Table S1. List of CDF proteins from *Bacteria*, *Archaea* and *Eukarya* included in the phylogenetic analysis. Their substrate specificity (in bold) is supported by two or more experimental evidences: 1) phenotypic analysis of mutants, 2) transcriptional gene induction by the target metal, 3) heterologous expression of the CDF-encoding gene, 4) differential metal-target accumulation in a mutant compared to wild type and 5) direct transport of the target metal into proteoliposomes. [a]The proteins not previously included by Montanini, et al. (2007) are gray-shaded. Proteins which only could be retrieved from the Montanini's dataset are written in blue. [b] ND = Not Determined

Organism	ID Protein	Genbank Accession number[a]	Substrates[b]	Reference
Clade I				
Bacillus subtilis	BsYeaB	P46348.2	ND	
Bacillus subtilis	BsYdbO	BAA19291.1	ND	
Lactococcus lactis subsp. lactis Il1403	L106965	NP_266464.1	ND	
Streptococcus gordonii str. Challis	SGO_1337	ABV10776.1	ND	
Streptococcus pneumoniae D39	**MntE**	**ABJ55467.1**	**Mn**	1
Streptococcus mutans UA159	SMU_1176	AAN58867.1	ND	
Streptococcus agalactiae A909	SAK_1232	YP_329849.1	ND	
Streptococcus gallolyticus UCN34	GALLO_1001	CBI13493.1	ND	
Bacillus cereus ATCC 14579	BC_4404	AAP11317.1	ND	
Clade II				
Ralstonia solanacearum GMI1000	RSc3077	CAD16786.1	ND	
Chromobacterium violaceum ATCC 12472	CV_1005	AAQ58679.1	ND	
Cupriavidus metallidurans CH34	**CmFieF**	**ZP_00593836.1**	**Fe, Co, Ni, Cd, Zn**	2
Phanerochaete chrysosporium	Phc_588	e_gww2.15.88.1, Phchr1:140863	ND	
Ustilago maydis 521	UM00151	XP_756298.1	ND	
Gibberella zeae PH-1	FG05506	XP_385682.1	ND	
Magnaporthe grisea 70-15	MGG_06247	XP_369732.2	ND	
Neurospora crassa OR74A	NCU07879	XP_962812.2	ND	
Sclerotinia sclerotiorum 1980	SS1G_07340	XP_001591894.1	ND	
Botrytis cinerea	BC1G_04719	BC1G_04719.1	ND	
Stagonospora nodorum	SNU00278	SNU00278.1	ND	
Aspergillus fumigatus Af293	Af5g09830	XP_753657.1	ND	
Aspergillus nidulans FGSC A4	AN8791	XP_682060.1	ND	
Ashbya gossypii ATCC 10895	AgAFL128	AAS53246.1	ND	
Saccharomyces cerevisiae	**ScMMT1**	**NP_013902.1**	**Fe**	3
Saccharomyces cerevisiae	**ScMMT2**	**NP_015100.1**	**Fe**	3
Clade III				
Corynebacterium glutamicum ATCC13032	Cgl2783	BAC00177.1	ND	
Streptomyces coelicolor A3(2)	SCO1310	CAC42857.1	ND	
Arthrobacter aurescens	AAS20133	AAS20133.1	ND	
Mycobacterium bovis AF2122/97	Mb2050c	NP_855700.1	ND	
Mycobacterium tuberculosis H37Ra	MtH3_21403	ZP_02552722.1	ND	
Mycobacterium tuberculosis H37Rv	Rv2025c	NP_216541.1	ND	
Mycobacterium vanbaalenii PYR-1	Mvan_3225	YP_954032.1	ND	
Mycobacterium gilvum PYR-GCK	Mflv_1902	YP_001133170.1	ND	
Mycobacterium sp. MCS	Mmcs_1489	ABG07600.1	ND	
Mycobacterium vanbaalenii PYR-1	Mvan_3726	YP_954515.1	ND	
Synechococcus sp. PCC 7335	S7335_4872	ZP_05038430.1	ND	
Rhodobacter sp. SW2	Rsw2_2021	EEW25041.1	ND	
Rhizobium leguminosarum bv. viciae 3841	RL1175	CAK06672.1	ND	

