Supplemental Information

Interplay between manganese and zinc homeostasis in the human pathogen *Streptococcus pneumoniae*[†]

Faith E. Jacobsen,[a] Krystyna M. Kazmierczak,[b] John P. Lisher,[a] Malcolm E. Winkler[b] and David P. Giedroc[a,*]

Bacterial strains and growth conditions. Strains used in this study are listed in Table S1. Bacteria were grown on plates containing trypticase soy agar II (modified; Becton-Dickinson) and 5% (vol/vol) defibrinated sheep blood (TSAII BA) and incubated at 37°C in an atmosphere of 5% CO_2. Strains were also cultured statically in Becton-Dickinson brain heart infusion (BHI) at 37 °C in an atmosphere of 5% CO_2, and growth was monitored by OD_{620} using a Spectronic 20 spectrophotometer fitted for measurement of capped tubes (outer diameter, 16 mm). Bacteria were inoculated into BHI broth from frozen cultures, serially diluted into the same medium, and propagated overnight. Overnight cultures that were still in exponential phase (OD_{620} = 0.1 to 0.3) were diluted back to an OD_{620} of ≈0.001 to start final cultures, which did not contain antibiotics.[1] Final cultures were grown in either BHI or BHI containing 200 μM $ZnSO_4$. For isotope experiments, bacteria were grown overnight in BHI and then diluted the next morning in BHI spiked with Zn^{67} isotope (Isoflex).

Construction and verification of *S. pneumoniae* mutants. Strains containing antibiotic markers were constructed by transforming linear DNA amplicons synthesized by overlapping fusion PCR into competent pneumococcal cells as described previously.[2, 3] Primers synthesized for this study are listed in Table S2 of the supplemental material. Transformations were carried out as described before.[4] TSAII BA plates were supplemented as appropriate with the following final concentrations of antibiotics: 250 μg kanamycin per mL, 150 μg streptomycin per mL. All constructs were confirmed by DNA sequencing of the region of interest amplified from genomic DNA, which was prepared from cell lysates (10 μL of culture plus 50 μL of 1x PCR buffer; heated for 10 min at 95°C) and amplified using KOD polymerase (EMD Biosciences). Amplicons were purified using a PCR cleanup kit (Qiagen) and sequenced in reaction mixtures containing 1 μL of Big Dye Terminator reagent (Applied Biosystems) as described previously.[5] Sequences were aligned and analyzed using Vector NTI software (Invitrogen).

ICP-MS analysis. 1 mL aliquots of *S. pneumoniae* IU1781 or other strains in exponential phase were centrifuged and washed once with BHI containing 1 mM nitrilotriacetic acid (Aldrich), then twice with PBS that had been treated overnight with chelex (Biorad) according to the manufacturer's protocol. The cell pellets were dried overnight in a rotary evaporator. 400 μL of 2.5% v/v nitric acid (Ultrapure, Sigma-Aldrich) with 0.1% v/v Triton-X 100 were added to solubilize the cell pellets which were then lysed for 10 min at 95 ºC with shaking at 500 rpm followed by vigorous vortexing for 20 s. 200 μL of the lysed cell solution (equivalent to 0.5 mL total cell culture) was

added to 1.3 mL of 2.5% v/v nitric acid for ICP-MS analysis. Analyses were performed using a Perkin Elmer ELAN DRCII ICP-MS. The instrument was equipped with a Microflow PFA-ST concentric nebulizer with a 100 μL/min self-aspiration capillary, a cyclonic spray chamber, a quartz torch and platinum sampler/skimmer cones. For iron measurements, DRC mode was used with ammonia as the reacting gas. Germanium at 50 ppb was added as an internal standard using an EzyFit glass mixing chamber. Metal concentration per mg protein was determined in the following way. ICP-MS gives [metal] in μg/L * 0.0015 L sample equals total μg metal in 0.5 mL of cell culture. 20 mL of an IU1781 culture at OD_{620}=0.20 equals 420 ± 20 μg total protein[6]; thus 0.5 mL equals 10.5 μg and total protein in 0.5 mL of mid-log growth phase cells equals 52.5 μg*OD_{620}, where optical densities used here were in the linear range of detection. An OD_{620}=0.20 is equivalent to $5.5x10^7$ CFU/mL or $1.1x10^8$ cells/mL (using 1 CFU=diploid cells). This corresponds to $2.75x10^8$ cells in 0.5 mL at OD_{620}=1.0 (191 μg protein in $1x10^9$ cells). Thus, $2.75x10^8$ cells*OD_{620} equals the number of cells analyzed in 0.5 mL; using 0.644×10^{-15} L as the cell volume allows determination of the molar metal concentration (see Figure S1). We note that this determination of molar metal concentration is a rough approximation that assumes an identical cell associated volume[7], an average chain lengthof two cells for all pneumococcal strains independent of the growth media[6], and does not distinguish between cytoplasmic and externally bound metal content.

Microarray analysis. Bacteria were grown in BHI broth as described[5] but were diluted 100-fold into BHI or BHI with added metals to start final cultures, which were harvested at an OD_{620} of ~0.2. RNA extraction and purification, cDNA synthesis, labeling and hybridization to *S. pneumoniae* R6 microarrays (Ocimum Biosolutions), array washing and data collection were performed as described previously.[5] Data were collected from three independent biological replicates, including one dye swap, and analyzed using software from the TM4 Microarray Software Suite (www.tm4.org). Results files generated by GenePix Pro 6.0 software (Molecular Devices) were converted to TIGR MultiExperiment Viewer file format using ExpressConverter 2.1 software. Lowess (block) data normalization was performed using TIGR MIDAS 2.21 software. Spots with background signals higher than foreground signals and spots that were flagged as bad by the investigator or MIDAS software were removed from the analysis. Expression ratios and Bayesian *P* values were calculated as described previously.[5] The cutoff for significant changes in relative transcript amounts was set at positive or negative 1.8-fold with a Bayesian *P* value of <0.001. Intensity and expression ratio data have been deposited in the GEO database (www.ncbi.nlm.nih.gov/geo/; accession no. GSE23504).

