

Table S1. Distribution of Sec-/Cys-containing sequences in different organisms

Selenoprotein family	Number of organisms containing both Sec form and Sec trait	Number of organisms containing only Cys form and Sec trait	Number of organisms containing Cys form but no Sec trait
FdhA	67	6	126
SelD	38	37	46
FrhD	10	5	9
HdrA	7	11	29
Peroxiredoxin	10	56	251
HesB-like	11	20	63
GrdA	10	0	0
GrdB	10	1	5
SelW-like	10	0	13
Prx-like	7	11	26
Thioredoxin	7	61	278
FrhA	4	12	24
GlpC	3	8	6
Proline reductase	5	1	7
DsbA-like	4	58	169
Glutaredoxin	3	45	237
Thiol:disulfide interchange protein	3	46	114
AhpD-like	2	59	228
ArsC-like	2	1	4
DsbG-like	2	10	31
Distant AhpD homolog	2	23	64
Homolog of AhpF, N-terminal domain	2	35	88
DsrE-like	1	0	4
NADH oxidase	1	59	231
GPx	1	63	244

Table S2. Complete list of sister and more distant species of selenoprotein-containing bacteria

Phylum	Organism	Sister organisms			Distant organisms			
		1	2	3	1	2	3	4
Actinobacteria	<i>Symbiobacterium thermophilum</i>	<i>Rubrobacter xylanophilus</i>	<i>Bifidobacterium longum</i>	<i>Tropheryma whippelii</i>	<i>Kineococcus radiotolerans</i>	<i>Mycobacterium avium</i>	<i>Brevibacterium linens</i>	<i>Corynebacterium diphtheriae</i>
	<i>Rubrobacter xylanophilus</i>	<i>Symbiobacterium thermophilum</i>	<i>Bifidobacterium longum</i>	<i>Tropheryma whippelii</i>	<i>Kineococcus radiotolerans</i>	<i>Mycobacterium avium</i>	<i>Brevibacterium linens</i>	<i>Corynebacterium diphtheriae</i>
	<i>Brevibacterium linens</i>	<i>Tropheryma whippelii</i>	<i>Leifsonia xyli</i>	<i>Arthrobacter sp.</i>	<i>Rubrobacter xylanophilus</i>	<i>Kineococcus radiotolerans</i>	<i>Mycobacterium avium</i>	<i>Corynebacterium diphtheriae</i>
	<i>Mycobacterium avium</i>	<i>Mycobacterium bovis</i>	<i>Mycobacterium leprae</i>	<i>Mycobacterium sp.</i>	<i>Brevibacterium linens</i>	<i>Kineococcus radiotolerans</i>	<i>Rubrobacter xylanophilus</i>	<i>Symbiobacterium thermophilum</i>
	<i>Kineococcus radiotolerans</i>	<i>Frankia sp.</i>	<i>Acidothermus cellulolyticus</i>	-	<i>Mycobacterium avium</i>	<i>Brevibacterium linens</i>	<i>Symbiobacterium thermophilum</i>	<i>Corynebacterium diphtheriae</i>
Acidobacteria	<i>Solibacter usitatus</i>	-	-	-	<i>Acidobacteria bacterium</i>	-	-	-
Aquificae	<i>Aquifex aeolicus</i>	-	-	-	-	-	-	-
Chloroflexi	<i>Chloroflexus aurantiacus</i>	<i>Chloroflexus aggregans</i>	<i>Roseiflexus sp.</i>	<i>Heliothrix oregonensis</i>	<i>Dehalococcoides ethenogenes</i>	<i>Thermomicrobium roseum</i>	<i>Candidatus Chlorothrix halophila</i>	-
Spirochaetes	<i>Treponema denticola</i>	<i>Treponema pallidum</i>	<i>Borrelia garinii</i>	<i>Borrelia burgdorferi</i>	<i>Leptospira interrogans</i>	-	-	-
Deltaproteobacteria	<i>Syntrophobacter fumaroxidans</i>	<i>Syntrophus aciditrophicus</i>	<i>Syntrophus buswellii</i>	-	<i>Desulfotalea psychrophila</i>	<i>Desulfovibrio vulgaris</i>	<i>Geobacter metallireducens</i>	<i>Desulfuromonas acetoxidans</i>
	<i>Desulfotalea psychrophila</i>	<i>Desulfobacterium autotrophicum</i>	<i>Nitrospina gracilis</i>	-	<i>Geobacter sulfurreducens</i>	<i>Desulfuromonas acetoxidans</i>	<i>Desulfovibrio vulgaris</i>	<i>Anaeromyxobacter dehalogenans</i>
