Supplementary Material

Supplementary Table S1. Metal content of *C. metallidurans* strains

Bacterial Strain	Metal atoms per cell[a]					
	Mg, 10^6	Fe, 10^3	Zn, 10^3	Cu, 10^3	Co, 10^3	Ni, 10^3
CH34(pMOL28, pMOL30)[b]	11.8±1.1	674±87	64.4±10.1	8.59±2.38	**1.21±0.48**	3.11±1.51
AE104 [b, c]	11.5±1.1	743±70	72.8±9.5	9.00±1.53	3.32±0.71	4.68±2.21
Δ*zupT* [b, c]	9.9±1.2	708±106	**21.1±5.8**	10.97±3.39	5.92±1.58	3.07±0.35
Δ*pitA*	**36.8±4.4**	649±68	58.3±5.4	11.14±3.65	**1.73±0.40**	**1.40±0.81**
Δ*hoxN*	12.7±0.8	816±151	61.2±4.3	14.18±3.65	2.48±0.49	1.75±1.35
Δ*zupT* Δ*pitA* [c]	**35.9±1.6**	760±144	**24.9±2.5**	9.55±2.76	2.81±0.86	3.02±2.55
Δ4 (Δ*zupT* Δ*corA*$_{1,2,3}$)	10.7±1.5	691±174	**30.5±4.1**	10.13±3.15	3.57±0.79	1.76±0.86
Δ5 (Δ4 Δ*pitA*)	**28.1±1.2**	**434±20**	**39.2±4.8**	**6.16±0.81**	4.25±0.58	**1.58±0.13**
Δ6 (Δ5 Δ*zntB*)	**29.9±2.1**	**486±34**	**36.9±4.4**	**6.93±0.80**	5.26±1.25	2.34±1.63
Δ7 (Δ6 Δ*hoxN*)	**43.5±3.9**	745±59	**31.6±3.9**	13.43±3.83	5.19±1.35	**1.48±0.30**
Δe4 (Δ*zntA* Δ*cadA* Δ*dmeF* Δ*fieF*) [c]	**36.3±2.0**	662±45	62.6±9.9	9.42±3.86	3.57±1.45	**1.37±0.97**
Δe4 (Δe4 Δ*zupT*) [c]	**35.3±5.6**	686±138	**31.9±7.4**	12.78±5.52	5.49±1.57	**1.29±0.43**

[a]As determined by ICP-MS, n ≥ 3. Some values are additional reproductions of published ([b]1 and [c]2) values under parallel conditions with the new mutant strain. **Bold-faced** number indicate differences in the metal content of a strain compared to strain AE104.

Supplementary Table S2. Minimal inhibitory concentrations of various *C. metallidurans* mutant strains up to Δ5 (*ΔzupT ΔcorA$_{123}$ ΔpitA*) on solid Tris-buffered mineral salts medium [a].