Expression and purification of PsaR. The *psaR* gene was PCR amplified using primers psaR001 and psaR006 from *S. pneumoniae* D39 genomic DNA prepared following the manufacturer's protocol (Master Pure Gram Positive DNA Purification Kit, Epicenter). Primers psaR001 and psaR006 introduced BamH1 and Nde1 restriction sites at the ends of the gene (see Table S2). The *psaR* amplicon and pET-3a plasmid DNA were BamHI- and NdeI-digested, treated with alkaline phosphatase, and ligated using standard molecular biology protocols. The resulting pET-3a *psaR* plasmid was transformed into Rosetta quick *E. coli* cells. The PsaR-overproducing strain was grown

in LB media containing both ampicillin (100 μg/mL) and chloramphenicol (37 μg/mL) at 37 C to an OD_{600} = 0.6, and induced for protein production with IPTG (0.4 mM) for an additional 2 h of growth. The cells were harvested at 8,000 rpm (11305 x g) for 25 min and stored at -20 ºC. Cell pellets were resuspended in lysis buffer (25 mM Tris-HCl, pH 8.0, 500 mM NaCl, 2 mM DTT and 10 mM EDTA). The resuspended cells were lysed by sonication and centrifuged at 13,000 rpm (25,885 x g) for 30 min to remove the cell debris. A 10% PEI solution (0.15% v/v) was added to the supernatant to remove any nucleic acids from solution and allowed to mix for 30 min at 4 C. The precipitate was removed through centrifugation at 11,000 rpm (18533 x g) for 20 min. Finally, ammonium sulfate cuts (30% and 70%) were done to isolate the fraction containing predominantly PsaR. The dehydrated proteins were pelleted at 11,000 rpm (18533 x g) for 15 min and resuspended in the low salt equilibration buffer (25 mM Tris, pH 8.0, 50 mM NaCl, 5% v/v glycerol, 2 mM DTT, 10 mM EDTA). To remove the remaining ammonium sulfate, the resuspended sample was dialyzed extensively into the low salt buffer. The dialyzed supernatant was loaded onto an anion exchange resin (Q Sepharose, flow rate 1.5 mL/min, NaCl gradient 0.05 – 1 M over 75 min) and PsaR-containing fractions were determined on the basis of SDS-PAGE gels and pooled for further chromatography. The pooled samples were concentrated using Centrifugal Filter Units (Millipore) and applied onto a size exclusion column (Superdex G200, flow rate 1.0 mL/min). PsaR protein was identified in the peaks by SDS-PAGE and analyzed by ESI-MS for purity, and the concentration of protein was calculated using a predicted extinction coefficient of 11920 M^{-1} cm^{-1} at 280 nm (ProtParam). These pooled fractions were stored at 4 ºC.

DNA binding experiments. Using the MerMade 4 instrument, two oligonucleotides were synthesized as representative of the reported PsaR operator region (5' – TTCAAA AATTAA CTTGAC TTAATT TTT TTT-Flu – 3' and 5' – AAA AAA AATTAA GTCAAG TTAATT TTTGAA – 3') with the first DNA containing the 3' fluorescein (Flu) group for the anisotropy experiment. The oligonucleotides were cleaved using AMA (1:1 methylamine and ammonium hydroxide), ethanol precipitated, and dried overnight in a speedvac. The dried precipitate was resuspended in 500 μL Milli-Q distilled water by heating the mixture to 65 ºC. The concentration of the primers was determined by their predicted extinction coefficients of 308,300 and 286,500 M^{-1} cm^{-1} for the fluorescein labeled and unlabeled strands, respectively. The primers were annealed with a slight excess of unlabeled primer by adding the two primers together in the presence of high salt (500 mM NaCl) and heating at 65 ºC for 10 minutes. The primers were allowed to cool in the dark for several hours for optimal annealing, and aliquots of the annealed DNA were diluted to approximately 1.5 μM.

Fluorescent anisotropy measurements were performed by using an ISS PC1 spectrofluorometer and exciting the fluorescein at 487 nm. A typical anisotropy experiment was done with 10 nM duplex DNA operator in 50 mM Tris, 100 mM NaCl, 1 mM TCEP, pH 8.0, at 25.0 ºC unless noted otherwise. With apo PsaR, 0.5 mM EDTA and 2 mM DTT was added to the cuvette and the protein was kept in the purification buffer to prevent oxidation and occupation of the regulatory sites by metals. For each experiment, an acid washed quartz cuvette was set up with the above conditions for the DNA and the anisotropy measured for each addition of PsaR as the average of five

replicate measurements. The PsaR protein was prepared by adding an excess of divalent metal (Mn(II), Zn(II) or Cd(II)) to the protein, and allowing the protein to equilibrate for at least 20 min before beginning the titration. The resulting data was fitted to a two-step model involving the dimerization of the protein *(*K_{dimer}), and binding of the dimer to the DNA using Dynafit. K_{dimer} was fixed to 2.0×10^6 M^{-1} as estimated by an unconstrained fit to the Mn(II)-PsaR titration. Using this model with K_{dimer} held constant for all PsaRs, the association constant, K_1, for the binding of the PsaR dimer to the DNA operator sequence was determined as follows: for apo, 8.0×10^6 M^{-1}; Zn-bound, 5.0×10^7 M^{-1}; Mn-bound, 6.0×10^8 M^{-1}; and Cd-bound, 2.0×10^9 M^{-1} (See Fig. 4, main text).

