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**Zinc disrupts central carbon metabolism and capsule biosynthesis in *Streptococcus pyogenes***

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Key words: zinc, group A streptococcus, *Streptococcus pyogenes*, glycolysis, capsule, tagatose-6-phosphate pathway

26 **Supplementary Information:**

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28 **Supplementary Tables S1-S2**

29 **Supplementary Figures S1-S5**

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52 **Table S1** Table of genes regulated by zinc, organized by gene number

Locus	Gene/Locus	Function	<i>gczA+</i> / $\Delta$ <i>gczA</i>	P- Value	q- Value
<i>Upregulated by zinc</i>					
M5005_Spy_0083	<i>rpoB</i>	DNA-directed RNA polymerase beta chain	2.290	0.001	0.050
M5005_Spy_0084	<i>rpoC</i>	DNA-directed RNA polymerase beta' chain	2.337	0.001	0.048
M5005_Spy_0087	<i>comYB</i>	ComG operon protein 2	2.052	0.003	0.109
M5005_Spy_0088	<i>comYC</i>	ComG operon protein 3	2.278	0.002	0.085
M5005_Spy_0089	<i>Spy0089</i>	ComG operon protein 4	2.267	0.001	0.059
M5005_Spy_0090	<i>Spy0090</i>	Hypothetical protein	2.266	0.001	0.060
M5005_Spy_0091	<i>comYD</i>	ComG operon protein 6	2.573	0.000	0.028
M5005_Spy_0092	<i>Spy0092</i>	ComG operon protein 6	2.381	0.001	0.047
M5005_Spy_0102	<i>Spy0102</i>	Single-strand DNA binding protein	2.853	0.002	0.085
M5005_Spy_0151	<i>Spy0151</i>	3-keto-L-gulonate-6-phosphate decarboxylase	2.028	0.004	0.123
M5005_Spy_0153	<i>araD</i>	L-ribulose-5-phosphate 4-epimerase	2.067	0.004	0.123
M5005_Spy_0157	<i>opuAA</i>	Glycine betaine transport ATP-binding protein	3.577	0.000	0.034
M5005_Spy_0158	<i>opuABC</i>	Glycine betaine transport system permease	3.361	0.001	0.048
M5005_Spy_0245	<i>nifU</i>	IscU protein	2.106	0.003	0.118
M5005_Spy_0246	<i>Spy0246</i>	ABC transporter-associated protein	2.145	0.003	0.110
M5005_Spy_0473	<i>Spy0473</i>	Multidrug resistance protein B	2.079	0.004	0.140
M5005_Spy_0653	<i>czcD</i>	Cobalt zinc cadmium resistance protein	28.799	0.000	0.005
M5005_Spy_0875	<i>Spy0875</i>	Sortase	3.047	0.002	0.073
M5005_Spy_0990	<i>dnaE</i>	DNA polymerase III alpha subunit	2.707	0.004	0.128
M5005_Spy_1021	<i>Spy1021</i>	Phage protein	2.597	0.003	0.112
M5005_Spy_1022	<i>Spy1022</i>	Portal protein	3.147	0.000	0.031
M5005_Spy_1472	<i>hit</i>	Bis(5'-nucleosyl)-tetraphosphatase	2.032	0.003	0.110
M5005_Spy_1629	<i>salX</i>	Lantibiotic transport ATP-binding protein	2.677	0.000	0.029
M5005_Spy_1630	<i>salB</i>	Serine (threonine) dehydratase	2.701	0.001	0.048
M5005_Spy_1631	<i>salA</i>	Lantibiotic salivaricin A	2.238	0.002	0.090
M5005_Spy_1632	<i>lacG</i>	6-phospho-beta-galactosidase	2.459	0.001	0.052
M5005_Spy_1633	<i>lacE</i>	PTS system, lactose-specific IIBC component	2.792	0.000	0.028
M5005_Spy_1634	<i>lacF</i>	PTS system, lactose-specific IIA component	2.754	0.000	0.031
M5005_Spy_1635	<i>lacD.2</i>	Tagatose-bisphosphate aldolase	2.609	0.000	0.030
M5005_Spy_1636	<i>lacC.2</i>	Tagatose-6-phosphate kinase	2.570	0.000	0.034
M5005_Spy_1637	<i>lacB.2</i>	Galactose-6-phosphate isomerase lacB subunit	2.700	0.000	0.030
M5005_Spy_1638	<i>lacA.2</i>	Galactose-6-phosphate isomerase lacA subunit	2.331	0.001	0.048
M5005_Spy_1639	<i>lacR.2</i>	Lactose phosphotransferase system repressor	2.016	0.003	0.119
M5005_Spy_1640	<i>Spy1640</i>	DNA-damage-inducible protein J	2.115	0.003	0.120
M5005_Spy_1641	<i>Spy1641</i>	Hypothetical cytosolic protein	2.380	0.001	0.060
M5005_Spy_1642	<i>Spy1642</i>	DNA integration/recombination/inversion	2.313	0.003	0.119
M5005_Spy_1643	<i>Spy1643</i>	DNA integration/recombination/inversion	2.379	0.001	0.047
M5005_Spy_1644	<i>Spy1644</i>	Hypothetical protein	3.024	0.000	0.034