	<i>Desulfovibrio desulfuricans</i>	<i>Desulfovibrio vulgaris</i>	<i>Lawsonia intracellularis</i>	-	<i>Geobacter sulfurreducens</i>	<i>Desulfotalea psychrophila</i>	<i>Desulfuromonas acetoxidans</i>	<i>Anaeromyxobacter dehalogenans</i>
	<i>Desulfovibrio vulgaris</i>	<i>Desulfovibrio desulfuricans</i>	<i>Lawsonia intracellularis</i>	-	<i>Geobacter sulfurreducens</i>	<i>Desulfotalea psychrophila</i>	<i>Desulfuromonas acetoxidans</i>	<i>Anaeromyxobacter dehalogenans</i>
	<i>Syntrophus aciditrophicus</i>	<i>Syntrophus buswellii</i>	<i>Syntrophobacter fumaroxidans</i>	-	<i>Desulfuromonas acetoxidans</i>	<i>Desulfotalea psychrophila</i>	<i>Desulfovibrio vulgaris</i>	<i>Geobacter sulfurreducens</i>
	<i>Pelobacter carbinolicus</i>	<i>Pelobacter propionicus</i>	<i>Geobacter sulfurreducens</i>	<i>Geobacter metallireducens</i>	<i>Desulfotalea psychrophila</i>	<i>Desulfovibrio vulgaris</i>	<i>Syntrophus aciditrophicus</i>	<i>Anaeromyxobacter dehalogenans</i>
	<i>Geobacter metallireducens</i>	<i>Geobacter sulfurreducens</i>	<i>Geobacter uraniumreducens</i>	<i>Pelobacter carbinolicus</i>	<i>Desulfotalea psychrophila</i>	<i>Desulfovibrio vulgaris</i>	<i>Syntrophus aciditrophicus</i>	<i>Desulfuromonas acetoxidans</i>
	<i>Geobacter sulfurreducens</i>	<i>Geobacter metallireducens</i>	<i>Geobacter uraniumreducens</i>	<i>Pelobacter carbinolicus</i>	<i>Desulfotalea psychrophila</i>	<i>Desulfovibrio vulgaris</i>	<i>Syntrophus aciditrophicus</i>	<i>Desulfuromonas acetoxidans</i>
	<i>Geobacter uraniumreducens</i>	<i>Geobacter sulfurreducens</i>	<i>Geobacter metallireducens</i>	<i>Pelobacter carbinolicus</i>	<i>Desulfotalea psychrophila</i>	<i>Desulfovibrio vulgaris</i>	<i>Syntrophus aciditrophicus</i>	<i>Desulfuromonas acetoxidans</i>
	<i>Desulfuromonas acetoxidans</i>	<i>Geobacter metallireducens</i>	<i>Geobacter sulfurreducens</i>	<i>Pelobacter carbinolicus</i>	<i>Desulfotalea psychrophila</i>	<i>Syntrophus aciditrophicus</i>	<i>Anaeromyxobacter dehalogenans</i>	<i>Desulfovibrio vulgaris</i>
	<i>Anaeromyxobacter dehalogenans</i>	<i>Myxococcus xanthus</i>	-	-	<i>Desulfotalea psychrophila</i>	<i>Syntrophus aciditrophicus</i>	<i>Desulfovibrio vulgaris</i>	<i>Geobacter metallireducens</i>
Firmicutes/Bacillales	<i>Bacillus sp.</i>	<i>Bacillus anthracis</i>	<i>Bacillus clausii</i>	<i>Bacillus thuringiensis</i>	<i>Listeria innocua</i>	<i>Oceanobacillus iheyensis</i>	<i>Staphylococcus aureus</i>	<i>Geobacillus kaustophilus</i>
Firmicutes/Clostridia	<i>Moorella thermoacetica</i>	<i>Thermoanaerobacter ethanolicus</i>	<i>Thermoanaerobacter tengcongensis</i>	<i>Ammonifex degensii</i>	<i>Carboxydotherrmus hydrogenoformans</i>	<i>Desulfotobacterium hafniense</i>	<i>Clostridium difficile</i>	<i>Desulfotomaculum reducens</i>
	<i>Syntrophomonas wolfei</i>	<i>Caldicellulosiruptor saccharolyticus</i>	<i>Anaerobranca gottschalkii</i>	-	<i>Moorella thermoacetica</i>	<i>Carboxydotherrmus hydrogenoformans</i>	<i>Thermoanaerobacter tengcongensis</i>	<i>Desulfotomaculum reducens</i>