Table S1. Strains used in the study [a]

Strain	Genotype (derivation)	Antibiotic resistance [b]	Reference or source
IU1781	D39 *rpsL1*	Str^r	[1]
IU2571	D39 *rpsL1* Δ*adcR*::[*kan^{R}rpsL$^+$*]	Kan^r	[7]
IU2594	D39 *rpsL1* Δ*adcR*	Str^r	[7]
IU3112	D39 *rpsL1* Δ*czcD*::[*kan^{R}rpsL$^+$*]	Kan^r	This paper
IU3114	D39 *rpsL1* Δ*adcR* Δ*czcD*::[*kan^{R}rpsL$^+$*]	Kan^r	This paper
	D39 *rpsL1* Δ*mntE*::[*kan^{R}rpsL$^+$*]	Kan^r	This paper
IU4024	D39 *rpsL1* Δ*mntE*	Str^r	This paper
IU4030	D39 *rpsL1* Δ*mntE* Δ*adcR*	Str^r	This paper
	D39 *rpsL1* Δ*psaA*::[*kan^{R}rpsL$^+$*]	Kan^r	This paper
IU4026	D39 *rpsL1* Δ*psaA*	Str^r	This paper
IU4036	D39 *rpsL1 AdcR-H108Q*	Str^r	[7]

[a] Strains were constructed by transformation of indicated recipients with PCR fragments synthesized by fusion PCR.
[b] Antibiotic resistance markers: Kan^r, kanamycin; Str^r, streptomycin

Table S2. Primers used in the study

Primer	Sequence (5' to 3')	Purpose
FJ czcD 001	GTCTTTTCAATCTATCTCTGGTGGGGAGAAAG CCTGCAAATTCAAGGGAA	*Construction of the ΔczcD::[kan^R rpsL$^+$] (Janus) amplicon*
FJ czcD 002	TATCCATTAAAAATCAAACGGATCCAACAATG GCATAAGTCAAATTTAAG	
FJ czcD 003	CTTAAATTTGACTTATGCCATTGTTGGATCCGT TTGATTTTTAATGGATA	
FJ czcD 004	TTTCGCTTATGGGTTTGGTGGGGCCCCTTTCC TTATGCTTTTGGA	
FJ czcD 005	TCCAAAAGCATAAGGAAAGGGGCCCCACCAA ACCCATAAGCGAAA	
FJ czcD 006	CATAGTGGAAGCGAATATATTCTGCCTGAAAAT CACCCCCCAAATTATTA	
FJ mntE 001	CAATTTTAAGAGAGTTGGCTTCTATCAAACGTA GGTCCGCTGGTACCAC	*Construction of the ΔmntE::[kan^R rpsL$^+$] (Janus) amplicon*
FJ mntE 002	CATTATCCATTAAAAATCAAACGGATCCCGAAA TACTGATAATGGCTCC	
FJ mntE 003	GGAGCCATTATCAGTATTTCGGGATCCGTTTGA TTTTTAATGGATAATG	
FJ mntE 004	GACGCCAGATGGGCCCCTTTCCTTATGCTTTTG GACGTTTAG	
FJ mntE 005	CTAAACGTCCAAAAGCATAAGGAAAGGGGCCC ATCTGGCGTC	

FJ mntE 006	CTAGATATTGCAACTTTGACAGTCTTAGAGAATT TCTTGCAAGGTTTTG	
FJ mntE 007	GTTTCGTGGCGACGCCAGATCGAAATACTGAT AATGGCTC	Construction of *ΔmntE*
FJ mntE 008	GAGCCATTATCAGTATTTCGATCTGGCGTCGC CACGAAAC	
psaA001	ATCTTGGGCCTTGACTTCTTTATCGGAGCCATT GTCTTTGGATTGCTAGC	*Construction of the ΔpsaA::[kan^R rpsL$^+$] (Janus) amplicon*
psaA007	ATTATCCATTAAAAATCAAACGGATCCAAACCA AGCATTGCCACCTGTTT	
psaA008	AAACAGGTGGCAATGCTTGGTTTGGATCCGTTT GATTTTTAATGGATAAT	
psaA009	GTTTTTCAACCAAGGTCTTGATTTGGGGCCCCT TTCCTTATGCTTTTGGA	
psaA010	TCCAAAAGCATAAGGAAAGGGGCCCCAAATCA AGACCTTGGTTGAAAAAC	
psaA004	GTCATCGTATCGGTTCTATTAGCTATTGTCATCC TTTACAATCTGACCAA	
psaAdelta001	GATAGAGTCAGTAAAGATTTGTGCAATATCAGC GATTGAGTTTGT	Construction of Δ*psaA*
psaAdelta002	ACAAACTCAATCATCGCTGATATTGCACAAATC TTTACTGACTCTATC	
psaR NdeI	GGCATATGACCCCAAACAAAGAAGACTATCTAA AATGTATTTATGAAATTGG	PsaR Cloning
psaR BamHI	CCCCTAGGTAGTTGATTTTCTCGACATAGAGTTG TTTTGCAATGTCC	

Table S3. Microarray analysis comparing relative transcript amounts from D39 *rpsL1* (IU1781) grown in BHI supplemented with 200 μM Zn(II) and in BHI without added Zn(II)[a]