Rhizobium etli CFN 42	**NepA**	**ABC93653.1**	**Ni, Co**	This work
Rhizobium etli CIAT 652	CIATCH3409	ACE92356.1	ND	
Methanosphaerula palustris E1-9c	Mpal_2329	ACL17614.1	ND	
Methanosarcina mazei Go1	MM0167	AAM29863.1	ND	
Methanosarcina acetivorans C2A	MA0805	AAM04244.1	ND	
Methanosarcina barkeri str. Fusaro	Mbar_A2147	YP_305656.1	ND	
Methanosarcina barkeri str. Fusaro	Mbar_A3649	YP_307093.1	ND	
Methanosarcina acetivorans C2A	MA4005	AAM07355.1	ND	
Methanosarcina barkeri str. Fusaro	Mbar_A1220	YP_304767.1	ND	
Clade IV				
Chlamydomonas reinhardtii	CrMTP2	110062	ND	
Ustilago maydis 521	UM05647	XP_761794.1	ND	
Gibberella zeae PH-1	FG01162	XP_381338.1	ND	
Magnaporthe grisea 70-15	MGG_04623	XP_362178.1	ND	
Neurospora crassa OR74A	NCU09368	XP_963778.1	ND	
Stagonospora nodorum	SNU09283	SNU09283.1	ND	
Sclerotinia sclerotiorum 1980	SS1G_04677	XP_001594869.1	ND	
Botrytis cinerea	BC1G_00092	BC1G_00092.1	ND	
Aspergillus nidulans FGSC A4	AN5049	XP_662653.1	ND	
Aspergillus fumigatus Af293	Af6g00440	XP_731511.1	ND	
Neurospora crassa OR74A	NCU05157	XP_956655.2	ND	
Gibberella zeae PH-1	FG05723	XP_385899.1	ND	
Magnaporthe grisea 70-15	MGG_04407	XP_361962.2	ND	
Botrytis cinerea	BC1G_05581	BC1G_05581.1	ND	
Stagonospora nodorum	SNU09445	SNU09445.1	ND	
Caenorhabditis elegans	CePDB1	AAA81718.3	ND	
Caenorhabditis elegans	CeF56C9	AAK18959.2	ND	
Rhizopus oryzae	RO3G_02629	RO3G_02629.1	ND	
Sclerotinia sclerotiorum 1980	SS1G_01056	XP_001598966.1	ND	
Botrytis cinerea	BC1G_13811	BC1G_13811.1	ND	
Neurospora crassa OR74A	NCU04818	XP_960444.2	ND	
Stagonospora nodorum	SNU01554	SNU01554.1	ND	
Magnaporthe grisea 70-15	MGG_04968	XP_359809.2	ND	
Gibberella zeae PH-1	FG01883	XP_382059.1	ND	
Magnaporthe grisea 70-15	MGG_07494	XP_367583.1	ND	
Neurospora crassa OR74A	NCU06699	XP_961014.1	ND	
Stagonospora nodorum	SNU02386	SNU02386.1	ND	
Aspergillus oryzae	Af1g14440	BAE64878.1	ND	
Aspergillus nidulans FGSC A4	AN1146	XP_658750.1	ND	
Ustilago maydis 521	UM03814	XP_759961.1	ND	
Paxillus involutus	PiMnT1	DQ515983	ND	
Phanerochaete chrysosporium	Phc_3213	e_gww2.13.213.1, Phchr1:135578	ND	
Entamoeba histolytica HM-1:IMSS	Eh152	XP_651682.2	ND	
Entamoeba histolytica HM-1:IMSS	Eh166	XP_651432.1	ND	
Arabidopsis thaliana	AtMTP8	NP_191365.2	ND	
Oryza sativa Japonica Group	Os02g53490	NP_001044741.1	ND	
Stylosanthes hamata	**ShMTP1**	**AAO38707.1**	**Mn**	[4]
Oryza sativa Japonica Group	Os03g12530	NP_001049439.1	ND	
Populus trichocarpa	PtrMTP8.4	FGENESH4_PM.C_LG_III000766	ND	

