	Aspartate kinase	39.5	18.9	–0.7
NP_344958.1	Aspartyl/glutamyl-tRNA amidotransferase subunit B	43.5	74.2	1.1
NP_346533.1	Aspartyl-tRNA synthetase	28.4	10.4	–1.1
NP_346451.1	Bifunctional acetaldehyde-CoA/alcohol dehydrogenase	64.2	144.9	1.5
NP_345899.1	Bifunctional GMP synthase/glutamine amidotransferase protein	85.5	48.6	–0.5
NP_345248.1	Branched-chain amino acid ABC transporter, amino acid-binding protein	35.3	14.4	–1.0
NP_344881.1	Capsular polysaccharide biosynthesis protein Cps4C	12.6	0	–4.4
NP_344891.1	Capsular polysaccharide biosynthesis protein Cps4J	78.4	36.3	–0.8
NP_345733.1	Choline kinase	18.4	0	–4.9
NP_345407.1	Cof family protein	0	9.8	4.7
NP_346047.1	Cytidylate kinase	26.6	5.2	–1.9
NP_344804.1	D-fructose-6-phosphate amidotransferase	116.5	49.3	–0.9
NP_346622.1	Elongation factor Ts	176.5	125.2	–0.2
NP_345941.1	Elongation factor Tu	643.0	437.1	–0.3
NP_345186.1	Elongation factor Tu family protein	25.0	8.8	–1.1
NP_346278.1	ExoDNase	29.2	7.3	–1.6
NP_345961.1	F0F1 ATP synthase subunit alpha	38.0	20.7	–0.6
NP_345959.1	F0F1 ATP synthase subunit beta	97.2	43.9	–0.8
NP_344979.1	Formate acetyltransferase	133.4	180.7	0.7
NP_345117.1	Fructose-bisphosphate aldolase	336.6	224.1	–0.3

Protein ID	Protein name	∑Xcorr *S. pneumoniae* TIGR4*	∑Xcorr *S. pneumoniae* TIGR4 *Δ potABCD**	log_2 ratio of abundance
NP_346237.1	General stress protein 24, putative	141.0	225.6	1.0
NP_344876.1	Glucan 1,6-alpha-glucosidase	12.0	2.3	−1.9
NP_345173.2	Glucokinase	38.9	15.3	−1.0
NP_345708.1	Glucose-6-phosphate 1-dehydrogenase	2.8	21.0	3.0
NP_346493.1	Glucose-6-phosphate isomerase	141.9	57.0	−1.0
NP_345769.1	Glutamate dehydrogenase	127.1	66.7	−0.6
NP_346439.1	Glyceraldehyde-3-phosphate dehydrogenase	892.4	607.6	−0.3
NP_345590.1	Glyceraldehyde-3-phosphate dehydrogenase, NADP-dependent	39.1	19.0	−0.7
NP_345552.1	GTPase ObgE	26.0	3.2	−2.5
NP_345039.1	HIT family protein	23.1	8.0	−1.2
NP_344642.1	Hypothetical protein SP_0095	2.3	39.6	4.1
NP_344644.1	Hypothetical protein SP_0097	16.5	46.1	1.8
NP_344899.1	Hypothetical protein SP_0372	26.4	0	−5.4
NP_344964.1	Hypothetical protein SP_0443	36.0	12.1	−1.2
NP_345279.1	Hypothetical protein SP_0782	9.3	0	−4.0
NP_345309.1	Hypothetical protein SP_0816	32.2	3.8	−2.6
NP_345314.1	Hypothetical protein SP_0822	7.6	0	−3.7
NP_345355.1	Hypothetical protein SP_0868	0	6.5	4.1
NP_345980.1	Hypothetical protein SP_1531	2.7	18.4	2.9
NP_344565.1	Hypoxanthine-guanine phosphoribosyltransferase	86.2	52.7	−0.4
NP_345358.1	Intein-containing protein	2.2	19.1	3.2
NP_345507.1	Iron-compound ABC transporter, iron compound-binding protein	29.6	8.1	−1.5
NP_346098.1	Isoleucyl-tRNA synthetase	2.1	22.9	3.5
NP_344968.1	Ketol-acid reductoisomerase	23.4	3.3	−2.3
NP_345362.1	Lactose phosphotransferase system repressor	17.9	3.1	−2.0

Protein ID	Protein name	∑Xcorr *S. pneumoniae* TIGR4*	∑Xcorr *S. pneumoniae* TIGR4 *Δ potABCD**	log_2 ratio of abundance
NP_345336.1	Lipoprotein	172.9	82.4	–0.8
NP_345686.1	L-lactate dehydrogenase	226.2	165.1	–0.1
NP_346527.1	Maltose/Maltodextrin ABC transporter, maltose/maltodextrin-binding pro	256.1	200.8	0.0
NP_345035.1	Molecular chaperone DnaK	163.3	118.9	–0.2
NP_346115.1	*N*-acetylneuraminate lyase, putative	64.0	40.5	–0.3
NP_346019.1	Non-haem iron-containing ferritin	223.1	162.6	–0.2
NP_346213.1	Oligoendopeptidase F, putative	0	10.2	4.7
NP_344894.1	Oligopeptide ABC transporter, oligopeptide-binding protein AliA	48.0	13.9	–1.5
NP_346322.1	Oligopeptide ABC transporter, oligopeptide-binding protein AmiA	135.7	84.9	–0.4
NP_345095.1	Phenylalanyl-tRNA synthetase subunit beta	14.4	0	–4.6
NP_345645.1	Phosphoenolpyruvate-protein phosphotransferase	65.6	41.5	–0.4
NP_345949.1	Phosphoglucomutase	123.2	78.0	–0.4
NP_345017.1	Phosphoglycerate kinase	662.3	548.6	0.0
NP_345598.1	Phosphopyruvate hydratase	1124.1	796.7	–0.2
NP_344663.1	Pneumococcal surface protein A	159.8	122.5	–0.1
NP_346351.1	Pneumolysin	48.5	23.2	–0.7
NP_344802.1	Prolyl-tRNA synthetase	52.8	30.7	–0.5
NP_345364.1	PTS system, fructose specific IIABC components	26.5	3.8	–2.3
NP_344822.1	PTS system, mannose-specific IIAB components	237.3	140.4	–0.5
NP_345384.1	Pyruvate kinase	191.2	125.5	–0.3
NP_345648.1	Ribonucleotide-diphosphate reductase subunit alpha	60.7	86.7	0.8
NP_346411.1	Ribosome-associated GTPase	0	12.3	5.0
NP_345546.1	RNA polymerase sigma factor RpoD	57.5	33.5	–0.5
NP_345151.1	Serine protease	0	8.8	4.5
NP_346646.1	Serine protease	48.1	18.1	–1.1