M5005_Spy_1646	<i>rpsI</i>	SSU ribosomal protein S9P	3.467	0.001	0.051
M5005_Spy_1647	<i>rplM</i>	LSU ribosomal protein L13P	2.471	0.001	0.069
M5005_Spy_1648	<i>Spy1648</i>	Transcriptional regulator, Cro/CI family	2.373	0.001	0.049
M5005_Spy_1649	<i>Spy1649</i>	Hypothetical membrane spanning protein	2.317	0.001	0.050
M5005_Spy_1670	<i>Spy1670</i>	Oxidoreductase	3.826	0.000	0.015
M5005_Spy_1671	<i>Spy1671</i>	Transcriptional regulator, MarR family	2.773	0.000	0.012
M5005_Spy_1807	<i>argR2</i>	Arginine repressor	5.380	0.000	0.028
M5005_Spy_1808	<i>argS</i>	Arginyl-tRNA synthetase	2.492	0.001	0.049
M5005_Spy_1810	<i>Spy1810</i>	Hypothetical membrane spanning protein	2.557	0.001	0.048
M5005_Spy_1811	<i>Spy1811</i>	Hypothetical membrane spanning protein	2.546	0.001	0.048
M5005_Spy_1812	<i>Spy1812</i>	Hypothetical membrane spanning protein	3.042	0.000	0.028
M5005_Spy_1813	<i>aspS</i>	Aspartyl-tRNA synthetase	3.720	0.000	0.013
M5005_Spy_1814	<i>hisS</i>	Histidyl-tRNA synthetase	3.154	0.000	0.029
M5005_Spy_1815	<i>rpmF</i>	LSU ribosomal protein L32P	4.444	0.000	0.010
M5005_Spy_1816	<i>rpmG</i>	LSU ribosomal protein L33P	3.685	0.000	0.017
M5005_Spy_1817	<i>cadD</i>	Cadmium resistance protein	3.603	0.000	0.015
M5005_Spy_1818	<i>cadC</i>	Cadmium efflux system accessory protein	3.162	0.000	0.031
M5005_Spy_1819	<i>Spy1819</i>	Hypothetical phage protein	3.036	0.001	0.053
<i>Downregulated by zinc</i>					
M5005_Spy_0350	<i>Spy0350</i>	Hypothetical protein	-3.484	0.000	0.013
M5005_Spy_0351	<i>spyA</i>	C3 family ADP-ribosyltransferase	-2.353	0.001	0.201
M5005_Spy_0437	<i>vicX</i>	Zinc-dependent hydrolase	-2.045	0.001	0.175
M5005_Spy_0534	<i>Spy0534</i>	(R,R)-butanediol dehydrogenase	-2.045	0.004	0.312
M5005_Spy_0582	<i>atpC</i>	ATP synthase epsilon chain	-2.169	0.004	0.332
M5005_Spy_0583	<i>Spy0583</i>	Hypothetical membrane associated protein	-2.300	0.003	0.317
M5005_Spy_0584	<i>murA</i>	UDP-N-acetylglucosamine-1-carboxyvinyltransferase	-2.439	0.002	0.229
M5005_Spy_0585	<i>epuA</i>	EpuA protein	-2.469	0.001	0.146
M5005_Spy_0586	<i>endA</i>	DNA-entry nuclease	-2.336	0.001	0.125
M5005_Spy_0885	<i>Spy0885</i>	DNA topoisomerase I	-6.060	0.000	0.000
M5005_Spy_0886	<i>Spy0886</i>	Transcriptional regulator, LysR family	-3.690	0.000	0.005
M5005_Spy_1034	<i>Spy1034</i>	Phage protein	-3.021	0.000	0.032
M5005_Spy_1036	<i>ssb</i>	Phage single-strand DNA binding protein	-2.353	0.000	0.117
M5005_Spy_1276	<i>Spy1276</i>	Transcription regulator, crp family	-3.300	0.000	0.013
M5005_Spy_1293	<i>Spy1293</i>	Hypothetical protein	-2.667	0.001	0.116
M5005_Spy_1358	<i>nadE</i>	Nicotinate phosphoribosyltransferase	-2.203	0.004	0.322
M5005_Spy_1678	<i>Spy1678</i>	Thioredoxin	-2.119	0.003	0.333