Mutant strain	Zn^{2+}, (µM)	Co^{2+}, (µM)	Ni^{2+}, (µM)	Cd^{2+}, (µM)	Cu^{2+}, (mM)
AE104[b]	127±43	500±100	360±83	150±39	1.5±0.2
Single Mutants					
Δ*zupT* [b]	133±58	**600±0**	350±50	160±7	1.4±0.1
Δ*pitA* [b]	120±35	400±0	380±84	140±35	1.3±0.1
Δ*corA*$_1$::*kan* [b]	113±42	400±0	**480±150**	100±56	1.3±0
Δ*corA*$_2$ [b]	120±35	400±0	340±55	150±34	1.5±0
Δ*corA*$_3$ [b]	113±42	400±0	320±84	150±34	1.5±0.1
Double mutants					
Δ*zupT* Δ*pitA* [b]	130±14	337±19	300±82	140±20	1.4±0.1
Δ*zupT* Δ*corA*$_1$::*kan*	130±14	200±141	**530±58**	150±12	1.5±0.1
Δ*zupT* Δ*corA*$_2$	120±20	193±209	370±58	140±20	1.5±0.1
Δ*zupT* Δ*corA*$_3$	120±20	193±209	330±58	140±20	1.6±0.1
Δ*pitA* Δ*corA*$_1$::*kan*	130±14	200±0	**470±58**	170±12	1.3±0.1
Δ*pitA* Δ*corA*$_2$	120±20	193±209	300±0	150±12	1.5±0.1
Δ*pitA* Δ*corA*$_3$	120±20	203±202	300±0	170±12	1.5±0.1
Δ*corA*$_1$::*kan* Δ*corA*$_2$	130±14	**60±0**	**530±58**	170±12	1.5±0.1
Δ*corA*$_1$::*kan* Δ*corA*$_3$	130±14	225±106	**600±100**	170±12	1.6±0.1
Δ*corA*$_2$ Δ*corA*$_3$	130±14	**73±23**	370±58	180±0	1.5±0.1
Triple mutants					
Δ*zupT* Δ*pitA* Δ*corA*$_3$	130±14	**107±81**	300±82	140±20	1.5±0.1
Δ*corA*$_1$::*kan* Δ*corA*$_2$ Δ*corA*$_3$	150±71	**600±0**	***450±132***	110±14	1.6±0.2
Quadruple mutants					
Δ4: Δ*zupT* Δ*corA*$_1$ Δ*corA*$_2$ Δ*corA*$_3$	130±14	362±19	**530±58**	140±20	1.6±0.1
Δ*zupT* Δ*pitA* Δ*corA*$_1$::*Kan* Δ*corA*$_2$	120±0	450±71	**570±115**	170±42	1.4±0.2
Δ*zupT* Δ*pitA* Δ*corA*$_1$::*Kan* Δ*corA*$_3$	120±0	275±177	***440±167***	140±20	1.5±0.1
Δ*zupT* Δ*pitA* Δ*corA*$_2$ Δ*corA*$_3$	150±71	**600±0**	**500±141**	120±0	1.4±0.1
Quintuple mutant					
Δ5 = Δ4 Δ*pitA* = Δ*zupT* Δ*pitA* Δ*corA*$_1$ Δ*corA*$_2$ Δ*corA*$_3$	135±19	400±0	***450±131***	150±27	1.5±0.1

[b] for reference as published [1]. B**old faced numbers** indicate decreased cobalt or increased nickel resistance, values in italics no significant results due to a high deviation. At least three independent experiments, deviations indicated.

Supplementary Table S3. Metal resistance of deletion strains in liquid culture[a]

Bacterial strain	IC_{50}, (µM)		
	Zinc	**Cobalt**	**Cadmium**
AE104 parent strain	442±40	94±6	93±10
AE104 Δ*zupT*	**156±16**	**34±6**	**16±3**
AE104 Δ*pitA*	**79±5**	**38±4**	**56±16**
Δ*zupT* Δ*pitA*	182±21	**14±1**	**9.1±1.3**
Δ4 (=Δ*zupT* Δ*corA*$_{123}$)	**374±16**	41±2	**6.7±1.4**
Δ5 (= Δ4 Δ*pitA*)	**70±5**	43±3	**19.7±2.2**
Δ5 Δ*hoxN*	**88±7**	30±6	**4.7±0.7**
Δ6 (=Δ5 Δ*zntB)*	**184±12**	41±3	**10.5±0.8**
Δ7 (=Δ6 Δ*hoxN)*	**53±4**	**8.3±1.6**	**2.19±0.4**
AE104 Δ*hoxN*	**323±23**	**31±4**	**15.8±1.4**
ΔzupT Δ*hoxN*	**93±9**	**20±2**	**5.9±1.2**
ΔzupT Δ*hoxN* Δ*zntB*	104±4	**9.6±2.4**	**2.6±0.6**
ΔzupT Δ*hoxN* Δ*zntB* Δ*pitA*	**53±4**	**4.1±0.4**	**1.2±0.3**
AE104 Δ*corA*$_1$*::kan* Δ*corA*$_2$ Δ*corA*$_3$	n.d.	**228±30**	104±8
<u>Efflux mutants</u>			
AE104 Δe4	**8.3±0.7**	**4.8±0.3**	**0.02±0.00**
AE104 Δe4 Δ*zupT*	**3.9±0.4**	4.8±0.4	**0.03±0.00**

[a] Dose response experiments were performed (n>3 per conditions) and the IC_{50} values calculated. Bold-faced values indicate significant derivations from metal resistance of the direct parent strain.