Populus trichocarpa	PtrMTP8.1	EUGENE3.00080435	ND	
Populus trichocarpa	PtrMTP8.2	GW1.I.1107.1	ND	
Oryza sativa Japonica Group	Os05g38670	NP_001055764	ND	
Oryza sativa Japonica Group	Os01g62070	NP_001041920.1	ND	
Arabidopsis thaliana	**AtMTP11**	**NP_181477.1**	**Mn**	5,6
Populus trichocarpa	PtrMTP11.1	GW1.I.5578.1	ND	
Populus trichocarpa	PtrMTP11.2	FGENESH4_PG.C_LG_VIII000724	ND	
Oryza sativa Japonica Group	Os01g03910	BAA99362.1	ND	
Oryza sativa Japonica Group	Os01g03914	NP_001041920.1	ND	
Stylosanthes hamata	ShMTP4	AAO38710.1	ND	
Arabidopsis thaliana	AtMTP10	NP_173081.2	ND	
Arabidopsis thaliana	AtMTP9	NP_178070.2	ND	
Stylosanthes hamata	ShMTP2	AAO38708.1	ND	
Stylosanthes hamata	ShMTP3	AAO38709.1	ND	
Populus trichocarpa	PtrMTP9	ESTEXT_GENEWISE1_V1.C_LG_XIV1924	ND	
Populus trichocarpa	PtrMTP10.3	GW1.I.6617.1	ND	
Caenorhabditis elegans	CeF41C6	AAA80448.2	ND	
Clade V				
Rickettsia prowazekii	RpP34	Q9ZCC5.1	ND	
Rickettsia felis URRWXCal2	RF_1320	YP_247336.1	ND	
Rickettsia rickettsii	RrP34	P21559.1	ND	
Thiomicrospira crunogena XCL-2	Tcr_0241	YP_390511.1	ND	
Pectobacterium atrosepticum	PaFIEF	Q6CZ45.1	ND	
Yersinia pseudotuberculosis	YpsFieF	Q66GA9.1	ND	
Klebsiella pneumoniae	KpFieF	Q8RR17.1	ND	
Escherichia coli	**EcYiiP**	**P69380.1**	**Fe, Zn, Cd**	7,8
Salmonella typhi	StiFieF	Q8Z2W4.1	ND	
Salmonella typhimurium	StyFieF	Q8ZKR4.1	ND	
Clade VI				
Thermus thermophilus	**TtCzrB**	**CAC83722.1**	**Zn, Cd**	9,10
Sinorhizobium meliloti 1021	SMc02724	NP_386492.1	ND	
Agrobacterium tumefaciens str. C58	Atu2274	NP_532947.1	ND	
Rhizobium etli CFN 42	CH3072	YP_470566.1	ND	
Rhizobium leguminosarum bv. viciae 3841	RL3518	YP_769098.1	ND	
Mesorhizobium loti MAFF303099	Mlr2775	NP_104036.1	ND	
Deinococcus radiodurans	**Dr1236**	**Q9RUZ4**	**Mn**	11
Azorhizobium caulinodans ORS 571	AZC_4559	YP_001527475.1	ND	
Mycobacterium vanbaalenii PYR-1	Mvan_5864	YP_956635.1	ND	
Nostoc punctiforme PCC 73102	NpF2455	ZP_00110704.1	ND	
Synechocystis sp. Strain PCC 6803	**Sll1263**	**P74068.1**	**Fe**	12
Clade VII				
Rhizopus oryzae	RO3G_08509	RO3G_08509.1	ND	
Drosophila melanogaster	Dm11163_PA	AAF57308.2	ND	
Cryptosporidium parvum Iowa II	Cgd1_3050	EAK88341.1	ND	
Homo sapiens	HsZnT4	O14863.2	ND	
Mus musculus	**MmZnT4**	**O35149.1**	**Zn**	13
Rattus novergicus	RnZnT4	O55174.1	ND	
Homo sapiens	HsZnT8	NP_776250.2	ND	
Drosophila melanogaster	Dm3994_PA	AAF53443.3	ND	