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60 **Table S2** List of primers used in this study

Primer	Gene / Function	Sequence (5'-3')
<i>Gene expression study</i>		
gyrA-F	<i>gyrA</i> / housekeeping	GAAGTGATCCCTGGACCTGA
gyrA-R		CCCGACCTGTTTGAGTTGTT
adh2-F	<i>adh2</i> / alcohol dehydrogenase	GCTATCCGCGTCATCTGGAA
adh2-R		ACCAACTGCGTTACGTCCAT
adhA-F	<i>adhA</i> / acetaldehyde dehydrogenase	ATTGGTCATGGTGAAGCGCT
adhA-R		TGACCAAAATCGCCATGTGC
pgmA-F	<i>pgmA</i> / phosphoglucomutase	TGCTTTTGAGTCTGCACAGG
pgmA-R		AGAGTTCCTGGTGTGGACGA
gapN-F	<i>gapN</i> / glyceraldehyde-3-P dehydrogenase	CAACGTTGGCCTGAGTAACC
gapN-R		CTTGGCGGTAAGGATTCTGC
pfk-F	<i>pfk</i> / phosphofructokinase	TGGCCAAGATTTGTCACACG
pfk-R		GCTGAAGGTGTTATGAGCGG

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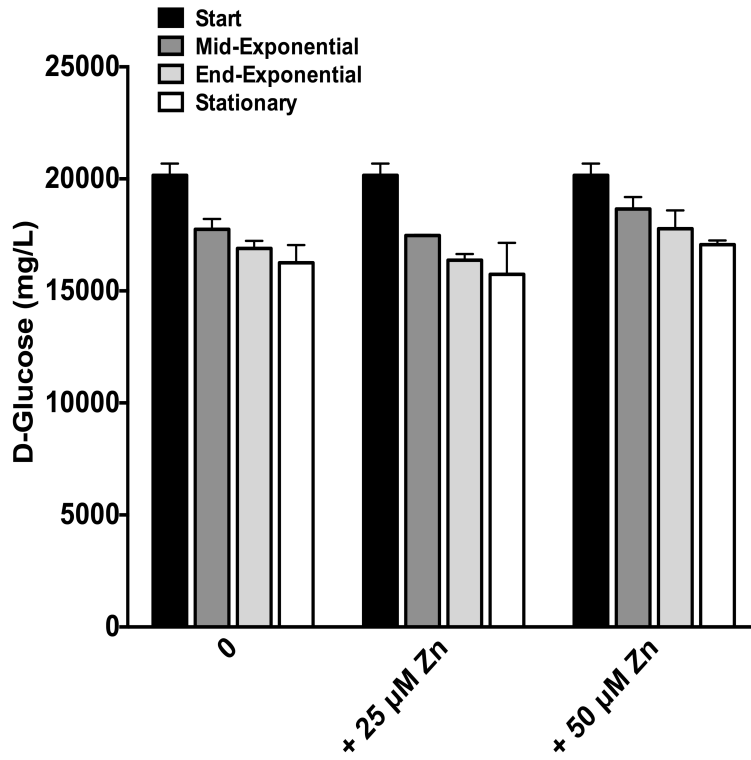
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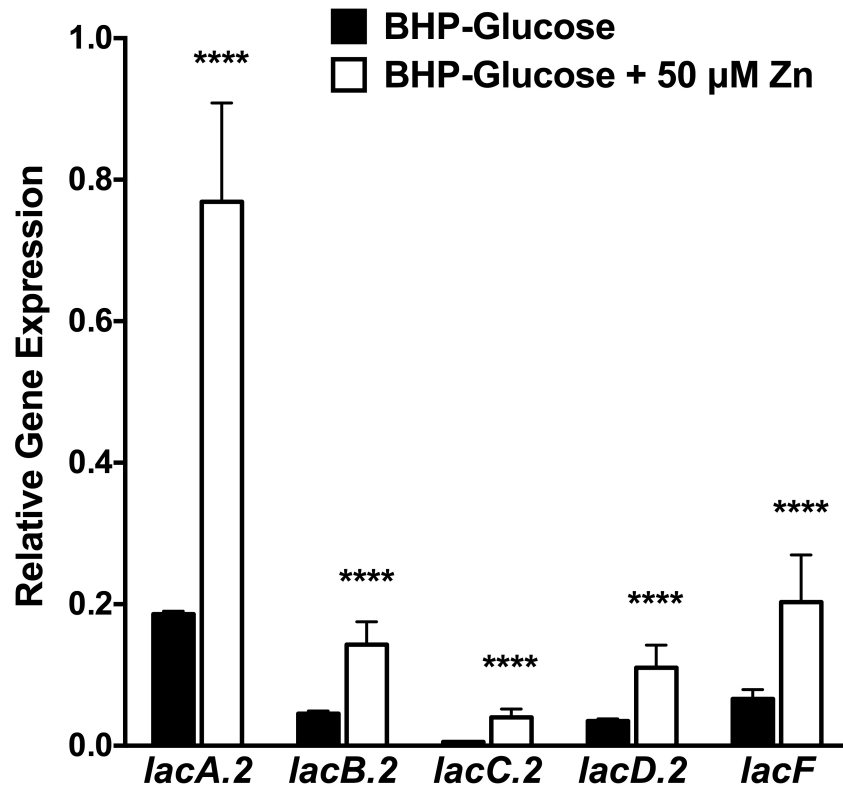
Supplementary Figures

Supplementary Figure 1.



Supplementary Figure 1 Total D-glucose measurement of cells grown in BHP-glucose in the presence and absence of 25 and 50 μM zinc. Supernatant was collected, filtered and measured for D-glucose at different stages of growth. Graph is a representative of three independent experiments.

97 **Supplementary Figure 2.**



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 99 **Supplementary Figure 2** Relative gene expression of the tagatose-6-phosphate metabolism  
 100 genes, *lacF* (encodes D-galactose phosphotransferase), *lacD.2* (encodes tagatose-6-phosphate  
 101 aldolase), *lacC.2* (encodes tagatose-6-phosphate kinase), *lacB.2* and *lacA.2* (encodes galactose-6-  
 102 phosphate isomerase A/B subunits). Cells were grown up to mid-exponential phase in BHP-  
 103 glucose in the presence and absence of 50  $\mu$ M zinc, error bars are indicative of the standard deviation  
 104 of 3 independent experiments. Relative gene expression was calculated using the  $2^{-\Delta\text{CT}}$  method, with  
 105 *proS* as the reference gene. Unpaired, 2-tailed *t* test was performed between relative gene expression  
 106 of cells grown in BHP-glucose in the absence and presence of 50  $\mu$ M zinc; \*\*\*\* $P$ <0.0001.