Supplementary Table S4. Metal resistance of deletion strains in liquid culture[a]

Bacterial strain	IC_{50}, (µM)			
	Copper	**Gold[b]**	**Nickel**	**EDTA**
AE104 parent strain	906±82	25.3±2.1	348±16	249±9
AE104 Δ*zupT*	**400±77**	**13.0±1.3**	**171±10**	**183±13**
AE104 Δ*hoxN*	**686±69**	**12.1±1.7**	**235±14**	221±11
Δ5 (=Δ*zupT* Δ*corA*$_{123}$ Δ*pitA*)	475±50	11.3±1.1	**328±27**	219±18
Δ6 (=Δ5 Δ*zntB*)	496±61	11.4±1.1	367±12	241±15
Δ7 (=Δ6 Δ*hoxN)*	**265±32**	14.8±2.6	**145±16**	205±22
Efflux mutants				
AE104 Δe4	**637±41**	23.1±1.9	n.d.	**1339±92**
AE104 Δe4 Δ*zupT*	**460±49**	**14.4±2.0**	n.d.	**951±69**

[a] Dose response experiments were performed (n>3 per conditions) and the IC_{50} values calculated. Bold-faced values indicate significant derivations from metal resistance of the direct parent strain. [b]$Au(III)Cl_4^-$.

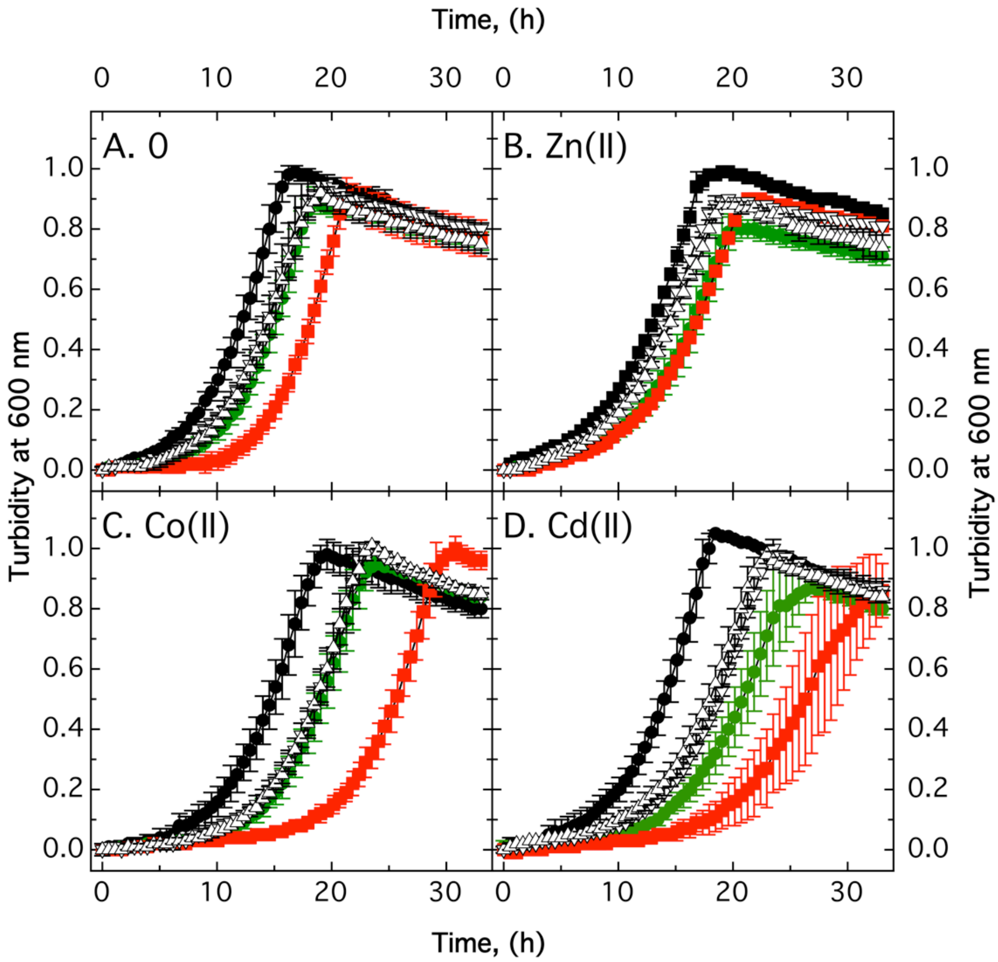
Supplementary Table S5. Oxidative stress resistance of deletion strains in liquid culture[a]