Drosophila melanogaster	Dm3994_PB	AAN10893.1	ND	
Homo sapiens	HsZnT3	Q99726.2	ND	
Mus musculus	**MmZnT3**	**P97441.1**	**Zn**	14
Mus musculus	MmZnT3-1	NP_035903.1	ND	
Homo sapiens	**HsZnT2**	**NP_001004434.1**	**Zn**	15,16
Rattus novergicus	RnZnT2	Q62941.1	ND	
Chlamydomonas reinhardtii	CrMTP1	670027	ND	
Oryza sativa Japonica Group	**OsMTP1**	**NP_001054539.1**	**Zn, Cd, Ni**	17
Hordeum vulgare	**HvMTP1**	**AM286795**	**Zn, Co**	18
Arabidopsis thaliana	AtMTP3	NP_191440.2	ND	
Populus trichocarpa	PtrMTP3.1	GW1.XIV.294.1	ND	
Populus trichocarpa	PtrMTP3.2	GW1.I.6615.1	ND	
Arabidopsis thaliana	AtMTP2	NP_191753.1	ND	
Nicotiana tabacum	**NtMTP1B**	**BAD89563.1**	**Zn, Co**	19
Nicotiana glauca	**NgMTP1**	**BAD89561.1**	**Zn, Co**	19
Nicotiana tabacum	**NtMTP1A**	**BAD89562.1**	**Zn, Co**	19
Thlaspi goesingense	**TgMTP1T2**	**AAK91871.2**	**Ni, Co, Zn, Cd**	20
Thlaspi goesingense	**TgMTP1c**	**AAS67026.1**	**Zn**	21
Thlaspi goesingense	**TgMTP1a**	**AAS67024.1**	**Zn**	21
Thlaspi goesingense	**TgMTP1b**	**AAS67025.1**	**Zn**	21
Arabidopsis halleri subsp. halleri	AhCDF1_3	CAD89013.1	ND	
Arabidopsis thaliana	**AtMTP1**	**NP_182203.1**	**Zn**	22
Arabidopsis lyrata subsp. lyrata	AlMTP1_1	CAG28982.1	ND	
Arabidopsis lyrata	HMTP1_1	AAR83908.1	ND	
Populus trichocarpa	PtrMTP2	FGENESH4_PM.C_LG_II000836	ND	
Populus balsamifera subsp. trichocarpa x Populus deltoides	**PtdMTP1**	**AAR23528.1**	**Zn**	23
Populus trichocarpa	PtrMTP1	ESTEXT_FGENESH4_PM.C_LG_X0839	ND	
Arabidopsis thaliana	AtMTP4	NP_180502.2	ND	
Populus trichocarpa	PtrMTP4.1	GW1.18419.4.1	ND	
Populus trichocarpa	PtrMTP4.2	FGENESH4_PM.C_SCAFFOLD_57000020	ND	
Clade VIII				
Mycobacterium leprae TN	ML0283	NP_301323.1	ND	
Mycobacterium smegmatis str. MC2 155	**Msmeg_ZitA**	**YP_885161.1**	**Zn**	24
Mycobacterium gilvum PYR-GCK	Mflv_0231	YP_001131514.1	ND	
Mycobacterium vanbaalenii PYR-1	Mvan_0673	YP_951517.1	ND	
Arthrobacter aurescens	AaCzcD	AAS20069.1	ND	
Cupriavidus metallidurans CH34	**CmCzcD**	**P13512.2**	**Zn, Co, Cd**	25,26
Corynebacterium glutamicum ATCC13032	Cgl1218	BAB98674.1	ND	
Bacillus cereus ATCC 14579	BC_1701	AAP08677.1	ND	
Bacillus subtilis subsp. subtilis str. 168	**BsCzcD**	**NP_390542.1**	**Zn, Co, Cu, Ni**	27
Staphylococcus aureus	**StaCzrB**	**BAA36686.1**	**Zn**	28
Staphylococcus aureus	**StaRzcB**	**AAC32485.1**	**Zn, Co**	29
Clade IX				
Mesorhizobium loti MAFF303099	Mll2984	NP_104195.1	ND	
Chromobacterium violaceum ATCC 12472	CV_3677	AAQ61339.1	ND	
Escherichia coli	**EcZitB**	**P75757.1**	**Zn**	30,31
Salmonella typhimurium	StyZitB	Q8ZQT3.1	ND	
Pectobacterium atrosepticum	PaZitB	Q6D7E5.1	ND	