Gene locus (SPD_/ spr)[b]	Average fold change	Bayesian P value	Description	Changing in Kloosterman *et al*[c]
0187/ 0183	2.2	7.5E-06	*nrdD*; Anaerobic rigonucleoside-triphosphate reductase	Increases
0189/ 0184	1.9	5.1E-04	Acetyltransferase, GNAT family	Increases
0191/ 0186	2.0	3.2E-04	Conserved hypothetical protein	Increases
0308/ 0307	1.8	2.3E-05	*clpL*; ATP-dependent Clp protease, ATP-binding subunit	Decreases
0558/ 0561	6.0	5.0E-10	*prtA*; Cell wall-associated serine protease PrtA	Increases
0616/ 0622	3.5	3.2E-08	Amino acid ABC transporter, ATP-binding protein	
0617/ 0623	2.0	6.9E-06	Amino acid ABC transporter, permease protein	
0618/ 0624	1.9	1.5E-04	Amino acid ABC transporter, permease protein	
0701/ 0707	1.8	4.3E-05	*ciaR*; DNA-binding response regulator CiaR	
0767/ 0776	-1.9	1.5E-05	*dacA*; D-alanyl-D-alanine carboxypeptidase	
0775/ 0782	2.7	1.2E-07	Conserved hypothetical protein	Decreases
0803/ 0810	2.1	3.1E-06	Conserved hypothetical protein	
0913/ 0931	2.6	1.5E-07	Conserved hypothetical protein	
1461/ 1492	4.2	2.2E-08	*psaB*; Manganese ABC transporter, ATP-binding protein	Increases
1462/ 1493	3.3	5.7E-08	*psaC*; Manganese ABC transporter, permease protein	Increases
1463/ 1494	2.9	1.2E-06	*psaA*; Manganese ABC transporter, substrate binding lipoprotein	
1504/ 1536	-1.8	7.0E-05	*nanA*; Sialidase A precursor	
1506/ 1538	1.8	6.4E-05	*axe1*; Acetyl xylan esterase, putative	
1577/ 1612	1.8	2.2E-05	Conserved hypothetical protein	
no SPD/ 1613	1.8	7.1E-05	Hypothetical protein	
1637/ 1671	10.3	1.1E-11	*nmlR*; Transcriptional regulator, MerR family	Increases
1638/ 1672	9.8	1.1E-09	*czcD*; Zn, Co, Cd cation efflux system protein	Increases

1643/ 1678	2.1	8.9E-05	*proV*; Choline transporter	
1717/ 1730	2.2	1.3E-06	Membrane protein, putative	
1718/ 1731	1.9	3.7E-05	Conserved hypothetical protein	
1871/ 1873	2.0	6.7E-04	Conserved hypothetical protein	
1874/ 1875	1.9	3.6E-05	LysM domain protein	
1933/ 1917	2.8	1.0E-05	*malM*; 4-α-glucanotransferase	
1965/ 1945	2.2	8.7E-04	*pcpA*; Choline-binding protein PcpA	Increases
1990/ 1968	1.8	3.2E-04	PTS system, IIC component	
1991/ 1969	2.2	4.1E-04	PTS system, IIB component	
1992/ 1970	2.5	1.1E-03	PTS system, IIA component	
1993/ 1971	3.0	3.0E-05	*fucU*; RbsD/ FucU transport protein family	
1994/ 1972	4.5	7.9E-10	*fucA*; L-fuculose phosphate aldolase	
1995/ 1973	3.8	2.8E-09	*fcsK*; L-fuculose kinase	
2068/ 2045	2.4	7.7E-07	*htrA*; Serine protease HtrA	
2069/ 2046	1.8	7.6E-05	*spo0J*; Spo0J protein	

[a]Microarray analyses were performed as described in Material and Methods. Fold changes are expressed relative to IU1781 grown in BHI without added Zn(II) and are the average of three independent biological replicates, including one dye swap. Changes in relative transcript amounts of positive or negative 1.8 fold with Bayesian P value of <0.001 were considered significant.
[b]SPD_gene tags refer to strain D39 used in this study. Corresponding spr gene tags for laboratory strain R6 are shown for convenience.
[c]Changes in relative transcript amounts observed for D39 grown in GM17 supplemented with 250 μM Zn(II) compared to D39 grown in GM17 without added Zn(II)[7] are shown for comparison.

Table S4. Microarray analysis comparing relative transcript amounts from D39 *rpsL1 AdcR-H108Q* (IU4036) grown in BHI supplemented with 200 μM Zn(II) and its parent strain D39 *rpsL1 adcR*$^{+}$ (IU1781) grown in BHI supplemented with 200 μM Zn(II)[a]

Gene locus (SPD_/ spr)[b]	Average fold change	Bayesian P value	Description	Changing in Δ*adcR*/ *adcR*$^{+}$[c]
0189/ 0184	1.9	1.1E-03	Acetyltransferase, GNAT family	
0265/ 0262	-2.1	1.6E-06	*adh*; Alcohol dehydrogenase, zinc-containing	Decreases
0451/ 0445	-4.2	4.4E-09	*hsdS*; Type I restriction-modification system, S subunit (putative)	
0453/ 0448	3.1	1.2E-08	*hsdS*; Type I restriction-modification system, S subunit	
0888/ 0906	13.2	8.1E-13	*adcA2*; Putative zinc-binding lipoprotein	Increases
0889/ 0907	65.8	7.4E-12	*phtD*; Pneumococcal histidine triad protein D	Increases
0890/ 0908	26.6	4.8E-10	*phtE*; Pneumococcal histidine triad protein E	Increases
0891/ 0909	9.9	3.3E-08	Pneumococcal histidine triad protein, frameshift	Increases
0892/ 0910	21.7	7.3E-13	Pneumococcal histidine triad protein precursor, truncation	Increases
1036/ 1059	1.8	5.7E-04	Conserved hypothetical protein	
1037/ 1060	8.8	9.2E-11	*phpA*; Streptococcal histidine triad protein	Increases
1038/ 1061	4.1	2.1E-04	*phtA*; Pneumococcal histidine triad protein A precursor	Increases
1637/ 1671	-1.0[d]	NA	*nmlR*; Transcriptional regulator, MerR family	
1638/ 1672	-1.0[d]	NA	*czcD*; Zn, Co, Cd cation efflux system protein	
1865/ 1866	-13.6	1.0E-11	Alcohol dehydrogenase, zinc-containing	Decreases
1997/ 1975	8.7	1.5E-11	*adcA*; Zinc ABC transporter, zinc-binding lipoprotein	Increases
1998/ 1976	9.5	2.1E-11	*adcB*; Zinc ABC transporter, permease protein	Increases
1999/ 1977	9.6	6.8E-12	*adcC*; Zinc ABC transporter, ATP-binding protein	Increases
2000/ 1978	10.0	1.3E-11	*adcR*; *adc* operon repressor	Increases