Protein ID	Protein name	∑Xcorr *S. pneumoniae* TIGR4*	∑Xcorr *S. pneumoniae* TIGR4 *Δ potABCD**	log_2 ratio of abundance
NP_346570.1	SPFH domain-containing protein/band 7 family protein	11.6	25.9	1.4
NP_344874.1	S-ribosylhomocysteinase	19.0	7.0	−1.1
NP_346325.1	Sucrose phosphorylase	12.4	30.9	1.6
NP_345659.1	Tagatose 1,6-diphosphate aldolase	52.4	80.0	0.9
NP_346455.1	Transketolase	86.0	53.6	−0.4
NP_344772.1	Translation initiation factor IF-1	9.7	0	−4.0
NP_344923.1	Trigger factor	253.5	105.8	−1.0
NP_344628.1	Trk family potassium uptake protein	26.5	9.0	−1.2
NP_345491.1	tRNA modification GTPase TrmE	0	6.5	4.1
NP_346637.1	Tryptophanyl-tRNA synthetase II	21.3	6.8	−1.3
NP_344893.1	UDP-*N*-acetylglucosamine 2-epimerase	8.1	0	−3.8

*Number of peptides identified at a peptide probability of $P \leq 0.05$

Polyamine biosynthesis and transport mechanisms are crucial for fitness and pathogenesis of *Streptococcus pneumoniae*

By: Pratik Shah, Bindu Nanduri, Edwin Swiatlo, Yinfa Ma and Ken Pendarvis

Supplementary Table S1. Tandem MS analysis of *S. pneumoniae* TIGR4 and TIGR4 Δ*potABCD*

Protein ID	Protein name	Number of peptides	
		S. pneumoniae* TIGR4	***S. pneumoniae* TIGR4 Δ*potABCD***
NP_345598.1	Phosphopyruvate hydratase	291	206
NP_346439.1	Glyceraldehyde-3-phosphate dehydrogenase	251	173
NP_345017.1	Phosphoglycerate kinase	201	166
NP_345231.1	Pyruvate oxidase	181	168
NP_345941.1	Elongation factor Tu	176	122
NP_344823.1	Alcohol dehydrogenase	105	80
NP_344811.1	Elongation factor G	98	80
NP_345812.1	50S ribosomal protein L7/L12	88	72
NP_345117.1	Fructose-bisphosphate aldolase	85	60
NP_346527.1	Maltose/Maltodextrin ABC transporter, maltose/maltodextrin-binding pro	76	62
NP_344923.1	Trigger factor	69	29
NP_344761.1	50S ribosomal protein L5	68	50
NP_345350.1	30S ribosomal protein S1	67	45
NP_344822.1	PTS system, mannose-specific IIAB components	61	37
NP_345686.1	L-lactate dehydrogenase	56	41
NP_344767.1	30S ribosomal protein S5	56	38
NP_346089.1	Manganese ABC transporter, manganese-binding adhesion liprotein	54	51
NP_346122.1	Sugar ABC transporter, sugar-binding protein	53	54
NP_346019.1	Non-heme iron-containing ferritin	52	37
NP_346479.1	Alcohol dehydrogenase, zinc-containing	51	37
NP_345384.1	Pyruvate kinase	51	34
NP_345336.1	Lipoprotein	51	24
NP_345035.1	Molecular chaperone DnaK	49	37
NP_344902.1	6-phosphogluconate dehydrogenase	49	36
NP_346094.1	Phosphoglyceromutase	46	44
NP_344765.1	50S ribosomal protein L6	43	35
NP_344663.1	Pneumococcal surface protein A	43	34
NP_345584.1	DNA-binding protein HU	42	36
NP_346622.1	Elongation factor Ts	42	33
NP_346336.1	Chaperonin GroEL	41	40
NP_345216.1	Lactate oxidase	41	38
NP_346623.1	30S ribosomal protein S2	39	20
NP_344810.1	30S ribosomal protein S7	38	35
NP_345923.1	NADH oxidase	37	44
NP_344945.1	3-oxoacyl-(acyl carrier protein) synthase II	37	26
NP_346493.1	Glucose-6-phosphate isomerase	37	17
NP_344979.1	Formate acetyltransferase	36	49
NP_345499.1	Serine hydroxymethyltransferase	36	29
NP_346322.1	Oligopeptide ABC transporter, oligopeptide-binding protein AmiA	36	23
NP_346020.1	Triosephosphate isomerase	35	35
NP_345769.1	Glutamate dehydrogenase	35	22
NP_346237.1	General stress protein 24, putative	34	55
NP_345989.1	30S ribosomal protein S6	34	35
NP_345763.1	50S ribosomal protein L31 type B	33	36
NP_346105.1	Cell division protein FtsZ	33	33
NP_344764.1	30S ribosomal protein S8	32	24
NP_345949.1	Phosphoglucomutase	32	23
NP_344804.1	D-fructose-6-phosphate amidotransferase	31	14
NP_346636.1	Inositol-5-monophosphate dehydrogenase	30	23
NP_345383.1	6-phosphofructokinase	30	18
NP_345646.1	Phosphocarrier protein HPr	29	39
NP_345141.1	50S ribosomal protein L11	29	17
NP_346026.1	Sugar ABC transporter, ATP-binding protein	28	34
NP_346209.1	Thioredoxin	28	31
NP_344760.1	50S ribosomal protein L24	28	30
NP_345748.1	Hypothetical protein SP_1284	27	25
NP_345142.1	50S ribosomal protein L1	27	16
NP_345959.1	F0F1 ATP synthase subunit beta	27	14