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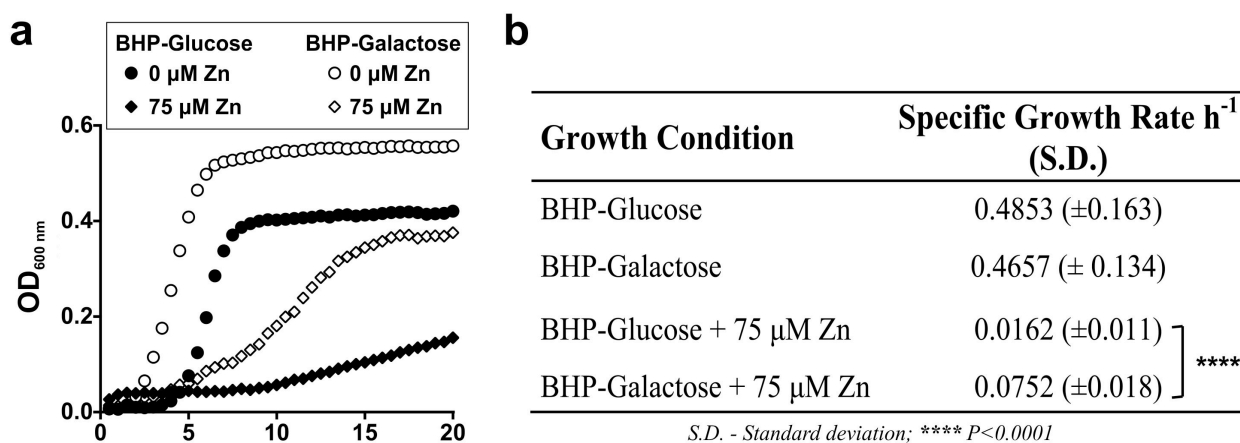
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112 **Supplementary Figure 3.**



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**Supplementary Figure 3 Growth curve and specific growth rate analysis of Group A**

***Streptococcus* grown in BHP-glucose, or BHP-galactose in the absence and presence of zinc. (a)**

Growth curve analysis in the absence and presence of 75 μM zinc. Graph is a representative of five

independent experiments. **(b)** Table of the specific growth rates (h<sup>-1</sup>) of GAS cells grown in the

absence and presence of 75 μM zinc. Values shown are the mean specific growth rates of five

independent experiments. Unpaired, 2-tailed *t* test was performed between growth rate of cells grown

in BHP-glucose in the presence of 75 μM zinc and cells grown in BHP-galactose in the presence of

75 μM; \*\*\*\*P<0.0001.