2017/ 1995	2.1	1.3E-06	*pspC*; Pneumococcal surface protein C; also called choline binding protein A (CbpA)	Increases
2018/ 1996	2.5	1.4E-07	Putative isoprenylcysteine carboxyl methyltransferase family protein	Increases

[a]Microarray analyses were performed as described in Material and Methods. Fold changes are expressed relative to IU1781 grown in BHI with added Zn(II) and are the average of three independent biological replicates, including one dye swap. Changes in relative transcript amounts of positive or negative 1.8 fold with Bayesian P value of <0.001 were considered significant.
[b]SPD_gene tags refer to strain D39 used in this study. Corresponding spr gene tags for laboratory strain R6 are shown for convenience.
[c]Changes in relative transcript amounts observed for a Δ*adcR* mutant grown in BHI compared to its *adcR*$^{+}$ parent grown in the same media[8] are shown for comparison.
[d]*nmlR* and *czcD* transcript amounts increased to comparably high levels in the *AdcR-H108Q* mutant and wild-type strains grown in BHI with 200 μM Zn(II) (see Table S3).

Table S5. Microarray analysis comparing relative transcript amounts from D39 *rpsL1* (IU1781) grown in BHI supplemented with 200 μM Zn(II) and 300 μM Mn(II) and in BHI without added Zn(II) and Mn(II)[a]

Gene locus (SPD_/ spr)[b]	Average fold change	Bayesian P value	Description
0558/ 0561	-2.7	3.9E-07	*prtA*; Cell wall-associated serine protease precursor
0775/ 0782	2.0	8.1E-05	Conserved hypothetical protein
0913/ 0931	2.4	9.6E-07	Conserved hypothetical protein
1028/ 1051	1.8	2.7E-04	*acoA*; TPP-dependent acetoin dehydrogenase, alpha subunit
1461/ 1492	-3.1	8.1E-09	*psaB*; Manganese ABC transporter, ATP-binding protein
1462/ 1493	-3.4	2.1E-08	*psaC*; Manganese ABC transporter, permease protein
1463/ 1494	-2.1	5.2E-06	*psaA*; ABC transporter, substrate-binding lipoprotein
1637/ 1671	7.9	3.4E-10	*nmlR*; Transcriptional regulator, MerR family
1638/ 1672	5.4	1.3E-08	*czcD*; Zn, Co, Cd cation efflux system protein
2068/ 2045	2.3	8.1E-06	*htrA*; Serine protease HtrA
2069/ 2046	2.1	6.7E-06	*spo0J*; Spo0J protein

[a]Microarray analyses were performed as described in Material and Methods. Fold changes are expressed relative to IU1781 grown in BHI without added Zn(II) and Mn(II) and are the average of three independent biological replicates, including one dye swap. Changes in relative transcript amounts of positive or negative 1.8 fold with Bayesian P value of <0.001 were considered significant.
[b]SPD_gene tags refer to strain D39 used in this study. Corresponding spr gene tags for laboratory strain R6 are shown for convenience.

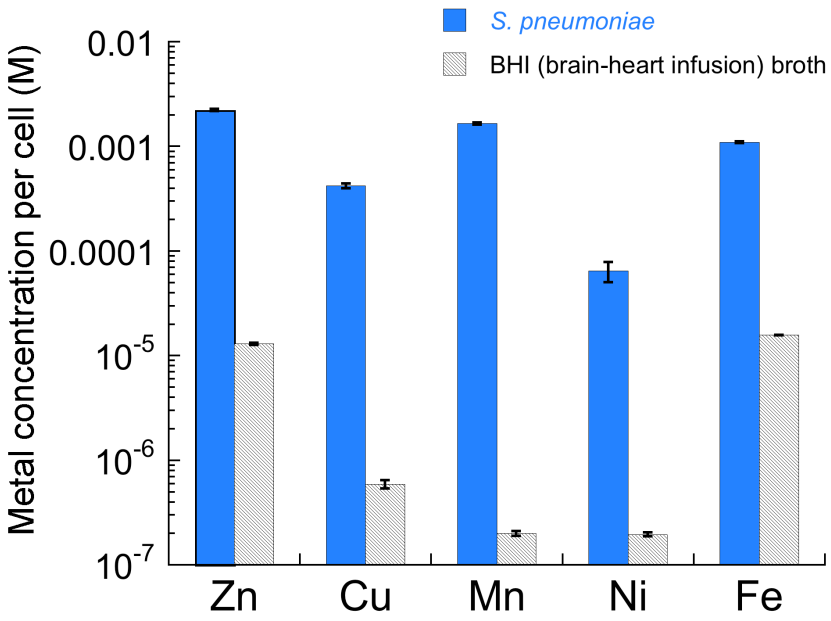

Figure S1. ICP-MS analysis of total cell-associated transition metal concentration grown in BHI media compared to the concentrations found in the growth media. The calculations were done assuming an OD_{620}=0.2 is 5.5 x 10^7 CFU/mL or 1.1 x 10^8 cells/mL (one CFU=diploid cells) and a total cell volume of an encapsulated cell to be 6.44 x 10^{-16} L based on previously determined cell dimensions.[4] We note that these concentrations are ≈5-10-fold higher than reported for *E. coli* grown on minimal M9 media[8] and ≈5-fold larger than we reported previously for Zn.[9] This correction is due to an estimate of 10 cells per CFU (chain) in the previous work[9] versus 2 cells per CFU used here, which reflects chain dissociation detected during serial dilution.[6] These calculations do not distinguish between cytoplasmic and extracellular metal content.