Protein ID	Protein name	Number of peptides	
		S. pneumoniae **TIGR4***	***S. pneumoniae*** **TIGR4 Δ*potABCD***
NP_346397.1	Asparagine synthetase AsnA	26	23
NP_345950.1	Bacterocin transport accessory protein	26	23
NP_345294.1	Aminopeptidase N	26	16
NP_345244.1	Uracil phosphoribosyltransferase	25	23
NP_345899.1	Bifunctional GMP synthase/glutamine Amidotransferase protein	25	14
NP_346426.1	Catabolite control protein A	24	29
NP_345813.1	50S ribosomal protein L10	24	24
NP_346499.1	Arginyl-tRNA synthetase	24	21
NP_346617.1	Ribosomal subunit interface protein	24	18
NP_344565.1	Hypoxanthine-guanine phosphoribosyltransferase	24	16
NP_345273.1	30S ribosomal protein S16	23	32
NP_346087.1	Endopeptidase O	23	29
NP_344957.1	Elongation factor P	23	18
NP_346455.1	Transketolase	23	15
NP_344891.1	Capsular polysaccharide biosynthesis protein Cps4J	23	12
NP_344759.1	50S ribosomal protein L14	22	14
NP_346115.1	N-acetylneuraminate lyase, putative	22	14
NP_345910.1	Peptide deformylase	21	16
NP_344748.1	30S ribosomal protein S10	20	18
NP_346451.1	Bifunctional acetaldehyde-CoA/alcohol dehydrogenase	19	41
NP_345572.1	Phosphotransacetylase	19	20
NP_346519.1	Tyrosyl-tRNA synthetase	19	16
NP_345645.1	Phosphoenolpyruvate-protein phosphotransferase	19	12
NP_345648.1	Ribonucleotide-diphosphate reductase subunit alpha	18	28
NP_344972.1	Hypothetical protein SP_0451	18	18
NP_344776.1	DNA-directed RNA polymerase subunit alpha	18	13
NP_345573.1	Hypothetical protein SP_1102	18	12
NP_344802.1	Prolyl-tRNA synthetase	18	11
NP_345428.1	Ribosome recycling factor	17	22
NP_346238.1	Hypothetical protein SP_1805	17	20
NP_344755.1	30S ribosomal protein S3	17	18
NP_344944.1	3-ketoacyl-(acyl-carrier-protein) reductase	17	17
NP_344832.1	50S ribosomal protein L13	17	17
NP_345830.1	Hypothetical protein SP_1372	17	16
NP_345968.1	Hypothetical protein SP_1518	17	12
NP_345320.1	Phosphopentomutase	16	15
NP_346511.1	UTP-glucose-1-phosphate uridylyltransferase	16	14
NP_345982.1	Putative manganese-dependent inorganic pyrophosphatase	16	13
NP_344941.1	Acyl carrier protein	16	12
NP_346297.1	Glutamyl-aminopeptidase	16	10
NP_345659.1	Tagatose 1,6-diphosphate aldolase	15	23
NP_345322.1	Purine nucleoside phosphorylase	15	13
NP_346285.1	Galactokinase	15	12
NP_346328.1	Sugar ABC transporter, sugar-binding protein	15	9
NP_345363.1	1-phosphofructokinase, putative	15	8
NP_346351.1	Pneumolysin	15	8
NP_346646.1	Serine protease	15	6
NP_344894.1	Oligopeptide ABC transporter, oligopeptide-binding protein AliA	15	4
NP_344775.1	30S ribosomal protein S11	14	11
NP_345285.1	Methionyl-tRNA synthetase	14	10
NP_345020.1	Glutamine synthetase, type I	14	9
NP_345546.1	RNA polymerase sigma factor RpoD	14	9
NP_346037.1	Proline dipeptidase	13	13
NP_345967.1	Transcription elongation factor GreA	13	13
NP_344959.1	Aspartyl/glutamyl-tRNA amidotransferase subunit A	13	11
NP_346014.1	Endoribonuclease L-PSP	13	11
NP_346615.1	50S ribosomal protein L9	13	10
NP_346421.1	Aminotransferase AlaT	13	10
NP_346480.1	N-acetylglucosamine-6-phosphate deacetylase	13	10
NP_344934.1	Seryl-tRNA synthetase	13	10
NP_344571.1	Adenylosuccinate synthetase	13	8
NP_344936.1	Aspartate kinase	13	7
NP_346051.1	UDP-glucose 4-epimerase	12	11
NP_346469.1	Acetate kinase	12	8
NP_345462.1	Foldase protein PrsA	12	8
NP_345961.1	F0F1 ATP synthase subunit alpha	12	6
NP_345590.1	Glyceraldehyde-3-phosphate dehydrogenase, NADP-dependent	12	6
NP_344964.1	Hypothetical protein SP_0443	12	5
NP_345173.2	Glucokinase	12	4