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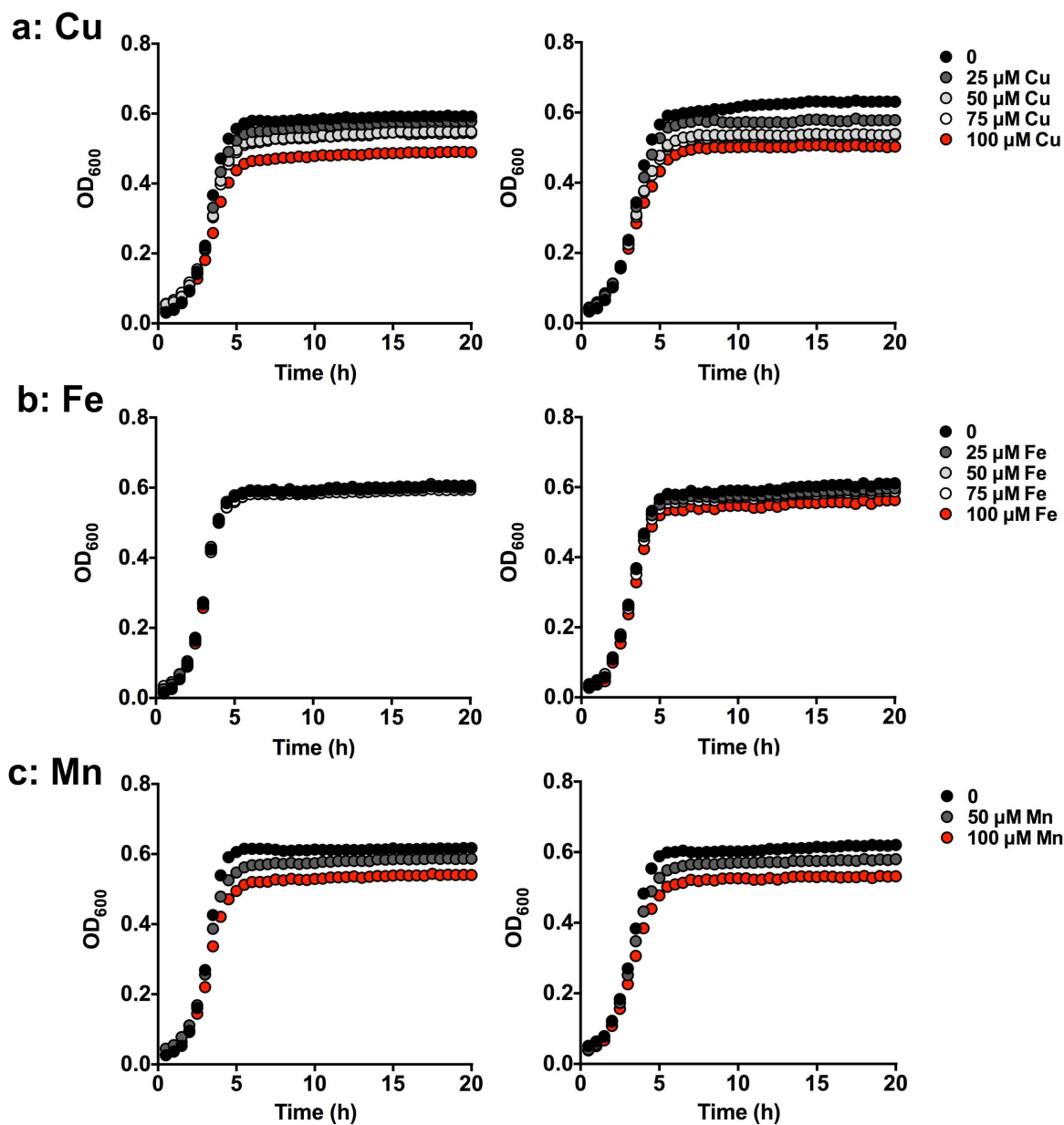
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Supplementary Figure 4 Growth curve analysis of Group A *Streptococcus* grown in BHP-

138 glucose, or BHP-galactose in the absence and presence of divalent metal ions. (a) copper, (b)

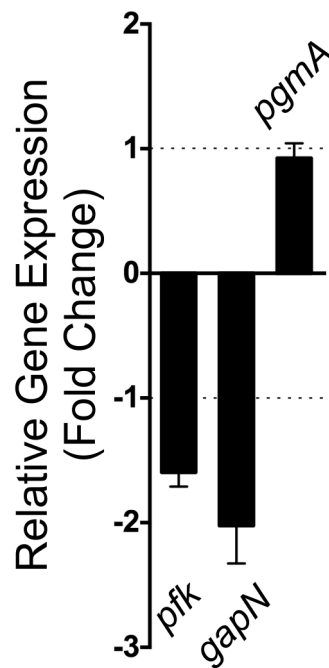
139 iron and, (c) manganese. Graph is a representative of three independent experiments.

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143 **Supplementary Figure 5.**



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146 **Supplementary Figure 5** Relative gene expression of the glycolytic genes, *pfk* (encodes  
147 **phosphofructokinase**), *gapN* (encodes **glyceraldehyde-3-phosphate dehydrogenase**) and *pgmA*  
148 **(encodes phosphoglucomutase)**. Cells were grown up to mid-exponential phase in BHP-glucose in  
149 the presence and absence of 50  $\mu$ M zinc, error bars are indicative of the standard deviation of 3  
150 independent experiments. Relative gene expression was calculated using the  $2^{-\Delta\text{CT}}$  method, with *gyrA*  
151 as the reference gene, and results are represented as fold change of expression from growth in zinc  
152 compared to growth without zinc.

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