	<i>Desulfotomaculum reducens</i>	<i>Carboxydotherrmus hydrogenoformans</i>	<i>Desulfitobacterium hafniense</i>	-	<i>Moorella thermoacetica</i>	<i>Syntrophomonas wolfei</i>	<i>Clostridium difficile</i>	<i>Thermoanaerobacter tengcongensis</i>
	<i>Carboxydotherrmus hydrogenoformans</i>	<i>Desulfotomaculum reducens</i>	<i>Desulfitobacterium hafniense</i>	-	<i>Moorella thermoacetica</i>	<i>Syntrophomonas wolfei</i>	<i>Clostridium difficile</i>	<i>Thermoanaerobacter tengcongensis</i>
	<i>Thermoanaerobact er tengcongensis</i>	<i>Moorella thermoacetica</i>	<i>Thermoanaerobact er ethanolicus</i>	<i>Ammonifex degensii</i>	<i>Carboxydotherrmus hydrogenoformans</i>	<i>Desulfitobacterium hafniense</i>	<i>Clostridium difficile</i>	<i>Desulfotomaculum reducens</i>
	<i>Clostridium perfringens</i>	<i>Clostridium difficile</i>	<i>Clostridium tetani</i>	<i>Clostridium botulinum</i>	<i>Thermoanaerobacter tengcongensis</i>	<i>Moorella thermoacetica</i>	<i>Desulfitobacterium hafniense</i>	<i>Carboxydotherrmus hydrogenoformans</i>
	<i>Clostridium difficile</i>	<i>Clostridium perfringens</i>	<i>Clostridium tetani</i>	<i>Clostridium botulinum</i>	<i>Thermoanaerobacter tengcongensis</i>	<i>Moorella thermoacetica</i>	<i>Desulfitobacterium hafniense</i>	<i>Carboxydotherrmus hydrogenoformans</i>
	<i>Alkaliphilus metalliredigenes</i>	<i>Clostridium difficile</i>	<i>Clostridium perfringens</i>	<i>Clostridium tetani</i>	<i>Carboxydotherrmus hydrogenoformans</i>	<i>Thermoanaerobacter tengcongensis</i>	<i>Desulfotomaculum reducens</i>	<i>Moorella thermoacetica</i>
	<i>Desulfitobacterium hafniense</i>	<i>Carboxydotherrmus hydrogenoformans</i>	<i>Desulfotomaculum reducens</i>	-	<i>Clostridium perfringens</i>	<i>Alkaliphilus metalliredigenes</i>	<i>Thermoanaerobacter tengcongensis</i>	<i>Moorella thermoacetica</i>
	<i>Sulfitobacter sp.</i>	<i>Jannaschia sp.</i>	<i>Paracoccus denitrificans</i>	<i>Rhodobacter sphaeroides</i>	<i>Xanthobacter autotrophicus</i>	<i>Sinorhizobium meliloti</i>	<i>Silicibacter sp.</i>	<i>Rickettsia bellii</i>
Alphaproteobac teria	<i>Paracoccus denitrificans</i>	<i>Sulfitobacter sp.</i>	<i>Jannaschia sp.</i>	<i>Rhodobacter sphaeroides</i>	<i>Xanthobacter autotrophicus</i>	<i>Sinorhizobium meliloti</i>	<i>Silicibacter sp.</i>	<i>Rickettsia bellii</i>
	<i>Xanthobacter autotrophicus</i>	<i>Brucella suis</i>	<i>Bartonella quintana</i>	<i>Sinorhizobium meliloti</i>	<i>Paracoccus denitrificans</i>	<i>Silicibacter sp.</i>	<i>Rickettsia bellii</i>	<i>Acidiphilium cryptum</i>
	<i>Sinorhizobium meliloti pSymA</i>	<i>Rhizobium etli</i>	<i>Agrobacterium tumefaciens</i>	<i>Xanthobacter autotrophicus</i>	<i>Paracoccus denitrificans</i>	<i>Silicibacter sp.</i>	<i>Rickettsia bellii</i>	<i>Acidiphilium cryptum</i>
	<i>Dechloromonas aromatica</i>	<i>Azoarcus sp.</i>	-	-	<i>Bordetella bronchiseptica</i>	<i>Burkholderia pseudomallei</i>	<i>Chromobacterium violaceum</i>	<i>Thiobacillus denitrificans</i>
Betaproteobacte ria	<i>Burkholderia fungorum</i>	<i>Burkholderia mallei</i>	<i>Burkholderia pseudomallei</i>	<i>Burkholderia vietnamiensis</i>	<i>Dechloromonas aromatica</i>	<i>Bordetella bronchiseptica</i>	<i>Chromobacterium violaceum</i>	<i>Thiobacillus denitrificans</i>
	<i>Burkholderia thailandensis</i>	<i>Burkholderia mallei</i>	<i>Burkholderia pseudomallei</i>	<i>Burkholderia ambifaria</i>	<i>Dechloromonas aromatica</i>	<i>Bordetella bronchiseptica</i>	<i>Chromobacterium violaceum</i>	<i>Thiobacillus denitrificans</i>
	<i>Burkholderia pseudomallei</i>	<i>Burkholderia mallei</i>	<i>Burkholderia ambifaria</i>	<i>Burkholderia fungorum</i>	<i>Dechloromonas aromatica</i>	<i>Bordetella bronchiseptica</i>	<i>Chromobacterium violaceum</i>	<i>Thiobacillus denitrificans</i>
	<i>Burkholderia mallei</i>	<i>Burkholderia pseudomallei</i>	<i>Burkholderia vietnamiensis</i>	<i>Burkholderia cenocepacia</i>	<i>Dechloromonas aromatica</i>	<i>Bordetella bronchiseptica</i>	<i>Chromobacterium violaceum</i>	<i>Thiobacillus denitrificans</i>
	<i>Burkholderia ambifaria</i>	<i>Burkholderia mallei</i>	<i>Burkholderia pseudomallei</i>	<i>Burkholderia cenocepacia</i>	<i>Dechloromonas aromatica</i>	<i>Bordetella bronchiseptica</i>	<i>Chromobacterium violaceum</i>	<i>Thiobacillus denitrificans</i>
	<i>Burkholderia vietnamiensis</i>	<i>Burkholderia mallei</i>	<i>Burkholderia pseudomallei</i>	<i>Burkholderia dolosa</i>	<i>Dechloromonas aromatica</i>	<i>Bordetella bronchiseptica</i>	<i>Chromobacterium violaceum</i>	<i>Thiobacillus denitrificans</i>
	<i>Burkholderia dolosa</i>	<i>Burkholderia mallei</i>	<i>Burkholderia ambifaria</i>	<i>Burkholderia fungorum</i>	<i>Dechloromonas aromatica</i>	<i>Bordetella bronchiseptica</i>	<i>Chromobacterium violaceum</i>	<i>Thiobacillus denitrificans</i>
	<i>Burkholderia cenocepacia</i>	<i>Burkholderia pseudomallei</i>	<i>Burkholderia dolosa</i>	<i>Burkholderia mallei</i>	<i>Dechloromonas aromatica</i>	<i>Bordetella bronchiseptica</i>	<i>Chromobacterium violaceum</i>	<i>Thiobacillus denitrificans</i>
	<i>Burkholderia sp.</i>	<i>Burkholderia mallei</i>	<i>Burkholderia pseudomallei</i>	<i>Burkholderia cenocepacia</i>	<i>Dechloromonas aromatica</i>	<i>Bordetella bronchiseptica</i>	<i>Chromobacterium violaceum</i>	<i>Thiobacillus denitrificans</i>
Epsilonproteoba cteria	<i>Wolinella succinogenes</i>	<i>Helicobacter hepaticus</i>	<i>Helicobacter pylori</i>	<i>Thiomicrospira denitrificans</i>	<i>Campylobacter jejuni</i>	<i>Campylobacter upsaliensis</i>	<i>Nautilia sp.</i>	-
	<i>Helicobacter hepaticus</i>	<i>Helicobacter pylori</i>	<i>Wolinella succinogenes</i>	<i>Thiomicrospira denitrificans</i>	<i>Campylobacter jejuni</i>	<i>Campylobacter upsaliensis</i>	<i>Nautilia sp.</i>	-
	<i>Thiomicrospira denitrificans</i>	<i>Wolinella succinogenes</i>	<i>Helicobacter hepaticus</i>	<i>Helicobacter pylori</i>	<i>Campylobacter coli</i>	<i>Campylobacter jejuni</i>	<i>Nautilia sp.</i>	-
	<i>Campylobacter fetus</i>	<i>Campylobacter jejuni</i>	<i>Campylobacter coli</i>	<i>Campylobacter upsaliensis</i>	<i>Wolinella succinogenes</i>	<i>Helicobacter hepaticus</i>	<i>Nautilia sp.</i>	<i>Thiomicrospira denitrificans</i>