Protein ID	Protein name	Number of peptides	
		S. pneumoniae* TIGR4	***S. pneumoniae* TIGR4 Δ*potABCD***
NP_344958.1	Aspartyl/glutamyl-tRNA amidotransferase subunit B	11	16
NP_344633.1	30S ribosomal protein S4	11	15
NP_344771.1	Adenylate kinase	11	12
NP_344766.1	50S ribosomal protein L18	11	9
NP_346544.1	Dihydroxy-acid dehydratase	11	3
NP_344833.1	30S ribosomal protein S9	10	18
NP_344754.1	50S ribosomal protein L22	10	16
NP_344690.1	ABC transporter, substrate-binding protein	10	11
NP_344819.1	Aminopeptidase C	10	11
NP_344820.1	PTS system, mannose-specific IID component	10	11
NP_344942.1	Enoyl-(acyl-carrier-protein) reductase	10	7
NP_345248.1	Branched-chain amino acid ABC transporter, amino acid-binding protein	10	4
NP_345175.1	Hypothetical protein SP_0670	9	8
NP_346434.1	Transcription antitermination protein NusG	9	7
NP_345134.1	Nitroreductase family protein	9	5
NP_345309.1	Hypothetical protein SP_0816	9	1
NP_346090.2	Thiol peroxidase	8	14
NP_344816.1	Aminopeptidase PepS	8	9
NP_346337.1	Co-chaperonin GroES	8	9
NP_344892.1	Capsular polysaccharide biosynthesis protein Cps4K	8	8
NP_345878.1	Nicotinate phosphoribosyltransferase	8	8
NP_344639.1	ABC transporter, substrate-binding protein	8	6
NP_346153.1	Hypothetical protein SP_1715	8	6
NP_345348.1	Pyrrolidone-carboxylate peptidase	8	6
NP_345757.1	50S ribosomal protein L19	8	5
NP_346492.1	Glutamyl-tRNA synthetase	8	5
NP_346533.1	Aspartyl-tRNA synthetase	8	3
NP_346278.1	Exodeoxyribonuclease	8	2
NP_345507.1	Iron-compound ABC transporter, iron compound-binding protein	8	2
NP_344628.1	Trk family potassium uptake protein	8	2
NP_345354.1	ABC transporter, ATP-binding protein	7	16
NP_346638.1	ABC transporter, ATP-binding protein	7	10
NP_344948.1	Acetyl-CoA carboxylase biotin carboxylase subunit	7	8
NP_346106.1	Cell division protein FtsA	7	8
NP_345467.1	UDP-N-acetylglucosamine pyrophosphorylase	7	8
NP_345819.1	Homoserine dehydrogenase	7	7
NP_344943.1	acyl-carrier-protein S-malonyltransferase	7	6
NP_345988.1	Single-strand DNA-binding protein	7	6
NP_345557.1	Methionine aminopeptidase	7	5
NP_345329.1	30S ribosomal protein S20	7	4
NP_345707.1	Amino acid ABC transporter, ATP-binding protein	7	4
NP_344585.1	Aromatic amino acid aminotransferase	7	4
NP_344779.1	Hypothetical protein SP_0239	7	4
NP_344950.1	Acetyl-CoA carboxylase subunit alpha	7	3
NP_345853.1	Phosphate transport system regulatory protein PhoU, putative	7	3
NP_344874.1	S-ribosylhomocysteinase	7	3
NP_346047.1	Cytidylate kinase	7	2
NP_346637.1	Tryptophanyl-tRNA synthetase II	7	2
NP_345552.1	GTPase ObgE	7	1
NP_344968.1	Ketol-acid reductoisomerase	7	1
NP_345364.1	PTS system, fructose specific IIABC components	7	1
NP_344899.1	Hypothetical protein SP_0372	7	0
NP_344777.1	50S ribosomal protein L17	6	10
NP_345262.1	Dihydroorotate dehydrogenase 1A	6	9
NP_345264.1	Superoxide dismutase, manganese-dependent	6	9
NP_344753.1	30S ribosomal protein S19	6	8
NP_345101.1	Polynucleotide phosphorylase/polyadenylase	6	8
NP_346023.1	Adenine phosphoribosyltransferase	6	7
NP_346002.1	Dihydrodipicolinate reductase	6	6
NP_345269.1	Peptidyl-prolyl cis-trans isomerase, cyclophilin-type	6	6
NP_345334.1	Deoxyribose-phosphate aldolase	6	5
NP_345034.1	Heat shock protein GrpE	6	4
NP_345926.1	Oxidoreductase, putative	6	3
NP_346006.1	Phosphoglucomutase/phosphomannomutase family protein	6	3
NP_345186.1	Elongation factor Tu family protein	6	2
NP_345039.1	HIT family protein	6	2
NP_344757.1	50S ribosomal protein L29	6	0
NP_344644.1	Hypothetical protein SP_0097	5	13
NP_345877.1	NAD synthetase	5	10