Strain	**IC_{50}, (µM)**	
	H_2O_2	**Paraquat**
AE104 parent strain	2650±279	169±10
AE104 Δ*zupT*	**894±133**	**111±6**
AE104 Δ*pitA*	1915±205	**255±7**
Δ*zupT* Δ*pitA*	**1507±125**	94±10
Δ*corA*$_1$*::kan* Δ*corA*$_2$ Δ*corA*$_3$	**1142±132**	**142±7**
Δ4 (=Δ*zupT* Δ*corA*$_{123}$)	1105±160	69±2
Δ7	989±102	**135±4**
Efflux mutants		
AE104 Δe4	**1059±111**	**257±12**
AE104 Δe4 Δ*zupT*	**953±191**	**147±10**

[a] Dose response experiments were performed (n>3 per conditions) and the IC_{50} values calculated. Bold-faced values indicate significant derivations from metal resistance of the direct parent strain

Supplementary Table S6. Primers used in this study

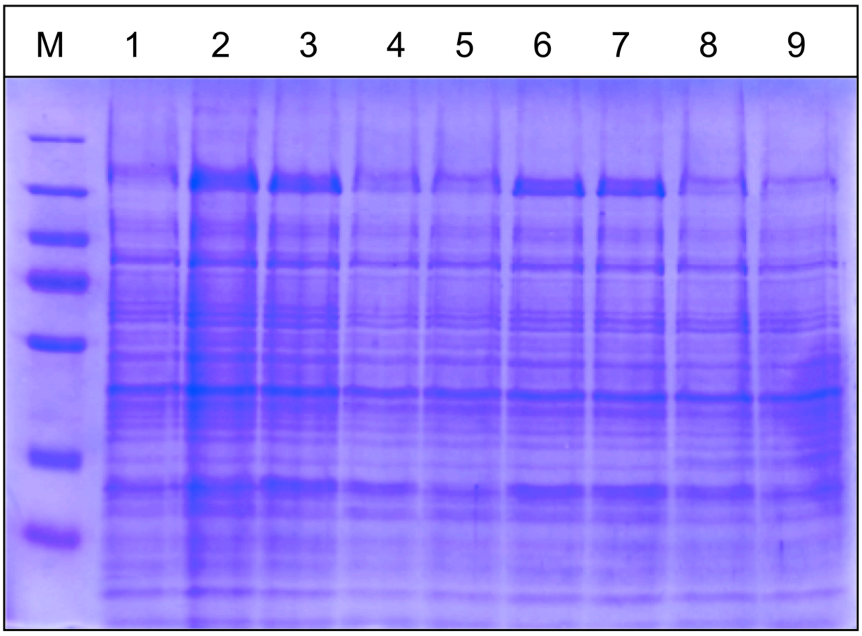
Name	5´→3´-sequence	comment	work
zupTRm 3´ Xba	AAA TCT AGA AGT CGC CAC CGG CAG CTA AGC	LacZ primer *zupT*	[1]
zupTRm 5´ PstI	AAA CTG CAG CCG CAC ACG AAA TCC CGC AGG	LacZ primer *zupT*	[1]
pitARm 3´ Xba	AAA TCT AGA CCT GGC TTG TCA TTG GGA TTC	LacZ primer *pitA*	[1]
pitARm 5´ PstI	AAA CTG CAG GTT CGG TTG GCG GAT TGT	LacZ primer *pitA*	[1]
JS RmcorA-Xba	AAA TCT AGA GGG GAC CAG GAA GGC TAT CAG	LacZ primer $corA_1$	[1]
JS RmcorA-Pst	AAA CTG CAG CGA ACA GCA GGA CGA AGC G	LacZ primer $corA_1$	[1]
JS corAIIXbaI	AAA TCT AGA CGA AAC CGT CAG ATC CAG CCC	LacZ primer $corA_2$	[1]
JS corAII 5´SphI	AAA GCA TGC CCT GCG CAA CGT GGT CTA TCC	LacZ primer $corA_2$	[1]
JS corAIII 3´XbaI	AAA TCT AGA GTC CGC GTC CTA GCG ATC CAG	LacZ primer $corA_3$	[1]
JS corAIII 5´PstI	AAA CTG CAG CGC CGA AGA TGC CGA AGG TAT	LacZ primer $corA_3$	[1]
JS zntB 3´ XbaI	AAA TCT AGA TCG ACT GCC AGC CTA CTC GGC	LacZ primer *zntB*	this work
JS zntB 5´SphI	AAA GCA TGC TCC GTC TGC TGA ACC GTC CGC	LacZ primer *zntB*	this work
HoxN lacZ1 SalI	AAA GTC GAC-GCT CGG GCT GCT GTT CAC CCT C	LacZ primer *HoxN*	this work
HoxN lacZ2 XbaI	AAA TCT AGA-GGATGCGGC CCG GCT ATT CGA C	LacZ primer *HoxN*	this work
RpoC lacZ 1 PstI	AAACTGCAG-CCTGCTGCTGGGTATTACGAAG	LacZ primer *rpoC*	this work
RpoC lacZ 2 XbaI	GAG TCTAGA GCT GTA CTG CTT GGC GTG ATG	LacZ primer *rpoC*	this work
Eno lacZ 1 SalI	AAAGTCGAC-CGGCAACTCGATTCTCATCAAG	LacZ primer *eno*	this work
Eno lacZ 2 XbaI	AAATCTAGA-ACACTAACCGGCGATATCAGC	LacZ primer *eno*	this work
CorA1 Dis1 PstI	AAA CTG CAG TCC ACG ATG AAG ACC T	disruption primer $corA_1$	[1]
CorA1 Dis2 XbaI	AAA TCT AGA GCT TCG TCC TGC TGT	disruption primer $corA_1$	[1]
KO $corA_1$ 1 *Age*I	AAA ACC GGT ATG TCA CTG CGG GCT GCG TAA	*cre-lox* primer $corA_1$	[1]