	<i>Campylobacter lari</i>	<i>Campylobacter jejuni</i>	<i>Campylobacter coli</i>	<i>Campylobacter upsaliensis</i>	<i>Wolinella succinogenes</i>	<i>Helicobacter hepaticus</i>	<i>Nautilia sp.</i>	<i>Thiomicrospira denitrificans</i>
	<i>Campylobacter upsaliensis</i>	<i>Campylobacter jejuni</i>	<i>Campylobacter coli</i>	<i>Campylobacter fetus</i>	<i>Wolinella succinogenes</i>	<i>Helicobacter hepaticus</i>	<i>Nautilia sp.</i>	<i>Thiomicrospira denitrificans</i>
	<i>Campylobacter jejuni</i>	<i>Campylobacter coli</i>	<i>Campylobacter upsaliensis</i>	<i>Campylobacter upsaliensis</i>	<i>Wolinella succinogenes</i>	<i>Helicobacter hepaticus</i>	<i>Nautilia sp.</i>	<i>Thiomicrospira denitrificans</i>
	<i>Campylobacter coli</i>	<i>Campylobacter jejuni</i>	<i>Campylobacter upsaliensis</i>	<i>Campylobacter coli</i>	<i>Wolinella succinogenes</i>	<i>Helicobacter hepaticus</i>	<i>Nautilia sp.</i>	<i>Thiomicrospira denitrificans</i>
Gammaproteobacteria	<i>Photobacterium sp.</i>	<i>Photobacterium profundum</i>	<i>Vibrio cholerae</i>	<i>Vibrio vulnificus</i>	<i>Escherichia coli</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas aeruginosa</i>	<i>Haemophilus influenzae</i>
	<i>Photobacterium profundum</i>	<i>Photobacterium sp.</i>	<i>Vibrio cholerae</i>	<i>Vibrio vulnificus</i>	<i>Escherichia coli</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas aeruginosa</i>	<i>Haemophilus influenzae</i>
	<i>Vibrio angustum</i>	<i>Vibrio cholerae</i>	<i>Vibrio vulnificus</i>	<i>Photobacterium profundum</i>	<i>Escherichia coli</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas aeruginosa</i>	<i>Haemophilus influenzae</i>
	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas putida</i>	<i>Pseudomonas syringae</i>	<i>Escherichia coli</i>	<i>Shewanella oneidensis</i>	<i>Haemophilus influenzae</i>	<i>Vibrio cholerae</i>
	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas putida</i>	<i>Pseudomonas syringae</i>	<i>Escherichia coli</i>	<i>Shewanella oneidensis</i>	<i>Haemophilus influenzae</i>	<i>Vibrio cholerae</i>
	<i>Pseudomonas putida</i>	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas syringae</i>	<i>Escherichia coli</i>	<i>Shewanella oneidensis</i>	<i>Haemophilus influenzae</i>	<i>Vibrio cholerae</i>
	<i>Shewanella sp.</i>	<i>Shewanella oneidensis</i>	<i>Shewanella denitrificans</i>	<i>Cobwellia psychrerythraea</i>	<i>Haemophilus influenzae</i>	<i>Escherichia coli</i>	<i>Vibrio cholerae</i>	<i>Pseudomonas aeruginosa</i>
	<i>Shewanella oneidensis</i>	<i>Shewanella denitrificans</i>	<i>Shewanella sp.</i>	<i>Cobwellia psychrerythraea</i>	<i>Haemophilus influenzae</i>	<i>Escherichia coli</i>	<i>Vibrio cholerae</i>	<i>Pseudomonas aeruginosa</i>
	<i>Actinobacillus pleuropneumoniae</i>	<i>Actinobacillus succinogenes</i>	<i>Haemophilus influenzae</i>	<i>Mannheimia succiniciproducens</i>	<i>Escherichia coli</i>	<i>Vibrio cholerae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas putida</i>
	<i>Haemophilus ducreyi</i>	<i>Haemophilus influenzae</i>	<i>Mannheimia succiniciproducens</i>	<i>Pasteurella multocida</i>	<i>Escherichia coli</i>	<i>Vibrio cholerae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas putida</i>
	<i>Haemophilus influenzae</i>	<i>Mannheimia succiniciproducens</i>	<i>Pasteurella multocida</i>	<i>Haemophilus ducreyi</i>	<i>Escherichia coli</i>	<i>Vibrio cholerae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas putida</i>
	<i>Pasteurella multocida</i>	<i>Mannheimia succiniciproducens</i>	<i>Haemophilus influenzae</i>	<i>Actinobacillus succinogenes</i>	<i>Escherichia coli</i>	<i>Vibrio cholerae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas putida</i>
	<i>Actinobacillus succinogenes</i>	<i>Actinobacillus pleuropneumoniae</i>	<i>Haemophilus influenzae</i>	<i>Pasteurella multocida</i>	<i>Escherichia coli</i>	<i>Vibrio cholerae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas putida</i>
	<i>Mannheimia succiniciproducens</i>	<i>Haemophilus influenzae</i>	<i>Actinobacillus pleuropneumoniae</i>	<i>Pasteurella multocida</i>	<i>Escherichia coli</i>	<i>Vibrio cholerae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas putida</i>
	<i>Photorhabdus luminescens</i>	<i>Escherichia coli</i>	<i>Yersinia pestis KIM</i>	<i>Salmonella enterica</i>	<i>Vibrio cholerae</i>	<i>Haemophilus influenzae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas putida</i>
	<i>Yersinia pseudotuberculosis</i>	<i>Escherichia coli</i>	<i>Yersinia pestis KIM</i>	<i>Photorhabdus luminescens</i>	<i>Vibrio cholerae</i>	<i>Haemophilus influenzae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas aeruginosa</i>
	<i>Yersinia pestis KIM</i>	<i>Escherichia coli</i>	<i>Yersinia pseudotuberculosis</i>	<i>Photorhabdus luminescens</i>	<i>Vibrio cholerae</i>	<i>Haemophilus influenzae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas aeruginosa</i>
	<i>Yersinia intermedia</i>	<i>Escherichia coli</i>	<i>Yersinia pestis KIM</i>	<i>Photorhabdus luminescens</i>	<i>Vibrio cholerae</i>	<i>Haemophilus influenzae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas aeruginosa</i>
	<i>Yersinia frederiksenii</i>	<i>Escherichia coli</i>	<i>Yersinia pseudotuberculosis</i>	<i>Photorhabdus luminescens</i>	<i>Vibrio cholerae</i>	<i>Haemophilus influenzae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas aeruginosa</i>
	<i>Yersinia mollaretii</i>	<i>Escherichia coli</i>	<i>Yersinia pseudotuberculosis</i>	<i>Photorhabdus luminescens</i>	<i>Vibrio cholerae</i>	<i>Haemophilus influenzae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas aeruginosa</i>
	<i>Yersinia bercovieri</i>	<i>Escherichia coli</i>	<i>Yersinia pseudotuberculosis</i>	<i>Photorhabdus luminescens</i>	<i>Vibrio cholerae</i>	<i>Haemophilus influenzae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas aeruginosa</i>