Protein ID	Protein name	Number of peptides	
		S. pneumoniae **TIGR4***	***S. pneumoniae*** **TIGR4 Δ*potABCD***
NP_344566.1	Cell division protein FtsH	5	8
NP_345755.1	Cof family protein	5	5
NP_345135.1	Dipeptidase PepV	5	5
NP_346339.1	Hypothetical protein SP_1910	5	5
NP_345854.1	Phosphate ABC transporter, ATP-binding protein, putative	5	5
NP_344960.1	Aspartyl/glutamyl-tRNA amidotransferase subunit C	5	4
NP_346016.1	ATP-dependent protease ATP-binding subunit	5	4
NP_346350.1	Hypothetical protein SP_1922	5	4
NP_346321.1	Oligopeptide ABC transporter, permease protein AmiC	5	3
NP_345912.1	Thioredoxin reductase	5	3
NP_345662.1	Galactose-6-phosphate isomerase subunit LacA	5	2
NP_346525.1	Glycogen phosphorylase family protein	5	2
NP_344876.1	Glucan 1,6-alpha-glucosidase	5	1
NP_345362.1	Lactose phosphotransferase system repressor	5	1
NP_345733.1	Choline kinase	5	0
NP_346570.1	SPFH domain-containing protein/band 7 family protein	4	10
NP_346325.1	Sucrose phosphorylase	4	10
NP_346329.1	Alpha-galactosidase	4	8
NP_344578.1	Ribose-phosphate pyrophosphokinase	4	7
NP_346516.1	2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase, putati	4	6
NP_346066.1	30S ribosomal protein S15	4	6
NP_345990.1	Asparaginyl-tRNA synthetase	4	5
NP_346599.1	Hsp33-like chaperonin	4	5
NP_345649.1	Ribonucleotide-diphosphate reductase subunit beta	4	5
NP_345869.1	Hypothetical protein SP_1411	4	4
NP_345260.1	S-adenosylmethionine synthetase	4	4
NP_346624.1	Secreted 45 kd protein	4	4
NP_345841.1	Alanyl-tRNA synthetase	4	3
NP_345999.1	Cation efflux family protein	4	3
NP_346100.1	Cell division protein DivIVA	4	3
NP_346173.1	DNA-directed RNA polymerase subunit omega	4	3
NP_346510.1	NAD(P)H-dependent glycerol-3-phosphate dehydrogenase	4	3
NP_345932.1	Aldo/keto reductase family oxidoreductase	4	2
NP_345986.1	Cof family protein/peptidyl-prolyl cis-trans isomerase, cyclophilin ty	4	2
NP_345929.1	Glycyl-tRNA synthetase subunit alpha	4	2
NP_344733.1	Hypothetical protein SP_0192	4	2
NP_344963.1	Hypothetical protein SP_0442	4	2
NP_345957.1	Hypothetical protein SP_1506	4	2
NP_346311.1	Hypothetical protein SP_1879	4	2
NP_345326.1	Purine nucleoside phosphorylase	4	2
NP_344691.1	Lipoprotein	4	1
NP_345925.1	Oxidoreductase, putative	4	1
NP_344881.1	Capsular polysaccharide biosynthesis protein Cps4C	4	0
NP_345095.1	Phenylalanyl-tRNA synthetase subunit beta	4	0
NP_345578.1	50S ribosomal protein L27	3	7
NP_346521.1	Hypothetical protein SP_2102	3	5
NP_344557.1	Translation-associated GTPase	3	5
NP_345594.1	Glycogen biosynthesis protein GlgD	3	4
NP_345138.1	Hypothetical protein SP_0627	3	4
NP_345349.1	Hypothetical protein SP_0861	3	4
NP_346034.1	Pyridine nucleotide-disulfide oxidoreductase	3	4
NP_345427.1	Uridylate kinase	3	4
NP_345489.1	Aspartate-semialdehyde dehydrogenase	3	3
NP_345817.1	Bifunctional methionine sulfoxide reductase A/B protein	3	3
NP_345450.1	GTP-binding protein Era	3	3
NP_346394.1	Hypothetical protein SP_1967	3	3
NP_346386.1	Nucleoside diphosphate kinase	3	3
NP_346406.1	Purine operon repressor	3	3
NP_344883.1	Capsular polysaccharide biosynthesis protein Cps4E	3	2
NP_344573.1	Deoxyuridine 5 -triphosphate nucleotidohydrolase	3	2
NP_344555.1	DNA polymerase III subunit beta	3	2
NP_344938.1	Enoyl-CoA hydratase	3	2
NP_346312.2	Fused deoxyribonucleotide triphosphate pyrophosphatase/unknown domain	3	2
NP_345943.1	Glycerol uptake facilitator protein, putative	3	2
NP_346152.1	GntR family transcriptional regulator	3	2
NP_345403.1	Hypothetical protein SP_0919	3	2
NP_346175.1	Hypothetical protein SP_1739	3	2
NP_346318.1	Oligopeptide ABC transporter, ATP-binding protein AmiF	3	2
NP_345484.1	Peptidase T	3	2