KO $corA_1$ 2 *Apa*I	AAA GGG CCC GAT TCC TTG TTA CTT GGC GGC G	*cre-lox* primer $corA_1$	[1]
KO $CorA_1$ 3 *Nco*I	AAA CCA TGG AGC CTT CCT GGT CCC CTC TCC	*cre-lox* primer $corA_1$	[1]
KO $corA_1$ 4 *Mun*I	AAA CAA TTG TTC CAC CAT CAC GCA GCA ACT	*cre-lox* primer $corA_1$	[1]
KO $corA_2$ 1 *Age*I	AAA ACC GGTGGT TTG CCC GCC TTC TCT TTA	*cre-lox* primer $corA_2$	[1]
KO $corA_2$ 2 *Apa*I	AAA GGG CCC CGG CCG TCC GAC ATT TGT T	*cre-lox* primer $corA_2$	[1]
KO $corA_2$3 *Nco*I	AAA CCA TGG CGG TTT CGT CGC CAT GAT CTC	*cre-lox* primer $corA_2$	[1]
KO $corA_2$ 4 *Mun*I	AAA CAA TTG TCC ATT CCA TTA CTG CCC CCT G	*cre-lox* primer $corA_2$	[1]
KO $corA_3$ 1 *Age*I	AAA ACC GGT AGC AAT CTG GGC AAG GGC AAC	*cre-lox* primer $corA_3$	[1]
KO $corA_3$ 2 *Apa*I	AAA GGG CCC GCT CAG GCC TGC AGC AGG C	*cre-lox* primer $corA_3$	[1]
KO $corA_3$3 *Nco*I	AAA CCA TGG ATC GCT AGG ACG CGG ACC G	*cre-lox* primer $corA_3$	[1]
KO $corA_3$ 4 *Mun*I	AAA CAA TTG GCC GCT TTC CCA TCT GAG TCT G	*cre-lox* primer $corA_3$	[1]
KO *pitA* 1 *Age*I	AAA ACC GGT TGG AGG ATGTTG CCG AGA CGA	*cre-lox* primer *pitA*	[1]
KO *pitA2Apa*I	AAA GGG CCC GCG CCT CAG GCG TTT TCC AG	*cre-lox* primer *pitA*	[1]
KO *pitA3Not*I	AAA GCG GCC GCG GCA AGC GCG AGC GGA AT	*cre-lox* primer *pitA*	[1]
KO *pitA4Mun*I	AAA CAA TTG GGC AATGTC CCT CGT CGG C	*cre-lox* primer *pitA*	[1]
*zup*T 1 *Nco*I	AAA CCA TGG CGG TGC TGG GTC TGT	*cre-lox* primer *zupT*	[1]
*zup*T 2 NotI	AAA GCG GCC GCG CAC CCC GAC TGA AAA GCT CA	*cre-lox* primer *zupT*	[1]
*zup*T 3 AgeI	AAA ACC GGT TCG AAC TGA TGT CGC TGG CGG	*cre-lox* primer *zupT*	[1]
*zup*T 4 *Sac*I	AAA GAG CTC GTG AAG ACC GTG GAT GCC GAC	*cre-lox* primer *zupT*	[1]
KO zntB1 MunI	AAA CAA TTG GCG AAC TGC GCA ATG TGC TG	*cre-lox* primer *zntB*	this work
KO zntB2 NotI	AAA GCG GCC GCA CGT TAG CAT GGC GCC GTG A	*cre-lox* primer *zntB*	this work
KO zntB3 ApaI	AAA GGG CCC TAC AAC GAC GAG GCG CTG AT	*cre-lox* primer *zntB*	this work
KO zntB4 AgaI	AAA ACC GGT TGA TCC GCT TGA TCA CAC CA	*cre-lox* primer *zntB*	this work
KO hoxN 1 AgeI	ACC ACC GGT AGT CAG CAG TTT GCG CAG CAG	*cre-lox* primer *hoxN*	this work
KO hoxN 2 ApaI	AAA GGG CCC ATC CGG CCA CGC GCC GCA TTC	*cre-lox* primer *hoxN*	this work
KO hoxN 3 NotI	AGG GCG GCC GC ATC GTT CGG CAG TGC TTC C	*cre-lox* primer *hoxN*	this work
KO hoxN 4 MunI	CAA CAA TTG-CTT CCC GCA GGC GGA TGT G	*cre-lox* primer *hoxN*	this work