Yersinia pestis	YpeZitB	Q8ZGY6.1	ND	
Yersinia pseudotuberculosis	YpsZitB	Q66D85.1	ND	
Clade X				
Caenorhabditis elegans	CeCDF1a	AAK39165.1	ND	
Caenorhabditis elegans	CeCDF1b	AAL13323.1	ND	
Entamoeba histolytica HM-1:IMSS	Eh005	EAL51642.1	ND	
Entamoeba histolytica HM-1:IMSS	Eh393	EAL43397.1	ND	
Drosophila melanogaster	Dm17723_PA	AAF47755.1	ND	
Aspergillus fumigatus Af293	Af4g04150	XP_746601.1	ND	
Gibberella zeae PH-1	FG09759	XP_389935.1	ND	
Aspergillus nidulans FGSC A4	AN7036	XP_664640.1	ND	
Aspergillus fumigatus Af293	Af7g06570	XP_748854.2	ND	
Stagonospora nodorum	SNU02584	SNU02584.1	ND	
Saccharomyces cerevisiae	**ScCOT1**	**CAA99636.1**	**Co**	32
Ashbya gossypii ATCC 10895	AgABR129	AAS50900.1	ND	
Saccharomyces cerevisiae	**ScZRC1**	**CAA88653.1**	**Zn**	33
Sclerotinia sclerotiorum 1980	SS1G_06298	XP_001593376.1	ND	
Botrytis cinerea	BC1G_15674	BC1G_15674	ND	
Gibberella zeae PH-1	FG00947	XP_381123.1	ND	
Neurospora crassa OR74A	NCU03145	XP_964194.1	ND	
Magnaporthe grisea 70-15	MGG_03634	XP_361091.1	ND	
Glomus intraradices	**GintZnt1**	**CAE00445.1**	**Zn**	34
Stagonospora nodorum	SNU04675	SNU04675.1	ND	
Aspergillus fumigatus Af293	Af2g14570	XP_755789.1	ND	
Aspergillus nidulans FGSC A4	AN1795	XP_659399.1	ND	
Schizosaccharomyces pombe	SpZHF1	O13918.3	ND	
Ustilago maydis 521	UM02906	XP_759053.1	ND	
Phanerochaete chrysosporium	Phc_2153	e_gwh2.12.153.1, Phchr1:124205	ND	
Phanerochaete chrysosporium	Phc_1228	e_gww2.11.228.1, Phchr1:134940	ND	
Homo sapiens	HsZnT10a	NP_061183.2	ND	
Takifugu rubripes	TrZnT_1	NP_001027895.1	ND	
Homo sapiens	HsZnT1	Q9Y6M5.2	ND	
Macaca fascicularis	MacfZnT1	Q4R6K2.1	ND	
Mus musculus	MmZnT1	Q60738.1	ND	
Rattus novergicus	**RnZnT1**	**Q62720.1**	**Zn**	35
Clade XI				
Oryza sativa Japonica Group	Os08g32650	BAC24961.1	ND	
Arabidopsis thaliana	AtMTP12	NP_178539.2	ND	
Populus trichocarpa	PtrMTP12	EUGENE3.00111272	ND	
Ashbya gossypii ATCC 10895	AgAGL102	AAS54389.1	ND	
Saccharomyces cerevisiae	**ScMSC2**	**Q03455.2**	**Zn**	36
Schizosaccharomyces pombe	**Cis4**	**Q9HGQ3.1**	**Zn**	37
Homo sapiens	**HsZnT5**	**AAM09099.1**	**Zn**	38
Homo sapiens	**HsZTL1**	**AAL84188.1**	**Zn**	39
Entamoeba histolytica HM-1:IMSS	Eh099	EAL47549.1	ND	
Gallus gallus	GgZnT6	AAY53770.1	ND	

Homo sapiens	**HsZnT6**	**NP_060434.2**	**Zn**	[40]
Mus musculus	MmZnT6	NP_659047.2	ND	
Arabidopsis thaliana	AtMTP5	NP_187817.2	ND	
Drosophila melanogaster	Dm6672_PA	AAF54604.1	ND	
Homo sapiens	**HsZnT7**	**AAM21969.1**	**Zn**	[41]
Rhizopus oryzae	RO3G_02565	RO3G_02565.1	ND	
Ustilago maydis 521	UM06139	XP_762286.1	ND	
Stagonospora nodorum	SNU09657	SNU09657.1	ND	
Aspergillus nidulans FGSC A4	AN5347	XP_662951.1	ND	
Aspergillus fumigatus Af293	Af6g14170	XP_751291.1	ND	
Magnaporthe grisea	MG10493.4	MG10493.4	ND	
Gibberella zeae PH-1	FG09632	XP_389808.1	ND	
Neurospora crassa OR74A	NCU07262	XP_962116.1	ND	
Sclerotinia sclerotiorum 1980	SS1G_02998	XP_001596775.1	ND	
Botrytis cinerea	BC1G_14239	BC1G_14239	ND	
Clade XII				
Pseudomonas aeruginosa	PsaPA1297	Q9I447	ND	[2]
Cupriavidus metallidurans CH34	**CmDmeF**	**ZP_00594243.1**	**Co, Ni, Fe, Cd, Zn**	
Shewanella oneidensis MR-1	SO_2045	NP_717648.1	ND	
Burkholderia cenocepacia AU 1054	Bcen_4761	ABF79640.1	ND	
Bradyrhizobium japonicum USDA 110	bll5050	NP_771690.1	ND	
Magnetospirillum magneticum AMB-1	Mmamb1013	YP_420376.1	ND	
Magnetospirillum magneticum AMB-1	Mmamb1234	YP_420597.1	ND	
Agrobacterium tumefaciens str. C58	Atu0891	NP_531589.1	ND	
Sinorhizobium meliloti 1021	SMc04167	NP_386102.1	ND	
Rhizobium etli CFN 42	**CepA**	**ABC90024.1**	**Co**	This work
Rhizobium leguminosarum bv. viciae 3841	RL1351	YP_766957.1	ND	
Clade XIII				
Kluyveromyces lactis	Kl_Q6CKZ6	CAG98101.1	ND	
Ashbya gossypii ATCC 10895	AgAGL227	AAS54264.1	ND	
Candida glabrata	CAGL07997g	CAG59637.1	ND	
Saccharomyces cerevisiae	**ScZRG17**	**NP_014437.1**	**Zn**	[42]
Schizosaccharomyces pombe	SpO14329	O14329.1	ND	
Candida albicans SC5314	CaO19_376	EAK98200.1	ND	
Debaryomyces hansenii	DEHA09933g	CAG86140.2	ND	
Yarrowia lipolytica	YALI02552g	CAG82647.1	ND	
Aspergillus fumigatus	Af1g12090	CAE47937.1	ND	
Aspergillus nidulans FGSC A4	AN1076	EAA66194.1	ND	
Gibberella zeae PH-1	FG07454	EAA77471.1	ND	
Neurospora crassa OR74A	NCU01254	EAA31338.2	ND	
Clade XIV				
Arabidopsis thaliana	AtMTP7	NP_564594.1	ND	
Populus trichocarpa	PtrMTP7	GW1.X.3157.1	ND	
Homo sapiens	HsZnT9	NP_006336.3	ND	
Streptomyces coelicolor A3(2)	SCO3024	CAB88908.1	ND	
Nostoc punctiforme PCC 73102	NpF1794	ZP_00109098.1	ND	
Nostoc punctiforme PCC 73102	NpR3104	ZP_00107840.1	ND	
Xanthomonas campestris pv. vesicatoria str. 85-10	XCV1414	YP_363145.1	ND	
Mesorhizobium loti MAFF303099	Mll4860	NP_105637.1	ND	