Protein ID	Protein name	Number of peptides	
		S. pneumoniae **TIGR4***	***S. pneumoniae*** **TIGR4 Δ*potABCD***
NP_346139.1	Phospho-2-dehydro-3-deoxyheptonate aldolase	3	2
NP_345256.1	PTS system, IIABC components	3	2
NP_346489.1	Threonine synthase	3	2
NP_344940.1	3-oxoacyl-(acyl carrier protein) synthase III	3	1
NP_345012.1	CTP synthetase	3	1
NP_345636.1	Dihydroorotase	3	1
NP_345873.1	Glucosamine-6-phosphate isomerase	3	1
NP_345038.1	Hypothetical protein SP_0520	3	1
NP_344896.1	Penicillin-binding protein 1A	3	1
NP_345073.1	Ribosome-binding factor A	3	1
NP_345751.1	Signal recognition particle protein	3	1
NP_345027.1	Type I restriction-modification system, M subunit	3	1
NP_345554.1	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	3	1
NP_345084.1	Valyl-tRNA synthetase	3	1
NP_345279.1	Hypothetical protein SP_0782	3	0
NP_345314.1	Hypothetical protein SP_0822	3	0
NP_344772.1	Translation initiation factor IF-1	3	0
NP_344893.1	UDP-N-acetylglucosamine 2-epimerase	3	0
NP_344774.1	30S ribosomal protein S13	2	4
NP_345952.1	Amino acid ABC transporter, ATP-binding protein	2	4
NP_346407.1	Cmp-binding-factor 1	2	4
NP_344875.1	Hypothetical protein SP_0341	2	4
NP_344780.1	Phosphoglycerate mutase family protein	2	4
NP_344587.1	Putative glycerol-3-phosphate acyltransferase PlsX	2	4
NP_346270.1	Capsular polysaccharide biosynthesis protein, putative	2	3
NP_345761.1	Flavodoxin	2	3
NP_345443.1	Lactoylglutathione lyase	2	3
NP_345214.2	Lysyl-tRNA synthetase	2	3
NP_344949.1	Acetyl-CoA carboxylase subunit beta	2	2
NP_344605.1	Adenylosuccinate lyase	2	2
NP_346174.1	Guanylate kinase	2	2
NP_345068.1	Hypothetical protein SP_0552	2	2
NP_346046.1	phnA protein	2	2
NP_346482.1	Queuine tRNA-ribosyltransferase	2	2
NP_345971.1	UDP-N-acetylmuramate--L-alanine ligase	2	2
NP_345740.1	Carbamoyl phosphate synthase small subunit	2	1
NP_346110.1	D-alanyl-alanine synthetase A	2	1
NP_345963.1	F0F1 ATP synthase subunit B	2	1
NP_345958.1	F0F1 ATP synthase subunit epsilon	2	1
NP_344720.1	Holliday junction DNA helicase motor protein	2	1
NP_345871.1	HPr kinase/phosphorylase	2	1
NP_346005.1	Hypothetical protein SP_1558	2	1
NP_346214.1	Hypothetical protein SP_1781	2	1
NP_346454.1	Preprotein translocase subunit YajC	2	1
NP_345660.1	Tagatose-6-phosphate kinase	2	1
NP_345069.1	Transcription elongation factor NusA	2	1
NP_345126.1	Tributyrin esterase	2	1
NP_344890.1	UDP-N-acetylglucosamine-2-epimerase	2	1
NP_345301.1	4-methyl-5(b-hydroxyethyl)-thiazole monophosphate biosynthesis protein	2	0
NP_344974.1	Amino acid ABC transporter, amino acid-binding protein/permease protei	2	0
NP_345490.1	Dihydrodipicolinate synthase	2	0
NP_346412.1	Dimethyladenosine transferase	2	0
NP_345868.1	Hypothetical protein SP_1410	2	0
YP_873931.1	Lysozyme	2	0
NP_344667.1	Metallo-beta-lactamase superfamily protein	2	0
NP_346319.1	Oligopeptide ABC transporter, ATP-binding protein AmiE	2	0
NP_345495.1	Peptide chain release factor 1	2	0
NP_346113.1	Phosphosugar-binding transcriptional regulator, putative	2	0
NP_345567.1	Ribose-phosphate pyrophosphokinase	2	0
NP_346340.1	Thioredoxin, putative	2	0
NP_346245.1	Tryptophan synthase subunit beta	2	0
NP_344642.1	Hypothetical protein SP_0095	1	11
NP_345708.1	Glucose-6-phosphate 1-dehydrogenase	1	7
NP_346098.1	Isoleucyl-tRNA synthetase	1	7
NP_345358.1	Intein-containing protein	1	6
NP_344751.1	50S ribosomal protein L23	1	4
NP_345706.1	Amino acid ABC transporter, amino acid-binding protein/permease protei	1	4
NP_345661.1	Galactose-6-phosphate isomerase subunit LacB	1	4
NP_345980.1	Hypothetical protein SP_1531	1	4