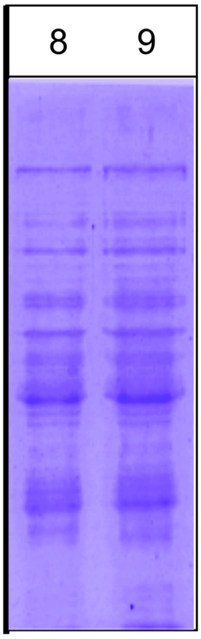
Supplementary Figure S1. Growth impairment of the mutant strains. The strains AE104 (closed circles), Δ*zupT* (**green** open circles), Δ7 (**red** open squares), Δ5 (open triangles) or Δ6 (open inverted triangles)were cultivated in TMM at 30°C without further additions (Panel A), in the presence of 10 µM Zn(II) (Panel B), 10 µM Co(II) (Panel C) or 2.5 µM Cd(II) (Panel D) and growth was measured as turbidity at 600 nm. The plots do not use a logarithmic y-axis to show the differences between the strains in the lag and the stationary phase more clearly. The table below give the growth rate (1/h)±deviation / the duration of the lag phase (h).

Strain	Growth rate µ±deviation, (1/h) / lag phase, (h)			
Metal added	none	Co(II)	Zn(II)	Cd(II)
AE104	0.29±0.03/ 0	0.26±0.07/ 0	**0.23±0.01/ 0**	0.23±0.06/ 0
ΔzupT	0.28±0.06/ 1.0	0.26±0.03/ 3.9	0.22±0.04/ 0	0.22±0.06/ 2.8
Δ5	0.29±0.06/ 0.5	0.25±0.03/ 2.6	0.24±0.01/ 0	0.20±0.02/ 0
Δ6	0.27±0.04/ 0	0.24±0.06/ 1.9	0.23±0.01/ 0	0.20±0.03/ 0
Δ7	0.34±0.15/ 6.1	0.21±0.02/ 7.0	0.23±0.01/ 0	0.20±0.07/ 6.2