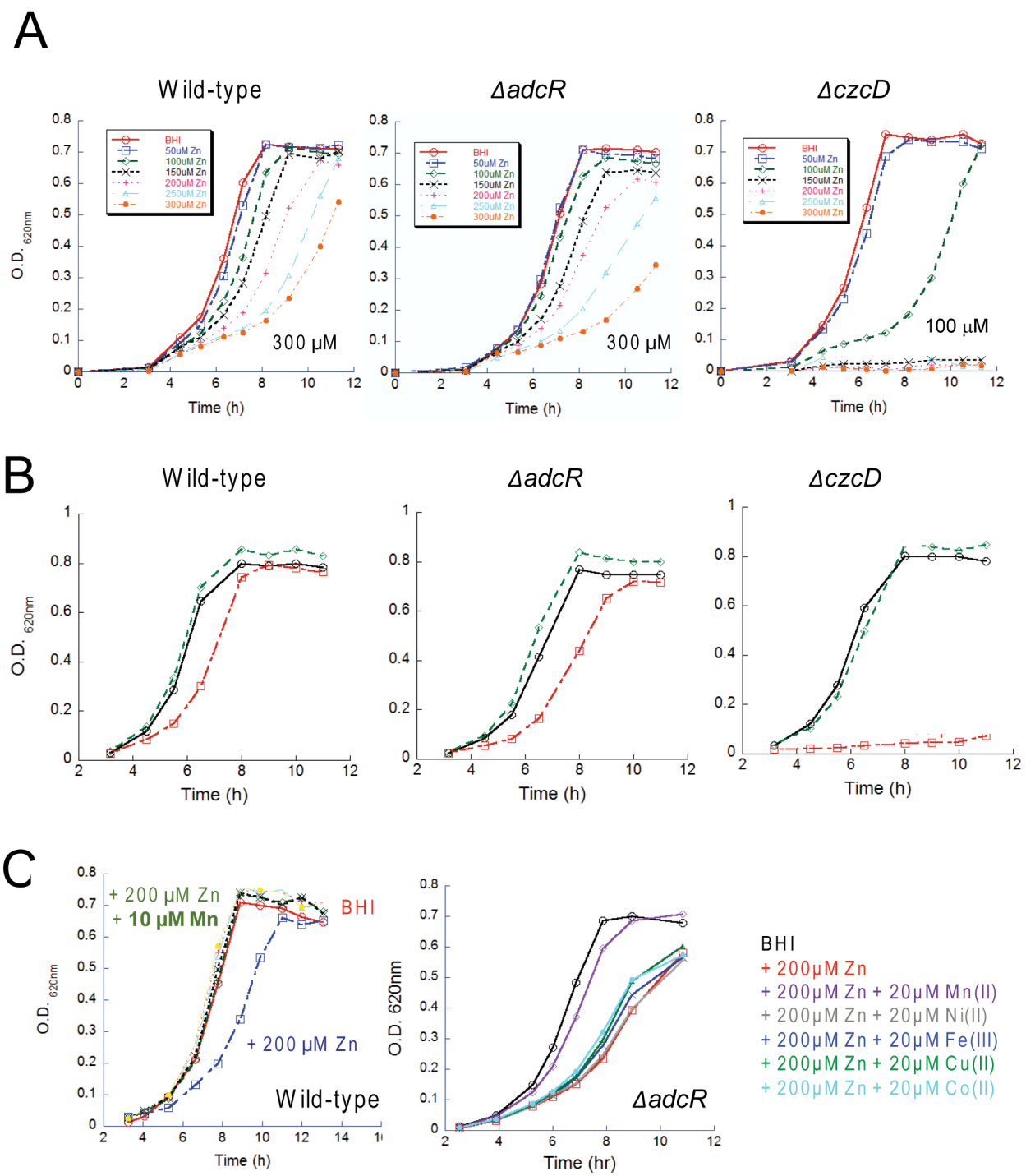

Figure S2. A) Growth curves of parent strain (left), Δ*adcR* (middle) and Δ*czcD::kan-rpsL* (right) with increasing zinc added to media. B) Growth of parent strain (left), Δ*adcR* (middle) and Δ*czcD::kan-rpsL* (right) in BHI (black), BHI with 200 μM Zn (red), and BHI with 200 μM Zn and 300 μM Mn (green). C) Growth of parent strain (left) in BHI (red), BHI with 200 μM Zn, and increasing Mn (10 μM to 300 μM). Growth of Δ*adcR* (right) in BHI (black), BHI with 200 μM Zn (red), and BHI with 200 μM Zn and 20 μM of other transition metals (Mn in purple).