Protein ID	Protein name	Number of peptides	
		S. pneumoniae **TIGR4***	***S. pneumoniae*** **TIGR4 Δ*potABCD***
NP_344793.1	Leucyl-tRNA synthetase	1	4
NP_345345.1	Branched-chain amino acid aminotransferase	1	3
NP_344645.1	Hypothetical protein SP_0098	1	3
NP_345183.1	Hypothetical protein SP_0678	1	3
NP_345125.1	Metallo-beta-lactamase superfamily protein	1	3
NP_346403.1	Pyruvate formate-lyase-activating enzyme	1	3
NP_345835.1	3-dehydroquinate dehydratase	1	2
NP_346526.1	4-alpha-glucanotransferase	1	2
NP_345492.1	4-oxalocrotonate tautomerase	1	2
NP_346158.1	Fructokinase	1	2
NP_345936.1	Gfo/Idh/MocA family oxidoreductase	1	2
NP_345818.1	Homoserine kinase	1	2
NP_344668.1	Hypothetical protein SP_0122	1	2
NP_346184.1	Hypothetical protein SP_1748	1	2
NP_345738.1	licD2 protein	1	2
NP_345093.1	Phenylalanyl-tRNA synthetase subunit alpha	1	2
NP_345831.1	Prephenate dehydrogenase	1	2
NP_345319.1	Ribose-5-phosphate isomerase A	1	2
NP_346215.1	Ribosomal protein L11 methyltransferase	1	2
NP_345337.1	Sugar ABC transporter, ATP-binding protein	1	2
YP_873932.1	Transketolase	1	2
NP_345735.1	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	1	1
NP_346608.1	ABC transporter, substrate-binding protein, putative	1	1
NP_345951.1	Amino acid ABC transporter, amino acid-binding protein	1	1
NP_345566.1	Aminotransferase, class-V	1	1
NP_345254.1	Cell division ABC transporter, ATP-binding protein FtsE	1	1
NP_346405.1	Diaminopimelate decarboxylase	1	1
NP_345704.1	Excinuclease ABC subunit B	1	1
NP_346284.1	Galactose-1-phosphate uridylyltransferase	1	1
NP_345865.1	HAD superfamily hydrolase	1	1
NP_344888.1	Hypothetical protein SP_0355	1	1
NP_345821.1	Hypothetical protein SP_1363	1	1
NP_345892.1	Hypothetical protein SP_1436	1	1
NP_345993.1	Hypothetical protein SP_1546	1	1
NP_346048.1	Hypothetical protein SP_1604	1	1
NP_346470.1	Hypothetical protein SP_2045	1	1
NP_345425.1	IS1381 transposase protein A	1	1
NP_345007.1	PAP2 family protein	1	1
NP_344716.1	Riboflavin synthase, beta subunit	1	1
NP_346400.1	spoU rRNA methylase family protein	1	1
NP_346606.1	Transcriptional regulator CtsR	1	1
NP_346370.1	Transcriptional regulator, putative	1	1
NP_345283.1	ABC transporter, ATP-binding protein	1	0
NP_346000.1	ABC transporter, ATP-binding protein	1	0
NP_346496.1	ABC transporter, ATP-binding/permease protein	1	0
NP_345479.1	Adhesion lipoprotein	1	0
NP_346136.1	Alanine racemase	1	0
NP_346250.1	Anthranilate synthase component I	1	0
NP_346249.1	Anthranilate synthase component II	1	0
NP_346565.1	Arginine deiminase	1	0
NP_345312.1	ATP-dependent Clp protease, ATP-binding subunit ClpE	1	0
NP_344606.1	Beta-N-acetylhexosaminidase	1	0
NP_345252.1	Branched-chain amino acid ABC transporter, ATP-binding protein	1	0
NP_345739.1	Carbamoyl phosphate synthase large subunit	1	0
NP_345998.1	Cation transporter E1-E2 family ATPase	1	0
NP_346310.1	CBS domain-containing protein	1	0
NP_346554.1	Choline binding protein PcpA	1	0
NP_344824.1	Cof family protein	1	0
NP_345583.1	degV family protein	1	0
NP_344714.1	DNA mismatch repair protein	1	0
NP_345352.1	DNA polymerase III subunits gamma and tau	1	0
NP_344914.1	DNA-binding response regulator	1	0
NP_345693.1	DNA-binding response regulator	1	0
NP_345446.1	Endo-beta-N-acetylglucosaminidase	1	0
NP_345962.1	F0F1 ATP synthase subunit delta	1	0
NP_346286.1	Galactose operon repressor	1	0
NP_345415.1	Gamma-glutamyl kinase	1	0
NP_346205.1	Glycosyl transferase family protein	1	0
NP_345824.1	Glycosyl transferase, group 1	1	0

Protein ID	Protein name	Number of peptides	
		S. pneumoniae* TIGR4	***S. pneumoniae* TIGR4 Δ*potABCD***
NP_344829.1	GTP cyclohydrolase I	1	0
NP_345640.1	HAD superfamily hydrolase	1	0
NP_345200.1	HesA/MoeB/ThiF family protein	1	0
NP_346540.1	Histidyl-tRNA synthetase	1	0
NP_344575.1	Hypothetical protein SP_0024	1	0
NP_344813.1	Hypothetical protein SP_0275	1	0
NP_344907.1	Hypothetical protein SP_0380	1	0
NP_345085.1	Hypothetical protein SP_0570	1	0
NP_345131.1	Hypothetical protein SP_0619	1	0
NP_345241.1	Hypothetical protein SP_0742	1	0
NP_345247.1	Hypothetical protein SP_0748	1	0
NP_345502.1	Hypothetical protein SP_1027	1	0
NP_345565.1	Hypothetical protein SP_1093	1	0
NP_345744.1	Hypothetical protein SP_1280	1	0
NP_345956.1	Hypothetical protein SP_1505	1	0
NP_345995.1	Hypothetical protein SP_1548	1	0
NP_346077.1	Hypothetical protein SP_1637	1	0
NP_346091.1	Hypothetical protein SP_1652	1	0
NP_346342.1	Hypothetical protein SP_1914	1	0
NP_346376.1	Hypothetical protein SP_1948	1	0
NP_346550.1	Hypothetical protein SP_2132	1	0
NP_344980.1	IS1167, transposase	1	0
NP_346355.1	IS1381 transposase protein A	1	0
NP_346029.1	Isochorismatase family protein	1	0
NP_346465.1	jag protein, putative	1	0
NP_346171.1	Methionyl-tRNA formyltransferase	1	0
NP_346600.1	NifR3 family TIM-barrel protein	1	0
NP_346033.1	Oxalate:formate antiporter	1	0
NP_346112.1	Penicillin-binding protein 2B	1	0
NP_344728.1	Peptidase M24 family protein	1	0
NP_346417.1	Primase-related protein	1	0
NP_345801.1	Prolyl oligopeptidase family protein	1	0
NP_346548.1	PTS system, IIB component, putative	1	0
NP_345154.1	PTS system, IIC component, putative	1	0
NP_346575.1	PTS system, IID component	1	0
NP_344997.1	PTS system, lactose-specific IIBC components	1	0
NP_346010.1	Pyridine nucleotide-disulphide oxidoreductase family protein	1	0
NP_346368.1	Recombinase A	1	0
NP_345782.1	ROK family protein	1	0
NP_345180.1	Short chain dehydrogenase/reductase family oxidoreductase	1	0
NP_344931.1	Sodium:alanine symporter family protein	1	0
NP_344986.1	Sortase, putative	1	0
NP_345402.1	Spermidine synthase	1	0
NP_345368.1	Thiamine biosynthesis protein ThiI	1	0
NP_344664.1	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase	1	0
NP_346423.1	Universal stress protein	1	0
NP_345773.1	V-type ATP synthase subunit D	1	0
NP_344752.1	50S ribosomal protein L2	0	14
NP_344756.1	50S ribosomal protein L16	0	8
NP_344769.1	50S ribosomal protein L15	0	4
NP_346552.1	50S ribosomal protein L32	0	4
NP_344946.1	Acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	0	4
NP_346411.1	Ribosome-associated GTPase	0	4
NP_344749.1	50S ribosomal protein L3	0	3
NP_345407.1	Cof family protein	0	3
NP_345355.1	Hypothetical protein SP_0868	0	3
NP_346213.1	Oligoendopeptidase F, putative	0	3
NP_345151.1	Serine protease	0	3
NP_345491.1	tRNA modification GTPase TrmE	0	3
NP_345991.1	Aspartate aminotransferase	0	2
NP_346063.1	Cation transporter E1-E2 family ATPase	0	2
NP_345867.1	Coproporphyrinogen III oxidase	0	2
NP_346388.1	DNA-directed RNA polymerase subunit beta	0	2
NP_346147.1	GTP-binding protein EngA	0	2
NP_346561.1	Hypothetical protein SP_2144	0	2
NP_345731.1	licC protein	0	2
NP_345235.1	Mannose-6-phosphate isomerase	0	2
NP_345227.1	Phosphomethylpyrimidine kinase	0	2
NP_345153.1	PTS system, IIB component, putative	0	2