<i>Salmonella typhimurium</i>	<i>Escherichia coli</i>	<i>Yersinia pestis KIM</i>	<i>Photorhabdus luminescens</i>	<i>Vibrio cholerae</i>	<i>Haemophilus influenzae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas aeruginosa</i>
<i>Salmonella enterica</i>	<i>Escherichia coli</i>	<i>Yersinia pestis KIM</i>	<i>Photorhabdus luminescens</i>	<i>Vibrio cholerae</i>	<i>Haemophilus influenzae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas aeruginosa</i>
<i>Escherichia coli</i>	<i>Yersinia pestis KIM</i>	<i>Salmonella enterica</i>	<i>Photorhabdus luminescens</i>	<i>Vibrio cholerae</i>	<i>Haemophilus influenzae</i>	<i>Shewanella oneidensis</i>	<i>Pseudomonas aeruginosa</i>

Table S3. Selenoprotein loss events identified in 25 bacterial selenoprotein families

Selenoprotein	Phylum	Organism	Sister organisms			Distant organisms			
			1	2	3	1	2	3	4
FdhA	<i>Firmicutes/Clostridia/Clostridium</i>	<i>Clostridium difficile</i>	<i>Clostridium perfringens</i> (-)	<i>Clostridium tetani</i> (-)	<i>Clostridium botulinum</i> (-)	<i>Thermoanaerobacter tengcongensis</i> (-)	<i>Moorella thermoacetica</i> (U)	<i>Desulfitobacterium hafniense</i> (U)	<i>Carboxydotherrnus hydrogenoformans</i> (U)
	<i>Firmicutes/Clostridia/Thermoanaerobacteriales</i>	<i>Moorella thermoacetica</i>	<i>Thermoanaerobacter ethanolicus</i> (-)	<i>Thermoanaerobacter tengcongensis</i> (-)	<i>Ammonifex degensii</i> (-)	<i>Carboxydotherrnus hydrogenoformans</i> (U)	<i>Desulfitobacterium hafniense</i> (U)	<i>Clostridium difficile</i> (U)	<i>Desulfotomaculum reducens</i> (U)
SeID	<i>Actinobacteria</i>	<i>Symbiobacterium thermophilum</i>	<i>Rubrobacter xylanophilus</i> (U)	<i>Bifidobacterium longum</i> (-)	<i>Tropheryma whipplei</i> (-)	<i>Kineococcus radiotolerans</i> (C)	<i>Mycobacterium avium</i> (C)	<i>Brevibacterium linens</i> (C)	<i>Corynebacterium diphtheriae</i> (-)
	<i>Actinobacteria</i>	<i>Rubrobacter xylanophilus</i>	<i>Symbiobacterium thermophilum</i> (U)	<i>Bifidobacterium longum</i> (-)	<i>Tropheryma whipplei</i> (-)	<i>Kineococcus radiotolerans</i> (C)	<i>Mycobacterium avium</i> (C)	<i>Brevibacterium linens</i> (C)	<i>Corynebacterium diphtheriae</i> (-)
	<i>Firmicutes/Clostridia/Clostridium</i>	<i>Clostridium difficile</i>	<i>Clostridium perfringens</i> (U)	<i>Clostridium tetani</i> (-)	<i>Clostridium botulinum</i> (-)	<i>Thermoanaerobacter tengcongensis</i> (U)	<i>Moorella thermoacetica</i> (U)	<i>Desulfitobacterium hafniense</i> (C)	<i>Carboxydotherrnus hydrogenoformans</i> (U)
	<i>Firmicutes/Clostridia/Clostridium</i>	<i>Clostridium perfringens</i>	<i>Clostridium difficile</i> (U)	<i>Clostridium tetani</i> (-)	<i>Clostridium botulinum</i> (-)	<i>Thermoanaerobacter tengcongensis</i> (U)	<i>Moorella thermoacetica</i> (U)	<i>Desulfitobacterium hafniense</i> (C)	<i>Carboxydotherrnus hydrogenoformans</i> (U)
	<i>Firmicutes/Clostridia/Thermoanaerobacteriales</i>	<i>Moorella thermoacetica</i>	<i>Thermoanaerobacter ethanolicus</i> (-)	<i>Thermoanaerobacter tengcongensis</i> (U)	<i>Ammonifex degensii</i> (-)	<i>Carboxydotherrnus hydrogenoformans</i> (U)	<i>Desulfitobacterium hafniense</i> (C)	<i>Clostridium difficile</i> (U)	<i>Desulfotomaculum reducens</i> (U)
	<i>Firmicutes/Clostridia/Thermoanaerobacteriales</i>	<i>Thermoanaerobacter tengcongensis</i>	<i>Moorella thermoacetica</i> (U)	<i>Thermoanaerobacter ethanolicus</i> (-)	<i>Ammonifex degensii</i> (-)	<i>Carboxydotherrnus hydrogenoformans</i> (U)	<i>Desulfitobacterium hafniense</i> (C)	<i>Clostridium difficile</i> (U)	<i>Desulfotomaculum reducens</i> (U)