Streptomyces coelicolor A3(2)	SCO0776	CAC14341.1	ND	
Streptomyces coelicolor A3(2)	SCO2772	CAB87209.1	ND	
Clade XV				
Nitrosospira multiformis ATCC 25196	Nmul_A1744	ABB75041.1	ND	
Thiomicrospira crunogena XCL-2	Tcr_1014	ABB41609.1	ND	
Rhodopseudomonas palustris CGA009	RPA0220	CAE25664.1	ND	
Bradyrhizobium japonicum USDA 110	blr4938	NP_771578.1	ND	
Azorhizobium caulinodans ORS 571	AZC_3457	YP_001526373.1	ND	
Clade XVI				
Methanosarcina acetivorans C2A	MA0549	AAM03993.1	ND	
Methanosarcina mazei Go1	MM1711	AAM31407.1	ND	
Synechococcus sp. CC9605	Synsp9605	YP_381353.1	ND	
Synechococcus elongatus PCC 7942	Synel_7942	YP_401006.1	ND	
Nostoc punctiforme PCC 73102	NpF0707	ZP_00109054.1	ND	
Clade XVII				
Thiomicrospira crunogena XCL-2	Tcr_2193	ABB42781.1	ND	
Pseudomonas fluorescens Pf-5	PfPFL_0604	AAY96011.1	ND	
Yersinia enterocolitica subsp. enterocolitica 8081	YE2833	YP_001007022.1	ND	
Stenotrophomonas maltophilia R551-3	Smal_0225	YP_002026613.1	ND	
Pseudomonas putida KT2440	PP_1227	NP_743387.1	ND	
Sinorhizobium meliloti 1021	SMa0683	NP_435608.2	ND	
Rhizobium etli CFN 42	PD193	NP_659845.1	ND	
Agrobacterium tumefaciens str. C58	Atu0991	NP_531689.1	ND	
Clade XVIII				
Methanosarcina acetivorans C2A	MA4394	AAM07736.1	ND	
Methanosarcina mazei Go1	MM1076	AAM30772.1	ND	
Methanosarcina acetivorans C2A	MA3366	AAM06735.1	ND	
Methanosarcina mazei Go1	MM2778	AAM32474.1	ND	
Methanosarcina acetivorans C2A	MA0617	AAM04061.1	ND	
Methanosarcina mazei Go1	MM1778	AAM31474.1	ND	
Unassigned				
Geobacter sulfurreducens PCA	GSU0487	AAR33819.1	ND	
Azorhizobium caulinodans ORS 571	AZC_2899	YP_001525815.1	ND	
Azorhizobium caulinodans ORS 571	AZC_3738	YP_001526654.1	ND	
Enterococcus faecalis	EfEF0859	Q837I0	ND	
Clostridium tetani E88	CTC_02458	AAO36923.1	ND	
Magnetospirillum gryphiswaldense	MgMamB	CAJ30127.1	ND	
Rhodopseudomonas palustris CGA009	RPA1939	CAE27380.1	ND	
Aquifex aeolicus	AQU_2073	O67851	ND	
Methanosarcina acetivorans C2A	MA2085	AAM05485.1	ND	
Geobacter sulfurreducens PCA	GSU2613	AAR35985.1	ND	
Bacillus cereus ATCC 14579	BC_2261	AAP09225.1	ND	
Methanocaldococcus jannaschii	Mj0449	Q57891.1	ND	
Arabidopsis thaliana	AtMTP6	NP_182304.2	ND	
Populus trichocarpa	PtrMTP6	FGENESH4_PM.C_LG_X000667	ND	
Oryza sativa Japonica Group	Os03g22550	NP_001050094.1	ND	
Bacillus subtilis subsp. subtilis str. 168	BsYdfM	NP_388428.2	ND	
Clostridium tetani E88	CTC_01013	AAO35599.1	ND	
Desulfovibrio desulfuricans subsp.	Dde_1511	YP_388005.1	ND	