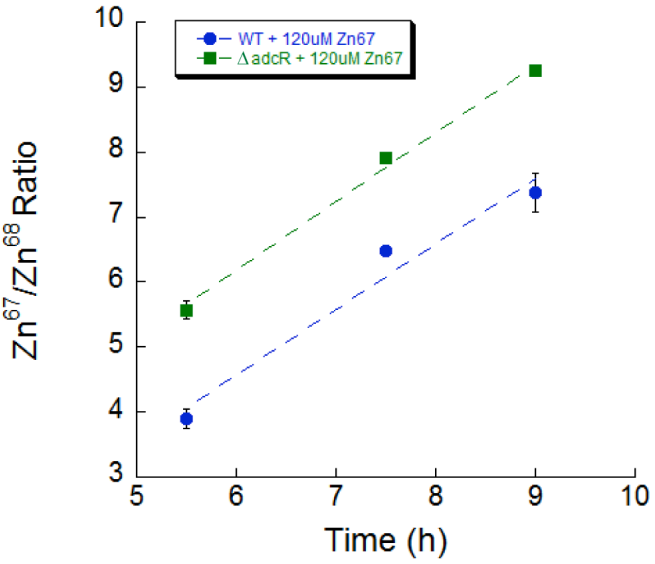

Figure S3. Change in Zn^{67}/Zn^{68} ratio for parent (*blue*, initial OD_{620nm} = 0.143) and Δ*adcR* (*green*, initial OD_{620nm} = 0.072) over time during the exponential growth phase. See Fig. S2A for comparative growth curves.

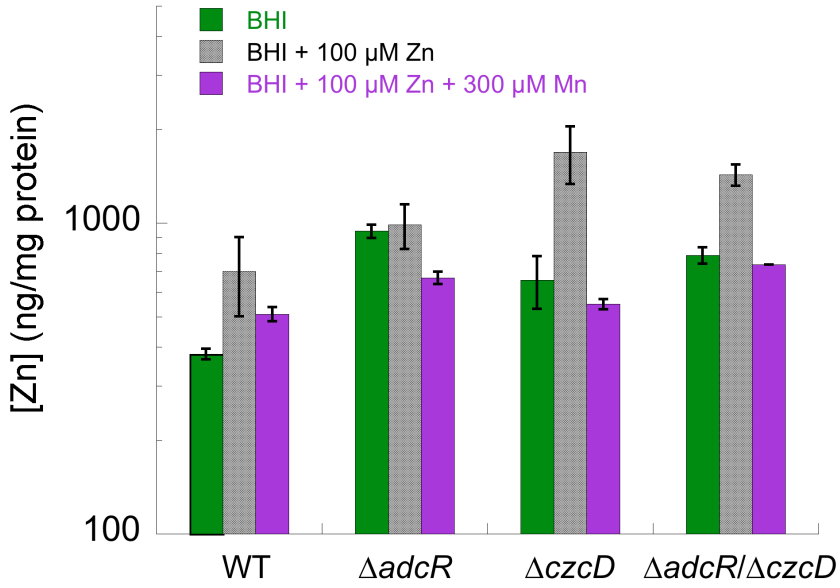

Figure S4. Zinc concentration expressed in ng/mg protein of parent WT, Δ*adcR*, Δ*czcD::kan-rpsL* and Δ*adcR* Δ*czcD::kan-rpsL* strains. *Green* bars represent total cell-associated [Zn] when grown in BHI alone, *gray* bars represent [Zn] when grown in BHI with 100 µM zinc added, while *purple* bars are [Zn] when grown in BHI with 100 µM zinc and 300 µM manganese added.

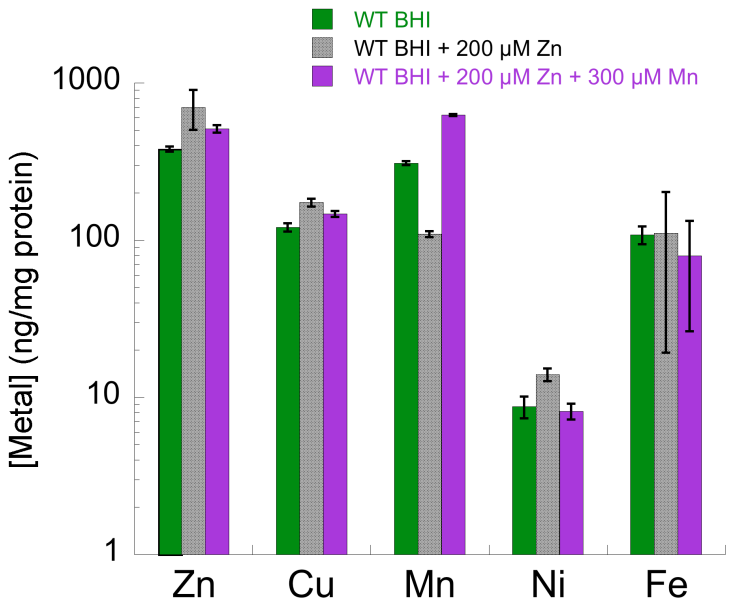

Figure S5. Transition metal concentrations expressed as ng metal/mg protein for parent wild-type strain grown in BHI (*green*), BHI with 200 µM zinc (*black dash*), and BHI with 200 µM zinc and 300 µM manganese (*magenta*).