	<i>Firmicutes/Clostridia/Thermoanaerobacteriales</i>	<i>Moorella thermoacetica</i>	<i>Thermoanaerobacter ethanolicus</i> (-)	<i>Thermoanaerobacter tengcongensis</i> (-)	<i>Ammonifex degensii</i> (-)	<i>Clostridium difficile</i> (-)	<i>Desulfotomaculum reducens</i> (U)	<i>Desulfitobacterium hafniense</i> (-)	<i>Carboxydotherrnus hydrogenoformans</i> (U)
FrhD	<i>Firmicutes/Clostridia/Peptococcaceae</i>	<i>Carboxydotherrnus hydrogenoformans</i>	<i>Desulfotomaculum reducens</i> (U)	<i>Desulfitobacterium hafniense</i> (-)		<i>Moorella thermoacetica</i> (U)	<i>Syntrophomonas wolfei</i> (U)	<i>Clostridium difficile</i> (-)	<i>Thermoanaerobacter tengcongensis</i> (-)
	<i>Firmicutes/Clostridia/Peptococcaceae</i>	<i>Desulfotomaculum reducens</i> (2)	<i>Carboxydotherrnus hydrogenoformans</i> (U)	<i>Desulfitobacterium hafniense</i> (-)		<i>Moorella thermoacetica</i> (U)	<i>Syntrophomonas wolfei</i> (U)	<i>Clostridium difficile</i> (-)	<i>Thermoanaerobacter tengcongensis</i> (-)
	<i>Firmicutes/Clostridia/Syntrophomonadaceae</i>	<i>Syntrophomonas wolfei</i>	<i>Caldicellulosiruptor saccharolyticus</i> (-)	<i>Anaerobranca gottschalkii</i> (-)		<i>Moorella thermoacetica</i> (U)	<i>Carboxydotherrnus hydrogenoformans</i> (U)	<i>Thermoanaerobacter tengcongensis</i> (-)	<i>Desulfotomaculum reducens</i> (U)
	<i>Firmicutes/Clostridia/Thermoanaerobacteriales</i>	<i>Moorella thermoacetica</i>	<i>Thermoanaerobacter ethanolicus</i> (-)	<i>Thermoanaerobacter tengcongensis</i> (-)	<i>Ammonifex degensii</i> (-)	<i>Clostridium difficile</i> (-)	<i>Syntrophomonas wolfei</i> (U)	<i>Desulfotomaculum reducens</i> (U)	<i>Carboxydotherrnus hydrogenoformans</i> (U)
HdrA	<i>Firmicutes/Clostridia/Peptococcaceae</i>	<i>Carboxydotherrnus hydrogenoformans</i>	<i>Desulfotomaculum reducens</i> (U)	<i>Desulfitobacterium hafniense</i> (-)		<i>Moorella thermoacetica</i> (U)	<i>Syntrophomonas wolfei</i> (U)	<i>Clostridium difficile</i> (-)	<i>Thermoanaerobacter tengcongensis</i> (-)
	<i>Firmicutes/Clostridia/Peptococcaceae</i>	<i>Desulfotomaculum reducens</i>	<i>Carboxydotherrnus hydrogenoformans</i> (U)	<i>Desulfitobacterium hafniense</i> (-)		<i>Moorella thermoacetica</i> (U)	<i>Syntrophomonas wolfei</i> (U)	<i>Clostridium difficile</i> (-)	<i>Thermoanaerobacter tengcongensis</i> (-)
	<i>Firmicutes/Clostridia/Syntrophomonadaceae</i>	<i>Syntrophomonas wolfei</i>	<i>Caldicellulosiruptor saccharolyticus</i> (-)	<i>Anaerobranca gottschalkii</i> (-)		<i>Moorella thermoacetica</i> (U)	<i>Carboxydotherrnus hydrogenoformans</i> (U)	<i>Thermoanaerobacter tengcongensis</i> (-)	<i>Desulfotomaculum reducens</i> (-)
	<i>Actinobacteria</i>	<i>Symbiobacterium thermophilum</i>	<i>Rubrobacter xylanophilus</i> (-)	<i>Bifidobacterium longum</i> (-)	<i>Tropheryma whipplei</i> (-)	<i>Kineococcus radiotolerans</i> (C)	<i>Mycobacterium avium</i> (C)	<i>Brevibacterium linens</i> (C)	<i>Corynebacterium diphtheriae</i> (C)
HesB-like	<i>Deltaproteobacteria/Geobacteraceae</i>	<i>Geobacter sulfurreducens</i>	<i>Geobacter metallireducens</i> (-)	<i>Geobacter uraniumreducens</i> (-)	<i>Pelobacter carbinolicus</i> (-)	<i>Syntrophobacter fumaroxidans</i> (U)	<i>Desulfovibrio vulgaris</i> (U)	<i>Syntrophus aciditrophicus</i> (U)	<i>Desulfuromonas acetoxidans</i> (U)
	<i>Firmicutes/Clostridia/Peptococcaceae</i>	<i>Desulfitobacterium hafniense</i>	<i>Carboxydotherrnus hydrogenoformans</i> (-)	<i>Desulfotomaculum reducens</i> (-)		<i>Clostridium perfringens</i> (U)	<i>Alkaliphilus metalliredigens</i> (U)	<i>Thermoanaerobacter tengcongensis</i> (-)	<i>Moorella thermoacetica</i> (-)
GrdA	<i>Firmicutes/Clostridia/Peptococcaceae</i>	<i>Carboxydotherrnus hydrogenoformans</i>	<i>Desulfotomaculum reducens</i> (-)	<i>Desulfitobacterium hafniense</i> (-)		<i>Thermoanaerobacter tengcongensis</i> (U)	<i>Clostridium difficile</i> (U)	<i>Moorella thermoacetica</i> (-)	<i>Syntrophomonas wolfei</i> (-)