desulfuricans str. G20				
Sulfolobus solfataricus P2	SulfsCzcD	AAK42415.1	ND	
Streptococcus agalactiae A909	SAK_0514	ABA44896.1	ND	
Streptococcus pneumoniae R6	Srp1672	AAL00475.1	ND	
Methanosarcina acetivorans C2A	MA1117	AAM04538.1	ND	
Thiomicrospira crunogena XCL-2	Tcr_1429	ABB42022.1	ND	
Pyrobaculum aerophilum str. IM2	PAE2715	NP_560205.1	ND	
Pyrococcus horikoshii OT3	PhCzcD	NP_142822.1	ND	
Removed Sequences				
Ralstonia solanacearum GMI1000	RSc2772	CAD16479.1	ND	
Thiomicrospira crunogena XCL-2	Tcr_1855	ABB42447.1	ND	
Magnaporthe grisea 70-15	MGG_04407	XP_361962.2	ND	
Homo sapiens	HsZnT10b	NP_001004433.1	ND	
Oryza sativa Japonica Group)	Os0035B13	CAD40428.3	ND	
Rhizopus oryzae	RO3G_09636	RO3G_02565.1	ND	
Rhizopus oryzae	RO3G_09126	RO3G_09126	ND	
Chlamydomonas reinhardtii	CrMTP3	110067	ND	
Chlamydomonas reinhardtii	CrMTP4	110212	ND	
Chlamydomonas reinhardtii	CrMTP5	290110	ND	
Bradyrhizobium japonicum USDA 110	bll4927	NP_771567.1	ND	

Table S2. Summary of MUMSA[43] analysis for 318-protein alignments. The alignment case difficulty (AOS score) was 0.72.

Input Alignment:	Alignment accuracy (MOS Score):
ClustalOhmm	0.87
MAFFT L-INS-i	0.87
hmmalign	0.85
MUSCLE	0.84
ClustalW	0.81
Msaprobs	0.70

Table S3. Orthologous CDF proteins among *Rhizobiales*. Orthology was determined by bidirectional best BLAST hit analysis. NepA protein was the only rhizobial plasmid-encoded CDF.

Organism	**Query protein**	
	RHE_PE00218 (NepA)	**RHE_CH01219 (CepA)**
***Rhizobium etli* CFN42**	NepA	CepA
***Rhizobium phaseoli* CIAT 652**	RHECIAT_CH0003409	RHECIAT_CH0001307
***Rhizobium leguminosarum* 3841**	RL1175	RL1351
***Rhizobium leguminosarum* WSM1325**	-	Rleg_0977
***Sinorhizobium meliloti* 1021**	-	Smc04167
***Sinorhizobium medicae* WSM 419**	-	Smed_1792
***Sinorhizobium fredi* NGR 234**	-	NGR_b07440
***Bradyrhizobium japonicum* USDA 110**	-	bll5050
***Agrobacterium tumefaciens* C58**	-	atu0891
Mesorhizobium loti MAFF 303099	-	mll2984
Azorhizobium caulinodans ORS 571	-	AZC_3457