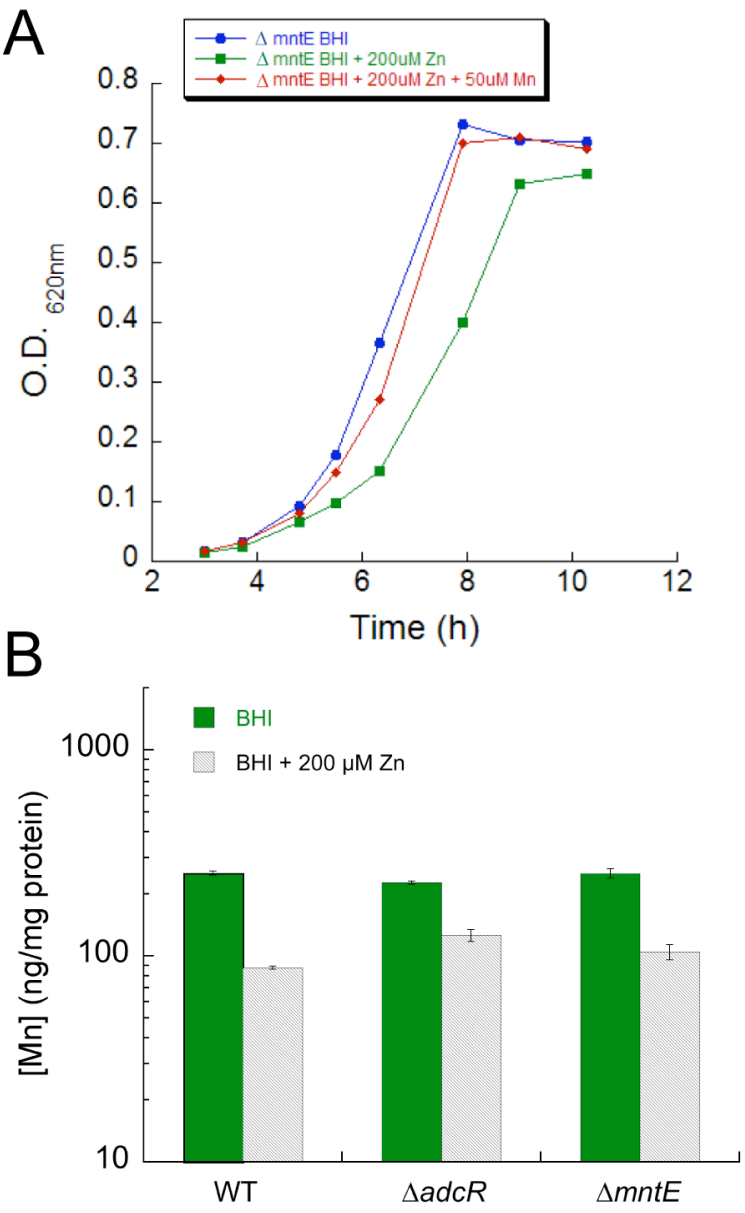

Figure S6. A) Growth curve for Δ*mntE* strain grown in BHI (blue), BHI + 200 μM Zn (green), and BHI + 200 μM Zn + 50 μM Mn. B) Manganese concentrations parent strain, Δ*adcR*, and Δ*mntE* strains grown in BHI (*green* bars) and BHI with 200 μM zinc (*gray* bars).

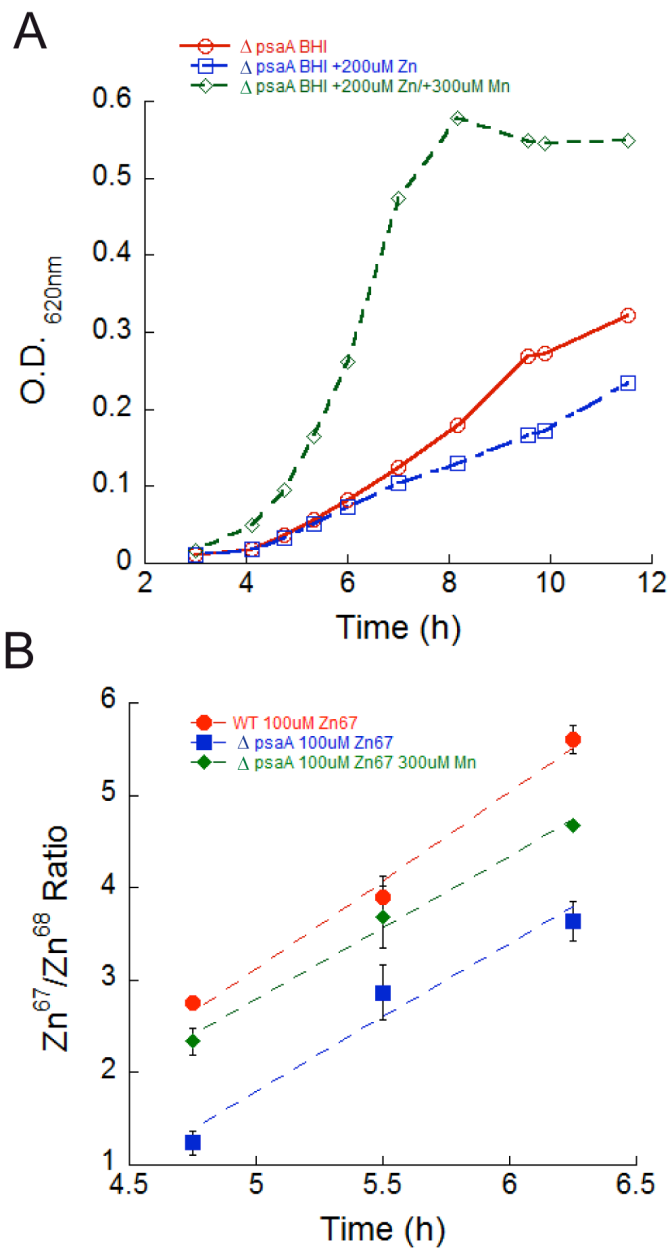

Figure S7. (A) Growth curves for Δ*psaA* strain in BHI alone (*red* symbols), BHI plus 200 μM added Zn (*blue* symbols) and BHI plus 200 μM Zn and 300 μM Mn (*green* symbols). (B) Change in Zn^{67}/Zn^{68} ratio for wild-type parent strain grown in 100 μM Zn^{67} (*red symbols*, initial OD_{620nm} = 0.087), Δ*psaA* strain grown in 100 μM Zn^{67} (*blue*, initial OD_{620nm} = 0.057), and Δ*psaA* grown in 100 μM Zn^{67} and 300 μM Mn (*green*, initial OD_{620nm} = 0.093).

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