	<i>Firmicutes/Clostridia/Thermoanaerobacteriales</i>	<i>Thermoanaerobacter tengcongensis</i>	<i>Moorella thermoacetica</i> (-)	<i>Thermoanaerobacter ethanolicus</i> (-)	<i>Ammonifex degensii</i> (-)	<i>Carboxydotherrnus hydrogenoformans</i> (U)	<i>Clostridium difficile</i> (U)	<i>Desulfitobacterium hafniense</i> (-)	<i>Desulfotomaculum reducens</i> (-)
	<i>Firmicutes/Clostridia/Clostridium</i>	<i>Clostridium difficile</i>	<i>Clostridium perfringens</i> (-)	<i>Clostridium tetani</i> (-)	<i>Clostridium botulinum</i> (-)	<i>Moorella thermoacetica</i> (-)	<i>Carboxydotherrnus hydrogenoformans</i> (U)	<i>Thermoanaerobacter tengcongensis</i> (U)	<i>Desulfitobacterium hafniense</i> (-)
	<i>Firmicutes/Clostridia</i>	<i>Alkaliphilus metalliredigenes</i>	<i>Clostridium difficile</i> (U)	<i>Clostridium perfringens</i> (-)	<i>Clostridium tetani</i> (-)	<i>Carboxydotherrnus hydrogenoformans</i> (U)	<i>Thermoanaerobacter tengcongensis</i> (U)	<i>Desulfotomaculum reducens</i> (-)	<i>Moorella thermoacetica</i> (-)
	<i>Deltaproteobacteria/Syntrophobacterales</i>	<i>Syntrophus aciditrophicus</i>	<i>Syntrophus buswellii</i> (-)	<i>Syntrophobacter fumaroxidans</i> (-)		<i>Desulfuromonas acetoxidans</i> (U)	<i>Desulfotalea psychrophila</i> (-)	<i>Desulfovibrio vulgaris</i> (-)	<i>Geobacter sulfurreducens</i> (-)
	<i>Deltaproteobacteria/Desulfuromonadales</i>	<i>Desulfuromonas acetoxidans</i>	<i>Geobacter metallireducens</i> (-)	<i>Geobacter sulfurreducens</i> (-)	<i>Pelobacter carbinolicus</i> (-)	<i>Syntrophus aciditrophicus</i> (U)	<i>Desulfotalea psychrophila</i> (-)	<i>Anaeromyxobacter dehalogenans</i> (-)	<i>Desulfovibrio vulgaris</i> (-)
	<i>Firmicutes/Clostridia/Peptococcaceae</i>	<i>Carboxydotherrnus hydrogenoformans</i>	<i>Desulfotomaculum reducens</i> (-)	<i>Desulfitobacterium hafniense</i> (-)		<i>Thermoanaerobacter tengcongensis</i> (U)	<i>Clostridium difficile</i> (U)	<i>Moorella thermoacetica</i> (-)	<i>Syntrophomonas wolfei</i> (-)
GrdB	<i>Firmicutes/Clostridia/Thermoanaerobacteriales</i>	<i>Thermoanaerobacter tengcongensis</i>	<i>Moorella thermoacetica</i> (-)	<i>Thermoanaerobacter ethanolicus</i> (-)	<i>Ammonifex degensii</i> (-)	<i>Carboxydotherrnus hydrogenoformans</i> (U)	<i>Clostridium difficile</i> (U)	<i>Desulfitobacterium hafniense</i> (-)	<i>Desulfotomaculum reducens</i> (-)
	<i>Firmicutes/Clostridia/Clostridium</i>	<i>Clostridium difficile</i>	<i>Clostridium perfringens</i> (-)	<i>Clostridium tetani</i> (-)	<i>Clostridium botulinum</i> (-)	<i>Carboxydotherrnus hydrogenoformans</i> (U)	<i>Thermoanaerobacter tengcongensis</i> (U)	<i>Moorella thermoacetica</i> (-)	<i>Desulfitobacterium hafniense</i> (-)
	<i>Firmicutes/Clostridia</i>	<i>Alkaliphilus metalliredigenes</i>	<i>Clostridium difficile</i> (U)	<i>Clostridium perfringens</i> (-)	<i>Clostridium tetani</i> (-)	<i>Carboxydotherrnus hydrogenoformans</i> (U)	<i>Thermoanaerobacter tengcongensis</i> (U)	<i>Desulfotomaculum reducens</i> (-)	<i>Moorella thermoacetica</i> (-)
	<i>Actinobacteria</i>	<i>Symbiobacterium thermophilum</i>	<i>Rubrobacter xylanophilus</i> (-)	<i>Bifidobacterium longum</i> (-)	<i>Tropheryma whipplei</i> (-)	<i>Kineococcus radiotolerans</i> (C)	<i>Streptomyces avermitilis</i> (C)	<i>Mycobacterium avium</i> (-)	<i>Corynebacterium diphtheriae</i> (-)
SelW-like	<i>Deltaproteobacteria/Geobacteraceae</i>	<i>Geobacter sulfurreducens</i>	<i>Geobacter metallireducens</i> (-)	<i>Geobacter uraniumreducens</i> (-)	<i>Pelobacter carbinolicus</i> (-)	<i>Desulfotalea psychrophila</i> (U)	<i>Desulfovibrio vulgaris</i> (-)	<i>Syntrophus aciditrophicus</i> (-)	<i>Desulfuromonas acetoxidans</i> (C)
	<i>Deltaproteobacteria/Desulfobacterales</i>	<i>Desulfotalea psychrophila</i>	<i>Desulfobacterium autotrophicum</i> (-)	<i>Nitrospina gracilis</i> (-)		<i>Geobacter sulfurreducens</i> (U)	<i>Desulfuromonas acetoxidans</i> (C)	<i>Desulfovibrio vulgaris</i> (-)	<i>Anaeromyxobacter dehalogenans</i> (-)
DsbA-like	<i>Firmicutes/Clostridia/Peptococcaceae</i>	<i>Desulfotomaculum reducens</i>	<i>Carboxydotherrnus hydrogenoformans</i> (-)	<i>Desulfitobacterium hafniense</i> (-)		<i>Clostridium difficile</i> (C)	<i>Thermoanaerobacter tengcongensis</i> (C)	<i>Moorella thermoacetica</i> (-)	<i>Syntrophomonas wolfei</i> (-)
ArsC	<i>Deltaproteobacteria/Desulfuromonadales</i>	<i>Desulfuromonas acetoxidans</i>	<i>Geobacter metallireducens</i> (-)	<i>Geobacter sulfurreducens</i> (-)	<i>Pelobacter carbinolicus</i> (-)	<i>Desulfotalea psychrophila</i> (U)	<i>Syntrophus aciditrophicus</i> (-)	<i>Anaeromyxobacter dehalogenans</i> (-)	<i>Desulfovibrio vulgaris</i> (-)
	<i>Deltaproteobacteria/Desulfobacterales</i>	<i>Desulfotalea psychrophila</i>	<i>Desulfobacterium autotrophicum</i> (-)	<i>Nitrospina gracilis</i> (-)		<i>Desulfuromonas acetoxidans</i> (U)	<i>Anaeromyxobacter dehalogenans</i> (-)	<i>Geobacter sulfurreducens</i> (-)	<i>Desulfovibrio vulgaris</i> (-)
AhpF	<i>Firmicutes/Clostridia/Peptococcaceae</i>	<i>Carboxydotherrnus hydrogenoformans</i>	<i>Desulfotomaculum reducens</i> (-)	<i>Desulfitobacterium hafniense</i> (C)		<i>Clostridium difficile</i> (C)	<i>Moorella thermoacetica</i> (-)	<i>Syntrophomonas wolfei</i> (-)	<i>Thermoanaerobacter tengcongensis</i> (-)
NADH oxidase	<i>Deltaproteobacteria/Geobacteraceae</i>	<i>Geobacter metallireducens</i>	<i>Geobacter sulfurreducens</i> (C)	<i>Geobacter uraniumreducens</i> (-)	<i>Pelobacter carbinolicus</i> (-)	<i>Desulfotalea psychrophila</i> (C)	<i>Desulfovibrio vulgaris</i> (C)	<i>Syntrophus aciditrophicus</i> (C)	<i>Desulfuromonas acetoxidans</i> (-)