Table S4. Strains and plasmids used in this study

Strain	Relevant feature (s)	Source or reference
Rhizobium etli		
CFN42	Wild type strain. Nal^r, Sm^r.	[44]
nepA-	CFN42 RHE_PE00218::pCACM218. Nal^r, Sm^r, Gm^r.	This work
cepA-	CFN42 RHE_CH01219::pCACM1219. Nal^r, Sm^r, Gm^r.	This work
nepA-/nepAc	*nepA*- complemented with pCAC*nepA*c. Nal^r, Sm^r, Tc^r, Gm^r.	This work
cepA-/cepAc	*cepA*- complemented with pCAC*cepA*c. Nal^r, Sm^r, Tc^r, Gm^r.	This work
Escherichia coli		
MC4100	Wild type strain	[45]
MC4100/ TOPO	MC4100 containing plasmid pCR 2.1-TOPO	This work
MC4100/ *nepAc*	MC4100 containing plasmid pCACT*nepA*c	This work
MC4100/ *cepAc*	MC4100 containing plasmid pCACT*cepA*c	This work
MC4100/ *nepA*H87D	MC4100 containing plasmid pCACT*nepA*H87D	This work
MC4100/ *nepA*N88A	MC4100 containing plasmid pCACT*nepA*N88A	This work
MC4100/ *nepA*R197A	MC4100 containing plasmid pCACT*nepA*R197A	This work
DH5α	Nal^r, Sm^s, Tc^s, host for recombinant plasmids.	Stratagene
Plasmids		
pCR 2.1-TOPO	Cloning vector, Km^r.	Invitrogen
pT218m	pCR 2.1-TOPO derivative carrying 0.5kb internal fragment of the *nepA* gene, Km^r.	This work
pRK7813	Broad-host-range cosmid vector; Mob IncP; Tc^r.	[46]
pRK2013	Conjugation helper, Km^r.	[47]
pPDGm	pSK+ derivative, Gm^r, *mob*, promoterless *gusA*.	[48]
pCAC*nepA*c	1.7-kb *EcoRI* fragment containing whole *nepA* gene flanked with 200bp of both sides, cloned in pRK7813, Tc^r.	This work
pCACT*nepA*c	1.7-kb EcoRI fragment containing whole nepA gene flanked with 200bp of both sides, cloned in pCR 2.1-TOPO, Km^r, Cb^r.	This work
pCACT*nepA*H87D	1.7-kb *EcoRI* fragment containing *nepA* single mutant gene at H87 residue, cloned in TOPO, Km^r.	This work
pCACT*nepA*N88A	1.7-kb *EcoRI* fragment containing *nepA* single mutant gene at N88 residue, cloned in TOPO, Km^r.	This work
pCACT*nepA*R197A	1.7-kb *EcoRI* fragment containing *nepA* single mutant gene at R197 residue, cloned in TOPO, Km^r.	This work
pCAC*cepA*c	1.5-kb *EcoRI* fragment containing RHE_CH01219 gene, cloned in pRK7813, Tc^r.	This work
pCACT*cepA*c	1.5-kb EcoRI fragment containing RHE_CH01219 gene, cloned in pCR 2.1-TOPO, Km^r.	This work
pCACM218	pPDGm derivative carrying *EcoRI* 0.5-kb internal fragment of RHE_PE00218 from pT218m.	This work
pCACM1219	pPDGm derivative carrying *EcoRI-HindIII* 0.3-kb internal fragment of RHE_CH01219.	This work

Table S5. Oligonucleotides used in this study. Restriction sites and point mutations are indicated in bold.

Name	Sequence 5'-3'	Use	Gene	Source
C	TCCGCAGCCAATCACCCAACT	Mutation	*nepA*	This work
D	TCCAAGCGAGGGCAGATGTCC	Mutation	*nepA*	This work
E	G**GAATTC**CCAAATGACCGCCCTCGAAC	Mutation	*cepA*	This work
F	GGC**AAGCTT**GATGGCGCTGGCGAAACC	Mutation	*cepA*	This work
I	CGACAAGGAGGAAGGGGTTTT	Complementation	*nepAc*	This work
J	CGGTCCGCTCATCGTGTCCAT	Complementation	*nepAc*	This work
M	AGA**GGTACC**TCGCCCGCATCAGCCGCC	Complementation	*cepAc*	This work
N	TCG**TCTAGA**TCCTCGCTCTTGTCCTGG	Complementation	*cepAc*	This work
R	GGCGACGAAGACTTTCAACT	qRT-PCR	*nepA*	This work
S	ACGGTCAATCGCTTCATAGC	qRT-PCR	*nepA*	This work
T	CGAACACGATCATGTCTTCCT	qRT-PCR	*cepA*	This work
U	GATTTCAGCCACCATCATCAC	qRT-PCR	*cepA*	This work
Y	CGATGGCGAGACAGCTAAAT	qRT-PCR	*hisCd*	[49]
Z	ATCATCGCAACGCTATCTCC	qRT-PCR	*hisCd*	[49]
H87D-P1	CTTGCGGACACCATC**GAC**AATGTCGGCGACGCC	Single Mutant	*nepA*	This work
N88A-P1	GCGGACACCATCCAC**GCT**GTCGGCGACGCCGCG	Single Mutant	*nepA*	This work
R197A-p1	GACGGTTACCATGCC**GCC**ACCGACGGCCTGACG	Single Mutant	*nepA*	This work

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