Protein ID	Protein name	Number of peptides	
		S. pneumoniae **TIGR4***	***S. pneumoniae*** **TIGR4 Δ*potABCD***
NP_346559.1	ROK family protein	0	2
NP_346071.1	Threonyl-tRNA synthetase	0	2
NP_345193.1	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	0	2
NP_345169.1	Zinc metalloprotease ZmpB, putative	0	2
NP_345987.1	30S ribosomal protein S18	0	1
NP_345833.1	3-dehydroquinate synthase	0	1
NP_345442.1	50S ribosomal protein L20	0	1
NP_344750.1	50S ribosomal protein L4	0	1
NP_345839.1	ABC transporter, ATP-binding protein	0	1
NP_345915.1	Amino acid ABC transporter, permease protein	0	1
NP_344742.1	Anaerobic ribonucleoside triphosphate reductase	0	1
NP_346365.1	Autolysin	0	1
NP_344884.1	Capsular polysaccharide biosynthesis protein Cps4F	0	1
NP_344650.1	Capsular polysaccharide biosynthesis protein, putative	0	1
NP_346390.1	CBS domain-containing protein	0	1
NP_345814.2	Chlorohydrolase	0	1
NP_345710.1	Cof family protein	0	1
NP_346387.1	DNA-directed RNA polymerase subunit beta	0	1
NP_346281.1	DpnD protein	0	1
NP_345695.1	Formate--tetrahydrofolate ligase	0	1
NP_345672.1	Geranyltranstransferase	0	1
NP_345548.1	Glycosyl transferase CpoA	0	1
NP_344679.1	Glycosyl transferase family protein	0	1
NP_346201.1	Glycosyl transferase family protein	0	1
NP_344646.1	Hypothetical protein SP_0099	0	1
NP_344901.1	Hypothetical protein SP_0374	0	1
NP_345075.1	Hypothetical protein SP_0559	0	1
NP_345286.1	Hypothetical protein SP_0789	0	1
NP_345439.1	Hypothetical protein SP_0958	0	1
NP_345500.1	Hypothetical protein SP_1025	0	1
NP_345542.1	Hypothetical protein SP_1069	0	1
NP_345712.1	Hypothetical protein SP_1247	0	1
NP_345785.1	Hypothetical protein SP_1327	0	1
NP_345930.1	Hypothetical protein SP_1476	0	1
NP_345934.1	Hypothetical protein SP_1480	0	1
NP_346013.1	Hypothetical protein SP_1566	0	1
NP_346053.1	Hypothetical protein SP_1609	0	1
NP_346296.1	Hypothetical protein SP_1864	0	1
NP_346377.1	Hypothetical protein SP_1949	0	1
NP_345054.1	IS1381 transposase protein A	0	1
NP_345771.1	IS1381 transposase protein A	0	1
NP_344838.1	IS630-Spn1, transposase Orf2	0	1
NP_345311.1	IS630-Spn1, transposase Orf2	0	1
NP_345617.1	IS630-Spn1, transposase Orf2	0	1
NP_344921.1	Mannitol-1-phosphate 5-dehydrogenase	0	1
NP_346108.1	MutT/nudix family protein	0	1
NP_344961.1	Peptide chain release factor 3	0	1
NP_345855.1	Phosphate ABC transporter, ATP-binding protein, putative	0	1
NP_346504.1	Phosphate ABC transporter, phosphate-binding protein	0	1
NP_345858.1	Phosphate ABC transporter, phosphate-binding protein, putative	0	1
NP_346502.1	Response regulator	0	1
NP_344697.1	Sensor histidine kinase, putative	0	1
NP_345304.1	Septation ring formation regulator EzrA	0	1
NP_345911.1	spoU rRNA methylase family protein	0	1
NP_346030.1	Transcriptional repressor CodY	0	1
NP_344559.1	Transcription-repair coupling factor	0	1
NP_345675.1	Uridine kinase	0	1
NP_345381.1	x-prolyl-dipeptidyl aminopeptidase	0	1

*Number of peptides identified at a peptide probability of $P \leq 0.05$.