Table S4. Relationship between selenium utilization traits and oxygen requirement

	Sec trait (only)	Sec and Selenouridine traits	Selenouridine trait	No selenium utilization trait s	Total
Anaerobic	13	11	5	27	56
Facultative	12	14	17	73	116
Microaerophilic	3	6	2	21	32
Aerobic	5	11	22	107	145
Total	33	42	46	228	349

Table S5. Relationship between selenium utilization traits and optimal growth temperature

	Sec trait (only)	Sec and Selenouridine traits	Selenouridine trait	No selenium utilization trait s	Total
< 20 °C (Psychrophilic)	0	2	3	6	11
20~30 °C	13	17	40	117	187
30~40 °C	15	21	3	93	132
> 40 °C (Thermophilic)	5	2	0	12	19
Total	33	42	46	228	349

Table S6. Relationship between Sec-/Cys-containing Prx and oxygen requirement

	Prx (Sec)	Prx (Cys in Sec⁺)*	Prx (Cys in Sec⁻)*	Prx lacking	Total
Anaerobic	7	10	33	6	56
Facultative	2	15	90	9	116
Microaerophilic	0	14	6	12	32
Aerobic	1	17	122	5	145
Total	10	56	251	32	349

Sec⁺: Organisms containing the Sec-decoding trait

Sec⁻: Organisms lacking the Sec-decoding trait

Table S7. Relationship between Sec-/Cys-containing HesB-like and oxygen requirement

	HesB-like (Sec)	HesB-like (Cys in Sec⁺)	HesB-like (Cys in Sec⁻)	HesB-like lacking	Total
Anaerobic	7	5	3	41	56
Facultative	2	4	14	96	116
Microaerophilic	2	5	20	5	32
Aerobic	0	6	26	113	145
Total	11	20	63	255	349