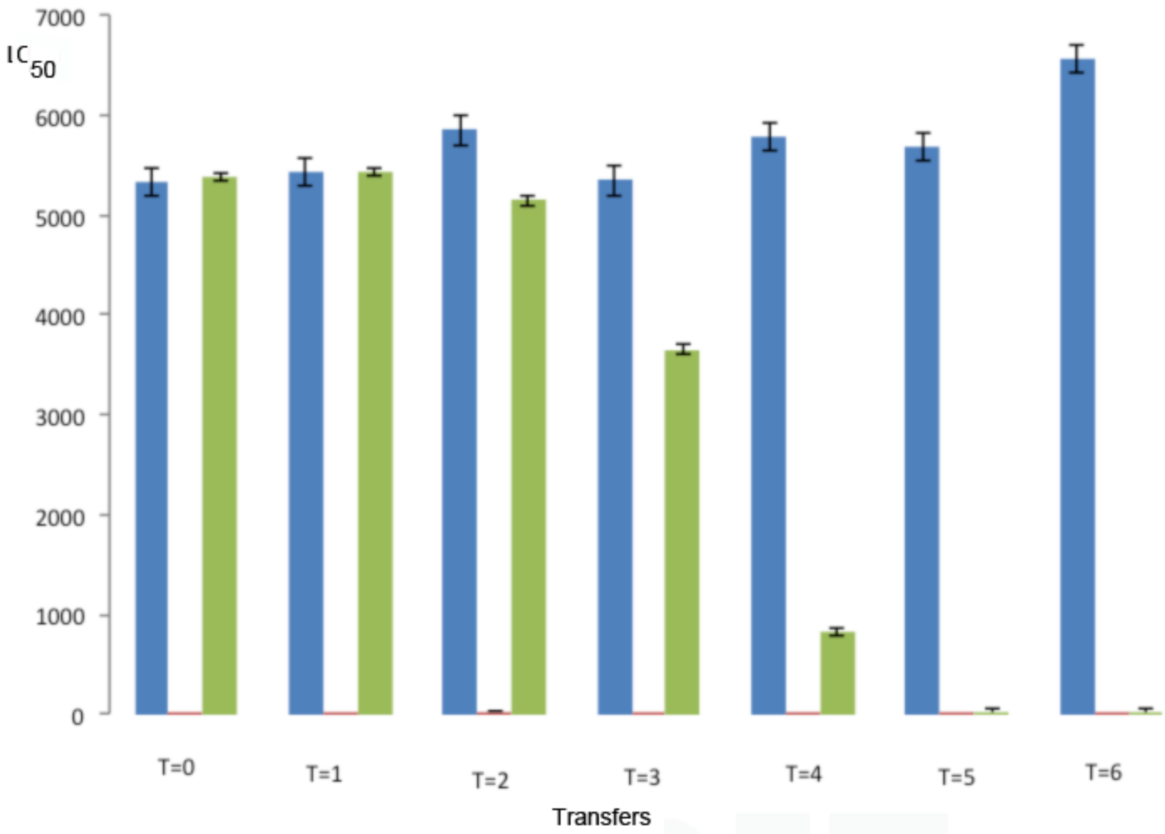
A.



B.



Supplementary Figure S2. Presence of RpoC and CzcA proteins in mutant strains of *C metallidurans* AE104. Accumulation of the RpoC subunit of the RNA polymerase was demonstrated in crude extracts in Coomassie-stained SDS gel shown here. Samples corresponding to 20 µg cellular dry mass were used per lane. The plasmid pDNA130 that expressed the *czcCBAD'* determinant constitutively in *C. metallidurans* cells had been transferred by conjugation into the mutant strains and their ability to produce CzcA, the central part of the CzcCBA efflux pump, as determined by a subsequent Western blot using anti-CzcA antibodies. The gel shown in Panel A was used for both experiments. Absence of CzcA in the ∆e4 ∆*zupT* strain could only be shown after six transfers on culture plates (Suppl. Fig. 3). Therefore, a second series of experiments was performed and the SDS gel used here is show in Panel B. Marker (M); AE104 negative control (1); ∆*zupT* positive control (2); ∆*pitA* (3); ∆*zupT* ∆*pitA* (4), ∆4 (5); ∆5 (6); ∆6 (7); ∆e4 (8); ∆e4 ∆*zupT* (9).



Supplementary Figure S3. Zinc resistance of ∆e4 ∆*zupT*(pDNA130) strains decreases with the number of transfers on strain collection plates. Similar to a published experiment [2], plasmid pDNA130 [3] that carries a constitutively expressed *czcCBAD'* region was freshly transferred into the ∆e4 ∆*zupT* mutant strain and zinc resistance of this strain (green bars) was compared to that of strain AE104(pDNA130)(blue bars) and an already existing ∆e4*zupT*(pDNA130) (small red bars between the two others). Resistance was determined in dose-response curves and the IC_{50} values were determined as published [4]. All strains were kept on TMM plates with antibiotic but without additional zinc as selection pressure, and after each monthly transfer, zinc resistance was determined again. Three experiments per transfer, deviation bars shown.

References for the Supplementary Material

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